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AGE AT MENARCHE IN RELATION TO BODY SIZE AND PHYSIOLOGICAL VARIABLES AMONG RAJPUT GIRLS

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Abstract

Body size and physiological variables of Rajput adolescent girls have been studied in relation to their age at menarche. Cross-sectional data based on four anthropometric measurements, three physiological traits and two derived measures were collected from 200 Rajput girls aged 10 to 17 years, belonging to Solan District of Himachal Pradesh. Information on age at menarche was obtained using status quo method. Results indicate that girls in whom menarche had occurred were taller, heavier, had higher mean value of body mass index and lower value of waist-hip ratio than those who had not experienced this phenomenon. Early maturing girls also exhibited higher mean values for systolic and diastolic blood pressure, higher pulse rate and greater grip strength than their late maturing peers during adolescence.

Key words: Menarche, Physiological variables, Rajput girls.

INTRODUCTION

A reliable marker of sexual development in girls is the commencement of menstruation, which is also associated with skeletal development (Bailey and Garn, 1986). Age at menarche is influenced by genetics but social class differences, diet, physical activity, anthropometric characteristics and various physiological parameters have all been shown to be associated with it (Berkey *et al.*, 2000; Burke *et al.*, 1992; Dare *et al.*, 1992; Elizondo, 1992; Fisher and Yates, 1957; Forbes, 1992; Garn and LaVelle, 1983; Merzenich *et al.*, 1993; Meyer *et al.*, 1990; Rimpela and Rimpela, 1993; Santon *et al.*, 1992; Talwar and Kaur, 2003). A review of literature reveals numerous studies on the relationship between anthropometric measurements and menarcheal status, but very little has been reported concerning possible changes in physiological variables in relation to age at menarche, especially on regional populations in India. Therefore, the present study has been conducted on Rajput adolescent girls from District Solan to evaluate the effect of menarcheal status on physical and physiological traits.

MATERIALS AND METHODS

The present study is based on a cross-sectional sample of 200 Rajput girls ranging in age from 10 to 17 years, belonging to Dharampur, Kumarhatti, Dagshai and Jabli areas of Solan District, Himachal Pradesh. Data were largely obtained from various Government schools located in the area. The anthropometric measurements taken included height, weight, waist circumference, and hip circumference. Physiological variables studied were systolic and diastolic blood pressures, pulse rate, and grip strength. Information on age at menarche was obtained using *status quo* method. The date of birth of girls was recorded from their school registers. All doubtful cases were excluded. The ages were calculated using the decimal age calendar. The body measurements were taken by following standard techniques recommended by Wiener and Lourie (Wiener and Lourie, 1969).

RESULTS AND DISCUSSION

The total number of girls in each age group and the number and percentage of girls who attained menarche in that group is given in Table 1. In our sample no girl had experienced menarche before the age of 12 years and all the girls of 16 or above had experienced this event. Using the probit transformation (Fisher and Yates, 1957) the median age of onset of menarche of Rajput girls has been found to be 13.00 ± 0.06 years.

Comparisons between the early and late maturing Rajput girls for body weight, stature, waist circumference, hip circumference, systolic and diastolic blood pressure, pulse rate and grip strength have been made in Table 2. Our results clearly indicate that the menstruating girls are heavier, taller, and also possess greater values of body mass index than the non-menstruating girls

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between 12 to 15 years. However, they reveal significant differences for height and weight at 12 and 13 years and for body mass index at 13 years. These findings are in accordance with earlier studies (Beunen, 1989; Ghai, 1977; Kaul and Corruccini, 1985; Moisan *et al.*, 1990; Singh *et al.*, 1998; Talwar and Singh, 1994; Zacharias and Rand, 1983). The early maturing girls from 12 to 15 years have greater mean values for waist as well as hip circumference as compared to their late maturing counterparts. However, the magnitude of difference is more in hip circumference than in waist circumference as is evident from the statistically significant t-values obtained at 12 and 13 years. Due to significantly greater hip circumference menstruating girls show lower value for waist-hip ratio than the non-menstruating girls.

It may be generalised from the foregoing results that early maturing girls are taller, heavier, and possess larger body dimensions than the late maturing girls, thereby, indicating that the girls who are ahead in sexual maturity are also ahead in physical maturity.

The onset of puberty is associated with increase in blood pressure. Greater body size seems to be the element that best predicts both earlier sexual maturation and increased blood pressures. Among the girls of the present study systolic and diastolic pressure was found to be greater in early maturing girls as compared to late maturing girls. Statistically significant differences have been reported at 14 years only. Data analysed from the United States Health Examination Survey on adolescents aged 12 to 17 years revealed the importance of physiological maturity to blood pressure (Cornoni-Huntley *et al.*, 1979). Girls blood pressure increased markedly during the pre-pubescent growth spurt at age 10 to 11 years and stabilized after puberty. Menstruating girls seem to reach their adult or near adult blood pressure earlier than the late maturerers due to their head start in puberty.

It appears that body size and body mass measured in various studies in terms of height, weight and skinfold thickness, influence both the timing of sexual maturation and the level of blood pressure (Gerber and Stern, 1999; Daniels *et al.*, 1996) demonstrated that neither ethnicity nor sexual maturation is associated with blood pressure independent of body size. On the other hand, skeletal age was an independent measure of physiological maturity that predicted systolic blood pressure in both boys and girls when weight was omitted from the regression models (Roche *et al.*, 1976). Sexual maturation as measured by Tanner's criteria (Tanner, 1962) predicted blood pressure only when weight and skeletal age were omitted from the analysis.

Early maturing girls of our study also show greater mean values for pulse rate and grip strength than their late maturing counterparts from 12 to 15 years. During adolescence, maturity relationships with strength are more apparent for boys than are for girls. However, early maturing girls are stronger than their late maturing peers (Beunen *et al.*, 1976; Carron *et al.*, 1977). The differences are most apparent in girls aged 11 to 15 years and are reduced somewhat by 16 and 17 years of age. Girls in the present sample also exhibit similar findings.

Table 1: Total number and percentage of Rajput girls who attained menarche

Age Group	No.	Menarche	%	Probit value
9.500-10.499	25	-	-	-
10.500-11.499	25	-	-	-
11.500-12.499	25	5	20.00	4.16
12.500-13.499	25	10	40.00	4.75
13.500-14.499	25	16	64.00	5.36
14.500-15.499	25	23	92.00	6.40
15.500-16.499	25	25	100.00	-
16.500-17.499	25	25	100.00	-

Table 2: Means and standard deviations of Anthropometric measurements, Physiological variables, BMI and WHR of Rajput girls in relation to their age at menarche.

	12 years			13 years		
	Menarche occurred	Menarche not occurred		Menarche occurred	Menarche not occurred	
	(N=5)	(N=20)		(N=10)	(N=15)	
	Mean ±SD	Mean ± SD	t-value	Mean ± SD	Mean ± SD	t-value
Weight (kg)	38.00 ± 4.24	31.80 ± 5.77	2.16*	39.05 ± 6.19	32.73 ± 6.60	2.35*
Height (cm)	148.70 ± 6.79	137.41 ± 10.74	2.15*	149.87 ± 4.05	143.74 ± 7.93	2.21*
BMI	17.28 ± 2.05	16.90 ± 1.21	0.52	17.55 ± 1.15	15.77 ± 2.38	2.17*
Waist Circumference (cm)	57.25 ± 5.03	53.85 ± 4.92	1.32	57.62 ± 4.73	56.08 ± 5.62	0.70
Hip Circumference (cm)	77.75 ± 4.62	68.10 ± 8.08	2.46	76.75 ± 5.17	71.83 ± 5.99	2.08*
WHR	0.73 ± 0.03	0.79 ± 0.04	4.00**	0.75 ± 0.04	0.78 ± 0.04	-1.87
Systolic Blood Pressure (mm/hg)	114.00 ± 3.16	111.30 ± 7.17	0.78	119.00 ± 3.16	117.20 ± 4.52	1.07
Diastolic Blood Pressure (mm/hg)	77.60 ± 4.33	75.35 ± 8.56	0.54	80.90 ± 2.68	78.06 ± 5.71	1.44
Pulse Rate	77.20 ± 3.70	75.90 ± 2.90	0.81	79.00 ± 2.90	75.26 ± 4.41	2.32*
Grip Strength (kg)	17.90 ± 1.97	16.75 ± 4.00	0.59	18.00 ± 2.44	17.00 ± 1.61	1.21
*p<0.05 **p<0.01 ***p<0.001						

	14 years			15 years		
	Menarche occurred	Menarche not occurred		Menarche occurred	Menarche not occurred	
	(N=16)	(N=9)		(N=23)	(N=2)	
	Mean ± SD	Mean ± SD	t-value	Mean ± SD	Mean ± SD	t-value
Weight (kg)	41.46 ± 5.80	37.77 ± 1.71	1.82	43.78 ± 4.45	40.00 ± 7.07	1.05
Height (cm)	151.30 ± 5.16	146.00 ± 4.66	2.50*	154.02 ± 3.77	151.30 ± 2.82	0.96
BMI	18.20 ± 2.26	17.72 ± 1.26	0.57	18.45 ± 0.19	17.49 ± 1.73	1.26
Waist Circumference (cm)	60.31 ± 5.81	58.75 ± 2.79	0.73	59.07 ± 3.69	58.75 ± 5.30	0.10
Hip Circumference (cm)	80.54 ± 5.86	76.52 ± 4.36	1.75	80.82 ± 4.75	79.37 ± 4.41	0.40
WHR	0.74 ± 0.03	0.76 ± 0.05	-1.25	0.73 ± 0.03	0.74 ± 0.02	-0.50
Systolic Blood Pressure (mm/hg)	118.37 ± 2.33	115.70 ± 2.53	2.61*	119.00 ± 3.08	116.00 ± 5.65	1.20
Diastolic Blood Pressure (mm/hg)	78.87 ± 3.00	75.11 ± 3.01	2.93*	80.13 ± 4.42	76.00 ± 2.82	1.29
Pulse Rate	72.31 ± 2.32	70.00 ± 3.67	1.87	72.00 ± 4.30	70.00 ± 1.41	0.63
Grip Strength (kg)	18.18 ± 2.09	17.16 ± 2.92	0.99	20.42 ± 3.10	18.75 ± 1.27	0.73
*p<0.05 **p<0.01 ***p<0.001						

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MOLECULAR BASIS OF NUTRIENT ABSORPTION FROM INTESTINE

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Abstract

Absorption of nutrients from small intestine follows a number of pathways. Among these sodium dependent, carrier mediated uphill transport mechanism plays a major role in the uptake process. Transport proteins involved in the absorption of nutrients have been cloned and expressed in various _expression systems. The kinetic characteristics of the expressed Na-Solute cotransporter are identical to that in the intact tissue. These advances have greatly helped in understanding the molecular basis of nutrient absorption and malabsorption from intestine.

Key words : Na-Solute cotransporter, Intestinal absorption, Active transport.

The intestinal tissue is remarkable in carrying out the digestive and absorptive functions in the body. Complex macromolecules such as polysaccharides and proteins moving across the length of the digestive tract are progressively degraded into simple molecules such as amino acids and sugars (monosaccharides), which are then taken up by the epithelial cells lining the lumen of small intestine. In humans, the length of intestine runs into several meters. Luminally, it is folded into tiny projections called villi. The surface of the villi is covered with a single layer of epithelial cells or "enterocytes". Nearly 90% of all the cells lining the villi are the absorbing epithelial cells. Mature enterocytes are columnar bipolar cells(Madara et al, 1994). The surface protruding into the lumen is folded into finger like processes called 'microvilli' and the contraluminal surface is embedded into the underlying muscular tissue. The presence of nearly 600 to 1000 microvilli per cell markedly enhances the absorbing surface of the enterocytes(Tier,1967; Cheng and Leblond,1974)..

The plasma membrane lining the microvilli is a mosaic of lipoprotein complexes, which in addition to organic solute transport proteins contains many digestive enzymes such as disaccharidases, peptidases, alkaline phosphatase and ATPases. Electron microscopic studies have shown that microvillus membrane is a typical lipid bilayer with a width of around 110Å. The luminal surface of the microvilli is covered with a carbohydrate rich fuzzy coat known as 'glycocalyx'(Moog, 1981). This structure is highly hydrophilic and is known to trap small ions and forms unstirred water layer (60Å width) in the vicinity of the microvilli.

Techniques to study nutrient absorption:

A number of *in vivo* and *in vitro* techniques have been used to establish the mechanisms responsible for the absorption of nutrients from the intestine. *In vivo* studies, involve the feeding of solutes in diet and their determination in blood or in the excretory products. The difference in the amount of the solute fed and that appearing in blood or being excreted may indicate the absorption of solute from intestine. Such a method, however does not, give any clue about the mode of solute transport or its metabolic degradation, the source of energy and structural requirements of the solute molecule being absorbed from the intestine. However, the use of *in vitro* techniques has immensely helped in elucidating the molecular mechanisms of absorption from intestine. Various *in vitro* techniques include the use of: (a) Everted sacs or blind sacs, (b) Everted rings, (c) scrapped mucosal layer, (d) Isolated epithelial cells and (e) Inside out or right side out vesicles made from microvillus membranes. The tissue preparations are incubated with the radiolabelled solutes and the amount of radioactivity taken up is determined. The rate of solute transport is determined taking into consideration the tissue weight, time of incubation and amount of the solute absorbed by the tissue. Solute transport rate is corrected for the extracellular space (ecs) determined using radiolabelled non-metabolizable and non-absorbable molecules such as H^3- mannitol or H^3- inulin. In intact rodent intestinal tissue, ecs comprises

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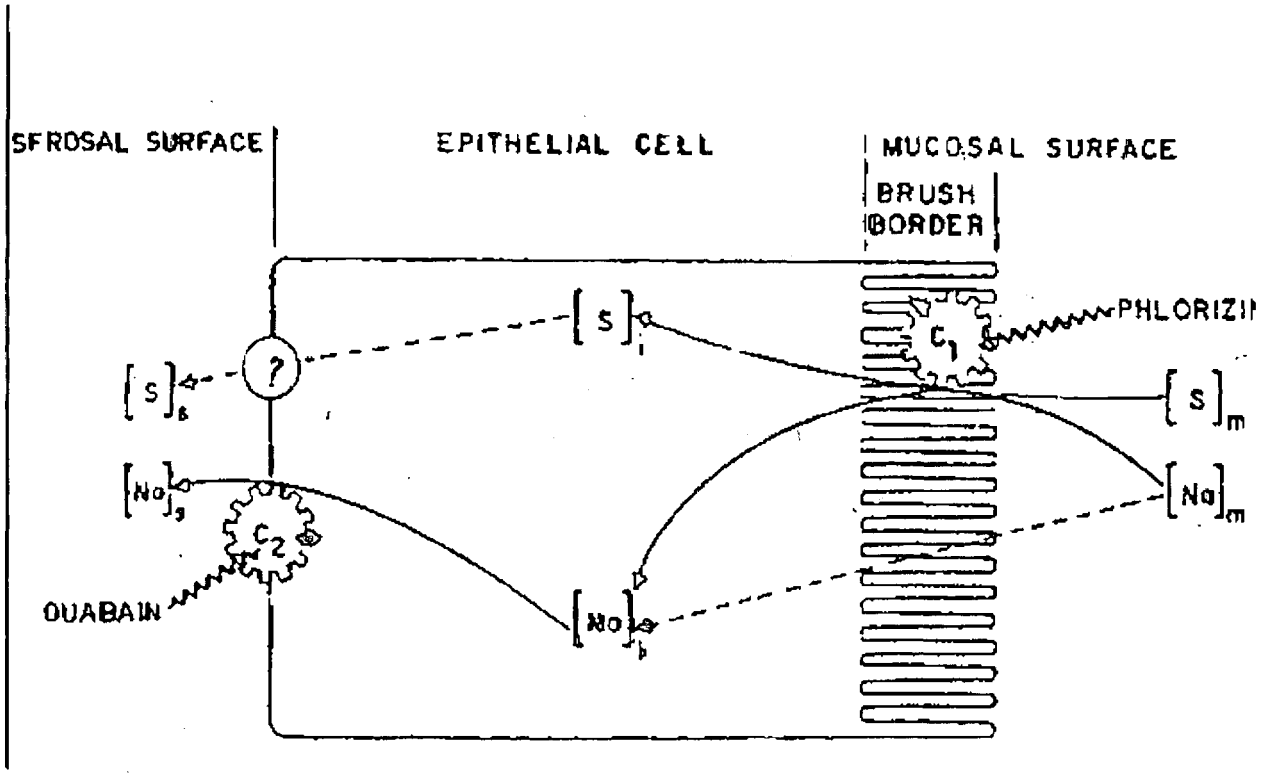

Figure 1: A model for Na^+- gradient hypothesis for the Na^+- substrate co-transport in epithelial cell.

On mucosal surface (m), substrate (S) binds the transporter (C) in presence of Na^+ ions to form the ternary complex S-C- Na complex. This complex translocates to the cell interior, where Na^+ ions dissociate resulting in low affinity of the carrier for the S. Substrate thus dissociates inside the cell. To maintain electroneutrality, Na^+ ions are pumped out of the cell by Na^+- K^+ ATPase (C2) located at the basolateral side of the enterocyte.

between 5-12% of the solute concentration (Robinson and Alvarado, 1971).

Use of radiolabelled non- metabolizable structural analogs of sugars such as alpha-methyl glucose, 3-O-methyl glucose or amino acid analog, H^3-cycloleucine have greatly helped the elucidation of transport mechanisms. It has been established that a majority of nutrients (sugars, amino acids, water soluble vitamins, choline, bile salts, calcium, phosphate and trace metal) are absorbed by passive diffusion, facilitative diffusion, water drag and by carrier mediated active transport processes. Among these, active transport mechanisms play a major role in the absorption of these solutes from intestine. Oils and fats being insoluble in aqueous media follow a different pathways for their absorption from intestine (Mahmood *et al*, 1993).

Mechanism of nutrient absorption:

Elegant studies employing both *in vivo* and *in vitro* techniques have suggested that a majority of nutrients are absorbed by a carrier mediated, energy dependent uphill transport systems from intestine (Crane, 1977; Alvarado and Mahmood, 1974; Mahmood and Alvarado, 1975, Kramer, 1994). The energy for the process is provided by the chemical and potential difference due to unequal distribution of sodium ions across microvillus membrane (Crane, 1965). Sodium concentration outside the cell is around 110-120 mM, whereas intracellularly, it is of the order of 5-10mM. The sodium ions coupled to solute molecule move towards their gradient (high to low) and each cycle results in the transport of a single solute molecule, inside the cell. The process is rheogenic, since each cycle results in accumulation of a positively charged sodium ion. Binding of sodium ions to the transporter enhances its affinity for the solute molecules, which is translocated into the cell interior. To maintain electroneutrality, excess of Na^+ ions are removed from inside the cell by sodium pump, located at the basolateral side of the enterocytes (Figure 1). The solute molecules enter the blood stream by a facilitative transport mechanism from the basolateral side of the cell. It has been estimated that about 300 molecules of the glucose are absorbed per second per cell (Moog, 1981). Sodium gradient across the

microvillus surface provides nearly 60% of the energy involved in uphill transport of the solutes from intestine. In addition to sodium gradient, the role of proton gradient in active transport of nutrients has also been suggested (Mahmood *et al*, 1986; Vadivel and Leibach, 1985; Kaur *et al*, 1995). *p*H inside the enterocytes is around 7.2-7.4 but in the vicinity of luminal membrane it is around 6-6.5. Thus under physiological conditions, there exists a proton gradient across the microvillus surface. Imposing proton gradient has been shown to stimulate the transport of glucose and amino acids in intestine. Thus pH gradient is also implicated in the uphill transport of organic solutes from intestine.

The molecules responsible for the active transport of nutrients from intestine have been isolated and characterized (Turk and Wright, 1997; Bissonnette *et al*, 1999). The purified carrier molecule (co-transporter) from a number of animal species and from human intestine is protein with a molecular weight of 69-73 kDa (Mackenzie *et al*, 1994). More than 30 such transport proteins have been identified for various solute molecules (Turk and Wright, 1997). These proteins bind solute molecule and the binding is dependent upon Na^+ ions. The kinetics of solute binding and stimulation by sodium ions are identical to that observed in intact intestinal tissue. The binding of solute and sodium to the transporter protein exhibit stiochemetery of 1:1 or 1:2, as seen in the intact intestinal preparation. The purified transport proteins have been reconstituted into proteoliposomes and the vesicles so produced; transport the solute molecules by a sodium dependent process against the concentration gradient. As expected the process is dependent upon the chemical and potential energy due to sodium ions. Amino acid sequence of the transport carrier has shown that protein is highly hydrophobic in nature and is made up of 662 amino acid residues (sodium dependent glucose transporter SGLT1). The N-terminal residues protrude to the luminal surface of the enterocytes, whereas the C-terminal is cytoplasmic. Hydropathy plots have indicated that the transporter molecules span the lipid bilayer 11-14 times and protein polymers seem to form a channel in the membrane (Turk and Wright, 1997). They undergo conformational changes upon binding and translocation of solute molecules during absorption (Figure 2).

Sodium-Solute Cotransporter:

Transporters for a variety of organic solutes in humans and various animal species have been cloned (You *et al*, 1995; Hirayama *et al*, 1996). The gene SGLT1 responsible for glucose absorption in humans is located on chromosome number 22 having localization at 11.2q. The identified genes for amino acids and sugars show 60-89% homology in base sequence. Several isoforms of the solute transporters have been identified, e.g. Sodium-glucose cotransporter has four isoforms as SGLT1, SGLT2, SGLT3, SAAT1 which differ in their affinity for various solutes and response to temperature (Martin *et al*, 2000). Na-glucose cotransporter (SGLT1)

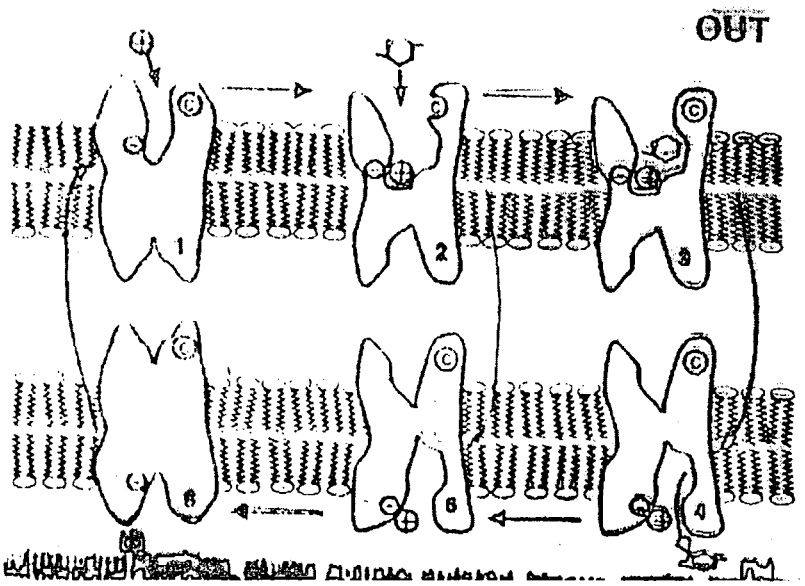

Figure 2: Na^+- solute co-transporter undergoes conformational changes, as the solute and Na^+ ions bind the transporter. This translocates the Na^+ ions and the substrate into the cell interior (Taken from Turk and Wright, 1997).

also transports certain neutral amino acids and the transport system has been named as SAAT1 (Kong *et al*, 1993). Veyhl *et al* (1998) demonstrated that SAAT1 can transport tumor suppressing drug, D19575 in human tumor cells. These observations suggest multiple functions of the transport protein in cell systems. Based on mutual interactions between sugars and amino acids (Alvarado, 1966) proposed polyfunctional nature of the transport carrier. Studies on Na- dependent transport systems, their cloning and _expression of the gene encoding the transport protein suggests, that indeed the transporter is polyfunctional, and translocates not only sugars and amino acids but certain anti -tumor drugs also (Veyhl *et al*,1998). The _expression of transporter genes in a number of mammalian cells and in frog oocytes has been achieved (Umbach *et al*, 1990). Electrophysiological and transport measurements have shown, the process is sodium dependent uphill transport (Sempedra *et al*, 2000).

The identification of genes for glucose- galactose transport in intestine has further shown genetic variations in the base sequences, which are responsible for glucose-galactose malabsoption, a genetic disorder. Studies with patients of glucose- galactose malabsorption syndrome indicated that mutation at position 92, in which Guanine (G) is replaced by Adenine (A), associated with a change of Asp28 to Ans28, is responsible for this genetic disorder (Turk *et al*, 1991).

Glucose transport systems responsible for facilitative transport represent a family of GLUT transporters, which are located at the basolateral site of the enterocytes. Several isoforms of GLUT systems have been identified and cloned from various systems (Thorens, 1996). These include a large family of GLUT isoforms (Joost and Thorens, 2001).

All the evidence accumulated so far, namely (a) functional activity, (b) chemical structure, isolation and reconstitution *in vitro* and (c) cloning of the transporter genes and there expressions in cell systems indicate that solute transporters are proteins. They exhibit kinetic properties similar to an enzyme system. Thus their characteristics can be explained by classical bisubstrate-enzyme kinetics.

Conclusions and unresolved Questions:

The identification of the solute transport mechanisms in intestine has helped to understand the pathophysiology of malabsorption and diarrhoea in human populations. The genetic basis of the sugar malabsorption syndrome has also been defined. However, some questions still remain unresolved such as: (i) How such a large transport protein molecule undergoes conformational change in response to organic solute and sodium ions binding, (ii) Does the energy provided by the chemical and potential gradient of sodium ions is sufficient for the conformational change that transporter molecules undergo, (iii) Role of the lipid micro environment surrounding the transport protein needs to be defined, (iv) Identification of the amino acid residues at the solute and the metal ion binding sites need further studies. How the activities of the transport molecules are modulated in diseased conditions is an important area of investigations. Modifications by site-directed mutagenesis can help to resolve some of these questions and can provide further insight into the molecular mechanisms of organic solute absorption and malabsorption.

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WHY HAVE OPEN SOURCE APPLICATIONS CAUGHT THE EYE OF HIGHER EDUCATION?

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Abstract

The choices of software in an educational environment are based upon a range of issues; much of which involve weighing the risks against the benefits about the purposes to which the software is to be put. The educational institutes are interested in increasing the interoperability between software systems for teaching and learning and administrative purposes. The question of how to achieve interoperability hinges on the nature of the standards upon which the software is based. This is akin to determining the nature of the foundations upon which a house is to be built. Open standards that allow the standard to be seen, provide the capacity for interoperability between both open and proprietary software. Open source software (OSS) provides a concrete way of developing open standards. Rather than making open standards theoretical exercises, OSS provides the practical means for determining these standards.

This paper looks at open source, a new model of software development that brings millions of developers into a collaborative, productive community and provides an introduction to OSS in the context of higher education. It is intended to provide the basis for developing some shared understandings about what OSS is; its benefits; its limitations; and it provides a brief description of popular open source licenses common to educational applications. We also focus on the use of OSS in computer science education. The Indian Department of Information Technology has announced that it wishes to move the whole country to Linux as its' 'platform of choice'. This paper may provide the basis for future discussions at state and national levels.

Key words: Open Source, GNU, Linux, Web browser, Apache.

I. INTRODUCTION AND BACKGROUND

Several surprising waves of growth and innovation have swept over the computer industry during the past 35 years, such as the first microcomputers in the 1970s, mass-produced commodity PCs from the mid-1980s through the present, and the rise of the Internet, and it may soon face a deluge of software based upon open source development models. The idea of Open Source Software (OSS) has been around for over 30 years, but it has really garnered attention in the past three years, both from the business and academic world and there is a mad flurry of news on OSS in just the past 8 months in various computer magazines and journals. In 1984, Richard Stallman founded the Free Software Foundation [19], a tax-exempt charity that raises funds for work on the GNU Project [20]. GNU is a recursive acronym for "GNU's Not Unix" and for "new". The GNU Project seeks to develop Unix-compatible software and return software to a state of freedom. Some projects in different application domains like Linux together with the suite of GNU utilities, GNOME, KDE, Apache, sendmail, bind, and several programming languages have achieved huge success in their respective markets. Open Source Development is a revolutionary new model for software development. Or at least that is what a lot of people want us to think. Most of the Open Source community, which is quite large at this point, cites Linus Torvald, developer of the Linux operating system, as the messiah of this new model.

The advantage of OSS which one hears most frequently is that it is free and can be adapted and extended to meet ones own needs. More important, in my opinion, is the advantage educational institutes can obtain by applying OSS: to shape teaching and learning and administration according to a clear vision of the educational and administrative methods one plans to apply. When Open Source e-learning and administrative components are applied; the future roadmap of development can be influenced for relatively low and controllable costs. Companies such as RedHat achieve financial gains related to OSS from the research developed through the collaborative processes which underpin OSS development, and from associated products such as the packaging of products, associated technical services, conferences, books, and promotional materials.

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It is indeed a strange world when educators need to be convinced that sharing information, as opposed to concealing information, is a good thing. The advances in all of the arts and sciences, indeed the sum total of human knowledge, are the result of the open sharing of ideas, theories, studies and research. Yet throughout many institutes, the software in use on computers is closed and locked, making educators partners in the censorship of the foundational information of this new age. This software not only seeks to obscure how it works, but it also entraps the users' data within closed, proprietary formats which change on the whim of the vendor and which are protected by the bludgeon of the End User License Agreement. This entrapment of data is a strong, punitive incentive to purchase the latest version of the software, regardless of whether it suits the educational purposes better, thereby siphoning more of the institute's limited resources away from the institute's primary purpose. The use of such closed software in education may be justified only where no suitable open source solution exists.

Educators have been called upon throughout history to combat censorship imposed by various powers over the flow of information. The censorship being applied today comes in the form of licenses that lock away the tools to build the information age and laws that limit fair use in ways that are unprecedented in the modern era. The powers imposing this censorship attempt to create an artificial scarcity of information and the tools to work with that information to feed their greed.

We are well into the beginning of the Information Age. It stands to affect the people of the world at least as profoundly as the Industrial Age. It is time for the opening of the tools that will be needed to build this new age. Teaching our children to be passive purchasers of closed, proprietary solutions to problems is not enough. Constraining students to move the mouse within the confines of the instruction set of a few closed, proprietary programs merely cages those students and constrains their future.

Students should, at least, be given the opportunity to see how their new tools work. They should be given the opportunity to examine the inner workings of software. They should be given the opportunity to extend the functions of their tools, where they see or imagine possibilities. They should not be held back by locking the toolbox of the Information Age and told they must not peer inside, must not try to discover how it works, must not share their tools with others, and must not use their tools, under penalty and punishment of law.

A computer science teacher constantly seeks new channels, strategies, and technologies to reach and intrigue his/her students. He/she hopes to first capture their interest, then maximize their understanding and retention of the material, and finally encourage their own independent creative work. Throughout this process, he/she tries to teach them skills that they can apply in the real world. The breadth of computer science field and the variety of pedagogical approaches make this process very difficult.

It is believed that OSS can serve as a channel, strategy, and technology to teach and learn computer science. OSS has the potential to expand group work beyond the classroom to include much larger projects and more distributed teams. OSS can also be used to introduce our students to the larger computer science community and to the practice of peer-review. Finally, OSS can often provide us with free or lower-cost technology in the classroom, permitting us to use technology that we might otherwise be unable to afford.

Conversations with students who complain of broken networks, un-repaired computers, too few computers, too few choices in programming languages, overworked and (so far as computers are concerned) under trained teachers are the inspiration leading to this document. The main purpose of this paper is to provide the information about OSS to those who wish to bring open source to their institutes and will have some 'ammunition' with which to persuade those who are in charge. Costs, benefits, limitations and managing risks of OSS are also discussed.

II. WHAT IS OPEN SOURCE SOFTWARE?

Source code is the language used by programmers to create software. Open source code means the code is available. OSS uses source code that is open, unrestricted and freely available by downloading it from the Internet. The 'open' in OSS is intended in the philosophical context of 'open or free speech' rather than as a free (that is, no cost) product. OSS forms the basic infrastructure of the Internet. As OSS is publicly accessible and viewable, it promotes interoperability. Open standards underpin the operations of different software systems' ability to exchange information, which is referred to as interoperability [2].

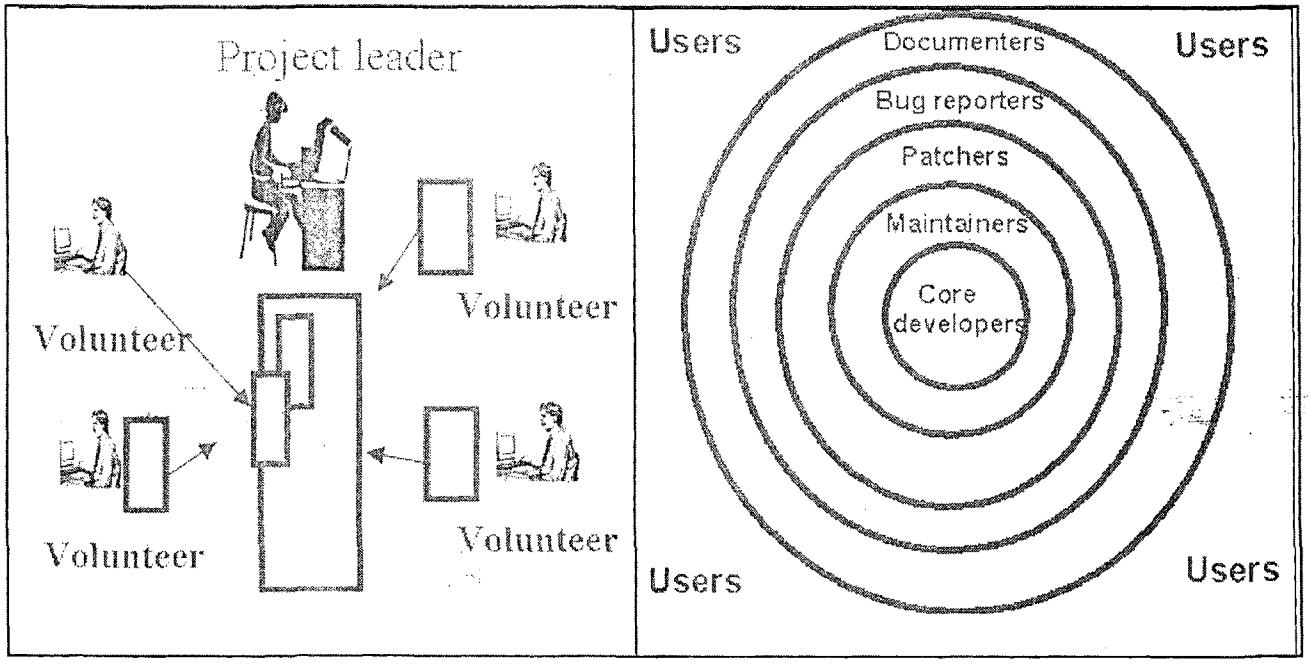
Without a fee, open source code can be moved around by those with sufficient expertise, to fix bugs and customize software, so that the software meets the specific demands placed on it. As such, OSS can be structured to meet institutional requirements. OSS both

encompasses a philosophy about the way software is produced; and at the same time is a product with a particular licensing agreement associated with that product. Figure 1 illustrates two major facets of OSS.

Fig. 1: Two major facets of OSS

III. WHAT IS OSS DEVELOPMENT MODEL?

The basic idea underpinning OSS is that when programmers and technicians can create, read, modify and redistribute the source code of a piece of software, then the software evolves: people improve the software; adapt it and people fix the bugs in it. Figure 2(a) and 2(b) illustrate the open source software development model.



Figs 2(a) and 2(b): Global network of software developers

OSS leads to greater reliability because with a global support community, OSS is stress-tested on a larger set of conditions [2]. IBM is a recognised and trusted member of the Linux community. IBM and SGI have made major contributions to the Linux community by making improvements to the Linux operating system, in particular in relation to Linux storage volume management and in strengthening the Linux file system. Fujitsu, Hitachi, IBM and NEC have contributed to the development of better tools for analyzing Linux performance and troubleshooting Linux kernels

IV. WHAT IS OPEN SOURCE LICENSING?

When OSS is downloaded from the Internet, the users of that software are required to adhere to the licence agreements of the software. Licenses for OSS provide an unconditional right of any party to modify and redistribute the software for free [5]. The copyright of OSS belongs to the author rather than the vendor. The licenses are premised on a view originally forwarded by

Richard Stallman that people should have more freedom in software construction and use [4]. As such he designed and codified a set of users' rights for open source software that he named 'copyleft' because the right to copy was left in place [6].

There are a variety of OSS licenses but they are all premised on the author (the 'licensor') giving some fundamental freedoms to the user (the 'licensee') inside a licence agreement [7]. These freedoms are:

- the freedom to study how the program works
- the freedom to access the software code
- the freedom to modify the code according to specific requirements
- the freedom to run the software for any purpose on any number of machines
- the freedom to redistribute copies of the software to others.

Examples of OSS licenses common to educational applications are

- the GNU General Public License (GPL)
- the GNU Library or 'Lesser' Public Licence
- the BSD License
- the Netscape Public License
- the Mozilla Public License

The following Table illustrates the comparison of OSS licenses [7].

License	Can be mixed with non-free software	Modifications can be taken private and not returned to you	Can be relicensed by anyone	Contains special privileges for the original copyright holder over your modifications
General Public License (GPL)	No	No	No	No
GNU Library General Purpose License	Yes	No	No	No
Berkeley System Distribution	Yes	Yes	No	No
Netscape Public License	Yes	Yes	No	Yes
Mozilla Public License	Yes	Yes	No	No
Public Domain	Yes	Yes	Yes	No

The Open Source Initiative has collected copies of various open source licenses [3]. The Open Source Initiative is a non-profit corporation that manages and promotes open source software licenses by providing certification of these licenses and hosts the details of the licenses on its website.

V. COSTS, BENEFITS AND RISK MANAGEMENT

The use of OSS in educational institutes tends to be idiosyncratic and piecemeal rather than coordinated. The costs and benefits of OSS for various institutes have not been subjected to research. Costs and benefits of OSS however have been researched in other countries in relation to government and private enterprise.

Determining costs

In considering the provision of an IT infrastructure, there are direct and indirect costs. Direct costs include the cost of software and hardware, telecommunications, operations and administration. Indirect costs include downtime, user satisfaction, support costs and the level or reliability the software demonstrates. While some of the costs can be quantitatively determined, many require qualitative rather than quantitative analysis. An environment that strikes a balance between the costs and benefits is required for a smooth functioning IT environment.

The direct quantitative costs of open source are lower than that of proprietary software. There is debate however, about the qualitative comparative aspects of the indirect costs of OSS when applied within an educational environment. No research exists that addresses the indirect costs of OSS as they apply to an educational environment. In order to gain some common points from which to conduct discussions, research work on this matter would make a useful contribution to the debate.

Management of risks

There are risks in leading and managing an institute's

software requirements, irrespective of whether the software is open or closed. The more decentralized an education sector is however, the less controls there are on software costs and the more diverse the local environments are likely to be. One of the most contentious issues in distributed organizations is deciding which IT issues are reserved exclusively for central office and those that can be decided at the local level. Generally, the more freedom is given at the local level for the purchase of software, the more expensive the total proprietary software costs are likely to be for the organisation; and the higher the risks individual sites have to manage in order not to compromise licensing arrangements.

The nature of the resolution concerning the degree of autonomy provided to the local level for software provision will affect the degree of risk an institute and/ or a system is to experience.

The management of software is emerging as an important part of the work of institutes. Institutes' requirements of software include the following:

- It is financially sustainable
- It is not overly time consuming to manage
- It ensures security and privacy
- It improves collaboration within and across agencies
- It has short development times
- It is able to accommodate uncertain futures
- It enables the ability to manage costs and reduce risks over time.

Risks for institutes with proprietary software include the following:

- Administrative costs of software licence compliance and maintenance of records
- Adding and deleting software to machines consistent with licence agreements
- Lack of professional development for staff concerning the regulations associated with software compliance and non compliance
- The provision of appropriate technical support

Costs and Benefits

Strengths of open source software include:

- Open licenses and control over the intellectual property
- Minimizing project start up costs
- Minimizing supplier licence management
- Supporting skill transfer through technical support across the organisation
- Peer support groups are available on the Internet for free
- Vendor support is available for a price
- It avoids lock-in and enables flexibility
- Collaboration and learning organizations are supported in philosophy and practice
- It allows cheap prototyping and develops the local industry
- It increases software reuse across the institute at lower cost which sees a greater return on investment.

Costs of OSS include:

- Training costs and other internal support costs
- Determining which software to use
- External support costs.

Figure. 3: provides an overview of some of the costs and benefits of OSS.

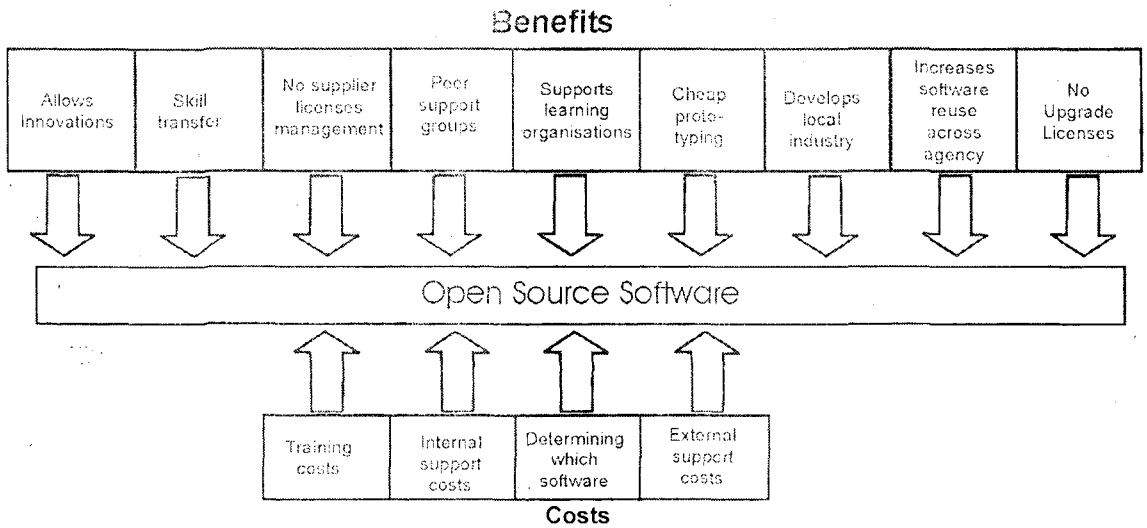


Fig. 3: Costs and benefits of OSS

Netcraft [17] conducted a survey of 40 million sites in April 2003 and found that 70% of the world's web servers run open source apache [18] as shown in Figure 4.

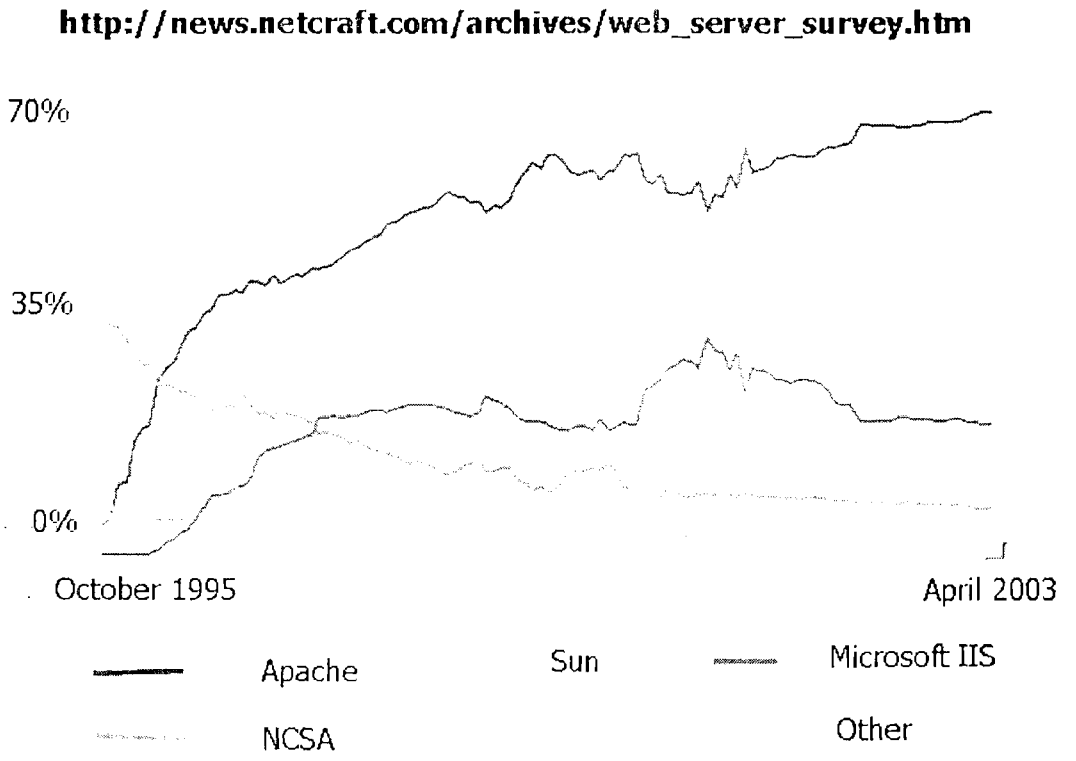


Fig. 4: Survey of web-sites

Limitations

Despite the growing success of the Open Source movement, most of the general public continues to feel that Open Source software is inaccessible to them. There are five fundamental problems with the current Open Source software development trend:

- The lack of focus on user interface design causes users to prefer proprietary software's more intuitive interface.
- The lack of complete and accessible documentation
- Developers focus on features in their software, rather than ensuring that they have a solid core.
- Open Source programmers tend to program with themselves as an intended audience, rather than the general public.
- There is a widely known stubbornness by Open Source programmers in refusing to learn from what lessons proprietary software has to offer.
- There is no single organisation with a responsibility for supporting it;

If Open Source software wishes to become widely used and embraced by the general public, all five of these issues will have to be overcome.

Minimizing risk

Whether the software is open or proprietary, the institutes require methods to minimize their risks with acquiring software. One of the most effective ways of doing this requires 'people' rather than 'technical' solutions. All institutes using IT have to ensure that there are effective IT governance arrangements in place within the institute. These governance arrangements have to be thoughtfully and carefully designed with the appropriate declarations of rights management in place. In distributed systems, clear statements about the roles the central agencies have in relation to the local sites,

are required.

The following issues also contribute to how an institute is able to minimize its risks in relation to acquiring software. Each component requires a discussion to determine the best decisions for that particular institute:

- Are there standards, criteria and guidelines specified and are there mechanisms for ensuring adherence to these specifications?
- Is there an asset register of hardware and software that is maintained and updated?
- What is the correct balance between standardization and complexity?
- Are policies and procedures regularly reviewed and updated?
- Do end users understand and comply with software licenses?
- What tracking mechanisms are in place to ensure licenses are adhered to and the organisation is not put at risk?
- Can software and hardware be reused and if so at what cost?
- What is the balance to be struck between the ability to provide technical support and computer down time?

The surprising thing is that OSS programs are very successful. Some of the facts are as follows:

- 65 % of the world's servers run UNIX/LINUX with Linux accounting for 26% and growing fast.
- 70% of the world's web servers are open source (apache)
- LINUX is now the second most popular desktop
- Benchmarks show that Linux is two and a half times faster than other equivalent operating systems at the data transport level
- LINUX has niche or large share markets in Cluster computing, The movies and Embedded systems

Below is a partial list of some (open source) applications that either come free of charge and inclusive with most distributions of LINUX or can be obtained:

- Apache web server, Mozilla web browser (firefox)
- Open Office (office suite)
- SAMBA (Microsoft integration)
- MySQL (very fast relational database) and Ingress (Full application from Computer Associates (CA))
- A full set of compilers for most languages including scripting and text formatting
- IPCOP, Smoothwall (security, firewall)
- GIMP (similar to adobe Photoshop), Multi-media toolset
- TOra (Toolkit for ORACLE)

Software Reliability greatly increases because of the following:

- "Treating users as co-developers is the least-hassle route to rapid code improvement and effective debugging."
- Linus's Law: "Given enough eyeballs, all bugs are shallow."

VI. ROLE OF OPEN SOURCE SOFTWARE IN EDUCATION

There is a growing interest in the education community in the development and use of OSS. Many groups have been established to develop and promote OSS as a viable technological solution for educational uses. By using OSS, educational institutes can free themselves of licensing costs and put their (often scarce) resources to other uses. Open source applications have caught the eye of Higher Education institutions as (a) state support for Higher Education has decreased (b) IT has become pervasive for administration, teaching and learning (c) the Open Source movement correlates well with academic freedom, and (d) now there is a choice for installation of a software such as in the Linux environment, users can pick from different user interfaces (Gnome, KDE), Web browsers (Mozilla, Nautilus), Office suites (Open, Star) etc. As one might expect, the focus is on Linux, and tools that work on Linux platforms.

The Indian Linux Project strives to bring the benefits of Information Technology down to the Indian masses by creating a Linux distribution that supports Indian Languages at all levels [8]. The goal of this project is to make technology accessible to the majority of India that does not speak English.

Moodle Project is a student centered course management system designed to help educators who want to create quality online courses [9]. The software

is used all over the world by universities, schools, companies and independent teachers.

LON-CAPA is a full featured, mature application that includes content manager [10].
Sakai Project is a community source software development effort to design, build and deploy a new Collaboration and Learning Environment (CLE) for higher education [11].

Open Source Now project developed by Red Hat, a company best known for its commercial support of GNU/Linux, is an advocacy group designed to advance the use of OSS in both education and public policy [12]. For educators, they provide an introduction to OSS in education, a discussion list, and links to a wide variety of other OSS in education sites.

Simple End User Linux - Education is a discussion list about all aspects of educational uses of Linux [13]. The site also provides a database of case studies, and links to current projects and a wide variety of software from astronomy to social studies.

The K-12 Linux Project provides information to schools to enable them to make use of OSS [14]. For example, their Linux Terminal Server project has produced an open source terminal server Linux distribution targeted at a lab of low-powered diskless computers and a high-powered server. This allows a school to take advantage of outdated hardware they may already own, as well as save money by reducing the software licenses the schools must purchase. They claim to cut a lab's hardware and software costs by more than a third. Open source software is not limited to low-cost computer labs in schools, but also is being actively used and developed by higher education institutions.

The ***OpenScience*** project is dedicated to developing scientific software that can be taken advantage of freely by anyone [15].

OSS is having an increasingly profound impact on education and training: Social and cultural impact, Economic impact, Impact on educational technology and Pedagogic impact [16].

Social and Cultural impact - Open source is changing the relationships between producers and consumers, systems administrators, managers and practitioners. Open source is changing the way in which we view software - software to support work processes rather than work processes supporting software.

Economic impact - Open source is leading to (i) the development of new business models, (ii) new opportunities for small enterprises and organizations, and (iii) to the formation of new communities. Open source software may be cheaper.

Impact on educational technology - Open source has resulted in a period of rapid innovation in educational technology. There is increasing diversity and choice of software to support different pedagogic approaches and increasing adoption of open standards and component architectures. The biggest impact of open source may be in contributing to changing ideas and practice about how people learn using Information and Communication Technologies (ICT).

Pedagogic impact - With social recognition of the potential for sharing and the recognition of new and different types of knowledge and contexts of learning, we are beginning to recognise a literacy of information navigation. Open source applications are supporting discovery based learning and experimental learning leading to new forms of reasoning. We are recognizing that interaction is a social process and are using the web to explore, act, make, construct and develop knowledge.

Relying on the flexibility, low-cost, standards compliance, and stability of OSS to strengthen its credibility as an educational solution, we must also recognize the factors that inhibit its widespread acceptance. Today's educational institutes are more-or-less Windows-Intel reliant, possibly due to historical vested interests, a steep learning curve, or masterful marketing. Another factor, which is sure to surface as another inhibitor, is the current mucky situation of open source licensing. While supplying a great amount of flexibility for developers, this plethora of licenses has a steep learning curve of its own. It also breeds confusion and uncertainty for users and neophyte developers.

VII. ROLE OF OPEN SOURCE SOFTWARE IN COMPUTER SCIENCE EDUCATION

OSS offers tremendous benefits to the computer science education community. By using OSS, computer science educators and students can develop (or further the development of existing) software that can be used and improved upon by an international community. This not only provides the student with a world-size laboratory and support staff, but also gives them experience in

large-scale software collaboration and development. *Distributed software collaboration has proven itself* effective in the educational setting through the use of Internet-driven collaboration tools such as web pages and email lists. These Internet-driven collaboration tools are the same as those that power open source development.

Thus, we can look to the open source community to expose our students to large-scale distributed software development. By using and developing OSS, not only is the student participating in a large distributed software community, but is also interacting with large, real, software code-bases.

GNU/Linux, an open source operating system, has also been presented as an educational technology to teach networking, databases, and system administration at the university level.

Students who are encouraged to build projects on top of OSS bases can build more interesting and exciting systems than they might have developed from scratch. Further, in order to succeed, they must work with a large code-base and associated documentation. If the code is well written and well documented, students learn how much can be accomplished with careful preparation. If it is not, students may take more care with their own work in the future. The best of these systems can be contributed back to the open source community.

Finally, due to the open and freely available source code, a high degree of verifiability can be attained. Just as we look to conferences and journals for peer-review of our ideas, we can look to the open source community for the same type of scrutiny concerning our software. *By taking advantage of the open source development* model we extend the methodology by which we learn, apply, and teach computer science, to include peer-review. We may look away from the pragmatic properties of the open source code to the more "moral" argument a free software advocate may pose. Free software assures everyone will have access to your code, which as a computer scientist, are your ideas. As a scientist it is your duty to assure everyone can understand, modify, and apply those ideas.

VIII. CONCLUSIONS

The use of open source software, and GNU/Linux in particular, is growing in all directions. OSS evolves as a result of 'many eyeballs' viewing the software, testing it, debugging it and contributing the improved software back to the software community. Ushered in by cost savings, better support, rapid software development, greater reliability and more security, Open Source solutions are making inroads at work places, schools, colleges, universities, and in homes. Ranging in application from the embedded world to corporate infrastructure, OSS also gives educators a type of flexibility and intellectual freedom often absent from software. OSS can serve as a channel, strategy, and technology to teach and learn computer science. As a channel, OSS can expand teamwork past the classroom to include much larger projects and more distributed teams. As a strategy, OSS can be used to introduce our students to the larger computer science community and to the practice of peer-review. Finally, acting as a technology, OSS can provide us with free or lower-cost technology in the classroom that we might *otherwise be unable to afford. People today are* considering Open Source software alternatives to proprietary software more than ever. There is a Digital Divide in the proprietary software market...those who own software legally, and those who have obtained the software illegally. Providing free, Open Source applications to the world will help to close the legal-illegal gap.

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BIOINFORMATICS APPROACH FOR SECONDARY STRUCTURE IDENTIFICATION IN BETA-BARREL FOLD PROTEINS

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Abstract

There is a need for knowing the structure of proteins for various reasons in biomedical applications. However, the rate at which the structures of proteins determined is not at par with the sequences of proteins derived. Therefore, theoretical methods using knowledge based techniques became popular to predict structures. Bioinformatics techniques are used for sequence alignment and comparison in the past and several algorithms were used in doing so. Structure based sequence information will be useful in making a link between the structure and sequence. In the present paper attempts were made to make a database of sequences of known secondary structure elements of a popular protein fold, beta-barrel or TIM-barrel. A string of sequence would be screened against the database strings to find out the secondary structural score of the input sequence. Provision has been made to give the input and obtain the output through a web interface.

Key words : Bioinformatics, protein structure, secondary structure, structure prediction, Needleman-Wunsch algorithm, TIM-barrel fold.

INTRODUCTION

The usage of computers in various fields of science is increasing year by year. The development of computer programs to ease the design and development of equipment was the beginning and it continued into handling much complicated tasks. Although it started late, the usage of computers in the field of biology slowly getting acceptance with huge problems being solved by means of simple algorithms, much credit goes to the rapid development of sophisticated hardware technology. It started with structural chemistry in solving the minimized structure of a chemical molecule with the help of basic physical principles. Computers played a big role in solving the structures of not only chemical molecules, but also quite complex biological assemblies.

Due to rapid developments in hardware and software technology the structural biology has seen tremendous growth. At present there are more than 22,000 structures of proteins/enzymes deposited in the Protein Data Bank (PDB), a depository which is helpful in understanding the structure and function relationship of protein/ enzymes (Berman et al., 2002). Proteins/enzymes are biomolecules which are responsible for most of the biological reactions happening in all the organisms. Knowing the protein structure is an important part of structural biology, to understand the function and thereby design drugs which will inhibit the function of proteins. Protein structure depends on the sequence of amino acids (the building units of proteins) which is coded by a gene. Currently various genome sequencing projects are unraveling the genomes of different organisms. It is nothing but identifying the nucleotide sequences of all DNA present in any organism. The DNA sequences are annotated into gene sequences which are responsible for the coding of amino acid sequences of different proteins/enzymes in any organism. For example there are 6500 genes present in yeast, a small organism used in baking industry (Guldener et al, 2005). Human genome project identified about 35000 genes to be present in humans. However, all the protein/enzyme structures could not be derived experimentally, otherwise the structures could be directly used in drug designing. Therefore molecular modeling those proteins/enzymes whose structure is not known, uses computers in deriving the structural model based on the information from existing experimentally determined models. The task is huge because the experimental determination of structures is not catching up with the speed at which the data from genome projects are releasing.

The development of methods to determine the structures of all proteins is a marathon task and is not solved

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through a single method. One of the methods could be to attempt to derive the structure of a set of proteins which will fold into a certain topology. It is known to protein chemists and structural biologists that proteins fold into different topological units. Each fold contains certain secondary structural units called alpha-helices and beta-strands connected by coiled loops. Like any structural unit, proteins use these helices and strands as skeleton of the body and the amino acids present on the loops as the functional units. According to a theoretical study there are about 1000 folds that all the proteins could be folded in to (Chotia, 1992). It was assumed that about 20% of total enzymes in all organisms will fold in to a famous TIM-barrel fold (Wierenga, 2001). This is a fold in which there are eight beta-strands form a central barrel surrounded by eight alpha-helices. The loops connecting the strands to helices contain amino acids which are responsible for the function. The TIM-barrel fold is observed to form from quite non-homologous protein sequences. Different parts of a protein sequence form different secondary structural elements which in turn fold to form a globular folded protein. It is difficult to predict which parts of sequences form helices or strands. Therefore, a knowledge base of sequences forming strands and helices, particularly from known TIM-barrel structures, will help in identifying unknown secondary structures of a protein sequence which can form a potential TIM-barrel.

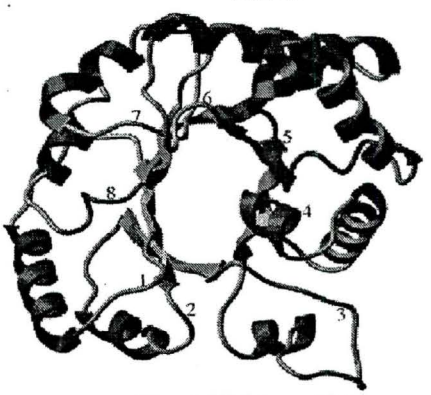

Fig. 1: TIM-barrel fold.

The first step would be to create a database of sequences forming helices, strands and loops from known TIM-barrel proteins. PDBSUM (Laskowski, 2001) link available from the PDB website has been used to identify the secondary structural elements of a set of redundant TIM-barrel protein structures. The sequences belonging to the secondary structural elements have been noted. These sequences are nothing but strings containing alphabets. These sequences could be used as search targets for protein sequences whose structure is not known. A string-pattern-search program would identify secondary structure from a problem sequence. To facilitate the user, the whole process would be automated where a user inputs a string (sequence) from a problem protein and gets back the information about whether the sequence will form a particular secondary structure of the TIM-barrel. In this process Needleman-Wunsch algorithm would help to recognize the sequence homology among the proteins using standard amino acid match scoring matrices.

The process

The steps in the process are as follows:

1. Data of secondary structural elements and loop sequences of TIM barrel fold proteins from known TIM barrel protein structures were collected.
2. Database of amino acid sequences of above mentioned elements were created.
3. A 'C' program to search the databases of sequences (as created in step 2) using dynamic programming algorithm on test sequences to predict secondary structure of an unknown sequence from genomic sequence data was written.
4. An interface was developed between the input form, database, the search program and the output form in PERL language, so that the search method could be automated.

Database creation

The data here involves obtaining stretches of amino acid sequences forming different secondary structural elements from different TIM-barrel proteins. For this the source is a major database called Protein Data Bank

(PDB), which is available as a public domain resource. A resource link was present in PDB called PDBSUM where the sequences of secondary structural elements of all the PDB entries are presented. The data was filtered from these major databases. The data, which looks like alphabetical strings were stored into a database which could be retrievable easily through a program. It also contains the information about a sequence derived from a secondary structural element (either helix, loop or strand) of a PDB code.

Protein name	PDB code secondary structure	Sequence
Triosephosphate Isomerase	1cb7H8	KEANAAGIKATKMALNM
Triosephosphate Isomerase	1cb7H3	RLLAEIIHAG

Table 1: An example of entries in PDB.

The 'C' program (alignment program) retrieves and compares input string against the strings in the database and gives a best alignment score based on a scoring matrix. The alignment of these strings was done using Needleman-Wunsch algorithm (Needleman and Wunsch, 1970). The Needleman-Wunch algorithm is nothing but optimization of matching a pair of sequences. It involves 3 steps, initialization, scoring or matrix fill up and trace back or alignment.

Details of Logic and Implementation

For example consider two sequences which are to be best aligned. When one of the sequences has M letters and the other has N, a matrix with M+1 columns and N+1 rows will be created. The first column and row will be filled with 0 (zeros). Next the matrix will be filled with appropriate scores which depend on a scoring scheme. A scoring scheme is generally a scoring matrix which will be derived from one of the existing matrices, for example BLOSUM62. These scores were assigned for appropriate pairs of amino acids based on the relative comparable chemical and physical properties of amino acids involved (Henikoff and Henikoff, 1992).

In the matrix the amino acids are single letter coded and the scores are a self-diagonal matrix. The MxN matrix will be filled from the upper left hand corner by finding the maximal score $M_{i,j}$ for each position in the matrix. To find the score of $M_{i,j}$ for any i,j it is necessary to know the score of the matrix positions above, left and diagonal to i,j. In terms of matrix positions they are $M_{i,j-1}$, $M_{i-1,j}$ and $M_{i-1,j-1}$.
So the maximum score for a position i,j will be,

$$M_{i,j} = \text{Maximum} [\ M_{i-1,j-1} + S_{i,j},\ M_{i,j-1} + W,\ M_{i-1,j} + W\ ]$$

Where, $S_{i,j}$ would be pairing score between the amino acids taken from the scoring matrix and W would be gap penalty. Gap penalty can be assumed to be any arbitrary number, for example between -5 to -15. By filling up the whole matrix through picking up the maximum score at each matrix position the matrix will be ready for next step. The next step is trace back or alignment of sequences. The trace back will determine the actual alignment that results in maximum score for the matrix. The trace back will start at the M,N position of the matrix. Moving backwards, taking the path where the matrix positions have maximum score, will give the trace back, depending on whether a diagonal climb (match) or a horizontal/vertical path (mismatch) will lead to alignment of both the sequences. In this way the top best score for all the alignments from the database sequences will be given showing that the query sequence has some hits with known secondary structures.

It will be easy for a user to give the input sequence through a front-end form like perl-form and the output also will be put on to a front-end form. This also will help to port this analysis on different platforms.

The flow of the data were processed. A query sequence will be input to the alignment program. The alignment program works on the basis of Needlemann-Wunsch algorithm. The alignment program takes data of sequences from a database which was made from data analysis and classification. The alignment requires information from a scoring matrix derived by experts in the field. Finally the top best aligned sequence will be output.

The program is tested with known input and output sequences. The results are satisfactory. The output is the best scoring sequence from the database which tells whether a particular sequence is forming alpha-helix or beta-strand or front-loop or back-loop of a TIM-

barrel protein. For example, it gives information about the sequence, secondary structure and the PDB code of the protein from which it has been derived. Verification checks were performed by 'omit-sequence' method in which a version of database was used where a secondary structure known protein sequence was omitted and its sequence was used as a test set of sequences.

CONCLUSIONS

Identifying the structure of proteins is very important in Bio-medical applications. Bio-informatics techniques with database of sequences of known structures are providing very useful results. For a given input of a small string of sequence the output obtained is satisfactory.

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RISK ABATEMENT THROUGH CONFIGURATION MANAGEMENT

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Abstract

As the software developmental processes generally cannot handle a change request at any arbitrary point in time, to handle the inevitable change and rework request, configuration control process is generally used. The software developmental processes are exposed to certain risks which may hamper the end result This paper presents common software development activities and their associated risks while exploring the role of configuration management in controlling or reducing those risks.

Key words : Risk Management, Configuration Management.

INTRODUCTION

The object of this paper is to examine the role that configuration management plays in risk reduction during the software development process. To do this we must identify the activities within the software development process, and the configuration management process, as well as define the terms to be used in this evaluation. Once the terms and activities have been identified, the configuration management activities can be cross referenced into the software development process. An evaluation can then be made as to access the effectiveness of configuration management in limiting project risk during the software development process.

2. Defining the Software Development Process to be used

Three software development process models widely used today are Waterfall [Royce 11], Spiral [Boehm 4], and Evolutionary [Gilb 5]. The waterfall is a linear progression of project activities, where an input is received by an activity, processed, and the output is delivered to the next sequential activity as the input to that activity. The final output is the product for delivery to the customer. The Spiral process model bends the planning, requirements, and design activities of the waterfall model back around itself three times to allow these three activities to be injected with activities of evaluation, risk, verification and planning based on the results of the previous spiral. The implementation activities of the Spiral process return to the sequential activities as in the Waterfall model. The Evolutionary process model is where each development activity feeds the output both forward as the waterfall model and also backwards to any activity as necessary to correct or improve the product being developed. When needed this process can go all the way back to the beginning to reevaluate and rework itself.

In all three of these process models, the same basic software development activities are taking place. These activities may be implemented differently, their relationship to other activities may change, and the process models themselves offer their own unique management issues [Bersoff 3], but the general software development function of these activities remains unchanged.

These general software development activities, not the process models, are the focus of this evaluation as to know what role configuration management plays in reducing project risk. Therefore, for the purpose of this paper the development process models will not be considered as factors but rather the basic software development activities that they all use. However, to avoid undesirable complexity in the evaluation, these software development activities will be discussed within the framework of the Waterfall process model.

3.Defining terms used in the evaluation

3.1 Risk

Before going further a definition for project risk needs to be established. Risks are those factors that may prevent the attainment of a set goal. H. Bersoff has defined the goal of a software development process as,

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"Producing a product that meets or closely matches the needs of the people for whom it is developed "[2]. He goes on to say that product integrity must also include the additional goals of meeting the planned cost, and meeting the planned schedule for producing that product. Therefore, project risk would involve anything that may compromise the attainment of project integrity. It is interesting to note that the current version of the Software Engineering Institute's (SEI) Software Capability Maturity Model (SW/CMM) does not include a risk process area, although a level 3 process area has been included in the draft SW/CMM Version 2 [Paulk 9].

3.2 Software Development

The term software development can apply to the total software development project as well as the specific project function of implementing the software product. Paulk in his paper on project control [9], presents a multi-dimensional model of the software development process. In his model he depicts project control activities matrixes upon project activities. Project management, configuration management, quality assurance, and others are part of project control activities. Software development constitutes the majority of the project development activities. A somewhat different model is used in the BDM software development methodology [1]. This model presents project management as the basic foundation with total oversight and support responsibility. The foundation is rooted on configuration management and quality assurance support and oversight. These three form the project control and oversight platform needed for software engineering activities.

For the purpose of this paper, the term software development will characterize the actual implementation stages and their respective activities in the software development project.

3.3 Project Management

The primary oversight role in a software development project belongs to project management. Project management is responsible for the planning, monitoring, controlling and evaluating the status of the project. Project management is generally assisted in this regard by configuration management and the quality assurance.

3.4 Configuration Management

The SW/CMM Version 1.1 [Paulk 8] defines four goals for configuration management as part of its level 2 key process areas. Three of the four goals can be mapped into the four activities as identified by H. Bersoff. Configuration management is responsible for identifying configuration items, controlling and tracking changes to those items, providing configuration status accounting reports to the Project Manager about those items, and performing configuration audits [Bersoff 2]. Although Bersoff and others identify configuration audits as a configuration management activity, it is not identified as such in the IEEE standards [IEEE 6]. Alexa Marshall [7] has opined that a project that has implemented configuration management as discipline reduces the need for certain verifications. In those cases, configuration audits may become more the domain of quality assurance.

3.5 Quality Assurance

Quality assurance, although confused at times with configuration management, is only concerned that products meets the baseline requirements and that the prescribed processes as set out in the software development plan are followed during the software development process. In the draft version of the SEI SW/CMM Version 2 [Paulk 9], Quality assurance has separate process assurance and product assurance goals.

4. Configuration Management Activities

As mentioned above, the configuration management is a project control activity. Software configuration management activities are designed for control and over sight into software development activities and not into other project control activities of project management and quality assurance. However, the results of configuration management activities can provide project management and quality assurance with information that can help identify developing risks related to schedule and process.

The following configuration management activities will be used for evaluating the effectiveness of configuration management activities in risk management.

4.1 Identification of configuration items

This activity is primarily dealt with during the project planning stage of a software development project. At that time, items are identified for which configuration management will be responsible. Some of these items will make up the project baseline, such as requirements documents, and source code. Other item not part of

the project baseline, such as project archive documents, development tools, and libraries needed to generate the product, should be identified and also come under configuration management control.

4.2 Change control and tracking

Once items have been identified for configuration management control, changes to these items and the processes involved with changing these items must be controlled and tracked. The control process is most formal with baseline items. Changes to baseline items generally are presented as Engineering Change Requests (ECR) and are not acted upon without the permission of a Configuration Control Boards approval (CCB). Non- baseline items have less formal control processes, but do require some form of authorization before changes are allowed. Tracking is the documentation part of the controlling activity. Configuration management must record all actions of the CCB as well as the revision history of each configured item. A second change control process is software problem tracking. These problems depending on when they are reported during the software development process may or may not require CCB involvement.

4.3 Configuration status accounting - version control

As a project becomes more complex and the number baselines and proposed ECR increase on a project, the activity of configuration status accounting becomes crucial. Configuration management is charged with keeping and providing the project with exact version information on all configured items for each baseline. This activity should be extended to include non-baseline configuration items as mentioned above in the configuration item section.

5. Software development activities

For the purpose of this evaluation a set of standard activities need to be defined. After reviewing the IEEE Standards for Developing Software Life Cycle Processes [6], Royce's waterfall process model [11], and Boehm's spiral model [4], the following software development activities presented themselves as the basic activities for any process.

5.1 Identification of risk Factors and mitigation in Software Development Activities

This section addresses software development activities according to the standard waterfall development model. As the following information is to be applied to both initial work and ongoing maintenance work, there are risks cited for failure to establish a baseline or use the correct existing baseline.

SD Activities	Risk Factor	Mitigation
Requirement Analysis		
	Misunderstanding customer's needs	**Use version control tools**
	Failure to baseline results	**Configuration Item / status accounting**
	Failure to identify all requirements	QA Review
	Implying that all requirements will be met	PM Issue
	Failure to version control draft work products	**Use version control tools**
	Failure to maintain requirements traceability matrix	QA Review -
Design Architecture design		
	Failure to design all system requirements	QA Review
	mistakes in design	QA Review
	Failure to use the correct design baseline	**Configuration status accounting**
	Failures to create an implement able design	QA Review
	Failure to version control draft work products	**Use version control tools**
Data Design	Failure to completely define data dictionary	QA Review
	Failure to use the correct baseline	**Configuration status accounting**
	Failure to completely define record formats	QA Review
	Failure to properly define primary keys mistakes in design	QA Review QA Review
Interfaces		
	Failure to design all system requirements	QA Review
	Mistakes in design	QA Review

	Failure to use the correct baseline	**Configuration status accounting**
	Failure to create an implement able design	QA Review
	Failure to version control draft work products	**Use version control tools**
	Failure to baseline results	**Configuration Item/status accounting**
Detail design		
	Failure to design all system requirements	QA Review
	Failure to use the correct baseline	**Configuration status accounting**
	Failure to create an implement able design	QA Review
	Failure to version control draft work products	**Use version control tools**
Implementation		
Create source code		
	Failure to implement all requirements	QA Review
	Failure to use the correct document baseline	**Configuration status accounting**
	Failure to use the correct source code baseline	**Configuration status accounting**
	Failure to implement requirement correctly	QA Review
	Unable to implement design	PM Issue
	Failure to baseline results	**Configuration Item/status accounting**
Reuse of available source code	Failure to use the right baseline	**Configuration status accounting**
	Lack of documentation	**Configuration Item**
	Unanticipated side effects	SWD Issue
Unit testing		
	Failure to test the correct software versions	***Configuration*** **status accounting**
	Failure to test the correct system configuration	**Configuration status accounting**
	Failure to test the correct requirement baseline	**Configuration status accounting**
	Failure to test all requirements	QA Review
	Failure to test requirement correctly	QA Review
Integration Creation of test plans		
	Failure to use the correct test plan baseline	**Configuration item/status accounting**
	Failure to use the correct requirements baseline	**Configuration status accounting**
	Failure to use the correct design baselines	**Configuration status accounting**
	Failure to correctly plan requirement test	QA Review
Integration testing		
	Failure to test the correct software versions	**Configuration status accounting/audit**
	Failure to test the correct system configuration	**Configuration status accounting/audit**
	Failure to track test results	**Audit**
Problem reporting		
	Failure to track problems	**CM Control & Tracking**
	Failure to track status of problems	**CM Control & Tracking**
	Failure to track fixes to software revision	**CM Control & Tracking**
	Failure to report status to management	**Configuration status accounting**
Problem resolution testing		
	Failure to test the correct software revisions	**CM Configuration status accounting**
	Failure to test the correct system configuration	**Configuration status accounting**
	Failure to report status to management	**Configuration status accounting**
	Failure to track test results of fixes	**QA Audit**

Delivery		
Building software		
	Failure to use the tested baselines	**Configuration status accounting/audit**
	Failure to use the correct tools or version of tools/libraries to build software release	**Configuration status accounting/audit**
	Failure to include all the required files	**Configuration status accounting/audit**

6.Evaluating effects of configuration management activities in limiting risk

In the previous section software development activities and associated risk factors where presented along with some mitigating activities. The list of risk factors is not meant to be exhaustive. They are taken from problems experienced by software engineers.

What can be seen from the breakout of configuration management activities versus quality assurance activities is that in many places they both tend to work in parallel. Configuration management providing the correct tools and work products for the activity, while quality assurance verifies and validates that the work done meets requirements and has followed the correct processes before the work product is rebase lined. There are slight shifts in this oversight balance throughout the software development process. Quality assurance appears to be the more dominant oversight in the initial activities of analysis and design. During implementation, configuration management takes a more lead role, but during integration testing, they return to shared oversight. Finally, configuration management is just slightly more involved in the delivery activity. This would support the BDM Methodology model of parallel support by configuration management and quality assurance for the software engineering activities [1].
Most of the identified risks that can be mitigated by adherence to configuration management principles can be grouped and reduced to a small set of generalized risk factors:

- Failure to identify work products that need to be controlled
- Failure to baseline controlled items
- Failure to use the correct/complete baseline version.

In addition there are the specific risk factors:

- Failure to track problems
- Failure to inform management
- Failure to use version control tool.

These risk factors map directly to configuration management activities.

6.1 Identification of configuration items is a SW/ CMM level 2 goal that mitigates risk by identifying all important project work products needed for delivery or future modification. Without a list of identified controlled items, work products can be lost and would have to be totally recreated, if at all possible, when needed. The majority of the work for this activity occurs during project planning, but like most project artifacts, it needs to be periodically updated to reflect changes.

Responsibility for mitigating the Failure to baseline work falls to both the identification activity, is it missing from the list of configured items, and the **configuration status accounting** activity

Another aspect of this activity is also identification of configuration management tools to be used on the project. These tools include version control tools to be used with draft work products, and system development tools that will be used for generating work products for final delivery to the customer. Tool identification is a risk mitigation factor for the software development team to be used during work on draft work products that are prone to the same errors and rework issues in much the same manner as the configuration management controlled baseline work products. Configuration management usually does not take responsibility for version control of draft work products.

Configuration status accounting is the primary version control activity for configuration management to avoid errors and rework due to using the wrong work product version when planning, designing, modifying and decision making. This is a common error that I have seen occur on projects. The use of an out of date baseline can usually be caught through QA reviews and testing, but this again involves unplanned rework. I have also witnessed a case where, in a multiple customer environment, a customer received a maintenance release that included modules containing other customers variations, not their own, and more recently a case where the current modification where applied to a version that was three releases out of date.

Configuration status accounting is a SW/CCM level 2 goal. The SW/CCM goal however, combines the activities of status accounting with change control and version tracking.

6.2 Control and tracking is regular baseline change control activity involving ECRs, and normally take place prior to software development in the project planning, or release planning activities of the project. In this evaluation control and tracking are represented by means of the control and tracking sub activity of problem reporting during the integration testing. In the problem tracking process, change control focuses on controlling the configuration of the current work products during testing. The configuration management risk mitigation role that is played during problem tracking has two aspects. The first is to prevent the inclusion of new work product version that have not been successfully tested into the baseline, and second is to inform project management as to the status of problems being tracked as a result of testing. In the more quality conscience projects, problems/defects are also tracked as a result of quality assurance work product reviews. These status reports can be clear indicator to project management of possible schedule, process, quality, and resource issues that could adversely affect the project integrity, risk.

6.3 Configuration audit is the risk mitigation activity that verifies that all't's are crossed and 'i's dotted by way of verifying that all baseline configurations are what configuration management believes them to be. This activity should be done periodically when supporting a production system, but especially when preparing a work product for customer delivery. The risk mitigation is obvious here. Without this activity it is possible that a configuration mismatch could be introduced into the product. The effect of such an occurrence can leave a project open to a myriad of possible errors and problems.

CONCLUSION

Certain software development risk factors can be effectively mitigated by configuration management. This is not really a surprising conclusion since configuration management evolved to address certain software integrity issues, i.e., risks.

Failure to mitigate the risk factors listed above leaves the software development project open to issues of credibility and loss of customer confidence, as well as additional unplanned costs in time and resources to perform rework.

Risk factors are often given the aura of potentially project shattering events and as a result the big ticket items usually get the primary focus. There are day to day risks that tend to be over looked or ignored because it is assumed that everyone knows the right thing to do. Oversight of some of these day by day activities falls to configuration management, an area that is also often under rated or under appreciated. However, the existence of configuration management on a project is not enough. Without proper implementation and project acceptance, the projects risks erosion of its credibility and project management loses a valuable insight into the project. Day by day things slip through the cracks and get lost or forgotten. Customer confidence begins to fade. Small mistakes become embarrassments. Hopefully large mistakes won't happen.

Configuration management, when done well, is a process area that is over looked. When configuration management personnel leave a project without qualified replacements it can become painfully clear of the day to day importance a well implemented configuration management program is in maintaining basic project integrity.

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SURFACE ALTERATIONS OF PLACENTA IN *PLASMODIUM BERGHEI* INFECTED PREGNANT MICE

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Abstract

The severity of malaria infection during pregnancy is correlated with the parasitaemic levels, placental infection and foetal abnormalities. Malaria is more severe and frequent in pregnant woman, than in non-pregnant women. Placenta of pregnant Plasmodium berghei infected mice showed alterations on surface of placenta. Trophoblastic membrane of the infected placenta was severely damaged. The bulbous microvillus ends were severely necrosed that resulted in aggregation of trophoblastic membrane. These altered placental surface may be responsible for internal damage of placenta which in turn might be responsible for abnormal foetal developments like pre-term delivery, low body weight of new born, foetal death or abortions in pregnant women.

Key words : Plasmodium berghei, placenta, pregnancy, malaria.

INTRODUCTION

Plasmodium falciparum is more severe and frequent in pregnant women than in non-pregnant women residing in endemic areas (Brabin, 1991; Dafallah et al, 2003; Adam and Elbashir, 2004). Primigravidae are more prone to malarial infection than multigravidae (Riley et al, 1989; Steketee et al, 1996; Adam et al, 2005). Malaria during pregnancy often leads to abortion, reduced birth weight, premature delivery, and still birth (McGregor, 1986 ; Nosten et al, 1991; Okoko et al. 2003). The premature delivery or reduced birth weight of new-borns may be either due to the impaired intrauterine growth or placental infection. Earlier studies have clearly shown that placental infections are always associated with pathological as well as ultrastructural alterations (Galbraith et al, 1980; Galbraith et al, 1980 a; Vinayak et al, 1986; Shukla et al, 1990). But the surface of such infected placenta has not been studied and warrants further study. Thus, it is pertinent to study the surface alteration of the placenta in *P.berghei* infected pregnant mice.

Parasite - Plasmodium berghei (NICD) was maintained in laca mice by intra-peritoneal (ip) inoculation of infected red blood cells. Percent parasitaemia was quantified on every alternate day in Giemsa stained tail blood film.

Animals - Close, bred laca female mice, 6-7 weeks old were employed. These animals were maintained in the animal house of Panjab University and were provided pellet diet and water ad libtum. Care and use of animals were followed in accordance with the guidelines of the institutional ethical committee.

Assessment of first gestational day - Female mice were kept for mating with male mice of the same strain in 2:1 ratio. First gestational day was determined by the presence of vaginal plug or cornfield squamous epithelial cells in papanicolaou stained vaginal smear (Papanicolaou, 1942).

Animals and infection - The animals were divided into 3 groups, Group I (n=12) non-pregnant infected mice; Group II (n=6) pregnant uninfected mice; and Group III (n=12) pregnant mice infected on gestational day 10 (GD-10)

Mice belonging to group III and I were inoculated with 10^6 parasitized erythrocytes intra-peritoneally. After the inoculation of the parasite, percent parasitaemia, survival time and alterations occurring on the placental surface were studied.

Scanning electron microscopy (SEM) - The structural alterations in infected and uninfected placenta was studied using the SEM. Mice having parasitaemia in the range of 50-60% were sacrificed with anaesthetic ether. Placenta from Group-III and Group-II mice was

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obtained after sacrificing on GD-16. The placenta was washed and fixed in glutaraldehyde buffer (pH 7.2) for 1 hr. After fixation, placenta was rinsed in 50, 70, 90 and 100% acetone for 15, 20 and 60 min respectively. Finally the placenta was dried, fixed and coated with gold and viewed under JEOL Electron Microscope (Model JSM 6100 SMJER) at 20 KV

The data was analysed by Students paired '**t**' test with equal numbers of observations.

RESULTS AND DISCUSSION

The pattern of parasitaemia in pregnant mice infected on GD-10 was similar to that in non-pregnant mice but had high percent parasitaemia (77.63 ± 1.49; Group III) as compared to non-pregnant mice (72.30 ± 3.00; Group I, Fig. 1). Pregnant infected mice (Group III) had lower survival duration (10.67 days) than non-pregnant infected (12.67 days) mice (Group I, Fig.1, Table 1). Mortality was 100% in both the groups of animals. The high parasitaemia in pregnant GD-10 infected mice has been related to depressed immune response, occurring due to hormones, that induces profound alteration in lymphoid organs responsible for immunological aberrations resulting in the increased diseased state of host or due to severe anaemia in pregnant women that causes abnormal cell division and defective lymphocyte functions, thereby affecting the cell mediated immunity (Brabin et al, 1990; Menendez, 1995; Okoko et al 2003 and Adam et al 2005).Thus high parasitaemia in pregnant mice could either be due to depressed state of host due to which parasite multiplied rapidly resulting in severe anaemia and reticulocytosis.

Placenta acts as a selective barrier for the various

Table 1: Peak Parasitaemia, survival period and percent MORtality in non-pregnant and pregnant (gd-10) mice infected with P.berghei.

Group of Animals	Number of Animals	Peak Parasitaemia (%)	Survival Period (days)	Mortality (%)
Non-pregnant infected	6	72.30 ± 3.00	12.67 ± 1.49	100
Pregnant infected on GD-10	6	77.63 ± 1.49	10.67 ± 1.25	100

Mean ± Standard Deviation; GD = Gestational Day,

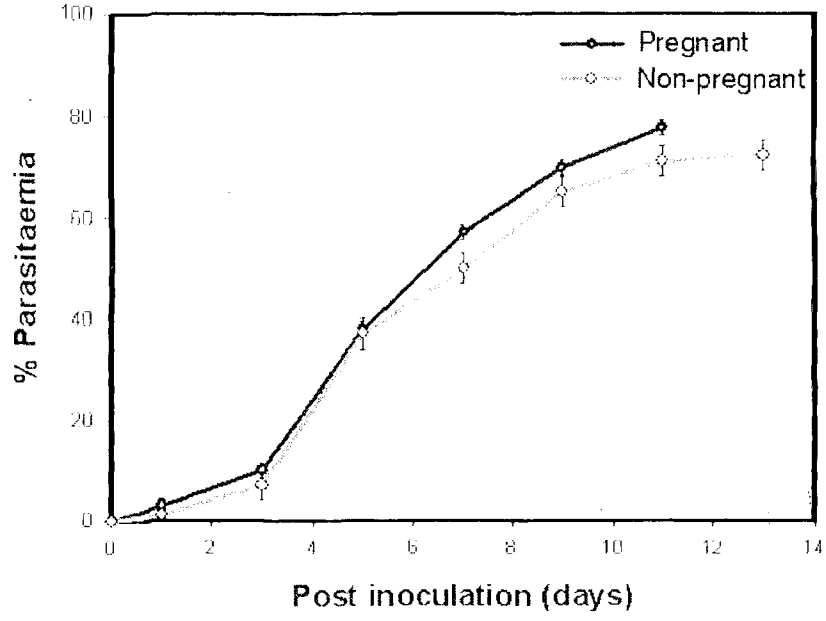

Fig. 1: Parasitaemic levels in pregnant (GD-10) and non-pregnant mice infected with P.berghei. [•-• Pregnant, o-o Non-pregnant, GD-gestational day].

indigenous materials. It protects the foetus from various infectious agents like bacteria, parasite, viruses etc. Normally the trophoblastic membrane in placenta is a metabolically complex cell system that covers the placental villi. The chronic villi appear as the voluminous cylinders with a complex interlacing patterns (Ferenczy and Richart, 1972; King and Menton, 1975). Therefore it is suggested that any alterations occurring either on the surface, internal structure or at ultra structural level of the placenta may limit the availability of essential nutrients for normal foetal development and growth which in turn culminates in abortions, reduced birth weight of foetus or foetal deaths in malaria infection.

Various pathological and ultra microscopy studies have documented that the placenta is severely damaged and is responsible for compromised foetal growth (Vinayak et al, 1986 ; Shukla, 1990; Brabin 1990; Menendez ,1990). The present study clearly indicated that there was a significant alterations occurring on the surface

Fig. 2a

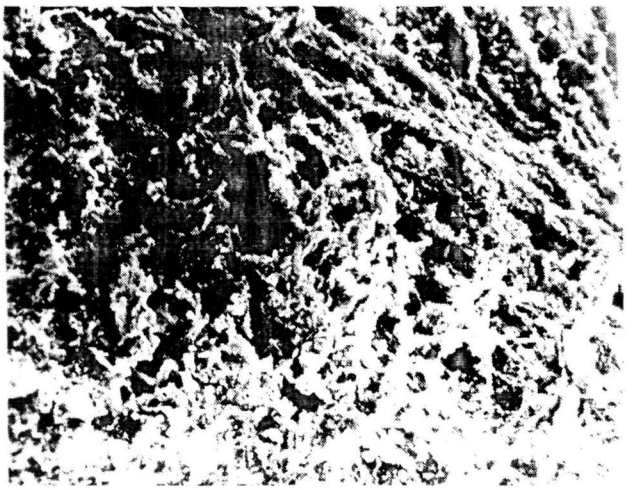

Fig. 2b

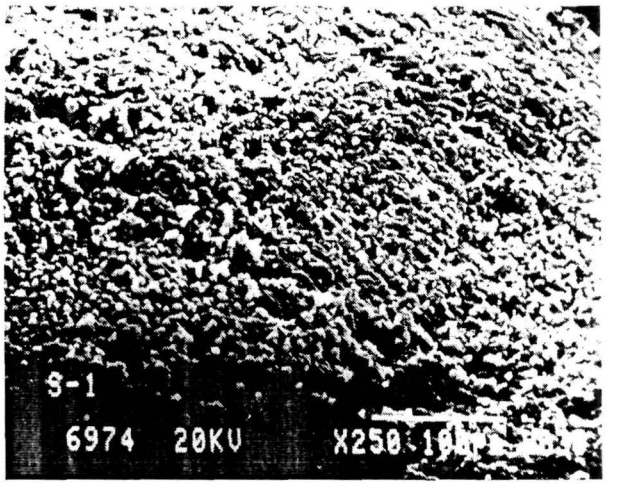

Fig. 2: Placenta of pregnant P.berghei (a) infected mice showing damaged trophoblastic membrane. Note the absence of bulbous microvilli ends (250x) and (b) uninfected mice showing abundant microvillus surface with bulbous end (250x).

Fig. 3a

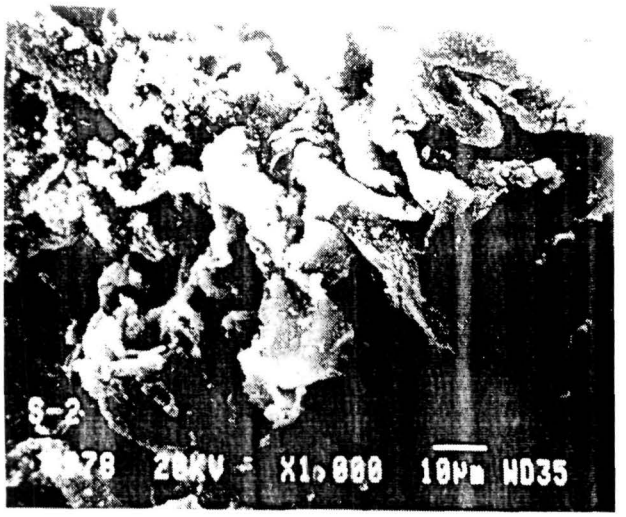

Fig. 3b

Fig. 3: Placenta of pregnant P.berghei (a) infected mice showing damaged surface of microvillus process (1000x) and (b) uninfected mice showing prominent chorionic villi giving rise to numerous trophoblastic sprouts suggesting an active trophoblastic growth (1000x).

of infected placenta as compared to the placenta of pregnant non-infected mice. The placenta obtained from all pregnant mice showed damaged trophoblastic membrane (Fig.2a) and loss of bulbous microvillus end (Fig.3a) that resulted in aggregation of trophoblastic membrane, whereas the surface of placenta obtained from uninfected pregnant mice showed the presence of abundant microvillus surface with bulbous end (Fig.2b), trophoblastic sprouts or numerous budding (Fig.3b). The altered structure of infected placenta might be either due to certain cytolytic substances or hydrolytic enzymes being produced by the parasite.

Conclusively, the present study clearly reveals the foetal abnormalities occurring during pregnancy in malaria infected mice damaged the placenta both externally and internally in addition to high parasitaemia, anaemia. Because of the severely damaged placenta, the early pregnancies often leads to foetal death, abortion or still birth and may explain why malaria is more severe in first and second trimester than in third trimester.

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PRODUCTION OF CARBON NANOTUBES USING ARC IGNITION OF GRAPHITE IN DE-IONIZED WATER

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Abstract

In this paper we report about the continuous production of carbon nanotubes using the arc ignition of graphite. The arc was generated under water at an arcing current of 60 amps A.C and the usual cumbersome requirements of vacuum and other details were not required. The resultant carbon soot was analyzed by SEM(Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy) which have shown the successful production of carbon nanotubes.

Key words : Nanomaterials, carbon nanotubes, SEM, TEM, fullerenes.

INTRODUCTION

Ever since the discovery of carbon nanotubes (CNT) in fullerenes soot by Ijima [1] there has been a tremendous interest in the scientific community. The carbon nanotubes have shown remarkable properties [2] which have lead to immense speculation about their diverse uses[3] in various fields; nanoelectronics [4], nanobiology [5], CNTs could act as field emitters in panel displays [6], scanning probe microscope tips [7], gas storage [8], single-molecular transistors [9] and many more applications. Shortly after the discovery of nanotubes they were produced [10] by the arc ignition of graphite which was similar to the one reported for the production of fullerenes [11]. The techniques require inert atmospheres and intensive labour and difficult preparation techniques of the catalyst. Thus easier production methods for the production of carbon nanotubes were required which are easily upgradeable and less cumbersome. Recently the carbon nanotubes have been reported by Biro et.al [12] to be formed by using an under water arc ignition of graphite. This method employs the benefits of an A.C and the arc is carried out under water leading to successful production of carbon nanotubes. In this paper we report the production of carbon nanotubes in a similar fashion albeit with some modifications.

EXPERIMENTAL

In this method two carbon rods were mounted in such a manner so that their ends just touch. This system is then mounted inside a water trough. The apparatus is then filled with triple deionized water. A large electrical signal is then passed across the rods thereby generating a brilliant arc. When the arc is running, black soot like material is sputtered throughout in the water. The arc process is a very high temperature process ~ 4000 K and a lot of heat is generated thus the water trough was surrounded by appropriate cooling. After the rods have been consumed the soot is isolated from the water and dried before further analysis. The experimental set up for carbon nanotube requires the following components; a water trough, a mechanical set up for supporting the rods, a low voltage high current power supply (A.C). The schematic diagram of the carbon nanotube generator along is as shown in FIG 1. The production was found to be more for higher arcing current-60 amps. For the arcing current of 40 amps practically no nanotubes were found. The experiments were also performed with normal tap water and deionized water. In the case of normal tap water no nanotubes were found even at 60 amps, whereas when deionized water was used there was some production but then the amount of other carbonaceous material was also more. On using triple deionized water the amount of tubes being produced also increased and further the amount of debris found is also reduced. The rods used for the experiment should be pure. The production of the tubes was found to be significantly less when impure carbon rods were used.

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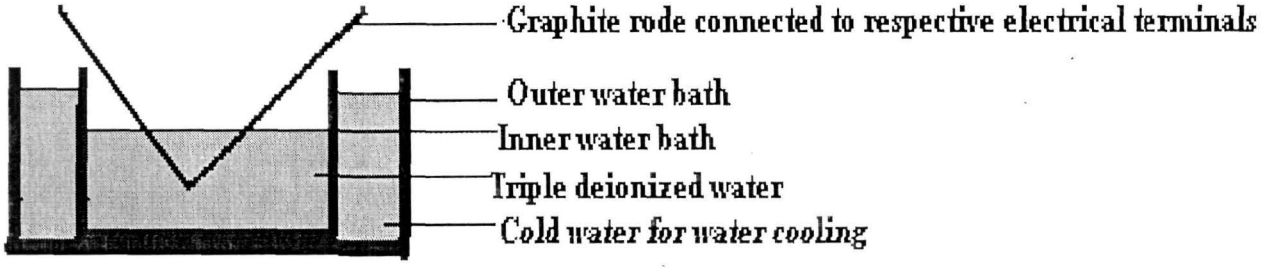

Fig. 1 : The Schematic Diagram of the Experimental Set Up

ANALYSIS AND RESULTS

As a result of our experiments we then collected the carbon soot. This soot was then first tested by SEM performed at RSIC, Panjab University, Chandigarh. The SEM photographs are as shown in fig 2. We also applied magnetic field on the arc to see the production of CNT under magnetic field. The SEM results of such production is shown in Fig. 3. Subsequently the TEM was also performed at IOP, Bhubaneshwar. The TEM photographs are as shown in Fig 3. From these SEM and TEM figures it is possible to conclude that the production of carbon nanotubes by using arc ignition of graphite is possible. Further, magnetic field seems to enhance the quantity and quality of CNT's. More work in this direction is in progress.

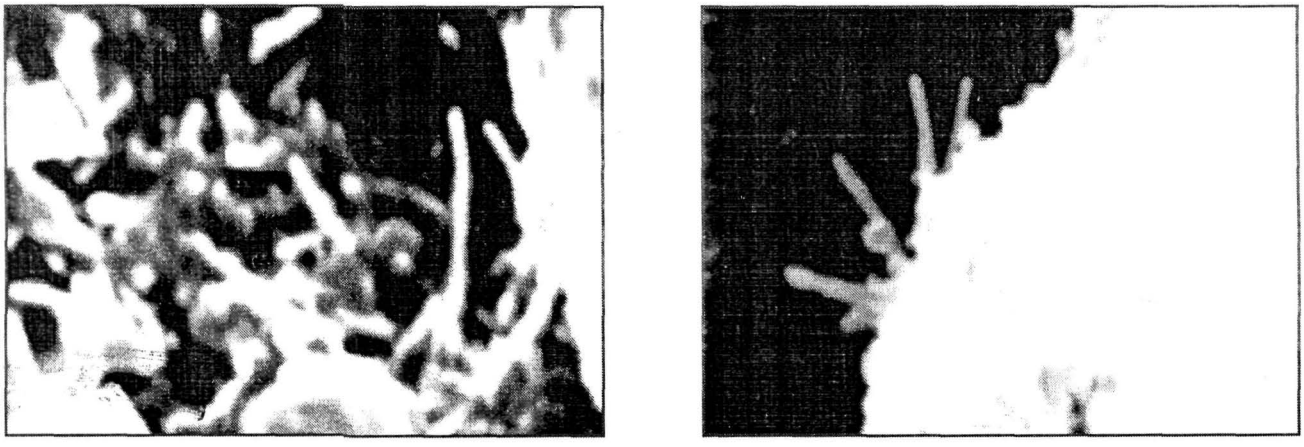

Fig. 2: SEM of carbon soot (without magnetic field)

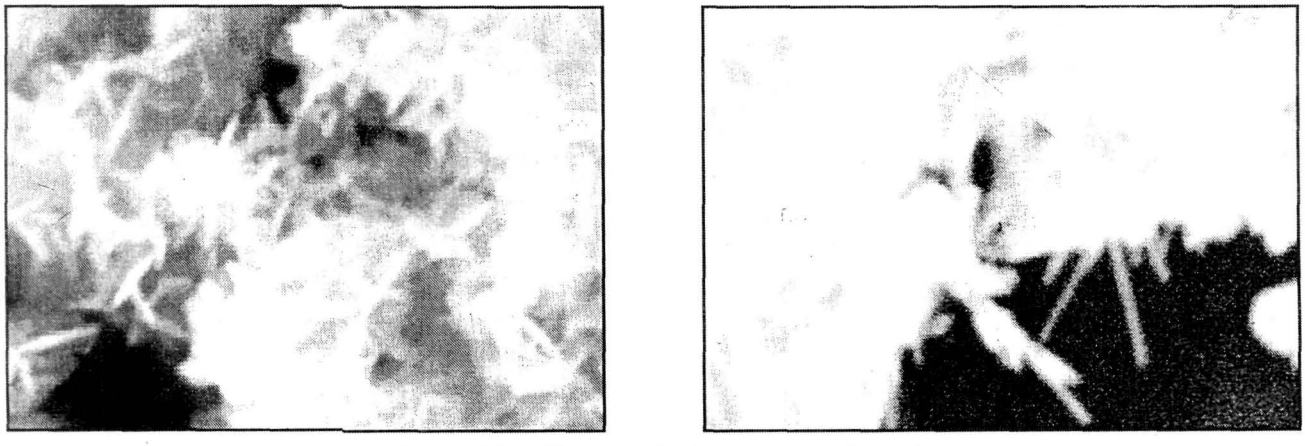

Fig. 3: SEM of Carbon soot (under magnetic field)

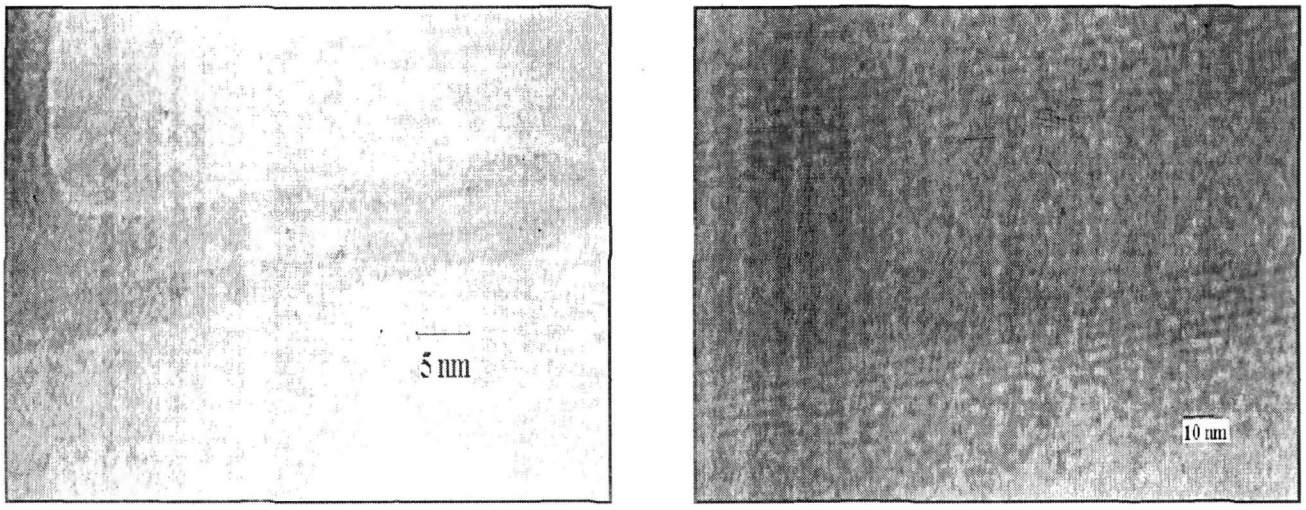

Fig. 4: TEM photograph showing a Single wall(left) and a multiwall (right) nanotube

CONCLUDING REMARKS

In spite of recent advancements there has been still a bottleneck relating to their easy methods of production. It appears that an under water arc generation might be an easy substitute for the production of carbon nanotubes. However experiments to increase the yield of nanotubes and efficient separation of desired kind are still being carried out.

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DETERMINATION OF THE EOQ UNDER PERMISSIBLE DELAY IN PAYMENTS ALLOWING PARTIAL PAYMENT WHEN QUANTITY RECEIVED IS UNCERTAIN

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Abstract

This paper deals with an inventory problem when delay in payments is permissible and yield is random. In practice, the supplier offers some credit period for settlement of accounts which is advantageous for the retailer in the sense that he can sell the product during that time and can earn some interest on the sales revenue during the permissible period of delay. In this problem facility of a part payment is introduced. The effect of part payment on the optimal solution is studied. The working of the model is illustrated with the numerical example.

Key words : Deterministic model, partial payment privilege, random input.

INTRODUCTION

The conventional EOQ model assumes that the payment for the units received has been made immediately after the receipt of the order. In practice, supplier offers some credit period to the retailer for making payments. This amounts to giving a kind of incentive in the form of supplying goods as an interest free loan to the buyer. The buyer in turn can make use of this incentive and earn some interest by keeping the amount in an interest earning account. With this idea, Goyal (1985) has studied a simple EOQ system with deterministic demand. Chand and Ward (1987) reinvestigated the same model. Mandal and Phaujdar (1989) extended the results of Goyal (1985) by allowing shortages. Shah (1991) studied this problem when quantity received was uncertain. Using first two moments of the distribution of the quantity received, Shah (1991) derived modified results of Goyal (1985) by the way of introducing probabilistic aspects. Shah (1992) developed probabilistic order level system when delay in payments is permissible. Shah (1997) extended the model when there is a lead-time. Shah *et al.* (1991) developed a model when demand increases with time and delay in payments is permissible. The aspect of admissible delay in payments has been extended to the case of two levels of storage by Shah and Shah (1992), which combined the ideas of Goyal (1985), and that of Hartely (1976).

In this paper, the main results of Goyal (1985) are reexamined by introducing practical aspects regarding part payment required by the supplier prior to the complete settlement of the accounts and when units ordered do not match with that replenished (Silver, 1976). It is a common practice that a part of the purchase amount is to be made sometime during the period of the permissible delay in payments. The part to be paid and the time at which it has to be made are to be mutually settled between the supplier and buyer at the time of purchasing the goods.

In this paper, we develop a deterministic model with above features (section 2) and provide c computational procedure (section 3) to determine the EOQ. Section 4 deals with sensitivity analysis of the model with respect to different parameters and results are interpreted. The paper concludes with conclusion section 5.

2. Mathematical Model:

The following notations and assumptions are used in developing the model :

- A : fixed ordering cost / order.
- C : unit cost of an item.
- I : inventory carrying charge fraction (excluding interest charged by the supplier) /annum
- R : annual demand, a deterministically known constant.
- I_c = rate of interest charged by the supplier/rupee / year.

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- I_e = rate of interest earned by the retailer / rupee / year.
- M : credit period expressed as a known fraction of a year.
- Y : a random variable which is received when Q – units are replenished with mean and variance E(Y) = bQ and $V(Y) = \sigma_0^2 + \sigma_1^2 Q^2$ (1)
- Q : procurement quantity (a decision variable).
- T_1 : time at which a part payment is to be made.
- α : part of the purchase amount to be paid at T_1 , $0 < \alpha < 1$.
- T(Y) : (Y / R) cycle time when Y units are received.

Assumptions are

- The lead-time is assumed to be zero.
- Rate of replenishment is infinite.
- Demand is deterministic.
- Shortages are not allowed.

The cost function for the problem comprises of a set-up cost, non-finance holding cost, interest paid and interest earned. Since M is a constant and T(Y) depends on the quantity received three case may arises viz.

1. $RT_1 < RM < Y$
2. $RT_1 < Y < RM$, and
3. $Y < RT_1 < RM$.

Here,

a. OC = ordering cost = A.

b. IHC = inventory holding cost = $CIY^2 / 2R$.

The interest charges (paid and earned) for the items kept in stock differ in the three case and will be computed as given below.

Case 1. $RT_1 < RM < Y$.

In this case, the settlement of the account has to be done before the cycle ends and a part payment will be made at time T_1 . The amount to be made will be αCRT_1. The following are the components of the interest earned.

i. Interest earned during $(0, T_1) = CI_eRT_1^2 / 2$.

ii. The cash on hand at $T_1 = (1-\alpha)CRT_1 + CI_eRT_1^2 / 2$. where the second term is due to interest earned during $(0, T_1)$ on the sale amount.

iii. The interest earned during (T_1, M) = $[(1 - \alpha)CRT_1 + CI_eRT_1^2 / 2] Ie(M-T_1) + CI_eR(M - T_1)^2 / 2$.

Hence,

c. Total interest earned in this case

$IE = CI_eRT_1^2 / 2 + [(1 - \alpha)CRT_1 + CI_eRT_1^2 / 2] Ie(M-T_1) + CI_eR(M - T_1)^2 / 2$.

d. Interest charged during (M, T(Y))

$IC = CI_c(Y – RM)^2 / 2R$.

Hence, total cost of an inventory system during (0, T(Y)) is

$K_1(Y) = OC + IHC + IC – IE$

Using Ross's renewal theory and eq. (1), the total cost

$K_1(Q) = E(K_1(Y)) / E(T(Y))$

$= E_1 / Q + E_2Q + E_3$ (2)

where

$$E_1 = \frac{AR}{b} + \frac{C(I + I_c)\sigma_0^2}{2b} + \frac{CI_cR^2M^2}{2b} - \frac{CI_cR^2(M - T_1)^2}{2b} - \frac{CI_cR^2T_1^2}{2b}$$

$$- \left\{(1 - \alpha)CRT_1 + \frac{CI_cRT_1^2}{2}\right\}\frac{I_c(M - T_1)R}{b}$$

$$E_2 = \frac{C(I + I_c)(\sigma_1^2 + b^2)}{2b}$$

$$E_3 = -CI_cRM$$

Minimizing $K_1(Q)$ with respect to Q gives the optimal value of Q_{10} as

$Q_{10} = \sqrt{E1 / E2}$ (3)

When T_1 = M i.e. part payment is not allowed and the entire payment has to be made at the end of the permissible credit period, the interest earned in this case has to be calculated on sales amount and not on the cash on hand. If we put σ_0^2 , $\sigma_1^2 = 0$ and b = 1 then developed model agrees with Goyal (1985).

When the permissible delay period is more than the cycle time, the retailer will settle the account of the current cycle only at the time M, after placing another order at T. There are again two cases : $RT_1 < Y < RM$ and $Y < RT_1 < RM$. These two case are discussed separately below :

Case 2 : $RT_1 < Y < RM$.

In this situation, a part payment will be made at T_1. The retailer earns interest on cash on hand until M. Hence, the interest earned in this case, following the same logic as in case 1, consists of the following components:

i. Interest earned during $(0, T_1) = CI_eRT_1^2 / 2$.

ii. The cash on hand at $T_1 = (1 - \alpha)CRT_1 + CI_eRT_1^2/2$.

iii. The interest earned during (T1, T(Y))

$= CI_eR(T(Y) - T_1)[2(1 - \alpha)T_1 + T(Y) - T_1 + T_1^2I_e] / 2$.

iv. The cash on hand at T(Y)

$= CR(T(Y) - T_1) + CI_eR(T(Y) - T_1)[2(1 - \alpha)T_1 + T(Y) - T_1 + T_1^2I_e] / 2$.

v. Interest earned during (T(Y), M)

$= \{ CR(T(Y) - T_1) + CI_eR(T(Y) - T_1)[2(1 - \alpha)T_1 + T(Y) - T_1 + T_1^2I_e] / 2 \}I_e$.

Hence, total interest earned

e. $IE = CI_eRT_1^2 / 2 + CI_eR(T(Y) - T_1)[2(1 - \alpha)T_1 + T(Y) - T_1 + T_1^2I_e] / 2$

$+ \{ CR(T(Y) - T_1) + CI_eR(T(Y) - T_1)[2(1 - \alpha)T_1 + T(Y) - T_1 + T_1^2I_e] / 2 \}I_e$.

f. Interest charged = 0.

Hence, total cost of an inventory system during (0, T(Y)) is

$K_2(Y) = OC + IHC - IE$

The total expected cost of an inventory system per time unit is

$K_2(Q) = E(K_2(Y)) / E(T(Y))$

$= E_1 / Q + E_2Q + E_3Q^2 + E_4$ (4)

where

$X = \sigma_1^2 + b^2$.

$$E_1 = \frac{1}{b}\left\{\begin{aligned} &AR - CI_eR^2T_1^2 + C(1-\alpha)I_eR^2T_1^2 + \frac{CI_e^2R^2T_1^3}{2} + CI_eRMT_1 \\ &+ C(1-\alpha)I_e^2R^2T_1^2M + \frac{CI_e^2R^2T_1^3M}{2} - \frac{CI_e^2R^2T_1^2M}{2} + \frac{C(I+I_e)\sigma_0^2}{2} \\ &+ C(1-\alpha)I_e^2T_1\sigma_0^2 + \frac{CI_e^3T_1^2\sigma_0^2}{2} - \frac{CI_eM\sigma_0^2}{2} - \frac{CI_e^3T_1\sigma_0^2}{2} - \frac{CI_eT_1\sigma_0^2}{2} \end{aligned}\right\}$$

$$E_2 = \frac{X}{b}\left\{\frac{C(I+I_e)}{2} + C(1-\alpha)I_e^2T_1 + \frac{CI_e^3T_1^2}{2} - \frac{CI_eM}{2} - \frac{CI_eT_1}{2} - \frac{CI_e^2T_1}{2}\right\}$$

$$E_3 = \frac{CI_e^2}{2R}(3\sigma_1^2 + b^2)$$

$$E_4 = CI_e^2RT_1M - C(1-\alpha)I_eRT_1 - CI_e^2RT_1^2 - CI_eRM - C(1-\alpha)I_e^2RT_1M -$$

$$C(1-\alpha)I_e^2RT_1^2 - \frac{CI_e^3RT_1^2M}{2} - \frac{CI_e^3RT_1^3}{2} + \frac{3CI_e^2\sigma_0^2}{2R}$$

Minimizing $K_2(Q)$ with respect to Q yields the optimal value of Q involved in cubic equation

$$2E_3Q^3 + E_2Q^2 - E_1 = 0 \qquad (5)$$

Case 3 : $Y < RT_1 < RM$.

Here, the settlement of accounts is made at M and part payment will be made at T_1. The interest charged is zero. The various components of interest earned are as follows:

i. Interest earned during $(0, T(Y)) = CI_eRT(Y)^2 / 2$.

ii. The cash on hand at $T(Y) = CRT(Y) + CI_eRT(Y)^2 / 2$.

iii. The interest earned during $(T(Y), T_1)$

$= CI_eRT(Y)(T_1 - T(Y)) + CI_e^2RT(Y)^2(T_1 - T(Y)) / 2$.

iv. The cash on hand at T_1

$= C(1-\alpha)RT(Y) + CI_eRT(Y)(T_1 - T(Y)) + CI_e^2RT(Y)^2 (T_1 - T(Y)) / 2$.

v. Interest earned during (T_1, M)

$=C(1-\alpha)I_eRT(Y)(M-T_1) + CI_e^2RT(Y)(T_1 - T(Y)) (M - T_1) + CI_e^3RT(Y)^2(T_1 - T(Y))(M - T_1) / 2$.

Hence, total interest earned

j. $IE = CI_eRT(Y)^2 / 2 + CI_eRT(Y)(T_1 - T(Y)) + CI_e^2RT(Y)^2(T_1 - T(Y)) / 2 + C(1-\alpha)I_eRT(Y)(M-T_1) + CI_e^2RT(Y)(T_1 - T(Y)) (M - T_1) + CI_e^3RT(Y)^2(T_1 - T(Y))(M - T_1) / 2$.

Hence, total cost of an inventory system during (0,T(Y)) is

$$K_3(Y) = OC + IHC - IE$$

The total expected cost of an inventory system per time unit is

$$K_3(Q) = E(K_3(Y)) / E(T(Y)) = F_1 / Q + F_2Q + F_3Q^2 + F_4 \qquad (6)$$

where

$X = \sigma_1^2 + b^2$.

$$F_1 = \frac{1}{b}\left\{AR + \frac{C(I + I_e)\sigma_0^2}{2} - \frac{3CI_e^2T_1\sigma_0^2}{2} + CI_e^2\sigma_0^2M - \frac{CI_e^3T_1M\sigma_0^2}{2} + \frac{CI_e^3T_1^2\sigma_0^2}{2}\right\}$$

$$F_2 = \frac{X}{b}\left\{\frac{C(I + I_e)}{2} - \frac{3CI_e^2T_1}{2} + CI_e^2M - \frac{CI_e^3T_1M}{2} + \frac{CI_e^3T_1^2}{2}\right\}$$

$$F_3 = \frac{1}{2R}\left\{CI_e^2(3\sigma_1^2 + b^2) + CI_e^3(3\sigma_1^2 + b^2)(M - T_1)\right\}$$

$$F_4 = -CI_eRT_1 - C(1-\alpha)RI_eM - C(1-\alpha)RI_eT_1 - CI_e^2RT_1M + CI_e^2RT_1^2 + \frac{3CI_e^2\sigma_0^2}{2R} + \frac{3CI_e^3\sigma_0^2M}{2R} - \frac{3CI_e^3\sigma_0^2T_1}{2R}$$

The optimal value which minimizes $K_3(Q)$ can be found by solving the cubic equation

$$2F_3Q^3 + F_2Q^2 - F_1 = 0 \qquad (7)$$

The expression in (4) reduces to Goyal's(1985) model when $T_1 = 0$, I.e. the part payment is not allowed.

3. Search for the Optimum Solution:

Since there are three cases, depending on the relative values of M and $T = Q / \rho$, the feasibility of each case

will be checked before evaluating the cost function. When T = M (i.e. the cycle time coincides with permissible delay period, the cost functions of all the cases reduce to

$$K(Q) = AR/Q + CIQ/2 - CI_eR^2T_1^2/2Q - CI_e(Q - RT_1)^2/2Q - C(1-\alpha)I_eT_1(Q - RT_1) \quad (8)$$

The optimum solutions can be obtained using the following step – wise method.

Step 1. Given the input parameters, evaluate Q_{10}, Q_{20}, and Q_{30}. If $Q_{10}/R \geq M$ then evaluate $K_1(Q_{10})$ using (2) otherwise evaluate K(M) using (8).

Define $J_1 = \min\{K_1(Q_{10}), K(M)\}$ and the corresponding interval by I_1.

Step 2. If $RT_1 < Y < RM$, evaluate $K_2(Q_{20})$ using (4). If $Q_{20}/R < M$ and Q_{20}/R does not lie between T_1 and M then evaluate $K_2(T_1)$ using (5).

Define $J_2 = \min\{K_2(Q_{20}), K_2(RT_1), K(M)\}$ and the corresponding interval by I_2.

Step 3. If $Q_{30} < RM$ and T_1 lies between Q_{30}/R and M, evaluate $K_3(Q_{30})$ using (6). If

$Q_{30} < RM$ and T_1 does not lie between Q_{30}/R and M then evaluate $K_3(RT_1)$ using (6) and K(M) using (8).

Define $J_3 = \min\{K_3(Q_{30}), K_3(RT_1), K(M)\}$ and the corresponding interval by I_3.

Step 4. $J = \min\{J_1, J_2, J_3\}$ and L denote the replenishment interval that gives J. Hence, the optimum solution given by $Q_0 = RL$ and the minimum cost is J.

1. Numerical Example:

The working of the model is illustrated with the following parameters:

$$[C, I, A, \rho, Ic, Ie, \alpha, M, \sigma_0^2, \sigma_1^2, b] =$$

[20, 10%, 100, 1000, 15%, 10%, 0.2, 0.1, 5, 0.1, 0.80]

$T_1 = 0.5 * M$ which means that the part payment is to be made at the middle of the credit period.

For the particular set of parameters chosen, Case1 is feasible and the optimum purchase quantity $Q_0 = 237$ – units and the minimum cost $K(Q_0) = 847.49$. To study the effect of the time at which part payment is made on the inventory system, consider $T1 = \beta M$ for $0 < \beta < 1$, i.e. the time, at which the part payment is made, is expressed as a fraction of the credit period.

Table 1: Sensitivity of the model w.r.t. α and β

α	β	0.25	0.50	0.75
0.00	Q_{10}	221.98	221.97	221.97
	$K(Q_{10})$	915.42	915.39	915.38
0.20	Q_{10}	222.54	222.72	222.54
	$K(Q_{10})$	918.54	919.29	918.32
0.40	Q_{10}	223.09	223.45	223.09
	$K(Q_{10})$	921.27	923.19	921.23

It is observed that for the particular value of α, when $\beta < 0.50$ or $\beta > 0.50$, the optimum purchase quantity and the total cost of an inventory system are less than that at $\beta = 0.50$. While for fixed value of β, increase in α result increase in optimum purchase quantity and total cost of an inventory system.

Table 2: Sensitivity of the model w.r.t. α and σ_0

α	σ_0	5	10	15
0.00	Q_{10}	221.98	222.00	222.01
	$K(Q_{10})$	915.42	915.49	915.56
0.20	Q_{10}	222.54	222.56	222.57
	$K(Q_{10})$	918.54	918.42	918.49
0.40	Q_{10}	223.10	223.11	223.13
	$K(Q_{10})$	921.27	921.34	921.41

The effect of the part payment α and σ_0^2 is studied here. It is observed that increase in α increases procurement quantity to be purchased and total cost of an inventory system. The model is not very sensitive to changes in σ_0^2 even though increase in σ_0^2 results increase in purchase quantity and total inventory cost of the system.

Table 3: Sensitivity of the model w.r.t. α and σ_1

α	σ_1	0.1	0.2	0.3
0.00	Q_{10}	236.50	221.98	209.85
	$K(Q_{10})$	843.85	915.42	982.84
0.20	Q_{10}	237.10	222.54	210.37
	$K(Q_{10})$	846.60	918.35	985.94
0.40	Q_{10}	237.70	223.10	210.90
	$K(Q_{10})$	849.34	921.27	989.03

The model is very sensitive to changes in σ_1^2, keeping α fixed. It is observed that increase in σ_1^2 results significant decrease in procurement quantity and increase in total inventory cost of a system. Similar pattern is observed for fixed σ_1^2 and increase in α. The increase in α also increases optimum purchase units.

Table 4: Sensitivity of the model w.r.t. α and b

α	b	0.6	0.7	0.8
0.00	Q_{10}	299. 97	264.87	236.51
	$K(Q_{10})$	899.90	866.25	843.85
0.20	Q_{10}	300.73	265.54	237.10
	$K(Q_{10})$	902.79	869.05	846.60
0.40	Q_{10}	301.48	266.20	237.70
	$K(Q_{10})$	905.67	871.85	849.34

The increase in bias factor b for fixed value of α, decreases optimum purchase quantity and total inventory cost significantly. Similar observations are for fixed value of b and increase in α results.

Table 5: Sensitivity of the model w.r.t. β and σ_0

σ_0	β	0.25	0.50	0.75
5.00	Q_{10}	237. 10	237.30	237.10
	$K(Q_{10})$	846.60	847.49	846.57
10.00	Q_{10}	237.12	237.31	237.11
	$K(Q_{10})$	846.67	847.56	846.64
15.00	Q_{10}	237.13	237.32	237.13
	$K(Q_{10})$	846.73	847.62	846.70

The sensitivity are studied here. It is observed that for $\beta < 0.50$ and $\beta > 0.50$ optimum procurement quantity and total inventory cost is less than that for $\beta = 0.50$. Increase in σ_0^2 result increase in optimum procurement quantity and total cost.

Table 6: Sensitivity of the model w.r.t. β and σ_1

σ_1	β	0.25	0.50	0.75
0.10	Q_{10}	237. 10	237.17	237.23
	$K(Q_{10})$	846.60	846.93	847.18
0.20	Q_{10}	222.54	222.61	222.66
	$K(Q_{10})$	918.35	918.69	918.96
0.30	Q_{10}	210.37	210.43	210.48
	$K(Q_{10})$	985.94	986.30	986.59

The effect of changes in β and σ_1^2 are studied in this table. For fixed value of σ_1^2, the increase in β does not have significant change in purchase quantity or total cost. While for fixed of β, increase in σ_1^2 reduces purchase quantity and increases total cost significantly.

Table 7: Sensitivity of the model w.r.t. β and b

b	β	0.25	0.30	0.35
0.70	Q_{10}	265.54	265.62	265.68
	$K(Q_{10})$	869.05	869.38	869.64
0.75	Q_{10}	250.59	250.66	250.72
	$K(Q_{10})$	856.76	857.09	857.34
0.80	Q_{10}	237.10	237.17	237.23
	$K(Q_{10})$	846.60	846.93	847.18

It is observed that model is insensitive to changes in β and b.

Table 8: Sensitivity of the model w.r.t. M

M	0	2/12	4/12	6/12	8/12
Q_{10}	225.03	243.00	290.10	354.68	428.70
$K(Q_{10})$	1111.09	699.81	432.39	251.23	116.71

It is observed that optimum purchase quantity and total cost are very sensitive to changes in permissible delay period. Increase in delay period, results increase in purchase units and decrease in total cost significantly.

CONCLUSIONS

It is observed that the optimal cost will be larger when $\beta = 0.5$ instead of $\beta \neq 0.5$. In other words, it would be advantageous to the retailer if the supplier insists a part payment either towards the beginning or the end of the permissible delay period instead of paying it at the middle.

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COMPARATIVE STUDIES ON THE INTERNAL GENITALIC ORGANS OF THREE INDIAN SPECIES OF GENUS *CALLIMORPHA* LATERILLE (ARCTIIDAE : LEPIDOPTERA)

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Abstract

Studies have been conducted on the internal genitalic organs of three Indian species i.e., nyctemerata Moore, equitalis Kollar and principalis Kollar under genus Callimorpha Laterille of family Arctiidae for the first time. A key to the presently studied species has also been formulated by incorporating their internal genitalic attributes.

Key words : Internal genitalic organs, male, female, genus, *Callimorpha* species.

INTRODUCTION

Extensive and intensive collection-cum-survey tours were conducted during September 1999 to September 2003 to different localities of North-East India for the study of internal genitalic organs of tiger moths belonging to family Arctiidae. The genus *Callimorpha* Laterille is represented by six Indian species i.e., *Callimorpha principalis* Kollar, *C. similes* Moore, *C. plagiata* Walker, *C. equitalis* Kollar, *C. nyctemerata* Moore and *C. lichenigera* Moore as described by Hampson (1894). Koda (1987) discussed and illustrated the male and female genitalia of the type species i.e. *dominula* Linnaeus of the referred genus. During the present investigations, internal male and female genitalia of *Callimorpha equitalis* Kollar and *C. nyctemerata* Moore and only female of *C. principalis* Kollar have been examined and illustrated. The identification of the above said species were made from the relevant literature (Hampson, 1894) and by comparison from the museum of Zoological Survey of India, Kolkata. Nomenclature for naming different internal genitalic organs has been followed from the works of current workers like Buntin and Pedigo (1983), Rose and Singh (1985, 1988, 1989a, 1989b), Justus and Mitchell (1999) and Gowda *et al.*, (2002).

MATERIALS AND METHODS

The adult Arctiid moths were collected from the fluorescent lights with the help of a light trap fitted at different places during night time. After starving the moths for about 7-10 hours in small insect breeding cages, the abdomens of anaesthetized moths were detached and descaled. The dissections were performed in physiological saline solution and the internal male and female genitalic organs were preserved in a mixture of 70% alcohol and 0.5% glycerol in the ratio of 1:4 in homoeopathic vials for further studies.

OBSERVATIONS

GENUS *CALLIMORPHA* LATERILLE

Laterille, 1809, *Gen. Crust. Ins.*, **4** : 220.

Type species : *Callimorpha dominula* Linnaeus from Europe.

Distribution : Europe; China; Himalayas, Assam; Myanmar.

Key to the species of Genus *Callimorpha* Laterille

1. Internal male genitalic organs with cuticular simplex curved, length 5.50 mm, width 0.50 mm; internal female genitalic organs with lateral oviduct short length 2.0 mm, width 0.50 mm; common oviduct long, length 1.25 mm x 0.25 mm.....**2**

— Internal male genitalic organs with cuticular simplex coiled, length 5.0 mm, width 0.50 mm; internal female genitalic organs with common oviduct short, length 0.66 mm x 0.41 mm.....***C. nyctemerata* Moore**

2. Internal female genitalic organs with lateral and common accessory glands reservoirs clearly differentiated; each ovary with common terminal

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filament long transparent, length 6.25 mm, width 0.25 mm..... ***C. equitalis* Kollar**

— Internal female genitalic organs with lateral and common accessory gland reservoirs completely fused into a single structure; each ovary with common terminal filament short translucent, length 3.0 mm, width 0.16 mm.....***C. principalis* Kollar**

***Callimorpha nyctemerata* Moore**
Moore, 1879, *Lep. Atk.*, **1879** : 38.
(Figs. 1, 2)

Male : Testis ellipsoidal, dark reddish-brown, opaque, length 1.0 mm, width 1.50 mm; seminal vesicle-I opaque, originating separately from testis, length 1.10 mm, width 0.20 mm; seminal vesicle-II dark opaque, crossed, length 0.80 mm, width 0.40 mm; vasa deferentia translucent, length 2.60 mm, width 0.10 mm, entering ductus ejaculatorius duplex beyond middle towards accessory glands; ductus ejaculatorius duplex dark opaque, S-shaped, length 4.10 mm, width 0.70 mm; accessory glands entirely free but slightly fused at tip, divided into three sections; ductus ejaculatorius simplex with primary simplex divided into five sections; constrictor muscular area with cuticular simplex dark opaque, coiled, length 5.0 mm, width 0.50 mm; cuticular tube long and slender, length 5.70 mm, width 0.20 mm, enters aedeagus apically.

Table 1: Intraspecific range of measurements of internal male genitalic organs- *Callimorpha nyctemerata* Moore.

Sr. No.	Organ	Length (mm)	Width (mm)
1.	Testis	0.93-1.05	1.47-1.53
2.	Seminal vesicle-I	1.07-1.13	0.18-0.23
3.	Seminal vesicle-II	0.78-0.83	0.37-0.44
4.	Vasa deferentia	2.53-2.63	0.09-0.12
5.	Ductus ejaculatorius duplex	4.02-4.15	0.60-0.76
6.	Ductus ejaculatorius simplex	42.70-42.95	0.30-0.65
7.	Constrictor muscular area	4.90-5.06	0.43-0.53
8.	Cuticular tube	5.63-5.72	0.18-0.23
9.	Accessory glands	37.80-37.96	0.20-0.35

Female : Each ovary with common terminal filament dark opaque, length 9.0 mm, width 0.16 mm; egg tube filled with yellowish opaque ova, length 30.22 mm, width 0.16-0.50 mm; pedicel yellowish opaque, length 1.16 mm, width 0.16 mm; lateral oviduct dark yellow opaque, length 16.66 mm, width 0.55 mm; common oviduct opaque, length 0.66 mm, width 0.33 mm; complex sperm storage system with spermathecal gland simple, dark opaque, bifurcated near tip, length 24.00 mm, width 0.16 mm; infundibulum length 0.25 mm, width 0.16 mm; spermathecal duct opaque, length 1.16 mm, width 0.16 mm; utriculus length 3.83 mm, width 0.50 mm; lagena dark opaque, length 0.83 mm, width 0.66 mm; spermathecal duct dark opaque, length 24.00 mm, width 0.16 mm; corpus bursae dark yellowish, opaque, diameter 17.00 mm, two long signa present in the corpus bursae; ductus bursae length 2.16 mm, width 1.0 mm; ostium bursae diameter 1.50 mm; ductus seminalis tube-like, originating from ductus bursae; common accessory glands reservoir length 0.33 mm, width 0.33 mm; lateral accessory gland reservoirs dark opaque, pear-shaped, length 1.16 mm, width 0.32 mm; accessory gland duct-I short, dark opaque, length 0.65 mm, width 0.25 mm; accessory gland duct-II length 8.33 mm, width 0.16 mm; vestibulum opaque, length 1.16 mm, width 0.32 mm; vagina 0.83 mm, width 0.50 mm.

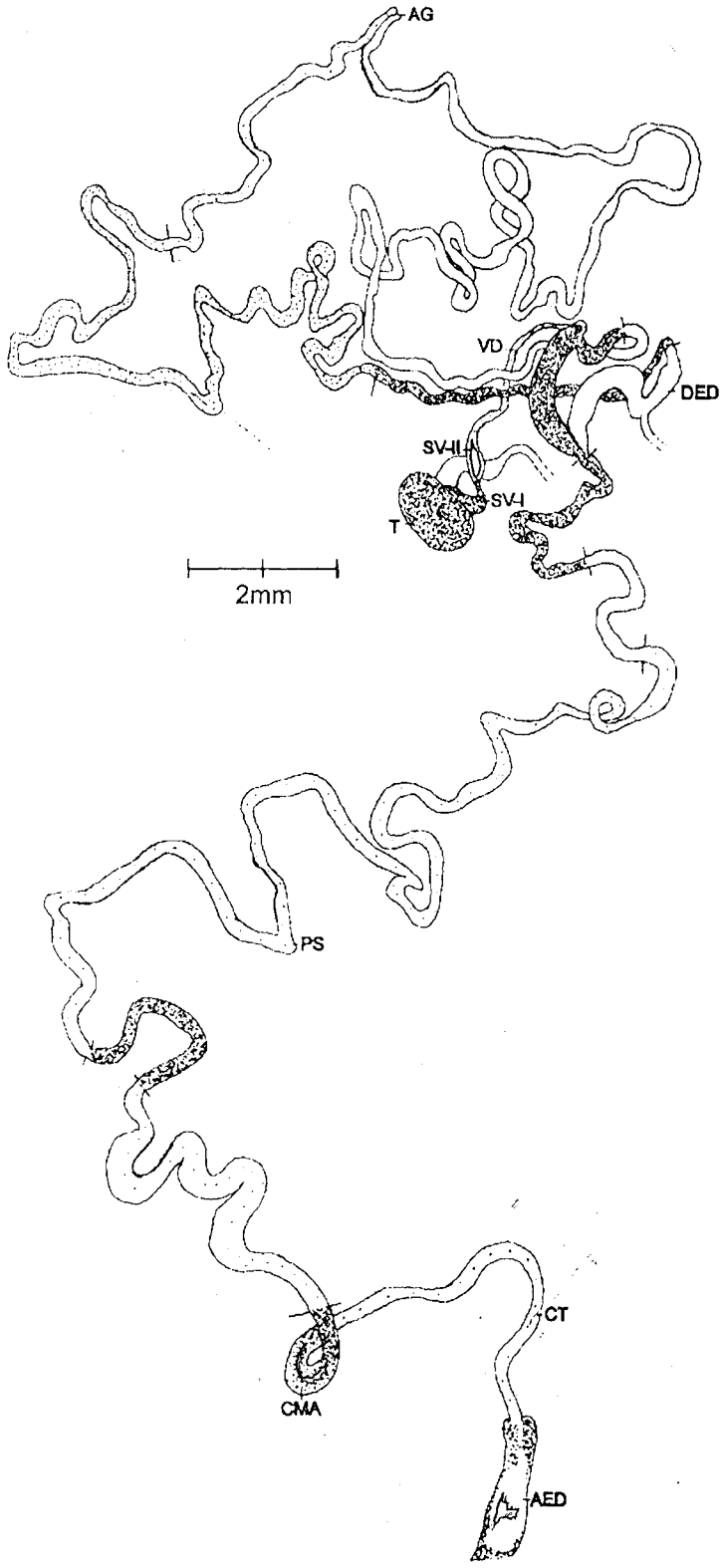

Fig. 1: Male Reproductive System of *Callimophra nyctemerata* Moore.

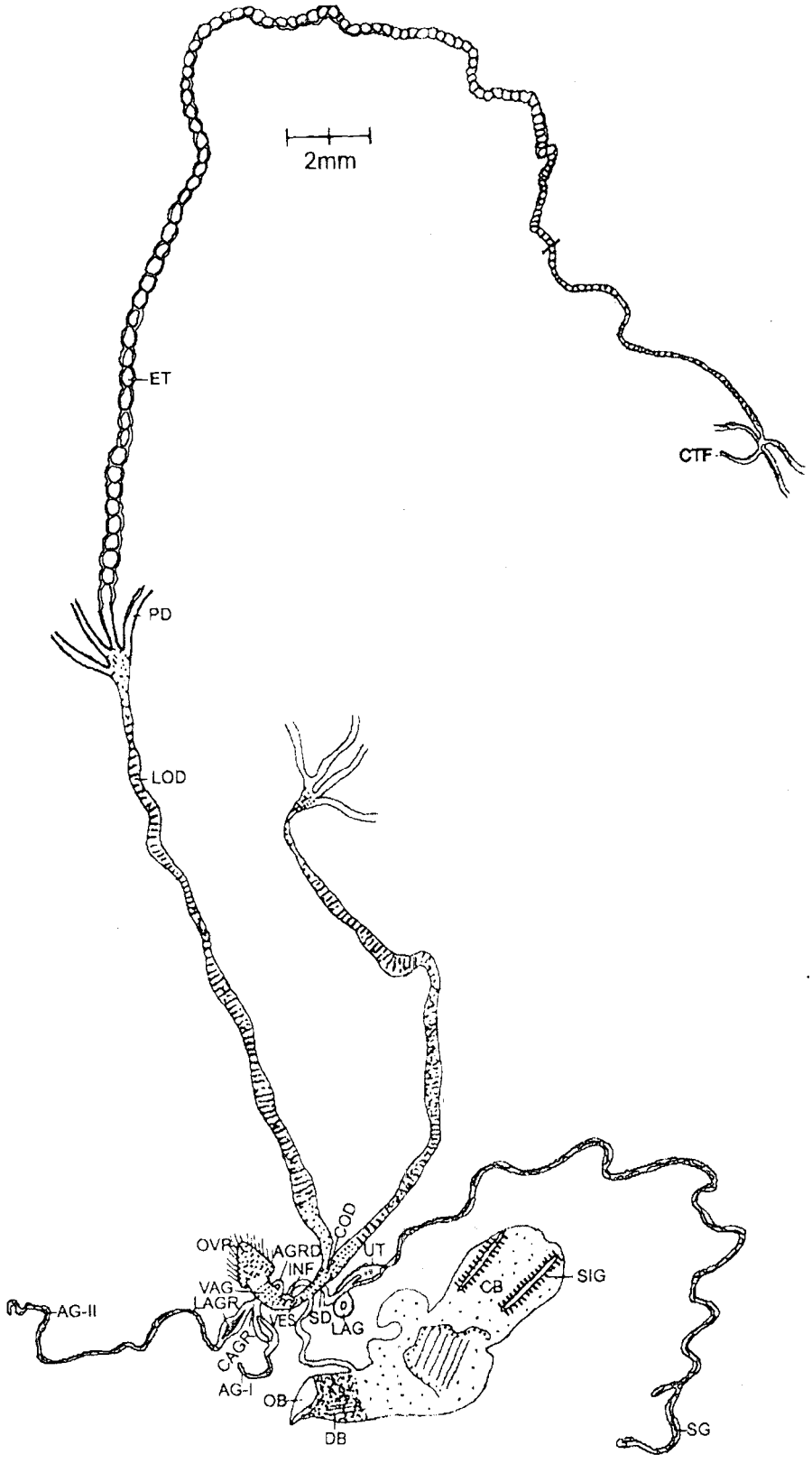

Fig. 2: Female Reproductive System of *Callimophra nyctemerata* Moore.

Table 2: Intraspecific range of measurements of internal female genitalic organs-*Callimorpha nyctemerata* Moore.

Sr. No.	Organ	Length (mm)	Width (mm)
1.	Common terminal filament	8.91-9.10	0.10-0.18
2.	Egg tube	30.10-30.55	0.15-0.55
3.	Lateral oviduct	16.10-16.70	0.45-0.55
4.	Common oviduct	0.60-0.70	0.30-0.35
5.	Pedicel	1.10-1.20	0.10-0.20
6.	Infundibulum	0.20-0.26	0.10-0.19
7.	Utriculus	3.80-3.90	0.40-0.55
8.	Lagena	0.80-0.85	0.60-0.70
9.	Spermathecal duct	1.15-1.18	0.14-0.19
10.	Spermathecal gland	23.10-24.10	0.10-0.19
11.	Corpus bursae-diameter	16.30-17.15	-
12.	Ductus bursae	2.10-2.20	0.90-1.07
13.	Ostium bursae-diameter	1.40-1.60	-
14.	Ductus seminalis	3.10-3.60	0.10-0.18
15.	Bulla seminalis	absent	
16.	Accessory gland reservoir duct	1.60-1.70	0.10-0.20
17.	Lateral accessory gland reservoir	1.10-1.20	0.30-0.35
18.	Common accessory gland reservoir	0.30-0.40	0.30-0.40
19.	Accessory gland duct-I	0.60-0.70	0.20-0.30
20.	Accessory gland duct-II	8.20-8.40	0.13-0.18
20.	Vestibulum	1.10-1.20	0.30-0.35
21.	Vagina	0.80-0.85	0.45-0.55

Material examined : Sikkim : Gangtok, 17.5.01, 1♀ ; 20.5.01, 1♂; Mangan, 18.9.2000, 2♂♂, 1♀; Arunachal Pradesh : West Kameng District, Bomdila, 13.5.01, 2♂♂, 2♀♀; Dirang, 14.5.01, 2♂♂, 1♀.

Old distribution : Sikkim.

Remarks : The present species is a new record from Arunachal Pradesh.

***Callimorpha equitalis* Kollar**

Kollar, 1844, *Hügel's Kaschmir,* **4** : 465.

(Figs. 3, 4)

Male : Testis oval, dark crimson red, length 1.0 mm, width 1.50 mm; seminal vesicle-I frothy opaque, crossed, length 6.0 mm, width 0.50 mm; seminal vesicle II pear-like, dark opaque, length 1.80 mm, width 0.80 mm; vasa deferentia light opaque, length 4.50 mm, width 0.33 mm, entering ductus ejaculatorius duplex beyond middle towards accessory glands; ductus ejaculatorius duplex dark opaque, S-shaped, length 8.30 mm, width 0.80 mm; accessory glands divided into three sections; section I dark opaque, fused distally, length 5.80 mm, width 0.33 mm; section II light opaque, length 63.33 mm, width 0.33 to 0.50 mm; section III dark opaque, yellowish-black, fused, length 12.50 mm, width 0.33 to 0.50 mm; ductus ejaculatorius simplex with primary simplex divided into six sections; constrictor muscular area with cuticular simplex dark yellow opaque, curved, length 5.50 mm, width 0.50 mm; cuticular tube light translucent, length 7.50 mm, width 0.33 mm, enters into aedeagus apically.

Table3: Intraspecific range of measurements of internal male genitalic organs- *Callimorpha equitalis* Kollar.

Sr. No.	Organ	Length (mm)	Width (mm)
1.	Testis	0.90-1.10	1.22-1.55
2.	Seminal vesicle-I	5.70-6.10	0.45-0.55
3.	Seminal vesicle-II	1.60-1.85	0.75-0.85
4.	Vasa deferentia	4.10-4.60	0.30-0.35
5.	Ductus ejaculatorius duplex	8.10-8.39	0.75-0.85
6.	Ductus ejaculatorius simplex	55.10-56.10	0.60-0.70
7.	Constrictor muscular area	5.40-5.60	0.45-0.55
8.	Cuticular tube	7.10-7.60	0.30-0.35
9.	Accessory glands	70.70-72.10	0.45-0.55

Female : Each ovary with common terminal filament transparent, length 6.25 mm, width 0.25 mm; egg tube filled with translucent whitish ova distally and light opaque ova proximally, length 30.50 mm, width 0.50 mm; pedicel light opaque, length 1.25 mm, width 0.25 mm; lateral oviduct length 2.0 mm, width 0.50 mm; common oviduct length 1.25 mm, width 0.25 mm; complex sperm storage system with spermathecal gland bifurcated deeply, length 59.25 mm, width 0.25 mm; infundibulum length 0.25 mm, width 0.25 mm; utriculus opaque, length 2.0 mm, width 0.12 mm; lagena opaque, length 2.0 mm, width 0.13 mm; spermathecal duct length 1.25 mm, width 0.25 mm; ductus seminalis white, translucent, length 9.0 mm, width 0.50 mm, originating from ductus bursae; Signum horse-shoe shaped; bulla seminalis well developed, translucent, length 4.75 mm, width 2.50 mm; ductus bursae length 4.75 mm, width 2.0 mm; ostium bursae diameter 2.25 mm; cement gland system with lateral accessory gland reservoir dark yellowish, opaque, length 2.50 mm, width 0.50 mm; common accessory gland reservoir opaque, length 0.20 mm, width 0.10 mm; accessory gland duct light opaque, length 14.0 mm, width 0.10 mm; accessory gland reservoir duct opaque, length 0.75 mm, width 0.10 mm; vestibulum opaque, length 1.25 mm, width 0.25 mm; vagina length 0.75 mm width 0.25 mm.

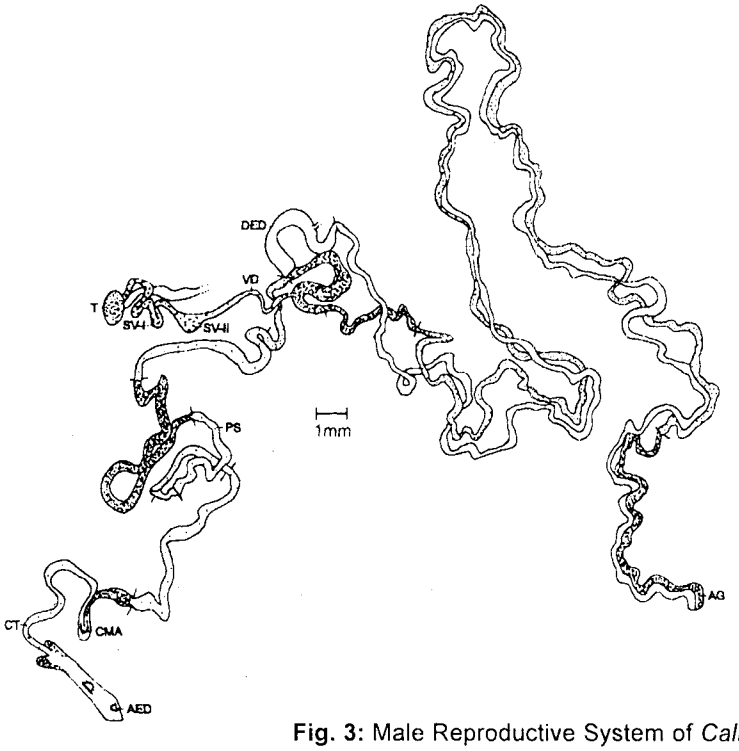

Fig. 3: Male Reproductive System of *Callimophra equitalis* Kollar.

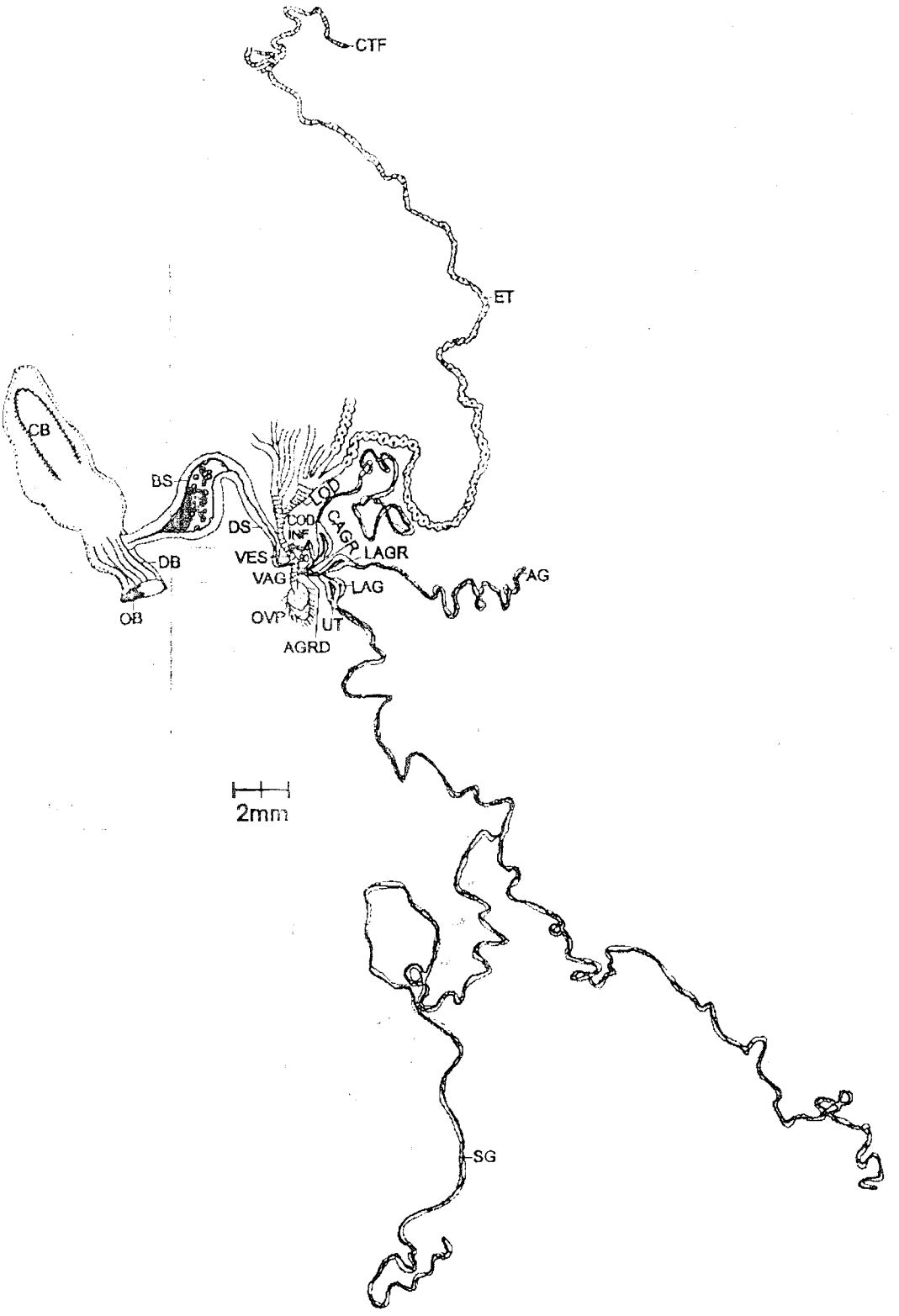

Fig. 4: Female Reproductive System of *Callimophra equitalis* Kollar.

Table 4: Intraspecific range of measurements of internal female genitalic organs- *Callimorpha equitalis* Kollar.

Sr. No.	Organ	Length (mm)	Width (mm)
1.	Common terminal filament	6.15-6.30	0.20-0.30
2.	Egg tube	30.10-30.60	0.25-0.55
3.	Lateral oviduct	1.90-2.10	0.39-0.52
4.	Common oviduct	1.20-1.30	0.20-0.30
5.	Pedicel	1.20-1.30	0.20-0.30
6.	Infundibulum	0.20-0.30	0.20-0.30
7.	Utriculus	1.90-2.10	0.10-0.15
8.	Lagena	1.90-2.10	0.10-0.15
9.	Spermathecal duct	1.20-1.30	0.20-0.30
10.	Spermathecal gland	58.95-60.00	0.20-0.30
11.	Corpus bursae-diameter	16.70-17.10	-
12.	Ductus bursae	4.60-4.80	1.90-2.10
13.	Ostium bursae-diameter	2.10-2.30	-
14.	Ductus seminalis	8.75-9.10	0.45-0.55
15.	Bulla seminalis	4.65-4.85	2.40-2.55
16.	Accessory gland reservoir duct	0.70-0.80	0.09-0.10
17.	Lateral accessory gland reservoir	2.35-2.45	0.45-0.55
18.	Common accessory gland reservoir	0.20-0.25	0.09-0.12
19.	Accessory gland duct	13.60-14.10	0.09-0.10
20.	Vestibulum	1.20-1.30	0.20-0.30
21.	Vagina	0.70-0.80	0.20-0.30

Material examined : Sikkim : Gangtok, 17.5.01, 2♂♂; Arunachal Pradesh : West Kameng District, Bomdila, 13.5.01, 2♂♂, 1♀; Meghalaya: Cheerapunjee, 6.5.01, 1♂, 1♀.

Old distribution : Himalayas from Shimla to Sikkim, Khasi hills.

***Callimorpha principalis* Kollar**
Kollar, 1844, *Hügel's Kaschmiri,* **4** : 465.
(Fig. 5)

Male : Not studied.
Female : Each ovary with common terminal filament translucent, length 3.0 mm, width 0.16 mm; egg tube filled with light yellowish opaque ova and dark yellowish opaque ova at their distal and proximal halves respectively, length 32.83 mm, width 0.16 to 0.50 mm; pedicel light opaque, length 2.16 mm, width 0.16 mm; lateral oviduct translucent, length 11.33 mm, width 0.83 mm; common oviduct light opaque, length 2.33 mm, width 0.50 mm; complex sperm storage system with spermathecal gland simple and opaque, length 22.99 mm, width 0.16 mm; infundibulum length 0.50 mm, width 0.50 mm; spermathecal duct opaque length 2.30 mm, width 0.16 mm; utriculus dark opaque, pear-like, length 1.83 mm, width 0.50 mm; lagena translucent, length 0.33 mm, width 0.16 mm; corpus bursae dark brownish opaque, diameter 16.66 mm; ductus bursae length 4.0 mm, width 0.66 mm; ostium bursae diameter 1.0 mm; ductus seminalis opaque, length 4.30 mm, width 0.16 mm, originating from the corpus bursae laterally; bulla seminalis opaque, length 9.0 mm, width 1.66 mm; cement gland system with lateral and common accessory gland reservoirs fused translucent, length 3.83 mm, width 0.83 mm; accessory gland reservoir duct opaque, length 0.33 mm, width 0.16 mm; accessory gland duct opaque, length 12.16 mm, width 0.16 mm; vestibulum length 2.0 mm, width 0.33 mm; vagina length 0.50 mm, width 0.33 mm.

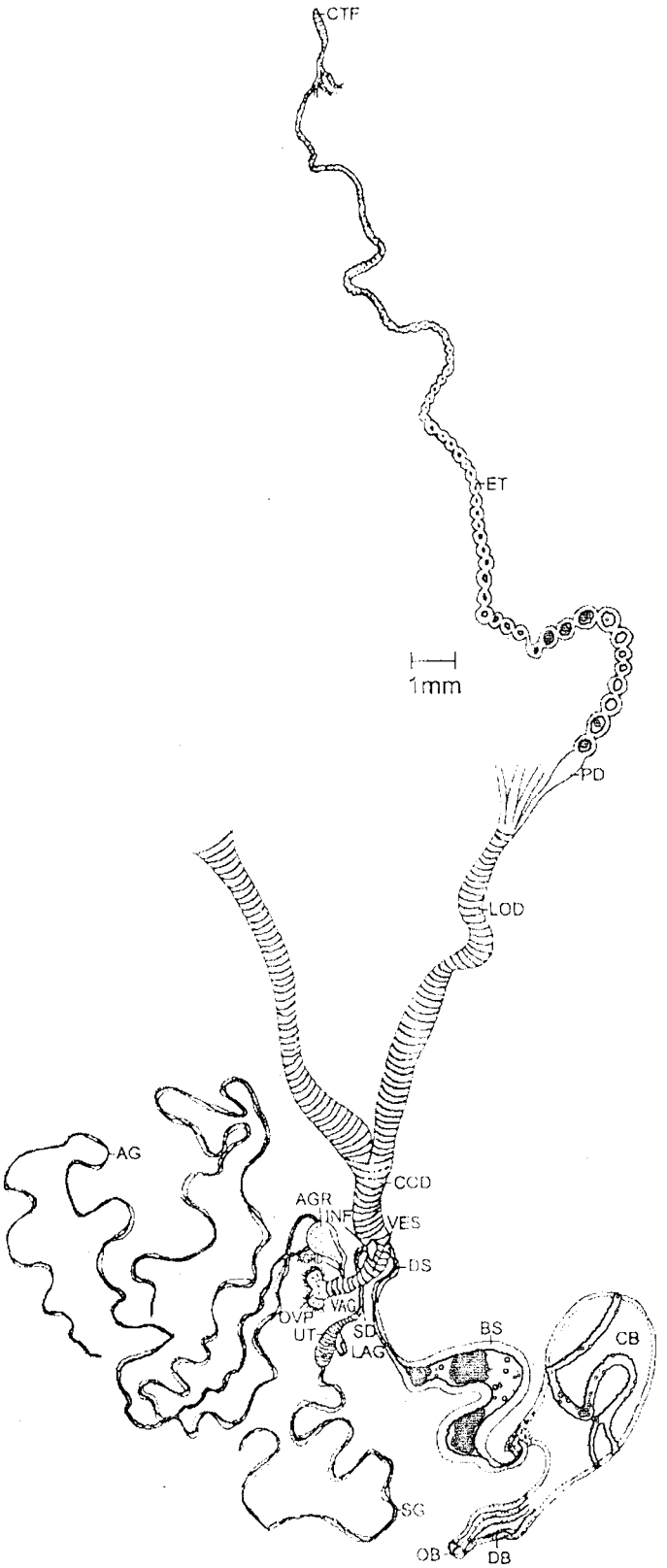

Fig. 5: Male Reproductive System of *Callimophra principalis* Kollar

Table 5: Intraspecific range of measurements of internal female genitalic organs-*Callimorpha principalis* Kollar.

Sr. No.	Organ	Length (mm)	Width (mm)
1.	Common terminal filament	2.90-3.10	0.13-0.19
2.	Egg tube	31.90-32.99	0.45-0.55
3.	Lateral oviduct	10.39-11.50	0.80-0.90
4.	Common oviduct	2.30-2.40	0.45-0.55
5.	Pedicel	2.10-2.20	0.15-0.20
6.	Infundibulum	0.45-0.55	0.45-0.55
7.	Utriculus	1.80-1.90	0.45-0.55
8.	Lagena	0.30-0.40	0.13-1.9
9.	Spermathecal duct	2.20-2.35	0.13-0.18
10.	Spermathecal gland	21.80-23.10	0.12-0.19
11.	Corpus bursae-diameter	15.99-16.78	-
12.	Ductus bursae	3.90-4.07	0.60-0.70
13.	Ostium bursae-diameter	0.70-1.07	-
14.	Ductus seminalis	4.20-4.35	0.12-0.18
15.	Bulla seminalis	8.80-9.10	1.56-1.70
16.	Accessory gland reservoir duct	0.30-0.40	0.15-0.20
17.	Accessory gland reservoir	3.70-3.89	0.75-0.85
18.	Accessory gland duct	12.10-12.30	0.10-0.15
19.	Vestibulum	1.85-2.07	0.30-0.35
20.	Vagina	0.45-0.55	0.30-0.40

Material examined : Sikkim : Chungthang, 19.9.2000, 1♀.

Old distribution : North-West Himalaya; Sikkim; Myanmar.

DISCUSSION

The internal male genitalia of two species studied here reveals that vasa deferentia open into the ductus ejaculatorius duplex beyond middle towards accessory gland in *Callimorpha nyctemerata* Moore and *C. equitalis* Kollar and entrance of cuticular tube in aedeagus is apical in both cases, which is a constant and stable character. On the other hand, in the internal female genitalia lagena is present in all the studied species. Bulla seminalis is present in *C. principalis* Kollar and *C. equitalis* Kollar and absent in *C. nyctemerata* Moore. Lateral accessory gland reservoirs and common accessory gland reservoirs fused in *C. principalis* Kollar while clearly separated in *C. equitalis* Kollar and *C. nyctemerata* Moore. Spermathecal gland bifurcated in *C. nyctemerata* Moore and *C. equitalis* Kollar but no bifurcation of spermathecal gland is observed in *C. principalis* Kollar. Ductus seminalis originating from corpus bursae laterally in *C. principalis* Kollar on the other hand its entrance in *C. nyctemerata* Moore and *C. equitalis* Kollar is directly into ductus bursae. The study on the basis of male genitalia reveals that all species conform to a natural group. However, the female genitalia do not show any such similarity and there is variability of different genitalic characters in these species.

ACKNOWLEDGEMENTS

The financial help rendered through a project on ***"Taxonomic Revision of Indian Arctiidae (Lepidoptera)"***, by Department of Science and Technology, New Delhi is heartily acknowledged.

ABBREVIATIONS

Aedeagus-AED, Accessory gland-AG, Accessory gland duct-AGD, Accessory gland reservoir duct-AGRD, Bulla seminalis-BS, Common accessory gland reservoir-CAGR, Corpus bursae-CB, Contrictor muscular area-CMA, Common oviduct-COD, Cuticular tube-CT, Common terminal filament-CTF, Ductus bursae-DB, Ductus ejaculatorius duplex-DED, Ductus seminalis-

DS, Egg tube-ET, Infundibulum-INF, Lagena-LAG, Lateral accessory gland reservoir-LAGR, Lateral oviduct-LOD, Ostium bursae-OB, Ovipositor-OVP, Pedicel-PD, Primary simplex-PS, Spermathecal duct-SD, Spermathecal gland-SG, Signum-SIG, Seminal vesicle I-SV I , Seminal vesicle II-SV II, Testis-T, Utriculus-UT, Vagina-VAG, Vas deferens-VD, Vestibulum-VES,

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MORPHOMETRIC AND MERISTIC ANALYSIS OF *TOR CHILINOIDES* (PISCES : CYPRINIDAE) FROM THE RIVER WESTERN NAYAR OF GARHWAL, CENTRAL HIMALAYA

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Abstract

The paper deals with the interrelationships of morphometric characters and their significance in the taxonomic raciation and identification of Tor chilinoides (McClelland), a hillstream detritivore fish collected from the river Western Nayar in the Garhwal, Central Himalaya. It was observed that all the body parts grow in accordance with the total length of the body. The maximum total length of the fish was observed to be 23.6 cm, which is the highest size collected so far in this region. The head length was 20.45 ±0.71 percent of the total length. Females were larger in size than males. The eyes were situated just ahead of the middle of head length, and the body depth was slightly less than the head length. Regression analyses were made between different dependent and independent variables by using the method of least squares and were tested by the analysis of variance (ANOVA).

Key words : Morphometrics, meristic analysis, *T. chilinoides*, Garhwal.

INTRODUCTION

The morphometric characters play an important role in the identification of a fish species. There are great variations in the morphometric characters of different fish species. Such intra-specific variations may occur either due to different habitat conditions or due to difference in genetic makeup. The fish is identified with a set of morphological characters taking into consideration the variations exhibited by each character. In some instances considerable biological information may be available and could further confirm the identification of fish in one way or the other. But in general the identification of fish is based on the morphometric, meristic, and some descriptive characters. Day (1878) produced a monumental monograph on the fishes of India, Pakistan, Ceylon and Burma. In the recent past many other workers (Beaven,1990; Chondar, 1974; Jayaram, 1959; Tandon *et al.*, 1993; Misra, 1962; Singh and Dobriyal, 1983; Srivastava, 1968; Talwar and Jhingran, 2001) contributed significantly to the fish taxonomy. The present communication deals with the racial analysis of the Dark mahseer *Tor chilinoides* (McClelland).

MATERIALS AND METHODS

The study was conducted on 222 specimens of *Tor chilinoides* (McClelland) collected with the help of local fishermen from the river Western Nayar at Pabau (900 masl) in Pauri Garhwal during January, 2001 to December 2003. For morphometric analysis, the body parameters considered were: Total length (TL), Standard length (SL), Head length (HL), Maximum body depth (MBD), Snout length (Snt.L), Eye diameter (ED), Pre-dorsal length (PDL), Pre-pelvic length (PPL), Pre-anal length (PAL), and Caudal length (CL). These variables were measured in centimeters and studied in relation to the independent variables - total length, standard length and head length. The regression analysis was made by the method of least squares and tested by the analysis of variance (F test). Correlation coefficients among different variables were calculated with the help of an electronic calculator.

OBSERVATIONS

The present study showed that the fish *Tor chilinoides* (McClelland) attains a maximum total length of 23.6 cm. Different body parameters were studied in percentage of total length, standard length and the head length and the results are presented in Table 1. Regression analysis of various body measurements with total length, standard length, and head length were calculated individually. The statistical values of intercept (a), regression coefficient (b) and coefficient of

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Table 1: Body parts in percentage of total length, standard length and head length of *Tor chilinoides* (McClelland).

SN	BODY PARTS	IN PERCENTAGE OF		
		TOTAL LENGTH	STANDARD LENGTH	HEAD LENGTH
1	STANDARD LENGTH	81.3-85.47* (83.15±1.39)	—	—
2.	PRE-DORSAL LENGTH	38.61-40.49 40.08± 0.5	45.45-49.75 (47.94±11.14)	—
3.	PRE-PELVIC LENGTH	38.02-41.49 (40.04±1.06)	43.86-51.02 (47.53±2.25)	—
4.	PRE-ANAL LENGTH	61.35-63.69 (62.55±1.05)	74.07-76.34 (75.25±0.73)	—
5.	HEAD LENGTH	19.68-21.74 20.45±0.71	23.58-26.8 (24.53±1.2	—
6	CAUDAL LENGTH	14.26-18.52 (15.77±1.42)	16.64-23.26 (20.07±2.12)	
7.	EYE DIAMETER	3.8-7.86 (5.23± 1.38)	4.49-9.69 (6.24± 1.77)	20.16-35.71 (26.01±5.47)
8.	SNOUT LENGTH	6.19-8.34 (6.85± 0.43)	7.3-10.28 (8.38± 0.91)	30.67-38.17 (34.1±2.2)
9.	MAXIMUM BODY DEPTH	18.51-19.49 (18.92±0.38)	21.79-23.58 (22.65±0.58)	84.75-1.0 (92.3±4.68)

* RANGE (MEAN ± STANDARD DEVIATION)

Table 2: Relationships of Dependent body parameters with the Independent variables.

Dependent body parameters	Independent body parametersTotal length								
	Total length			Standard length			Head Lenth		
	"a"	"b"	"r"	"a"	"b"	"r"	"a"	"b"	"r"
Standard length	-0.391	0.8634	0.9998	-	-	-	-	-	-
Pre-dorsal length	0.0478	0.3966	0.9985	0.2293	0.4592	0.9984	-	-	-
Pre-pelvic length	0.2754	0.3803	0.9970	0.4497	0.4403	0.9969	-	-	-
Pre-anal length	-0.263	0.6476	0.9998	0.0332	0.7498	0.9997	-	-	-
Head length	0.183	0.193	0.9993	0.2254	0.2237	0.9995	-	-	-
Caudal length	0.4054	0.13598	0.9898	0.474	0.1569	0.9865	-	-	-
Max. body depth	-0.023	0.1918	0.9976	0.0679	0.2219	0.9965	-0.1527	0.9909	0.9960
Snout length	0.1073	0.0617	0.9874	0.1359	0.0714	0.9869	0.0638	0.3192	0.9876
Eye diameter	0.3671	0.0225	0.9741	0.3789	0.0259	0.9694	0.3523	0.1162	0.9711

Table 3: Analysis of variance (ANOVA) between independent and dependent Morphometric characters in *Tor chilinoides*. (McClelland)

Parameters	S^2B	S^2W	Observed "F"	Remarks
TL X SL	*2.897*	*28.894*	*0.1003*	*NS*
TL X HL	69.47	17.169	4.046	NS
TLX MBD	71.604	17.160	4.173	NS
TL X Snout length	94.682	16.613	5.699	*
TL X ED	99.124	16.557	5.978	*
TL X PDL	39.329	19.159	2.0353	NS
TL X PPL	39.444	18.958	2.080	NS
TL X PAL	38.656	23.494	1.645	NS
TL X CL	76.434	16.861	4.533	NS
SL X HL	43.992	17.167	2.563	NS
SL X MBD	45.696	17.160	2.663	NS
SL X Snout Length	64.456	16.613	3.880	NS
SL X ED	68.13	16.56	4.115	NS
SL X PDL	20.878	19.159	1.090	NS
SL X PPL	20.962	18.958	1.106	NS
SL X PAL	4.688	23.494	0.199	NS
SL X CL	49.570	16.861	2.940	NS
HL X ED	2.715	0.627	4.33	NS
HL X Snout Length	1.948	0.683	2.852	NS

Table $F_{0.05}$ = 5.12 (ndf = 1, ddf = 8)
ndf = numerator degree of freedom, ddf = denominator degree of freedom, S^2B and S^2W are two independent estimates of population variance.
* = Significant at 5 % level.

correlation (r) are presented in Table 2. The linearity of the regression was tested by the analysis of variance (ANOVA, F test) for all the relationships (Table 3).
On the basis of meristic analysis conducted on 222 specimens, the fin formula was summarized as follows: **D = 10 -11 (3/7 or 3/8), P = 15-17, V = 9, A = 7 (2/5), C = 19, LI. 32-34, Ltr. 5 ½-6**

DISCUSSION

The intra-specific variations are important in the economic management of fish populations. A commercially exploited fish species may come from a single or multiple stocks. If the fish species comes from the similar stock, the intensity of fishing at any one place is likely to be effective in due course at other centers also. The fishes are known to have intra-specific variations in morphometric characters due to different ecological niche and of course through genetic variations. So, it is essential to know the stock of fish prior to the study of its biology and thus morphometric and meristic studies are the best way to do it.

The taxonomic investigations are based on the hypothesis that certain meristic and non-meristic characters vary significantly at specific level and can be useful in identifying a species, the lowest taxon in the hierarchial system. There are many small characters which some times become an important mean for raciation in the given species. The morphometric analyses involve the measurement of various body parts and their inter relationships. All body parts are generally studied in relation to three main independent variables, the total body length, the standard length and the head length. The meristic study deals primarily with counts of fin rays, number of scales in lateral line and also above and below the lateral line. All these studies are essential as these provide a basis for differentiating one

species from the other and also the formation of sub-species.

Pioneering study in raciation for analyzing different stocks of fishes have been conducted by Jones (1954) who ascertained that the fish *Hilsa ilisha* belonged to a homogenous stock in the rivers Hooghly, Mahanadi and the lake Chilka. On the other hand Pillay (1958) observed that *Hilsa ilisha* collected from the river Hooghly and Chilka lake belongs to a heterogeneous population. He made certain interesting observations that among the morphometric characters significant differences were recorded in the standard length amongst the males, body height between the females, head length in both the sexes, thickness of caudal peduncle within males and body thickness in either sex. Sarojini (1957) observed no significant difference in *Mugil parsia* collected from Bengal waters. Jayaram (1959) distinguished four populations of *Rita chrysea* collected from river Mahanadi. Singh and Dobriyal (1983) studied the morphometric characters and their relationships in the hillstream catfish *Pseudecheneis sulcatus* (McClelland) collected in the river Alaknanda at Srinagar and found no second stock.

In the present investigations on *Tor chilinoides* (McClelland), it was observed that all the body parts grow in accordance with the total length of the body. The maximum total length of the fish was observed to be 23.6 cm, which is the highest size collected so far in this region. The head length is 20.45 ±0.71 % of the total length. Morphometric study revealed that in the fish up to 19 cm generally the dorsal fin originated slightly ahead than pelvic fin but in large female specimen (19-24 cm) the pelvic fin originated slightly ahead than the dorsal fin. The females were observed to be larger in size than males. The snout length was 34.1± 2.2 % of head length which indicated that the eye is situated just ahead of the middle of head length. Another important observation is that the body depth was slightly less than head length as the former is 18.92 ± 0.38 % and the latter is 20.45 ± % of the total length. The snout length (0.53 – 1.65 cm) was always more than the eye diameter (0.5 – 0.85 cm). In smaller fishes the difference is narrow (about 1.1 – 1.2 times) but in larger fish the difference is more (1.5 – 1.9 times). Body depth was also slightly more than caudal length except in very small specimens (5-9 cm).

According to Day (1878), who described it as *Barbus chilinoides*, length of head is 20 %, of caudal 16.67 % and height of body 18.18 % in the total length. Mouth directed forward with upper jaw slightly longer than lower jaw. The rostral and maxillary pairs of barbel were reported to be of same size and equal to 1½ diameter of the orbit. Dorsal fin commences midway between end of snout and base of the caudal fin. Lateral line is complete, but three rows of scales between it and the base of the caudal fin were reported. Fin Formula as reported is B. iii; D, 10-11 (3/7-8); P, 17; V,9; A 7(2-5); C,19; LI- 32-35; Ltr- 5½/6. According to Beaven (1990), who also named the fish as *Barbus chilinoides*, the maximum total length is up to 20 cm. Head length was 22.2-25 % in the total length. Scales 32-35. Dorsal ray is very stout.

In the present study on *Tor chilinoides*, it was observed that the head length is 20.45±0.71, of caudal 15.77±1.42 and height of body 18.92±0.38 in percentage of total length. Mouth directed forward with upper jaw slightly longer than lower jaw. The maxillary pair of barbels was always larger than the rostral pair which is different from that reported by Day (1878). Dorsal fin commences midway between end of snout and base of the caudal fin. Lateral line is complete and there are three rows of scales between it and the base of the caudal fin. Fin Formula is B. iii; D, 10-11 (3/7-8); P, 15-17 (again showing difference to that reported by Day, op. cit.); V,9; A 7(2-5); C,19; LI- 32-34; Ltr- 5½/6. The maximum length of fish was recorded to be 23.6 cm.

The significance of growth relationship between the independent and dependent variables was tested statistically by analysis of variance and was found to be non significant (TL and SL- $F_{0.05}$ = 0.1003, TL and HL- $F_{0.05}$ = 4.046, TL and MBD- $F_{0.05}$ = 4.173, TL and PDL- $F_{0.05}$ = 2.0353, TL and PPL- $F_{0.05}$ = 2.08, TL and PAL- $F_{0.05}$ = 1.645, TL and CL- $F_{0.05}$ = 4.533, SL and HL- $F_{0.05}$ = 2.563, SL and MBD- $F_{0.05}$ = 2.663, SL and SnTL- $F_{0.05}$ = 3.88, SL and ED- $F_{0.05}$ = 4.122, SL and PDL- $F_{0.05}$ = 1.090, SL and PPL- $F_{0.05}$ = 1.106, SL and PAL- $F_{0.05}$ = 0.199, SL and CL- $F_{0.05}$ = 2.94, HL and ED- $F_{0.05}$ = 4.33, HL and SnTL- $F_{0.05}$ = 2.852 and HL and MBD- $F_{0.05}$ = 0.132) except in case of total length-snout length and total length-eye diameter when it was observed significant at 5 % level ($F_{0.05}$ = 5.699 and 5.987 respectively). The correlation coefficient was recorded high, indicating a high degree of correlation in growth between the independent and the dependent variables (r =0.9694 to 0.9998).

Thus on the basis of present study it is concluded that there was only one stock of the population of *Tor chilinoides* (McClelland). A close similarity in the present investigation was observed with that of the previous

available literature. However, certain differences were also recorded but they were not sufficient enough to be considered as a separate stock of the population.

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PHYSICO - CHEMICAL ANALYSIS OF SEWAGE WATER AND ITS EFFECTS ON PARAMETERS OF SOIL AND *SOLANUM TUBEROSUM* LINN.

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Abstract

The use of sewage water for commercial crop like potato (Solanum tuberosum) has gained importance throughout India due to limited water resources, high cost of fertilizers and expensive technology for the treatment of waste water. The present investigation was conducted to analyze the physico-chemical characteristics of sewage water and its effects on parameters of soil and S. tuberosum. Certified seed tubers of potato, S. tuberosum Linn. var. Kufri Chandramukhi (Solanaceae), sown in three sets of pots, were treated with different fertilizer doses of N, P and K (0,50 and 100%) and 0, 25, 50, 75 and 100% sewage water concentrations to study morphological and biochemical parameters. The analysis of sewage water and soil revealed that these were rich in major nutrients. The effects on growth, morphology and yield using 50% sewage water with 50% recommended fertilizer (N, P and K) dose gave the best results in all the cases. Therefore, the combination can be used for commercial potato crop irrespective of the effects of heavy metals present in sewage water on potato crop.

Key words : Sewage water, *Solanum tuberosum*, Physico-chemical analysis, Soil parameters.

INTRODUCTION

Potato (*Solanum tuberosum* Linn.var.Kufri Chandramukhi; family Solanaceae), native of Andean Plateau of America, is abundantly produced as edible food crop in the world which is richest source of calories for human energy. Due to wide spread apathy or possible ignorance concerning cultural practices, irrigation facilities and fertilizers, average yield of potato in India is comparatively lesser than developed countries. Factors, like healthy seed tubers, timely irrigation and fertilizer, play an important role for efficient growth, development and yield of potato. Now-a-days, there has been increase in practice of applying sewage water to agricultural land to increase irrigation facilities due to water crisis and high prices of chemical fertilizers. The application of sewage water improves the physical properties of soil and provides nutrients to crop plants, hence increases the yield. Besides nutrients, other heavy metals, pathogens and other undesirable substances present in sewage water may affect the physical properties of soil, plant growth, yield and quality of food products. Hence, successful working of sewage water irrigation for valuable food crops requires an understanding of characteristics of soil, inter-relationship of soil and sewage water and useful as well as harmful effects of constituents of sewage water on soil and crop plants. The evaluation of effects of sewage water has been done in edible crops. Aziz (1991) studied the effects of treated refinery waste water on physico-morphological characteristics of wheat. The effect of municipal solid waste on growth, yield and mineral concentration in tomato cultivar Sunny and on squash (*Cucurbita maxima* cv. Dixe) was analyzed by Ozores *et al.* (1994). Waste water could be safely used for irrigation. The effect of sewage water irrigation on mineral composition of corn and sorghum plants was observed by Al-jalaud *et al.* (1995). An experiment was carried out by Dahdoh and Hassanin (1997) to estimate the effect of different sewage sludge rates to calcareous loam and loamy soils irrigated with water of different salinity using broad bean plants. The impact of sewage sludge on cabbage, potatoes and lettuce was determined by Chipeva and Merinova (1998). This paper deals with the physico-chemical analysis of sewage water and its effects on parameters of soil and *S. tuberosum* Linn.

Certified seed tubers for the present investigation were procured from Central Potato Research Institute (CPRI), Jalandhar (Punjab) and seed tubers preferably of uniform size (2.5 cm in diameter) were sown in sandy loam soil in three sets of earthen pots. Each set with five pots

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was irrigated with 0, 25, 50, 75 and 100% concentrations of sewage water. Different N, P and K fertilizer treatments (0, 50 and 100% of recommended doses) were given to three sets of pots. The fungicide dithane M-45 (copper oxychloride) and dimecron -85 were used to prevent the attack of disease and pests, respectively. Sewage water samples used for irrigating crop were collected from the pumps installed on sewage drain at Ram Tirath disposal site, Amritsar. Sewage water was analyzed for its physico-chemical characteristics following AWWA (American Waste Water Analysis), APHA (American Public Health Association), WEF (1995) and organic nitrogen by using semi micro-Kjeldhal method (1983). The estimation of phosphate and potassium content was determined by flame photometric method given by Yadav and Khera (1993). The soil collected from Agriculture Farm of Guru Nanak Dev University was used for sowing potato crop in pots. Soil samples before crop sowing and after crop harvesting were used to study their different parameters under the influence of different sewage water concentrations. The preliminary investigation of role of different parameters like electrical conductivity, nitrogen content, phosphate content was done. Physico-chemical analysis of irrigation water was also studied. The samples of sewage water were mixed with irrigation water for 24 hours to have the average mixed sample. The physical characteristics of soil were determined following the methodology given by AWWA, APHA, WEF. The nitrogen content in soil samples was determined by using semi-micro-Kjeldhal method (1983), and phosphorus and potassium content by following the methods suggested by Olsen *et al* (1954) and Merwin and Peech (1950), respectively. The critical limits of these contents for soil analysis were compared according to the standards given by Punjab Agriculture University, Ludhiana (Punjab) as given below:

	Low	Medium	High
Available nitrogen(Kg/ha)	<272	272-422	>422
Available phosphorus(Kg/ha)	<12.4	12.4-22.4	>22.4
Available potassium (Kg/ha)	114	114-277	>277

Source: Department of Soil Sciences, Punjab Agriculture University, Ludhiana.

The parameters of potato (*S. tuberosum*) studied under the effect of different sewage water concentrations and fertilizer doses included morphology (length and number of shoots per plant), yield and biochemical studies (protein and starch content). The protein content of potato tubers was estimated following the protocol of Lowry (1951). Starch content of potato tubers was estimated following the methodology of Loewus (1951). Physico-chemical analysis of sewage water, soil samples and effects of various parameters analyzed on *S. tuberosum* Linn. are discussed below:-

Sewage water was analyzed for its various physical and chemical parameters (Table1). As per the standards given by Metcalf and Eddy (1991), sewage water of this disposable site was found to be of medium type. Soil samples taken before crop sowing for its different physical and chemical characteristics (Table 2). Soil analysis showed that, it was medium in nitrogen and phosphorus contents, but has higher value of potassium content than critical limits. Soil samples collected after harvesting from three sets of pots treated with different concentrations of sewage water with respect to different fertilizer doses were analyzed for its physico-chemical properties (Table 3). No change in colour was observed in soil collected from different pots. The observations revealed that continuous use of 100% sewage water for irrigation increased the pH of soil. The analysis of soil before crop sowing and after crop harvesting also showed that the nitrogen, phosphorus and potassium contents in soil collected from pots treated with fully recommended fertilizer dose and sewage water concentrations (50, 75 and 100%) were higher than the critical limits. Thus the prolonged use of this combination for soil would result in excessive accumulation of these nutrients (N, P and K) in soil and cause adverse effects on crop grown in it. The effect of different concentrations of sewage water was studied on morphological and biochemical parameters, like no. of shoots, shoot length and yield. The observations made in all parameters of soil and *S. tuberosum* showed that the combination of 50% sewage water concentration and 50% of recommended dose of fertilizer proved best for fertility, growth and yield (Table 4) of potato crop. No change in the starch and protein content was observed in potato tubers. So, sewage water should be diluted to 50% before using it for

Table 1 : Physico-chemical parameters of sewage water

Parameter	Concentration
Colour	Greyish black
Odour	Foul smell
PH	8.6
Electrical conductivity (mmhos)	33.6
Total solids content (mg/l)	1370
Total dissolved solids content (mg/l)	590
Suspended solids (mg/l)	780
DO (mg/l)	0.95
COD (mg/l)	654
BOD(mg/l)	244
Nitrogen content (mg/l)	200
Phosphate content (mg/l)	17.5
Potassium content (mg/l)	25

DO: Dissolved oxygen; COD: Chemical Oxygen Demand
BOD: Biochemical Oxygen Demand

Table 2: Physico-chemical parameters of soil before crop sowing (*S.tuberosum*).

Parameter	Concentration
Colour	Light brown
PH	6.3
Electrical conductivity (mmhos)	19.0
Available nitrogen content (kg/ha)	375.3
Available phosphate content (kg/ha)	18.3
Available potassium content (kg/ha)	330.8

Table 3: Effect of different concentrations of sewage water and fertilizer (N, P and K) doses on physico-chemical properties of soil after crop harvesting.

Fertilizer dose (%)	Concentration of sewage water (%)	Soil properties		
		pH	Electrical conductivity (mmhos)	Available nitrogen content (kg/ha)
Control	0	6.63±0.04	25.27±0.03	91.65±1.19
	25	6.67±0.06	26.20±0.09	117.91±.0.04
	50	6.71±0.01	26.43±0.07	146.12±2.67
	75	6.73±0.01	27.34±0.03	179.65±0.41
	100	6.79±0.01	28.50±0.01	209.42±0.25
50	0	6.65±0.12	26.03±0.04	221.15±2.69
	25	6.69±0.07	27.80±0.07	253.63±0.41
	50	6.72±0.02	28.23±0.29	289.59±3.33
	75	6.75±0.06	28.67±0.09	317.39±0.64
	100	6.80±0.01	29.23±0.13	344.57±0.41
100	0	6.71±0.02	27.33±0.03	356.72±1.79
	25	6.76±0.03	28.23±0.12	386.64±2.32
	50	6.79±0.01	29.15±0.20	432.86±1.19
	75	6.83±0.09	29.53±0.21	447.31±1.47
	100	6.87±0.11	30.73±0.03	476.14±4.89

Data shown are Mean ± SE of three replicates.

Table 4: Yield of potato tubers (g/pot) under the effect of different concentrations of sewage water and fertilizer (N, P and K) doses.

Concentration of sewage water	Yield of potato tubers (g/pot)		
	Fertilizer doses (%)		
(%)	Control	50	100
0	253.47±2.14	375.06±1.54*	482.98±2.01*
25	285.81±1.84*	473.65±2.71*	496.86±1.89*
50	365.53±2.78*	557.88±8.23*	393.19±4.30*
75	477.21±2.46*	499.60±2.12*	262.09±3.51
100	346.10±1.90*	369.94±0.07*	187.87±4.13

Data shown are Mean±SE of three replicates.
*Significant at $p \leq 0.05$ by applying Student's 't' test.

irrigation purposes for crops. The results are in confirmation with McLaughlin (2000). In the end it can be concluded that 50% sewage water with 50% recommended fertilizer (N, P and K) can be safely used for irrigating potato crop.

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ABBREVIATIONS

APHA - American Public Health Association
AWWA - American Waste Water Analysis

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TAXONOMIC STUDIES ON INDIAN GELECHIIDAE (LEPIDOPTERA : GELECHIOIDEA) X. TWO NEW SPECIES OF GENUS *THYRSOSTOMA* MEYRICK FROM NORTH INDIA

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Abstract

Two new gelechiid species i.e., Thyrsostoma shivai and T. albilustra have been described and illustrated in this manuscript. Both the new species are first records of genus Thyrsostoma Meyrick from North India.

Key words : Lepidoptera, Gelechiidae, *Thyrsostoma*, New species, North India.

INTRODUCTION

Genus *Thyrsostoma* was proposed on the basis of *glaucitis* (Meyrick, 1907) from India and Ceylon. Although closely allied to *Thiotricha* Meyrick, yet emphatically differs from the latter in the secondary sexual characters of male viz., expanded fringe of expansible tufts of hairs enclosed in a furrow on under side of second segment of labial palpi and long hair scales along under surface of costa of forewing. In addition to South-Europe and South-East Asian species, this genus is represented by five species from India (Gaede, 1937; Clarke, 1969). Barring *T. nesoclera* Meyrick from South Andamans, remaining all Indian species are from Assam. In view of no representative of this genus having been recorded from North India, intensive and extensive surveys were conducted which resulted in the procurement of two species. Both of these could not be identified after following the relevant literature (Meyrick, 1907, 1908, 1916-23, 1923-30, 1925; Gaede, 1937; Clarke, 1969) and have been named as *Thyrsostoma shivai* sp. nov. and *T. albilustra* sp. nov. In the present communication, both the new species have been described in detail along with relevant illustrations.

SYSTEMATIC ACCOUNT

Genus *Thyrsostoma* Meyrick

Thyrsostoma Meyrick, 1907, *J. Bombay nat. Hist. Soc.* **17** : 736.

Type-species : *Thyrsostoma glaucitis* Meyrick, 1907, *J. Bombay nat. Hist. Soc.* **17** : 736, by monotype.

Key to the species of genus *Thyrsostoma* Meyrick

1. Costa of forewing bearing long ochraceous hair scales on under surface, M_1 connate with stalk of R_3 and R_4; second segment of labial palpi in male with a thick tuft of long hair scales enclosed in a furrow beneath; male genitalia with gnathos large and sickle- shaped, each valva comprising of two extremely unequal-sized processes..... *shivai* sp. nov.

   Costa of forewing without hair scales on under surface, M_1 stalked with R_3 and R_4; tuft of hair scales few and short in a furrow beneath second segment of labial palpi in male; male genitalia with gnathos small and slightly up curved, each valva distinguished into three densely setose processes of varying lengths. ..... *albilustra* sp. nov.

Thyrsostoma shivai sp. nov.

(Pl. 1)

Male: Head adorned with shining white scales. Antenna ciliated, cilia long; suprascape about one-third of scape, both shining white without pecten; flagellum shining white dorsally, fuscous ventrally. Labial palpi white scaled, with a large expansible tuft of hair scales enclosed in a furrow beneath, hair scales reaching apex of third segment; third segment as long as second, creamish-white, with apex fuscous and pointed. Proboscis and maxillary palpi white.

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Thorax shining white dorsally, somewhat pale white ventrally. Forewing very narrow, widest near base, costa straight; apex pointed; termen highly oblique; tornus inconspicuous (Fig.1). Ground colour ochraceous, tinged with brown dorsally; costal margin black on basal one-fourth; an indistinct basal patch, oblique band at one-third from base and a large patch at one-third before tip white. Under surface ochraceous at base, fuscous in rest; long ochraceous hair scales originating from costal margin in basal half. Discal cell three-fourth of wing length; M_1 connate with base of R_3+R_4; CuA_1 basely connate with M_2; 1A+2A forming a moderately short basal fork, with accessory retinaculum beyond it represented by a row of closely placed fifteen strong setae. Hindwing with a small sub basal costal bulge beset with a row of setae matching in number with those present above 1A+2A of forewing (Fig.2). Ground colour greyish-ochraceous on upper and under surfaces; cilia along costa grey, tinged with fuscous; cilia along inner margin dull grey, three times of wing width. Discal cell three-fifth of wing length; Sc+R_1 joined by an oblique bar to anterior margin of discal cell one-fifth away from base; CuA_2 from distal one-fourth of posterior margin of discal cell. Hind tibia whitish-ochraceous, with dorsal tuft of long stiff scales intercepted twice by fuscous, also with a whorl of fuscous, long stiff scales behind middle and at distal end.

Abdomen with upper surface ochraceous, suffused with fuscous, its distal half showing distinct white transverse bands; under surface shining white. Male genitalia with uncus hood-like; gnathos large, sickle-shaped, up curved, acutely pointed at apex; tegumen broad; vinculum strap-like; saccus rounded at tip (Fig.3); each valva more or less simple; costa serrated along basal half; cucullus densely setose; sacculus produced into short thumb-like process (Fig.4). Aedeagus short, broad proximally, gradually narrowing distally, ductus ejaculatorius opening antero-laterally.

Alar expanse: Male : 10 mm
Female : Not studied.

Material examined: Holotype: ♂, 20.ix.2003, Kalesar (Forest Rest House), Haryana; Paratypes: 2♂♂, 22.ix.2000; 1♂, 20.ix.2003, Kalesar (Forest Rest House), Haryana. Coll : V.K.Walia and D. Wadhawan. (Type specimens deposited in reference collection of Entomology section, Department of Zoology, Panjab University, Chandigarh)

Flight period: September
Type locality : Kalesar (Haryana)
Larval host Plant : Unknown

Etymology: *Thyrsostoma shivai* is named in honour of Lord Shiva of Hindu religion, the destroyer of the world.

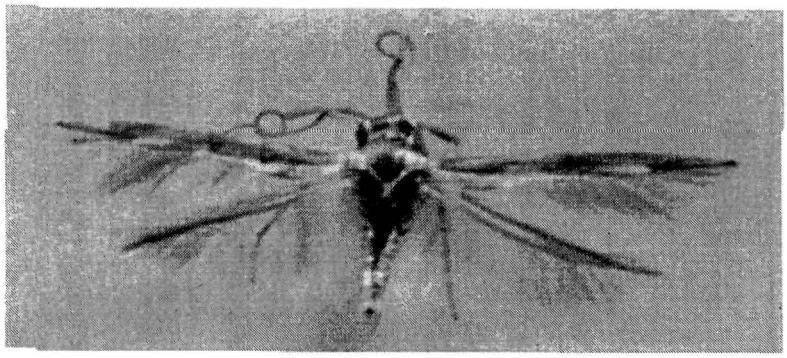
Remarks: *Thyrsostoma shivai* sp. nov. resembles *pylartis* (Meyrick) in shining white colouration of head, labial palpi and thorax, in addition to structure of uncus, gnathos and valva. However, the closely allied species differs in having vein R_5 of forewing reaching the apex and hindwing without vein CuA_1. Whereas, R_5 in the forewing joins costa and CuA_1 is present in the hindwing of *T. shivai* sp.nov. Moreover, saccus in male genitalia is acuminate at tip in *pylartis* (Meyrick, 1916-23; Clarke, 1969) but rounded in *shivai* sp. nov.

Thyrosotoma albilustra sp. nov.
(Pl. 2)

Male: Head shining white. Antenna ciliated, cilia moderately long; scape white; flagellum white above, apex and under surface fuscous. Labial palpi white, second segment adorned with few and short white hair scales in furrow beneath; third segment as long as second, tip acutely pointed, fuscous. Proboscis and maxillary palpi white.

Thorax shining white. Forewing very narrow, oblong, costa nearly linear; apex pointed; termen highly oblique; tornus inconspicuous (Fig.5). Ground colour creamish-white on upper surface, densely suffused with fuscous in basal half, with a white basal patch and obliquely straight line slightly away and a diffused fuscous spot near tornus. Under surface fuscous, without long hair scales from costa; cilia along margin pale grey. Discal cell two-third of wing length; R_2 close to upper end of discal cell; R_3, R_4 and M_1 stalked; CuA_1 close to M_2 than CuA_2; accessory retinaculum represented by a row of nine strong setae just beyond basal fork of 1A+2A,. Hindwing with a slight sub basal costal bulge bearing a row of strong setae matching with those present above 1A+2A of forewing; apex produced, pointed; termen oblique, sinuate; tornus obtusely angulate (Fig.6). Ground colour on upper surface dark grey, tinged with fuscous; under side dark grey, with apex somewhat whitish; cilia along inner margin pale grey, three times of wing width. Discal cell four-seventh of wing length; Sc+R_1 joined by an oblique bar to anterior margin of discal cell one-fourth away from base; CuA_2 from about distal one-third of posterior margin of discal

PLATE 1



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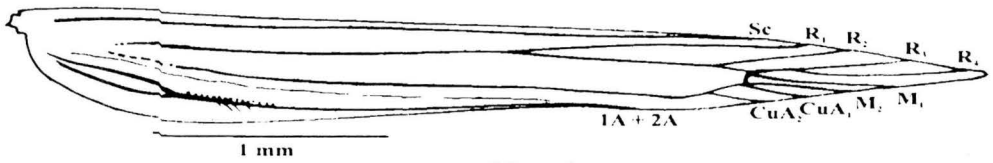


Fig. 1

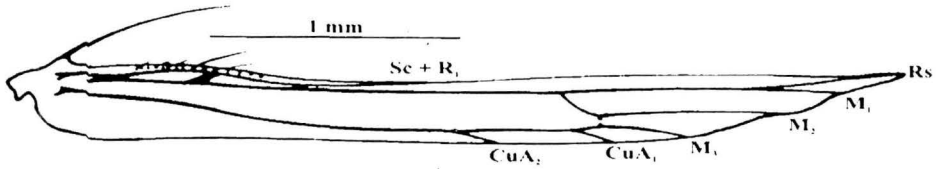


Fig. 2

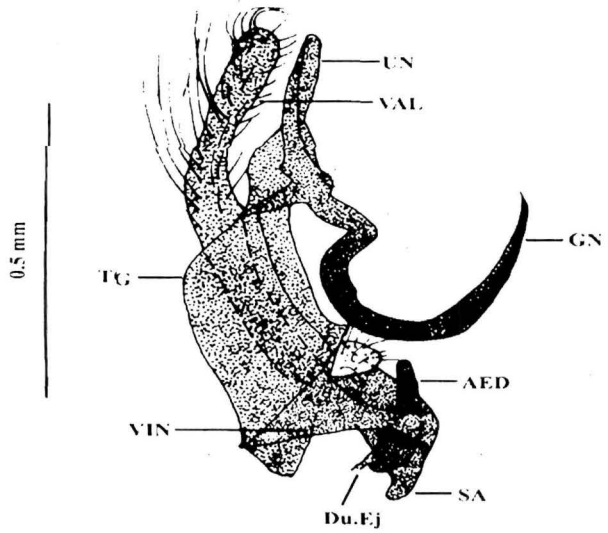


Fig. 3

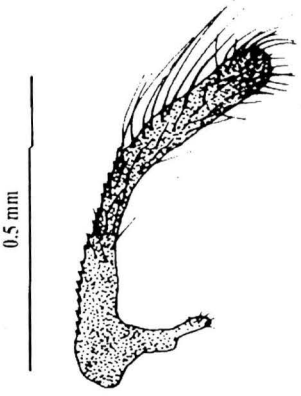
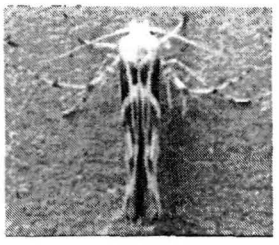


Fig. 4

Plate I : Ph.1 and Figs. 1 – 4 : *Thyrsostoma shivai* sp. nov.; Ph. 1 stretched moth; Figs. (1) forewing; (2) hindwing; (3) male genitalia; (4) valva.

PLATE 2



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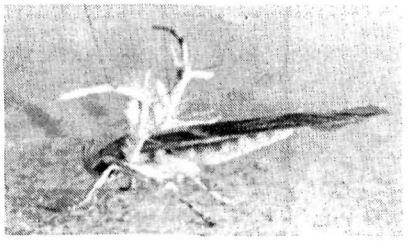

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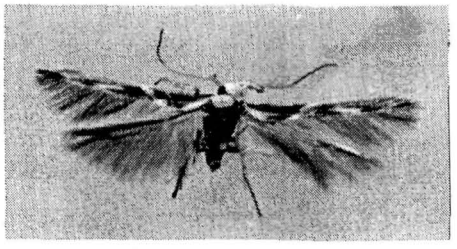

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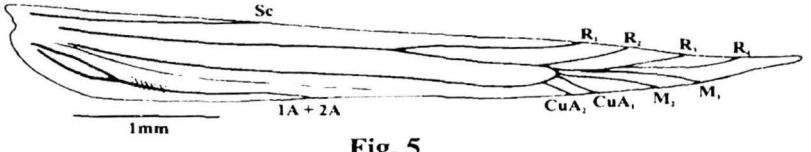

Fig. 5

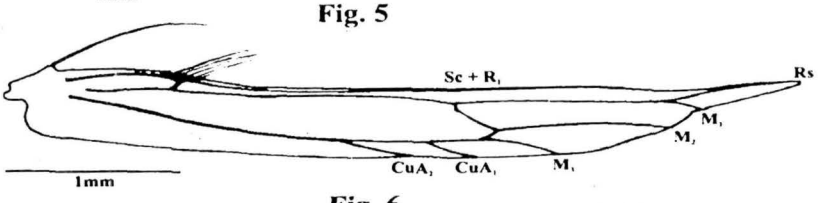

Fig. 6

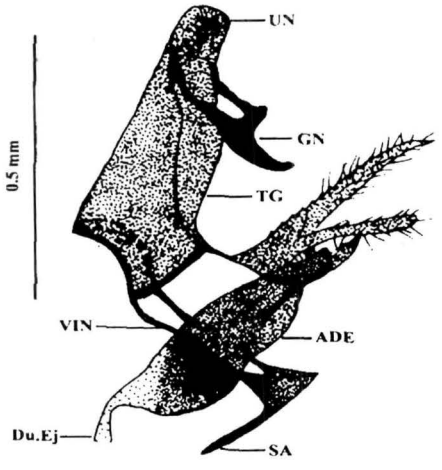

Fig.7

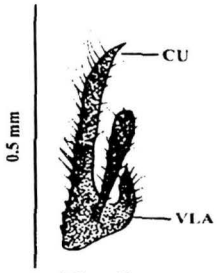

Fig. 8

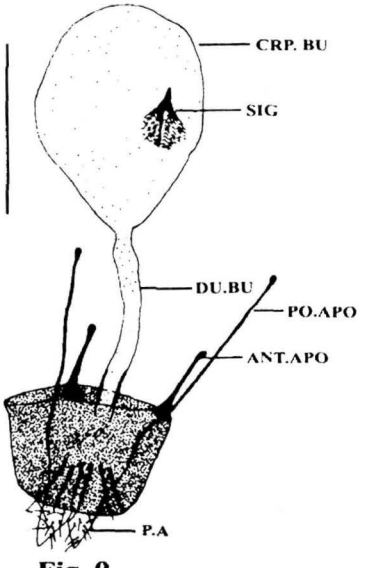

Fig. 9

Plate 2 : Phs. 2 – 4 and Figs. 5 – 9 : *Thyrsostoma albilustra* sp. nov.; Phs. (2) dorsal view of live moth; (3) lateral view of live moth; (4) stretched moth; Figs. (5) forewing; (6) hindwing; (7) male genitalia; (8) valva; (9) female genitalia.

cell. Legs white, hind tibia beset with white dorsal tuft of long stiff scales up to middle interrupted twice by fuscous, also with a whorl of pale ochraceous long stiff scales at distal end.

Abdomen blackish-ochraceous on upper surface with segmental white bands; under surface shining white. Male genitalia with uncus broad, U-like; gnathos comparatively more sclerotised, with medial upturned structure short and blunt at apex; tegumen broad; vinculum narrow strap-like; saccus narrowly elongate, acuminate at tip (Fig.7); each valva with costa serrate; cucullus narrowly elongate, curved and acutely pointed at tip, its dorsal margin adorned with long setae; valvula about one-third of cucullus, acute at apex; sacculus distinctly prolonged into a sparsely setose and clavate process (Fig.8). Aedeagus highly sclerotised, extremely broad anteriorly, narrowed posteriorly, entry of ductus ejaculatorius antero-lateral. Female genitalia with corpus bursae ovoid; signum spine-like anteriorly, abruptly broadened posteriorly; ductus bursae slightly shorter than corpus bursae, its distal one-third part sclerotised; anterior apophyses about one-fourth of posterior, both pairs broadened at tips; papillae anales foliate, bearing setae of varying lengths (Fig.9).

Alar expanse : Male : 12 – 13 mm
Female : 13 mm

Material examined: Holotype: ♂, 5.iv.2002, Kalesar, Haryana; Paratypes:1♀, 4.viii.2002, 1♂, 6.iv.2002 Haryana, Kalesar, Haryana; 1♂, 17.viii.2001; 1♂, 1♀, 18.viii.2002, Sairighat, Himachal Pradesh; 1♀, 30.viii.2002, Subathu, Himachal Pradesh. Coll: V.K.Walia and D. Wadhawan. (Type specimens deposited in reference collection of Entomology section, Department of Zoology, Panjab University, Chandigarh)

Flight period : April and August.
Type locality : Kalesar (Haryana).
Larval host plant : Unknown.

Etymology: The name of the new species *Thyrsostoma albilustra* denotes predominantly shiny white colouration of the body.

Remarks: This species resembles *nephelochtha* Meyrick from Upolu and Malololelei (Samoan Is.) as far as shape of signum and both pairs of apophyses is concerned. However, presence of an oblique white band one-third away from base in the forewing of *albilustra* sp. nov. distinctly differentiates it from the closely allied species (Clarke, 1969).

DISCUSSION

While dealing with *Thyrsostoma* Meyrick, various workers have documented venation of forewing in different ways with regards to number and presence of specific veins. At the time of characterising this genus, Meyrick (1907) stated absence of veins R_3 and M_3 along with R_4 and R_5 stalked, with R_5 reaching the costa. The same author in 1925 mentioned absence of R_5 and M_1 along with R_3 and R_4 as stalked. In contrast to his version, illustration in catalogue of type specimens by Clarke (1969) shows presence of 11 veins, revealing absence of only one of the radials. However, during the course of present investigations, 10 veins have been observed in both the studied species which is in agreement with Meyrick (1925) as far as number and absence of aforementioned veins is concerned.

ACKNOWLEDGEMENTS

The authors are sincerely thankful to the Ministry of Environment and Forests for sanctioning an All India Coordinated project on Taxonomy for research on Microlepidoptera (AICOPTAX) No. J-22018/58/99-CSC (BC) and to the Forest Department in the concerned states for rendering cooperation during collection of material. We are also grateful to Prof. S. Chaudhry, Chairman, Department of Zoology, Panjab University, Chandigarh for providing necessary facilities and secretarial assistance.

ABBREVIATIONS USED IN FIGURES

1 A + 2 A = fused first and second anal veins; AED = aedeagus; ANT. APO = anterior apophysis; CRP. BU = corpus bursae; CU = cucullus; Cu A_1 = first anterior cubital vein; Cu A_2 = second anterior cubital vein; DU. BU = ductus bursae; Du. Ej = ductus ejaculatorius; GN = gnathos; M_1 = first median vein; M_2 = second median vein; P. A = papilla analis; PO. APO = posterior apophysis; R_1 = first radial vein; R_2 = second radial vein; R_3 = third radial vein; R_4 = fourth radial vein; Rs = radial sector vein; SA = saccus; Sc = subcosta; Sc+R_1 fused subcosta and first radial; SIG = signum; TG = tegumen; UN = uncus; VAL = valva; VLA = valvula; VIN = vinculum.

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Pb. Univ. Res. J (Sci.), Vol. 55, 2005, pp. 81-85

TAXONOMIC STUDIES ON INDIAN GELECHIIDAE IX. TWO SPECIES OF GENUS *THIOTRICHA* MEYRICK INCLUDING ONE NEW FROM NORTH INDIA (LEPIDOPTERA : GELECHIOIDEA)

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Abstract

Thiotricha albicephalata sp. nov. along with male genitalia of T. animosella (Walker) is described in detail. In addition to this, various morphological features and genitalic structures in both the species strengthening congeneric status have been commented upon.

Key words: Lepidoptera, Gelechiidae, *Thiotricha*, New species, North India.

INTRODUCTION

Thiotricha Meyrick is characteristically an Indo-Malayan genus with sizeable number of species mainly from Queensland in Australia and a few species from Europe, Africa, South America and New Zealand (Meyrick, 1925). Out of a large number of species pertaining to this genus from different corners of the world, as many as 37 have been recorded from India, chiefly from Assam and South India as compared to only one from Punjab (Rawalpindi) of pre-partition India (Gaede, 1937). Intensive and extensive surveys conducted between 2000 and 2003 resulted into collection of an already described species *T. animosella* (Walker) recorded from Chandigarh by Pajni and Mehta (1986) and a new species i.e., *T. albicephalata*. In this communication, detailed account of the new species along with male genitalia of already known species has been dealt with. The descriptions have been corroborated by relevant illustrations, including photograph of adult and diagrams of wing venation of already described species *animosella* which has not been published so far.

SYSTEMATIC ACCOUNT

Genus *Thiotricha* Meyrick

Thiotricha Meyrick, *Trans. N. Zeal. Inst.*, **18** : 164.

Type-species : *Thiotricha thorybodes* Meyrick, 1886, *Trans. N. Zeal. Inst.*, **18** : 164.

Key to the species of genus *Thiotricha* Meyrick

1. Labial palpi with third segment as long as second, both jointly forming 3-like shape; ground colour of forewing greyish-white above; vein R_2 free, R_3 connate with R_4+M_1; male genitalia without sicae. .....***albicephlata* sp. nov.**

– Labial palpi with third segment longer than second not forming 3-like shape; ground colour of forewing white above; vein R_2 connate with common stalk of R_3, R_4 and M_1; male genitalia with sicae present.....***animosella* (Walker)**

Thiotricha albicephalata sp. nov.

(Pl. I)

Male : Head shining white. Antenna with scape whitish-ochraceous; flagellum ochraceous, incompletely ringed with greyish-fuscous. Labial palpi whitish-ochraceous, sparingly tinged with greyish-fuscous along ventro lateral edges; second segment bearing tuft of long white hair scales above in distal half, creamish at tip; third segment as long as second, both jointly forming 3-like shape. Proboscis and maxillary palpi white.

Thorax shining white. Forewing (Fig. 1) oblong, extremely narrow; costa nearly straight; apex acute, produced; termen highly oblique; tornus poorly defined; inner margin nearly parallel to costa. Ground colour light greyish-white on upper surface; costal

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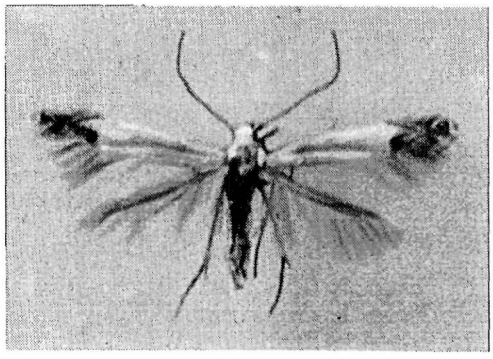
PLATE I



Ph. 1

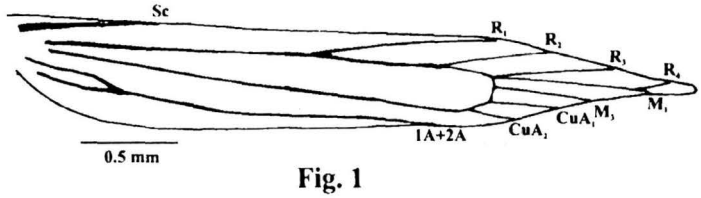


Fig. 1

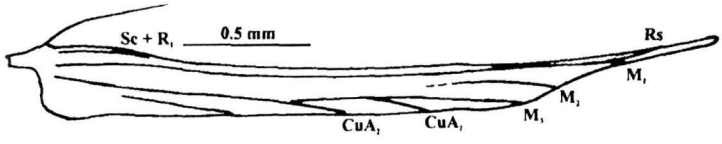


Fig. 2

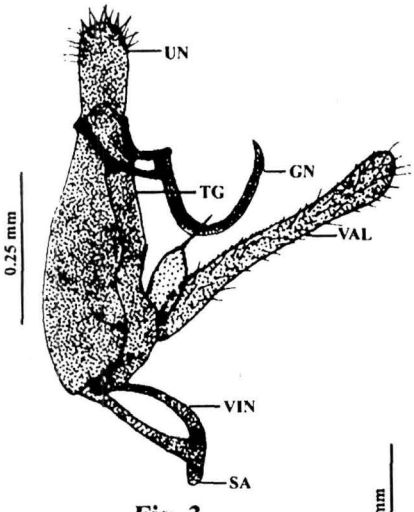


Fig. 3

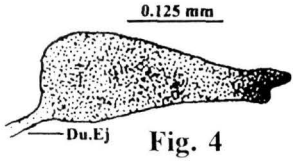


Fig. 4

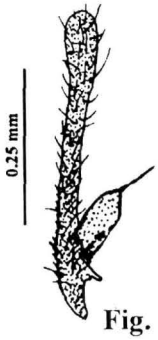


Fig. 5

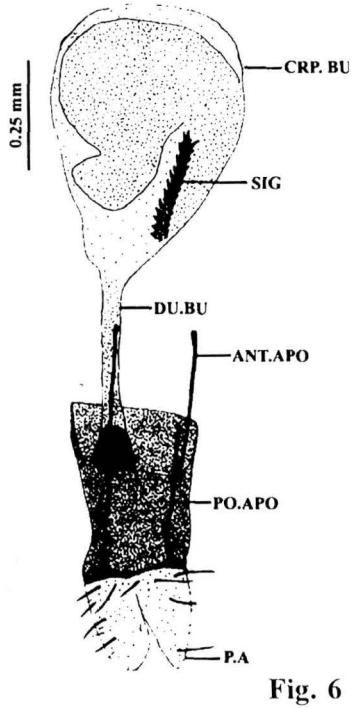
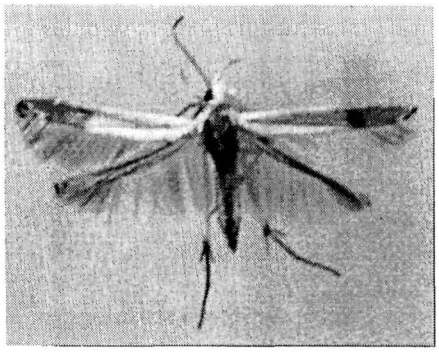


Fig. 6

Pl. I : Ph.1 and Figs. 1 – 6 : *Thiotricha albicephalata* sp. nov.; Ph. 1 stretched moth; Figs. (1) forewing; (2) hindwing; (3) male genitalia ; (4) aedeagus; (5) valva; (6) female genitalia.

PLATE II



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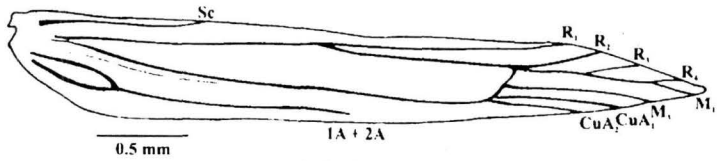

Fig. 7

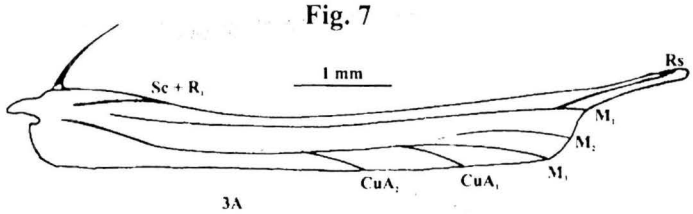

Fig. 8

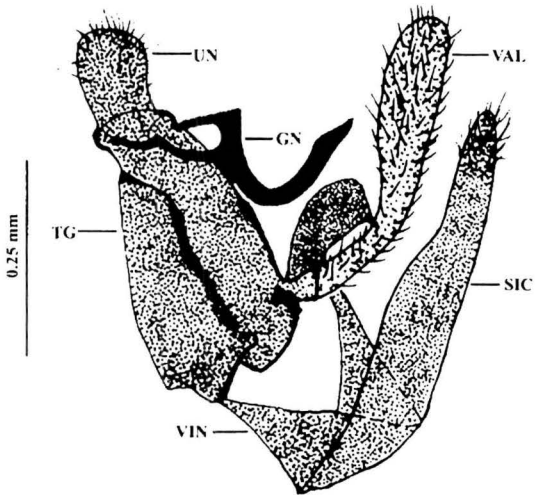

Fig. 9

Fig. 10

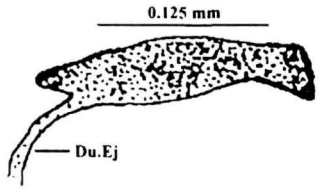

Fig. 11

Pl. II : Ph. 2 and Figs. 7 –11 : *Thiotricha animosella* (Walker); Ph. 2 stretched moth; Figs. (7) forewing; (8) hindwing; (9) male genitalia; (10) valva; (11) aedeagus.

Flight period : March

Old distribution : Maskeliya; Ceylon: N. Coorg, Khasi Hills, Chandigarh; India : Queensland; Australia (Meyrick, 1908; Gaede, 1937; Pajni and Deepak Mehta, 1986).

Larval host plant : Unknown.

DISCUSSION

Keeping in view elegantly marked wings or sometimes with orange or rosy colouration as a distinctive feature of the species belonging to genus *Thiotricha* Meyrick, both the investigated species viz., *albicephalata* sp. nov. and *animosella* (Walker) are absolutely in agreement with it as remarked by Meyrick (1925). Furthermore, narrow at base and broadened distally valvae in both the investigated species bearing a pointed process as in type species of the genus i.e., *T. thorybodes* Meyrick also corroborate the homogenous status of the studied species (Clarke, 1969).

ACKNOWLEDGEMENTS

The authors are sincerely thankful to the Ministry of Environment and Forests for sanctioning an All India Coordinated project on Taxonomy for research on Microlepidoptera (AICOPTAX) No. J-22018/58/99-CSC(BC). Authors are indebted to the Forest Department of Haryana for rendering cooperation during the collection of material. We are also grateful to Prof. S. Chaudhry, Chairman, Department of Zoology, Panjab University, Chandigarh for providing necessary facilities and secretarial assistance.

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SOURCE–SINK MANIPULATION AND ITS EFFECT ON POTENTIAL GRAIN YIELD OF RICE (*ORYZA SATIVA* L.)

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[2]*Department of Botany, Panjab University, Chandigarh - 160 014*

Abstract

A field experiment was conducted to investigate the hypothesis that yield of some cultivars of rice (Oryza sativa L.) may be limited by potential seed size. In this investigation, the source – sink ratio was manipulated to examine the performance of source-sink interactions after anthesis and the factor(s) limiting grain filling. Plants of three rice cultivars were artificially modified to give different source – sink ratios. The treatments were; I. control, II. cutting flag leaf blade, III. thinning half of the plants, IV. cutting flag leaf blade and thinning. The distribution of dry matter between kernels was analyzed at harvest in all the three cultivars. For determining potential grain weight in each plot, 50 per cent of grains at anthesis in mean shoot was reduced. Modification of source – sink ratio led to different patterns of allocation of dry matter between cultivars. The pattern of partitioning of dry matter observed in plants suggests both source and sink limitation in different rice cultivars. Also, in one of the cultivars there was a relative balance between source and sink and none of the two factors were limited.

Key words : Thinning, Flag Leaf, Source, Sink, Manipulation, Rice.

INTRODUCTION

In striving to increase the yield potential of cultivated plants it is important to determine the physiological factors limiting grain yield. The first step towards this is to assess whether the growth of harvested organs is limited by the availability of substrates (source limited) or by the capacity of the organ to assimilate and utilize the available substances for growth (sink limited) (Patrick, 1988). Attempts to identify physiological factors limiting yield must integrate source-sink interactions both spatially and temporally (Patrick, 1988). Results from the response of kernel weight and grain set to source-sink manipulations suggested yield limitation by both the sink (the number of grains m^{-2} and grain size) and the source, depending on the seasons, time of day etc. (Evans and Wardlaw, 1996). However, the time courses of source and sink control have not been well documented. Some authors (Cock and Yoshida, 1973 and Richards, 1996) suggested that grain yield in rice and wheat may not be limited by the supply of carbon at any time during grain filling. However, there are data showing significant increase in mass per grain associated with reduction in grain number (Fischer and HilleRisLambers,1978; Ledent and Stoy, 1985; Koshkin and Tararina, 1989) implying source limitation at least on some occasions after anthesis. Analysis of source-sink interactions should also consider the role of alternative sinks in the plant (Schnyder, 1993). In wheat, particularly, special attention should be given to the stems, since competition exists between the growing upper internodes and reproductive organs in the weeks before anthesis (Wardlaw, 1968; Bingham, 1972; Brooking and Kirby, 1981; Siddique *et al.*, 1989) the out come of which depends on both genotype and environment. Besides, there is good evidence that temporary storage is very important under stress conditions (Bidinger *et al.*, 1977; Blum *et al.*, 1994; Seidel, 1996).

Manipulation of source-sink ratios by artificial reduction in grain number per inflorescence has been used in several cereal grain species to estimate potential kernel weight and study the grain filling process (Blum *et al.*, 1983; Simmons and Busch, 1984; Cruz-Aguado *et al.*, 1999). Actual kernel weight is less than potential kernel weight because of competition among kernels for available assimilate and interplant competition for light, water and

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nutrients (Peterson, 1983). Kernel weight in cereal spikes generally increases in response to reduced kernel number per spike (Fischer and HilleRisLambers, 1978 and Peterson, 1983), although kernel weight reductions have been reported (Fischer and HilleRisLambers, 1978). It is assumed that such kernel weight increase occurs because assimilate available to each remaining kernel increases (Peterson, 1983 and Simmons and Busch, 1984). Potential kernel weight is obtained when kernel number is reduced to a point at which competition among kernels for assimilate no longer exists (Fischer and HilleRisLambers, 1978; Peterson, 1983). Fischer and Laing (1976) and Martinez-Carrasco and Thorne (1979) have used thinning as a technique for increasing photosynthate supply for developing kernels and increasing kernel weight. Removal of the flag leaf (Walpole and Morgan, 1974) or a portion of it (Winzeler *et al.*, 1989) has been used to reduce the amount of photosynthate available to developing kernels. It is possible that small-seeded cultivars are more sensitive to photosynthate supply. If this is true, treatments such as thinning, flag leaf removal, and spikelet removal should have different effects on small– and large-seeded cultivars. The aim of this study was to examine the performance of source-sink interactions after anthesis to evaluate the possible factors limiting grain yield in rice cultivars. Artificial manipulations of the source-sink ratio and evaluation of the variation in dry matter partitioning in three rice cultivars was used to assess the existence of genotypic differences in the response of availability of photo-assimilates.

MATERIALS AND METHODS

Plant material and Treatments

A field experiment was carried out at the Rice Research Institute of Rasht, Iran during wet season of 1999. This site has Mediterranean climate and a silty loamy soil.

The experiment was arranged in a randomized complete block design with a split plot arrangement with four replicates. Plots were fertilized at sowing with 90-20-60-20-3.5 kg N, P, K, S and Zn ha^{-1}. Entire amount of all the fertilizers except N was applied prior to transplanting while N was applied in three equal splits 4, 21 and 52 days after transplanting. Plots were occasionally sprayed with fungicides to avoid disease and were hand weeded.

Thirty-five days old rice seedlings of three cultivars comprising; bejar, khazar and binam were transplanted in plots consisted of seven rows, 7 meters long spaced 20cm. The distance between plants was 20 centimeter. Treatments consisted of a factorial combination of three cultivars and four source-sink manipulations arranged in a randomized split-plot design. Main plots consisted of the three cultivars and the sub-plots consisted of the four source-sink manipulation comprising; I. control, II. flag leaf blade removed, III. thinning consisted of cutting rows 2, 4 and 6 to ground level and IV. thinning and cutting flag leaf blade.

For determining potential grain weight 20 main shoots from the central rows of each plot were tagged and detillered to avoid the tillers becoming alternative sinks for mobilized carbohydrates (Slafer and Savin, 1994), sink strength of mainstems was decreased by half at anthesis by sterilizing 50 percent of fertile basal and lateral florets of the panicle. Total treatment was applied at anthesis stage, the day when anthers were extruded in 50 percent of the panicle in a plot (Hanft and Wych, 1982).

Measurements

At maturity, for determining the final grain yield and biological yield one square meter from central lines in each plot after removing boundary harvested and hand threshed. For each plot ten plants randomly selected and component of yield and agronomic traits were measured. At maturity the potential grain weight were recorded by measuring the dry mass of grains in degraining panicle. Harvest index calculated from the following formula:

$$HI = \frac{\text{Economical yield}}{\text{Biological yield}} \times 100$$

At anthesis, the area of the main stem leaves and flag leaf was measured according to Yoshid (1981) (Length × maximum width × 0.74). This formula represented the actual leaf area in the cultivars used in this experiment. Leaf area duration (LAD) was estimated according to Armas *et al.* (1988) using the following equation:

$$LAD = [(A2 - A_1)/ (LnA_2 - LnA_1)] (t_2 - t_1)$$

Where A_2 and A_1 represent shoot leaf area at anthesis and physiological maturity respectively, and $t_2 - t_1$ represents the time between anthesis and physiological maturity.

STATISTICAL ANALYSIS

Data were analyzed statistically by analysis of variance (ANOVA) and means were compared by least significant difference (LSD) test (Gomez and Gomez, 1984). The results of statistical analysis were considered significant when they were outside 95% confidence intervals.

RESULTS AND DISCUSSION

Table 1 shows the analysis of variance of grain yield, component of yield and potential grain weight of rice cultivars. In this experiment grain yield is significantly affected by cultivars that bejar and binam have highest and lowest grain yield respectively. Thinning at anthesis stage resulted in an average increase of 24 percent grain yield (Table 2). We observed a significant cultivars×thinning interaction for grain yield. Thus concluded that response of cultivars was not the same and bejar has highes,t increase in yield. An average thinning treatment resulted in increase of grain yield by 16, 38 and 15 percent in khazar, bejar and binam respectively (Table 2). On the other hand, if there is enough supply of assimilate bejar cultivar could utilize of it more than other cultivars. Therefore, it is necessary to determine optimum density and other inputs for this cultivar in different environments. But the extent of the source at the time of anthesis sets an upper limit to potential sink size in cv.binam and khazar.

Removal of the flag leaf blade at anthesis resulted in a decrease of 12 percent in grain yield (Table 2). This more considerable decrease in grain yield shows that flag leaf has an important role in grain filling. Supply more assimilates from other sources such as flag leaf.sheath and the leaves below the flag leaf can some what compensate the lack of flag leaf, on the other hand contribution of flag leaf in grain filling is more than 12 percent (Das and Mukharjee, 1989). The flag leaf blade is the principal source of photoassimilates imported by grains during grain filling (Rawson *et al.*, 1976). In spite of this, removal of the flag leaf may lead, in some circumstances, to enhancement of the photosynthetic activity of other leaves and green parts of plant (Koch, 1996) and remobilization of stored carbohydrates (Schnyder, 1993). These mechanisms avoid the restriction of grain filling in such a manner that often no source limitation occurs (Richards, 1996).

The comparison between control and defoliated plants in three cultivars indicates that cultivars differed in the ability to remobilize reserves from the stems to the grains. Such differences in the response to availability of photoassimilates seem to be the consequence of different patterns of photoassimilate partitioning between cultivars with varying source-sink ratios (Evans and Wardlaw, 1996). In khazar, bejar and binam cultivars, removal of flag leaf resulted in a decrease of 10, 18 and 6.5 percent grain yield respectively (Table 2). These results represent the possibility of remobilization of assimilates from secondary sources to grains in khazar and binam are more than bejar. One of the reasons for more decreased yield in bejar relation to other cultivars is related to large flag leaf area in this cultivar as compared to others (data not shown) as one of the most important factors in photosynthesis rate and supply assimilate to ear is flag leaf area (Matsushima, 1977). Removal of spikelet did not alter the pattern of senescence of photosynthetic tissues, and hence there were no significant differences in Leaf area duration (LAD) between control and degraining plants. LAD in flag leaf removal treatment was reduced due to lack of the flag leaf blade (Fig.1).

Table 1 shows that there were significant effects between thinning treatments and cutting of flag leaf blade. Whenever these two treatments occurred synchronized, grain yield increased only about 9 percent (Table 2). It is suggested that the role of increasing thinning is more than the role of decreasing removal of flag leaf in grain yield, because stored materials in stems and leaf sheath at removed flag leaf conditions compensate of low assimilate.

Also the study of components of yield revealed that thinning and removal of flag leaf blade treatments had no significant effects on number of panicle per unit area because of the time of treatments application. However, thinning treatment at anthesis time increased tillering but most of them were infertile and thinning merely only increased biomass (Fig.2), Cock and Yoshida (1973) have similar conclusions.

Table 1 shows the number of grains per ear affected by cultivars. Highest and lowest number of grains per ear belongs to khazar and binam respectively. According to Xu and Vergara (1986) variability of total grain number in cultivars have a genetical basis and depends on growth length and plant height, thus binam with taller height and weaker stem has lower number of grains per ear (Fig.3).

Since number of grains per ear are determined before

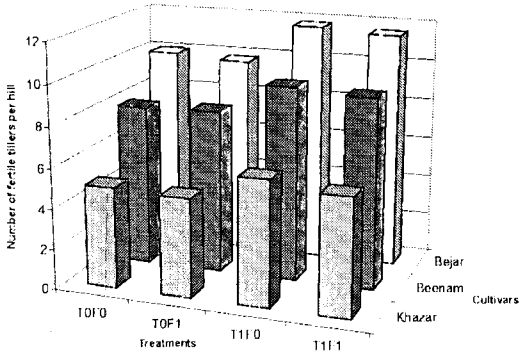

Fig. 1: Effect of source-sink manipulation on number of fertile tillers per hill

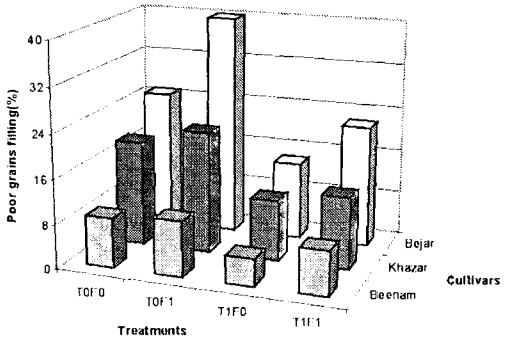

Fig. 2: Effect of source-sink manipulation on percentage of poor grain filling

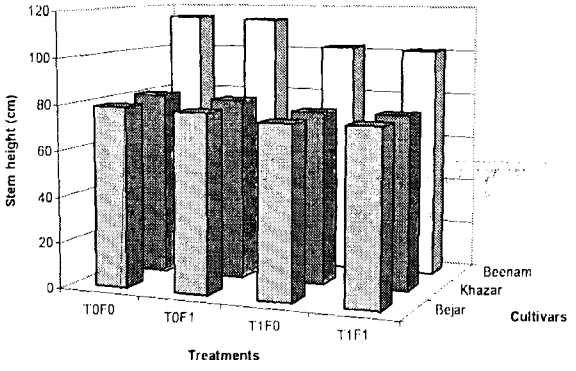

Fig. 3: Effect of source-sink manipulation on stem height

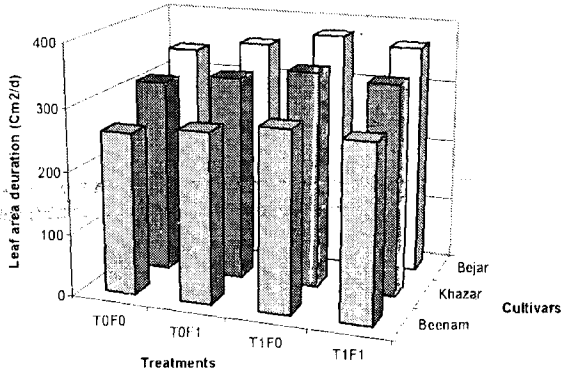

Fig. 4: Effect of source-sink manipulation on leaf area duration (LAD)

panicle initiation thus after determining primary grains, continued growth and filling grain depends on supply assimilate from different parts of plant (Venkateswarlu and Visperas, 1987). By manipulate of source potential ratio such as removal of flag leaf or thinning, some of physiological indices as source and sink capacity, carbohydrates stored and potential translocation of assimilates during stress conditions can be determined (Matsushima, 1977).

Our study have shown that in control plants of cv. binam nearly 91 percent of grains fully filled and matured (Fig.4), these results are consistent with findings of Matsushima (1977) and Murty and Muty(1981) of which concluded that if matured grain ratio is more than 80 percent, capacity of sink is limiting factor, which revealed that the limiting factor of yield in this cultivar is sink capacity. In this direction total of filled grains per plant on cv. khazar was about 80 percent (Fig.4) and showed that none of the factors i.e. capacity of sink and assimilate content are not limiting in this cultivar. On the other hand, in khazar cultivar there is a balance between source and sink. In this study, in bejar cultivar about 71 percent of grains in control plant matured (Fig.4). According to the definitions of Matsushima (1977) and Murty and Muty (1981) since number of filled grains in this cultivar are less than 80 percent, therefore, supply of assimilate is a limiting factor of yield.

Grain weight has more pronounced effects on grain yield, as we considered grain weight is different within the cultivars and binam and khazar with 28 and 24.2 mg had higher and lower grain weight respectively (Table 2). Thinning increased grain weight approximately 11 percent (Table 2). This increase was mainly due to supply of more assimilates to grains and a decrease of competition between plants. Thus after thinning the remaining plants may have more ability of using current photoassimilates, Zia (1987) in rice had similar conclusion.

In three cultivars of khazar, bejar and binam thinning

Table 1 : Analysis of variance of grain yield, components of yield and potential grain weight of three rice cultivars and four source – sink manipulations.

S.O.V.	df	Grain yield g/m²	Biomass g/m²	HI (%)	No. of panicles (m²)	No. of grains per panicle	1000 Grain weight (g)	Potential grain weight (g)
Replication	3	176.250$^{n.s}$	840.927$^{n.s}$	0.646$^{n.s}$	11.556$^{n.s}$	11.048$^{n.s}$	0.047$^{n.s}$	0.026$^{n.s}$
Cultivars (V)	2	733551.396**	461602.083**	1013.666**	44984.313**	28340.746**	72.171**	55.043**
Thinning (T)	1	488840.333**	1573252.083**	17.400**	30.083$^{n.s}$	1534.541**	84.005**	0.047$^{n.s}$
Flag leaf (F)	1	179585.333**	315252.083**	51.460**	14.083$^{n.s}$	424.830**	41.255**	0.422*
V × T	2	84039.521**	94808.333**	23.443**	3.771$^{n.s}$	17.341**	25.318**	0.019$^{n.s}$
V × F	2	34739.521**	45033.333**	8.631**	12.521$^{n.s}$	61.114**	51.106**	0.013$^{n.s}$
T × F	1	396.750*	1102.083$^{n.s}$	8.927**	12.000$^{n.s}$	1.401$^{n.s}$	3.050*	0.075$^{n.s}$
V × T × F	2	51.062$^{n.s}$	64.583$^{n.s}$	1.177$^{n.s}$	8.063$^{n.s}$	0.120$^{n.s}$	0.073$^{n.s}$	0.020$^{n.s}$
C.V. %		10.9	13.3	3.3	9.8	9.9	6.9	6.5

N.s, * and ** = Non significant, significant at the 5% and significant at 1% levels respectively.

Table 2 : Comparison of mean triple interaction between cultivars, thinning and flag leaf cutting on yield and components of yield in rice cultivars.

Cultivar	Thinning	Flag Leaf	Grain yield g/m²	Biomass g/m²	HI (%)	No. of panicles (m²)	No. of grains per panicle	1000 Grain weight (g)	Potential grain weight (g)
	*T_0	**F_0	896	1750	51.1	314	162	24.2	25.4
Khazar		F_1	850	1694	50.2	313	160	23.5	25.3
	T_1	F_0	963	1868	51.6	315	168	25.0	25.4
		F_1	918	1812	50.6	315	165	24.4	25.4
	T_0	F_0	1114	2044	54.2	325	142	24.0	28.8
Bejar		F_1	1005	1902	52.4	326	136	22.4	28.6
	T_1	F_0	1298	2310	56.2	327	148	26.8	28.8
		F_1	1189	2168	54.4	326	143	25.2	28.6
	T_0	F_0	714	1801	39.6	228	80	28.2	28.3
Binam		F_1	690	1757	39.2	229	81	27.6	28.2
	T_1	F_0	764	1959	39.0	230	85	28.5	28.4
		F_1	740	1915	38.7	229	84	28.0	28.3
	(LSD)	(5%)	16.1	33.00	0.82	3.0	3.5	0.25	0.3

* T_0 and T_1 = represent unthinned and thinning treatments respectively.

** F_0 and F_1 = represent no cutting and flag leaf blade cutting treatments respectively.

treatment increased grain weight by 7, 25 and 3 percent respectively (Table 2). Bejar and binam cultivars with 5.5 and 0.7 mg had highest and lowest increases respectively. Thus in bejar cultivar adjustment of yield with decreased composition between plant can occur through grain weight variation. If grain weight increased in response to more supply of assimilate, it can be said that grains are under source limited (Yoshida and Ahn, 1986). In general, thinning treatment showed that bejar related to other cultivars is source limited and if this cultivar had more assimilate it can produce heavier grains.

Cutting flag leaf resulted in decrease of grain weight by 7 percent and represents the importance of flag leaf supply material for grain growth. Of course more amount of decrease was compensated by flag leaf sheath, internodes and other leaves and even spikes photosynthesis (Yoshida, 1981). If kernel weight was limited in some cultivars due to sink capacity and not in others, reductions of photosynthate availability would cause greater decrease in nonlimited cultivars than in limited cultivars. An interaction between cultivar and flag leaf removal would be further evidence of limitations in sink size. In three cultivars of khazar, bejar and binam with removed flag leaf grain weight decreased by 4, 12 and 4 percent respectively (Table 2).These results show that, may be, remobilization from secondary sources in khazar and binam is more than cv. bejar and higher photosynthetic activity of ear in these two cultivars as compared to cv. bejar. Source limitation in cv.bejar could be the cause of the significant decrease in specific mass of grain in cutting flag leaf treatment as compared to other cultivars.

In general grain weight more than number of grain is under photoassimilate stress (Xu and Vergara, 1986). On the other hand, under stress conditions or lack of assimilates with a balanced distribution of carbohydrate grains with less weight reached to final growth. According to Cruz-Aguado *et al.* (1999) a linear relationship exist between endosperm cell number and grain weight. Shortage of photoassimilates during endosperm cell proliferation possibly resulted in decreased endosperm cell number and grain weight (Fukoshima *et al.*, 1985).

Across all cultivars, spikelet removal at anthesis resulted in a large increase in some cultivars (Table 1, 2). Sink reduction not only decrease the competition for assimilates among growing grains, but eliminated, if existing, physical size constraints affecting the development of florets. The effects of spikelet removal were not the same for all cultivars, as indicated highly significant difference between these cultivars in analyses of variance. Spikelet removal at anthesis resulted a significant increase in potential grain weight for cv. bejar and cv. khazar but a small increase in average potential grain weight in cv. binam. The significant increase in potential grain weight of bejar (4.8 mg) and khazar (1.2 mg) suggested that small-seeded cultivars may have greater grain weight response to spikelet removal than large-seeded cultivars, as insignificant increased in grain weight for large-seeded like cv. binam (0.1 mg) support such a thesis. However, there are data in the literature showing a significant increase in mass of grains associated with reductions in grain number after anthesis, implying source limitation, at least some time during grain filling (Cruz-Aguado *et al.*, 1999).In this study, small-seeded cultivars (bejar and khazar) tended to show the greatest response to spikelet removal.These results agree with those of Ma *et al.* (1990) and Blum *et al.* (1994).

Finally, results of this study indicated that sink limitation could explain the lack of growth of the remaining grains in half-panicle plants in binam cultivar. Whereas grain yield in cv. bejar was limited by source activity rather than sink size. There is a relative balance between source and sink in khazar cultivar and none of the two factors were limited in grain growth of this genotype.

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SOME NEW AQUATIC FUNGI FROM NORTHERN INDIA

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Abstract

In the present paper ten species of water moulds belonging to Oomycota and Chytridiomycota have been described from Chandigarh and adjoining areas. Saprolegnia turfosa, S. unispora, S. australis, Achlya racemosa, A. spiraccaulis and Apodachlya minima are recorded for the first time from Chandigarh and are new records for India. Pythium Khulbe Prasher and Sharma is being described as a new species. Nowakowskiella elegans and Pythium multisporum are being reported for the first time from Chandigarh and adjoining area.

Key words: Aquatic fungi, Chandigarh, India.

INTRODUCTION

Water moulds include zoosporic fungi belonging to Chytridiomycota (Fungi) and Oomycota (Chromista), found in fresh and polluted waters (Kirk *et al.*, 2000). Review on the status of zoosporic fungi of India (Sarbhoy, 1997) revealed that major work on the systematics of water moulds of India have been carried out in Uttar Pradesh, Bihar, W. Bengal, Andhra Pradesh, Haryana and Chandigarh. A review of the above literature reveals that little work has been done on the aquatic fungi of Chandigarh and adjoining areas (Prasher,1983; Rawla and Prasher, 1986; Prasher, 1998) . These reports constitute a fraction of the aquatic fungi which may be occurring in Chandigarh and adjoining areas. In light of above mentioned fact, studies were initiated to describe the water moulds of Chandigarh and adjoining area.

MATERIALS AND METHODS

The water moulds included in this work are those that have been isolated from fresh water bodies of Chandigarh in the months September 2002 – September 2003 and that deposited in the herbarium of Botany Department, Panjab University, Chandigarh (PAN). The water moulds included in this paper have been collected from fresh water tanks of Botanical Gardens, from standing water of Gandhi Bhavan and the tank adjacent to the student center, Panjab University, Chandigarh, Sukhna Lake and seasonal drain in Rose Garden, Sector 16, Chandigarh.

The fungi were isolated by a method described by Prasher (1983). The morphological details of the hyphal tip isolates of the fungi (bacteria free) were microscopically observed on sterilized hamp seeds in distilled water (Seymour, 1970). The brief procedure is as follows: A sterilized hemp seed half was placed, cut surface down, on the margin of the growing colony on PDA in petriplates. After 48 hours, the seed was removed, transferred to a petriplate containing 25 ml of sterilized distilled water and incubated at 18-22°C (found optimum for the development of reproductive structure in the fungi studied). Daily observations were taken. Normally the gemmae are produced after 3 days, sporangia after 4-5 days and oogonia after 12 days depending upon the species. The temporary preparations for microscopic study were made in water mounts. Semi permanent microscopic preparations were made after staining in cotton blue Le Gal, 1947 (Lactic acid 30.00 g, cotton blue 0.05 g). All measurements were made from fresh specimens in water mounts. The illustrations have been made with camera lucida manufactured by American optical Co. U.S.A. All the specimens have been preserved in mineral oil (Buell and Weston, 1947) and have also been kept in water cultures.

The fungi included on this paper have been deposited in the PAN (Herbarium Botany Department, Panjab University, Chandigarh).

TAXONOMY:

Saprolegnia turfosa (Minden) Gaumann (Fig. 1 A-G)

Botaniska Notiser, 1918-19: 154. 1918.
Hyphae stout, sparingly branched 15-25 mm in diameter. Gemmae laking or sparse; pyriform or irregular; single and terminal, rarely intercalary. Zoosporangia abundant,

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filiform or clavate; 156-390 X 21-25mm; renewed by internal proliferation; encysted spores 8-10 mm in diameter. Oogonia abundant; lateral, rarely terminal, spherical, 15-80mm in diameter. Oogonial wall thick; pits numerous and conspicuous. Oogonial stalks short. Oospores centric, 5-10 in number; 25-26 mm in diameter. Antheridial branches androgynous. Antheridial cells long, tubular or cylindrical and simple.

Collection Examined: Nisha 26047 (PAN), isolated on hemp seeds, Rose Garden (Temp 13°C), Sector 16, Chandigarh, January 10, 2003.

The above collection resembles the type species except for minor variation in the size of the zoosporangia and in having only androgynous antheridial branches. It is a new record for India.

***Saprolegnia unispora* (Coker & Couch) Seymour. (Fig. 2 A-H)**

The Genus *Saprolegnia*. Nova Hedwigia, XIX: 57-58, 1970 Hyphae stout, sparingly branched 12-26 mm in diameter. Gemmae variable abundant, spherical. Zoosporangia abundant; fusiform, filiform or pyriform; variable in length, renewed internally, 80-420 x 20-40 mm in size; encysted zoospores 6-8 mm in diameter. Oogonia abundant; lateral, occasionally terminal; sometimes clustered on a hypha; spherical or pyriform; 20-64.5 mm in diameter. Oogonial wall pitted or unpitted, smooth; Oogonial stalk variable in length; straight or curved. Oospores subcentric or centric; spherical 1-2 in number; 20-30 mm in diameter. Antheridial branches and cells not observed.

Collection Examined: Nisha 26046 (PAN), isolated on hemp seeds, Rose Garden, (Temp. 13°C), Sector 16, Chandigarh, October 17, 2002.

The morphological details of the above specimen are similar to that given by Seymour (1970) except for thinner hyphal branches. It is a new record for Chandigarh and India.

***Saprolegnia australis* Elliott (Fig. 3 A-G)**

New Zealand J. Bot., 6: 103, figs. 2,3,4c-f. 1968.

Hyphae stout straight about 30 mm in diameter; sparingly branched. Gemmae abundant; filiform, clavate, spherical or irregular. Zoosporangia filiform to clavate; 50-90 mm long; zoospore discharge saprolegnoid; encysted spores 8.6 mm in diameter. Oogonia abundant; pyriform to obovate, sometimes with long neck; usually terminal; 50-60 mm in diameter. Oogonial wall conspicuously pitted; smooth. Oogonial stalks usually long, 2 to many times diameter of oogonium. Oospheres often not maturing. Oospores subcentric or sub-eccentric; 5-9 in number; 16-24 mm in diameter. Antheridial branches predominately diclinous; persistent, much branched. Antheridial cells simple; laterally appressed.

Collection Examined: Nisha 26045 (PAN), isolated on hemp seeds, fresh water tank (Temp. 14°C) botanical garden, Panjab University, Chandigarh (November 30, 2002).

The above collection resembles the type specimens in morphological details except for slightly smaller oogonia. It is a new record for India.

***Achlya racemosa* Hildebrand (Fig. 4 A-E)**

Jahrb. wiss. Bot., 6: 249, pl. 15. 1867-68.
Mycelium extensive, principal hyphae stout, 30-80 mm in diameter at base. Gemmae abundant; filiform, irregular, and usually single, rairly in chains. Zoosporangia abundant; filiform, 150-300 X 17-30 mm in size, renewed sympodially or in basipetalous succession. Zoospore discharge achlyoid; spore cluster persistent at exit pore; encysted spores 8-10 mm in diameter. Oogonia abundant; lateral, spherical or pyriform; 50-110 mm in diameter. Oogonial wall smooth only pitted under point of attachment of antheridial cell. Oogonial stalk, 1½ times the size of the oogonium. Oospores centric to subcentric, 1-10 in number; 15-20 mm in diameter. Antheridial branches androgynous to diclinous. Antheridial cells simple.

Collection Examined: Nisha 26048 (PAN), isolated on hemp seeds, Gandhi Bhavan (Temp. 13°C), Panjab University, Chandigarh, September 10, 2003.

The above collection resembled the description given by Johnson (1956), except for smaller zoosporangia (150-300 X 17-30 mm instead of 200-900 X 15-45 mm). It is a new record for India.

***Achlya spiraccaulis* Johnson (Fig. 5 A-F)**

Mycologia, 41: 678, figs.1,2, 1949.
Mycelium extensive, diffuse. Gemmae sparse; spherical, filiform. Zoosporangia abundant; filiform, 250-300 mm long by 20-30 mm in diameter. Zoospore discharge achlyoid; spore cluster persistent at exit pore; encysted spores 10-12 mm in diameter. Oogonia abundant; spherical or subglobose, 40-100 mm in diameter.

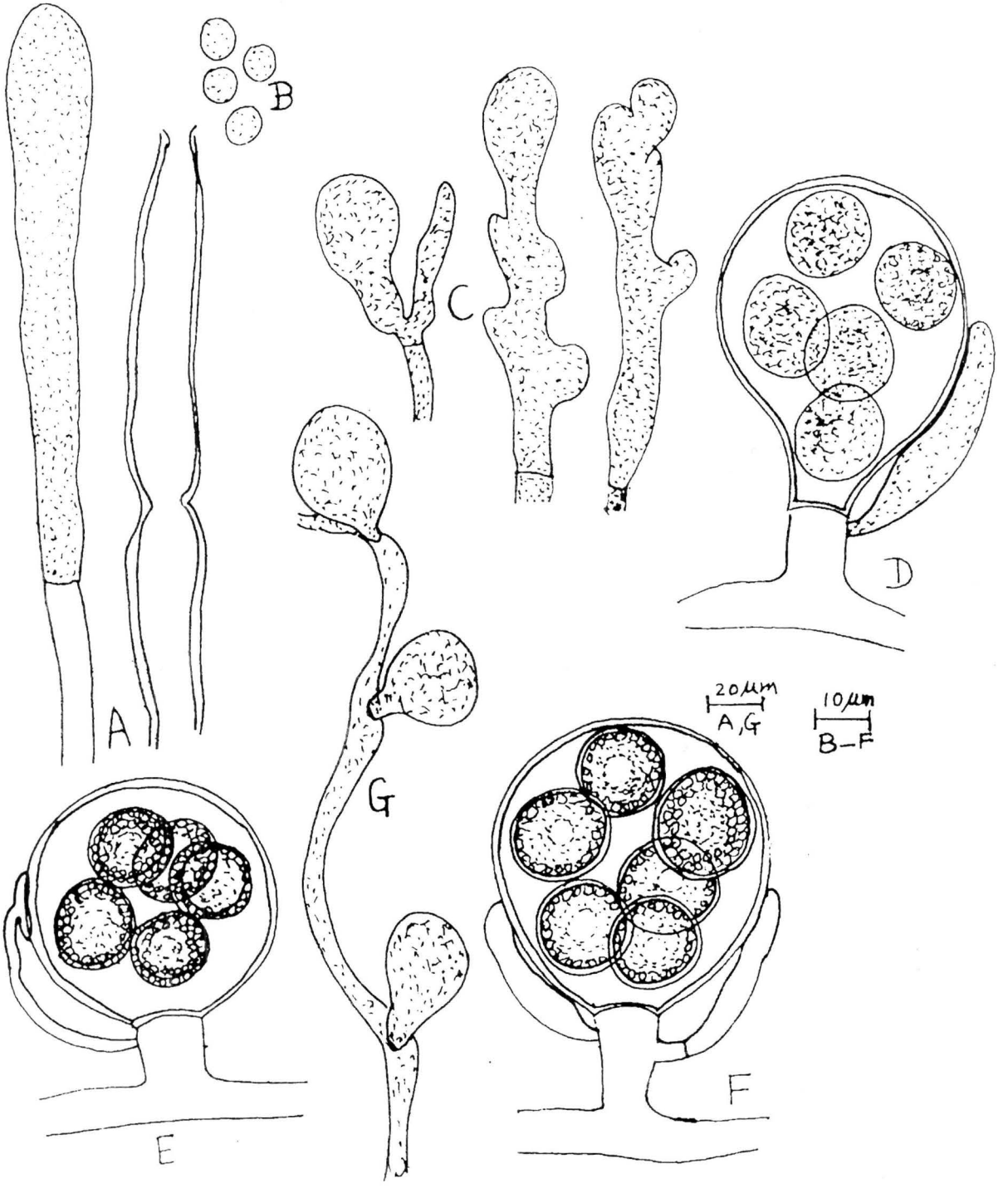

Fig. 1: ***Saprolegnia turfosa*** (Minden) Gaumann : A - Zoosporangia; B - Encysted zoospores; C - Gemmae, D-G - Oogonia and antheridia

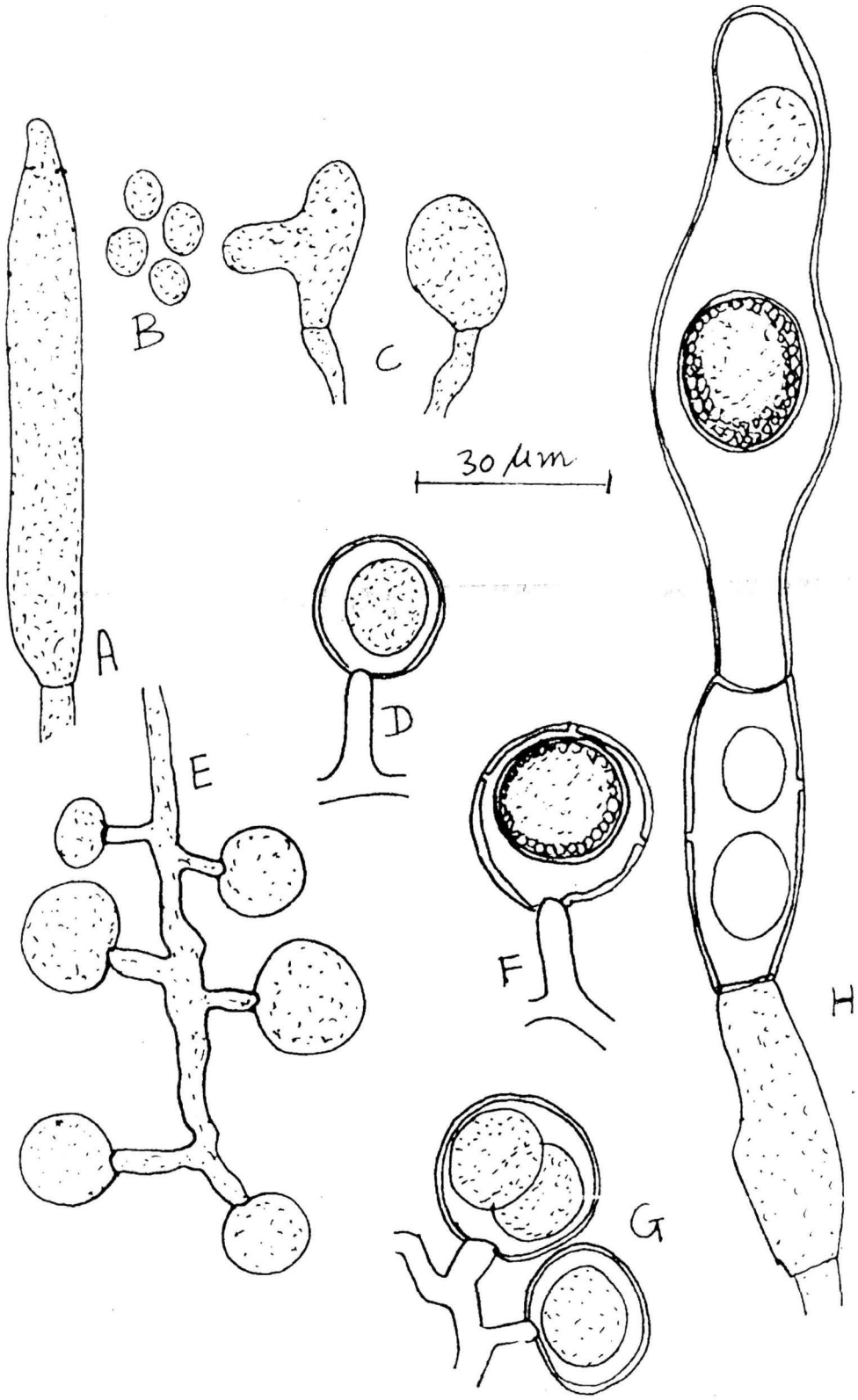

Fig. 2: ***Saprolegnia unispora*** (Coker & Couch) Seymour : A - Zoosporangia, B - Encysted zoospores, C- Gemmae, D-G - Oogonia

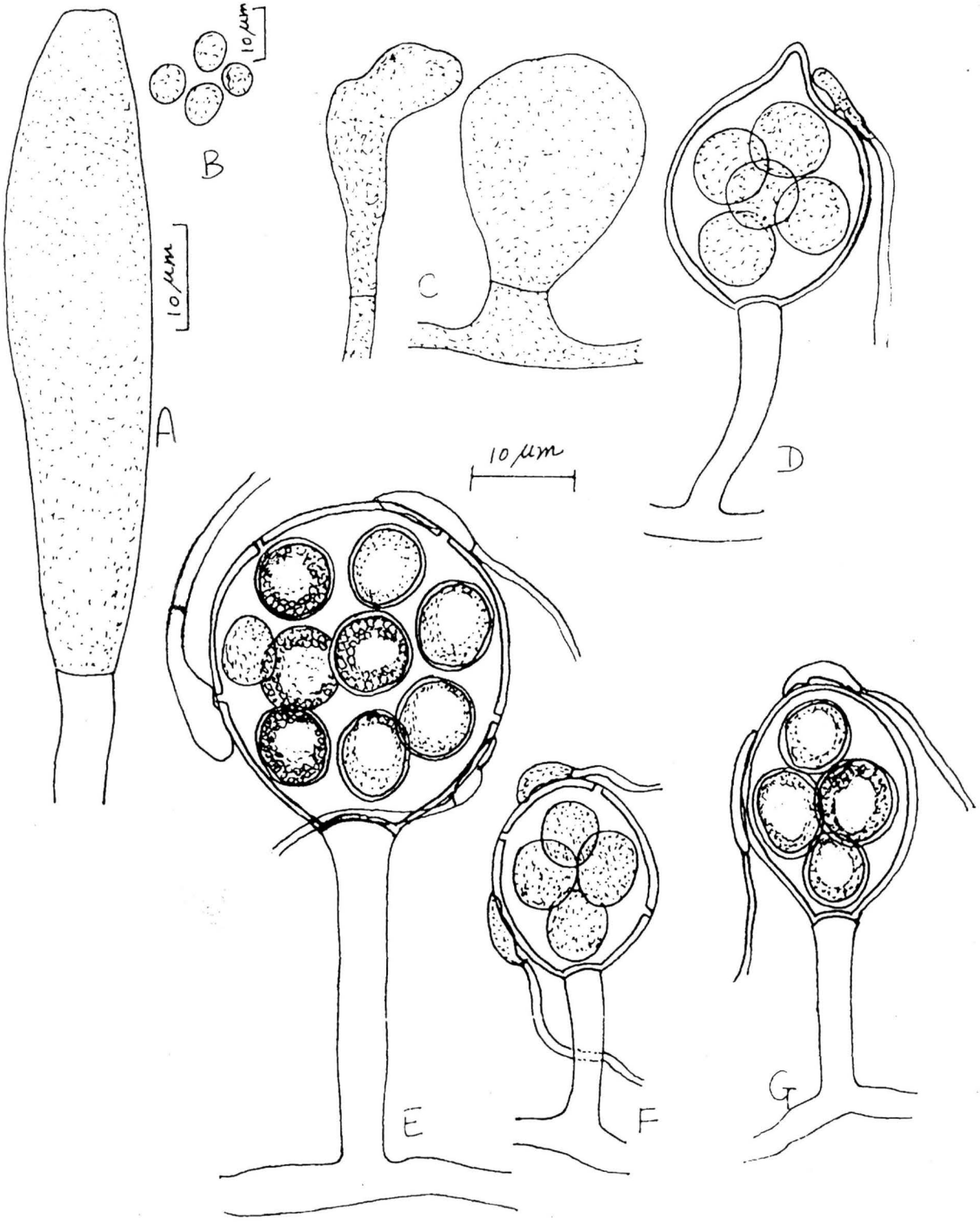

Fig. 3: ***Saprolegnia australis*** Elliott : A - Zoosporangia, B - Encysted, Zoospores, C-Gemmae, D-G - Oogonia and antheridia

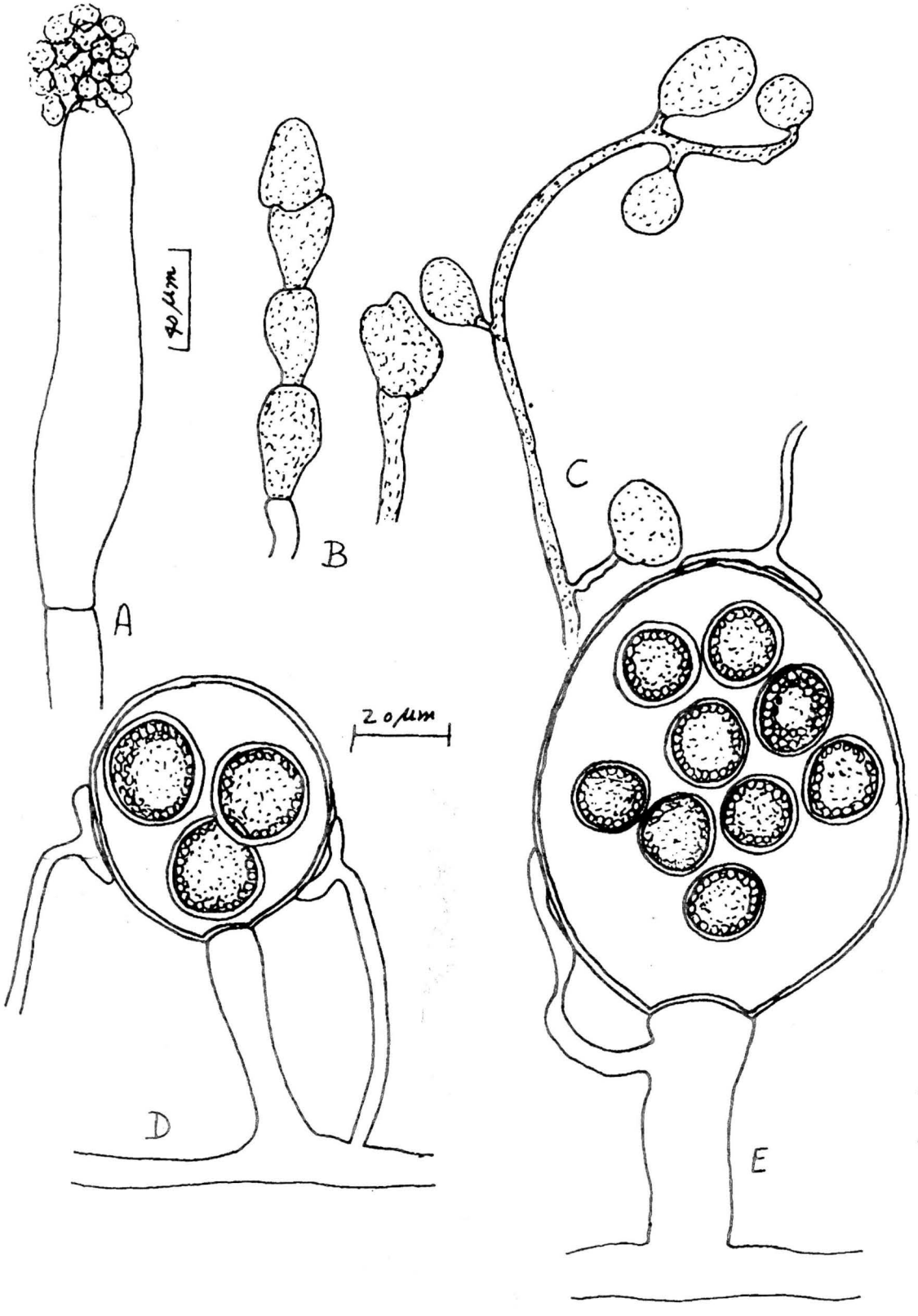

Fig. 4: ***Achlya racemosa*** Hildebrand : A - Zoosporangia and encysted zoospores, B - Gemmae, C-Oogonial initials, D-E - Oogonia and antheridia

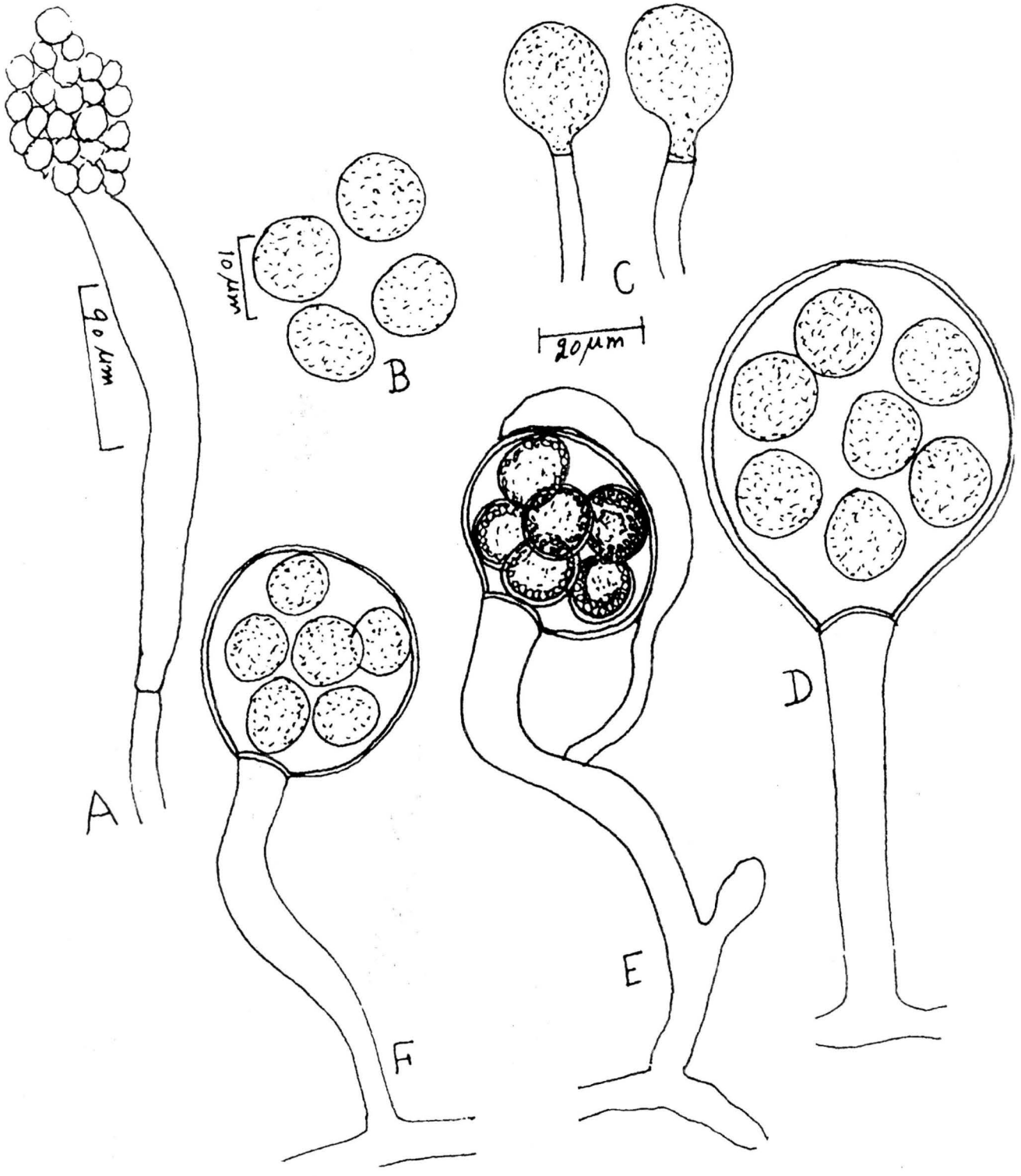

Fig. 5: ***Achlya spiraccaulis*** Johnson : A - Zoosporangia and encysted zoospores, B - Encysted zoospores, C-Gemmae, D-F - Oogonia and antheridia

Oogonial wall unpitted. Oospores centric to subcentric; 4-6 in number; 12-25 mm in diameter. Oogonial stalk 1-5 times the size of the oogonium, some times branched, rarely short. Antheridial branches usually lacking; when present, androgynous, monoclinous or diclinous. Antheridial cells long, tubular, apically appressed.

Collection Examined: Nisha 26049 (PAN), isolated on hemp seeds, Rose Garden (Temp.12°C), Sector 16, Chandigarh, September 5, 2002.

The description of above collection resembles the type description except for smaller zoosporangia (280-640 mm in type specimen) and slightly smaller size of the oospores (12-25 mm instead of 13-52 mm). It is a new record for India.

***Dictyuchus* sp. (Fig. 6 A-B)**

Mycellium well developed, branched. Zoosporangia long cylindrical, 80-350 x 15 mm – 30 mm in size. Zoospores encysting inside the sporangium. Secondary encysted zoopores 8-12 mm in diameter, emerging later on leaving their cysts in the form of a net. Oogonia and antheridia are not observed.

Collection Examined: Nisha 26050 (PAN), isolated on hemp seeds, Sukhna Lake (Temp. 13°C), Chandigarh, September 20, 2003.

The above specimen resembles *Dictyuchus monosporus* Leitgeb, except for the absence of oogonia. Studies are in progress to find the optimum conditions for the development of sex organs.

***Nowakowskiella elegans* (Nowak) J. Schroeter (Fig. 7 A-E)**

Engler and Prantl, *Naturlichen Pflanzenfam*, 1(1): 82, 1892 (1893).

Sporangium terminal, occasionally intercalary, spherical, ovoid, pyriform, 18-32mm in width, apophysate or nonapophysate, with or without a discharge tube, thin walled, smooth, colourless. Zoospores spherical, 5-7mm in diameter, forming a mass at the orfice, resting spores with smooth wall.

Collection Examined: **Nisha 26051 (PAN), isolated on hemp seed, Rose Garden, Sector 16, Chandigarh, May 19, 2003.**

The description of the above specimen resembles the type description except for minor differences. It is a new record for Chandigarh.

***Pythium Khulbe* Prasher et Sharma sp. nov. (Fig. 7 F-J)**

Mycelium extensum, 2-4 mm diametro, nonseptatem. Zoosporangia sphaericus to obovata, 14-30 mm diametro, intercalares et sessilis, emmissio tube upto 6 mm. Zoosporae incystatae 7-11 mm diametro. Oogonia sphaericus, terminalia 13-10 mm diametro, laevigata, oosporae, plerotice 10-15 mm diametro. Antheridia nulla.

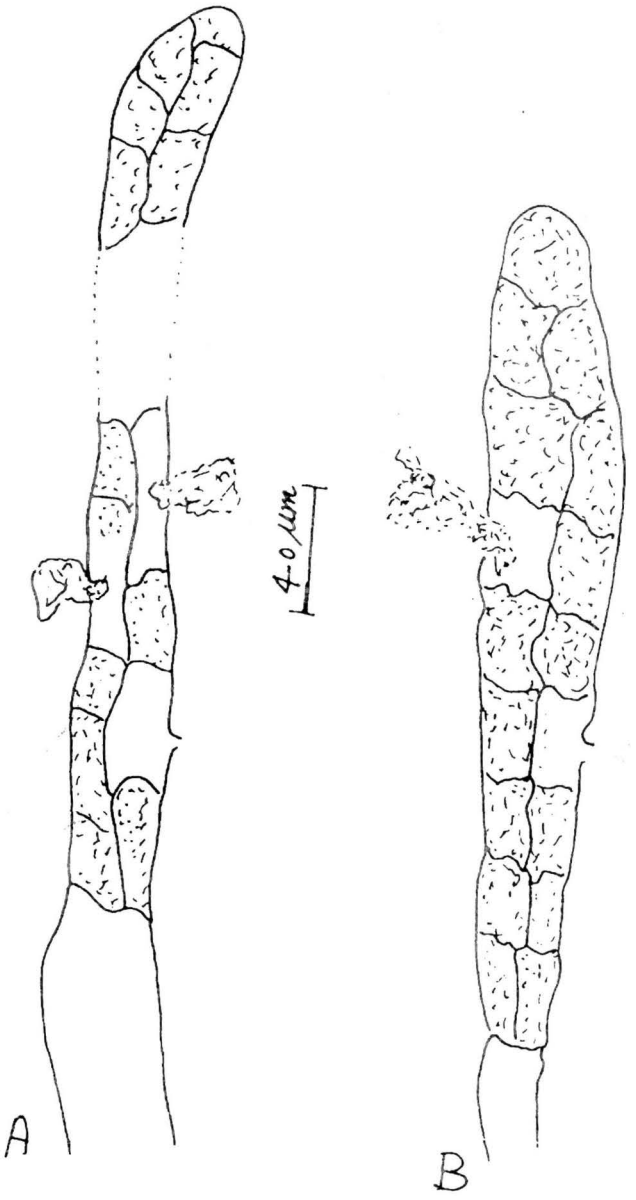

Fig. 6: *Dictyuchus* sp : A-B - Zoosporangium with empty cells and zoospores in the process of discharge

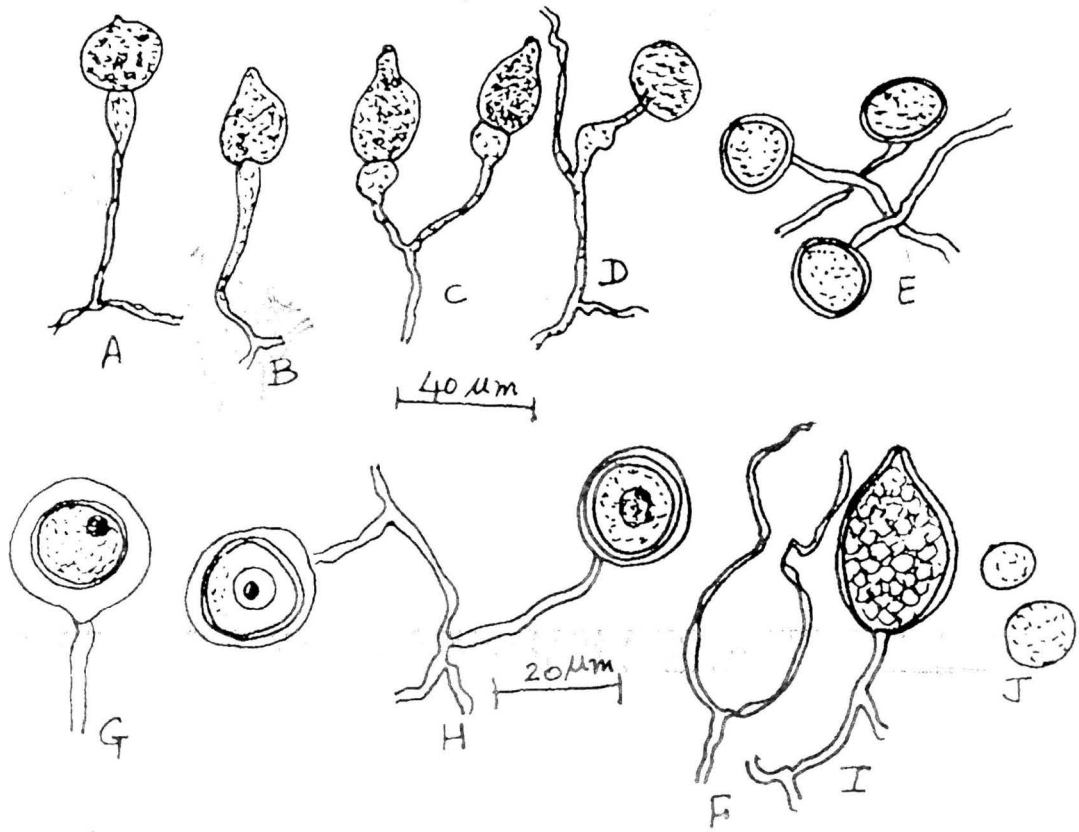

Fig. 7: (A-E):***Nowakowskiella elegans*** (Nowak) J. Schroet : A-D - Mycelium and sporangia, E - Resting spores; (F-J):***Pythium Khulbe*** **Prasher** et Sharma sp. nov. : F - Sporangium and Vesicle (empty), G-H - Oogonia, I - Sporangium, J - Zoospores (encysted)

Mycelium well developed extensive, branched, 2-4 mm in diameter. Zoosporangium spherical to ovoid, 14-30 mm in diameter, intercalary or laterally sessile with an emmission tube up to 6 mm in length. Zoopores expelled from the sporangium into the vesicle, 15-28 mm in width, encysted zoopores 7-11 mm in diameter. Oogonia typically spherical, terminal, 13-18 mm in diameter, smooth, oospore single, plerotic 10-15 mm in diameter, wall thick. Antheridia absent.

Etymology: In honour of late Prof. R.D. Khulbe who contributed immensely to water moulds of Uttranchal and adjacent areas.

Collection Examined: Supti 26036 (PAN), isolated on hemp seeds, artificial lake, Vatika, Panchkula, April 9, 2000.

This specimen which has ovoid, spherical sporangia and oogonia is characterized by the absence of antheridia. It does not resembled any of the known species of *Pythium* Mathews (1931), Sparrow (1943).

Pythium multisporum **Poitras (Fig. 8 A-E)**

Mycologia 41: 171, fig. 1-17, 1949.

Mycelium well developed, branched, 2-5 mm in diameter, sporangia spherical, subspherical to ellipsoid, terminal on lateral branches, 30-38 mm in diameter; encysted zoospores 9-10 x 11-15 mm in size. Oogonia irregular in shape, 25-48 x 35-54 mm in size. Oospore 1-4 per oogonium, aplerotic, 11-26 mm in diameter, with smooth wall. Antheridia monoclinous and diclinous, one to several; stalk of variable length.

Collection Examined: Supti 26037 (PAN), isolated on hemp seeds, artificial lake, Vatika, Panchkula, April 9, 2000.

The description of the above specimen resembles with the standard description of *Pythium multisporum* Poitras except for slightly smaller oogonia and having only more than one oospore per oogonium. It has already been recorded from India (Bilgrami *et al*, 1991) but it is a new record for this area.

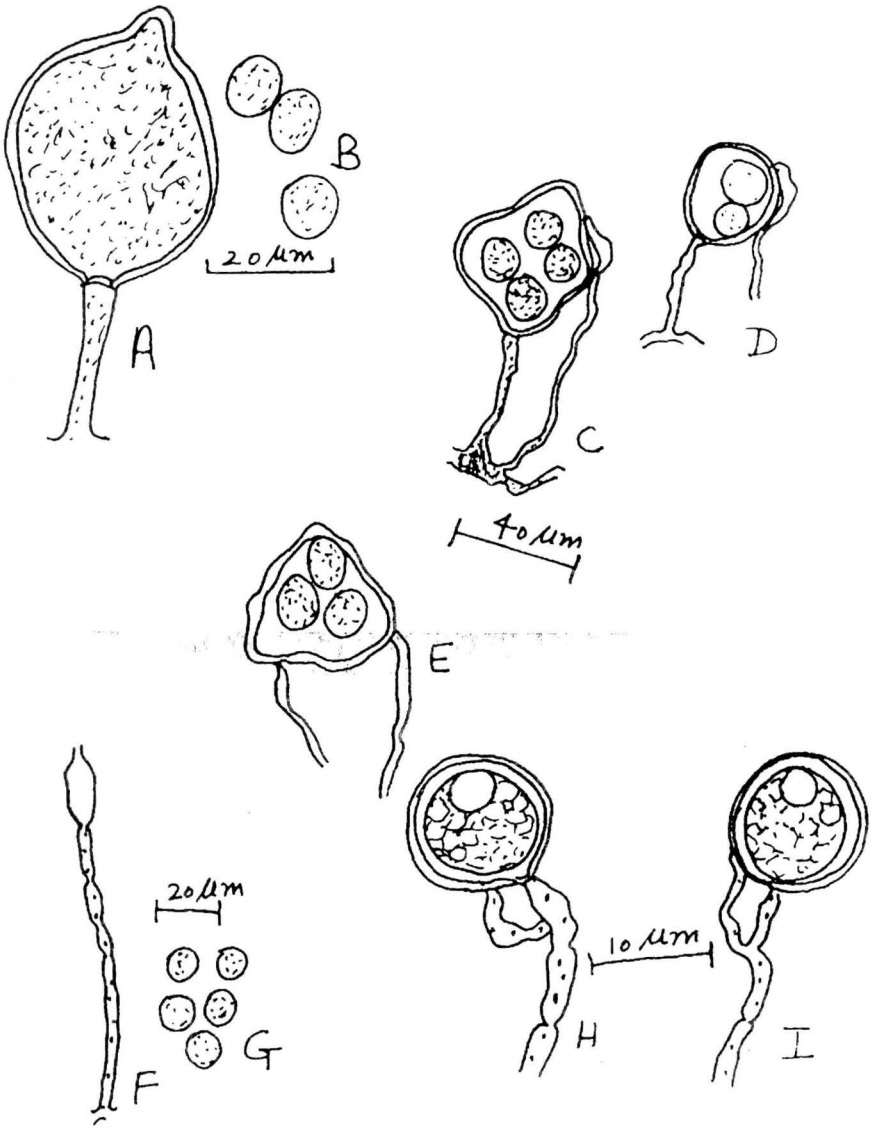

Fig. 8 (A-E): ***Pythium multisporum*** Poitras : A - Sporangium, B - Encysted zoospores, C-E - Oogonia and antheridia, **(F-I):*Apodachlya minima*** Coker and Leitner : F - Sporangium, G - Encysted zoospores, H-I-Oogonia and antheridia

Apodachlya minima **Coker and Leitner (Fig. 8 F-I)**

J. Elisha Mitchell Sci Soc 54: 313, 1938.

Mycelium extensive, branched, segments long or short, contents with cellulin bodies in each segment, especially near the constrictions. Sporangia terminal, single, ovoid to ellipsoidal, 15-23 mm long by 19-20 mm in diameter, papilla usually terminal or some what lateral; Oogonia spherical, 10-16 mm in diameter, generally terminal or lateral on a short segment near a constriction. Antheridia lateral or sub-oogonial; oospore spherical, filling the oogonium, contents with a large oil globule.

Collection Examined: Supti 26039 (PAN), isolated on hemp seeds, artificial lake, Vatika, Panchkula, July 18, 2000.

The description of the specimen resembles the type description of *Apodachlya minima*. It is a new record for India.

ACKNOWLEDGEMENTS

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Pb. Univ. Res. J (Sci.), Vol. 55, 2005, pp. 109-129

LICHENS OF HIMACHAL PRADESH – I

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Abstract

Thirty species of lichens belonging to the lichen families Candelariaceae, Chrysothricaceae, Cladoniaceae, Hymeneliaceae, Lecanoraceae, Parmeliaceae, Pertusariaceae, Physciaceae, Porpidiaceae, Ramalinaceae, Teloschistaceae and Verrucariaceae are being described and illustrated from Kullu district of Himachal Pradesh.

Key words : Lichens, Himachal Pradesh, Kullu district, floristics.

INTRODUCTION

This paper gives a detailed morphotaxonomical investigations of the thirty species of lichens collected from diverse substrata from different localities of Kullu district which have not been explored for lichens previously. A checklist of lichens of Great Himalayan National Park has been given by Upreti (2000). However no detailed account has been provided by earlier workers from this area.

MATERIALS AND METHODS

The collections were made from different localities of Kullu district (H.P.). The specimens were collected from different substrates like bark of trees, wood, rocks and soil. In all, 92 specimens were collected from different localities at altitudes varying between c 1100–c 2864 m. All the specimens were collected along with part of the substrate by using a knife (for corticolous and terricolous lichens), chisel and hammer (for saxicolous lichens). A hand lens (25X) was used for recording macroscopic characters. The field data recorded for each specimen included : substratum, colour of the specimen, size and shape of the specimen, locality (with approximate altitude in meters), date of collection and name of collector. The specimens were studied morphologically after Prasher *et al.* (2003). The chemical tests were performed after Walker and James (1980, 1985). The collections cited in the text have been deposited in PAN (Herbarium, Department of Botany, Panjab University, Chandigarh).

1. Taxonomy

CANDELARIACEAE

Candelaria concolor (Dicks.) Arn., *Flora* **62**: 364, 1879.

(PLATE 1, Fig. A; PLATE 6, Figs. A-C)

MORPHOLOGY– Thallus corticolous and saxicolous, foliose, yellow to bright yellow in colour, 1-3 cm in diameter, lobed, lobes 2-3 mm wide, crenate; upper surface non-pseudocyphellate, sorediate and isidiate; lower surface pale brown in colour, rhizinate, rhizinae 0.5-0.7 mm long, pale brown, thallus lacinulate, lacinae 0.2-0.4 mm wide; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 30.1-43.0 µm wide, plectenchymatous; algal layer 86.0-107.5 µm wide, algal cells green, circular, 6.4-8.0 µm in diameter, photobiont a green alga; medullary layer 64.5-86.0 µm wide, medulla lax, hyphae septate and branched; lower cortex 51.6-77.4 µm wide. SPOT TESTS /CHEMISTRY- Medulla K-, C-, KC- and P-. SUBSTRATUM- Bark and Rock (Quartzite).

COLLECTION EXAMINED- PAN 28121, on bark of *Quercus semecarpifolia* Sm., in forest, Jagatsukh, c 2000 m, Kullu (H.P.), October 20, 2003, leg. Hem Chander; PAN 28134, on bark of *Quercus* sp., in forest, Kullu, c 1500 m, Kullu (H.P.), October 23, 2003, leg. Hem Chander; PAN 28144, on bare rock (Quartzite), in forest, Chansari, c 1900 m, Kullu (H.P.), October 25, 2003, leg. Hem Chander; PAN 28161, On bark of *Morus alba* Linn., in forest, Kasol, c 2000 m, Kullu (H.P.), October 29, 2003, leg. Hem Chander; PAN 28183, on bark of *Pyrus* sp., in forest, Banjar, c 1600 m, Kullu (H.P.), November 2, 2003, leg. Hem Chander; PAN 28185, on bark of *Prunus* sp., in forest, Sainj, c 1400 m, Kullu (H.P.), November 3, 2003, leg. Hem Chander.

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CHRYSOTHRICACEAE

Chrysothrix chlorina (L.) J.R. Laundon, *Lichenologist* **13**: 110, 1981.

(PLATE 1, Fig. B; PLATE 6, Fig. D)

MORPHOLOGY– Thallus corticolous, leprose, greenish yellow, thick, 5-10 cm in diameter, forming pulverulent mass; apothecia not observed in the specimen examined.

ANATOMY– Thallus ecorticate, algal cells green, circular, 6.4-8.0 µm in diameter, photobiont a green alga; medulla lax, hyphae septate and unbranched.

SPOT TESTS /CHEMISTRY - Medulla K-, C-, KC+ red and P-.SUBSTRATUM- Rock (Bonded Quartzite)

COLLECTION EXAMINED- PAN 28175, on bare rock (Bonded Quartzite), in forest, Bajaura, c 1100 m, Kullu (H.P.), November 1, 2003, leg. Hem Chander.

CLADONIACEAE

Cladonia chlorophaea (Flörke ex Sommerfelt) Sprengel, *Syst. Veg.* **4 (1)**: 272, 1827.

(PLATE 1, Fig. C; PLATE 6, Figs. E-G)

MORPHOLOGY– Thallus terricolous, dimorphic, stratose-radiate-fruticose; primary thallus squamulose, squamules 2-3 mm wide, erect, incised, upper surface greenish grey, sorediate, lower surface white, erhizinate; secondary thallus podatia, cylindrical, 0.7-1.5 cm long, sorediate, greyish, hollow, bearing apothecia, apothecia brown, scyphi goblet shaped, 3-5 mm in diameter and 1-3 mm deep, apothecia lacking at maturity.

ANATOMY– Primary thallus heteromerous, lower cortex absent, upper cortex 43.0-51.6 µm wide, plectenchymatous; algal layer 64.5-77.4 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 86.0-111.8 µm wide, lax, hyphae septate and branched; secondary thallus (podetia) heteromerous, lower cortex absent, upper cortex 12.9-21.5 µm wide, algal layer 86.0-107.5 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 43.0-86.0 µm wide, lax, hyphae septate and branched.

SPOT TESTS /CHEMISTRY- Podetia K-, C-, KC+ red, P- and UV-, fumarprotocetraric acid present.

SUBSTRATUM- Soil.

COLLECTION EXAMINED- PAN 28105, on soil, in forest, Gulaba, c 2864 m, Kullu (H.P.), October 15, 2003, leg. Hem Chander; PAN 28173, on soil, in forest, Thela, c 1500 m, Kullu (H.P.), October 23, 2003, leg. Hem Chander.

Cladonia coniocraea (Flörke) Sprengel, *Syst. Veg.* **4 (1)**: 272, 1827.

(PLATE 1, Fig. D; PLATE 6, Figs. H-J)

MORPHOLOGY– Thallus terricolous and corticolous, dimorphic, stratose-radiate-fruticose; primary thallus squamulose, squamules 2-3 mm wide, erect, incised, upper surface greenish grey, sorediate; lower surface white, erhizinate; secondary thallus podatia, cylindrical, 0.7-1.5 cm long, 1-2 mm in diameter, greyish, pointed at tips, unbranched, curved, sorediate, apothecia not observed in the specimen examined.

ANATOMY– Primary thallus heteromerous, lower cortex absent, upper cortex 30.1-43.0 µm wide, plectenchymatous; algal layer 86.0-107.5 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 86.0-107.5 µm wide, lax, hyphae septate and branched; secondary thallus (podetia) heteromerous, lower cortex absent, upper cortex 17.2-25.0 µm wide, algal layer 64.5-77.4 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 86.0-107.5 µm wide, lax, hyphae septate and branched.

SPOT TESTS /CHEMISTRY- Cortex K+ light yellow, C-, KC-, P+ red and UV-, fumarprotocetraric acid present.

SUBSTRATUM- Bark and Soil.

COLLECTION EXAMINED- PAN 28111, on Bark of *Acer acuminiatum* D. Don, in forest, Kothi, c 2500 m, Kullu (H.P.), October 17, 2003, leg. Hem Chander; PAN 28146, on soil, in forest, Chansari, c 1900 m, Kullu (H.P.), October 25, 2003, leg. Hem Chander.

HYMENELIACEAE

Aspicilia calcarea (L.) Mudd., *Man. Brit. Lich.* :161, 1861.

(PLATE 1, Fig. E; PLATE 6, Fig. K)

MORPHOLOGY– Thallus saxicolous, crustose, pale grey, areolate; thallus fertile with apothecia, apothecia grayish, pruinose, 0.5-1 mm in diameter, flat, blackish; apothecia lacking at maturity.

ANATOMY– Heteromerous, lower cortex absent, upper cortex 30.1-43.0 µm, algal layer 51.6-73.1 µm, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 73.1-94.6 µm,

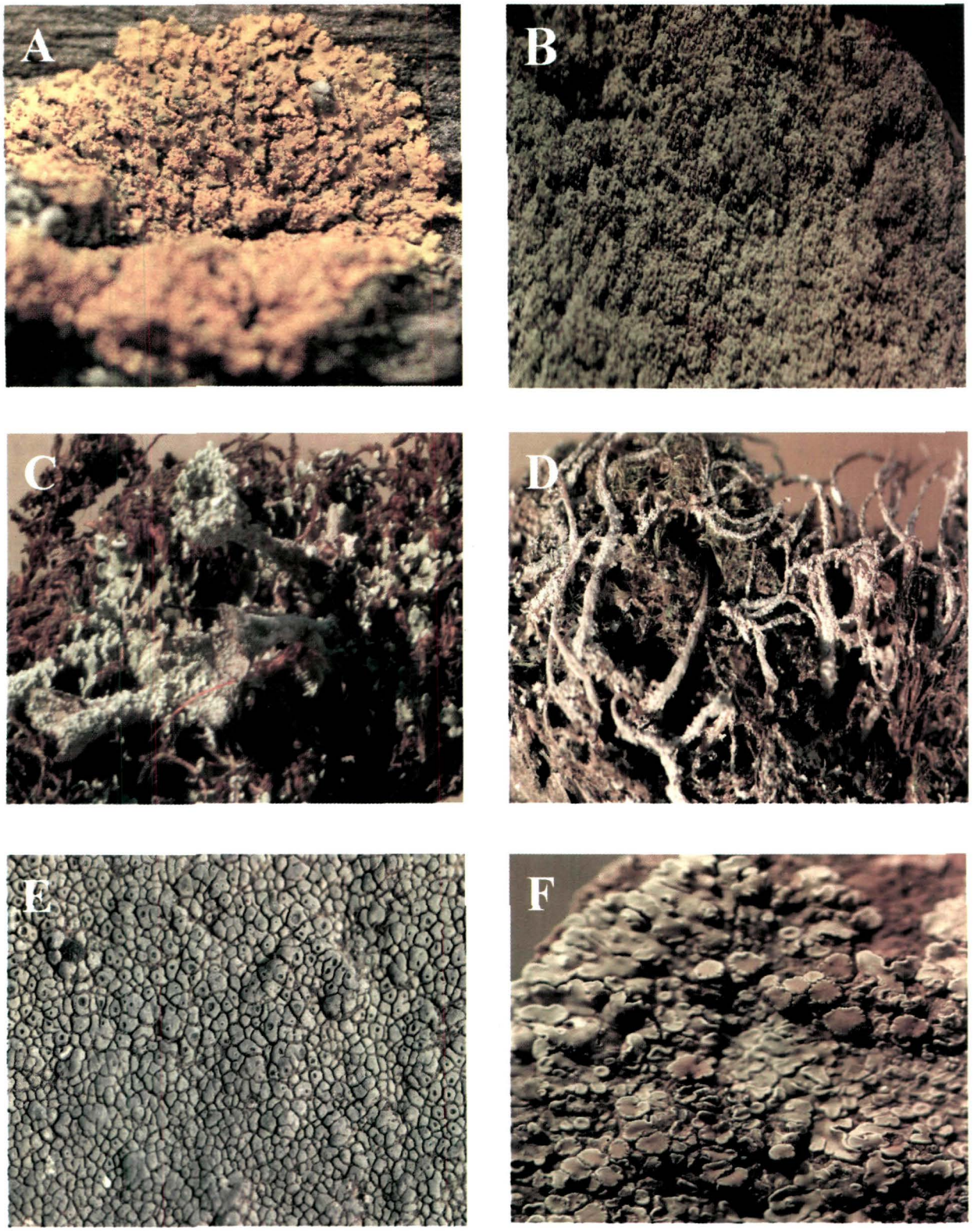

PLATE 1 (Figs. A-F): (A) *Candelaria concolor* (B) *Chrysothrix chlorina* (C) *Cladonia chlorophaea* (D) *Cladonia coniocraea* (E) *Aspicilia calcarea* (F) *Lecanora muralis.*

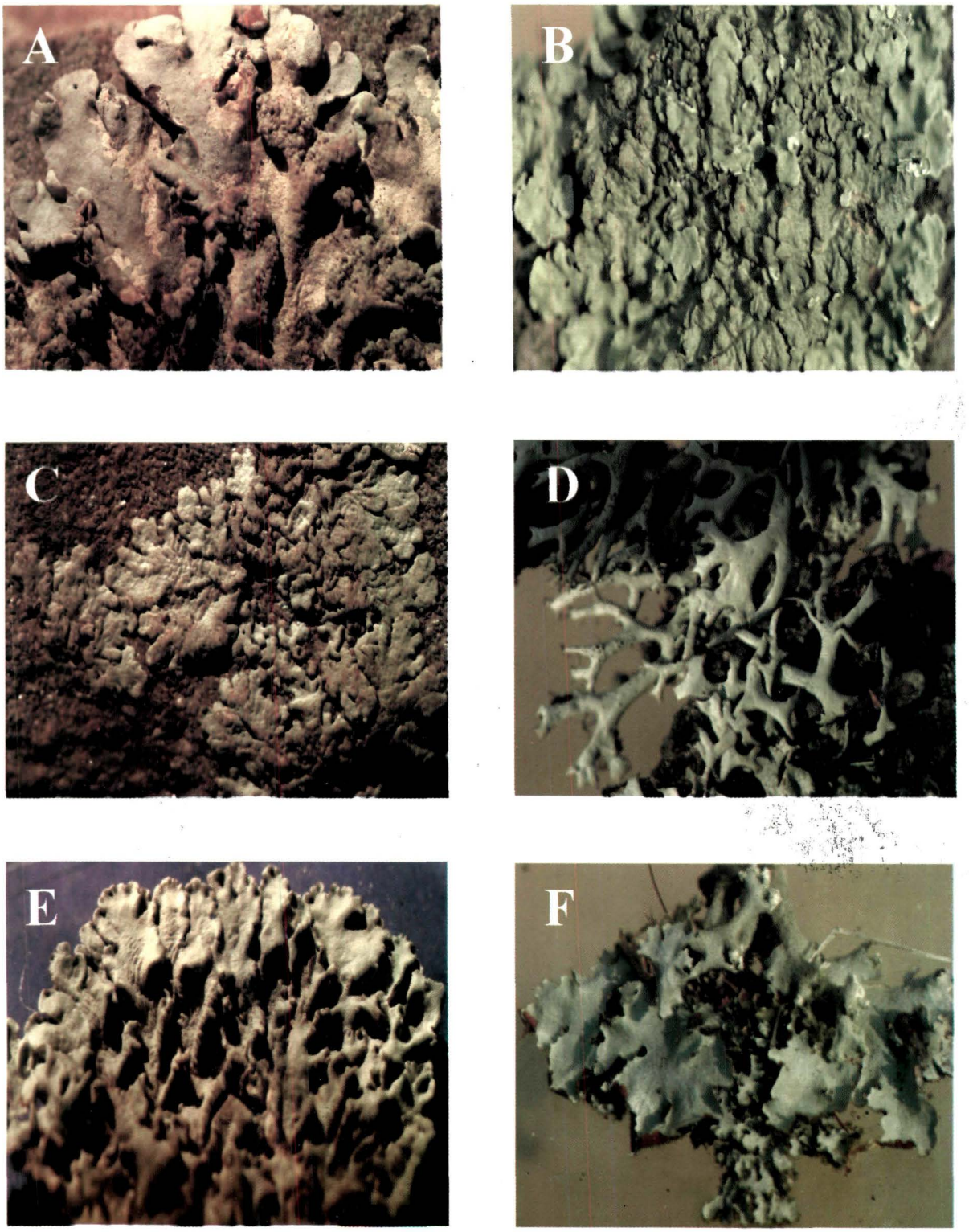

PLATE 2(Figs. A-F): (A) *Canomaculina subsumpta* (B) *Canomaculina subtinctoria* (C) *Canoparmelia texana* (D) *Everniastrum cirrhatum* (E) *Flavoparmelia caperata* (F) *Hypotrachyna radiculata.*

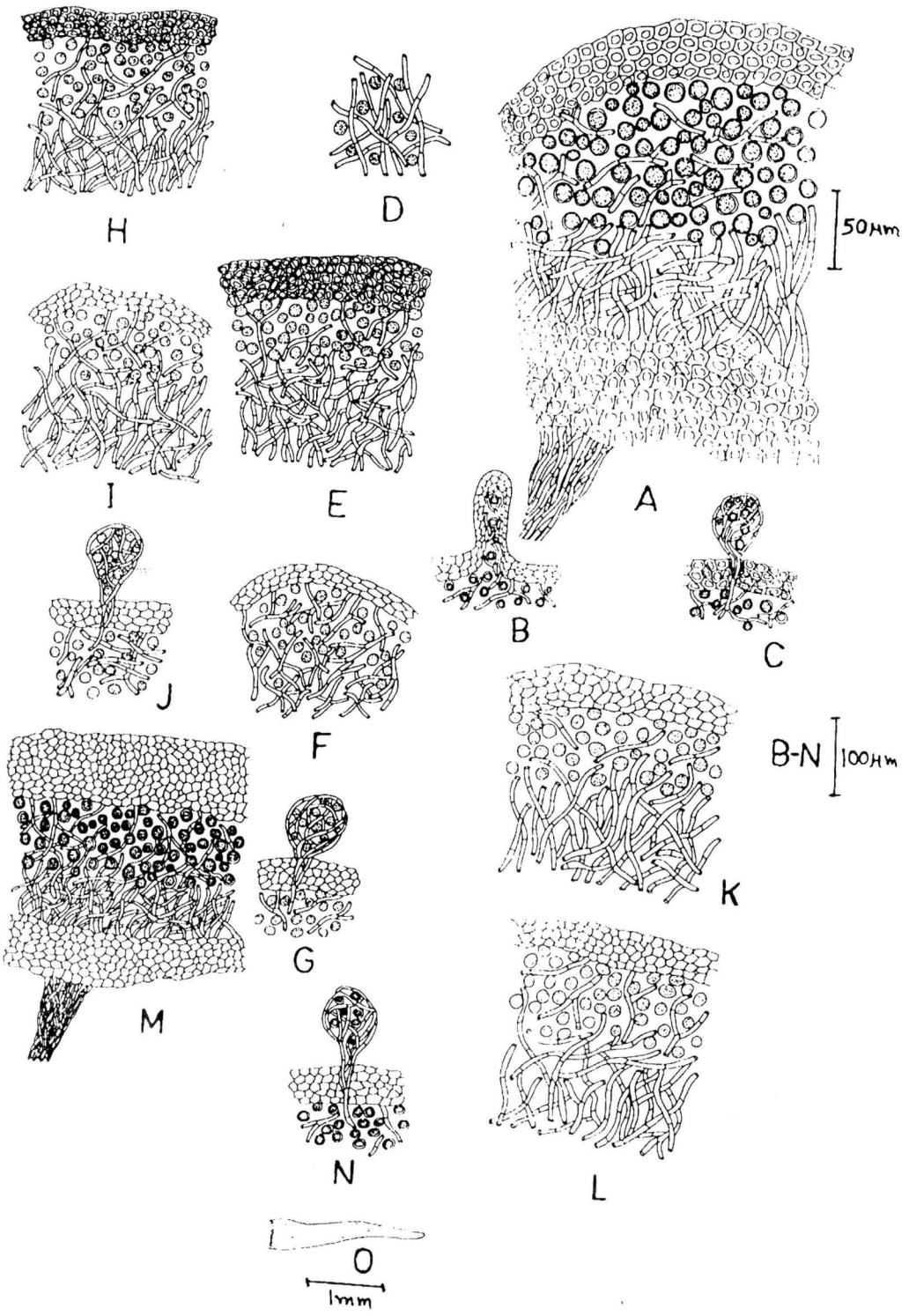

PLATE 6 (Figs. A-O) :*Candelaria concolor* (Dicks.) (A) T. S. of Thallus, (B) A portion of T. S. of Thallus through isidia, (C) A portion of T.S. of Thallus through soredia; ***Chrysothrix candelaris*** (L) J.R. Laundon, (D) T. S. of Thallus; ***Cladonia chlorophaea*** (Flörke ex Sommerfelt) Sprengel (E) T.S. of Squamule. (F) T.S. of Podetia. (G) A portion of T.S. of Thallus through soredia ***Cladonia coniocraea*** (Flörke) Sprengel (H) T.S. of Squamule, (I) T.S. of Podetia, (J) A portion of T.S. of Thallus through soredia; ***Aspicilia calcarea*** (L.) Mudd. (K) T.S. of Thallus, ***Lecanora muralis*** (Müll. Arg) Poelt.(L) T.S. of Thallus; ***Canomaculina subsumpta*** (Nyl.) Elix (M) T.S. of Thallus,(N) A portion of T.S. of Thallus through soredia,(O) W.M. of cilia.

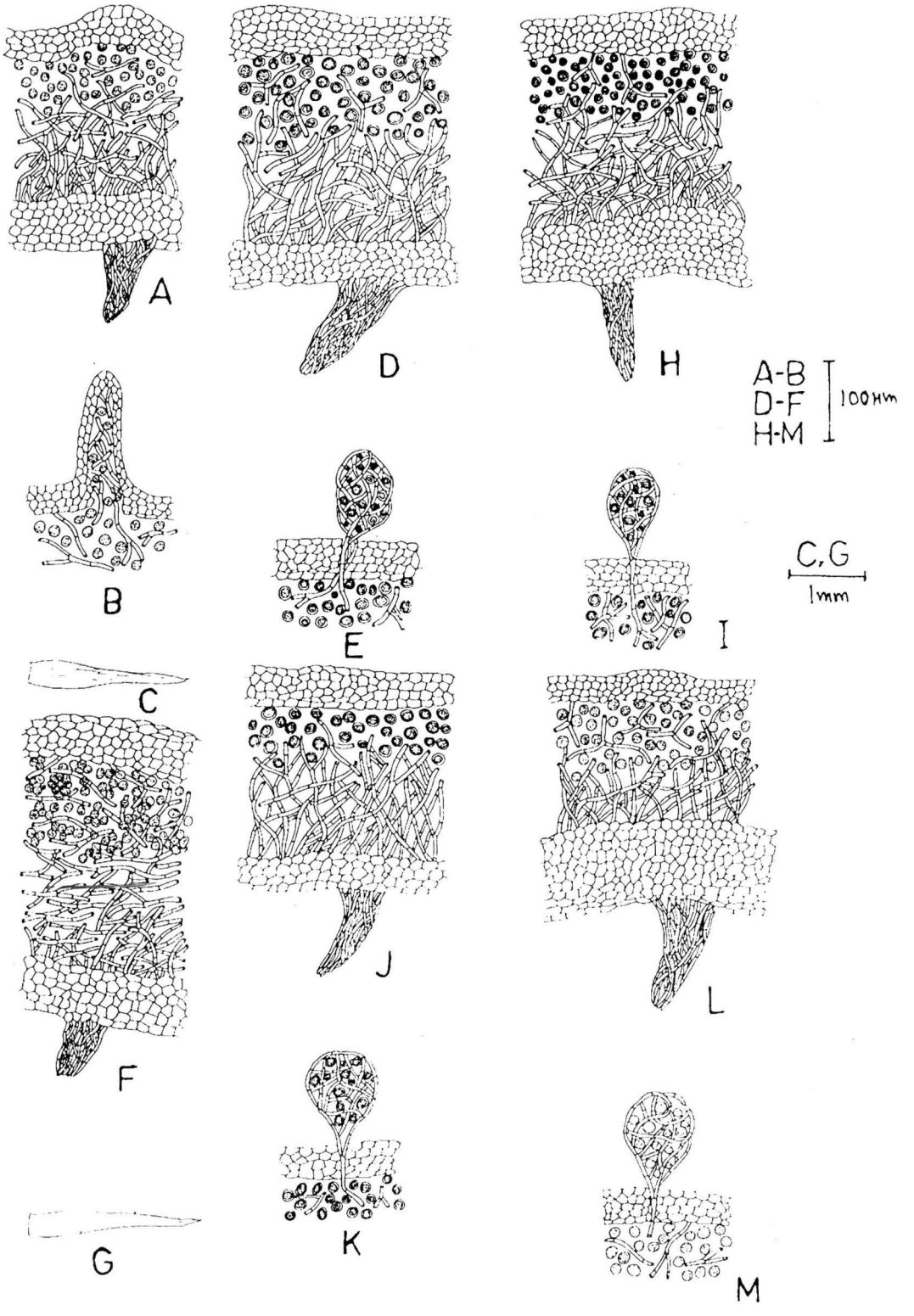

PLATE 7 (Figs. A-M): ***Canomaculina subtinctoria*** (Zahlbr.) Elix and Hale (A) T.S. of Thallus, (B) A portion of T.S. of Thallus through isidia, (C) W.M. of cilia; ***Canoparmelia texana*** (Tuck.) Elix and Hale (D) T.S. of Thallus, (E) A portion of T.S. of Thallus through soredia; (F) T.S. of Thallus, (G) W.M. of cilia; ***Flavoparmelia caperata*** (L.) Hale(H) T.S. of Thallus, (I) A portion of T.S. of Thallus through soredia; (J) T.S. of Thallus, (K) A portion of T.S. of Thallus through soredia; ***Parmotrema austrosinense*** (Zahlbr.) Hale - (L) T.S. of Thallus; (M) A portion of T.S. of Thallus through soredia.

composed of septate, unbranched and loosely interwoven hyphae.

SPOT TESTS /CHEMISTRY- Medulla K-, C-, KC-, P-, aspicilin present.

SUBSTRATUM- Rock (Mycaschist and Schist).

COLLECTION EXAMINED- PAN 28128, on bare rock (Mycaschist), in forest, Patalikuhl, c 1600 m, Kullu (H.P.), October 22, 2003, leg. Hem Chander; PAN 28129, on bare rock (Schist), in forest, Patalikuhl, c 1600 m, Kullu (H.P.), October 22, 2003, leg. Hem Chander.

LECANORACEAE

Lecanora muralis (Müll. Arg) Poelt, *Mitt. Bot. Staatssamml. München*, **2**: 499, 1958.

(PLATE 1 Fig. F; PLATE 6, Fig. L)

MORPHOLOGY– Thallus saxicolous, squamulose, rossette, yellow brown with reticulate cracks, lobulate at periphery, convex, eciliate, lobes radiating at periphery, peripheral lobes longer than broad; upper surface non-isidiate and non-sorediate; apothecia lacking at maturity.

ANATOMY– Thallus heteromerous, lower cortex absent, upper cortex 30.1-38.7 µm wide, plectenchymatous; algal layer 64.8-86.0 µm, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 77.4-98.9 µm wide, lax, hyphae septate and branched.

SPOT TESTS /CHEMISTRY - Medulla K-, C-, KC-and P-. SUBSTRATUM- Rock (Mycaschist).

COLLECTION EXAMINED- PAN 28179, on bare rock (Mycaschist), in forest, Banjar, c 1600 m, Kullu (H.P.), November 3, 2003, leg. Hem Chander.

PARMELIACEAE

Canomaculina subsumpta (Nyl.) Elix, *Mycotaxon* **29**: 239, 1987.

(PLATE 2, Fig. A; PLATE 6, Figs. M-O)

MORPHOLOGY– Thallus saxicolous and corticolous, foliose, greenish grey, 8-12 cm in diameter, loosely adnate to the substratum, irregularly lobed, lobes rotund, 10-14 mm wide, margins crenate, ciliate, simple cilia present in the dents, cilia 1-2 mm long; upper surface white, maculate, maculae effigurate, rough, non-isidiate, sorediate; lower surface pale brown at margins and dark brown in the center, rhizines simple, brown, 0.5-1.0mm long, restricted in the central part resulting in broad bare marginal zone; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 68.0-86.0 µm wide, plectenchymatous; algal layer 81.7-98.9 µm wide, algal cells green, circular, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 51.6-73.1 µm wide, lax, hyphae septate and branched; lower cortex 43.0-64.0 µm wide.

SPOT TESTS /CHEMISTRY - Cortex K+ yellow, medulla K+ yellow - brownish red, C-, KC+ yellow-orange, P+ orange-red, salazinic acid and usnic acid present.

SUBSTRATUM- Bark and Rock (Schistose quartzite and Quartzite).

COLLECTION EXAMINED- PAN 28117, on bark of *Quercus* sp., in forest, Manali, c 2050 m, Kullu (H.P.), October 19, 2003, leg. Hem Chander; PAN 28170, on bare rock (Schistose quartzite), in forest, Thela, c 1500 m, Kullu (H.P.), October 31, 2003, leg. Hem Chander; PAN 28176, on bare rock (Quartzite), Bajaura, c 1100 m, Kullu (H.P.), November 2, 2003, leg. Hem Chander.

Canomaculina subtinctoria (Zahlbr.) Elix and Hale, *Mycotaxon* **55**: 447, 1997

(PLATE 2, Fig. B; PLATE 7, Figs. A-C)

MORPHOLOGY– Thallus terricolous and corticolous, foliose, brownish grey, 6-10 cm in diameter, loosely adnate to the substratum, irregularly branched, lobed, lobes rotund, 10-14 mm wide, margins dentate, ciliate, simple cilia present in the dents, cilia 1.5-2 mm long; upper surface white, maculate, maculae effigurate, non-sorediate, non-pseudocyphellate, isidiate, isidia simple, laminal, filiform, dense in the central part, medulla rust coloured; lower surface pale brown at margins and dark brown in the center, rhizinate, rhizines simple, brown, 0.5-1.0 mm long, restricted in the central part resulting in broad bare marginal zone; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 25.8-43.0 µm wide, plectenchymatous; algal layer 55.9-77.4 µm wide, algal cells green, circular, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 86.0-111.8 µm wide, lax, hyphae septate and branched; lower cortex 51.6-64.5 µm wide.

SPOT TESTS /CHEMISTRY- Cortex K+ yellow, medulla K+ yellow - orange, C-, KC-, P+ orange, atranorin and salazinic acid present.

SUBSTRATUM- Bark and soil.

COLLECTION EXAMINED- PAN 28110, on bark of *Acer caesium* Brandis, in forest, Kothi, c 2500 m, Kullu (H.P.), October 17, 2003, leg. Hem Chander; PAN 28116, on bark of *Acer caesium* Brandis, in forest, Solang, c 2200 m, Kullu (H.P.), October 18, 2003, leg. Hem Chander; PAN 28126, on rock soil, in forest, Naggar, c 1760 m, Kullu (H.P.), October 21, 2003, leg. Hem Chander; PAN 28131, on rock soil, in forest, Patalikuhl, c 1600 m, Kullu (H.P.), October 22, 2003, leg. Hem Chander; PAN 28138, on bark of *Cedrus deodara* G. Don, in forest, Ram Shilla, c 1500 m, Kullu (H.P.), October 24, 2003, leg. Hem Chander; PAN 28140, on bark of *Cedrus deodara* G. Don, in forest, Ram Shilla, c 1500 m, Kullu (H.P.), October 24, 2003, leg. Hem Chander; PAN 28160, on bark of *Morus* sp., in forest, Bhunter, c 1500 m, Kullu (H.P.), October 28, 2003, leg. Hem Chander; PAN 28168, on bark of *Prunus* sp., in forest, Manikaran, c 2000 m, Kullu (H.P.), October 2, 2003, leg. Hem Chander; PAN 28172, on bark of *Cedrus deodara* G. Don, in forest, Thela, c 1500 m, Kullu (H.P.), October 24, 2003, leg. Hem Chander.

Canoparmelia texana (Tuck.) Elix and Hale, *Mycotaxon* **27**: 279, 1986.

(PLATE 2, Fig. C; PLATE 7, Figs. D-E)

MORPHOLOGY– Thallus saxicolous, foliose, yellowish grey, 6-10 cm in diameter, loosely adnate to the substratum, irregularly lobed, lobes almost linear, 2-3 mm wide, compact and closely packed in central part but discrete at periphey, crenate – dentate apically, eciliate; upper surface lacunose, non-pseudocyphellate, non-isidiate, profusely pustulate– sorediate, soralia laminal to nearly marginal protruding out from verruca like protruberances, soralia ash grey; lower surface black, sparsely rhizinate, rhizines thick, black, 0.5-2.0 mm long, reach upto margins; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 43.0-51.6 µm wide, plectenchymatous; algal layer 73.1-94.6 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 107.5-129.0 µm wide, lax, hyphae septate and branched; lower cortex 43.0-55.9 µm wide.

SPOT TESTS /CHEMISTRY- Cortex K+ yellow, medulla K-, C-, KC+ red, P, atranorin and divaricatic acids present.

SUBSTRATUM- Rock (Schist).

COLLECTION EXAMINED- PAN 28171, on bare rock, in forest, Thela, c 1500 m, Kullu (H.P.), October 31, 2003, leg. Hem Chander.

Everniastrum cirrhatum (E. Fr.) Hale ex Sipman, *Mycotaxon* **26**: 239, 1986.

(PLATE 2, Fig D; PLATE 7, Figs. F-G)

MORPHOLOGY– Thallus corticolous, foliose, grey coloured, 3-5 cm in diameter, loosely adnate to the substratum, slightly erect, thick, coriaceous, thallus lacinate, 3.5 mm wide, lacinae divaricate, canaliculate, apically tapering, lobed, lobe margins revolute; upper surface smooth, non-pseudocyphellate, non-sorediate, non-isidiate, margins ciliate, cilia 1-2 mm long, brown; lower surface, rhizinate, rhizines simple, brown, 0.5-1.0 mm long, irregularly scattered; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 38.7-55.9 µm wide, plectenchymatous; algal layer 77.4-120.4 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 111.8-129.0 µm wide, lax, hyphae septate and branched; lower cortex 55.9-64.5 µm wide.

SPOT TESTS /CHEMISTRY- Cortex K+ yellow, medulla K+ yellow – brownish red, C-, KC-, P+ orange, salazinic acid and protolichesterinic acid present.

SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28118, on bark of *Quercus semecarpifolia* sm., in forest, Manali, c 2050 m, Kullu (H.P.), October 19, 2003, leg. Hem Chander.

Flavoparmelia caperata (L.) Hale, *Mycotaxon* **25**: 604, 1986.

(PLATE 2, Fig. E; PLATE 7, Figs. H-I)

MORPHOLOGY– Thallus corticolous and saxicolous, foliose, greenish yellow, 7-12 cm in diameter, closely adnate to the substratum, thick, coriarios, lobed, lobes elongate, ascending, confluent, roughly crenate, 7-12 mm wide, apices nearly rotund, margins entire to crenate, eciliate; upper surface rough, faintly maculate, longitudinaly plicate at the margins, rugose in the center, smooth at periphery, non-isidiate, non-pseudocyphellate, pustulate and sorediate, soralia laminal to marginal, capitate, sorediate, soralia granular; lower surface pale yellow, smooth, rhizinate papillae sparsely distributed throughout leaving a norrow 2-3 mm wide brownish erhizinate zone, rhizinae simple, brown, 1-1.5 mm long; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 34.4-47.3 µm wide, plectenchymatous; algal layer 60.2-73.1 µm wide, algal cells green, spherical,

6.4-8.0 µm in diameter, photobiont a green alga; medulla 107.5-124.7 µm wide, lax, hyphae septate and branched; lower cortex 60.2-73.1 µm wide.

SPOT TESTS /CHEMISTRY- Cortex K-, medulla K-, C-, KC+ rose red, P+ orange-red, usnic, protocetraric and caperatic acids present.

SUBSTRATUM- Bark and Rock (High grade mycaschist and Schistose quartzite).

COLLECTION EXAMINED- PAN 28156, on bark of *Pinus smithiana* Boss, in forest, Pahnullah valley, c 1600 m, Kullu (H.P.), October 27, 2003, leg. Hem Chander; PAN 28158, on bare rock (High grade mycaschist), in forest, Bhunter, c 1500 m, Kullu (H.P.), October 28, 2003, leg. Hem Chander; PAN 28174, on bare rock (Schistose quartzite), in forest, Thela, c 1500 m, Kullu (H.P.), October 27, 2003, leg. Hem Chander.

Hypotrachyna radiculata (Kurok.) Elix, *Australian Lichenology* **48**: 16, 2001.

(PLATE 2, Fig. F; PLATE 7, Figs. J-K)

MORPHOLOGY– Thallus terricolous and corticolous, foliose, greyish, 5-7 cm in diameter, lobed, lobes crenate, 0.5-10.0 mm wide, curved, margins eciliate; upper surface smooth, non-pseudocyphellate, non-isidiate, sorediate along margins, soralia greyish white, capitate, globose; lower surface pale brown, rhizinate, rhizinae brown, 0.5-1.0 mm long, dichotomously branched; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 43.0-51.6 µm wide, plectenchymatous; algal layer 43.0-64.5 µm wide, algal cells green, sherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 103.2-107.0 µm wide, lax, hyphae septate and branched; lower cortex 34.4-43.0 µm wide.

SPOT TESTS /CHEMISTRY- Cortex K-, medulla K-, C-, KC-, P+ orange-red, usnic and protocetraric acids present.

SUBSTRATUM- Soil and Bark.

COLLECTION EXAMINED- PAN 28154, on soil, in forest, Pahnullah valley, c 1600 m, Kullu (H.P.), October 27, 2003, leg. Hem Chander; PAN 28155, on bark of *Pinus roxburghii* Sarg., in forest, Pahnullah Valley, c 1600 m, Kullu (H.P.), October 27, 2003, leg. Hem Chander.

Parmotrema austrosinense (Zahlbr.) Hale, *Phytologia* **28**: 335, 1974.

(PLATE 3, Fig. A; PLATE 7, Figs. L-M)

MORPHOLOGY– Thallus corticolous, foliose, greenish grey, 5-10 cm in diameter, loosely adnate to the substratum, lobed, lobes rotund, eciliate, 0.5-10.0 mm wide, foamy, soft to touch; upper surface smooth, emaculate, lobe margins ascending, imbricate, sinuous, non-pseudocyphellate, sorediate, soralia marginal, soredia white, farinose, linear, wavy, non-isidiate non pustulate; lower surface brown, minutely reticulate, rugose, rhizinate, rhizinae brown, 0.5-1.0 mm long, sparse, simple, characteristically restricted to central part, marginal zone erhizinate, white mottled ; apothecia not observed in the specimen examined.
ANATOMY– Thallus heteromerous, corticate, upper cortex 21.5-55.9 µm wide, plectenchymatous; algal layer 60.2-86.0 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 55.9-68.8 µm wide, lax, hyphae septate and branched; lower cortex 86.0-98.8 µm wide.

SPOT TESTS /CHEMISTRY- Cortex K+ yellow, medulla K-, C+ red, KC+ red, P-, atranorin and lecanoric acid present.

SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28159, on bark of *Morus alba* Linn., in forest, Bhunter, c 1500 m, Kullu (H.P.), October 28, 2003, leg. Hem Chander; PAN 28165, on bark of *Prunus* sp., in forest, Manikaran, c 2000 m, Kullu (H.P.), October 30, 2003, leg. Hem Chander; PAN 28182, on bark of *Pyrus* sp., in forest, Banjar, c 1600 m, Kullu (H.P.), November 2, 2003, leg. Hem Chander.

Punctelia borreri (Sm.) Krog, *Nordic J. Bot.* **2**: 291, 1982.

(PLATE 3, Fig. B; PLATE 8, Figs. A-B)

MORPHOLOGY– Thallus corticolous, foliose, greyish, 4-8 cm in diameter, loosely adnate to the substratum, radialy lacinate giving a bluish tinge, lobed, lobes 5-10 mm wide, ascending, imbricate rotund, lobe margins crenate to dentate, eciliate; upper surface rough, pseudocyphellate, pseudocyphellae white, distinct at marginal area, punctiform, non-isidiate, sorediate, soredia capitate, soralia both laminal and marginal, laminal soralia confluent, marginal sorelia sinuous, pseudocyphellae continguos, coalescent and form long eroded patches; lower surface black, rhizinate, rhizinae pale brown, simple, 0.5-1.0 mm, forked towards tips, marginal area pale brown, papillate; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 25.8-38.7 µm wide, plectenchymatous; algal layer 64.5-94.6 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 64.5-81.7 µm wide, lax, hyphae septate and branched; lower cortex 68.8-86.0 µm wide.

SPOT TESTS /CHEMISTRY- Cortex K+ yellow, medulla K-, C+ red, KC+ red, P-, atranorin and gyrophoric acids present.

SUBSTRATUM- Bark and Rock (Quartzite).

COLLECTION EXAMINED- PAN 28149, on bark of *Pyrus* sp., in forest, Kasol, c 2000 m, Kullu (H.P.), October 29, 2003, leg. Hem Chander; PAN 28164, on bark of *Prunus* sp., in forest, Manikaran, c 2000 m, Kullu (H.P.), October 30, 2003, leg. Hem Chander; PAN 28167, on bark of *Pinus wallichiana* A.B. Jacks, in forest, Manikaran, c 2000 m, Kullu (H.P.), October 30, 2003, leg. Hem Chander; PAN 28169, on bark of *Cassia tora* Linn., in forest, Banjar, c 1600 m, Kullu (H.P.), November 2, 2003, leg. Hem Chander; PAN 28180, on bark of *Prunus* sp., in forest, Banjar, c 1600 m, Kullu (H.P.), November 2, 2003, leg. Hem Chander; PAN 28184, on bark of *Prunus* sp., in forest, Sainj, c 1400 m, Kullu (H.P.), November 3, 2003, leg. Hem Chander.

Punctelia ruducta (Ach.) Krog, *Nordic J. Bot.* **2**: 291, 1982.

(PLATE 3, Fig. C; PLATE 8, Figs. C-D)

MORPHOLOGY– Thallus corticolous, foliose, greyish, 7-10 cm in diameter, loosely adnate to the substratum, crisp and fragile, lobed, lobes 5-10 mm wide, roughly rotund, lobe margins crenate, eciliate; upper surface rough, reticulately rugose near the lobe margins, isidiate, pseudocyphellate, pseudocyphellae white, punctiform, non-sorediate, isidiate, isidia laminal, simple, coralloid – flattened lacinulate, 0.3 x 0.8 mm in size; lower surface pale brown, densely rhizinate, rhizinae brown, simple, slender, 0.5-1.0 mm; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 51.6-68.8 µm wide, plectenchymatous; algal layer 77.4-98.9 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 86.0-103.2 µm wide, white, lax, hyphae septate and branched; lower cortex 55.9-129.0 µm wide.

SPOT TESTS /CHEMISTRY - Cortex K-, medulla K-, C+ red, KC+ red, P-, atranorin and lecanoric acids present.

SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28137, on bark of *Cedrus deodara* G. Don., in forest, Ram Shilla, c 1500 m, Kullu (H.P.), October 24, 2003, leg. Hem Chander.

Punctelia subrudecta (Nyl.) Krog, *Nordic J. Bot.* **2**: 291, 1982.

(PLATE 3, Fig. D; PLATE 8, Figs. E-F)

MORPHOLOGY– Thallus corticolous and terricolous, foliose, greyish, 4-10 cm in diameter, loosely adnate to the substratum, crisp and fragile, lobed, lobes 5-10 mm wide, linear - rotund, lobe margins crenate - dentate, eciliate; upper surface lacunose, non-isidiate, pseudocyphellate, pseudocyphellae white, punctiform, sorediate, soralia marginal and laminal, confluent; lower surface pale brown, rhizinate, rhizinae brown, simple, 0.5-1.0 mm long, marginal area erhizinate; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 43.0-60.2 µm wide, plectenchymatous; algal layer 129.0-146.2 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 129.0-137.6 µm wide, white, lax, hyphae septate and branched; lower cortex 47.3-55.9 µm wide.

SPOT TESTS /CHEMISTRY– Cortex K+ yelow, Medulla K-, C+ blood red, KC+ red, P-, atranorin and lecanoric acid and protolichesterinic acid present.

SUBSTRATUM- Bark and Soil.

COLLECTION EXAMINED- PAN 28136, on bark of *Cedrus deodara* G. Don., in forest, Kullu, c 1500 m, Kullu (H.P.), October 23, 2003, leg. Hem Chander; PAN 28139, on bark of *Cedrus deodara* G. Don., in forest, Ram Shilla, c 1500 m, Kullu (H.P.), October 24, 2003, leg. Hem Chander; PAN 28141, on bark of *Cedrus deodara* G. Don., in forest, Ram Shilla, c 1500 m, Kullu (H.P.), October 24, 2003, leg. Hem Chander; PAN 28148, on bark of *Picea smithiana* Boss., in forest, Lag Valley, c 1500 m, Kullu (H.P.), October 26, 2003, leg. Hem Chander; PAN 28152, on soil, in forest, Pah nullah, c 1600 m, Kullu (H.P.), October 27, 2003, leg. Hem Chander; PAN 28157, on bark of *Pinus roxburghii* Sarg., in forest, Bhunter, 1500 m, Kullu (H.P.), October 28, 2003, leg. Hem Chander; PAN 28186, on bark of *Prunus* sp., in forest, Sainj, 1400 m, Kullu (H.P.), November 3, 2003, leg. Hem Chander.

Rimelia reticulata (Taylor) Hale and Fletcher, *Bryologist* **93**: 28, 1990.

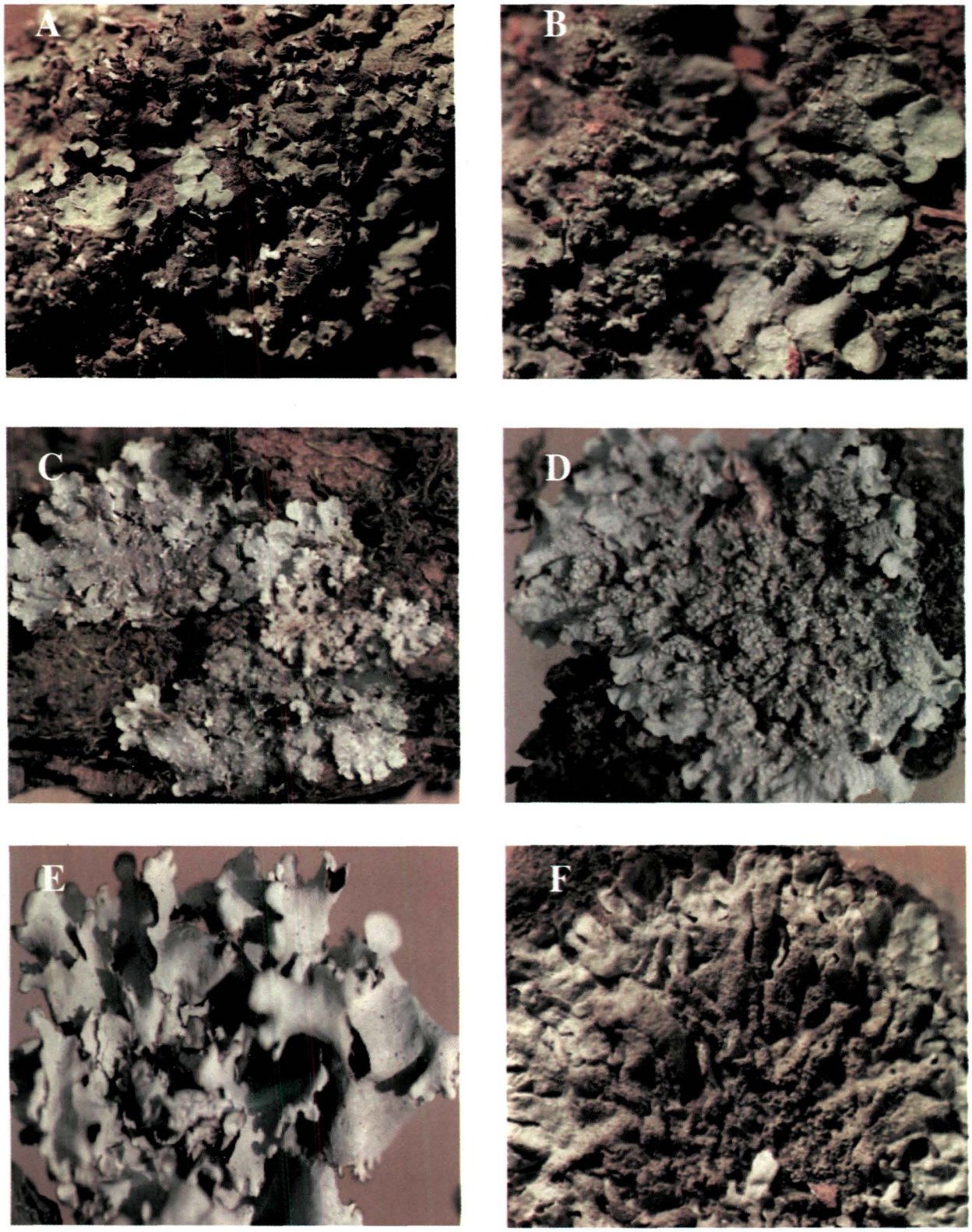

PLATE 3 (Figs. A-F): (A) *Parmotrema austrosinense* (B) *Punctelia borreri* (C) *Punctelia ruducta* (D) *Punctelia subrudecta* (D) *Punctelia subrudecta* (E) *Rimelia reticulata* (F) *Xanthoparmelia tinctina.*

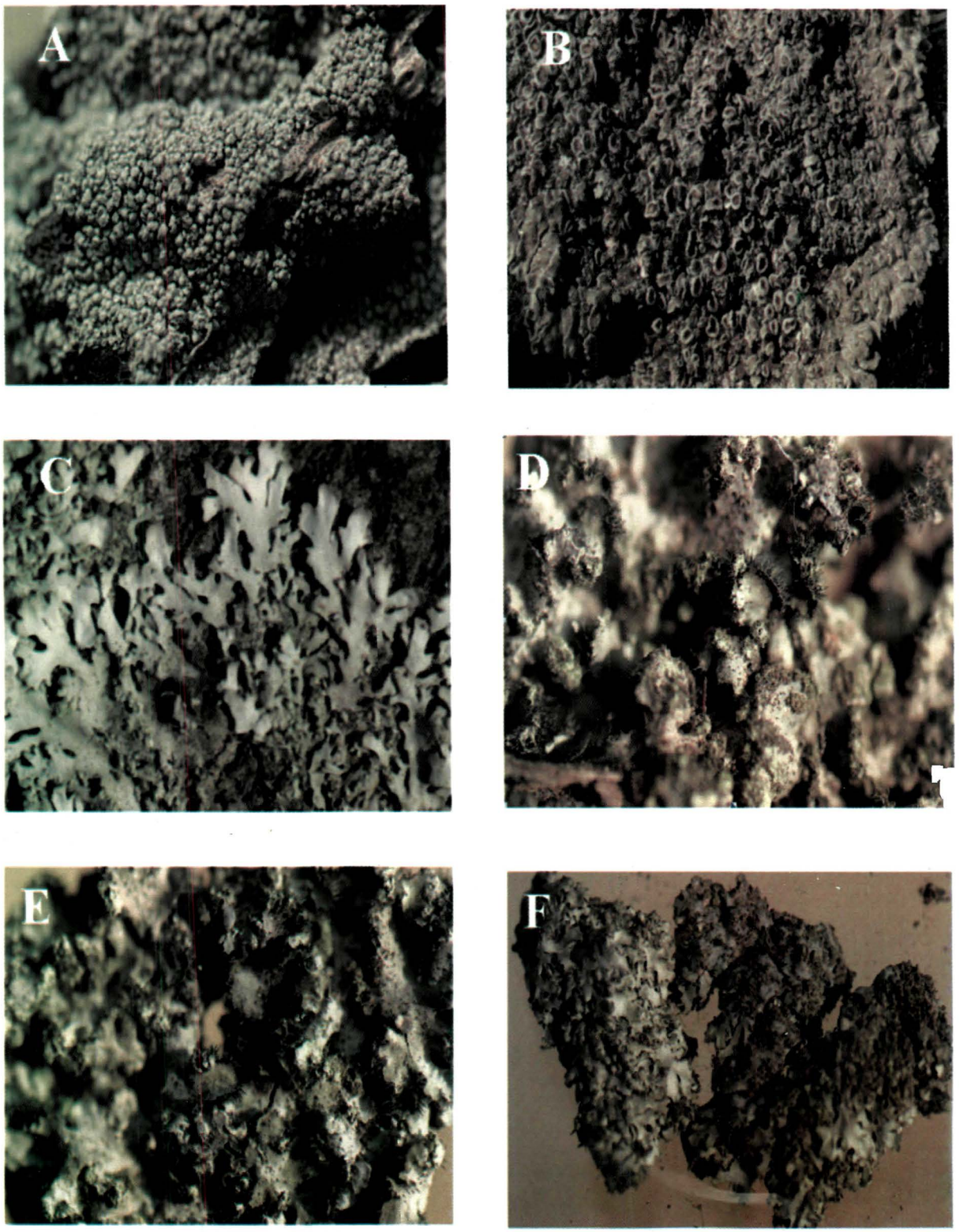

PLATE 4 (Figs. A-F): (A) *Pertusaria leucosorodes* (B) *Dirinaria applanata* (C) *Heterodermia leucomela* (D) *Phaeophyscia hispidula* (E) *Phaeophyscia orbicularis* (F) *Phaeophyscia pyrrhophora.*

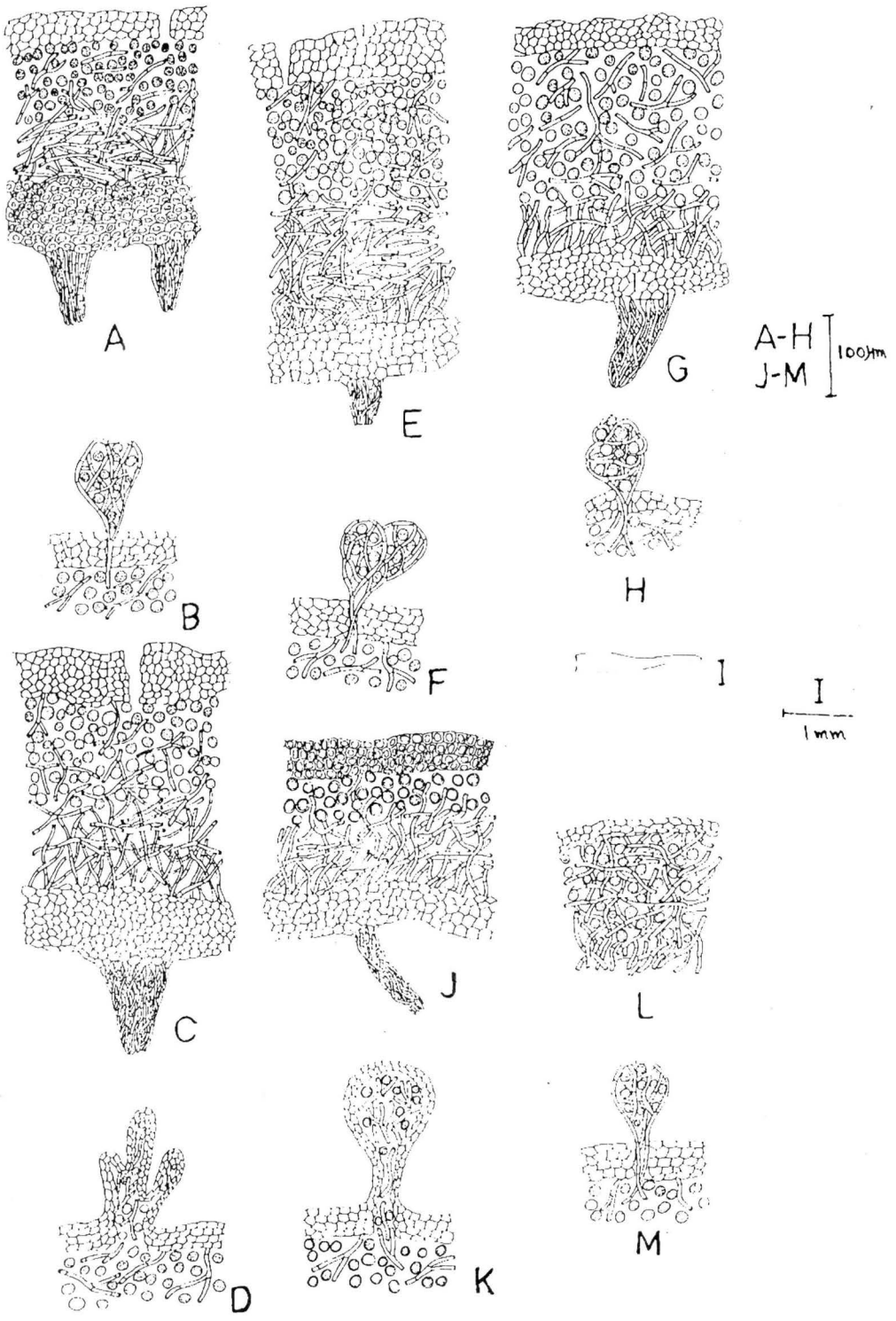

PLATE 8 (Figs. A-M): ***Punctelia borreri*** (Sm.) Krog (A) T.S. of Thallus, (B) A portion of T.S. of Thallus through soredia; ***Punctelia ruducta*** (Ach.) Krog (C) T.S. of Thallus, (D) A portion of T.S. of Thallus through isidia; ***Punctelia subrudecta*** (Nyl.) Krog(E) T.S. of Thallus, (F) A portion of T.S. of Thallus through soredia; ***Rimelia reticulata*** (Taylor) Hale and Fletcher, (G) T.S. of Thallus, (H) A portion of T.S. of Thallus through soredia, (I) W.M. of cilia; ***Xanthoparmelia tinctina*** (Mahe and Gill) Hale(J) T.S. of Thallus, (K) A portion of T.S. of Thallus through isidia; ***Pertusaria leucosorodes*** Nyl. (L) T.S. of Thallus, (M) A portion of T.S. of Thallus through soredia.

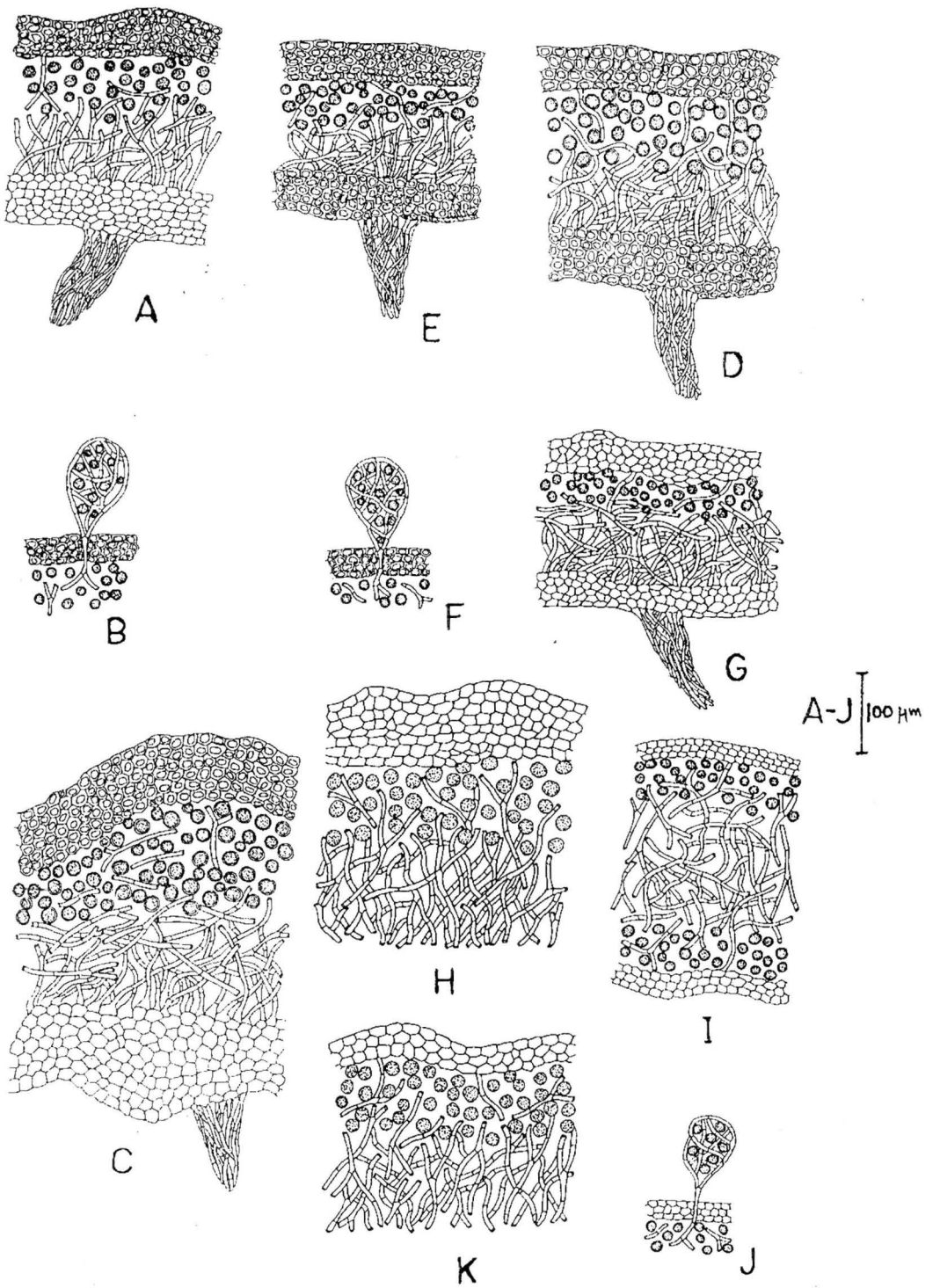

PLATE 9 (Figs. A-K) : ***Dirinaria applanata*** (Fée) Awasthi (A) T.S. of Thallus, (B) A portion of T.S. of Thallus through soredia; ***Heterodermia leucomela*** (L.) Poelt (C) T.S. of Thallus; ***Phaeophyscia hispidula*** (Ach.) Moberg (D) T.S. of Thallus; ***Phaeophyscia orbicularis*** (Necker) Moberg (E) T.S. of Thallus,(F) A portion of T.S. of Thallus through soredia; ***Phaeophyscia pyrrhophora*** (Poelt) Awasthi and Joshi(G) T.S. of Thallus; ***Porpidia albocaerulescens*** (Wulfen) Hertel and Knoph (H) T.S. of Thallus; ***Ramalina roesleri*** (Hochst. In Schaerer) Hue (I) T.S. of Thallus, (J) A portion of T.S. of Thallus through soredia; ***Ramalina sinensis*** Jatta (K) T.S. of Thallus.

(PLATE 3, Fig. E; PLATE 8, Figs. G-I)

MORPHOLOGY– Thallus corticolous, foliose, greyish, 8-12 cm in diameter, loosely adnate to the substratum, dichotomously branched, lobed, lobes 1-15 mm wide, lobe margins wavy, raised, indented, ciliate, lobe apices down turned; upper surface rough, non isidiate, non-pseudocyphellate, sorediate, soralia marginal, common on marginal involute lacinae, erose, irregular, capitate, upper surface maculate, maculae forming reticulate cracks; lower surface pale brown, rhizinate, rhizinae brown, simple, 0.5-1.0 mm long, present through out; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 25.8-34.4 µm wide, plectenchymatous; algal layer 159.1-172.0 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 34.4-55.9 µm wide, white, lax, hyphae septate and branched; lower cortex 25.8-68.8 µm wide.

SPOT TESTS /CHEMISTRY– Cortex K+ yellow, medulla K+ yellow - red, C-, KC+ orange - red, P+ orange, UV-, salazinic acid, chloratranorin, atranorin and consalazinic acid present.

SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28145, on bark of *Cedrus deodara* G. Don., in forest, Chansari, c 1900 m, Kullu (H.P.), October 25, 2003, leg. Hem Chander.

Xanthoparmelia tinctina (Mahe and Gill) Hale, *Phytologia* **28**: 489, 1974.

(PLATE 3, Fig. F; PLATE 8, Figs. J-K)

MORPHOLOGY– Thallus saxicolous, foliose, greenish grey, 4-8 cm in diameter, loosely adnate to the substratum, lobed, lobes 2-5 mm wide, separate to imbricate, flat to strongly convoluted, irregular to linear, lobe apices incised, round, sub ascending, eciliate, lobe margins black rimmed; upper surface smooth, rugose, non-pseudocyphellate, non-sorediate, isidiate, isidia globular, simple, inflated, pale tipped; lower surface black, canaliculate, smooth to rugose, rhizinate, rhizinae black, simple, 0.5-1.0 mm long; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 34.4-43.0 µm wide, plectenchymatous; algal layer 38.7-51.6 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 77.4-98.9 µm wide, white, lax, hyphae septate and branched; lower cortex 43.0-55.9 µm wide.

SPOT TESTS /CHEMISTRY– Cortex K-, medulla K+ yellow - red, C-, KC-, P+ orange, usnic acid, salazinic acid and consalazinic acids present.

SUBSTRATUM- Rock (Mycaschist).

COLLECTION EXAMINED- PAN 28178, on bark rock, in forest, Bajaura, c 1100 m, Kullu (H.P.), November 1, 2003, leg. Hem Chander.

PERTUSARIACEAE

Pertusaria leucosorodes Nyl., *Acta Soc. Sci. Fenn.* **26 (10)**: 16, 1900.

(PLATE 4, Fig. A; PLATE 8, Figs. L-M)

MORPHOLOGY– Thallus corticolous, crustose, granular, yellowish–grey, sorediate, non-isidiate, medulla soredia white.

ANATOMY– Thallus heteromerous, lower cortex absent, upper cortex 12.9-17.2 µm wide, plectenchymatous; algal layer 77.4-94.6 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 38.7-51.6 µm wide, white, hyphae septate and unbranched.

SPOT TESTS /CHEMISTRY- Medulla K+ yellow - red, C-, KC-, P+ orange - red, thamnolic, protocetraric and stictic acid.

SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28122, on bark of *Quercus semecarpifolia* Sm., in forest, Jagatsukh, c 2000 m, Kullu (H.P.), October 20, 2003, leg. Hem Chander.

PHYSCIACEAE

Dirinaria applanata (Fée) Awasthi, *J. Indian Bot. Soc.* **49**: 135, 1970.

(PLATE 4, Fig. B; PLATE 9, Figs. A-B)

MORPHOLOGY– Thallus saxicolous, foliose, yellowish glaucous grey, suborbicular, 3-6 cm in diameter, appressed, lobed, lobes narrow, 1-2 mm wide, entire, eciliate, upper surface rough, non isidiate, non-pseudocyphellate, sorediate, soralia laminal, globose – capitate, soradia farinose, lacinate, lacinae irregularly divided, oblong, plicate – rugose, confluent; lower surface pale, 0.5-0.7 mm wide, rhizinate, rhizinae pale brown, 0.3-0.5 mm long; apothecia brown, sub-sessile, lacking in maturity.

ANATOMY– Thallus heteromerous, corticate, upper cortex 38.7-51.6 µm wide, plectenchymatous; algal layer 47.3-68.8 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 68.8-86.0 µm wide, white, lax, hyphae septate and branched; lower cortex 51.6-60.2 wide.

SPOT TESTS /CHEMISTRY- Medulla K+ yellow, C-, KC-, P-, atranorin and divaricatic acids present.

SUBSTRATUM- Rock (Mycaschist).

COLLECTION EXAMINED- PAN 28106, on bare rock, in forest, Gulaba, c 2864 m, Kullu (H.P.), October 15, 2003, leg. Hem Chander.

Heterodermia leucomela (L.) Poelt, *Nova Hedwigia* **9**: 31, 1965.

(PLATE 4, Fig. C; PLATE 9, Fig. C)

MORPHOLOGY– Thallus saxicolous, foliose, light grey, 6-10 cm in diameter, loosely adnate to the substratum, lobed, lobes 3-6 mm wide, ribbon like, linear, rosulate, ascending, eciliate, thallus laciniate, lacinae linear, elongate, ribbon like, dichotomously branched, 0.9-1.0 mm wide, entangled at periphery; upper surface non-pseudocyphellate, non-isidiate and non-sorediate; lower surface white, rhizinate, rhizinae black, simple, conspicuous, 2-5 mm long; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 55.9-64.5 µm wide, plectenchymatous; algal layer 47.3-98.9 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 64.5-81.7 µm wide, white, lax, hyphae septate and branched; lower cortex 17.2-30.1 µm wide.

SPOT TESTS /CHEMISTRY- Medulla K+ yellow – red, C-, KC+ yellow -red, P+ orange – red, zeorin and salazinic acids present.

SUBSTRATUM- Rock (Mycaschist – biovite and granite).

COLLECTION EXAMINED- PAN 28127, on bare rock (Mycaschist– biovite), in forest, Naggar, c 1760 m, Kullu (H.P.), October 21, 2003, leg. Hem Chander; PAN 28142, on bare rock (Granite), in forest, Ram Shilla, c 1500 m, Kullu (H.P.), October 24, 2003, leg. Hem Chander.

Phaeophyscia hispidula (Ach.) Moberg, *Bot. Notiser* **131**: 260, 1978.

(PLATE 4, Fig. D; PLATE 9, Fig. D)

MORPHOLOGY– Thallus terricolous and corticolous, foliose, light greyish, 2-7 cm in diameter, loosely adnate to the substratum, dichotomously branched, lobed, lobes 2-4 mm wide, radiating, entire, flat; upper surface smooth, non-pseudocyphellate, non-sorediate, non isidiate, lobe margins eciliate; lower surface black, rhizinate, rhizinae black, simple, 1-2 mm long, projecting beyond lobe margins; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 34.4-43.0 µm wide, plectenchymatous; algal layer 51.6-73.1 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 86.0-111.8 µm wide, yellow, lax, hyphae septate and branched; lower cortex 43.0-51.6 µm wide.

SPOT TESTS /CHEMISTRY- Medulla K-, C-, KC-, P-.

SUBSTRATUM- Soil and Bark.

COLLECTION EXAMINED- PAN 28124, on soil, in forest, Naggar, c 1760 m, Kullu (H.P.), October 21, 2003, leg. Hem Chander; PAN 28125, on soil, in forest, Naggar, c 1760 m, Kullu (H.P.), October 21, 2003, leg. Hem Chander; PAN 28153, on soil, in forest, Pah nullah Valley, c 1600 m, Kullu (H.P.), October 27, 2003, leg. Hem Chander; PAN 28162, on bark of *Morus alba* Linn., in forest, Kasol, c 2000 m, Kullu (H.P.), October 29, 2003, leg. Hem Chander.

Phaeophyscia orbicularis (Necker) Moberg, *Lichen genus Physcia and allied genera in Fennoscandia*, New York, 1977

(PLATE 4, Fig. E; PLATE 9, Figs. E-F)

MORPHOLOGY– Thallus corticolous, foliose, light greyish, 2-5 cm in diameter, closely adpressed, orbicular–suborbicular, dichotomously branched, lobed, lobes 2-4 mm wide, separate–overlapping, entire, radiating, flat; upper surface smooth, non-pseudocyphellate, non isidiate, lobe margins eciliate, soradiate, soralia laminal and orbicular; lower surface black, rhizinate, rhizinae black, simple, 1-2 mm long, projecting beyond lobe margins; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 34.4-43.0 µm wide, plectenchymatous; algal layer 34.4-51.6 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 43.0-68.8 µm wide, white, lax, hyphae septate and branched; lower cortex 43.0-55.9 µm wide.

SPOT TESTS /CHEMISTRY- Medulla K+ yellow, C-, KC-, P-. SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28109, on bark of *Acer caesium* Brandis, in forest, Kothi, c 2500 m, Kullu (H.P.), October 29, 2003, leg. Hem Chander; PAN 28135, on bark of *Quercus leucotrichophora* A. Camus, in forest, Kuilu, c 1500 m, Kullu (H.P.), October 23, 2003, leg. Hem Chander.

Phaeophyscia pyrrhophora (Poelt) Awasthi and Joshi, *Indian J. Mycol. Res.* **16(2)**: 278, 1978.

(PLATE 4, Fig. F; PLATE 9, Fig. G)

MORPHOLOGY– Thallus corticolous terricolous and saxicolous, foliose, light greyish, 4-8 cm in diameter, closely adpressed, rossette forming, dichotomously branched, lobed, lobes 2-3 mm wide, entire, radiating, flat; upper surface smooth, non-pseudocyphellate, non-isidiate, lobe margins eciliate, non - soradiate; lower surface black, rhizinate, rhizinae black, simple, 1-2 mm long, projecting beyond lobe margins; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, corticate, upper cortex 25.8-38.7 µm wide, plectenchymatous; algal layer 21.5-34.4 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 55.9-68.8 µm wide, orange, lax, hyphae septate and branched; lower cortex 17.2-25.8 µm wide.

SPOT TESTS /CHEMISTRY- Medulla K+ violet, C-, KC, P-.SUBSTRATUM- Bark, soil and rock (Schist).

COLLECTION EXAMINED- PAN 28115, on bark of *Abies pindrow* Royle, in forest, Solang, c 2500 m, Kullu (H.P.), October 18, 2003, leg. Hem Chander; PAN 28147, on soil, in forest, Kullu, c 1900 m, Kullu (H.P.), October 25, 2003, leg. Hem Chander; PAN 28177, on rock (Schist), in forest, Bajaura, c 1100 m, Kullu (H.P.), November 1, 2003, leg. Hem Chander.

PORPIDIACEAE

Porpidia albocaerulescens (Wulfen) Hertel and Knoph, *Nova Hedwigia* **79**: 433, 1984.

(PLATE 5, Fig. A; PLATE 9, Fig. H)

MORPHOLOGY– Thallus saxicolous, crustose, greenish grey, 4-8 cm in diameter, rimose areolate, 0.15-1.5 mm thick, non-sorediate, non-isidiate; apothecia scattered, hemi-angiocarpic, sessile, partially sunken in thallus, 0.2-1.5 mm in diameter, disc black, apothecia lacking in maturity.

ANATOMY– Thallus heteromerous, lower cortex absent, upper cortex 55.9-68.8 µm wide, plectenchymatous; algal layer 60.2-86.0 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 68.8-98.8 µm wide, white, lax, hyphae septate and branched.

SPOT TESTS /CHEMISTRY- Medulla K+ yellow, C-, KC-, P+ yellow – orange, stictic acid present.

SUBSTRATUM- Rock (Pure quartz and Schist).

COLLECTION EXAMINED- PAN 28130, on rock (Pure Quartz), in forest, Patalikuhl, c 1600 m, Kullu (H.P.), October 22, 2003, leg. Hem Chander; PAN 28133, on rock (Schist), in forest, Kullu, c 1500 m, Kullu (H.P.), October 23, 2003, leg. Hem Chander.

RAMALINACEAE

Ramalina roesleri (Hochst. In Schaerer) Hue, *Rev. Bot.* **6**: 151, 1997.

(PLATE 5, Fig. B; PLATE 9, Figs. I-J)

MORPHOLOGY– Thallus corticolous, radiate-Fruticose, smooth, pale yellowish green, erect, 2-4 cm long, attached to substratum by a basal disc or hold fast, branched, branches flattened, apically finely divided, non-pseudocyphellate, non-isidiate, sorediate, soralia capitate and present on finely divided apices, tips attenuated, thallus corticate; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, cortices 17.2-21.5 µm wide, plectenchymatous; algal layer present below upper cortex and above lower cortex, 43.0-51.6 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 133.3-159.1 µm wide, white, lax, hyphae septate and branched.

SPOT TESTS /CHEMISTRY- Medulla K-, C-, KC-, P, diterpine and sekikaic acid present.

SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28119, on bark of *Abies acuminiatum* D. Don, in forest, Solang, c 2200 m, Kullu (H.P.), October 18, 2003, leg. Hem Chander; PAN 28120, on bark of *Quercus semecarpifolia* Sm., in forest, Lag Valley, c 1500 m, Kullu (H.P.), October 26, 2003, leg. Hem Chander.

Ramalina sinensis Jatta, *Nuov. Giorn. Bot. Ital.* **9**: 462, 1902.

(PLATE 5, Fig. C; PLATE 9, Fig. K)

MORPHOLOGY– Thallus corticolous, radiate-Fruticose, yellowish green, erect, 4-5 cm long, attached to substratum by a basal disc or hold fast, palmately

lobed, straped shaped with prominent nerves, erect, perforated, pseudocyphellae alternating with corticated ridges, lacking soredia, non-isidiate, branched, palmately lanceolate, medulla solid; apothecia terminal, discoid, shorlety stipitate, disc concave, yellowish brown, 5-9 mm in diameter, apothecia lacking in maturity.

ANATOMY– Thallus heteromerous, cortices 17.2-21.5 µm wide, plectenchymatous; algal layer present below upper cortex and above lower cortex, 38.7-55.9 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 77.4-90.3 µm wide, white, lax, hyphae septate and branched.

SPOT TESTS /CHEMISTRY- Medulla K-, C-, KC-, P-, diterpine and sekikaic acid present.

SUBSTRATUM- Bark.

COLLECTION EXAMINED- PAN 28113, on bark of *Abies acuminiatum* D. Don, in forest, Solang, c 2200 m, Kullu (H.P.), October 18, 2003, leg. Hem Chander; PAN 28123, on bark of *Quercus semecarpifolia* Sm., in forest, Naggar, c 1760 m, Kullu (H.P.), October 21, 2003, leg. Hem Chander; PAN 28166, on bark of *Prunus* sp., in forest, Manikaran, c 2000 m, Kullu (H.P.), October 30, 2003, leg. Hem Chander.

TELOSCHISTACEAE

Caloplaca flavorubescens (Huds.) J.R. Laundon, *Lichenologist* **8**: 139, 1976.

(PLATE 5, Fig. D; PLATE 10, Fig. A)

MORPHOLOGY– Thallus saxicolous and corticolous, crustose, squamulose, effigurate at margins, yellowish orange in colour, poorly developed, smooth, rimulose; apothecia 0.4-1.0 mm in diameter, laminal, crowded – scattered, sessile, epruinose, orange yellow in colour; apothecia lacking in maturity.

ANATOMY– Thallus heteromerous, upper cortex 21.5-25.8 µm wide, plectenchymatous; algal layer 47.3-64.5 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 51.6-64.5 µm wide, white, lax, hyphae septate and branched, lower cortex absent.

SPOT TESTS /CHEMISTRY- Medulla K+ violet, C-, KC-, P-, parietin present.

SUBSTRATUM- Rock (Mycaschist) and Bark.

COLLECTION EXAMINED- PAN 28107, on bare rock (Mycaschist), in forest, Gulaba, c 2864 m, Kullu (H.P.), October 15, 2003, leg. Hem Chander; PAN 28114, on bark of *Abies caesium* Brandis, in forest, Solang, c 2200 m, Kullu (H.P.), October 18, 2003, leg. Hem Chander.

Xanthoria candelaria (L.) Arn., *Flora* **62**: 364, 1979.

(PLATE 5, Fig. E; PLATE 10, Figs. B-C)

MORPHOLOGY– Thallus corticolous, foliose, yellowish orange, appressed to suberect, slightly raised towards margins, sub orbicular, 1-2 cm in diameter, lobed, lobes compact, clustered, incised, 1 mm wide, margins sorediate, soredia farinose; lower surface greyish white, rhizinate, rhizinae pale brown, 0.5 mm long; apothecia not observed in the specimen examined.

ANATOMY– Thallus heteromerous, upper cortex 34.4-55.9 µm wide, plectenchymatous; algal layer 51.6-77.4 µm wide, algal cells green, spherical, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 64.5-81.7 µm wide, white, lax, hyphae septate and branched, lower cortex 17.2-25.8 µm wide.

SPOT TESTS /CHEMISTRY- Medulla K+ red - purple, C-, KC-, P-, parietin present.

SUBSTRATUM- Bark and Rock (Quartzite).

COLLECTION EXAMINED- PAN 28112, on bark of *Abies acuminiatum* D. Don, in forest, Solang, c 2200 m, Kullu (H.P.), October 18, 2003, leg. Hem Chander; PAN 28150, on bark of *Picea smithiana* Boss., in forest, Lag Valley, c 1550 m, Kullu (H.P.), October 26, 2003, leg. Hem Chander; PAN 28151, on bark of *Pinus roxburghii* Sarg., in forest, Lag Valley, c 1500 m, Kullu (H.P.), October 26, 2003, leg. Hem Chander; PAN 28163, on bark of *Morus alba* Linn., in forest, Kasol, c 2000 m, Kullu (H.P.), October 29, 2003, leg. Hem Chander; PAN 28181, on bark of *Pyrus sp.*, in forest, Banjar, c 1100 m, Kullu (H.P.), November 2, 2003, leg. Hem Chander.

VERRUCARIACEAE

Dermatocarpon miniatum var. *miniatum* (L.) Mann., *Lich. Bohem. Observ. Dispos.* : 66, 1825.

(PLATE 5, Fig. F; PLATE 10, Fig. D)

MORPHOLOGY– Thallus saxicolous, foliose, monophyllous, ash grey coloured, 3-7 cm in diameter, loosely adnate to the substratum, by an umblicus, thick, more thick in the central part as copared to the marginal area, lobes imbricate, margins round, entire, ascending and recurved downward; upper surface rough, non-pseudocyphellate, non-isidiate and non-sorediate; lower

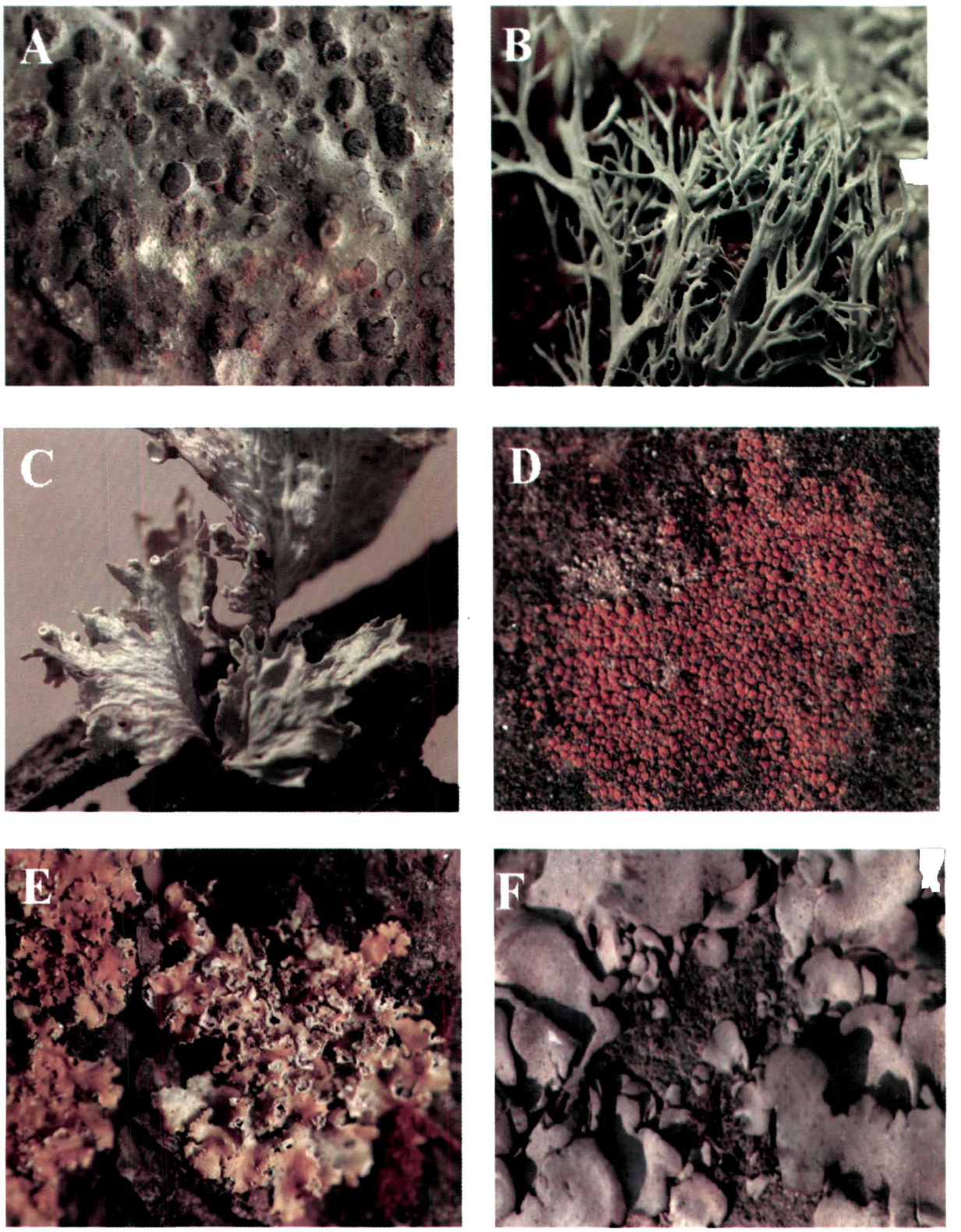

PLATE 5 (Figs. A-F): (A) *Porpidia albocaerulescens* (B) *Ramalina roesleri* (C) *Ramalina sinensis* (D) *Caloplaca flavorubescens* (E) *Xanthoria candelaria* (F) *Dermatocarpon miniatum* var. *miniatum*

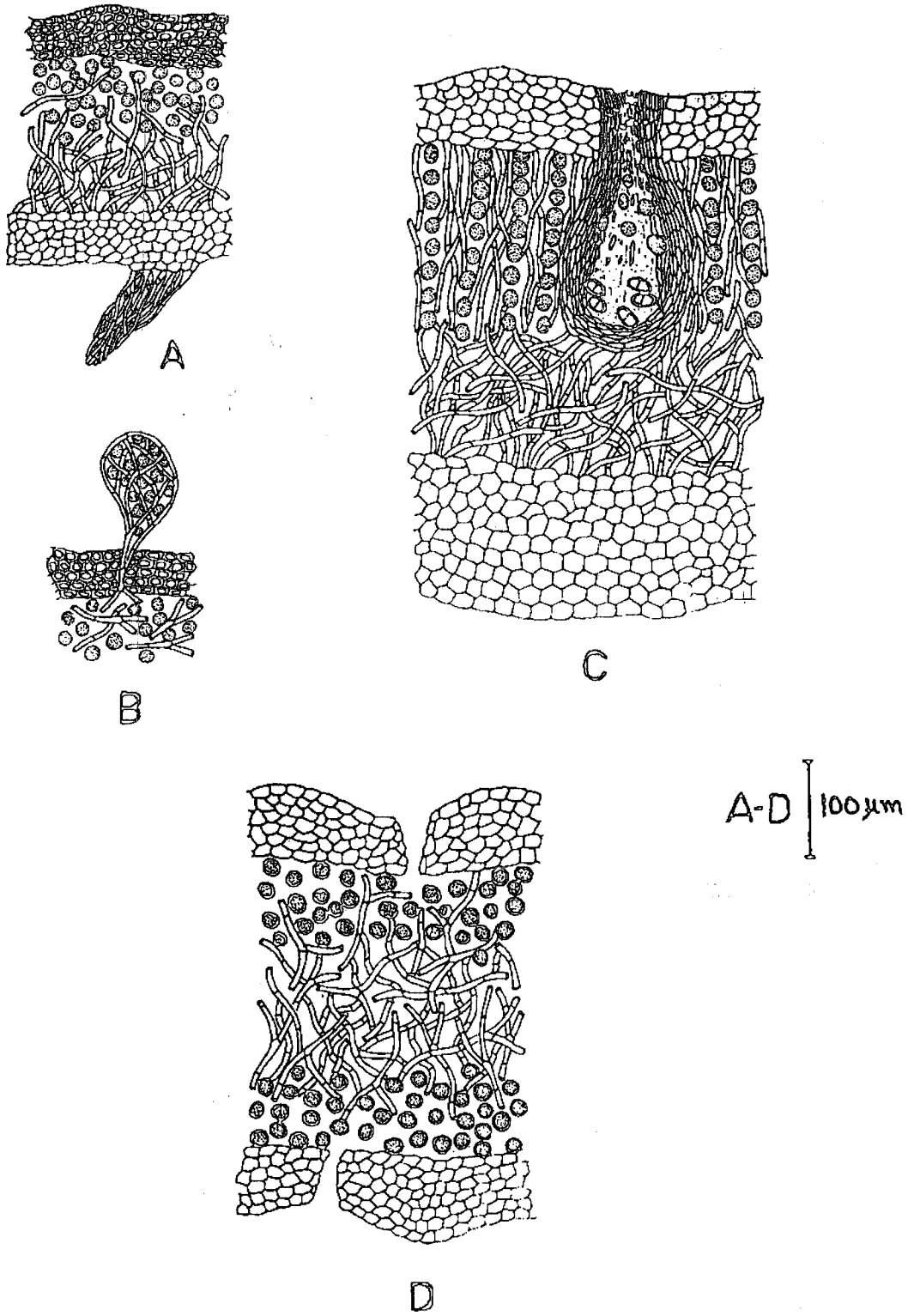

PLATE 10 (Figs. A-D): ***Caloplaca flavorubescens*** (Huds.) J.R. Laundon(A) T.S. of Thallus.
Xanthoria candelaria (L.) Arn, (B) T.S. of Thallus, (C) A portion of T.S. of Thallus through soredia; ***Dermatocarpon miniatum*** var. ***miniatum*** (L.) Mann,(D) T.S. of Thallus.

surface black, smooth, erhizinate, shining in the central part, marginal zone wrinkled.

ANATOMY– Thallus heteromerous, upper cortex 47.3-64.5 µm wide, plectenchymatous; algal layer 133.3-150.5 µm wide, algal cells green, round, 6.4-8.0 µm in diameter, photobiont a green alga; medulla 81.7-103.2 µm wide, white, lax, hyphae septate and unbranched, lower cortex 103.2-124.7 µm wide; perithecia immersed, globose, dimidiate, 96 µm high and 64 µm in diameter, ostiole brown, spores oval, hyaline, septate, 8.0 x 14.4 µm in size, paraphysis degenerated.

SPOT TESTS /CHEMISTRY– Medulla and cortex K-, C-, KC-, P-, no lichen substance found in T.L.C.

SUBSTRATUM- Rock (Mycaschist, schist and amphibolite).

COLLECTION EXAMINED- PAN 28108, on bare rock (Mycaschist), in forest, Gulaba, c 2864 m, Kullu (H.P.), October 15, 2003, leg. Hem Chander; PAN 28132, on bare rock (Schist), in forest, Kullu, c 1500 m, Kullu (H.P.), October 23, 2003, leg. Hem Chander; PAN 28143, on bare rock (Amphibolite), in forest, Chansari, c 1900 m, Kullu (H.P.), October 25, 2003, leg. Hem Chander.

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BIOCONVERSION OF WATER HYACINTH TO FUEL AND FERTILIZER THROUGH PRETREATMENT

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Abstract

Anaerobic digestion of water hyacinth may reduce the nuisance value and water pollution problem created by this waterweed and considerable amount of biogas can be obtained. The rate of biogas can be enhanced under a short period by giving certain fungal treatment prior to anaerobic digestion. Pretreatment of water hyacinth with Phanerochaete chrysosporium and Phanerochaete chrysosporium + pyrogallol red was found to increase the amount of gas production within short Hydraulic Retention Time (HRT), through solublization of lignocellulosic biomass in water due to which choking problem of the biogas plant could be sorted out. This paper deals with the efficient utilization of water hyacinth for fuel and fertilizer. Treatment of water hyacinth with Phanerochaete chrysosporium and Phanerochaete chrysosporium + pyrogallol red showed 243.82 and 314.07l/kgdm of gas production respectively i.e. about 143 % and 213.2 % more than the gas obtained from pure water hyacinth. Accordingly increase in manurial value with respect to NPK was also observed.

Key words : Anaerobic digestion, biogas, fungi.

INTRODUCTION

In India most of the biogas plants are cow dung based but due to less availability of dung at various places, people are unable to install these plants. Incorporation of other type of alternate feed material in the biogas plant can solve the problem. Most of the waterweeds responsible for creating pollution problem can be recycled for biogas generation alone or along with cow dung. Water hyacinth *(Eichhornia crassipes)* is one of the most predominant, persistent and troublesome aquatic weeds in India. This plant is considered as a nuisance since it spreads and clogs up lakes and waterways. As a result, there is a growing interest in the potential use of aquatic plants for nutrient removal from waste or polluted water and recycling of the resulting biomass for production of gaseous fuel, feed, fiber and other products. In particular the protein content of this plants is similar in chemical composition to leaf protein from crop plants (Boyed, 1970) and rich in amino acids similar to hen egg protein. A single plant is capable of infesting an area of one acre in a year. It is normally free floating, buoyed by bladder-like inflated leaf petioles.

The nuisance value of this plant can be reduced by utilizing it as high quality vegetable protein, minerals, energy (in the form of biogas), fertilizer and chemicals. The available dry matter (100-150 ton/ha/year) has the potential of producing about 30,000 cum of biogas and 70 tonnes of fertilizer through anaerobic decomposition (Chawla, 1986).

This surplus biomass are either thrown in compost pits or burnt in open air to reduce their volume, which create a severe environmental pollution. Calorific value of this material is also very low, thus it is not worthwhile using this as a fuel. The main problem associated with this type of material is their floatable nature having high lignin, cellulose and hemicellulose content due to which they do not form homogeneous mixture with water like cattle dung and cause choking problem in the biogas plant. The anaerobic decomposition of lignocellulosic complex is very slow in digester. For the degradation of any waste material breaking of lignocellulosic complex is a prerequisite. It is obvious then to think that if the rate of hydrolysis of the cellulose could be accelerated, there will be improvement in the rate of biogas generation, and as consequences the reduction in retention period of biogas plants. Biological and chemical pretreatment is one of the best methods of delignification of lignocellulosic biomass. In biological approach, waste can be treated with some selected microorganisms such as fungi and in chemical treatment some chemical inducers can be tried for lignin degradation. The microbial and chemical delignification

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of solid waste and further biogas formation would offer the possibility of utilizing lignocellulosic waste in an environment friendly approach.

MATERIALS AND METHODS

The present experiment was conducted at Department of Renewable Energy Sources, College of Technology and Engineering, Maharana Pratap University of Technology and Engineering, and at Department of Botany, College of Science, Mohanlal Sukhadia University Udaipur, India. Water hyacinth was collected from the lake Swaroopsagar, Udaipur.

The collected water hyacinth was sun dried initially and ground in a grinder to the size of 1" to 1.5". The total solid of water hyacinth was found to be 90.96% (Table 2). Thus the substrate to water ratio was kept 1:8 so as to bring the total solid (TS) concentration to the level of 8-10 %. Total ten sets with two replications were prepared.

For the microbial pretreatment, two-lignocellulolytic fungi taken were *Phanerochaete chrysosporium* and *Volveriella diplasia*. These were procured from the National Collection of Industrial Microorganisms (NCIM), Biochemical Sciences Division, National Chemical Laboratories, Pune (Maharashtra) India. These cultures were treated as parental cultures and sub – culturing of the parental cultures was done. Sub – culturing of fungi was done on Malt extract agar medium having the following composition (Vij and Subramanian, 1999).

Malt extract	20g.
Agar	20g.
Distilled water (Tempaque make)	1000ml
pH	6.5

From these cultures mother spawn was prepared on sorghum grains by adapting the method of spawn preparation (Suman *et al.*, 1988). About 125 g of water hyacinth (dried and crushed) was soaked in 1 liter of water and kept for over night. The water was previously mixed with 0.05 per cent bavastine and 0.25 per cent formaldehyde. This is required to sterilize the substrate(Doshi and Sharma, 1997). Next day the water was decanted by spreading the material over a polythene sheet thus the material was sterilized by chemical treatment. Two per cent by weight of substrate, spawn of both fungi (prepared previously) were spread separately on water hyacinth layer by layer in perforated polythene bags and the bags were tied as treatment T_1 and T_2 (Table 1). The bags were kept in incubator at 25 ± 1°C temperature for the optimum growth of fungi. T_3 and T_4 were treated with pyrogallol red (PR) 1ppm and 2ppm. Sets T_5 to T_8 were treated with fungi + PR 1ppm and 2ppm (Table 1). The fungi + PR treated polythene bags were kept in incubator at 25 ± 1°C temperature for 10 days. The material was kept in perforated polythene bags for 10 days for pre-digestion under partial anaerobic conditions. After physico-chemical, biochemical analysis (American Public Health Association, 1985; A.O.A.C., 1965) of undigested (fresh and dried) material, the required parameters were optimized for sufficient gas production. One set of pure water hyacinth (T_9) and one set of pure cattle dung (CD) without adding chemicals and fungi was prepared and kept under similar condition for comparative study.

The study was performed on laboratory models and the gas was collected using water displacement method. In this method airtight water filled aspirator bottle having outlet at the bottom is connected to another glass reagent bottle containing biomass culture. As the gas is formed it will increase the pressure on water surface and correspondingly water will be discharged through outlet tube at the bottom till the pressure balance is maintained on the water surface and at the outlet of aspirator bottle. The bottle is marked or graduated in liters. The amount of water displaced i.e. the amount of gas formed can be noted down in liters.

After pretreatment of the material as mentioned earlier, remaining water was added and 20% previously digested cattle dung slurry was added as seeding material to enhance the rate of reaction. The bottle, acted as digester was filled with the predigested material after 10 days predigesting period. The whole system was made airtight by applying araldite solution at all joints and at the cover lining. The gas outlet of digester and gas inlet of gas collector were connected by rubber tubing and sealed at the joints. The second outlet at the top of gas collector connected with a rubber tube was closed to airtight condition using a pinch clip as shown in Fig 1. For refilling of the gas collector the water outlet of gas collector at the bottom was connected by rubber tube to water source slightly above the top level of gas collector. The gas outlet was opened. Water from the source flowed under the gravity to the gas collector and forced the gas out through the gas outlet and this could be tested by burning the gas at the mouth of the tube for its combustible property. If it burns then the percentage of methane could be estimated using Orsate gas analyzer.

Preparation of conical flasks for analysis at different intervals of time

Similar sets in replication as described above were prepared in conical flasks of 250 ml capacity for analysis at 15 days interval during anaerobic digestion. Total 100 g of sample of substrate was taken in 45 flasks with two replications. The material was kept for predigestion under similar condition say for 10 days. The conical flasks were sealed with rubber cork having a hole of 3mm diameter at the top, after predigesting period of 10 days. The hole at the top of flask was connected with a glass tube of 2" length having a long rubber tube dipped in water filled beaker. Flasks were kept for anaerobic digestion in the laboratory along with the set prepared for gas production under similar condition and optimum parameters. The flasks were opened for analysis on their respective days. Cellulose, lignin, protein content of material before and after pretreatment of organic waste and after 15 days interval of digestion period (up to 60 days) was analyzed by opening the conical flasks.

Different physico-chemical characteristics i.e., total solid, moisture content, total volatile solid, carbon, nitrogen, C/N ratio, phosphate and potash were analyzed by using standard methods (American Public Heallth Association, 1985) shown in Tables 3, 4, 5.

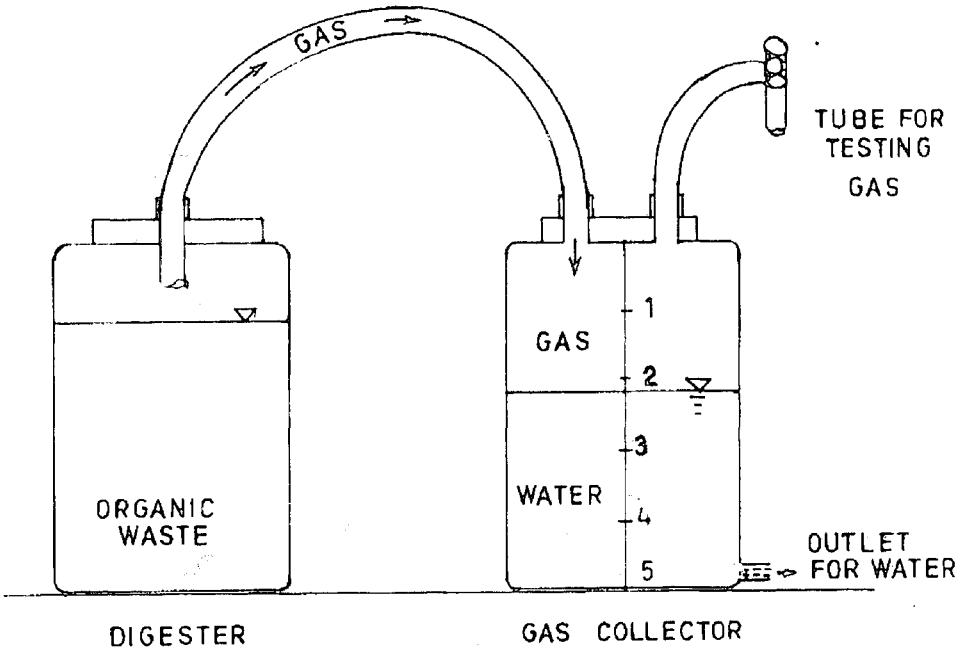

Fig.1 : Laboratory set up for studies on gas production

Table 1: Different treatment given to water hyacinth

S. No.	Treatments	Chemicals	Fungi
1.	T_1	-	*P.chrysosporium*
2.	T_2	-	*V.diplasia*
3.	T_3	*PR, 1ppm	-
4.	T_4	*PR, 2ppm	-
5.	T_5	*PR, 1ppm	*P.chrysosporium*
6.	T_6	*PR, 2ppm	*P.chrysosporium*
7.	T_7	*PR, 1ppm	*V.diplasia*
8.	T_8	*PR, 2ppm	*V.diplasia*
9.	T_9	Pure water hyacinth as control	
10.	CD	Pure cattle dung as control	

* = Pyrogallol Red

Table 2: Biochemical analysis of fresh water hyacinth and cattle dung in per cent

Constituents/substrate	TS	VS	C	N	C/N	P	K	Cellulose	Lignin	Protein
Water hyacinth	90.96	86.22	50.01	2.25	22:1	0.48	1.58	15.80	7.80	14.06
Cattle dung	19.78	83.69	48.50	1.40	35:1	0.58	0.70	21.72	19.88	8.75

TS= total solid, VS = volatile solid, C = carbon, N = nitrogen, P = phosphate, K = Potash

RESULTS AND DISCUSSION

The average total amount of gas produced (at 15 days interval and total 60 days) from the water hyacinth given different treatments is shown in Table 3. Accordingly biochemical analysis of material, having different treatments, with respect to total solid (TS), volatile solid (VS) carbon (C), nitrogen (N) cellulose and lignin etc. were also done as given in Table 3.

From the study it was observed that treatment T_1 and T_5 having *P.chrysosporium* treatment and *P.chrysosporium* + PR1ppm produced 243.66 and 314.07 l/kgdm of biogas respectively i.e. 142.9 per cent and 213.09 % respectively more than the gas obtained from the set (T_9) having pure water hyacinth (100.31 l/kgdm) and clear from Table 3 & figures 2 & 3. While in case of cattle dung the total gas obtained was 258.44 l/kgdm that is at par with set T_1 and 21.53 % less than the gas obtained from set T_5. In case of treatment T_5 the material having pyrogallol red (1ppm concentration) with *P.chrysosporium,* maximum gas production was observed in comparison to gas obtained from all other treatments (Table 3). This may be due to the beneficial combined effect of chemical and *P.chrysosporium* due to which more solubulisation of volatile matter could have taken place during pretreatment period. Accordingly per cent degradation of cellulose and lignin was also observed in increased pattern (Table 3 & Figs. 5,6). In case of T_3, T_4 having only pyrogallol red 1ppm & 2ppm the gas production observed to be 102.31 and 110.79 l/kgdm respectively, (Table 3) which is comparable to the gas obtained from pure water hyacinth i.e. 100.31 l/kgdm. Treatment T_6 in which PR concentration was increased, showed lesser gas production than the gas obtained from T_5. This indicates that higher concentration of chemicals reduces the gas production rate (Table 3). It is also clear from fig.3 that during 15-45 days period maximum biogas production was obtained from T_1-T_9. It increased up to 45 days of digestion and thereafter declined slightly up to 60 days that is similar to cow dung, also showing higher gas production up to 45 days (Table 3). Thus pretreatment of water hyacinth with fungi reduces the hydraulic retention time.

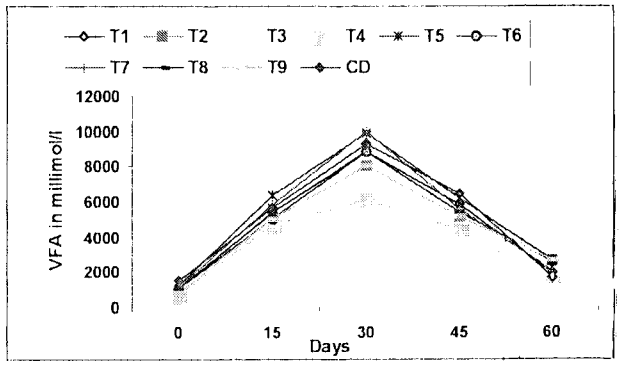

Fig. 2 : Effect of treatments on Volatile Fatty Acid in millimol/l with time

Effect of pretreatment of water hyacinth with *P.chrysosporium* on cellulose and lignin degradation and in turn on VS degradation is given in Table 3. From the comparative study it was observed that maximum lignin degradation was observed during pretreatment period of 10 days. In all treatments i.e. from T_1 to T_5 lignin degradation varied from 7.11 to 7.27 % during 10 days of pretreatment time in comparison to lignin content of fresh water hyacinth (Fig.6). In case of pure water hyacinth (digested) without any treatment 6.66 % degradation was observed in comparison to lignin content of fresh water hyacinth (undigested). During anaerobic digestion i.e. at 30 and 60 days decrease in percent degradation in lignin level was seen in each treatment which shows that under partial anaerobic pretreatment *P.chrysosporium* and pyrogallol red are capable of hydrolyzing the lignin content of water hyacinth. Many workers (Bousfield *et. al.* 1979; Dar and Tandon, 1987; Dar *et. al.*, 1989) have already reported wide variation in chemical composition. It slows down during anaerobic digestion but once it is solubilised during pretreatment it does not have any retarding effect during rest of the period of digestion period. Cellulose was also solubilised by chemicals

and *P.chrysosporium* during pretreatment period of 10 days. Maximum 40 to 33.99 per cent cellulose degradation was observed in treatment T_1 to T_6 respectively within 60 days of anaerobic digestion (Fig. 5). More solubilisation of cellulose and lignin tends to increase the rate of production of volatile fatty acids shown in Fig. 2 during initial phase of anaerobic digestion, which is a favourable environment for the growth of methane formers, and hence more gas production was obtained as stated by Dhawale (1996) Supplementation of chemical inducer along with fungi favoured the growth of fungi (T_3 to T_8) and hence the more solubilization of complex material took place as evident from Table 3. 1ppm concentration of chemical inducer is favourable for the hydrolysis while higher concentration i.e. 2 ppm resulted in lower hydrolysis. The reason for higher degradation of cellulose lignin in *P.chrysosporium* treated water hyacinth may be due to the extracellular enzymes released during growth of this species in response to lignocellulosic substrates as stated by Ghosh and Nandi (1995).

The less loss in solid indicates that the substrate lacks a balance of chemical components, which might be needed to translate decomposition of substrate by the fungus. An overall maximum reduction in per cent carbon and increase in nitrogen was observed during growth of *P.chrysosporium* during 10 days pretreatment periods (0 day analysis) in comparison to rest of anaerobic digestion period. Loss in substrate is due to loss of carbon in the form of CO_2 produced by the respiratory activity of fungus. During this growth period, the nitrogen component of substrate therefore increased in proportion (Gupta *et. al.*, 1999). It is reported that during microbial utilization of plant substrates, nitrogen becomes mobilized into the cells of the colonizer while most of the carbon is released as CO_2. As result of nitrogen being captive in organic combination, the percentage of nitrogen continuously rises in organic combination, and hence the C:N ratio shows a decreasing trend. The results obtained are also in conformity with earlier study by Moorthy (1981) and Vandel Wal (1979). C/N ratio decreased with the period of digestion. Initially higher C/N ratio was recorded in all the treatments (Table 4). C/N ratio decreased rapidly with increasing digestion period up to 60 days. Higher C/N ratio of the substrate suppressed decomposition at early stages due to lower nitrogen availability to bacteria but after 15 days of digestion the rate of biogas production in all the treatments was more as the resistant material becomes susceptible to decomposition with time. It indicates that higher C/N ratio may take comparatively more time for optimum gas production. The results are in conformity with Bardiya & Gaur (1997 a,b) who reported higher rate of biogas production with addition of chopped feed residue with dung raising C/N ratio between 25-32.

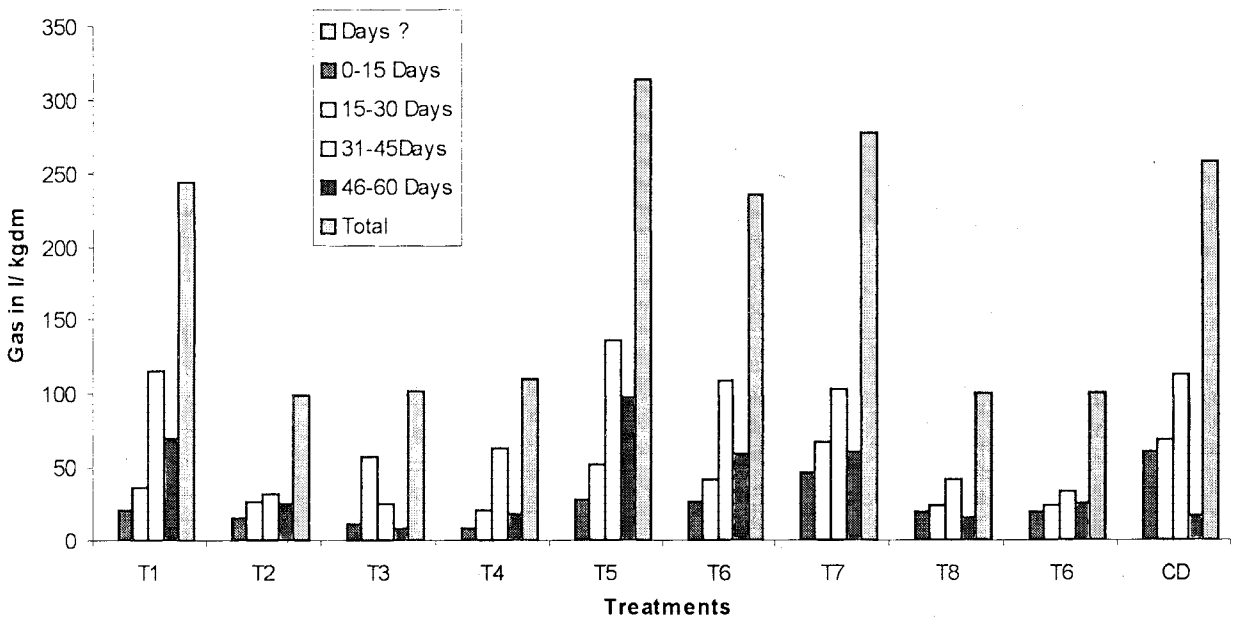

Fig. 3 : Fortnightly biogas production in l/kgdm

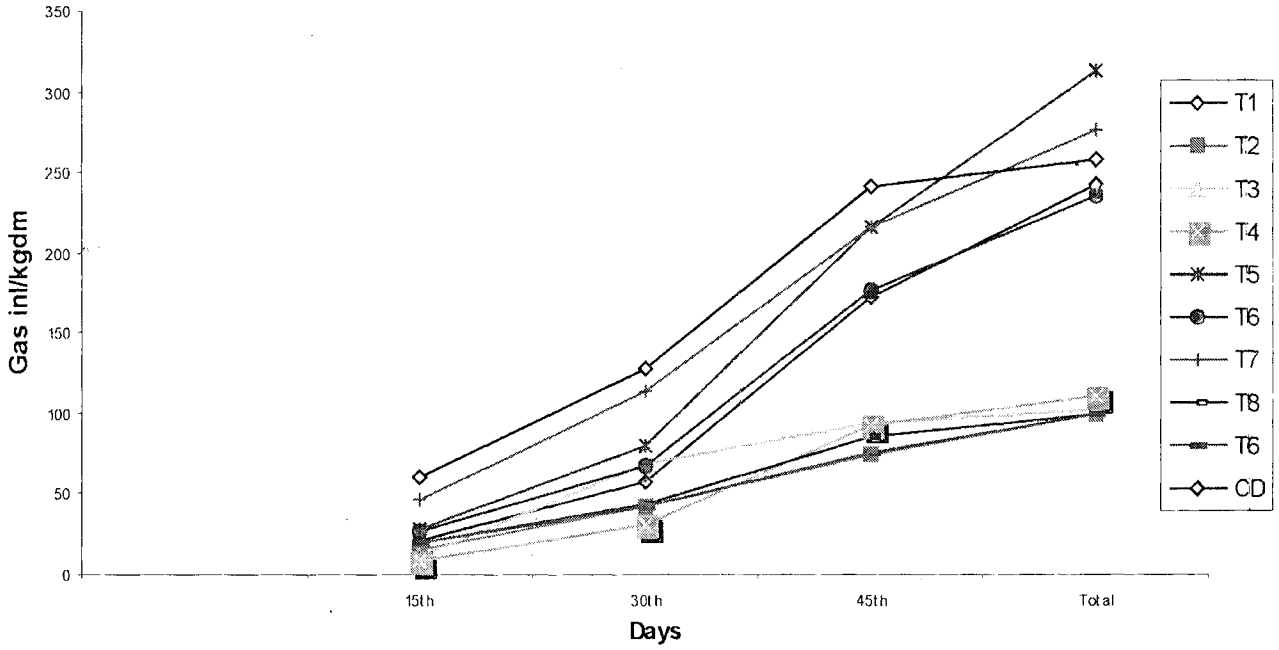

Fig. 4: Cumulative biogas in l/kgdm

Initial pH of the slurry ranged from 6.53 to 6.80 in treatment T_1 to T_6. There was sharp drop in pH during initial 15-30 days of digestion period. In each case drop in pH during first 15 days of digestion and reaction of the fluid was generally acidic during digestion. It might be due to the production of fatty acids and also possibly due to CO_2 pressure (Thakur and Singh, 2000).

Manurial value of slurry after anaerobic digestion with respect to NPK was also observed to be improved when compared to its initial NPK content (Table 3). Anaerobic digestion increases the available NPK in the digested slurry. Nitrogen has received the maximum attentions compared to all other nutrients contained in the digested material. It is also the single largest nutrient consumed from fertilizer in our country. In anaerobic fermentation the loss of organic matter leads to almost complete conservation of nitrogen. During anaerobic decomposition there is loss of 25-30% of organic material, which is converted to CO_2 and combustible gases and, 15 to 18% of the total nitrogen is converted to ammonia (Chawla, 1986). Romashkevich and Karelina (1961) found that saw dust after anaerobic digestion could be used as an effective manure in comparison to unfermented saw dust.

Methane percentage of treated water hyacinth was about 64 to 67 % in each treatment which was quite comparable with control (pure water hyacinth) and it was at par with cattle dung (Table 4). During pretreatment period the growth of *P.chrysosporium* was more than *V.diplasia*. The fungus seems to have utilized the free sugars and other smaller molecular weight nutrients like amino acids, organic acids and others for its initial growth. It may also be seen further from Table 4 that the percentage of dry weight decreased continuously till the last day of study. This may be due to the utilization of organic volatile matter. The same result was noted by Sharma *et.al.* (1996) with *Pleurotus* sajora–kaju. The microbial count of fungi, after pretreatment period are as shown in Table 4.

In case of *V.diplasia* treated water hyacinth the degradation was lesser due to the lesser production of enzymes in response to the presence of lignocellulosic molecules in the material. This fact favours the result obtained by Punia and Singh (1995 a,b).

Table 3: Effect of various treatments on different constituents in per cent after 60 days of digestion

Treatments → / constituents ↓	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	CD
TS	7.09	7.62	7.60	7.58	6.55	7.12	7.03	7.60	7.66	7.48
VS	65.08	73.40	73.08	75.20	59.29	66.20	65.80	73.16	73.02	68.71
C	65.08	73.40	73.08	75.20	59.29	66.20	65.80	73.16	73.02	68.71
N	2.81	2.51	2.64	2.69	2.86	2.80	2.85	2.56	2.52	2.05
Protein	17.56	15.69	16.25	16.56	17.88	17.50	17.81	16.00	15.76	12.82
C/N	13:1	17:1	16:1	16:1	12:1	14:1	13:1	17:1	17:1	19:1
P	1.01	0.68	0.79	0.86	1.00	0.99	0.97	0.82	0.66	0.76
K	2.24	2.11	2.21	2.22	2.31	2.27	2.25	2.05	2.10	0.89
Cellulose	10.89	12.42	11.85	11.43	9.57	10.43	9.64	12.00	11.85	17.62
Lignin	6.15	6.50	6.06	6.55	6.04	6.20	6.06	6.72	6.55	13.81
Total biogas in l/kgdm	243.66	99.45	102.31	110.79	314.07	235.69	277.07	100.45	100.31	258.44

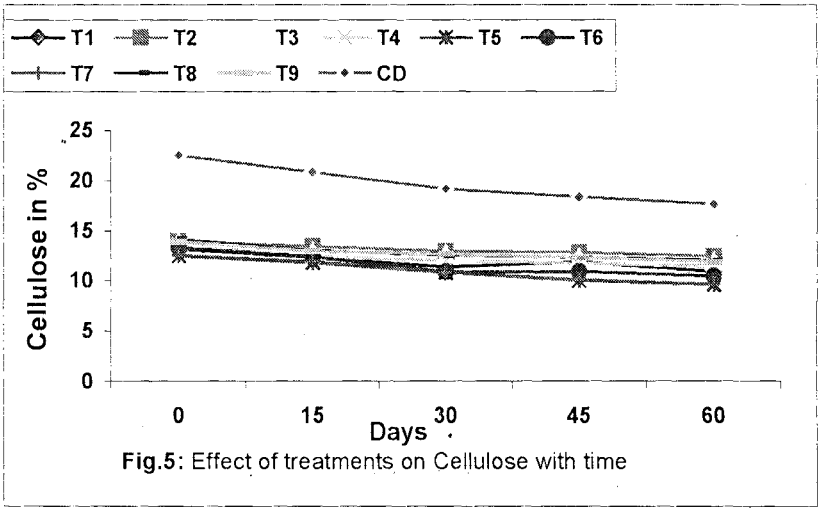

Fig.5: Effect of treatments on Cellulose with time

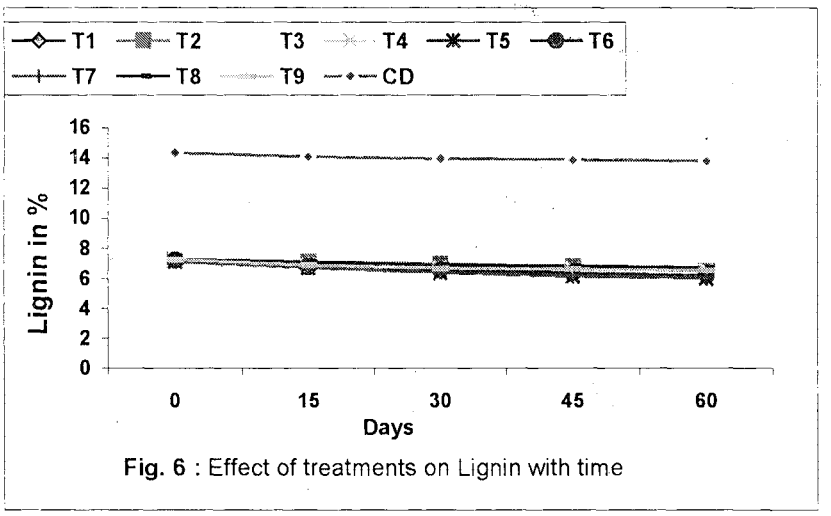

Fig. 6 : Effect of treatments on Lignin with time

Table 4: Biogas analysis and microbial population count in cfu/ml

Treatments	Biogas in %		Dilutions/ population cfu/ml		
	CH_4	CO_2	10^{-1}	10^{-2}	10^{-3}
Wh_1	66	33	Uncountable	Uncountable	994
Wh_2	64	35	Uncountable	990	678
Wh_3	65	33	-	-	-
Wh_4	66	33	-	-	-
Wh_5	67	32	Uncountable	Uncountable	998
Wh_6	64	35	Uncountable	994	365
Wh_7	66	33	Uncountable	996	983
Wh_8	66	33	Uncountable	996	448
Wh_9	60	39	-	-	-

cfu / ml = colony forming unit per milliliter.

CONCLUSIONS

From the above results it can be concluded that:-

Water weeds such as water hyacinth having high cellulose and lignin content can be used as best substrate for biogas production after giving pre-treatment with *P.chrysosporium* and *P.chrysosporium* + pyrogallol red in 1 ppm and 2ppm. More gas production was obtained from the water hyacinth with *P.chrysosporium* treatment (243.66 l/kgdm) that is 142.9% more gas obtained from pure water hyacinth (without any treatment) and it is at par with the gas production obtained from cattle dung. Maximum gas production was noted from *P.chrysosporium* + pyrogallol red 1ppm treatment which is 21.52 % more than the gas obtained from cattle dung and 213.09 % more than the gas obtained from pure water hyacinth.

Cellulose and lignin degradation percentage was also maximum in case of treatments *P.chrysosporium* and *P.chrysosporium* + pyrogallol red in 1 ppm and 2ppm in comparison to pure water hyacinth. Supplementation of substrate with PR was found to stimulatory over control. However, application of PR at 2 ppm concentration resulted in low or marginal gas production. Treatment with fungi and chemicals also showed higher concentration of CH_4 content over control. Average methane content was in the range of 64-67 %. Manurial value of slurry in terms of NPK was also found to be better in treated water hyacinth than NPK content found in untreated water hyacinth. It is also clear from the study that pretreatment with fungi reduces the HRT. Thus use of water hyacinth with pretreatment reduces the pollution load, gives better fuel and manure.

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EFFECT OF PERIPHYTON AND SUPPLEMENTARY FEEDING ON GUT INDICES OF TWO BRACKISHWATER FISH SPECIES

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Abstract

Present communication deals with the food and feeding habits of two aquaculturally important brackishwater fish species (Oreochromis niloticus L. and Etroplus suratensis Bloch) grown in ponds provided with additional substrate for the development of periphyton. Two controls (Control 1 with supplementary feeding and control 2 with no additional substrate and no supplementary feeding) were maintained for comparison. The gut content analysis have revealed that irrespective of the treatments, O. niloticus mainly depended on vegetable matter and is thus a herbivore, whereas E. suratensis appears to be omnivorous as well as deterivorous. High values of RLG and DSI in O. niloticus and low values in E. suratensis further confirm the differences in feeding habits of these two fish species. Values for forage ratio indicated Coelastrum, Closterium and Cladophora are the preferred food items for O. niloticus confirming its herbivore nature. However, the values of forage ratio for E. suratensis indicated that Navicula, Synedra and Coelastrum are the preferred food items and in addition to this forage ratio values (1.79) for zooplankton were also high, indicating that E. suratensis is an opportunistic omnivore.

Key words : Gut indices, periphyton, RLG, DSI, pearlspot, nile tilapia, supplementary feeding.

INTRODUCTION

Fish have adopted to many nutritional habits and thus have been classified as piscivores, insectivores, detritivores, herbivores, omnivores, molluscivores and carnivores etc. An analysis of stomach contents of a fish indicate the extent of competition among different fish species and their food preferences.

A wide variety of structural and physiological adaptations permit fishes to capture, digest and obtain their requirements from the available food, for example, the gut length of fish varies in different species in relation to their size, age and feeding habits etc. It is long in herbivorous, omnivorous and macrophages, while it is short in carnivorous fish species. According to Al Hussaini (1947) and Kapoor *et al.* (1976), this variation depends upon the proportion of indigestible to digestible materials in their diet. The estimation of overall length of the intestine, relative to the length of the fish body, seems to be related to their feeding habits and found to fit well to the above mentioned concepts (Bhatnagar *et al.*, 2003). It is also related to the duration of the intestinal digestion. In addition to this, determination of digestive somatic index also appears to be helpful in determining the feeding habits/behaviour of fish. Calculation of forage ratio evaluates the preferred food organisms for a particular species in its natural habitat. Keeping in view the importance of these studies in aquaculture, gut contents, relative length of the gut (RLG) and digestive somatic index (DSI) of two important brackishwater fish species, *Oreochromis niloticus* and *Etroplus suratensis* have been investigated when grown in ponds with and without the provision of additional substrate for periphyton growth and development and also in ponds where the fish were fed on supplementary diet.

MATERIALS AND METHODS

Etroplus suratensis and *Oreochromis niloticus* were grown in brackishwater ponds under mono and polyculture practices. Following three treatments were maintained :

1. Ponds provided with additional substrate (bamboo poles) and without supplementary feeding (Treated ponds).
2. Ponds provided with no additional substrate but with supplementary feeding (@ 5% BW d^{-1})- Control 1.
3. Ponds provided with no additional substrate and no supplementary feeding - Control 2.

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Before the termination of the experiment, water samples for plankton analysis were collected from different treatments by filtering 10L of pond water through a plankton net of 50 μm mesh and analysed quali- and quantitatively to determine the forage ratio. At the end of the experimental schedule (90 days), 10 fish from each treatment were collected and sacrificed for following investigations.

1. Gut content analysis : Immediately after collection, the fish were dissected and the digestive tract along with its contents were removed and preserved in 5 per cent formalin. Thereafter, the gut contents were analysed both quantitatively and qualitatively. Food items were identified mostly upto the generic level as most of the items were in advanced stage of digestion which rendered identification a little difficult.

2. Forage ratio was calculated as follows :

$$\text{Forage ratio} = \frac{\text{\% of organisms in the gut contents}}{\text{\% of organisms present in the water/bottom sample}}$$

If forage ratio is 1, it indicates random selection of food from the environment.

If forage ratio <1, it indicates avoidance of particular food item.

If forage ratio >1, it indicates that the item is actively preferred or chosen by the fish.

3. Relative length of the gut (RLG) and Digestive somatic index (DSI) : The fish was dissected to remove the alimentary canal and was separated from the mesenteries. After recording the weight, the length of uncoiled intestine was recorded. Relative length of the gut (RLG) and digestive somatic index (DSI) were calculated following Hepher (1988) and Hidalgo (1999) as follows :

$$\text{Relative length of gut} = \frac{\text{Length of gut}}{\text{Length of body}}$$

$$\text{DSI} = \frac{\text{Digestive tract wt.}}{\text{Body wt.}} \times 100$$

Statistical analysis:

P values between the experimental and control groups were calculated by student's 't' test (Snedecor and Cochran, 1982).

RESULTS

Food and feeding habits

(i) Gut content analysis of *O. niloticus* from monoculture ponds (Table 1), RLG and DSI values.

Analysis of gut contents indicated that chlorophyceae (40-44.52%), was the dominant group (Table 1). Detritus/debris was highest (45%) in fish collected from control 1 ponds where the fish were fed on supplementary feed. No zooplankton/insect or arthropod appendages were observed, indicating that the fish is a herbivore - phytoplankton feeder. Results have revealed significantly ($P<0.05$) high values of forage ratio (Table 2) for *Cladophora* (1.04-2.49) and *Coelastrum* (0.74-1.01). Whereas the values for *Navicula, Nitzschia* and *Synedra* were always less than one clearly revealing that *O. niloticus* is a herbivore and *Cladophora, Coclastrum* and *Closterium* are the preferred food items.

Significantly ($P<0.05$) high values of RLG (8.08±0.14) were observed in treated ponds where an additional substrate was provided (Table 3) revealing that *O. niloticus* is a microalgae feeder herbivore as also revealed by the gut content analysis and forage ratio. The values of RLG though were low in control 1 than control 2, however, the differences were not statistically significant. The digestive somatic index (DSI) values remained significantly ($P<0.05$) high in control 2 ponds (Table 3) where no supplementary feeding or an additional substrate was provided.

Gut content analysis of *E. suratensis* from monoculture ponds (Table 1)

Analysis of gut contents of *E. suratensis* collected from monoculture ponds indicated large quantities of mud/debris (41.0-43.3%) in the gut. The other gut contents consisted of chlorophyceae (32.19-35.02%) as the dominant group, followed by bacillariophyceae (18.19-20.00%) (Table 1). Appendages of some zooplanktons/copepods were also observed in the gut contents of this fish. High values of forage ratio were observed for *Synedra* (1.33) and *Navicula* (1.01-1.66) in ponds with additional substrate. The forage ratio for *Closterium* was high (1.42) in control ponds, indicating members of chlorophyceae were the preferred food items for this fish (Table 2). Forage ratio for zooplankton/copepod appendages was less than one (Table 2).

RLG values (7.18±0.08) for *E. suratensis* were significantly ($P<0.05$) high in control 2, followed by

Table 1: Gut content analysis (% composition of food components) of *Oreochromis niloticus* and *Etroplus suratensis* from different treatments under monoculture (A) and polyculture (B) - Duration of experiment 90 days

Food components	*Oreochromis niloticus*			*Etroplus suratensis*		
	With additional substrate	Control 1	Control 2	With additional substrate	Control 1	Control 2
A. MONOCULTURE						
Debris	40.34	45.00	41.05	41.00	43.30	41.00
Chlorophyceae	43.08	40.00	44.52	33.00	32.19	35.02
Bacillariophyceae	16.58	15.00	14.43	20.00	18.76	18.19
Zooplankton	-	-	-	6.00	5.85	5.79
B. POLYCULTURE						
Debris	44.02	31.50	40.00	35.20	40.00	47.89
Chlorophyceae	40.21	36.02	40.48	34.22	30.00	16.89
Bacillariophyceae	15.77	30.07	19.52	21.65	20.00	25.12
Zooplankton	-	1.41	-	8.93	10.00	10.10

Control 1 : Treatments where no additional substrate was provided, however, the fish were fed on supplementary diet
Control 2 : Treatments where no additional substrate and no supplementary diet were provided

Table 2: Forage ratio of *Oreochromis niloticus* and *Etroplus suratensis* from different treatments under monoculture (A) and polyculture (B) - Duration of experiment 90 days

Food components	*Oreochromis niloticus*			*Etroplus suratensis*		
	With additional substrate	Control 1	Control 2	With additional substrate	Control 1	Control 2
A. Monoculture						
Closterium	1.02	0.74	1.01	0.42	0.82	1.42
Coelastrum	1.33	0.97	1.29	1.05	0.89	1.38
Cladophora	2.49	1.42	1.04	0.62	-	-
Nitzschia	0.96	-	-	-	0.97	-
Navicula	0.91	0.75	0.54	1.01	1.66	1.46
Synedra	-	0.67	0.68	1.33	0.93	0.42
Frustulia	-	-	-	-	-	-
Enteromorpha	-	-	-	-	-	-
Cyclops/appendages	-	-	-	0.66	0.74	0.10
Brachionus	-	-	-	-	-	-
B. Polyculture						
Closterium	1.01	2.79	1.81	0.55	2.15	0.98
Coelastrum	1.41	1.02	1.03	2.37	0.92	1.09
Cladophora	0.72	-	2.98	-	-	-
Nitzschia	0.84	0.82	-	-	-	1.31
Navicula	1.02	1.61	0.43	1.85	1.68	0.61
Synedra	1.10	1.33	0.87	2.34	-	0.89
Frustulia	-	-	0.56	-	-	-
Enteromorpha	-	-	0.43	-	-	-
Cyclops/appendages	-	0.23	-	1.79	-	0.87
Brachionus	-	-	-	-	0.89	0.60

Control 1 : Treatments where no additional substrate was provided, however, the fish were fed on supplementary diet
Control 2 : Treatments where no additional substrate and no supplementary diet were provided

Table 3: Effect of additional substrate on relative length of the gut and digestive somatic index in *Oreochromis niloticus* and *Etroplus suratensis* under mono and polyculture

Treatments	Relative length of gut	Digestive somatic index
***Oreochromis niloticus* (Monoculture)**		
With additional substrate	8.08±0.14^{A}	6.57±0.18^{AB}
Control 1	6.88±0.38^{B}	6.14±0.52^{B}
Control 2	7.12±0.18^{B}	7.10±0.19^{AB}
***Etroplus suratensis* (Monoculture)**		
With additional substrate	6.51±0.14^{BC}	7.94±0.10^{A}
Control 1	6.25±0.22^{C}	6.13±0.28^{B}
Control 2	7.18±0.08^{A}	7.00±0.40^{A}
***Oreochromis niloticus* (Polyculture)**		
With additional substrate	9.11±0.17^{A}	6.25±0.10^{B}
Control 1	8.11±0.15^{B}	6.71±0.93^{A}
Control 2	6.38±0.32^{C}	7.23±0.26^{A}
***Etroplus suratensis* (Polyculture)**		
With additional substrate	6.06±0.17^{C}	6.55±0.40^{A}
Control 1	7.07±0.37^{A}	7.21±0.53^{A}
Control 2	6.67±0.28^{AB}	8.87±0.23^{A}

All values are mean±SE of mean of six observations
Values with the same superscripts in same column for each treatments are not significantly ($P<0.05$) different
Control 1 : Treatments where no additional substrate was provided, however, the fish were fed on supplementary diet
Control 2 : Treatments where no additional substrate and no supplementary diet were provided

treated ponds (treatment provided with additional substrate) and control 1 (Table 3). On the other hand, DSI was significantly ($P<0.05$) high in treated and control 2 ponds in comparison to control 1 ponds (Table 3), however, the differences were not statistically significantly.

Gut content analysis of *O. niloticus* and *E. suratensis* from polyculture ponds (Table 1), RLG and DSI values.

When *O. niloticus* was reared under polyculture along with *E. suratensis*, chlorophyceae (36.02-40.48%) was the dominant group, followed by bacillariophyceae (15.77-30.07%). However, debris was also found in large quantity (31.5-44.02%). Calculation of the forage ratio revealed that the values for *Closterium* were high (1.01-2.79), followed by *Coelastrum* (1.02-1.41) and *Cladophora* (2.98 in control 2 ponds) (Table 2). *Navicula* (1.02-1.61) and *synedra* also appears to be the preferred food items for *O. niloticus* in treated and control 1 ponds. RLG values also showed significantly ($P<0.05$) high values in treated ponds (9.11±0.17) in comparison with the two controls (Table 3). DSI values were, however, high in the two controls in comparison with the fish collected from treated ponds (Table 3).

Analysis of gut contents of *E. suratensis* under polyculture revealed that the fish prefers the members of chlorophyceae (16.89-34.22) and bacillariophyceae (20.00-25.12) in comparison to zooplankton (8.93-10.0) (Table 1). Forage ratio indicated that *Navicula, Synedra* and *Coelastrum* appears to be the preferred food items

by the fish. However, the forage ratio for the zooplankton was high (1.79) in fish grown in treated ponds indicating that *E. suratensis* is an opportunistic omnivore (Table 2). RLG values in this treatment were also high in control 1 (7.07±0.37) where the fish were fed on supplementary feed, followed by control 2 (6.67±0.28) and treated ponds (6.07±0.17) (Table 3). No significant variations in DSI values among different treatments were observed (Table 3).

DISCUSSION

The relative length of the gut seems to be related to the food and feeding habits of the fish species under investigations. Irrespective of the treatments, high RLG and DSI values were observed for *O. niloticus*. The gut content analysis have revealed that *O. niloticus* mainly depends upon vegetable matter as animal matter was found to be completely absent in the gut contents, advocating that the fish is a herbivore macroalgae feeder. Al Hussaini (1947), Kapoor *et al.* (1976) and Bhatnagar *et al.*, (2003) have also concluded that the relative length of the gut is largest in macroalgae feeders, followed by herbivore, omnivore and carnivore fish species thus confirming the present results. RLG values in *O. niloticus* ranged between 6.38 to 9.11, which are close to the RLG values reported for *Labeo dero* (8.9) by Das and Nath (1965) and *Labeo gonius* (9.5) reported by Das and Moitra (1956, 1958, 1963).

On the other hand, significantly high value of RLG in *O. niloticus* in treated ponds than controls further indicate that the fish was mainly subsisting by grazing on periphytic community. Low RLG values in control 1 ponds indicates that even though the ponds were rich in phytoplankton, the fish appears to be mainly subsisting on the supplementary diet. Since no supplementary diet was available in control 2 ponds, RLG and DSI values remained significantly ($P<0.05$) higher than control 1 indicating that the fish had to depend on plant food material available as a result of primary productivity owing to the absence of supplementary feeding.

Results of qualitative and quantitative analysis of gut contents clearly indicate the dominance of filamentous algae and decayed organic matter in the gut of *E. suratensis*. Among miscellaneous items, cyclops/ zooplankton appendages (crustaceans) etc. were observed in considerable quantity again indicating omnivore nature of the fish. These results are in agreement to those of Keshava *et al.* (1988) on food and feeding habits of pearlspot inhabiting Nethravati-Gurpur estuary. Forage ratio and low values of RLG and DSI indicate that *E. suratensis* is a bottom feeder as fairly high percentage of decayed organic matter along with filamentous algae was observed from the gut contents of the fish. Low RLG values of *E. suratensis* kept in treated ponds (6.50±0.14) in comparison to two controls (6.25±0.22, 7.18±0.08) have further indicated the poor devouring habit of the fish on periphytic community and the fish may be preferring more decayed organic matter from the bottom of the pond. Poor growth performance in comparison to *O.niloticus* both under mono and polyculture in fish from treated ponds and control 1 ponds further support these results.

The present study also shows that *E. suratensis* feeds mostly on decayed organic matter, filamentous algae and miscellaneous food items. Sand grains were also present in the stomach indicating a tendency of the fish to feed at the bottom. Earlier reports had indicated that the young ones of this species are herbivores (Alikunhi, 1957; Hora and Pillay, 1962). Prasadam (1971) and Jhingran and Natarajan (1969) have observed that though it feeds on micro and macro vegetation, its food mainly consists of invertebrates such as insect larvae, bivalves, mysids and decayed organic matter. Devaraj *et al.* (1975) stated that in the estuarine habitat the major food items of this species are filamentous algae and they also recorded detritus (24.39%) and sand particles (9.83%) in appreciable quantities.

Semidigested/decayed organic matter which formed a good percentage in the gut of *E. suratensis* was constituted by various macroplankters. Sand particles found in small quantities were probably related to the bottom feeding habit of the fish. However, Thompson (1954) believes that the function of sediments is to act as grinding mill in the degradation of plant cell wall which otherwise is hard to rupture. A detritus feeder is bound to ingest good quantities of sediments and sand particles. Alikunhi (1957), Hora and Pillay (1962), Prasadam (1971) and Jhingran and Natarajan (1969) had also indicated a mixed diet for this species.

Results of gut content analysis of *O. niloticus* also indicated the dominance of chlorophycean algal matter and debris in the gut both under monoculture and polyculture experiments. However, no zooplankton/ insect/ arthropod appendages were observed indicating that this species is a herbivore and primarily feeds on phytoplankton. Significantly ($P<0.05$) high value of RLG

in *O. niloticus* further confirms its long intestine. The values were high in ponds where additional substrate was provided in comparison to other treatments confirming the availability of algal matter for the herbivore fish. Dempster *et al.* (1993) and Huchette *et al.* (2000) also advocated the herbivory of *O. niloticus* and showed that the grazing on periphyton communities developed on additional substrate promotes growth rate.

High values of RLG and DSI in *O. niloticus* and low values in *E. suratensis* indicates the differences in feeding habits of the two fish species under investigation clearly revealing that *O. niloticus* is a herbivore while *E. suratensis* is an omnivore when grown/cultured in inland saline ground waters, irrespective of the experimental conditions.

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PHYSIOLOGICAL BASIS OF VARIATION IN THE DEVELOPMENT OF *TRITICUM AESTIVUM* GRAINS WITH REFERENCE TO THE ROLE PLAYED BY SOME CATABOLIC ENZYMES.

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Abstract

This study analyses the positional differences in wheat grains in respect to dry matter accumulation (yield) and to correlate the same with some physiological and biochemical parameters. Significant variations were discernible in the two types of grains (bolder and smaller). Smaller grains possessed a higher activity of catabolic enzymes namely- amylase, invertase, protease and peroxidase as compared to the bolder grains. The exogenous application of growth promoters to smaller grains increased their dry matter precipitation, an event that was otherwise characteristic of the bolder grain.

Key words : Wheat, bolder and smaller grains, plant growth regulators, catabolic enzymes.

INTRODUCTION

Wheat (*Triticum aestivum*) of grass family – Poaceae (Graminae) – is the major source of food for human beings in the world. To sustain the stretching population, there is a need to study factors constituting the total yield i.e. number of tillers per m^{-2}, grain number per spike and 1000 grain weight and potential upgradation in any of these factors, will help in the grain production substantially. Grain yield may be influenced by the availability of reserve materials vis-a-vis their translocation to developing sinks (Mahapatra *et al.*, 1993). Growth promoters and a few biochemical components may be significant in determining the sink efficiency and/ or the grain yield as suggested by Reilly (1990), Jenner (1993), and Andrews (1994).

In the present study, attention has been directed in exploring the differential behavior of different grains, growing in the same spike, with regard to their capacity to mobilize the dry matter, pattern of key enzymes (catabolic) and few metabolites and to see if exogenous application of different plant growth regulators (PGRs) and their specific inhibitors can bring a change in sink efficiency vis a vis paving the way towards improving grain yield.

MATERIALS AND METHODS

Seeds of *Triticum aestivum L.* variety PBW -343 were obtained from Punjab Agricultural University, Ludhiana. Pots of 30cm size were filled with a mixture of clay, sand and compost in the ratio 1:1:1and seeds were sown at the rate of 8 seeds per pot. Thinning was done after the establishment of seedlings so as to have only three plants of uniform size in the pot. Development of different grains was determined with regular sampling at 10 days, 25 days and 40 days after anthesis (DAA) counting clockwise, the florets in the spike facing upper side of the flag leaf, the grains were numbered as Bold (B_I and B_{II}) and small (S). PGR's namely IAA (100 ppm), GA_3 (100 ppm) and kinetin (10 ppm) and their inhibitors, TIBA (50 ppm), AMO-1618 (50 ppm) and Hadacidin (10 ppm) were injected individually with the help of micropipette into individual spikelet on the day of anthesis.

Starch content in the grains was estimated by the method of Neilson and Gleason (1943) using potato starch as standard. Desired amount of samples were extracted with hot ethanol according to Yemms and Willis (1954) and reducing and non-reducing sugars were analyzed using anthrone reagent and compared using glucose as standard.

Protein concentration in the grains was quantified by Lowry *et al.* (1951) method using bovine albumin as standard.

Enzymes namely alpha- amylase, invertase, protease and peroxidase were assayed in fresh samples

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according to procedures described by Mc Cready *et al.* (1950), Nelson (1944), Dubey and Rani (1990), and Seevers *et al.* (1971), respectively.

RESULTS

Data pertaining to the growth and development pattern of different grains growing in the same ear depicted significant variation in their weight owing to their location as well as age (Table,1). Because of their unusual behavior amongst the growing grains the first grain was designated as B_I (bold), the second one S (small) and third as B_{II} (bold) and this nomenclature was retained for subsequent studies. The application of the three growth regulators (IAA, GA_3 kinetin) resulted in significant increase in dry weight of grains but the promotory effect of growth regulators was more in case of smaller grains as compared to bolder grains. Application of specific inhibitors of growth hormones specifically used in this study namely-TIBA, AMO-1618, Hadacidin, corroborated above finding.

The data on starch and protein contents in the grains are presented in table 2. It is evident that starch content revealed a sequential increase in grains as they progressed towards maturity and the bolder grains possessed relatively more starch than the smaller grains throughout the period of growth. However, under the influence of PGRs, their behavior differed. IAA and kinetin applications enhanced the mobilization of starch at all stages in both the grains nevertheless it was higher in smaller grains. On the other hand GA_3 decreased the starch content, the bolder grains showed reduction in starch level (16.1 per cent) over smaller grain (7.6 per cent) at maturity. The application of growth inhibitors reduced the level of starch content which was more pronounced in smaller grains as compared to bolder ones at 10 and 25 DAA, however this trend reversed at maturity. Protein content declined as grains progressed towards maturity. Depending upon the location the bolder grains possessed higher amount of protein in comparison to the smaller grains at all stages of development. PGRs treatments such as IAA, GA_3 and kinetin enhanced the protein content. Out of all the PGRs, kinetin proved to be relatively more efficient in enhancing protein level since there was increase to the tune of 29.8 to 78.2 per cent as compared to 16.7 to 58.2 per cent of auxin. Application of GA_3 resulted in insignificant increase as compared to IAA and kinetin. Protein content significantly decreased with application of PGR's inhibitors. The maximum reduction in protein content accounted for 90.2 per cent with the inhibitor of GA_3.

The reducing sugars were higher than the non-reducing sugars in the bolder grains (Table, 3), at all the stages of their development. However, this trend was reverse in smaller grains. These solutes accumulated with the application of PGR treatments and levels of accumulation were more in bolder grains. The reducing sugars content decreased on application of inhibitors of growth regulators of IAA and kinetin. The reduction was significantly high in bolder grains as compared to smaller grains at maturity.

With the application of IAA and kinetin the non-reducing sugars content declined notably in the range of 30.4 to 76.0 and 55.6 to 84.5 per cent for IAA and kinetin, respectively. Interestingly, GA_3 treatment enhanced the non-reducing sugar content; the increase was significant in bolder grains (80.4 per cent) than the smaller grains (3.2 per cent) at 10 DAA. Accumulation in non-reducing sugars was apparent with the inhibitors of IAA and kinetin in both types of grains, though increase was more significant in bolder grain as compared to smaller one. With auxin inhibitor TIBA the level of non-reducing sugars rose to 60.2 per cent in bolder grain as compared to 42.9 per cent in smaller grain. On the other hand, GA_3 inhibitor AMO1618 decreased the non-reducing sugar content at all stages of development, the reduction was to the tune of 72.9 to 90.4 per cent, in both types of grain.

It is evident from the data of table, 4 that bolder grains possessed relatively lower activity of alpha-amylase and invertase than smaller grains. There was a decrease in invertase activity in both two types of grains with their maturity. Among the PGRs tested, GA_3 significantly enhanced the activity of alpha amylase in both types of grain although application of IAA and kinetin elicited inhibition of the activity in both types of grains. Application of specific inhibitors resulted into differential effect on the activity of alpha-amylase and invertase in both types of grains. Inhibitor of IAA and kinetin (TIBA, HADACIDIN) increased the alpha-amylase and invertase activity significantly in bolder grain while gibberellin inhibitor caused reduction in enzyme activity in both types of grains. The inhibition was more apparent in smaller grains than in bolder grains at all stages of grain development.

The level of protease and peroxidase activity represented in table 5 revealed that their activity was unequal in both types of grains during all stages of development .The smaller grains had relatively higher activity of protease as compared to bolder grains at 10

Table 1: Effect of plant growth regulators and their specific inhibitors on dry weight of bolder and smaller wheat grains (mg/ grain) at different positions within the same ear.

Mean of 3 replications (parenthesis values show per cent increase (+) and decrease (-) over control).

DAA*	GRAIN TYPE	CONTROL	AUXIN	TIBA	GIBBERELLIN	AMO-1618	KINETIN	HADACIDIN
	B_1	8.20	10.32	6.00	9.34	6.99	9.97	6.17
			(+25.8%)	(-26.8%)	(+13.9%)	(-14.7%)	(+21.5%)	(-24.7%)
10	S	5.00	6.66	3.30	6.09	3.82	7.34	3.56
			(+33.2%)	(-34.0%)	(+21.8%)	(-23.6%)	(+46.8%)	(-28.8%)
	B_{11}	9.50	11.86	6.56	10.63	7.34	12.04	6.34
			(+24.8%)	(-30.9%)	(+11.8%)	(-22.7%)	(+26.7%)	(-33.2%)
	B_1	20.50	22.51	14.17	23.02	16.09	24.03	15.33
			(+9.8%)	(-30.8%)	(+12.2%)	(-21.5%)	(+17.2%)	(-2.2%)
25	S	14.43	18.86	10.88	16.56	13.01	17.98	12.02
			(+30.6%)	(-24.6%)	(+14.7%)	(-9.8%)	(+24.6%)	(-16.7%)
	B_{11}	23.23	26.57	19.29	25.46	20.40	26.57	17.95
			(+14.3%)	(-16.9%)	(+9.5%)	(-12.1%)	(+14.3%)	(-22.7%)
	B_1	31.4	34.25	20.16	32.13	25.16	33.15	19.18
			(+9.9%)	(-35.2%)	(+3.1%)	(-19.2%)	(+4.4%)	(-38.4%)
*M**	*S*	*24.40*	*29.56*	*16.14*	*26.132*	*22.12*	*27.28*	*15.84*
			(+21.2%)	(-33.8%)	(+7.0%)	(-9.3%)	(+11.8%)	(-35.0%)
	B_{11}	30.17	31.16	21.17	31.12	24.15	32.80	20.18
			(+3.2%)	(-29.8%)	(+3.1%)	(-19.9%)	(+9.7%)	(-33.1%)

C.D at 5 per cent level due to Treatment = 0.73, Age = 1.35, Interaction =1.06

B = BOLDER GRAINS
S = SMALLER GRAINS
M*= MATURITY (40 Days)
DAA* = DAYS AFTER ANTHESIS

Table 2: Relative levels of starch and total proteins in bolder and smaller grains of same ear of wheat at different interval of time after anthesis on treatment with different growth regulators and their specific inhibitors.(parenthesis values shows per cent increase (+) and decrease (-) over control). Mean of three replications.

DAA* Starch	GRAIN TYPE	CONTROL	AUXIN	TIBA	GIBBERELLIN	AMO-1618	KINETIN	HADACIDIN
	B	2.300	2.532 (+10.0%)	2.100 (-8.6%)	2.226 (+3.2%)	1.896 (-17.5%)	2.309 (+0.3%)	1.931 (-16.0%)
10								
	S	1.502	1.875 (+24.7%	0.981 (-34.6%)	1.478 (-1.5%)	1.231 (-18.0%)	1.799 (+19.7%)	0.951 (-36.6%)
	B	2.710	3.036 (+12.0%)	2.010 (-25.8%)	2.273 (-16.1%)	1.897 (-30.0%)	3.100 (+14.3%)	0.212 (-92.1%)
25								
	S	2.150	2.651 (+23.3%)	(-25.8%) (-38.5%)	1.986 (-7.6%)	1.821 (-15.3%)	2.761 (+29.3%)	1.381 (-35.7%)
	B	3.381	3.881 (+14.7%)	2.531 (-25.1%)	3.314 (-1.9%)	2.123 (-37.2%)	3.698 (+9.3%)	3.100 (-8.3%)
M*								
	S	2.856	3.258 (+14.0%)	2.800 (-1.9%)	2.751 (-3.6%)	1.896 (-33.6%)	3.214 (+12.5%)	2.750 (-3.7%)
C.D. at 5 per cent level due to: Treatment =0.096, Age= 1.130, Interaction= 0.102								
Total Protein								
	B	15.74	18.37 (+16.7%)	4.21 (-73.2%)	16.9 (+7.3%)	2.31 (-85.3%)	20.44 (+29.8%)	3.88 (-75.3%)
10								
	S	13.82	17.84 (+29.0%)	4.69 (-66.0%)	15.6 (+12.8%)	3.26 (-76.4%)	19.8 (+43.2%)	3.98 (-71.2%)
	B	13.63	17.7 (+29.8%)	4.19 (-69.2%)	15.4 (+12.9%)	2.38 (-82.5%)	18.4 (+34.9%)	3.43 (-83.3%)
25								
	S	9.48	15.0 (+58.2%)	2.33 (-75.4%)	11.9 (+25.5%)	0.92 (-90.2%)	16.9 (+78.2%)	1.58 (-83.3%)
	B	7.34	9.2 (+25.3%)	2.56 (-65.1%)	8.1 (+10.3%)	1.40 (-80.9%)	10.7 (+45.7%)	3.99 (-45.6%)
M*								
	S	5.77	7.4 (+28.2%)	1.95 (-66.2%)	6.6 (+14.3%)	0.59 (-89.7%)	8.4 (+45.5%)	2.01 (-65.1%)

C.D at 5 per cent level due to: Treatment = 0.131, Age = 0.083, Interaction =0.918

B = BOLDER GRAINS S = SMALLER GRAINS DAA* = DAYS AFTER ANTHESIS
M*= MATURITY (40Days)

Table 3: Relative levels of non reducing and reducing sugars in bolder and smaller grains of same ear of wheat at different interval of time after anthesis on treatment with different growth regulators and their specific inhibitors.(parenthesis values shows per cent increase (+) and decrease (-) over control).Mean of 3 replications.

DAA*	GRAIN TYPE	CONTROL	AUXIN	TIBA	GIBBERELLIN	AMO-1618	KINETIN	HADACIDiN
Non Reducing Sugars								
	B	1.168	0.812 (-30.4%)	1.872 (+60.2%)	2.108 (+80.4%)	0.316 (-72.9%)	0.381 (-67.3%)	1.882 (+61.1%)
10								
	S	2.324	0.558 (-75.9%)	0.981 (+42.9%)	2.400 (3.2%)	0.316 (-86.4%)	0.358 (-84.5%)	2.334 (0.4%)
	B	1.558	0.621 (-60.1%)	1.959 (+25.7%)	1.681 (+7.8%)	0.189 (-87.8%)	0.628 (-59.6%)	1.689 (+8.4%)
25								
	S	2.743	0.658 (-76.0%)	3.780 (+37.6%)	2.862 (+4.3%)	0.363 (-19.4%)	0.651 (-76.2%)	2.884 (+5.1%)
	B	1.535	0.651 (-57.5%)	1.768 (+15.1%)	1.640 (+6.8%)	0.188 (-87.7%)	0.681 (-55.6%)	1.580 (+2.9%)
M*								
	S	2.324	0.658 (-71.6%)	3.338 (+43.6%)	2.364 (+1.7%)	0.251 (-89.1%)	0.651 (-71.9%)	2.328 (+0.1%)
C.D. at 5 per cent level due to: Treatment =0.121, Age= 0.093, Interaction= 0.097								
Reducing Sugars								
	B	1.325	1.873 (+41.3%)	0.886 (-33.1%)	1.863 (+40.6%)	0.812 (-38.7%)	1.889 (+42.5%)	0.881 (-33.5%)
10								
	S	1.369	1.443 (+5.4%)	0.741 (-45.8%)	1.731 (+26.4%)	0.781 (-42.9%)	1.837 (+34.1%)	0.781 (-42.9%)
	B	2.089	2.214 (+5.9%)	1.913 (-8.4%)	2.376 (+12.7%)	1.376 (-34.1%)	2.426 (+16.1%)	1.812 (-13.2%)
25								
	S	2.862	3.268 (+14.1%)	1.931 (-32.5%)	2.976 (+3.9%)	1.342 (-53.1%)	3.412 (+19.2%)	1.651 (-42.3%)
	B	2.114	2.218 (+4.9%)	0.915 (-56.7%)	2.268 (+7.2%)	1.364 (-35.4%)	2.296 (+8.6%)	0.812 (-61.5%)
M*								
	S	1.788	1.874 (+4.8%)	0.852 (-52.3%)	2.324 (+29.9%)	1.342 (-24.9%)	2.223 (+24.3%)	0.951 (-46.8%)

C.D at 5 per cent level due to: Treatment = 0.73, Age = 1.35, Interaction =1.06

B = BOLDER GRAINS S = SMALLER GRAINS DAA* = DAYS AFTER ANTHESIS

M*= MATURITY (40 Days)

Table 4: Effect of plant growth regulators and their specific inhibitors on the alpha amylase and invertase activity in the bolder and smaller grains of wheat. Mean of three replications. (parenthesis values shows per cent increase (+) and decrease (-) over control).

DAA*	GRAIN TYPE	CONTROL	AUXIN	TIBA	GIBBERELLIN	AMO-1618	KINETIN	HADACIDIN
Alpha Amylase								
	B	15.08	9.50 (-37.0%)	19.50 (+29.3%)	31.50 (+108.8%)	10.50 (-30.3%)	9.00 (-40.3%)	22.00 (+45.8%)
10								
	S	20.50	11.70 (-42.9%)	24.50 (+19.5%)	38.50 (+87.8%)	8.00 (-60.9%)	12.00 (-41.4%)	21.30 (+3.9%)
	B	14.80	9.60 (-35.1%)	21.30 (+43.9%)	32.60 (+120.2%)	9.10 (-98.5%)	9.50 (-38.5%)	21.30 (+43.9%)
25								
	S	19.50	14.80 (-24.1%)	25.20 (+29.2%)	41.10 (+110.7%)	8.30 (-57.4%)	12.30 (-36.9%)	21.40 (+9.7%)
	B	11.30	6.50 (-42.4%)	14.40 (+27.4%)	34.50 (+205.3%)	7.30 (-35.3%)	7.40 (-34.3%)	14.40 (+27.4%)
M*								
	S	16.50	5.70 (-65.4%)	20.40 (+23.6%)	39.50 (+139.3%)	7.40 (-55.1%)	5.70 (-65.4%)	20.40 (+23.6%)
C.D. at 5 per cent level due to: Treatment =1.15, Age= 0.998, Interaction= 1.083								
Invertase	**B**	12.10	10.10 (-16.5%)	15.10 (+24.7%)	11.80 (-2.4%)	11.50 (-4.9%)	9.80 (-19.0%)	15.40 (+27.2%)
10								
	S	17.10	14.50 (-15.2%)	20.20 (+18.1%)	16.50 (-3.5%)	16.50 (-3.5%)	14.20 (-16.9%)	20.101 (+17.5%)
	B	7.80	6.20 (-20.5%)	9.20 (+17.9%)	7.60 (-2.5%)	6.80 (-12.8%)	9.10 (-21.7%)	9.10 (+16.6%)
25								
	S	12.80	7.50 (-41.4%)	14.10 (+10.1%)	12.70 (-0.7%)	11.90 (-7.0%)	740 (-42.1%)	14.50 (+13.2%)
	B	5.20	4.10 (-21.1%)	7.20 (+38.4%)	5.10 (-1.9%)	5.10 (-1.9%)	4.50 (-13.4%)	6.80 (+30.7%)
M*								
	S	12.10	8.20 (-32.2%)	15.20 (+25.6%)	11.40 (-5.7%)	11.30 (-6.6%)	8.10 (-33.0)	14.70 (+21.4%)

C.D at 5 per cent level due to: Treatment = 0.089, Age = 0.101, Interaction =0.093

B = BOLDER GRAINS S = SMALLER GRAINS DAA* = DAYS AFTER ANTHESIS M*= MATURITY (40Days)

Table 5: Effect of plant growth regulators and their specific inhibitors on the protease and peroxidase activity in the bolder and smaller grains of wheat. Mean of three replications. (parenthesis values shows percent increase (+) and decrease (-) over control).

DAA*	GRAIN TYPE	CONTROL	AUXIN	TIBA	GIBBERELLIN	AMO-1618	KINETIN	HADACIDIN
Protease								
	B	0.40	0.37 (-7.5%)	0.56 (+40.0%)	0.38 (-5.0%)	0.58 (+45.0%)	0.35 (-12.5%)	0.61 +52.5%
10	S	0.44	0.35 (-20.4%)	0.58 (+31.8%)	0.36 (-18.1%)	0.63 (+43.1%)	0.33 (-25.0%)	0.62 +40.9%
	B	2.48	1.92 (-22.5%)	3.41 (+37.5%)	2.17 (-12.5%)	2.96 (+19.3%)	2.00 (-19.3%)	3.52 (+41.9%)
25	S	1.88	1.51 (-19.6%)	3.81 (+102.6%)	1.60 (-14.8%)	3.59 (-90.9%)	1.53 (-18.6%)	3.71 (+58.3%)
	B	1.49	1.25 (-16.1%)	2.56 (+71.8%)	1.38 (-7.3%)	2.61 (+75.1%)	1.118 (-20.8%)	2.36 (+58.3%)
M*	S	1.60	1.39 (-13.1%)	2.81 (+75.6%)	1.48 (-7.5%)	2.93 (+83.1%)	1.39 (-13.1 %)	2.65 (+65.6%)
C.D. at 5 per cent level due to: Treatment =0.017, Age= 0.019, Interaction= 0.021								
Peroxidase								
	B	3.75	2.59 (-30.9%)	3.91 (+4.2%)	2.63 (-29.8%)	3.96 (+5.6%)	2.69 (-28.2%)	3.87 (+3.2%)
10	S	4.31	3.41 (-27.1%)	4.51 (+4.6%)	3.12 (-27.6%)	4.52 (+4.8%)	3.04 (-29.4%)	4.43 (+2.7%)
	B	2.56	1.89 (-23.3%)	2.61 (+1.9%)	1.87 (-26.9%)	2.67 (+4.2%)	1.78 (-30.4%)	2.73 (+6.6%)
25	S	3.86	2.96 (-23.3%)	3.98 (+34.9%)	2.94 (-23.8%)	3.94 (+2.0%)	2.87 (-25.6%)	3.92 (+1.5%)
	B	2.12	1.60 (-24.5%)	2.86 (+34.9%)	1.63 (-23.1%)	2.84 (+33.9%)	1.72 (-18.8%)	2.82 (+33.0%)
M*	S	2.17	1.81 (-33.2%)	3.12 (+15.1%)	1.89 (-30.2%)	3.14 (+15.8%)	1.91 (-29.5%)	3.18 (+17.3%)

C.D at 5 per cent level due to: Treatment = 0.032, Age = 0.041, Interaction =0.060

B = BOLDER GRAINS S = SMALLER GRAINS DAA* = DAYS AFTER ANTHESIS

M*= MATURITY (40Days)

DAA and maturity, while at 25 days the activity was reverse. As such peroxidase activity decreased in both types of grains irrespective of their localization. Upon treatment of growth promoters a tendency in decrease of enzyme activity was noticed. Auxin caused inhibition in the range of 23.3- 33.2 per cent in both types of grains. With kinetin and GA_3, the decrease in activity was in the range of 18.8-30.4 and 23.1-30.2 per cent respectively for both types of grains. Application of inhibitors of PGRs enhanced the enzyme activity in both types of grains and all three inhibitors showed similar responses. At maturity, peroxidase activity increased to 34.9, 33.9 and 33.0 per cent for IAA, GA_3 and kinetin inhibitors, respectively.

DISCUSSION

The results show that bolder grains are conspicuous by a few prominent characteristics like higher level of starch, probably at the cost of non-reducing sugars, higher quantity of proteins, and lesser activity of some catabolic enzymes. These studies corroborate to the postulations that relatively lower endogenous levels of auxins and cytokinins in smaller grains were responsible for a variable metabolic profile thereby causing a reduction in yield potential. Furthermore, these selected physiological parameters in smaller grains, when studied under the influence of growth promoters, tend to follow the pattern existing in the bolder grains. Several workers in the past have reported interaction of growth regulating substances with sink efficiency and their involvement in influencing the yield potential in crops other than wheat (Klee and Estella, 1991; Chandler and Robertson, 1994: Andrews, 1994; Patrick and Offer, 1996). Based on the earlier findings and the extensive studies conducted in our laboratory (Dua *et al.*, 1990,1991) on this aspect it can be concluded that positional differences in the yielding ability among grains was inherent and was probably determined by the endogenous levels of hormones and it is through these regulators that metabolism, translocation and/ or resultant growth potentiality of grains are regulated.

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EXPLOITATION OF FIELD EGG PARASITOID *USCANA FEMORALIS* PAJNI AND SOOD FOR THE CONTROL OF STORE PEST, *CALLOSOBRUCHUS MACULATUS* (F.)

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Abstract

Uscana femoralis Pajni and Sood, an egg parasitoid of field bruchid pests has been made to adapt to the eggs of Callosobruchus maculatus (F.), a serious pest of stored legumes. The parasitoid lays its egg within the egg of the host, and completes its development on an average in 9 days at controlled conditions of 28±1°C and 78%R.H. The maximum individuals emerge during the first two days of emergence. The male and female sex ratio is 1: 4.8. The adults copulate within 1-2 hours after emergence. The parasitoid prefers freshly laid eggs of the bruchid for oviposition. On an average 45.86 eggs with a maximum of 72 eggs and minimum of 31 eggs are laid by a mated female. The average developmental mortality of the parasitoid is 4.2%. The males live longer than females with an average longevity of 2.47 and 1.4 days respectively.

Key words : *Uscana femoralis*, Egg Parasitoid, Biological Control, Store Pest, *Callosobruchus maculatus*.

INTRODUCTION

The members of family Bruchidae, are small sized phytophagous beetles which attack a variety of host seeds both in the field and in the stores. Major losses are caused to pulses, beans, grams and lentils by four species of bruchids viz., *Callosobruchus maculatus* (F.), *C.analis* (F.), *C.chinensis* (L.) and *Zabrotes subfasciatus* (Boh.). These bruchids are attacked by larval as well as egg parasitoids. As bruchids lay their eggs on the surface of seed, their destruction by egg parasitoids saves the seed, hence these egg parasitoids need to be investigated. In India, the store bruchids are attacked by a single species of egg parasitoids viz., *Uscana mukerjii* (Mani). Efforts were made to procure some more *Uscana* species from the eggs laid on wild legumes by field bruchids and check the probability of rearing them on the eggs of store bruchids. *Uscana femoralis* Pajni and Sood described from the eggs of *Conicobruchus albopubens* by Pajni and Sood (1999) has been found attacking several other species of field bruchids. *U.femoralis* collected from the field was made to infest the eggs of store bruchids and it has successfully completed its development on the eggs of *C.maculatus*. The general biology of *U.femoralis*, especially its reproductive behavior was studied in laboratory to evaluate its potential for biocontrol of store bruchids.

MATERIALS AND METHODS

The adults of *Uscana femoralis* were obtained from the parasitized eggs of field bruchids attacking the green pods of *Albizzia lebbek* Benth 'Sarin', a common tree which starts blooming in June-July with fresh pods appearing towards the end of August. The green pods of *A.lebbek* were collected during the surveys on the important roads in the interior of Punjab and Haryana states as well the roads connecting Chandigarh with Himachal Pradesh. Pods were procured from about two hundred trees. The trees showing attack by bruchids were marked for repeated collections. The parasitized bruchid eggs turn yellow, orange, brown and black during the development of the parasitoid in them. Such coloured eggs were kept in separate culture tubes and emerging wasps were collected to make the parasitoid breed on the eggs of store bruchid pest, *Callosobruchus maculatus*. The stock cultures of *C.maculatus* were maintained on healthy sterilized seeds of *Vigna radiata* (L) 'Moong'. The copulated female parasitoids were transferred to culture tubes containing pest cultures. After some reluctance, the parasitoid started laying eggs in the eggs of its new host and a progeny of the parasitoids was obtained which bred successfully on the eggs of *C. maculatus*.

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Blackish host eggs containing nearly fully formed adult wasps were kept singly in glass vials plugged with cotton to obtain fresh adults for different experiments. Adult wasps were easily sexed on the basis of abnormally broad hind femur in the male. The stock cultures of *U.femoralis* on the eggs of *C.maculatus* and the experimental sets to study the biological characteristics of the parasitoid were kept under controlled conditions (29 ±1°C and 70% R.H.).

RESULTS

U.femoralis attacks in nature the eggs of field bruchid *Conicobruchus albopubens* (Pic.), a pest of fodder plant, *Cyamopsis psoralioides* 'Guara' and four species of bruchids viz., *Bruchidius pygomaculatus* Arora, *B.saundersi* (Jek.), *B.aureus* Arora and *B.multilineolatus* Arora attacking the pods of *Albizzia lebbek*. These field bruchids lay their eggs on the surface of pod where the seed is developing inside it. The female parasitoid lays an egg inside the bruchid egg, which turns yellow, then orange then brown and finally black as the parasitoid grows. The larval and the pupal stages of the parasitoid wasp are passed inside the bruchid egg and the adult emerges through a hole in the host egg shell. The contents of the host egg are consumed by the parasitoid to complete its development and the seed is saved from the attack of the bruchid pest. This parasitoid of field has successfully adapted itself on the eggs of store bruchid, *Callosobruchus maculatus* during laboratory trials.

Emergence : *U.femoralis* completes its life cycle on the egg of *C.maculatus* and the adult emerges after cutting a circular hole in the chorion of the host egg. Position of the hole coincides with the head of the parasitoid within the egg. The adult first makes a small puncture and slowly enlarges it by cutting away thin strips of the chorion with its mandibles. The total time recorded for emergence is 5-30 minutes. The newly emerged adult rests for 1-3 minutes. Then it rubs its forelegs on wings in order to dry and clean the latter. It takes 5-20 minutes for wings to dry and expand. The first adult comes out on the 9th day of egg laying, emergence reaches maximum on 10th day and continues till 13th day (Table I). According to Huis and Appiah (1995) eclosion of offsprings of *U.lariophaga* took place from 7th –11th day. *U.femoralis* has been seen to emerge at any hour of day and males and females emerge randomly. Kapila and Agarwal (1995) also reported the emergence of *U.mukerjii* at any hour of the day. However, Sood (1999) observed that the adults of *U.mukerjii* usually emerge in early hours. According to Gagnepain and Rasplus (1989) the males of *U.caryedoni* emerge 20-24 hours before females. In most *Trichogramma* species males emerge shortly before the females (Waage and Ming, 1984). Generally, one adult of *U.femoralis* develops in an egg of *C.maculatus*. However, the emergence of two adults from one host egg is also common. Both the individuals emerging from same egg are viable and fertile but they are smaller in size than their counterparts emerging singly. Sood (1999) reported that only one *U.mukerjii* adult emerges from one egg of *C.maculatus*. More than one individual emerges from an egg in many species of *Trichogramma* as per reports of Stern and Atallah (1965).

Sex Ratio : A mated female of *U.femoralis* produces a progeny of both sexes. Female off-springs are always produced more in number than males. The average male: female ratio is 1:4.8 (Table 2). The female mates only once in its lifetime while male exhibits polygamy. This explains for the production of less number of males as compared to females. Also, the species is arrhenotokous, it often resorts to parthenogenetic mode of reproduction. According to Pajni *et al.*, (1997) the male\female ratio in *U. mukerjii* under similar optimum conditions (30°C &70% R.H.) is 1:2.63.

Longevity : The males live invariably longer than females. Average longevity of male and female is 2.47 days and 1.47 days respectively. The life span of *U.femoralis* differs not only in male and females but also in mated and unmated sexes. The mated females of *U.femoralis* have a longer life span than the virgin females where as reverse is true for the males. Average longevity of mated and unmated females provided with host eggs has been observed to be 40 hours and 28.5 hours respectively whereas unmated male lives for 66.6 hours as compared to 52 hours of mated male. Pajni *et.al.*(1997) also observed differences in longevity of two sexes as well as in mated and unmated individuals of both sexes in *U.mukerjii*. According to Sood (1999) longevity of mated and unmated females of *U.mukerjii* differed insignificantly from each other, whereas unmated males live longer than the mated males. Kapila and Agarwal (1995) reported that both unmated males and females live longer than the mated ones.

The longevity of *U.femoralis* varies with fluctuation in lab temperature during different months even when the adults are kept in controlled conditions of temperature and humidity. High temperature reduces the life span of parasitoid whereas the low temperature prolongs it. Longevity of wasp kept in BOD (29 ± 1°C and R.H.

Table 1: Emergence pattern of *U. femoralis* from the eggs of *C.maculatus* (29 ±1°C, R.H. 70%)

S.No.	Emergence on day										% Emergence in first two Days	
	9th		10th		11th		12th		13th			
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1.	-	1	2	22	-	-	-	-	-	-	100	100
2.	5	6	5	29	-	-	-	1	-	1	100	94.5
3.	1	1	6	33	-	-	-	5	-	2	100	82.9
4.	-	2	5	40	-	-	2	3	-	-	71.4	97.6
5.	1	-	6	35	-	-	-	1	-	-	100	97.2
6.	2	5	5	30	-	2	-	3	-	-	100	87.5
7.	2	7	3	19	-	-	1	2	-	2	83.3	86.6
8.	4	9	3	21	-	-	1	2	-	1	87.5	90.9
9.	2	9	4	22	-	-	-	6	-	3	100	77.5
10.	1	6	2	14	-	1	-	5	-	1	100	74.07

Table 2: Sex Ratio in U. femoralis reared on the eggs of C. maculatus (29 ±1°C and R.H. 70%)

S.No.	Emergence of Males		Emergence of Females		Male : Female
	Number	Percentage	Number	Percentage	
1.	15	25.42	44	74.58	1:2.9
2.	8	19.51	33	80.49	1:4.1
3.	14	18.42	62	81.58	1:4.4
4.	10	21.28	37	78.72	1:3.7
5.	7	14.89	40	85.11	1:5.7
6.	7	13.46	45	86.54	1:6.4
7.	7	16.28	36	83.72	1:5
8.	6	13.04	40	86.96	1:6.6
9.	3	10.00	27	90.00	1:9
10.	4	13.33	26	86.67	1:6.5
Avg.	**8.1**	**17.20**	**39.00**	**82.80**	**1:4.8**
S.D.	**3.90**	**4.61**	**10.29**	**4.61**	

70%) was observed to be maximum of 1-2 days in females and 2-3 days in males in colder months of December and January. As the outside temperature started rising, the longevity gradually started decreasing reaching a minimum of less than 1 day in females and less than 2 days in case of males in the month of April. According to Pajni *et.al.* (1997) the shortest life span of *U.mukerjii* was witnessed during the months of May and June and the longest in December.

Reproductive Behaviour : The virgin female produces only male offsprings whereas a mated female produces offsprings of both sexes.

Copulation : The males and females of *U.femoralis* are sexually mature on emergence. Adults procured from the field bruchid eggs initially seemed very reluctant to copulate in captive conditions. Many times the females laid eggs parthenogenetically even when they were kept along with the males. But once they were well adapted to the laboratory conditions, copulation took place without fail. They generally copulate within 1-2 hours after emergence.

When a freshly emerged virgin female is kept with a male in a vial, they move randomly with high mobility, passing by each other several times without halting. However after some time, the male on sensing the presence of female, runs towards her and mounts her body. It seems that after a short while of its emergence the female emits some pheromones, which guide a male in locating a virgin female unerringly even when they are confined together in a large petridish. If the female is not ready for copulation, she continues moving and finally dismounts the mounted male. A receptive female becomes still and allows the male to mount her body. The male clasps the female with its legs and starts fluttering its wings. It then, while still mounting on the female, assumes a nearby vertical position and bends the tip if its abdomen downwards and forwards to make the genital contact, leading to a successful coition. After copulation, the female pushes away the male with its hind legs or sometimes she moves ahead causing the male to fall on its back. The female then sweeps its abdomen and genitalia with its legs and moves away. The entire process from mounting to separation is completed in 70 sec. on an average, with a range of 40 sec. to 100 sec. The pair remains in genital contact for 3-5 sec. According to Chatterji (1953) and Pajni *et al.* (1997) copulation process in *U.mukerjii* is completed in 80 sec. and 94 sec. respectively.

The females of *U.femoralis* mate only once in their life whereas males may copulate upto 5 females. The minimum time period between two successive copulations by a male was noted to be 4 min. According

to Sood (1999) *U.mukerjii* male may copulate with upto 10 females while Kapila and Agarwal (1995), on the other hand observed the male mating with upto 4 females only.

Oviposition : The copulated females start ovipositing within an hour after copulation. While searching for a suitable host egg for oviposition, the female wasp moves about examining the area with its continuously vibrating, anteriorly or downwardly directed antennae. If the host egg is in her view, it takes a leap and successfully lands straight over the host egg. The females have been seen jumping to the host eggs from a distance of about 8-11 mm and it was amazing to watch that they never missed their target. Otherwise, if the egg is not visible to her, she goes all around the pulse seed examining the surface with her antennae in order to locate the host egg. After finding an egg on the seed, it carefully taps its surface with the tip of her antennae, the process being referred to as 'drumming' by Strand and Vinson, 1984. The purpose of drumming is to gather information regarding the age and health of the host eggs. The female of *U.femoralis* is very particular in selecting an egg for ovipositing and rejects an old, damaged egg.

After making the final selection, the female wasp taps the egg vigorously with her antennae, presumably to determine the proper place for the insertion of ovipositor. After 7-10 sec. the female stops, protrudes the ovipositor to touch the egg surface and starts drilling a hole in the chorion of the host egg by moving the ovipositor up and down. The forward and backward movements of abdomen are followed by lowering of the abdomen on to the host egg for depositing the egg. It takes 80 sec. to 155 sec. for the parasitoid to drill a hole in the freshly laid host egg, lay an egg and then withdraw its ovipositor. In *U.mukerjii*, the time taken for laying an egg varies from 5-8 min. (Pajni *et al.*, 1997). Huis *et. al.* (1991) have recorded the oviposition time of 25 sec. in *U.lariophaga*. The female wasp after laying an egg walks over the host egg presumably for marking the parasitized host egg with sticky host material adhering to her ovipositor. A mated female lays a maximum of 72 eggs and a minimum of 31 eggs with an average of 45.86 eggs (Table 3). Whereas an unmated female lays on an average 42.5 eggs with a maximum of 53 and

Table 3: Oviposition record of mated and unmated females of *U. femoralis* on the eggs if *C. maculatus* in the month of November

S.No.	Number of host eggs infected on different days				Total number of host eggs infested		Peak day of oviposition		Period of oviposition (days)	
	Ist day		IInd day							
	Mated	Unmated	Mated	Unmated	Mated	Unmated	Mated	Unmated	Mated	Unmated
1.	27	34	4	8	31	42	1st	1st	2	2
2.	26	36	9	12	35	48	1st	1st	2	2
3.	31	27	-	-	31	27	1st	1st	1	1
4.	55	37	16	14	71	51	1st	1st	2	2
5.	41	26	2	2	43	28	1st	1st	2	2
6.	54	31	18	2	72	33	1st	1st	2	2
7.	20	34	18	19	38	53	1st	1st	2	2
8.	30	44	7	3	40	47	1st	1st	1	2
9.	38	45	6	3	59	48	1st	1st	2	2
10.	40	45	15	3	38	48	1st	1st	2	2
Avg.	**36.2**	**35.9**	**10.55**	**7.33**	**45.8**	**42.5**			**1.8**	**1.9**
S.D.	**11.67**	**6.99**	**6.24**	**6.28**	**15.68**	**9.62**			**6.42**	**0.31**

Average no. of eggs laid by a female during its life time = 42.5
Average oviposition Period = 1.9 days

Table 4: Developmental Period of *U. femoralis* at controlled conditions (29 ±1°C, R.H.70%) during the months of April, October and November/ December

Months	Total Developmental Period	Stages of development on subsequent day of parasitization				
		Yellow	Orange	Brown	Black	Emergence
April	8	3	4	6	7	8
October	9	3	5	7	8	9
Nov./Dec.	10	3	5	8	9	10

Data based on 10 observations

minimum of 27 eggs. The time interval between successive egg layings observed during the laying of first 10 eggs by *U.femoralis* on *C.maculatus* host eggs varies from 1 minute to 8 minutes. The peak period for oviposition is between 12-24 hours of the start of egg laying. The average oviposition period of the mated and unmated female is 1.86 days and 1.9 days respectively.

It has been observed that the host eggs, which are not properly pasted on the pulse seeds, are also parasitized by *U.femoralis*. The females lay yellow coloured, rounded eggs on the sides of the test tube, in case they are not provided with host to lay their eggs in.

The female generally deposits one egg per host egg but laying of two eggs in the same host egg is very common even when sufficient number of host eggs are available. Both the larvae develop to maturity. Occurrence of a maximum of 25.8% of double emergence has been noticed in the eggs of *U.femoralis*. According to Pajni *et al.* (1997) *U.mukerjii* female normally deposits only one egg, however, in a no choice situation more than one egg can be laid in the same host egg, although only one larva finally develops to maturity.

Developmental Period : It takes on an average 9 days to complete its development at controlled conditions (29 ±1°C and R.H. 70%). The parasitized host eggs first turn yellow, then orange, then brown and finally black. The yellow and orange stages correspond to early and late larval stages whereas brown and black colour are early and late pupal stages. The host eggs turn yellow on 3rd day, orange on the 5th day, brown on 7th and black on 8th day of parasitization. The emergence of adult parasitoid occurs within 24 hrs. of the host egg turning black. The developmental period of *U.femoralis* shows variation during different months (Table 4). Fluctuations in the normal developmental period of 8-10 days of *U.mukerjii*, during the different months have been recorded by Sood (1999).

Developmental Mortality : The host eggs that turn yellow without the further development of the parasitoid indicate the mortality of the parasitoid during the larval stage. The brown/black eggs, which fail to produce the adult parasitoids, show the mortality at pupal stage. The average developmental mortality of *U.femoralis* under controlled conditions (29 ±1°C, 70% R.H.) is 4.2% with 2.44% early/larval mortality and 1.76% late/ pupal. Late mortality rarely occurred during emergence as very few parasitoids were observed stuck half way in the emergence hole of the egg. A relatively higher developmental mortality of 10-15% under optimum rearing conditions (30°C, 70% R.H.) has been recorded in *Uscana mukerjii* by Pajni *et al.*, 1997.

Conclusions : *Uscana femoralis*, an egg parasitoid of the bruchids attacking wild legumes when reared on the eggs of store pest *C. maculatus*, exhibits all the characters of a good biocontrol agent i.e. high fecundity, short development period and high detection power of the host.The parasitoid can thus be further exploited for biocontrol operations of store bruchid pests.

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ON THE VARIATIONS IN LOCATION OF GENITAL MARKINGS IN *AMYNTHAS MORRISI* BEDDARD (1892) AND *METAPHIRE POSTHUMA* VAILLANT (1868) (OLIGOCHAETA : MEGASCOLECIDAE)

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Abstract

The paper includes variations in location of genital markings of two species of earthworms, Amynthas morrisi Beddard (1892) and Metaphire posthuma Vaillant (1868) of Megascolecidae. A brief comparison of taxonomically important features for identification of these species is also added.

Key words : *Amynthas morrisi, Metaphire posthuma,* genital markings, Megascolecidae, Oligochaeta.

Gates (1972) reported genital markings as modifications of certain areas of the epidermis on the ventral surface of the sexually mature earthworms. Julka (1981) substantiated the systematic value of the shape, number and location of genital markings, although variable. Julka (1988), further well-documented that the genital markings are distinctly delimited and have slightly depressed central areas. This article presents observations on the variations in location of genital markings of two species of earthworms, *Amynthas morrisi* Beddard (1892) and *Metaphire posthuma* Vaillant (1868) both belonging to family Megascolecidae. The specimens of each species have been recorded from more than one locality and different ecological niches such as gardens, cultivated fields, orchards, margins of aquatic bodies, kitchen drainages, manure heaps, mud-soils etc. spread over Ludhiana, Doraha, Sirhind, Kapurthala, Hoshiarpur, Jalandhar, Gurdaspur, Faridkot, Phillaur from Punjab and Anandpur Sahib, Garhshankar forest area, Delhi, Ambala, Panipat and Bareilly.

These areas were explored for a period of more than three years i.e. from July, 1999 to August, 2003 except in the months of December and January due to non-availability of worms during extreme cold conditions in the upper soil profile. Live worms were brought to laboratory for identification. For diagnostic taxonomic characters, the worms were dissected after narcotizing in 30% ethyl alcohol, fixing in 10-15% formalin for 24 hours and finally preserving in 10-12% formalin. The anatomical details were examined under dissecting binocular microscope.

Although a consolidated account on taxonomic studies towards identification of earthworm species has been made by Michaelsen (1909), Stephenson, (1923,1930), Gates (1972) and Julka (1988), a limited comparative studies based on variations in diagnostic identification features has led to paucity of literature in this regard. The only reported species so far is *Amynthas diffringens* Baird, 1869 which has been discussed for location of genital markings (Julka, 1981). Important taxonomic characters and variations in location of genital markings examined in total 17 specimens of *A. morrisi* and 22 specimens of *M. posthuma* are recorded in Tables 1 & 2 respectively. One of the paired markings, either left or right side, is missing in some specimens (Figs. 1 to 7).

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Abbreviations used in figures:

CLI = Clitellum; FGP = Female Genital Pore; GM = Genital Marking; MGP = Male Genital Pore; PG = Prostate Gland; ST = Spermatheca

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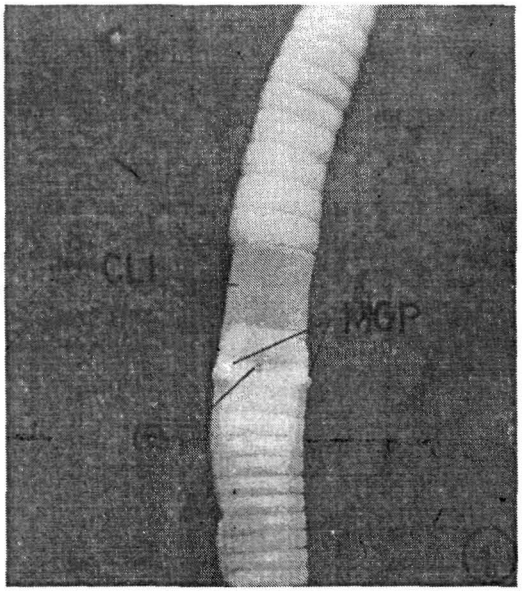
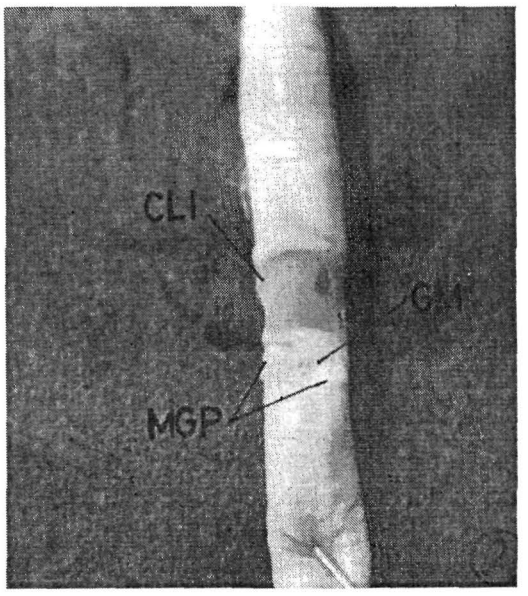
Table 1: Comparison of two earthworms from Megascolecidae.

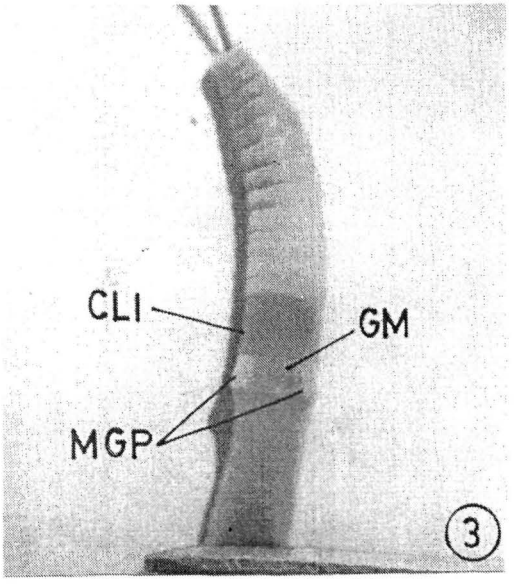
Species/Character	*Amynthas morrisi*	*Metaphire posthuma*
Length (mm)	95-115	80-130
Diameter (mm)	4.5-5.0	4.5-6.5
Segments	90-100	90-135
First functional dorsal pore	10/11	12/13
Clitellum	XIV-XVI	XIII ½-XVI ½or XVII
Male pores	XVIII	XVIII
Female pores	XIV	XIV
Spermathecal pores	2; 5/6,6/7	4; 5/6-8/9
Seminal vesicles	XI, XII	XI, XII
Prostate glands	XVII-XXII	XV-XXI

Table 2: Variation in location of genital markings in two earthworm species.

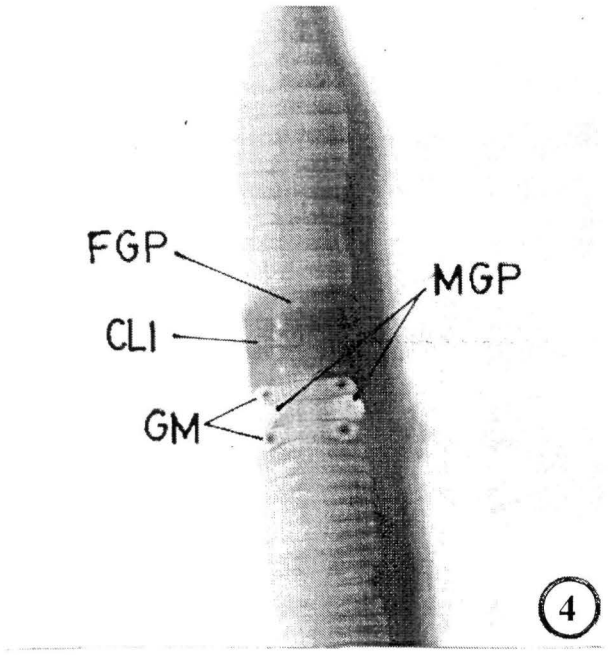
S. No.	Name of Species	Segment on which genital marking is present	Number of genital markings	Position of genital markings	Number of specimens
1.	*Amynthas morrisi* Beddard, 1892	VI	Paired	Ventro-lateral	2
		VII	Single	Ventro-median	7
		XVIII	Paired	Ventro-median	2
		XVIII	In triplicate	Ventro-median	4
		XVI	More than three	In continuous line	2
					Total: 17
2.	*Metaphire posthuma* Vaillant, 1868	XVII	Single	Ventro-Lateral	5
		XIX	Single	Ventro-Lateral	2
		XIX	Paired	Ventro-Lateral	9
		XX	Single	Ventro-Lateral	2
		XXI	Single	Ventro-Lateral	In same specimen
		XXII	Single	Ventro-Lateral	
		XXIII	Single	Ventro-Lateral	
					Total : 22

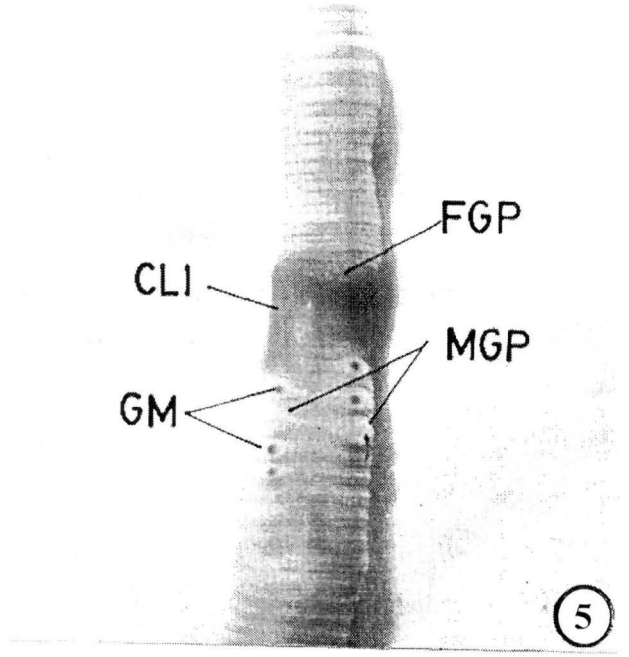


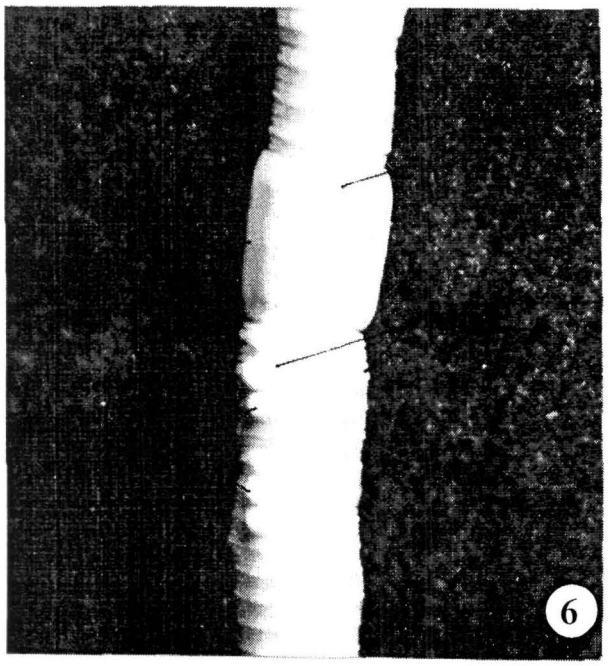


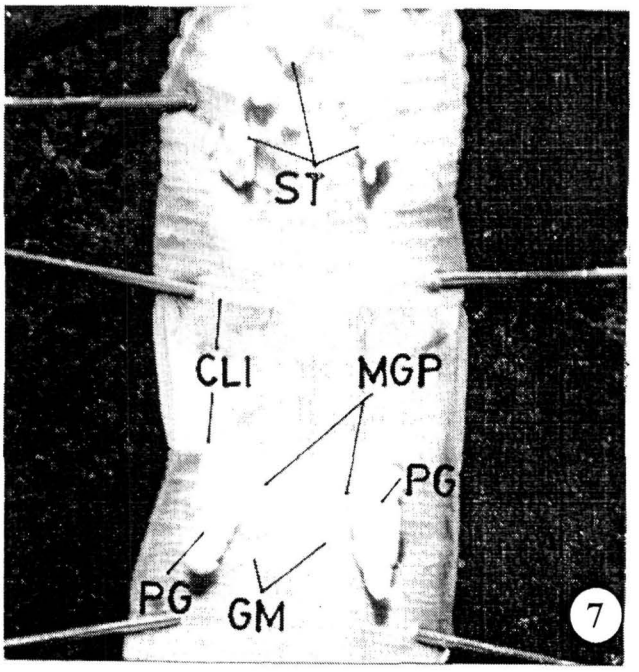


***Amynthas morrisi* Beddard: Figs.** (1) Genital markings paired and ventro-median; (2) Genital markings in triplicate and ventro-median; (3) Genital markings in a line, ventrally and before segment XVII









***Metaphire posthuma* Vaillant: Figs.** (4) Ventral side showing female genital pore, male genital pores. And genital markings on XVIII and XIX; (5 & 6) Ventral side showing variation in genital markings of two different specimens; (7) Location of genital markings in a dissected specimen (gut removed)

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A SURVEY OF THE MOSS FLORA OF CHANDIGARH AND SOME ADJOINING AREAS

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Abstract

Twenty-one moss species included in 14 genera and 7 families are reported from Chandigarh and some adjoining areas for the first time. Morphology of a rarely found taxon – Splachnobryum flaccidum (Harv.) Braithw. is given in detail.

Key words : Moss flora, Chandigarh, systematics.

INTRODUCTION

Systematic study of the moss flora of the Western Himalayas and the adjoining plains is one of the ongoing research projects in our department. The present study, a part of this project, deals with the survey of the moss flora of Chandigarh and some adjoining areas. The survey sites lying at about 30°43" latitude and 49°51" longitude are located in the foot - hills of the Shivaliks (which is a mountainous range of Himalayas) at 347 m to 362 m altitude. The area is bounded on the eastern side by 'Sukhna Cho' and on the North-Western side by "Patiali ki Rao'. It experiences an annual rainfall of 85-125 cm. Pending a detailed illustrated taxonomic account of the moss diversity of this interesting area, presently 21 moss species included in 14 genera and 7 families are being reported.

MATERIALS AND METHODS

The materials were collected from the different sites (shown in the map) of Chandigarh which is spread over 40 sq. km. The materials collected from the different sites were brought to the laboratory in paper packets. These were later soaked in water to enable the plants to regain turgidity. The mixtures were carefully separated under binocular and different plant organs were mounted in 'gum choral' (gum arabic 50 gm + chloral hydrate 50 gm + glycerine 50 c.c. + water 250 c.c.) for microscopic examination. The studied materials were compared with the details recorded for some of these taxa in the earlier manuals (Gangulee 1969-80; Chopra 1975; Chopra & Kumar 1981).

OBSERVATIONS

The taxa found from different sites are listed below:

Fissidens Hedw.

This taxon is easily recognized in the field by its distichous leaves observable with the naked eye and a unique leaf organization showing vaginant lamina, dorsal lamina and apical lamina, when seen with a hand lens.

1. ***F.curvato- involutus*** Dix.
   Rock Garden, on rocks, 8th Feb, 2004. PAN 5192; Mansa Devi, on soil, 15th Feb, 2004. PAN 5193.

Anoectangium Schwaegr.

This is another interesting moss genus commonly found in xeric or semi- xeric habitats. Soil collected on bridges, bricks and rocks offer a favoured substratum for its growth and spread.

2. ***A. stracheyanum*** Mitt.
   Mansa Devi, on rocks, 15th Feb, 2004. PAN 5194; Sector 46 (Ayurvedic College), on walls, 7th Feb, 2004. PAN 5195; Industrial area (Phase 1) CTU Workshop , on walls, 7th Feb, 2004. PAN 5196; Sector 30 (CSIO), on walls, 7th Feb, 2004. PAN 5197.

3. ***A. thomsonii*** Mitt.
   Mansa Devi, on rocks, 15th Feb, 2004, PAN 5198.

The two listed species of *Anoectangium* ascend the Western Himalayas up to nearly 2400 m altitude.

Hyophila Brid.

4. ***H. involuta*** (Hook.) Jaeg.
   Rock Garden, on rocks, 8th Feb 2004, PAN 5200; Kishangarh, on walls, 15th Feb, 2004. PAN 5201.

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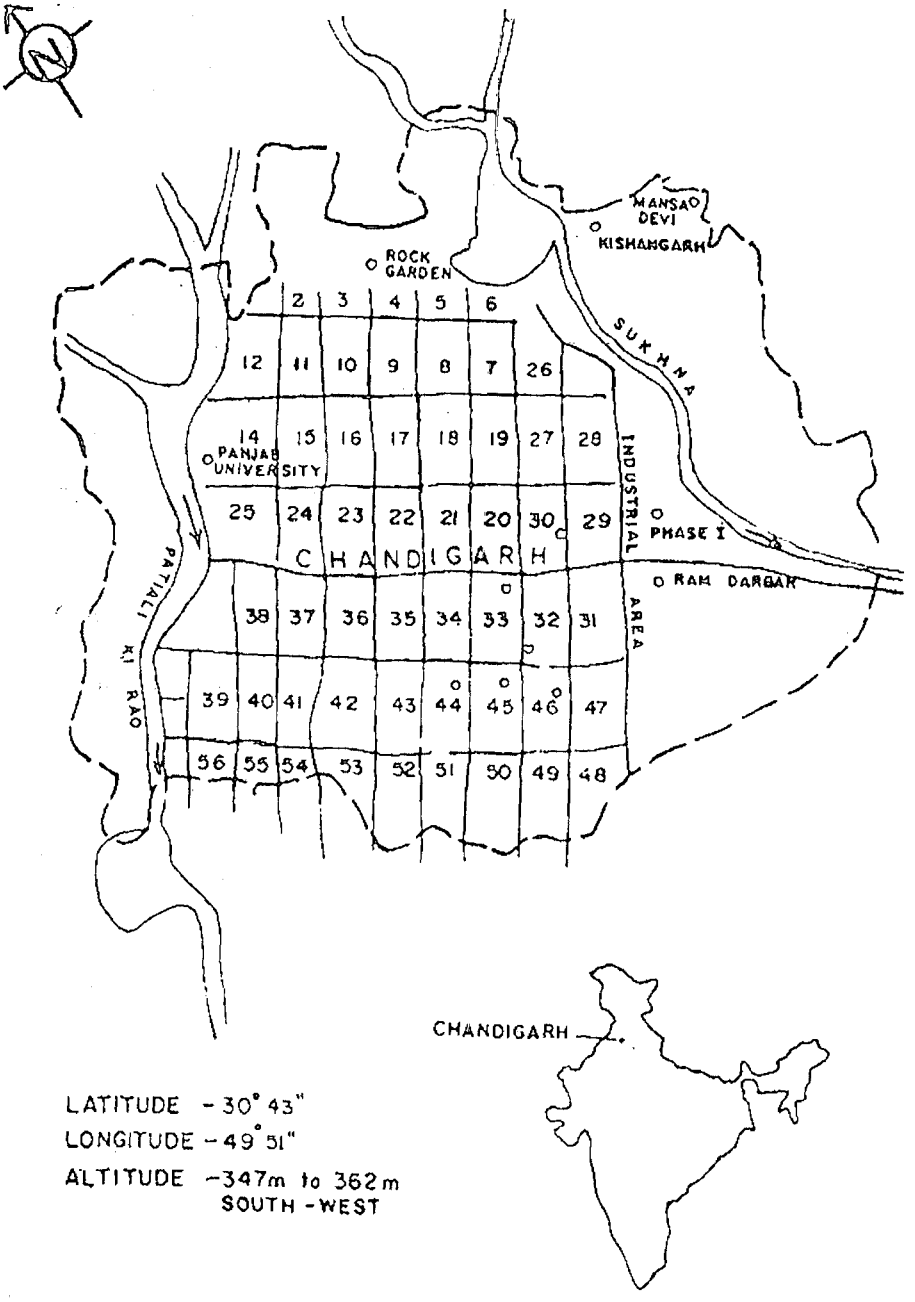
MAP - I

MAP OF THE VISITED LOCALITIES



LATITUDE - 30° 43"
LONGITUDE - 49° 51"
ALTITUDE -347m to 362m
SOUTH - WEST

This is one of the commonest mosses, found growing densely on walls and rocks in the plains of India during the monsoon months. The crenate leaf margins particularly towards the apex and involute leaves when in dry condition help ready recognition.

5. ***H. spathulata*** (Harv.) Jaeg.

Rock Garden, on rocks, 8th Feb, 2004. PAN 5202.

Semibarbula Herz.

6. ***S. orientalis*** (Web.) Wijk.

Rock Garden, on rocks, 8th Feb, 2004. PAN 5205; Mansa Devi, on walls, 15th Feb, 2004. PAN 5206; Sector 44, on walls, 22nd Feb, 2004. PAN 5207; Sector 45, on walls, 22nd Feb, 2004. PAN 5208; Sector 33, on walls, 22nd Feb, 2004. PAN 5209; Kishangarh, on walls, 15th Feb, 2004. PAN 5203.

Hymenostylium Brid.

7. ***H. recurvirostre*** (Hedw.) Dix.

Rock Garden, on rocks, 8th Feb, 2004. PAN 5199.

This taxon is very common in the Western Himalayas. It is very commonly found on rocky walls, cemented structures and other calcareous substrata.

Interestingly, it descends down to the plains. The characteristic cushions and small, nearly ovoid, non-peristomate capsules help recognition.

Barbula Hedw.

8. ***B.confertifolia*** Mitt.

Kishangarh, on walls, 15th Feb, 2004. PAN 5204.

Entosthodon Schwaegr.

9. ***E.wallichii*** Mitt.

Rock Garden, on rock, 24th Feb, 2000. PAN 5186.

Splachnobryum C.Muell.

10. ***S.flaccidum*** (Harv.) Braithw.

Rock Garden (Near waterfall), on soil, 8th Feb, 2004. PAN 5214.

(Plates 1-2).

Plants in lax tufts. Stems 0.5-0.7 cm long, laxly leafy. Leaves crisp when dry,spreading when moist , 520 - 830 μm long and 150-175 μm wide above base, lanceolate-ligulate, apex rounded to obtuse, margins plain, crenate due to projecting ends of cells in the apical portion; nerve strong, ending below or well below the apex ; Basal laminal cells broadly rectangular, 43 – 45 μm x 15 – 20 μm towards the nerve, gradually narrowing 20 – 40 μm x 7 - 9 μm towards the margins, marginal row of narrowly sub–rectangular to sub-quadrate, 7– 23 μm x 4 – 6 μm cells; the median laminal cells elongate hexagonal , 20 – 48 μm x 12 -14 μm tending to be narrowly rectangular, 17 – 34 μm x 4 - 7 μm towards margins; the upper laminal cells progressively shorter hexagonal , 11 – 30 μm x 4 -11μm; the cells of the marginal row protrude out to form crenations.

Sporophyte not observed.

This interesting taxon, with thin, laxly leafy stems, soft leaves with the nerve ceasing well below the apex, was collected from soil gathered on rocks along which the waterfall was running down.

In our country, previously this moss was reported from Delhi.

Physcomitrium (Brid.) Fuernr.

In the field this taxon looks like *Funaria* in habit, but is easily differentiated from that taxon by beaked operculum, non-peristomate capsule and erect calyptra lobed at base and covering about half the capsule.

11. ***P.repandum*** (Griff.) Mitt.
Botany Department (Panjab University), on soil, 19th Feb, 2004. PAN 5211; Sector 45, on soil, 22nd Feb, 2004. PAN 5212.

12. ***P. pulchellum*** (Griff.) Mitt.

Sector 33, on soil, 22nd Feb, 2004. PAN 5213.

13. ***P. eurystomum*** Sendtn.

Sector 32, on soil, 22nd Feb, 2004. PAN 5210.

Funaria Hedw.

14. ***Funaria hygrometrica* var. *calvescens*** (Schwaegr.) Mont.

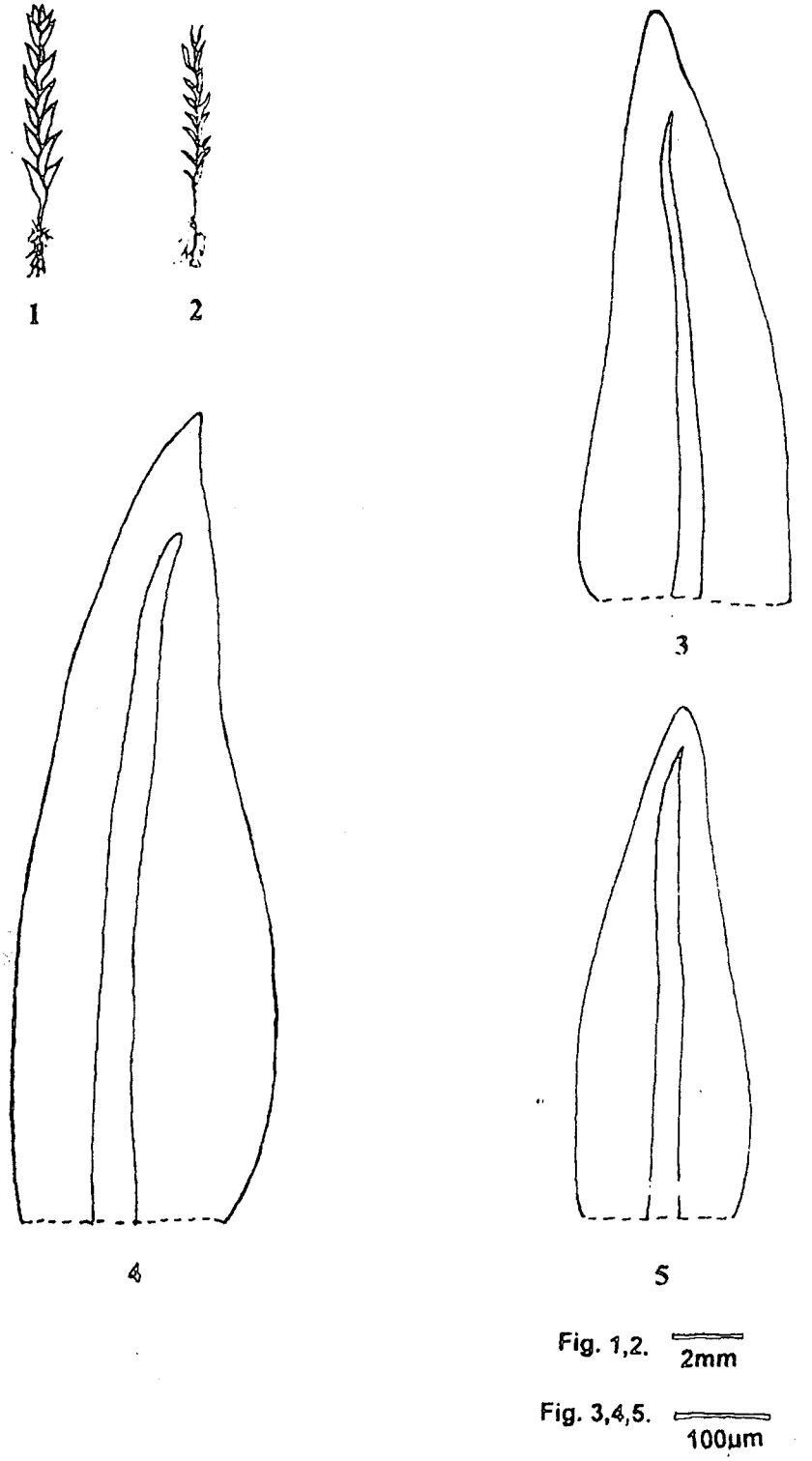
Panjab University Campus, on soil, 5th Nov 1999. PAN 5187.

Bryum Hedw.

15. ***B.capillare*** Hedw.
Rock Garden, on soil, 8th Feb, 2004. PAN 5215;

PLATE - 1

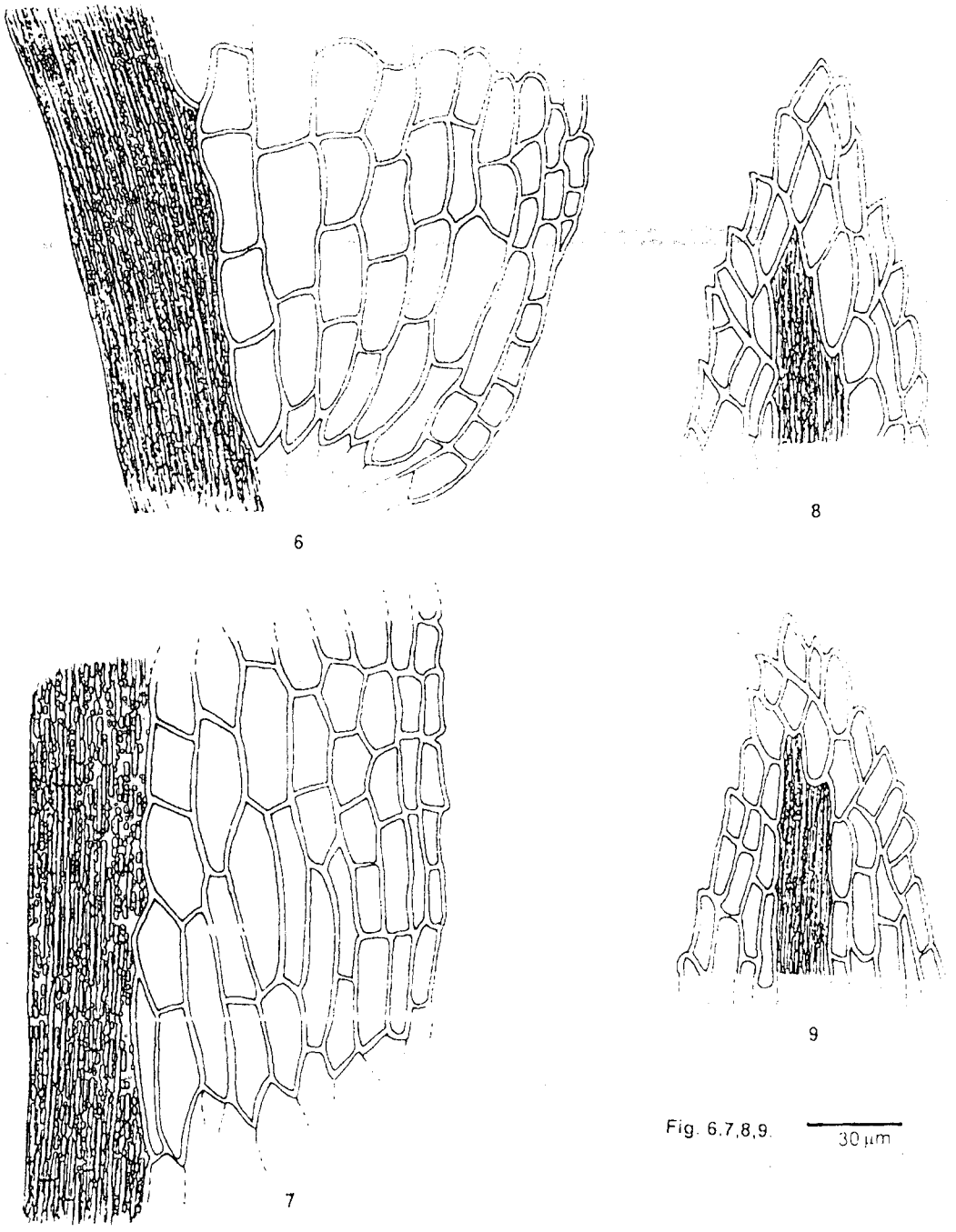
Splachnobryum flaccidum



Figs.1-5: (1) Wet plant; (2) Dry plant; (3-5) Leaf (whole mount)

PLATE - 2

Splachnobryum flaccidum



Figs.6-9: (6) Basal laminal cells; (7) Middle laminal cells; (8-9) Apical cells

Mansa Devi, on walls, 15th Feb, 2004. PAN 5216; Botany Department (Panjab University), on soil, 19th Feb, 2004. PAN 5217; Sector 46, on walls, 20th Feb, 2004. PAN 5218; Sector 45, on soil, 22nd Feb, 2004. PAN 5219.

16. ***B.pseudotriquetrum*** (Hedw.) Schwaegr.

    Sector 45, on walls, 22nd Feb, 2004. PAN 5220.

17. ***B. coronatum*** Schwaegr.

    Sector 45, on walls, 22nd Feb, 2004. PAN 5221; Mansa Devi, on walls, 15th Feb, 2004. PAN 5222.

18. ***B.argenteum*** Hedw.

    Sector *34, on bricks,* 12th Jan, 2000. PAN 5189.

Brachymenium Schwaegr.

19. ***B. nepalense*** Hook.

    Rock Garden, on soil, 8th Feb, 2004. PAN 5223; Ram Darbar, on soil 7th Feb, 2004. PAN 5224.

Brachythecium B.S.G.

20. ***B. kumounense*** (Harv.) Jaeg.

    Zoology Department (Panjab University Campus) 4th Feb, 2004. PAN 2225.

Isopterygium Mitt.

This taxon superficially agrees with *Fissidens* in the distichous arrangement of leaves, but its characteristic pleurocarpic habit, form of the leaves and the whitish tinge of the dry plants readily help recognition in the field.

21. ***I.serrulatum*** Fleisch.

    Rock Garden, on moist rocks, 8th Sep 1999, PAN 5191.

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SOMATIC EMBRYO ENCAPSULATION FOR SYNTHETIC SEED PRODUCTION IN *AZADIRACHTA INDICA*

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Abstract

A protocol has been developed for the production of synthetic seeds in neem. The calluses were initiated from various explants on MS medium supplemented with BAP, KN, IBA, and IAA. Embryogenic cultures were obtained on modified MS medium [MS + BAP (1mg/l) + KN (1mg/l) +sucrose (3%) + agar-agar (0.8%) at pH 5.74], which were transferred to another modified MS medium [MS + IBA (2mg/l) + agar-agar (0.8%) + sucrose (3%) at pH 5.74] for the induction of repetitive somatic embryogenesis. Viability of somatic embryos was tested with the help of TTC (2,3, 5-triphenyltetrazolium chloride) test. Somatic embryos were encapsulated using gel complexion via dropping procedure. Amongst various hydrogels like sodium alginate, carrageenan, tragacanth gum powder and locust bean gum, used with different complexing agents for the preparation of synthetic seeds, sodium alginate (3%) proved best. Complete plantlets were obtained from synthetic seeds under controlled conditions, and transferred to green house and field after hardening and acclimatization.

Key words : Neem, synthetic seeds, encapsulation

Short Communication

Azadirachta indica A.Juss (commonly known as Indian lilac/ neem, synonymous *Melia azadirachta* L., family Meliaceae), a native of Indian subcontinent, is a highly valuable and miraculous tree. Currently, neem has a place of pride in international scientific research and literature. Its derivatives have found use in agriculture, public health, medicine, toiletries, cosmetics and livestock production. It has a store of biologically active chemicals, including biopesticidal and anticancerous properties (Anonymous, 1992). The neem family is a challenge to tissue culturists (Sanyal and Datta, 1984). Conventional multiplication of neem through seeds is limited, since its seeds remain viable for a few weeks. Hence, there is an urgent need for evolving alternative means, such as, rapid multiplication through micropropagation and production of synthetic seeds for product development. Micropropagation through artificial seeds has the potential of being exploited commercially on a large scale so that millions of plantlets can be produced in less time. Synthetic seed is a new concept in seed biotechnological research for micropropagation and delivery of tissue cultured plants. This concept employs encapsulation of vegetative propagules like axillary buds, nodal segments, shoot apices, protocorms for the preparation of synthetic seeds which develop into seedlings under *in vitro* conditions. The present investigation represents a culture protocol for the production of synthetic seeds in *A. indica* by encapsulating somatic embryos employing nodal segments, shoot apices, and axillary buds as explants on MS medium.

The explants (nodal segments, axillary buds, shoot apices, leaves and young bark) were taken from coppiced branches of neem trees of 3-4 years age. Thoroughly washed explants were surface sterilized with 0.1% mercuric chloride. The calluses were raised on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations and combinations of BAP, KN, and IBA at 26±1°C. The callus masses formed after 28 days were transferred to different somatic embryogenic media (Table1). The suspension cultures of embryogenic callus were obtained on rotary shaker at a speed of 80-100 rpm at 26±1°C. The viability of somatic embryos was tested with the help of TTC test. Dormant somatic embryos were allowed to germinate on regeneration medium. Suspension cultures containing somatic embryos were encapsulated with various hydrogels (Table 2) using gel complexion via dropping procedure. Encapsulated beads were allowed to dry on filter paper and were rolled down in a mixture of talcum powder and antibiotic streptomycin. Thereafter, the beads were put into sterilized pharmaceutical capsules and stored at 4°C. Somatic embryos were dried at 26°C to reduce moisture content up to 10% to increase the storage capacity prior to encapsulation. The plantlets obtained from synthetic seeds were transferred to plastic pots/glass

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Table 1: Various media used for somatic embryogenesis in *A. indica*.

MS medium	SBID medium	SBIN medium	SBD medium	MI medium	MD medium
MS major salts	MS major salts	MS major salts	MS major salts	MS major salts	MS major salts
MS minor salts	MS minor salts	MS minor salts	MS minor salts	MS minor salts	MS minor salts
Iron EDTA	Iron EDTA	Iron EDTA	Iron EDTA	Iron EDTA	Iron EDTA
B_5 vitamins*	B_5 vitamins*	B_5 vitamins*	B_5 vitamins*	B_5 vitamins*	B_5 vitamins*
Sucrose (3%)	Sucrose (3%)	Sucrose (3%)	Sucrose (3%)	Sucrose (3%)	Sucrose (3%)
BAP (1 mg/l)	2,4-D (40 mg/l)	NAA (10 mg/l)	_	2,4-D (1 mg/l)	_
KN (2 mg/l)	_	_	_	$AgNO_3$	(10 mg/l)
Nobel agar	Nobel agar	Nobel agar	Gelrite	Agar	Gelrite
(0.8%)	(0.8%)	(0.8%)	(0.2%)	(0.8%)	(0.2%)
pH 5.74	pH 7.0	pH 7.0	pH 5.6	pH5.6	pH 5.8

SBID, SBIN, SBD, MI, and MD showed negative response towards somatic embryogenesis while modified MS medium showed positive response towards somatic embryogenesis.

Table 2:. Different types of hydrogels, complexing agents and their concentrations.

Gel concentration (% w/v)		Complexing agent concentrations (%)	
Sodium alginate	(0.5-4.0)	Calcium salts	(1.0-3.0)
Carrageenan	(0.2-0.8)	Potassium chloride	(0.2-1.2)
Locust bean gum	(0.4-1.0)	Ammonium chloride	(0.4-1.0)
Tragacanth gum	(0.5-3.0)	Calcium salts	(1.0-3.0)
Plantago	(0.5-2.0)	Calcium salts	(1.0-3.0)
Agar-agar	(0.5-2.0)	Calcium salts	(1.0-3.0)

Source : The Central Drug House (CDH), Delhi (India)

culture tubes containing a mixture of sterilized sand and garden soil (1:1). Successfully established plantlets were transferred to field after keeping them for one month in the glass house and survival rate was checked. For each treatment, the experiments were performed in triplicate. Photographs of various developmental stages were taken with Nikon F Camera with Micron Nikon Lens. Statistical computations were performed using computer software. To determine the significance of effect of various concentrations and combinations of plant growth regulators on a number of cultures producing calluses, shoots, roots and somatic embryos, the Chi square (χ^2) test was applied. Computer programme was made using the following formula:

$$\chi^2 = \frac{\Sigma (B^2/A) - (\Sigma B \times \Sigma B) / \Sigma A}{\Sigma B/\Sigma A \times (1 - \Sigma B/\Sigma A)}$$

Where A is the total number of cultures inoculated and B is the number of successful cultures showing callus/ shoot/root/somatic embryo/plantlet formation.

Callus induction was found maximum (87.50%) from nodal segments, followed by shoot apices (83.33%), axillary buds (66.66%), leaves (45.83%) and young bark (40.27%) and differences were statistically significant at $p \leq 0.001$. (Table 3). The amount of callus formed and its characteristics vary substantially with the auxin used. However, the combination of BAP and KN (0.5mg/l each)

Table 3: Percentage of callus induction from various explants taken from coppiced young branches of *A. indica* on MS medium supplemented with BAP and KN (0.5 mg/l each).

Explant	No. of explants Inoculated	No. of explants showing callus initiation	No. of days after which callus initiated	Callus induction (%)
Nodal segments	24	22	7-8	
	24	21	8-9	87.50
	24	20	7-8	
Shoot apices	24	18	7-8	
	24	20	7-8	83.33
	24	22	8-9	
Axillary buds	24	15	9-10	
	24	17	10-12	66.66
	24	16	11-12	
Leaves	24	10	10-15	
	24	11	10-12	45.83
	24	12	10-14	
Young bark with active	24	10	10-12	
Cambium	24	8	15-16	40.27
	24	11	10-14	

$\times^2 = 57.50$; df = 4; $p \leq 0.001$.

Table 4: Percentage frequency of callus initiation on MS medium supplemented with BAP and KN individually and in combination using nodal segments.

Cytokinins	Concentration (mg/l)	No. of explants Inoculated	No. of explants developing callus	No. of days after which callus initiated	Callus induction (%)
BAP	0.5	24	8	10-12	33.33
	1.0	24	12	12-14	50.00
	2.0	24	11	14-16	45.83
KN	0.5	24	13	12-14	54.16
	1.0	24	12	10-12	50.00
	2.0	24	9	14-16	37.50
BAP+KN	0.5+0.5	24	21	7-8	87.50
	1.0+1.0	24	20	8-9	83.30
	2.0+2.0	24	18	9-10	75.00

BAP: $\chi^2 = 1.99$; df = 2; $p \leq 0.10$.
KN: $\chi^2 = 4.16$; df = 2; $p \leq 0.05$.
BAP+KN: $\chi^2 = 1.69$; df = 2; $p \leq 0.10$..

proved best for callus induction using nodal segments (Table 4). Various somatic embryogenesis media, like SBID, SBIN, SBD, MI and MD, showed negative response towards direct somatic embryogenesis in neem. Friable callus masses, when transferred to modified MS medium [MS+BAP (1mg/l)+ sucrose (0.3%)+ agar-agar (0.8%) at pH 5.74], showed somatic embryogenesis (Fig.1). Embryogenic calluses were dark green in colour and also showed the presence of teratomas, and regeneration of shoots. Microscopic examination of embryogenic suspension cultures revealed turgid, round, globular embryogenic cells, which assured the nature of somatic cells. During second subculturing of embryogenic calluses, greenish globular structures (referred as somatic embryos) began to emerge from cell aggregates after 20 days (Fig.2). Repetitive somatic embryogenesis was observed on modified MS medium. Average percentage viability of somatic embryos was found to be 70% after storage of 4 months. Their viability was 80% after 2 months, 70% after 4 months and 60% after 6 months. Somatic embryos were encapsulated with different concentrations of sodium alginate, carrageenan, locust bean gum and tragacanth gum powder (Table2). However, sodium alginate (3%) proved best for suitable encapsulation to prepare synthetic seeds (Fig3) with 1% each calcium chloride and calcium nitrate used as complexing agents. There was no bead formation using various concentrations of locust bean gum and carrageenan and tragacanth gum powder, so unsuitable for encapsulation of different encapsulants. Isabgol and agar-agar were also not successful for encapsulation, because they remain in liquid form only at high temperatures where embryogenic suspension cells died. Somatic embryos were encapsulated directly by forcing them into solidified blocks of agar with the help of forceps under aseptic conditions. The blocks/cubes prepared from *Plantago* (Isabgol) could not be used for encapsulation because of their soft texture. Overall, sodium alginate proved the best hydrogel and calcium nitrate as the best complexing agent, which were used for further investigations. Sodium alginate (2.5 %) was found the best for encapsulation of suspension cultures (Kaur and Virk 2001, Kaur *et al* 2002) whereas 3% sodium alginate was better for encapsulating nodal segments, somatic embryos, zygotic embryos, etc. Encapsulated propagules were stored on MS medium enriched with hormones and were grown on regenerating medium (MS+IBA). The plantlets raised from encapsulated somatic embryos were grown in different types of pots in small earthen pots, plastic pots and thermocol pots containing a sterilized mixture of sand and soil (1:1). Plastic pots showed better results as compared to earthen pots and differences were statistically significant at $p< 0.001$. In order to get better multiplication and growth, plantlets regenerated from encapsulated somatic embryos were routinely irrigated with 20ml (per pot) of MS liquid medium and IBA (2mg/l) for 10 days to provide exogenous supply of plant growth regulators and nutrients. All the cultures were kept in culture room at 26±1°C for 25-30 days and transferred to large pots containing garden soil in glasshouse.

Sanyal and Datta (1984) cultured different explants, like bark, leaf, epicotyl and hypocotyls, on Sanyal and Datta medium comprising basal salts supplemented with vitamins, sucrose (30g/l), coconut milk (15%), IAA (1.5mg/l) and KN (0.5 mg/l). Among the various explants inoculated, only young bark containing cambium tissue responded best while during the present investigation, nodal segments responded best among various explants inoculated on MS medium supplemented with BAP and KN.

Shrikhande *et al.* (1993) induced somatic embryogenesis from cotyledons of neem. Globular shiny masses, which developed into somatic embryos, were visible on the immature cotyledon-derived calluses. Same structures were observed during the present investigation from calluses derived from nodal segments of neem on MS medium supplemented with BAP and KN. No such structures were observed over a period of five subcultures in cotyledon-derived callus cultures by Su *et al.* (1997). The formation of adventitious shoots and roots was observed in some calluses by them which was also observed in the present study. No suspension cultures were used by Shrikhande *et al.* (1993) while suspension cultures were successful for the induction of somatic embryogenesis during the present study. Murthy and Saxena (1998) induced somatic embryogenesis from mature seeds of neem on MS medium supplemented with TDZ while similar results were obtained from nodal segments on same medium during the present investigation. No attempt has been made on the production of synthetic seeds in neem earlier. However, successful encapsulation of somatic embryos was done using sodium alginate (3%) during the present study as prescribed by Redenbaugh and co-workers (1984). Similarly, Kulkarni *et al.* (2000) reported successful encapsulation of neem seeds using sodium alginate. Ara *et al.* (2000) reported that basic hindrance to synthetic seed technology was based on the fact that somatic embryos lack endosperm and

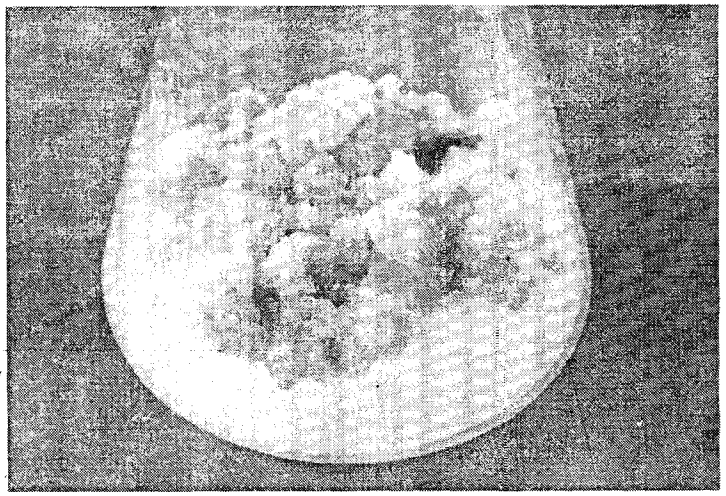

Fig. 1: Embryogenic callus masses on modified MS medium

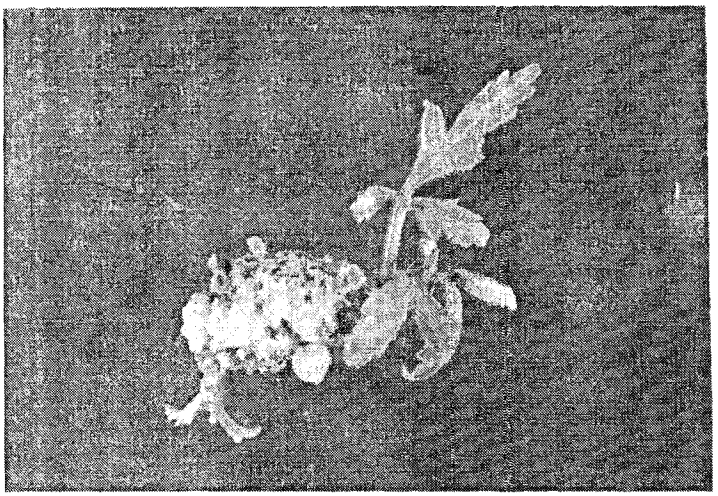

Fig. 2: Embryogenic callus showing somatic embryos and their regeneration in neem.

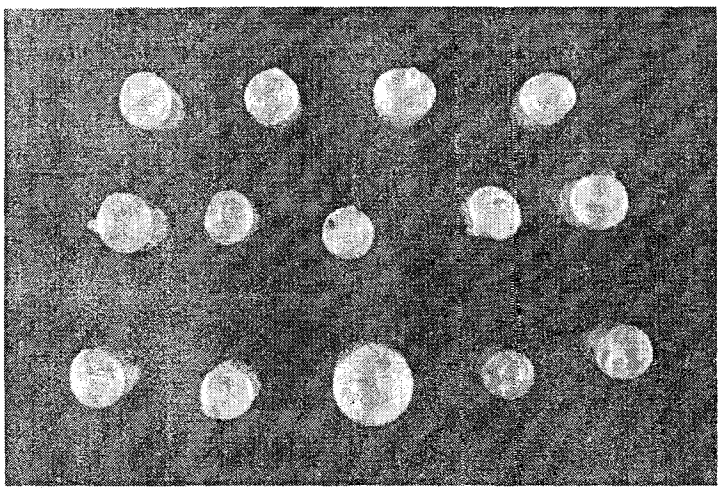

Fig. 3: **Synthetic seeds of neem**

protective coating make them convenient to store and handle. So, efforts were made to increase storage efficiency by providing artificial nutrients to achieve successful regeneration of synthetic seeds.

In summary, the best culture protocol developed in this study for the production of synthetic seeds is as follows. Embryogenic calluses were obtained from nodal segments on MS medium [MS+ BAP (1mg/l) + KN (1mg/l) +sucrose (3%) + agar-agar (0.8%) at pH 5.74]. Globular somatic embryos were obtained from embryogenic masses on MS medium supplemented with IBA (2mg/l) and B5 vitamins. A successful regeneration was observed when somatic embryos were encapsulated with sodium alginate (3%). The results are reproducible and have the potential for probing the mechanism of synthetic seed production in neem.

ABBREVIATIONS

BAP- 6- benzylaminopurine; IBA-indole-3-butyric acid; KN- kinetin; SBID- soyabean induction medium; SBD- soyabean embryo development medium; MI maize embryo induction medium; MD- maize embryo development medium; TDZ- thidiazuron.

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EFFECT OF REPLACEMENT OF FISHMEAL WITH DEFATTED CANOLA ON GROWTH PERFORMANCE AND NUTRIENT RETENTION IN THE FINGERLINGS OF *CHANNA PUNCTATUS* (BLOCH.)

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Abstract

To study the growth performance of Channa punctatus, ten isonitrogenous diets (1-10) were formulated by replacing fish meal (FM) with defatted canola (DFC) (rapeseed) at four inclusion levels (25, 50, 75 and 100 gKg^{-1}) from the reference diet with and without supplementing the diets with a mineral premix and amino acids (MPA). Studies have revealed that live weight gain (g) growth per cent gain in body weight and specific growth rate (SGR % d^{-1}) were significantly ($P< 0.05$) enhanced in fish fed on diets containing DFC at high (75-100 gKg^{-1}) inclusion levels. Better growth performances of fishes were further enhanced on supplementing the diets with MPA. Protein efficiency ration (PER) and Apparent Nutrient Digestibility (APD) (%) values increased, while those of FCR, excretory levels of total ammonia (NH_3-N) and reactive phosphate (P) production decreased with the each increase in the inclusion levels of DFC in the diets.

Key words : *Channa punctatus*, defatted canola, protein, growth, digestibility, excretion, carcass.

INTRODUCTION

Increasing demand, high costs and uncertain availability of fish meal (FM), together with risk factors associated with diseases from animal protein sources, have resulted in studying alternative protein sources such as soybean, rapeseed meal, corn gluten meal, etc. which may replace FM either partially or completely.

Studies have revealed that the use of plant protein in fish feed not only reduces the cost of feed formulation but also reduces the post prandial excretion of total ammonia (NH_3-N) and reactive phosphate (P) in the treated water (Kim *et. al.* 1995; Vielman *et. al.* 2000; Kalla *et. al.* 2003; Singh *et. al.* 2003).

Canola is the registered name given to the genetically selected varieties of rapeseed of *Brassica napus* species that are low in both glucosinolates or antithyroid factors and erucic acid. The meal from these new varieties is called canola meal. Higgs *et. al.* (1994, 1990) used canola meal as a protein source in the diets of rainbow trout and *Oreochromis mossambicus* × *O. aureus* hybrids and achieved complete replacement of FM by the use of defatted processed canola (DFC). Kalla and Garg (2004) found high digestibility values in *C. mrigala* after complete replacement of FM by the use of DFC.

Forster *et. al.* (1999) reported that fish fed on canola protein concentrate diets, regardless of their phytase and P levels, exhibited higher growth, feed efficiencies and protein utilization comparable to those of control fish fed on commercial trout feed and concluded that dietary phytase has potential to improve the nutritive quality of canola protein concentrate for trout and availability of phytase P.

The present investigation was, therefore, planned to study the effect of DFC on growth, digestibility, body composition in the fingerlings of *Channa punctatus* (Bloch.). Effect of DFC diets on post prandial excretion of NH_3-N and reactive P in the treated water was also examined.

MATERIALS AND METHODS

Experimental Diets

A control diet was prepared using FM (as the chief protein source), ground-nut oil cake and rice bran. Canola was used as FM replacer. Canola was defattened to remove some anti-nutritional factors (ANF's) such as glucosides and tannins prior to inclusion in diets (Garg *et. al.*, 2002).

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Ten iso-caloric and iso-nitrogenous diets (1-10) were formulated by replacing FM with DFC at four inclusion levels (25, 50, 75 and 100 gKg^{-1}) from the control diet with and without supplementing the diets with a mineral premix and amino acids (MPA) (Table 1). Diets 1 and 6 were used as reference diets.

The proximate composition of the diets revealed that per cent content of crude protein ranged between 30 – 33 %, crude fat 6.0 - 6.7 %, crude fiber 9.3 – 10.6 %, ash 9.1 – 12.6 % and energy 16.95 – 17.23 KJg^{-1} (Table 1).

Experimental Design

Fingerlings of *C. punctatus* (Mean body weight 7.5 ± 0.05 g) were obtained from fish dealers of Hisar during April-May and acclimated in the laboratory for a minimum period of 7 days and were fed *ad libitum* on a feed containing FM as the protein source (Diet 1) between 2.00 – 4.00 p.m.

All treatments were conducted in transparent glass aquaria (60 × 30 × 30 cm), containing 30L of chlorine free water, in replicate of three, kept in the laboratory where the temperature was maintained at 20±1° C and a lighting schedule at 12:12h of light. All groups of fish were fed daily between 2.00 – 4.00 p.m., at a fixed feeding rate of 2% BWd^{-1} for the whole experimental duration of 45 days. Fish were bulk weighed every 10th day and feeding rates adjusted accordingly. In order to maintain water quality, water in the aquaria was replenished daily with the water which had been

Table 1: Ingredient and Proximate Composition (% dry weight basis) of experimental diets (1-10)

	Diet Number									
	1	2	3	4	5	6	7	8	9	10
Ingredients										
Groundnut Oil Cake	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Rice Bran	24.0	24.0	24.0	24.0	24.0	23.0	23.0	23.0	23.0	23.0
Fish Meal (FM)	10.0	7.5	5.0	2.5	-	10.0	7.5	5.0	2.5	-
Processed Canola (DFC)	-	2.5	5.0	7.5	10.0	-	2.5	5.0	7.5	10.0
Chromic Oxide (Cr_2O_3)*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Binder (Carboxyl methyl cellulose)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
MPA**	-	-	-	-	-	1.0	1.0	1.0	1.0	1.0
Proximate Composition (%)										
Crude Protein	33.8	31.8	31.5	30.9	30.6	33.8	33.5	32.4	31.8	31.5
Crude Fat	6.6	6.6	6.5	6.5	6.3	6.7	6.6	6.6	6.5	6.4
Crude Fiber	9.3	9.4	10.4	10.3	10.6	9.4	9.6	10.4	10.3	10.6
Total Ash	12.6	12.3	10.5	10.4	9.7	12.7	12.2	11.3	10.2	9.2
NFE	31.5	39.7	41.1	41.8	42.7	37.4	38.1	39.4	41.2	42.4
Gross Energy (KJg^{-1})	17.1	16.9	17.1	17.1	17.1	17.1	17.1	17.0	17.2	17.3

* - Cr_2O_3 was used as an external indigestible marker for estimating apparent digestibility

** - mineral premix and amino acid (MPA) supplemented @ $10gkg^{-1}$ diet

(each Kg contains Cu – 312 mg; Co – 45 mg; Mg – 2.114 g; Fe – 979 mg; Zn – 2.13 g; I – 156 mg; DL-Methionine – 1.92g; L-lysine mono hydrochloride – 4.4 g; Ca – 30%; P – 8.25%)

Diets 1-5 not supplemented with MPA; Diets 6-10 supplemented with MPA.

previously equilibrated to the desired temperature (20°C).

Fish fry were fed with respective diets and there after, the uneaten feed was siphoned out and stored separately for calculating the feed conversion ratio (FCR). Faecal matter was collected by pippeting following the method of Spyridakis *et. al.* (1989) every morning. The pooled faecal samples were dried in an oven maintained at 60° C for subsequent analysis. Individual weight of the fish fry was recorded at the beginning and at the end of the experiment.

Analytical Techniques

The feed ingredients, experimental diets, faecal samples and fish carcass (initial and final) were analysed following the procedure of AOAC (1995). Chromide oxide levels in the diets as well as in the faecal samples were estimated spectrophotometrically following the method of Furukawa and Tsukhara (1966).

Live weight gain (g), growth percent gain, specific growth rate (SGR, $\%d^{-1}$), protein efficiency ratio (PER) and gross conversion efficiency (GCE) were calculated using standard methods (Steffens, 1989). Apparent nutrient digestibility (APD) of the diets were calculated according to Cho *et. al.* (1982) as follows:

$$APD = 100 - \frac{100 \times \%\ Cr_2O_3 \text{ in diet} \times \%\ \text{nutrient in faeces}}{\%\ Cr_2O_3 \text{ in faeces} \times \%\ \text{nutrient in diet}}$$

At the end of feeding trials, water samples from each aquarium were collected at 2h interval for the estimation of excretory levels of total ammonia (NH_3-N) and reactive phosphate (P) following APHA (1998) and calculated as follows:

$$\text{Total ammonia excretion } (mgkg^{-1}BWd^{-1}) = \frac{NH_3\text{-N } (mgl^{-1}) \text{ in water}}{\text{fish biomass (kg) per L of water}}$$

$$\text{Reactive phosphate production } (mgkg^{-1}BWd^{-1}) = \frac{P\ (mgl^{-1}) \text{ in water}}{\text{fish biomass (kg) per L of water}}$$

ANOVA, followed by Duncan's multiple range test (Duncan, 1955) was applied to find out the significant differences between different treatments. Correlation coefficient was determined between different variables.

RESULTS AND DISCUSSION

Growth and digestibility

Feeding results have revealed low mortality in all dietary treatments. Live weight gain (g) and growth per cent gain in body weight increased significantly ($P < 0.05$) with each increase in the inclusion levels of DFC. Further, significantly high values of these parameters were observed in groups of fish fed on diets (6-10) supplemented with MPA (Table 2). These results are in agreement with the observations reported for *C. mrigala* (Singh *et. al.* 2003), silver perch (Allan and Rowland, 1994).

The apparent protein digestibility (APD) and energy retention increased with increase in the per cent inclusion levels of DFC in the diets and significantly ($P < 0.05$) highest values were found in fish fed on diet 10 containing DFC at highest inclusion level and supplemented with MPA. Studies have further revealed that APD had no effect on protein and energy retention of fish (Bureau *et. al.* 2000).

Protein efficiency ratio (PER), gross conversion efficiency (GCE) and specific growth rate (SGR) values increased with each increase in the inclusion levels of processed canola and significantly ($P < 0.05$) highest values were found in fish fed on diet 10 supplemented with MPA. These results are in agreement with the observations reported in *C. mrigala* (Kalla *et. al.*, 2003).

On the other hand, feed conversion ratio (FCR) values significantly ($P < 0.05$) decreased with each increase in the inclusion levels of DFC, which were further lowered when the diets were supplemented with MPA. Thus the lowest FCR values were observed in fingerlings fed on diet 10 containing DFC at highest inclusion level and supplemented with MPA. These results obtained in *C. mrigala* (Kalla *et. al.*, 2003; Kalla and Garg, 2004).

II. Postprandial Excretory Levels of Total Ammonia (NH_3-N) and Reactive Phosphate (P)

The daily NH_3-N and P excretion was also significantly affected by the dietary inclusion levels of DFC *i.e.* the levels decreased with each increase in the inclusion levels of canola. Supplementing the diets with MPA further reduced the post prandial excretory levels of NH_3-N and P in the treated water. Thus, significantly

Table 2: Growth performance of *Channa punctatus* fed on ten experimental diets containing fish meal and processed canola meal at various inclusion levels.

Diet No.	Weight gain (g)	Growth % gain in Body Weight	Specific Growth Rate (SGR)	Feed Conversion Ratio(FCR)	Apparent Protein Digestibility (APD)	Gross Conversion Efficiency (GCE)	Protein Efficiency Ratio (PER)	Total ammonia (NH_3-N) $mgKg^{-1}$ Bwd^{-1}	Reactive phosphate (P) $mgKg^{-1}$ Bwd^{-1}
1	2.177^{A} ± 0.018	25.260^{A} ± 0.367	0.500^{A} ± 0.006	3.713^{A} ± 0.032	74.086^{A} ± 0.056	0.269^{A} ± 0.002	0.064^{A} ± 0.000	0.703 ±0.003	0.266 ±0.006
2	2.920^{B} ± 0.011	33.360^{B} ± 0.274	0.640^{B} ± 0.004	2.866^{B} ± 0.011	75.343^{B} ± 0.158	0.349^{B} ± 0.001	0.092^{BG} ± 0.001	0.620 ±0.005	0.246 ±0.003
3	3.260^{C} ± 0.036	36.680^{C} ± 0.284	0.695^{C} ± 0.004	2.605^{C} ± 0.028	77.863^{C} ± 0.204	0.384^{C} ± 0.004	0.104^{C} ± 0.002	0.570 ±0.000	0.240 ±0.000
4	3.950^{D} ± 0.020	45.790^{D} ± 0.438	0.838^{D} ± 0.006	2.231^{D} ± 0.011	81.573^{D} ± 0.261	0.448^{D} ± 0.002	0.128^{D} ± 0.001	0.516 ±0.008	0.186 ±0.003
5	3.723^{E} ± 0.023	40.860^{E} ± 0.219	0.761^{E} ± 0.003	2.345^{E} ± 0.014	80.206^{E} ± 0.337	0.427^{E} ± 0.002	0.122^{E} ± 0.000	0.536 ±0.008	0.160 ±0.005
6	2.350^{F} ± 0.015	26.240^{A} ± 0.528	0.518^{A} ± 0.009	3.481^{F} ± 0.022	75.446^{B} ± 0.165	0.287^{F} ± 0.001	0.069^{F} ± 0.000	0.650 ±0.000	0.260 ±0.000
7	3.010^{G} ± 0.015	34.000^{B} ± 0.181	0.650^{B} ± 0.002	2.798^{G} ± 0.014	76.490^{F} ± 0.276	0.357^{G} ± 0.001	0.090^{G} ± 0.000	0.613 ±0.003	0.226 ±0.006
8	3.390^{H} ± 0.040	38.750^{F} ± 0.632	0.728^{F} ± 0.010	2.517^{H} ± 0.030	79.076^{G} ± 0.131	0.397^{H} ± 0.004	0.105^{C} ± 0.001	0.540 ±0.005	0.173 ±0.006
9	3.840^{I} ± 0.011	43.870^{G} ± 0.144	0.808^{G} ± 0.002	2.253^{D} ± 0.006	83.570^{H} ± 0.148	0.444^{D} ± 0.001	0.121^{E} ± 0.001	0.523 ±0.003	0.163 ±0.003
10	4.090^{J} ± 0.02	44.900^{DG} ± 0.450	0.824^{DG} ± 0.007	2.174^{D} ± 0.010	86.670^{I} ± 0.164	0.460^{I} ± 0.002	0.130^{D} ± 0.001	0.480 ±0.000	0.110 ±0.005

All values are mean ± S.E. of mean (n=3)
Mean with same letter in the same row are not significantly (P>0.05) different.
Data were analysed by Duncan's Multiple Range Test

($P < 0.05$) low levels were observed in fingerlings fed on diet 10 (Fig. 1), where the FM was completely replaced with DFC (Table 2). These results are similar to those reported by Lall (1991), Cho (1993), Singh *et. al.* (2003), Kalla *et. al.* (2004).

III. Effects of Diets on Carcass Composition

In general, a significant ($P < 0.05$) decrease in carcass moisture and an increase in carcass protein were noticed with increase in the inclusion levels of DFC, which was further high when diets were supplemented with MPA (Table 3). Further, an inverse relationship

Table 3: Proximate carcass composition (% wet weight) of *Channa punctatus* fed on ten experimental diets (1-10)

Diet No.	Moisture	Crude Protein	Crude Fat	Total Ash	Nitrogen Free Extract	Gross Energy KJg^{-1}
1	78.450^{B} ± 0.034	13.010^{B} ± 0.102	3.973^{B} ± 0.012	3.013^{B} ± 0.003	1.553^{B} ± 0.112	4.913^{B} ± 0.009
2	77.966^{C} ± 0.031	13.356^{C} ± 0.052	4.126^{C} ± 0.003	2.990^{B} ± 0.010	1.560^{B} ± 0.076	5.057^{C} ± 0.005
3	77.610^{D} ± 0.030	13.920^{D} ± 0.000	4.250^{D} ± 0.010	2.870^{C} ± 0.010	1.350^{C} ± 0.015	5.202^{E} ± 0.006
4	77.100^{E} ± 0.030	14.633^{E} ± 0.118	4.426^{E} ± 0.006	2.610^{D} ± 0.011	1.230^{C} ± 0.117	5.420^{E} ± 0.010
5	76.836^{F} ± 0.054	14.936^{F} ± 0.029	4.560^{F} ± 0.011	2.486^{E} ± 0.016	1.180^{C} ± 0.065	5.536^{F} ± 0.006
6	77.756^{G} ± 0.069	13.503^{C} ± 0.080	4.256^{D} ± 0.017	3.150^{F} ± 0.015	1.333^{C} ± 0.066	5.104^{G} ± 0.016
7	77.190^{E} ± 0.018	13.993^{D} ± 0.013	4.410^{E} ± 0.015	3.060^{G} ± 0.010	1.340^{C} ± 0.020	5.281^{H} ± 0.004
8	76.826^{F} ± 0.020	14.323^{G} ± 0.056	4.536^{F} ± 0.008	2.983^{B} ± 0.003	1.330^{C} ± 0.065	5.408^{E} ± 0.002
9	76.213^{H} ± 0.032	14.983^{F} ± 0.023	4.493^{G} ± 0.013	2.903^{C} ± 0.013	1.406^{BC} ± 0.003	5.560^{I} ± 0.005
10	75.816^{I} ± 0.003	15.440^{H} ± 0.010	4.563^{F} ± 0.008	2.850^{C} ± 0.017	1.330^{C} ± 0.015	5.682^{J} ± 0.003

All values are mean ± S.E. of mean (n=3)
Mean with same letter in the same row are not significantly (P>0.05) different.
Data were analysed by Duncan's Multiple Range Test

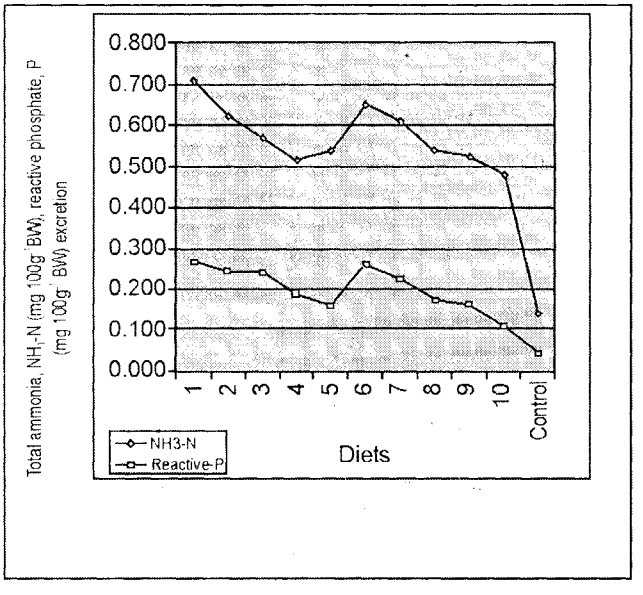

Fig. 1: Excretion pattern of total ammonia, NH^3-N (mg $100g^{-1}BW$) and reactive phosphate, P (mg $100g^{-1}BW$) in treated water in fish *Channa punctatus* fed on diets 1-10 containing processed canola as the main protein source

between the moisture contents and carcass fat were also observed during the present study as has also been observed by Mazid *et al.* (1994) and Shearer (1994).

Gross energy levels increased with increase in the inclusion levels of DFC in different experimental diets and this increase was much more in the groups of fish fed on diets supplemented with MPA. These results are same to those observed by Kalla *et. al.* (2003).

CONCLUSIONS

Defatted canola supplemented with MPA can be used as protein source in the diets of *Channa punctatus*. DFC can completely substitute the use of FM protein without compromising growth rate, nutrient retention and fish quality. The complete replacement of FM with DFC in the diets of fishes could save total feed costs but certainly would reduce excretion of nitrogenous and total organic matter, possibly also of P and alleviate the pollution problems associated with intensive aquaculture system.

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STUDIES ON INTERNAL MALE AND FEMALE GENITALIC ORGANS OF THE TYPE SPECIES OF GENUS *MAHAVIRA* MOORE AND ITS TAXONOMIC SIGNIFICANCE

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Abstract

The internal reproductive organs of the type species flavicollis Moore of genus Mahavira Moore have been studied and illustrated for the first time. The taxonomic significance of these organs has been commented upon in the present manuscript.

Key words : Internal reproductive organs, Genus, Species, Male, Female.

INTRODUCTION

The genus *Mahavira* Moore was erected on type species *flavicollis* Moore by Hampson in 1894. The internal male and female genitalia of this species has been described and illustrated here for the first time. Different surveys were conducted in North East India from March 2000 to September 2003 for the collection of Arctiid species. The present paper incorporates male and female genitalia of this species. The dissections of male and female specimens were performed for detailed examination of its internal reproductive organs. The present species was identified with the help of literature (Hampson, 1894) and by comparison from the museum of Zoological Survey of India, Kolkata. The nomenclature for naming different internal reproductive organs has been adopted from the recent workers like Amaldos and Hsue (1989), Kumar, (1990), Mitter, (1993), Hauser, (1993, 1993a), Miller (1993), Chaudhary and Sinha (1994), Kobayashi, (1994), Justus and Mitchell, (1999), Gowda *et al.* (2002) and Eveden *et al.* (2003).

MATERIALS AND METHODS

The collection of adult Arctiid moths were done from the flourescent lights and with the help of portable light trap at night time. In unelectrified areas, petromax lamps were used as a source of light. The collected moths were starved for about 7-10 hours in small insect breeding cages made of wire gauge. This was done to avoid the superfluous fat which otherwise hinders the process of dissection and clarity of male and female genitalic organs. The abdomens of the starved moths were detached and descaled after anesthetizing the adult insects with vapours of ethyl acetate. After this, their dissections were done in physiological saline solution. During the dissections various observations were made on the spot. The colour, transparency and shape of different internal genital organs were immediately noted in the field notebook. This is necessary to avoid any changes after preserving the internal male and female genitalia. The internal male and female genitalic organs were dissected out and preserved in a mixture of alcohol and glycerol in ratio of 1:4 in homoeopathic vials. The duplicate adult representatives of this species were also pinned, stretched and preserved in the insect cabinets, along with abdomenless specimens.

RESULTS AND DISCUSSION

Genus *Mahavira* Moore

Moore, 1878, *Proc. Zool. Soc.*, **1878** : 11.

Type-species : *Mahavira flavicollis* Moore.

Distribution : Sikkim.

***Mahavira flavicollis* Moore**

Moore, 1878, *Proc. Zool. Soc.*, **1878**, p. 11.

(Figs. 1, 2)

Male : Testis ellipsoidal, dark yellowish opaque, length 1.22 mm, width 0.88 mm; seminal vesicle-I light opaque, crossed, length 0.88 mm, width 0.33 mm; seminal vesicle-II dark opaque, length 1.88 mm, width 0.33 mm; vasa deferentia translucent, length 1.77 mm, width 0.11 mm, entering into ductus ejaculatorius duplex nearer to ductus ejaculatorius simplex; ductus ejaculatorius

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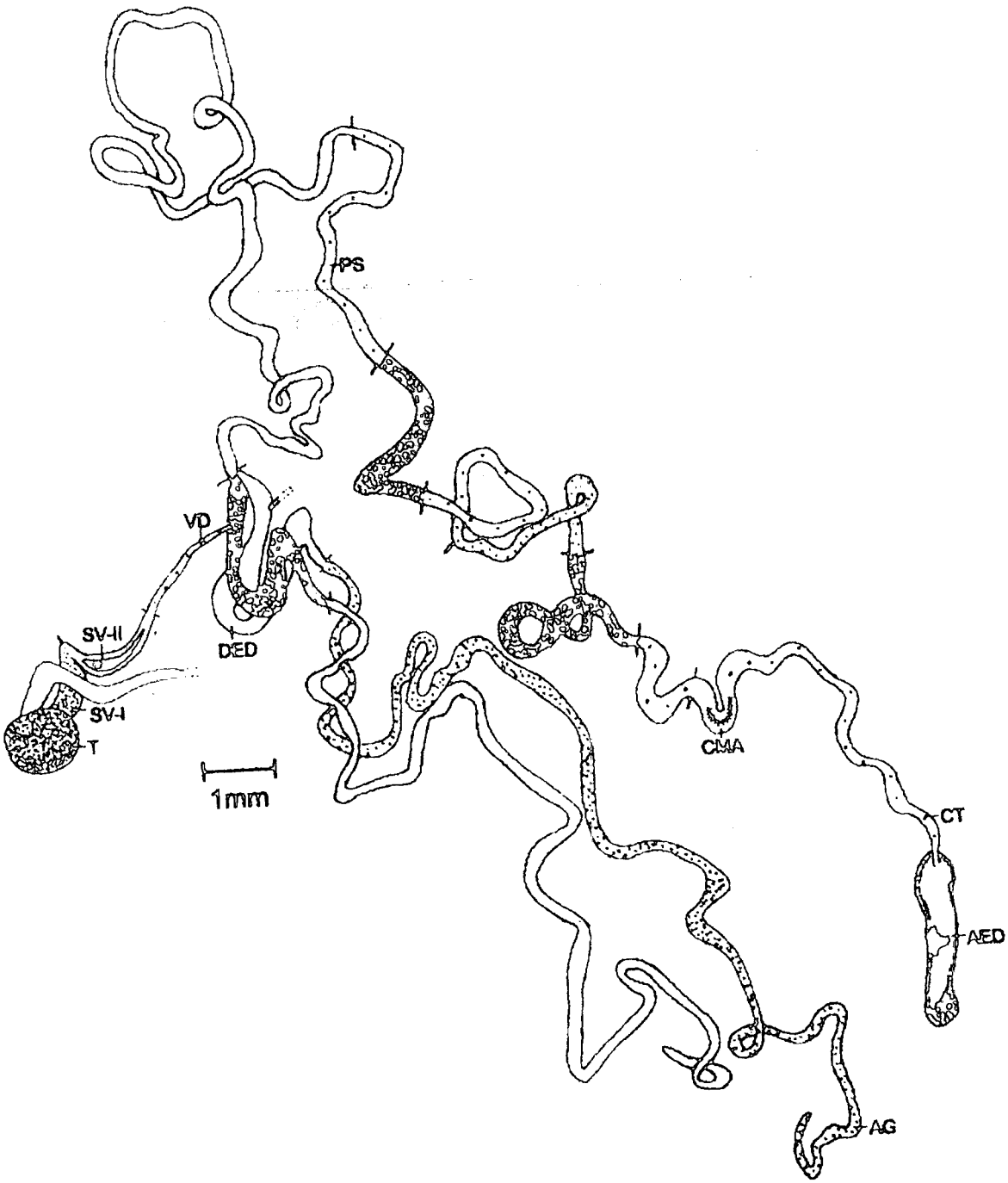

Fig. 1 : *Mahavira flavicollis* Moore

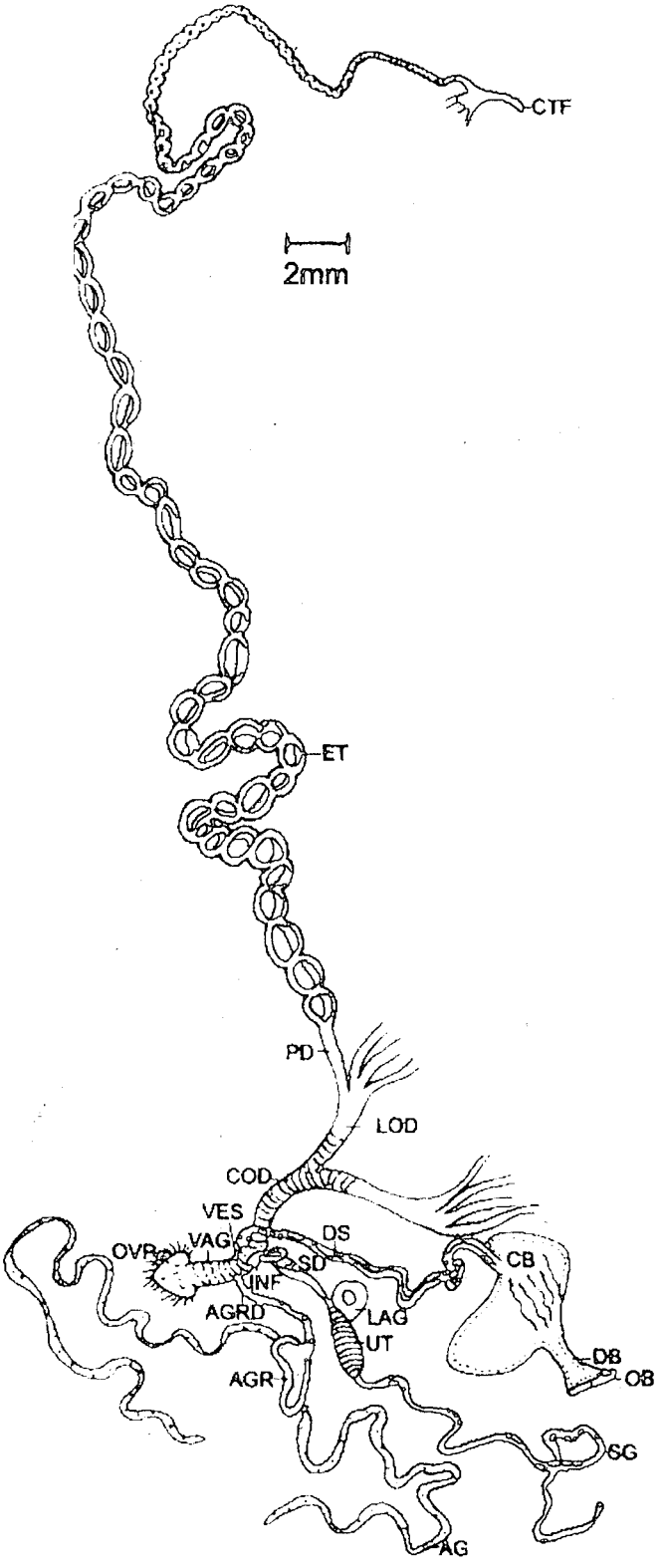

Fig. 2 : *Mahavira flavicollis* Moore

duplex opaque, S-shaped, crossed, length 4.55 mm, width 0.44 mm; accessory glands entirely free, bent like hook at tip, entire accessory glands dark yellow opaque, length 20.77 mm, width 0.22 to 0.33 mm; ductus ejaculatorius simplex with primary simplex divided into seven sections; section-I, transparent, coiled once, length 4.44 mm, width 0.22 mm; section-II yellowish translucent, length 5.0 mm, width 0.22 mm; section-III dark opaque, length 3.88 mm, width 0.22 mm; section-IV coiled once, translucent, length 4.44 mm, width 0.22 mm; section-V light opaque, coiled once, length 3.66 mm, width 0.22 mm; section-VI dark opaque, coiled twice, length 5.77 mm, width 0.33 mm; section-VII translucent, length 2.55 mm, width 0.33 mm; constrictor muscular area with cuticular simplex dark translucent yellow, curved, length 2.0 mm, width 0.33 mm; cuticular tube translucent, length 6.10 mm, width 0.22 mm, enters into aedeagus apically.

Female: Each ovary with common terminal filament transparent, length 1.85 mm, width 0.28 mm; egg tube filled with dark brownish, opaque ova in proximal half, light brownish opaque ova in distal half, length 34.20 mm, width 0.28 to 0.71 mm; pedicel translucent, length 1.57 mm, width 0.42 mm; lateral oviduct translucent, length 2.13 mm, width 0.57 mm; common oviduct length 1.71 mm, width 0.57 mm; complex sperm storage system with spermathecal gland simple, opaque, coiled once, length 1.57 mm, width 0.28 mm; infundibulum length 0.28 mm, width 0.13 mm; spermathecal duct coiled twice, opaque, length 1.30 mm, width 0.28 mm; utriculus dark opaque, length 1.71 mm, width 0.71 mm; lagena light opaque, length 0.57 mm, width 0.57 mm; corpus bursae light translucent, diameter 7.50 mm; ductus bursae length 2.80 mm, width 0.85 mm; ostium bursae diameter 1.70 mm; ductus seminalis light translucent, coiled once, originating from corpus bursae anteriorly, length 6.20 mm, width 0.42 mm; cement gland system with lateral and common accessory gland reservoir undifferentiated, heart-like, dark brownish opaque, length 1.70 mm, width 0.57 mm; accessory gland reservoir duct opaque, length 2.30 mm, width 0.28 mm; accessory gland ducts opaque, length 14.70 mm, width 0.28 mm; vestibulum length 1.0 mm, width 0.57 mm; vagina length 0.85 mm, width 0.71 mm.

Material examined: Arunachal Pradesh : West Kameng District, Bomdila, 11.9.02, 1♂, 1♀; Assam : North Cachar Hills, Jatinga, 10.9.01, 1♂, 1♀.

Old distribution: Sikkim.

Remarks: The present study reveals that in the internal male reproductive system the entrance of vasa deferentia into ductus ejaculatorius duplex is nearer towards ductus ejaculatorius simplex, the entrance of cuticular tube into aedeagus is apical. Study of internal female reproductive organs reveal that cement gland system with lateral and common accessory gland reservoir is undifferentiated. Another important character of taxonomic significance is of ductus seminalis which originates from the corpus bursae on anterior side. These characters of the type species can profitably be added to the diagnosis of genus *Mahavira* Moore. The reporting of the present species from different localities of Arunachal Pradesh and Assam are its new distributional areas.

ACKNOWLEDGEMENTS

The financial help rendered through a project on "Taxonomic Revision of Indian Arctiidae (Lepidoptera)", by Department of Science and Technology, New Delhi is heartily acknowledged.

ABBREVIATIONS

AED-Aedeagus; AG-Accessory glands; AGD-Accessory gland ducts; AGRD-Accessory gland reservoir duct; BS-Bulla seminalis; CAGR-Common Accessory gland reservoir; CB-Corpus bursae; CMA-Constrictor muscle area; COD Common oviduct; CT-Cuticular tube; CTF-Common terminal filament; DB Ductus bursae; DED-Ductus ejaculatorius duplex; DS-Ductus seminalis; ET Egg tube; INF-Infundibulum; LAG-Lagena; LAGR-Lateral accessory gland reservoir; LOD-Lateral oviduct; OB-Ostium bursae; OVP-Ovipositor; PD-Pedicel; DES-Ductus ejaculatorius simplex; SD-Spermathecal duct; SG-Spermathecal gland; SGD Spermathecal gland duct; SVI and SVII-Seminal vesciles I and II; T-Testis; UT-Utriculus; VAG-Vagina, VD-Vas deferens; VES-Vestibulum.

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MACROBOTHRIDIUM DJEDDENSIS NEW SPECIES (CESTODA:MACROBOTHRIDIIDAE) FROM *RHYNCHOBATUS DJEDDENSIS* FORSSKAL, 1775 FROM BAY OF BENGAL, AT DIGHA COAST, INDIA

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Abstract

Macrobothridium djeddensis n.sp. described from the spiral intestine of Rhynchobatus djeddensis, captured at Digha coastal waters , Bay of Bengal, India. This species differs from the only known species Macrobothridium rhynchobati in combination of characters, rostellum armed with 22 hooks, 42-46 testes per proglottid and vitellaria in three to four rows.

Key words : *Macrobothridium,* Bay of Bengal, Digha coast, *Rhynchobatus djeddensis.*

INTRODUCTION

During collection of helminth parasites of cartilagenous fishes from Bay of Bengal at Digha coast , West Bengal, India, thirty cestode parasites were recovered from the spiral intestine of *Rhynchobatus djeddensis*, in the month of November, 1994. The specimens after proper processing were identified as a new species of the genus *Macrobothridium* Khalil and Abdul-Salam, 1989. So far only one species under this genus has been described.

MATERIALS AND METHODS

The living tape worms recovered from a shark *Rhynchobatus djeddensis* Forsskal, 1775 caught in commercial fish traps of fishermen from the Bay of Bengal at Digha coastal waters, were fixed *in situ* by immersion in 10% formalin and a few of them were fixed in AFA, post fixed and preserved in 70% ethyl alcohol. Whole mounts were prepared using standard procedures (Pramanik and Manna, 2004). Drawings were made with the Camera lucida. Measurements of ten specimens were recorded and the ranges are mentioned in millimetre.

Description

***Macrobothridium djeddensis* n. sp.** (Figs. 1, 2)

Family : Macrobothridiidae Khalil & Abdul-Salam, 1989

Genus : *Macrobothridium* Khalil & Abdul-Salam, 1989

Total length of the parasite is 51-57, maximum breadth 1.2-1.4 and total number of proglottids are 97. The scolex is rectangular, bears two bothridia. The length of scolex is 3.06-3.3 and width 1.18-1.4. Each bothridium is more or less tongue-shaped, its one fourth is detached from the scolex wall and three fourth remain attached with the wall. The bothridium is 2.0-2.3 in length and 1.1-1.2 in width, spineless, projects backwards over the cephalic peduncle. Anterior portion of the scolex has a bowl-shaped rostellum of 0.82-1.1 length and with anterior portion 1.1-1.3 in bredth and posterior portion 0.94-1.13 in width. Just anterior to the rostellum is a dorsal and ventral group of apical hooks lying on lateral side of the rostellum. Each group consist of 22 hooks of different lengths. From the dorsal to ventral side 1st hook length 0.2-0.21, 2nd hook length 0.25-0.28, 3rd hook length 0.33-0.35, 4th hook length 0.44-0.45, 5th hook length 0.53-0.55, 6th hook length 0.46-0.47, 7th hook length 0.71-0.74, 8th hook length 0.49-0.52, 9th hook length 0.81-0.83, 10th hook length 0.61-0.62, 11th hook length 0.92-0.94, 12th hook length 0.53-0.54, 13th hook length 0.79-0.81, 14th hook length 0.53-0.55, 15th hook length 0.75-0.77, 16th hook length 0.49-0.51, 17th hook length 0.57-0.59, 18th hook length 0.45-0.47, 19th hook 0.34-0.35 and 20th hook length 0.25-0.28, 21st hook length 0.18-0.21 and 22nd hook length 0.14-0.15. Following the head proper is the spineless cephalic peduncle of 0.47-0.59 length and 0.37-0.47 width. Short narrow, unstrobilated 0.47-0.53 long neck region present, after the cephalic peduncle. The posterior proglottids are 1.8-2.2 in length and 0.63-0.74 in width. Proglottids are acraspedote, apolytic. Each proglottid

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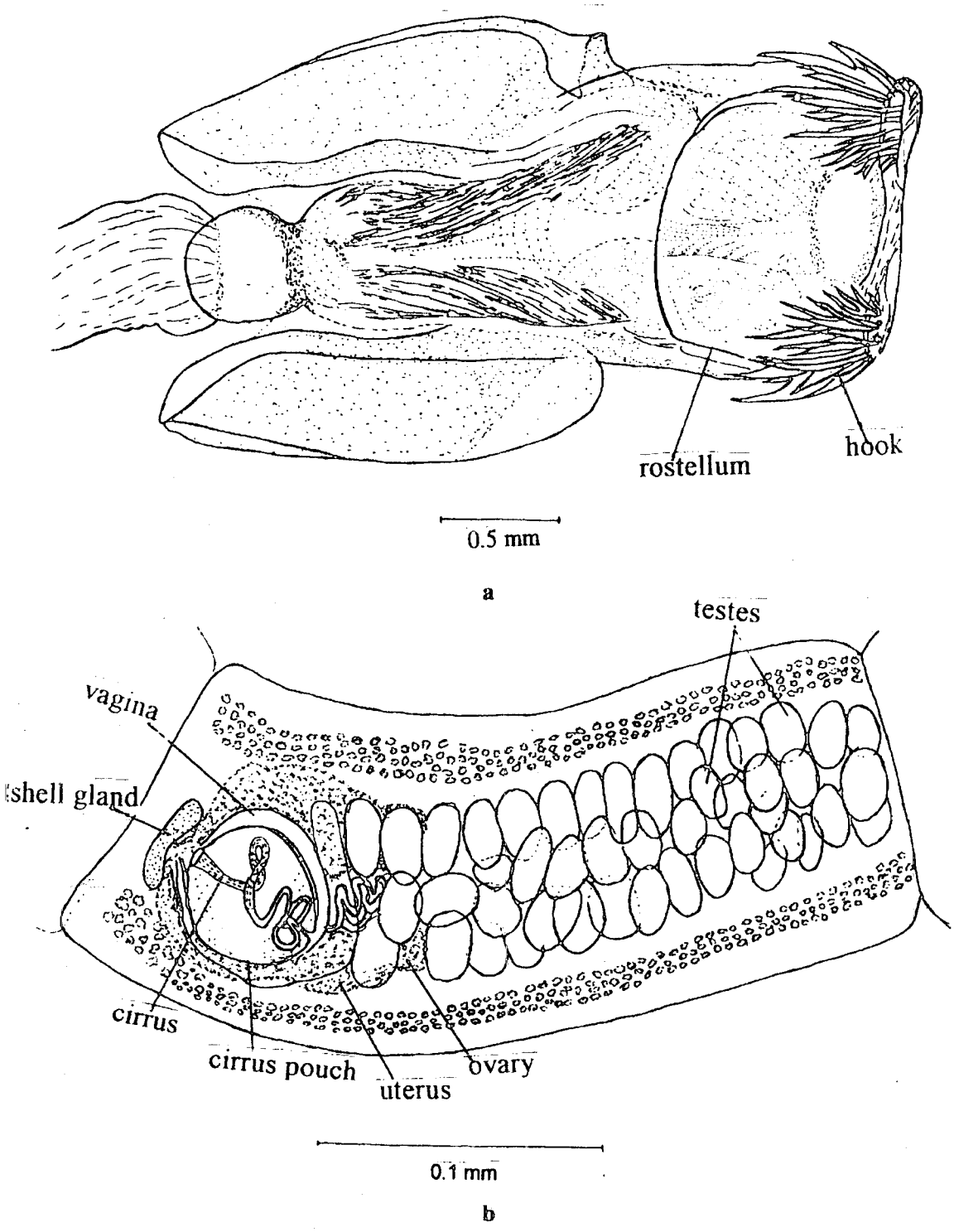

Fig.1: Macrobothridium djeddensis n.sp., Camera lucida drawing; a. scolex, b. mature proglottid

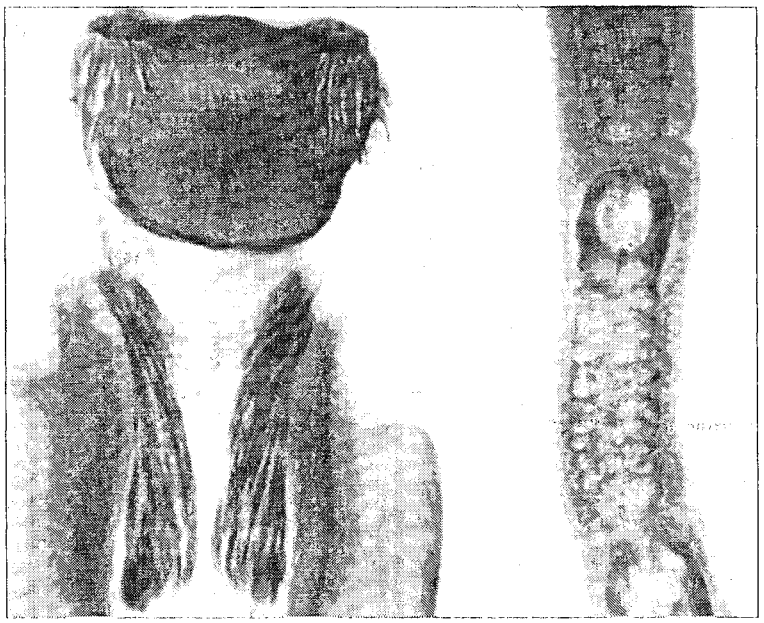

Fig.2: Macrobothridium djeddensis n.sp. , photomicrograph, a. scolex, b. mature proglottid

has 42-46 testes. The testes are more or less oval, 0.11-0.16 in length and 0.21-0.24 in breadth, placed medially. Cirrus pouch is more or less circular, length 0.35-0.38 and width 0.25-0.29, placed at one fourth of the proglottid. Cirrus is coiled, 0.5-0.52 in length, spinous; spines are rose thorn-shaped, 0.005 long. Ovary with two lobes, more or less 'U'-shaped and each arm follicular. Each half of ovary is 0.47-0.51 in length and 0.07-0.08 in width. The shell gland present between the ovary, 0.07-0.08 in length and 0.14-0.18 in width. The genital pore is placed more or less at the junction of two ovarian lobe. The vagina starts from genital pore and runs anterior to cirrus pouch.

Vitelline gland follicular, arranged in 3-4 rows, sub-marginal, present in two lateral field from anterior to posterior end of proglottid. Uterus start from the middle of ovarian isthmus and it bends above the cirrus pouch, its length is 1.2-1.3 and width is 0.16-0.17.

Host -	*Rhynchobatus djeddensis* Forsskal, 1775
Location -	Spiral valve
Locality -	Digha coast, Bay of Bengal, India
No. of specimen -	Thirty
Holotype -	in one slide
Paratype -	twenty in 10 slides
Date of collection -	24.11.1994
Accession No. -	000034/03

Deposited at present to the Parasitology Laboratory, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road. Kolkata 700 019, India

SPECIFIC CHARACTER

Rostellar hooks largest in the middle row, decreasing in size towards extremities. Two unarmed bothridia present. Cephalic peduncle unarmed. Proglottids acraspedote, posterior proglottids longer than wide; last proglottid with long extension terminating a small hole or sucker-like structure. Cirrus sac large; cirrus armed. Vitelline follicles in two lateral bands with 3-4 rows.

DISCUSSION

The order *Diphyllidea* van Beneden in Carus, 1863 has three families *Echinobothriidae* Perrier, 1897, *Ditrachybothridiidae* Schmidt, 1970 and *Macrobothridiidae* Khalil & Abdul-Salam, 1989. The *Macrobothriididae* family has one genus *Macrobothridium* erected by Khalil & Abdul-Salam, 1989 with type species *Macrobothridium rhynchobati* Khalil and Abdul-Salam, 1989 from guitar fish *Rhynchobatus granulatus* in Kuwaiti water in the Arabian Gulf. The validity of the *Diphyllidea* is now generally accepted with the three families, each with a single genus.

Macrobothridium rhynchobati differs from the present species, in spite of many similarities. *M. rhynchobati* has eleven hooks on each side of rostellum but the

present species has twenty two hooks on each side of rostellum. The proglottids are two times longer than breadth in *M. rhynchobati* but three to four times longer than width in the present species. Each proglottid has thirty eight testes in *M. rhynchobati* but 42-46 in the present species. The ovary is 'V'-shaped in *M. rhynchobati* but 'U'-shaped in the present species. Shell gland flower-like in *M. rhynchobati* but kidney-shaped in the present species. The vitelline follicles in two rows in *M. rhynchobati* but in 3-4 rows in the present species.

From the above discussion it is clear that the observed species differs from the existing valid species of the genus *Macrobothridium* in five to six vital characters. It is a new species and the author suggests its name as *Macrobothridium djeddensis* after the species name of its host. A comparative account of the characters of the present species and the existing valid species is given in Table 1.

Key to species

Rostellum with eleven hooks on each side .....M. rhynchobati *Khalil & Abdul-Salam, 1989*

Rostellum with twenty two hooks on each side ..... *M. djeddensis* n. sp.

Table-1: A comparative accounts of the valid species of the genus *Macrobothridium* Khalil & Abdul-Salam, 1989.

Character	*Macrobothridium rhynchobati* Khalil & Abdul-Salam, 1989	*Macrobothridium djeddensis* n. sp.
Bothridia	Start from the anterior part of rostellum	Start after a gap from the rostellum
Hook number	Eleven in number, spine- shaped	Twenty two in number some are dagger-shape and some are spine- shape
Cephalic peduncle	Elongated and narrow	Short and wide
Proglottid	Two times longer than breadth	Three to four times longer than broad
Ovary	V'-shaped	'U'-shaped
Shell gland	'Flower-like	Kidney-like
Cirrus pouch	Oval	More or less circular
Testes	Thirty eight	Forty two to forty six
Vitellaria	Two rows	Three to four rows
Host	*Rhynchobatus granulatus*	*Rhynchobatus djeddensis*

ACKNOWLEDGEMENTS

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CROSSABILITY STUDIES BETWEEN WHEAT-RYE ADDITION LINES AND BREAD WHEAT GENOTYPES

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Abstract

Crossablity of seventeen disomic /ditelosomic wheat rye addition lines with three bread wheat genotypes is determined. All cross combinations (51) are highly crossable except 4 and 5, which had seed set percentage below 60. It was also noticed that a specific combination (6R x HD29) had very high percentage of seed set (92.98). In some cases like 4R x PBW343, crossability is very poor (39.69%), which suggested that crossability of wheat-rye addition lines vary with different wheat genotypes. It is desirable to select highly crossable disomic/ ditelosomic lines and bread wheat's to transfer crossability genes of these genotypes to the attractive wheat parent, which can then be used in for further wheat improvement programme.

Key words: Crossability, wheat-rye addition lines, wheat genotypes

INTRODUCTION

Many species of *Triticum* and related genera are cross compatible and large number of hybrids have been produced. One combination of genotypes of two species may be compatible and others not. Two crossability genes Kr1 and Kr2 had been reported earlier on chromosomes 5B and 5A respectively, that determined crossability in wheat. These two loci/gene(s) are important for the crossability of wheat with many species as dominant alleles inhibit crossability and they have additive effect. Riley and Chapman (1957) reported that crossability depends on both wheat and rye parents being used. Fedak and Jui (1982) found Kr3 gene located on 5D chromosome, which had only small effect on crossability of wheat. Zevan (1987) determined that genotypes with one dominant gene showed crossability 10-30 per cent and the other dominant gene possessing genotypes had 30-50 per cent crossability. The genotypes with both the genes in recessive form (kr1 kr1, kr2 kr2) showed crossability more than 50 percent. In the present study, wheat-rye disomic/ditelosomic addition lines have been used to study the effect of additional rye chromosomes/chromosome arm on the crossability with the bread wheat genotypes.

MATERIALS AND METHODS

The material consisted of wheat-rye addition lines (Disomic 1R to 7R and ditelosomic1RS, 2RL, 3RS, 4RS, 4RL, 5RS, 5RL, 6RL, 7RS and 7RL) along with three bread wheat genotypes. *viz.*, HD29, PBW343 and WL711. The material was sown in two-meter long beds by keeping plant-to-plant distance of 25cm and each row is 100cm apart in the department of Genetics and Biotechnology, Punjab Agricultural University, Ludhiana during 1999-2001. Minimum 15 lines of each material were sown. Addition lines were taken as female parents whereas wheat genotypes represented pollen parent. The emasculations were done preferably in the evening (4.00pm-7.00pm) by removing the anthers with the help of fine forceps before the maturity of anthers and receptivity of stigma. Immediately after the emasculation, the spikes were covered by parchment bag to avoid open pollination. Two to three days after emasculation (when ovary become receptive), the spikes from the pollen parent just before anthesis were taken and shaken over emasculated spikes by cutting parchment bag from the top. Crossability percentage was calculated by noting the total number of crossed grains obtained from each cross as follows

$$\text{Crossability \%} = \frac{\text{No of crossed grains obtained}}{\text{No of spikelets pollinated x 2}} \times 100$$

RESULTS AND DISCUSSION

The crossability of disomic addition lines (DRAL-1 to DRAL-7) x HD29 was maximum in DRAL-6 (92.98%) and minimum in DRAL-4 (79.94%). DRAL-7 x PBW343 had maximum crossability i.e. 83.63% and DRAL-4 x

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PBW343 had minimum (39.69%). Similarly WL711 showed high crossability with DRAL-1 (89.11%) and minimum with DRAL-6 (64.00%). It is important to note here that 6R disomic addition line had maximum crossability with HD29 but minimum with WL711, indicating that the crossability of a particular line is genotype dependant. Similarly addition line DRAL-4 showed variable effect with different wheat genotypes whereas DRAL-7 showed almost equal crossability with three wheat genotypes (Fig-1). However, different wheat genotypes were observed to have maximum mean crossability percentage with DRAL-7 (85.76%) and minimum with DRAL-4 is 68.99% (Table1).

Crosses of ditelosomic lines with HD29 showed minimum crossability 51.97% in DTRAL-4RS, whereas maximum crossability 83.64% had been observed in DTRAL-4RL. It means that short arm of chromosome 4 of rye had the gene(s) which suppress the crossability. Crossability of PBW 343 was 85.03% with DTRAL-2RL and 59.06% with DTRAL-7RL. Crossability percentage with WL711 was found to be minimum in DTRAL-4RS and maximum in DTRAL-2RL (Table 2, Fig 2).

Disomic addition line-4R, have poor crossability with HD29 and PBW343. 6R addition line shows good combination with HD29 and performs inadequately with WL711. Disomic lines having rye chromosome 1 and 7 are highly crossable with WL711 and PBW343 respectively. Further the study of ditelosomic lines showed that 4RS i.e. short arm of chromosome 4 of rye possess some genes which suppress its crossability with HD29 and WL711. Similar observations were also recorded for DTRAL-7RL x PBW343.

Table I: Crossability percentage of disomic addition lines with wheat genotypes

DRAL	HD 29	PBW 343	WL 711	Mean
1R	86.46	78.78	89.11 ↑	84.78
2R	84.61	78.72	73.91	79.08
3R	89.26	56.64	87.86	77.92
4R	79.94 ↓	39.69 ↓	87.35	68.99 ↓
5R	88.15	82.84	75.68	82.22
6R	92.98 ↑	74.58	64.00 ↓	77.18
7R	89.41	83.63 ↑	84.26	85.76 ↑
Mean	87.25	70.68	80.31	

Arrows indicating maximum (↑) and minimum (↓) crossablity
DRAL=Disomic Rye Addition Lines

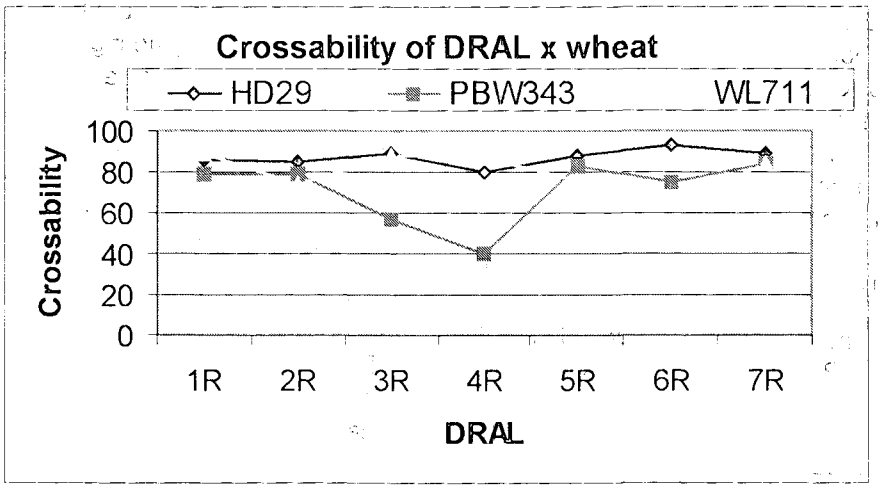

Fig. 1: Crossability of DRAL x wheat hybrids

Table 2 : Crossability percentage of ditelosomic addition lines with wheat genotypes

DTRAL	HD 29	PBW 343	WL 711	Mean
1RS	82.72	84.34	76.50	81.18
2RL	80.45	85.03 ↑	87.50 ↑	84.32
3RS	83.42	82.92	86.79	84.37 ↑
4RS	51.97 ↓	84.52	65.92 ↓	67.47
4RL	83.64 ↑	79.21	78.70	80.50
5RS	77.73	74.02	81.11	77.62
5RL	73.05	66.96	73.10	72.37
6RL	81.96	69.13	67.44	72.84
7RS	64.14	78.02	67.16	69.77
7RL	58.07	59.06 ↓	78.26	65.13 ↓
Mean	73.71	76.32	76.64	

Arrows indicating maximum (↑) and minimum (↓) crossability

DTRAL=Ditelosomic Rye Addition Lines

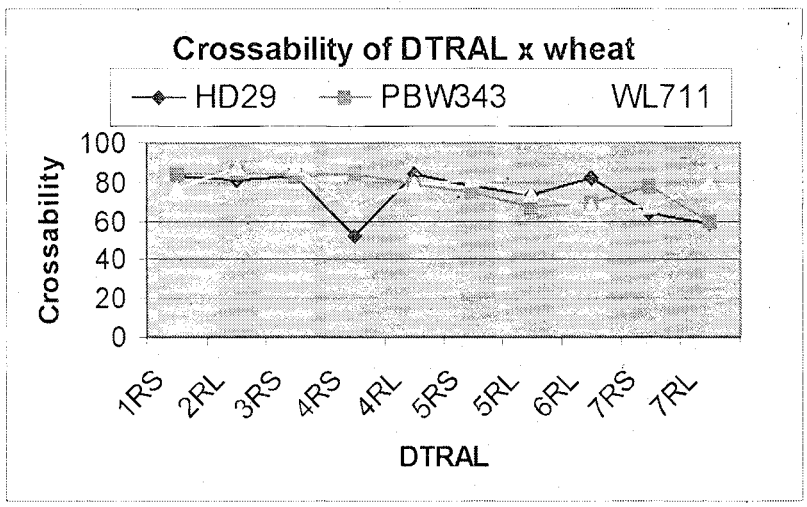

Fig. 2: Crossability of DTRAL x wheat hybrids

However, long arm of chromosome 2 and 4 of rye carry genes, which promote the crossability of wheat-rye addition lines with wheat genotypes under consideration (Table 3).

HD29 is recognized as superior genotype in crossability with addition line, where 11 out of 17 crosses possess crossability more than 80%. This is followed by WL711, which had 7 combinations with more than 80% crossability. PBW343 is inferior to both HD29 and WL711 and have only six cross combinations exceeding 80% crossability. There is only one case in PBW343 where crossability is perceived to be lesser than 40% (Table 4).

Mean crossability of ditelosomic addition lines with wheat genotypes recorded to be minimum in HD29 (73.71%) and maximum with WL711 (76.64%). Mean crossability percentage of addition lines with all wheat genotypes were maximum with DTRAL-3RS and minimum in DTRAL-7RL. Among the wheat genotypes, mean crossability was the highest with WL711 followed by PBW 343 and HD 29 (Table 2).

Sitch *et al.* (1985) suggested that both Kr1 and Kr2 genes control the crossability of wheat with rye and *Hordeum* species. Zeven and Waninge (1986) determined the crossability of bread wheat landraces from northern Africa with rye and reported poor, low, and moderate to very good crossability depending upon the area from where the landraces have been taken.

Table 3: Crossability trends between addition lines and wheat genotypes

	DRAL	
HD29	6R ↑	4 R ↓
PBW 343	7R ↑	4R ↓
WL711	1R ↑	6R ↓
	DTRAL	
HD 29	4RL ↑	4RS ↓
PBW 343	2RL ↑	7RL ↓
WL711	2RL ↑	4RS ↓

Arrows indicating maximum (↑) and minimum (↓) crossability

Table 4: Crossability range in different crosses

	0-20%	20-40%	40-60%	60-80%	80-100%
RAL x HD 29	0	0	2	4	11
RAL x PBW 343	0	1	2	8	6
RAL x WL 711	0	0	0	10	7

RAL = Rye Addition Lines

Zeven (1987) suggested that genotype with two dominant genes had lower crossability than the genotype having only one. The genotype with both the genes in recessive form showed crossability higher than 50 per cent. In the present study, almost all the lines showed crossability more than 50 per cent which indicated that these lines possess crossability genes in recessive form.

These observations suggested that the short and long arm of different rye chromosome varied in their expression as it was found that suppressor gene(s) present on short arm of 2R and 4R chromosomes had lesser influence on crossability percentage than the promoter gene on long arm of 2R and 4R chromosomes. Oettler (1985) observed crossability in wheat x rye hybrids and their nucleocytoplasmic interactions rather than any general compatibility of particular genotype.

Wheat-rye addition lines were synthesized in the background of Chinese Spring, which had kr1 and kr2 genes in recessive form and the rye chromosome also enhanced the crossability. It had also been observed that disomic/ ditelosomic wheat-rye addition lines showed variable crossability with different bread wheat genotypes.

Thus the present study suggested the screening of addition lines along with large number of wheat genotypes to identify specific genotypes, which have maximum crossability so as to have maximum hybrid seeds while transferring desirable attributes from rye chromosome through the use of disomic/ditelosomic wheat-rye addition lines.

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MANNOSE SPECIFIC LECTINS FROM EDIBLE GARLIC (*ALLIUM SATIVUM*)

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Abstract

Lectins constitute a heterogeneous group of carbohydrate binding proteins of non-immune origin, which agglutinate cells and/or precipitate glycoconjugates without affecting their covalent linkages. Mannose specific lectins have been reported from monocot species, namely Narcissus pseudonacissus, leucojum aestivum M, Leucojum vernum N, and Alliaceae. The mannose specific lectins from Alliaceae strongly resemble Amaryllidaceae lectins. Lectins specific for D-mannose differ from each other in their interactions with mono-oligosaccharides. This property enables them to distinguish D-mannose from D-glucose and thereby make them quite useful in biomedical research. The cDNA clones for two different lectins/agglutinins from garlic bulbs (Allium sativum), Allium sativum agglutinin-I (ASA-I) and ASA-II have been characterized. While the ASA-I, is a heterodimer composed of two different subunits of 11.5 kDa and 12.5 kDa, the ASA-II is a homodimer of two identical 12-kDa subunits. The use of an asialofetuin-silica affinity column has led to isolate agglutinin of 110 kDa besides lectins of 25 kDa. The ASA_{110} is a glycoprotein of two identical subunits of 47 kDa. In addition, a complex of 136 kDa comprising a polypeptide chain of 54 +/- 4 kDa and the subunits of ASA-I and ASA-III elutes earlier than these lectins on gel filtration. The physicochemical characterization, biological action, and nutritional toxicity of Allium sativum agglutinins have been reviewed.

Key words : Lectins; Garlic lectins; *Allium sativum*, Agglutinins, Mannose binding lectins.

INTRODUCTION

Lectins constitute a heterogeneous group of carbohydrate binding proteins of non-immune origin, which agglutinate cells and/or precipitate glycoconjugates without affecting their covalent linkages (Goldstein *et al.*, 1980). This definition implies that each lectin molecule has two or more carbohydrate binding sites to allow cross-linking between cells and between sugar containing macromolecules. During the last few decades, interest in lectins has been greatly intensified after realization that they act as mediators of cell recognition in biological system (Sharon, 1993). Lectins are used in wide variety of areas as in separation of glycoproteins and glycoconjugates, histochemistry of cells and tissues, cell differentiation, and in tracing cell surface pathways. These molecules are of great interest to immunologists mainly because of their ability to interact with lymphocytes and to induce blast cell transformation. All foods of plant origin contain specific lectins (Goldstein *et al.*, 1980) and other anti-nutritional factors. These lectins when consumed in raw form, both in food and feed, may have serious and deleterious effects (Liener, 1986). Early studies on lectins mainly belonged to dicotylednous plants and only a few monocot lectins such as WGA were reported from *Poaceae/Gramineae*. But in the last few decades, prevalence of very interesting lectins has been reported in other families: *Araceae* (Sandhu *et al.*, 1986, 1990), *Liliaceae* (Cammune *et al.*, 1986; Oda *et al.*, 1987), *Orchidaceae* (Van Damme *et al.*, 1986), and *Amaryllidaceae* (Van Damme *et al.*, 1987). Bulbs of snow drop (*Galanthus nivalis,* GNA) and leaves of Orchid twayblade contain lectins specific for D-mannose. The biosynthesis, primary structure and molecular cloning of snowdrop GNA have been studied by Van Damme *et al.*, (1991). Mannose specific lectins have also been reported from monocot species, namely *Narcissus pseudonacissus* (NPA), *leucojum aestivum M* (LAA), and *Leucojum vernum N* (LVA). These lectins show specific agglutination of trypsinized rabbit erythrocytes but not of human RBC. The representatives of family of *Alliaceae,* which also contain mannose specific lectins and strongly resemble *Amaryllidaceae* lectins have been discussed in this review. Lectins from *Alliaceae* show a complex mixture and cross-reactions with lectins from *Amaryllidaceae* (Van Damme *et al.*, 1991, Gupta and Sandhu, 1997a). *Allium sativum* commonly known as edible garlic is regarded as one of the most treasured food having medicinal value in variety of ailments.

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Although some lectin activity from edible garlic was reported in 1980 (Nacchbar and Oppenheim, 1980), a family of mannose-specific lectins from *Allium sativum* comprising proteins of low and high molecular weights have been reported by Van Damme *et al.*, (1991; 1992) and Gupta and Sandhu, (1997a). In addition, a complex of 136 kDa comprising a polypeptide chain of 54 +/- 4 kDa and the subunits of ASA-I and ASA-III elutes earlier than these lectins on gel filtration. The 54 kDa subunit is proven to be alliinase, which is known to form a complex with garlic lectins. Alliinase contains 5.5-6% of neutral sugars and binds to concanavalin A (Con A). It can form a complex with *Allium sativum* Agglutinin (ASA). The formation of such a complex is mediated by the interaction of the carbohydrate of the glycoprotein enzyme with the lectin. The ASA-I was shown as the main lectin present in the complex with alliinase (Rabinkov *et al.*, 1995). The potencies of the ligands for ASAs increase in the following order: mannobiose (Manα1-3Man) < mannotriose (Manα1-6Manα1-3Man) approximately mannopentaose <<Man9-oligosaccharide, suggesting that lectins specific for D-mannose differ from each other in their interactions with mono-oligosaccharides (Dam et al., 1998). This property enables them to distinguish D-mannose from D-glucose and thereby make them quite useful in biomedical research. For instance, murine IgM and human macroglobulin can be readily purified using affinity chromatography on immobilized mannose-specific lectin from snow drop (Shibuya *et al.*, 1988). Though, the *Allium sativum* is a complex mixture of lectins and their complexes whose composition varies with age of the bulb, only ASA-I and ASA-II have been thoroughly investigated.

CHARACTERIZATION

Mannose linked affinity columns have been generally used to isolate mannose specific lectins. Van Damme *et al.*, (1991; 1992) described two low molecular weight lectins, ASA-I and ASA-II employing mannose-linked Sepharose-4B column. Both these agglutinins are low molecular weight proteins of 25 kDa. The ASA-I is a heterodimer composed of two different subunits of 11.5 kDa and 12.5 kDa. In contrast, ASA-II is a homodimer of two identical subunits of 12 kDa. The amino activated silica is another matrix used for isolation of *Allium sativum* lectins. The matrix besides being rigid and highly stable facilitates rapid liquid flow and can be covalently linked to amino group of proteins or amino sugars via treatment with glutaraldehyde – a bifunctional agent (Singh *et al.*, 1993). Because of its higher specificity towards asialofetuin, it was preferred over D-mannose. Using activated silica as matrix it was possible to resolve the lectins into two separate molecular species by using stepwise gradient of glycine-HCl buffer. The presence of multiple isolectins on polyacrylamide gel electrophoresis (PAGE) at pH 8.3, some of which are present in higher concentrations than others confirmed the presence of different isoforms. In addition to low molecular weight agglutinins (ASAI and ASAII), a mannose-binding lectin ASA_{110} (with subunit of 47 kDa) was also identified in garlic bulbs. Besides ASA-I, ASA-II and ASA_{110}, a lectin of 48 kDa has also been reported from *Allium sativum*. The subunit structures of these lectins are different, but they display similar sugar specificities. Both ASA-I and ASA-III, with different Mr, are made up of 12.5- and 11.5-kDa subunits. Characterization of homodimeric and heterodimeric mannose-binding lectins indicated that despite their different molecular structures, the homodimeric and heterodimeric lectins resemble each other reasonably well with respect to their agglutination properties and carbohydrate-binding specificity. However, an analysis of the lectin-alliinase complexes demonstrated that only the heterodimeric lectins are capable of binding to the glycan chains of the alliinase molecules. Moreover, it appears that only a subpopulation of alliinase molecules is involved in the formation of lectin-alliinase complexes and that the complexed alliinase contains more glycan chains than the free enzyme (Smeets *et al.*, 1997a,b,c).

ASA-I and ASA-II cDNA from garlic bulbs: cDNA clones for two lectins from garlic bulbs, ASA-I and ASA-II, have been characterized. The first lectin, ASA-I, a heterodimer composed of two different subunits (11.5 kDa and 12.5 kDa), is translated from an mRNA of 1400 nt encoding a polypeptide of 306 amino acids with two very similar domains (**Fig.1**). N-terminal sequencing of the two polypeptides of the mature lectin showed that both subunits are derived from the same precursor and that each corresponds to one of the two domains in the sequence. In contrast to ASA-I, the ASA-II, a homodimer of two identical 12-kDa subunits, is translated from an mRNA of approximately 800nt encoding a polypeptide of 154 amino acids. However, the coding region of the ASA-II cDNA clones is almost identical to that of the second domain of the ASA-I cDNA clones (Van Damme *et al.*, 1992). The ASA-I and ASA-II, which are the most predominant bulb proteins, accumulate exclusively in the developing garlic cloves and progressively disappear when the old clove is consumed by the plant. Thus ASA-I and ASA-II can be regarded as typical vegetative storage proteins (Smeets et al, 1997c).

C1	MGRNTPSAKL	MSIAAVAAIL	TILASTCMAR	NLLTNGEGLY	AGQSLNVEPY	50
C2	MGRNTPSAKL	MSIAAVAAIL	TILASTCMAR	NILTNGEGLY	AGQSLDVEPY	50
C1	HFIMQEDCNL	VLYDHSTAVW	ATNTDIPGKK	ECRAVLQSDG	NFVVYDADGR	100
C2	HFIMQEDCNL	VLYDHNTAVW	ATNTDIPGKK	ECRAVLQSDG	NFVVYDADGR	100
C1	PLWASHSVBG	NGNYVLVLQE	DGNVVIYGSD	IWSTGTYVKA	ARGPVVVAMN	150
C2	PLWASHSVGG	NGNYVLVLQE	DGNVVIYGSD	IWSTGTYVKA	ARGPVVVAMN	150
C1	GTVDGGSLIR	PVIVNKNHVP	VIRKVGTLSR	NILRNDEGLY	AGQSLDVEPY	200
C2	GTVDGGSLIR	PVTVNKNRVP	VIRKVGTLAR	NILRNDEGLY	AGQSLDVEPY	200
C1	HFIMHEDCNL	VLYDHSTAVW	ASNTDIPGKK	GCKAVLQSDG	NFVVYDAEGR	250
C2	HFIMQEDCNL	VLYDHSTAVW	ASNTDIPGKK	GCKAVLQSDG	NFVVYDAEGR	250
C1	SLWASHSVRG	NGNYVLVLQE	DGNVVIYGSD	IWSTDTYRKS	ARGPVIRKVG	300
C1	SLWASHSVGG	NGNSVLVLQE	DGNVVIYGYD	IWSTDTYRKS	ARGPVIRKVG	300
C1	TLAGAA					306
C2	TLAGAA					306

Fig.1: Amino acid sequence of garlic ASA-I: Clone1 (C1: Accession S23494) and Clone 2 (C2: Accession S23495) (Source: NCBI). Variant amino acids in two clones are underlined (Van Damme et al, 1992).

Sequence comparison of two lectins isolated from ramsons (*Allium ursinum* L.) bulbs, AUA-I and AUA-II (AUA, *Allium ursinum* agglutinin) revealed three types of lectin clones called LECAUAG0, LECAUAG1 and LECAUAG2, which besides the obvious differences in their sequences also differed from each other in the number of potential glycosylation sites within the C-terminal peptide of the lectin precursor. Despite the similarities between the *Allium ursinum* and the *Allium sativum* (garlic) lectins at the protein level, the two lectin genes in *Allium ursinum* are organized differently. Whereas the lectin polypeptides of the heterodimeric ASA-I in *Allium sativum* are encoded by one large precursor, those of the heterodimeric AUA-I lectin are derived from two different precursors. Northern blot hybridization of *Allium ursinum* RNA revealed only one band of 800nt in contrast to *Allium sativum* RNA which gave two bands of 1400 and 800nt. Furthermore, it is shown that the two mannose-binding lectins are differentially expressed (Van Damme *et al.*, 1993). The carbohydrate-binding specificity of ASA and AUA lectins on comparison showed strong reaction between ASA and a synthetic linear (1→3)-α-D-mannan and *S. cerevisiae* mannan, weakly with a synthetic (1→6)-α-D-mannan, and failed to precipitate with galactomannans from *T. gropengiesseri* and *T. lactis-condensi*, a linear mannopentaose, and murine IgM. On the other hand, AUA gave a strong reaction of precipitation with murine IgM, and good reactions with *S. cerevisiae* mannan and both synthetic linear mannans, suggesting that the two lectins have somewhat different binding specificities for α-D-mannosyl units. Of the saccharides tested as inhibitors of precipitation, those with α-(1→3)-linked mannosyl units were the best inhibitors of ASA, the α-(1→2)-, α-(1→4)-, and α-(1→6)-linked mannobioses and biosides having less than one eighth the affinity of the α-(1→3)-linked compounds. The N-terminal amino acid sequence of ASA exhibits 79% homology with that of AUA, and moderately high homology (53%) with that of snowdrop bulb lectin, also an α-D-mannosyl-binding lectin (Kaku *et al.*, 1992).

ASA_{110}: Although erythrocyte agglutination by lectins from *Allium sativum* was inhibited only by mannose of the sugars tested, asialofetuin was more effective inhibitor of agglutination as compared to mannose (**Table-1**). The use of an asialofetuin-silica affinity column led to isolate agglutinins of 110 kDa besides lectins of 25 kDa (ASA_{110} and ASA_{25}). While ASA_{25} is a dimeric protein comprising subunits of 12.5 and 13.0 kDa, the ASA_{110} is a glycoprotein of 110 kDa containing two identical subunits of 47 kDa. The ASA_{110} revealed to have a high content of aspartic acid, glycine, leucine and serine but low content of cysteine and methionine. It contains 14 residues of neutral sugars in addition to

Table-1. Inhibition of *Alllium sativum* lectins (ASA) by oligosaccharides and glycoproteins.

S.No.	Sugar	Inhibitory Concentration
1	L (+) Arabinose	-
2	D(-) Arabinose	-
3	D(-) Ribose	-
4	Fructose	-
5	D-Galactose	-
6	D-Glucose	+ (50mM)*
7	D-Mannose	-
8	L-Sorbose	-
9	L-Fucose	-
10	L-Rhamnose	-
11	N-acetyl b-D-mannosamine	-
12	N-acetyl b-D-galactosamine	-
13	N-acetylb-D-glucosamine	-
14	a-methyl-D-pyranoside	-
15	l-O-methyl-b-D-galactoside	-
16	l-O-methyl-a-Dgalactoside	-
17	b-phenyl-D-glucoside	-
18	b-phenyl-D-galactoside	-
19	b-phenyl-D-glucopyranoside	-
20	b-phenyl-D-glucoside	-
21	Adonitol	-
22	Myoinositol	-
23	N-Acetylneuraminic acid	-
24	b-Gentibiose	-
25	Lactose	-
26	Maltose	-
27	L/D (+) Melibiose	-
28	Trehalose	-
29	Melizitose	-
30	Raffinose	-
31	Chitin	-
32	Fetuin	-
33	Asialofetuin	+(0.6mM)

(—) indicates no inhibition at 50 nM; (+) indicates inhibition at minimal (*) effective inhibitory concentration within ( ) (Gupta and Sandhu, 1997a).

43 residues of hexosamines per mole of lectin and required metal ions for its functional conformation. Though the metalloprotein nature of ASAs is still not known, garlic lectins show high agglutination of erythrocytes in presence of $CuCl_2$ and $MnCl_2$. Serological cross-reactions with other species showed some common epitopes of ASA_{110} and ASA_{25} present in *A. porrum, A. ascalonicum, Narcissus alba*, PHA and Con A but not *A. cepa*. The ASA_{110} was weakly cytotoxic to CHO cells with LD50 of 160 □g/ml (Gupta and Sandhu, 1997a). The ASA_{110}, which showed low cytotoxicity towards CHO cells is a metalloprotein whose haemagglutinin activity is significantly affected in the presence or absence of metal ions similar to the Stinkhorn mushroom lectin (Entlicher *et al.*, 1985).

ASA in Roots and Leaves of Garlic: Two lectins are present in roots and leaves of garlic. Characterization of these proteins indicated that the *Allium sativum agglutinin from* leaf (ASAL) is a dimer of two identical subunits of 12 kDa, which closely resembles the leaf lectins from onion, leek and shallot with respect to its molecular structure and agglutination activity. In contrast, the *Allium sativum agglutinin-I* from root (ASAR-I), which is a dimer of subunits of 15 kDa, strongly differs from the leaf lectin with respect to its agglutination activity. The cDNA cloning of the leaf and root lectins revealed that the deduced amino acid sequences of ASAL and ASAR-I are virtually identical. Since both lectins have identical N-terminal sequences, the larger molecular weight of ASAR-I subunits implies that the root lectin has an extra sequence at its C-terminus. These results demonstrated that virtually identical precursor polypeptides are differently processed at their C-terminus in roots and leaves and that the differential processing yields mature lectins with strongly different biological activities (Smeets *et al.*, 1997b). A study, on the temporal and spatial regulation of garlic lectins and alliinase through the life cycle of the plant, showed that the two bulb-specific lectins (ASA-I and ASA-II), which are the most predominant bulb proteins, accumulate exclusively in the developing garlic cloves and progressively disappear when the old clove is consumed by the plant. Thus ASAI and ASA-II can be regarded as typical vegetative storage proteins. The leaf-specific lectin (ASAL), on the contrary, is specifically synthesized in young leaves and remains present until withering. Because ASAL is only a minor protein, it probably fulfils a specific function in the plant. Unlike the lectins, alliinase is present in large quantities in bulbs as well as in leaves (Smeets *et al.*, 1997c).

Constituent subunits of ASA-I and ASA-III exhibited the same sequence at their amino termini. ASA-I and ASA-III recognize monosaccharides in mannosyl configuration. The potency of the ligands for ASAs increases in the following order: mannobiose (Man□1→3Man) < mannotriose (Man□1→6Man□1→3Man) approximately mannopentaose << Man_9-oligosaccharide. The addition of two GlcNAc residues at the reducing end of mannotriose or

mannopentaose enhances their potencies significantly, whereas substitution of both α1—▷3-and α1—▷6-mannosyl residues of mannotriose with GlcNAc at the nonreducing end increases their activity only marginally. The best manno-oligosaccharide ligand is $Man_9GlcNAc_2Asn$, which bears several α1—▷2-linked mannose residues. Interaction with glycoproteins suggested that these lectins could recognize internal mannose as well as bind to the core pentasaccharide of N-linked glycans even when it is sialylated. The strongest inhibitors are the high mannose-containing glycoproteins, which carry larger glycan chains (Dam *et al.*, 1998).

PHYSICOCHEMICAL PROPERTIES

The thermal unfolding of ASA-I is highly reversible and can be defined as a two-state process in which the folded dimer is converted directly to the unfolded monomers. The conformational stability determined as a function of temperature, guanidium hydrchloride (GdnCl) concentration, and pH yielded the heat capacity change upon unfolding (ΔC_p) and the temperature dependence of ΔG, ΔH, and ΔS. The thermodynamic parameters of protein suggested that the ASA-I attained a completely unfolded state irrespective of the method of denaturation. The quaternary interactions appeared to be the major contributors to the conformational stability of the protein, which correlated well with its X-ray structure. The unfolding of ASA-I reflects a relatively small, buried hydrophobic core in the folded dimeric protein (Bachhawat *et al.*, 2001a). The interaction of the bound lectin immobilized on a sensor chip with a selected group of high mannose oligosaccharides correlated with the special preference of garlic lectin for terminal α1,2—▷linked mannose residues. The association (k1) and dissociation rate constants (k2) for the binding of $Man_9GlcNAc_2Asn$ to ASAI are 6.1 x 10^4 $m^{-1}s^{-1}$ and 4.9 x $10^{-2}s^{-1}$, respectively, at 25° C. Whereas k1 increases progressively from Man_3 to Man_7 derivatives, and more dramatically for Man_8 and Man_9 derivatives, k2 decreased relatively much less gradually from Man_3 to Man_9 structures (Bachhawat *et al.*, 2001b). Trimannosides and higher oligomers can cross-link lectin dimers, amplifying the protein-oligosaccharide interactions several-fold, thus explaining the role of multivalency in enhancing affinity (Ramachandraiah *et al.*, 2003).

3D STRUCTURE

Crystals of the dimeric form of garlic protein are monoclinic C2 with unit-cell dimensions a = 203.2, b = 43.8, c = 79.3 Å and β = 112.4° and have two dimers in the asymmetric unit. The structure was solved at 2.4 Å resolution by molecular replacement using the coordinates of the snowdrop lectin as the search model. The structure reveals a β-prism II fold, similar to that in the snowdrop lectin, containing three antiparallel four-stranded β-sheets arranged as a 12-stranded β-barrel, with an approximate internal 3-fold symmetry. This agglutinin is, however, a dimer unlike snowdrop lectin which exists as a tetramer, despite a high degree of sequence similarity between them. A comparison of the two structures revealed that a few substitutions in the garlic lectin are responsible for its stability into a dimer and prevent it into tetramer formation. Three mannose molecules have been identified on each subunit. In addition, electron density is observed for another possible mannose molecule per dimer resulting in a total of seven mannose molecules in each dimer. Although the mannose binding sites and the overall structure are similar in the subunits of snowdrop and garlic lectin, their specificities to glycoproteins such as gp-120 vary considerably (Chandra, 1997; Chandra *et al.*, 1999; Ramachandraiah *et al.*, 2002).

Gastrodianin, an antifungal protein, also exists in two isoforms and belongs to the protein super-family of monocot mannose-specific lectins. Since all available structures in this super family are oligomers, the crystal structures of the both orchid lectins at 2.0Å revealed a novel monomeric structure. It resulted from the rearrangement of the C-terminal peptide inclusive of the 12th β-strand, which changes from the "C-terminal exchange" into a "C-terminal self-assembly" mode. Thus, the overall tertiary scaffold is stabilized with an intramolecular β-sheet instead of the hybrid one observed on subunit/subunit interface in all known homologous dimeric or tetrameric lectins. In contrast to the constrained extended conformation with a cis peptide bond between residue 98 and 99 commonly occurred in oligomers, a β-hairpin forms from position 97-101 with a normal trans peptide bond at the corresponding site in gastrodianin, which determines the topology of the C-terminal peptide and thereby its unique fold pattern. Sequence and structure comparison shows that residue replacement and insertion at the position where the β-hairpin occurs in association with cis-trans inter-conversion of the specific peptide bond (97-98) are possibly responsible for such a radical structure switch between monomers and oligomers. The observations revealed a structural mechanism by which the quaternary organization of monocot mannose-binding lectins could be governed (Liu *et al.*, 2005).

BIOSYNTHESIS AND PROCESSING

The biosynthesis and processing of the homodimeric and heterodimeric lectins from the bulbs of garlic (*Allium sativum*) and ramsons (wild garlic; *Allium ursinum*) have been studied on developing bulbs. The sequence of events leading from the primary translation products into the mature lectin polypeptides demonstrated that garlic and ramsons use different schemes of post-translational modifications in order to synthesize apparently similar lectins from totally different precursors. Both the homodimeric garlic lectin (ASA-II) and its homologue in ramsons (AUA-II) are synthesized on the endoplasmic reticulum (ER) as nonglycosylated 13.5 kDa precursors, which after their transport out of the ER are converted into the mature 12.0 kDa lectin polypeptides by the cleavage of a C-terminal peptide. The heterodimeric garlic lectin ASA-I is synthesized on the ER as a single glycosylated precursor of 38 kDa, which after its transport out of the ER undergoes a complex processing that gives rise to two mature lectin subunits of 11.5 and 12.5 kDa. In contrast, both subunits of the heterodimeric ramsons lectin AUA-I are synthesized separately on the ER as glycosylated precursors, which after their transport out of the ER are deglycosylated and further processed into the mature lectin polypeptides by the cleavage of a C-terminal peptide (Smeets *et al.*, 1994).

BIOLOGICAL ACTIONS

Immune Responses and Cytotoxicity: The anti-alliinase antibodies were highly specific, while anti-ASA antibodies were polyreactive. Whether normal human serum contains antibodies to dietary proteins that were never injected have been studied. The human serum contains antibodies to the two major proteins from cloves of garlic. The antibodies found were directed against alliinase and mannose-specific *Allium sativum* agglutinin (ASA) (Tchernychev *et al.*, 1995). Immunoglobulins were mainly of the IgG and IgM classes and could be divided into two categories: specific and cross-reactive.

Although, garlic lectin was not cytotoxic towards Chinese Hamster Ovarian cells, it showed mitogenic activity towards human and guinea pig peripheral blood lymphocytes (PBL). In addition ASA_{110} had an additive effect on phytoheme agglutinin (PHA) or Con A stimulation **(Fig.2)** (Gupta and Sandhu, 1996). However, in another study, a protein fraction (PF) from aged garlic extract enhanced cytotoxicity of human PBL against both natural-killer (NK)-sensitive K562 and NK-resistant M14 cell lines. Although PF treatment alone increased cytotoxicity, the effect was more remarkable when PF was combined with suboptimal doses of inter-leukine-2 (IL-2). The PF enhanced the IL-2-induced proliferation and IL-2 receptor (Tac) expression of PBL without increase of IL-2 production. The enhancement of cytotoxicity induced by PF alone or by PF plus IL-2 was abolished by anti-IL-2 antibody. The PF also enhanced ConA-induced proliferation of PBL. Nonetheless, deleterious effects of mitogen-induced liver damage in mice can be prevented by allicin, present in garlic, probably because of its immunomodulatory effects on T cells and adhesion molecules and inhibition of NF-κβ activation (Bruck et al, 2005). Thus PF and ASA_{110} are immunopotentiator and can be used for immunotherapy (Morioka *et al.*, 1993).

Effects on Intestinal Functions: Intragastric intubation of a mannose specific garlic lectin was found to bind the brush border membranes and epithelial cells of the intestine and arrested the growth of swiss albino rats. The arrested growth of the treated rats was associated with decrease in food intake (Gupta and Sandhu, 1997c). However, feeding of heat denatured lectin or heated garlic extracts did not significantly alter body weights. As lectin passed through the gut, it withstood all enzymatic reactions, though its binding to the mucosal epithelium of gastrointestinal tract was indicative of deleterious effects. Histological examination of jejunum of treated rats revealed alterations of brush border membrane, thinning and sloughing off of villus structure and vacuolization. While ASA_{110} and heat treated garlic extracts had no significant effect on nucleic acids, the enzyme activities of disaccharidases and acid phosphatase in these rats were higher. On the other hand alkaline phosphatase, lactate dehydrogenase and adenosine triphosphatase (including ouabain sensitive ATPase) declined in the jejunum of garlic/lectin treated rats; the ouabain sensitive ATPase was the most sensitive showing a diminution of about 70% after lectin treatment. In addition, ASA was found to be a competitive inhibitor of brush border membrane vesicular disaccharidases. Interestingly, crude garlic extracts showed increased levels of nucleic acids in jejunum. It was suggested that garlic components may influence regular biological functions of rat intestine by activating DNA replication and inhibiting enzyme activities in two discrete types of cell populations. The enhancement of DNA could be due to both cellular hypertrophy and hyperplasia as shown by lengthening of intestinal villi and proliferation of human and guinea pig lymphocytes by garlic lectins (Gupta and Sandhu, 1996) and Soybean

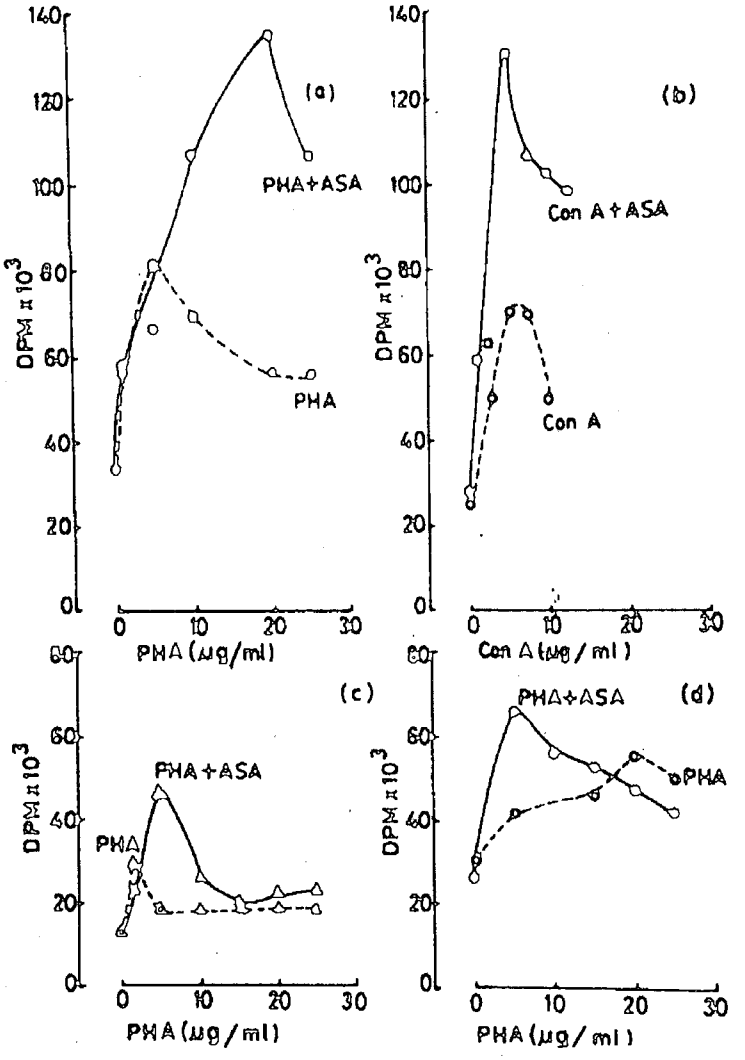

Fig. 2: Additive effect of ASA110 on PHA induced proliferation of peripheral blood lymphocytes (PBL) from human (a and d) and guinea pig (b) and Con A induced proliferation of guinea pig PBL (c).

Whey protein (Grant *et al.*, 1988). Heat denatured garlic extracts did not abolish the binding ability of ASA_{110} suggesting that the secondary structure of lectin was not essential in the inhibition of enzyme activity. These observations along with morphological changes in brush border membranes supported the possibility of direct interactions of lectins with intestinal mucosa (Gupta and Sandhu, 1997c; 1998). Lectins, which do not bind to the epithelial membranes of the intestine may or may not have any deleterious effect on absorptive functions of the gut. Perhaps cooking provides safe-guard against deleterious effects of such lectins. Though garlic is eaten in cooked form, but its consumption in raw form is not ruled out under diseased state.

The study designed to investigate the effect of garlic lectin on intestinal brush border disaccharidases and uptake of ^{14}C-glucose and ^{14}C-leucine by intestinal sacs *in vitro* showed enhanced incorporation of radioactivity in presence of lectin. The enhanced uptake of radio-labeled glucose and amino acid by intestinal sacs was associated with the decrease of brush border membrane vesicle (BBMV) sucrase and maltase. It appeared that the lectin inhibited maltase and sucrase by a competitive mechanism thus suggesting a stearic hindrance to the enzymes for interaction with the substrates. The Michaelis constant (Km) of maltase was enhanced from 0.9mM to 2.7mM whereas Km of sucrase increased from 0.9mM to 33.3 mM in presence of lectin.

Serum Chemistry: *In vivo* binding and intubation of mannose specific ASA resulted in hypercalcemia and hyperproteinemia with decreased levels of phosphate, amino acids and glucose in blood. Serum cholesterol

did not change following ASA administration. Decreased levels of serum-glucose could be due to uptake of the same by the hepatic cells where it was stored in the form of glycogen (Gupta A, unpublished). It seems that the ASA_{110} works to compensate the loss of serum glucose and amino acids as shown by enhanced uptake of radioactive glucose and amino acids by BBMV.

Binding with Tumor Cells: The distribution of a mannose specific lectin on formalin fixed paraffin sections of human breast carcinomas by immunocytochemical methods showed intense positive staining particularly at ductal and acinar epithelium. However, in case of astrocytoma, cervix, urinary bladder, kidney and lung carcinomas, the reaction varied from moderate to weak (A. Gupta, unpublished).

USES OF *ALLIUM SATIVUM* AGGLUTININS IN PEST CONTROL

Mannose-binding lectins from different monocotyledonous plants (*Allium sativum, Colocasia esculenta,* and *Diffenbachia sequina*) have a detrimental effect on the growth and development of the insect. Lectin from garlic bulb showed the highest mortality in particular. The same bulb lectin not only affected the growth and fecundity of the insect but also imparted drastic changes in the color, weight, and size, even on the second generation of the insects, which have been reared on artificial diet supplemented with a sub-lethal dose of the lectin. This finding opened a possibility of using garlic lectin as an important component in crop management (Roy *et al.*, 2002). Thus 25 kDa lectins from leaves of garlic, *Diffenbachia sequina* and tubers of *Colocasia esculanta* were evaluated for their effectiveness against chickpea aphids, mustard aphids and green leaf hoppers of rice. The binding of the lectin had been demonstrated at the epithelial membrane of the midgut of the lectin-treated insects. Receptor proteins of brush border membrane vesicle of the target insects, responsible for binding of the lectin to the midgut of the epithelial layer were suggested for the lectin-receptor interaction at molecular level (Majumder *et al.*, 2004)

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POTENTIAL OF STARCH AS A CHEAP GELLING AGENT IN PLANT TISSUE CULTURE MEDIA

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Abstract

In spite of its higher cost, agar is still used as most frequent gelling agent in commercialisation of plant tissue culture. The potential of tissue culture medium gelled with starch of two different varieties to support seed germination of Glycine max L. and morphogenetic response of various explants (shoot tip, leaf and root) was studied. Explants were obtained from one week old Glycine max L. plants raised in vitro. By analysing various responses, it was found that although there was no significant difference between the two gelling agents (agar and starch) in response to seed germination, yet the starch gelled medium was not found appropriate to replace agar for morphogenetic responses of various explants in culture.

Key words : Gelling agent, starch, agar, morphogenetic response

INTRODUCTION

Tissue culture technique has added new dimensions to the commercial exploitation of plants with desired genotypes. *In vitro* propagated plants are increasingly becoming the planting material of choice because of uniformity, disease control and possibility of rapid multiplication of a valuable genotype with desired characters. The propagation of most plants is presently carried out commercially through the organogenic pathways in agar gelled cultures, even though the protocols are large and costly. The properties of agar which make it the gelling agent of choice are its stability, high clarity and resistance to metabolism during culture (McLachlan, 1985; Henderson and Kinnersley, 1988). Moreover, the exclusive use of agar has resulted in the over exploitation of its sources. During last decade large number of attempts have been made to look for suitable substitutes for agar. Consequently a large number of substances viz. carrageenans (Lines, 1977), alginates (Scheurich *et al.*, 1980), ficoll (Kao, 1981), agarose (Johansson, 1988), gelrite (Pasqualleto *et al.*, 1988), isubgol (Jain *et al.* 1997; Babbar and Jain, 1998) have been tried as a substitute for agar. The unit cost of micropropagule production is critical in the use of any market driven technology. Since the price of production of plants in agar gelled medium was high, it was not worthwhile to take these plants out in the field.

It is against this economic problem background that potential agar substitute has been investigated. Taking this into consideration, two different varieties of starch were used as gelling agent to replace agar. *In vitro* germination response of *Glycine max* L. seeds was observed. In second part of the experiment, morphogenetic response of various explants (shoot, root and leaf) excised from the *in vitro* germinated plants was observed in media gelled with agar and starch of variety I and II.

MATERIALS AND METHODS

Simple basal medium, Knop's medium (1869) was used. The media used for the present investigation were either gelled with 0.8% (W/V) agar (Qualigens) or 6% starch. The media was autoclaved at 15lbs/ sq. inch pressure for 20 minutes. For all experiments, 25ml of culture medium was dispensed in Corning/ Borosil test tubes (6" × 1"), which were then closed with cotton plugs (cotton wrapped in muslin cloth). The cultures were exposed to 8 hours of illumination daily from fluorescent tubes (40W Phillips, India) and were maintained in an air conditioned room at 25° ± 2°C. For observing the germination response, seeds of *Glycine max* L. were sterilized by 0.25% mercuric chloride for 5 minutes in 100ml flasks. The seeds were then washed 4-5 times with autoclaved distilled

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water in order to remove the sterilizing agent. The inoculations were done manually under strictly sterile conditions in an inoculation chamber.

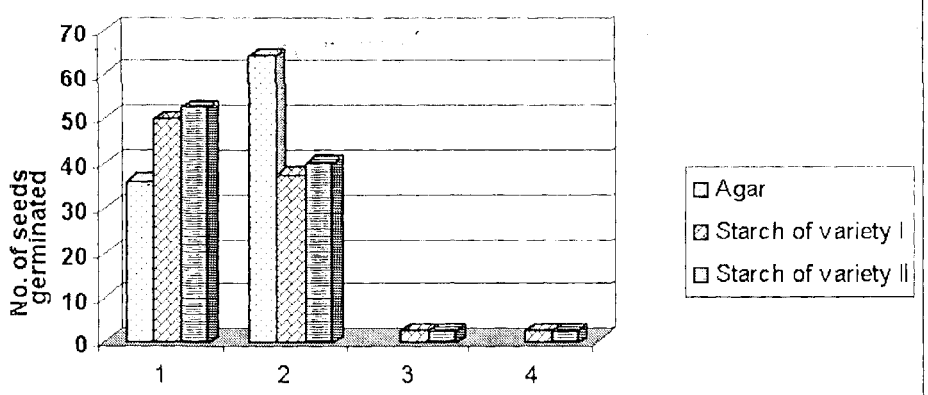
For experiments dealing with morphogenetic response, three different explants namely shoot, root and leaf were excised from one week old *in vitro* cultured plants.

OBSERVATIONS

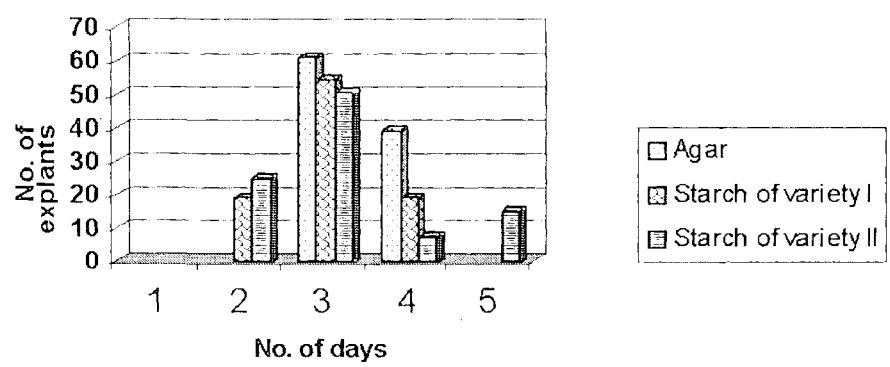
Time taken for initiation of germination response showed that there was no significant difference in media gelled with either agar or starch of variety I and II. The initial germination response of seeds inoculated on media gelled with starch was high as compared to agar but the total percentage of seed germination was high in the media gelled with agar. It was 100% as compared to 92.5% and 97.5% in media gelled with starch (I and II).

All the seeds inoculated on media gelled with agar and starch started developing root primordia within four days of inoculation. In 15% of the seeds however, the root primordia developed after five days. Initial response of development of leaf primordia was high in case of medium gelled with starch as compared to medium gelled with agar (Graph1, 2 and 3).

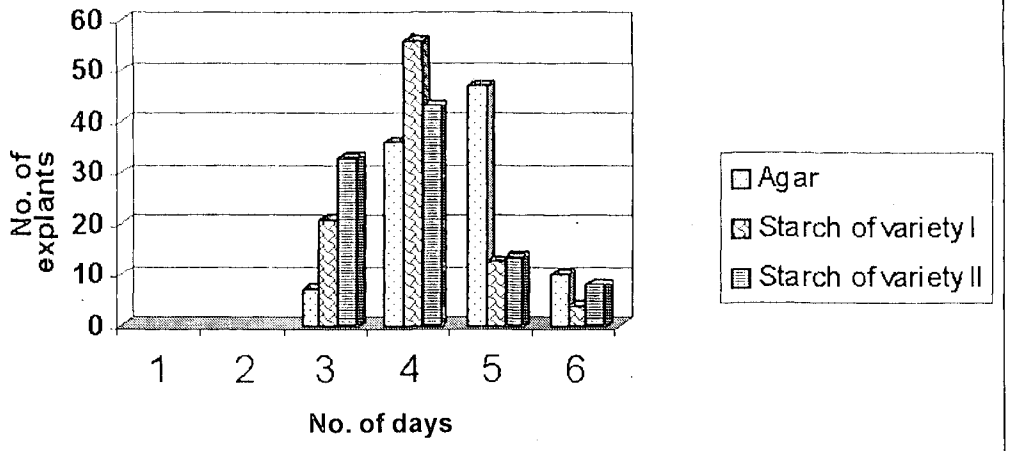
Graph 1: Comparison of germination response of *Glycine max* L. seeds on Knop's medium having different gelling agents



Graph 2: Comparison of development of root primordia in *Glycine max* L. on Knop's medium having different gelling agents



Graph 3 : Comparion of development of leaf primordia in *Glycine max* L. on Knop's medium having different gelling agents



Morphogenetic response

a. **Shoot culture** – The explants cultured on various proliferation media showed swelling initially. Two weeks after inoculation, callus initiation occurred from the cut ends of shoot tip cultured on medium containing agar as solidifying agent and supplemented with 2, 4-dichlorophenoxyacetic acid (1mg/l) and 2% sucrose. The callus was yellowish brown in colour. Shoot tips cultured on medium containing starch as gelling agent showed direct shoot formation.

b. **Leaf culture** – Colour of the leaf explants cultured on medium supplemented with 2, 4-dichlorophenoxyacetic acid (1mg/l) and 2% sucrose containing agar as gelling agent changed to yellow and cluster of roots developed from the cut petiolar end. Explants cultured on medium containing starch as gelling agent showed deformation and discolouration but did not show callusing or rooting response. The response of callus induction was poor on starch gelled medium as compared to the agar gelled medium.

c. **Root culture** – Root segment cultured on medium supplemented with 2, 4-dichlorophenoxyacetic acid (1mg/l) and 2% sucrose containing agar showed swelling initially. Three weeks after inoculation small lateral root formation occurred from the swollen explants. Contrasting results were obtained from explants cultured on medium containing starch as gelling agent, as they showed shrinkage instead of swelling. No callus formation was initiated in these explants. The morphogenetic response of these explants was poor as compared to agar gelled medium.

DISCUSSION

In the present study the main emphasis was to reduce the cost price of plants produced by tissue culture so that they can be cultured in large number and transferred to land. The most important contribution has been to replace the expensive agar with some suitable, cheaper gelling agent. Different experiments were set for seed germination and callus induction. But the success was achieved only in seed germination because no harmful effect of gelling agent was observed on seed germination. Even the germination percentage and time taken for initiation of root and leaf primordia was almost the same.

In the present study it was seen that there was no significant difference in seed germination on Knop's medium either gelled with agar or starch of two different varieties but it was not found appropriate for regeneration of callus. Maliro and Lameck (2004), while working with stem nodal sections of *Vapaca kirkirana* and *Faidherbia albida* found similar results.

The initial germination of seeds on media gelled with starches (I and II) was high as compared to media gelled with agar. Similar results were observed when cassava flour was used as gelling agent (Maliro and Lameck, 2004). Cassava starch forms a gelatinous matrix that can be autoclaved and stored or thereafter melted by heating (Kasandze,2000;Nene and Sheila,1994). Same properties were observed in starches used in the present study.

While working with gum katira as a cheap alternate for agar, Jain and Babbar(2002) found that shoots developed on gum gelled medium had yellow green leaves that were smaller than the dark green leaves of the shoots developed on medium containing agar. But the leaves were normal sized, green in colour on medium gelled with starch in the present study.

Starch in the present study was not found as suitable to replace the agar completely for morphogenetic response of various explants (shoot, root and leaf). Similarly Reed and Tsao (2002) while working with *Rubus* sp. found that three gelling treatments (agar, gelrite and combination) did not affect explant regeneration or the number of shoots per explant but did affect the callus production.

Usually 12 hours of light and 12 hours of dark period are required for the growth of cultures. We wanted to reduce the cost price of production. So we supplied 8 hours of light for the growth of cultures. Results were absolutely same without any adverse effect. Thus recommending 8 hours of light for plants to be cultured for commercial purpose.

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ABSTRACTS OF Ph.D. THESES AWARDED BY THE PANJAB UNIVERSITY CHANDIGARH, IN THE YEAR 2005

DEPARTMENT OF CHEMISTRY

(1) **Name of the Candidate : Suman Rathee** **Supervisor : S.S. Bari**

STUDIES IN THE ASYMMETRIC SYNTHESIS OF AZETIDIN-2-ONES AND RELATED BIOLOGICALLY ACTIVE COMPOUNDS

The work presented in this dissertation relates to the synthesis of C-3 functionalized, symmetrically as well as unsymmetrically disubstituted β-lactams, their structure-activity correlation (SAR) study through a Cambridge Structure Database (CSD) search. Apart from this studies on asymmetric synthesis of non-steroidal anti-inflammatory drugs (NSAID's) have also been included.

The introductory part gives a brief review of the developments in the area of symmetric synthesis of optically pure molecules; β-lactam antibiotics including their brief history, chemistry and biological activity; mechanism of action of β-lactam antibiotics, bacterial resistance and different methods of construction of this heterocycle, the β-lactam ring.

The first chapter has been further subdivided into three parts. The first part deals with the synthesis of C-3-aryl substituted β-lactams. These β-lactams, which are diversely substituted at C-3, are analogous of recently reported potent inhibitors of proteinase such as Thrombin inhibitor, Prostate specific antigen inhibitor and cholesterol absorption inhibitors.

The second chapter describes asymmetric synthesis of non-steroidal anti-inflammatory drugs (NSAID's) using asymmetric /Friedel-Crafts alkylation reaction of chiral chlorosulphides with aromatic nucleophiles in the presence of Lewis acids such as $SnCl_4$ and $TiCl_4$, and their conversion to chiral alcohols which are useful synthons for chiral expoxides and azridines.

The third chapter deals with study of a large number of β-lactam antibiotic crystal structures (both bicyclic and monocyclic) retrieved from Cambridge Structure Database (CSD) to investigate the range of favourable values of the empirical structural parameters defined by Woodward (**h**) and Cohel (**c**) for the antibacterial activity. It is found that neither **h** nor **c** alone can predict about the activity but joint analysis of **h** and **c** parameters provides a better though still empirical correlation of structure with biological activity.

The last part of this dissertation deals with the evaluation of antibacterial activity of some of the synthesized diversely substituted C-3 β-lactams as reported in Chapter 1. It has been observed that some of these β-lactams have a tendency to inhibit Stephylococci to some extent.

(2) **Name of candidate : Sandeep Kanwar** **Supervisor : Sanjay Trehan**

STUDIES TOWARDS THE ORIGIN AND DEVELOPMENT OF DIASTEREOSELECTIVE ADDITIONS ON SYSTEMS DEVOID OF 1,3-ALLYLIC STRAIN

The work in this thesis involves investigation on the mechanism and the observed diastereoselectivity in the Lewis acid catalyzed reaction of chiral acyclic mixed acetals with weak nucleophiles.These acetals undergo allylation via oxocarbenium ion intermediate. To gain insight into the structure of oxocarbenium ion intermediate ab initio molecular orbital and density functional calculation are carried.O-Aralkyl group have profound influence on the structure of oxocarbenium ion intermediate in such a way that aryl group occupies the syn position which is drastically different than predicated based on 1,3-allylic strain model. The study has established that oxocarbenium

ion can be exploited to carry out highly diastereoselective and enantio selective aldol and acetate aldol addition reactions. This adds new dimension to the aldol strategy for the synthesis of complex molecule .

(3) **Name of candidate : Gurmeet Kaur Nanda** **Suprevisor : Sanjay Trehan**

STUDIES TOWARDS THE SYNTHESIS OF ZARAGOZIC ACID

The work in this thesis describe investigation towards the synthesis of polyoxygenated molecules like zaragozic acids and cinatrins having high therapeutic value.The highly diastereoselective addition addition reaction on an oxygenatrd ketone, prepared from readily available D- tartaric acid for the synthesis of zaragozic acid which subsequently used for the synthesis of cinatrines analogue. The other key steps in the synthesis of epicinatrines C1 and C3 involves stereoselective epoxidation and subsequent ring opening using organocuprates.

(4) **Name of candidate : Manoj Bali** **Supervisor : Tejvir Singh**

STUDIES TOWARDS THE SYNTHESIS OF 9,10-SECOSTEROIDS

This dissertation includes the studies towards the liner synthesis of marine 9,10 secosteroids such as calicoferol E and astrogorgiadiol through their different ways. The methodology involves cleavage of C9-C10 bond in the ring B of 9α-hydroxy-3-(ethylenedioxide) cholestane using DDQ, resulting in the aromatisation of ring A through dienone phenol rearrangement to give Calicoferol A. The second route involves the introduction of hydroxy group at C-9 followed by dehydrogenation and aromatisation resulting in the formation of calicoferol E. The third synthesis based upon double bond maneuvering and free radical fragmentation of 3-keto-1,4-diene. The fluorine was first introduced at the C-11 of cholest-9(11)-ene-3-one. The dehydrogenation of C1-C2 and C4-C5 bonds was achieved by DDQ oxidation and clevage of C9-C10 was done with TBTH.The alkene was regenerated in the secosteroid by dehydrofluorination. Its hydroboration followed by oxidation gave astrogorgiadiol.

(5) **Name of candidate : Rani Jindal** **Supervisors : G.L. Kad and J.S. Brar**

USE OF CHEMOSELECTIVE STRATEGIES AND UNCONVENITIONAL TECHNIQUES FOR THE SYNTHES OF ORGANIC COMPOUNDS

The present dissertation record the synthesis of some naturally occurring compounds such as terpenoids, capsaicinoid, insect pheromones and the oxidative bromodecarboxylation of α, β-unsaturated carboxylic acids using green methodologies such as microwave energy, ultrasound, solid state reaction and chemoselective approach. The work in this thesis has been divided into three chapters.

Chapter 1 deals with the synthesis of 4-hydroxy-3-methoxybenzyl-7-methyloctanoate and 6-oxononan-1-ol using microwave and conventional chemistry.

Chapter 2 deals with the synthesis of 10-oxo-3,7,11-trimethyldodecanoic acid a sesquiterpene isolated from marine green algal species *Caulerpa racemosa*. Key step in the synthesis is Zn-Cu catalysed addition of alkyl halide to α, β- unsaturated carbonyl compounds under ultrasonic conditions.

Chapter 3 describe the synthesis of heneicos-6(Z)-en-11-one and des-5(Z)-en-1-yl acetate by utilizing chemoselective hydroboration iodination procedure as the key step.

(6) Name of the Candidate : Amita Wadehra **Supervisor : B.M. Deb**

TIME-DEPENDENT QUANTUM MECHANICS OF SYSTEMS FROM ONE TO THREE DIMENSIONS

The principal objective of this thesis is to obtain deeper insights into the structure and dynamics of various quantum mechanical systems through time-dependent quantum mechanics. The focus is on two fundamental and

mathematically challenging problems: (1) To devise a method for obtaining the energy eigenvalues of ground and excited states of nonlinear quantum mechanical systems with high accuracy and (2) study the behaviour of these systems under interaction with strong external fields, particularly intense lasers.

Chapter 1 gives an introduction to the above two problems and brief account of the various quantum mechanical approaches used to solve them including the ones employed in this thesis.

In Chapter 2, the energy eigenvalue problem is solved for an interesting class of nonlinear oscillators, namely self-interacting oscillator, which is a realistic model for describing those situations of prime interest where system-surrounding feedback interactions are important. We have obtained energy eigenvalues, probability densities and expectation values for one-dimensional self-interacting oscillators by evolving the time-dependent Schrödinger equation in imaginary time, coupled with the minimization of energy expectation values. For excited states, the orthogonality constraint with lower states is maintained. Probability density plots for ground and first three excited states are also presented. A comparison of energy eigenvalues is also made between oscillators with and without self-interaction which shows the self-interaction leads to stability of the system in the ground state.

Chapter 3 presents and analysis of the responses of an electron moving in one-dimensional symmetric and asymmetric double-well oscillator (DWO) potentials under intense and superintense laser fields by numerically solving the time-dependent Schrödinger equation and evolving the systems for 96 fs at a laser of wavelength 1064 and 800 nm and varying laser intensities. Emphasis is placed on the study of only those features which can arise from the response of a single system. A detailed investigation of multiphoton processes such as high harmonics generation and the energy spectrum obtained by fast fourier transform of autocorrelation function) is made. The applicability of these DWOs as model systems for the generation of attosecond pulses is also examined. Furthermore, a comparison is made with atoms and molecules under similar conditions, thereby establishing a qualitative parallelism in the behaviour of real atoms/molecules and these model DWO systems

Chapter 4 presents a numerical study of time-dependent Schrödinger equation for the multiphoton vibrational interaction of NO molecule, modeled as Morse oscillator, with intense far-infrared laser of 10503 nm and four different intensities. Starting from the vibrational ground state at zero time, various time-dependent quantities such as probability density, dissociation probability, potential energy curve and dipole moment are examined. Rich high-harmonics generation and above-threshold dissociation spectra, due to the multiphoton interaction of vibrational motions with the laser field, and consequently elevation to the vibrational continuum, have been obtained and analyzed.

Chapter 5 is an attempt to answer the question whether molecular dissociation is possible by a microwave radiation or not. By employing an intense microwave laser of wavelength 116.65 m and high intensities, a conclusion is reached theoretically that it is possible to dissociate the CO molecule, modeled as Morse oscillator. The essential condition is that the molecule should absorb 1050 photons in about 12 fs, in order to reach the vibrational continuum. An internally consistent analysis of the above-threshold dissociation (ATD) process is provided through the time-dependent probability density, dissociation probability, potential function, high-harmonics generation and above-threshold dissociation spectra.

Chapter 6 presents the femtosecond dynamics of hydrogen molecule in intense laser fields. We have adopted a quantum fluid density functional theoretical framework to explore the dynamics in terms of time-dependent density-based quantities. It becomes essential to follow the dynamical change of electron density in a molecule in order to study its behaviour in external fields because realistically all the dynamical phenomena originate from the displacement of electrons from one region of space to another. Interesting insights have been obtained into molecular time-dependent phenomena such as excitation, ionization, bond-softening, dipole formation and high-harmonics generation from the movement of electron cloud. We have thus ascertained that our method is a suitable and powerful technique to study laser-matter interactions and can yield attractive and transparent physical insights into such dynamical processes.

(7) **Name of the Candidate : Renu Arora** **Supervisor : S.S. Bari**

STUDIES IN ASYMMETRIC SYNTHESIS AND C-3 FUNCTIONALIZATION OF AZETIDIN-2-ONES

The work presented in the dissertation relates to the synthesis of C-3 functionalized β-lactams. The study is preceded by an introductory part, which gives a brief review of the history and chemistry of β-lactams.

The introduction part gives a brief review of the developments in the field of β-lactam antibiotics. It includes their activity, mechanism of action, bacterial resistance and different methods of construction of the β-lactam ring, including their asymmetric synthesis.

The first chapter consists of three parts. The first part deals with the developments of a new methodology for the synthesis of 3-allyl-βlactams. These β-lactams have been reported to be novel β-lactamase inhibitors. The strategy employs allylation of α-chlorosulphides in the presence of a Lewis acid.

The second part of this chapter describes synthesis of C-3-substitutedallyl-β-lactams. For this purpose, the desired substituted allylsilanes i.e. crotylsilanes, β-methylallylsilane were prepared by coupling of Grignard reagent of corresponding allyl chloride with trimethylchlorosilane. These allylated β-lactams were also subjected to desulphurisation reactions.

The third part of this chapter relates to an attempt made towards asymmetric allylation using chiral Lewis acid. Chiral Lewis acid was prepared *in situ* by reaction of BINOL and TiF_4 in the presence of molecular sieves.

The second chapter describes the synthesis of 3-allylidene-β-lactams employing thermolysis reactions of sulfoxide derivatives of 3α-allyl-3-phenylthio-β-lactams. The starting 3α-allyl-3-phenylsulfinyl-β-lactams were prepared by oxidation of 3α-allyl-3-phenylthio-β-lactams using sodium metaperiodate ($NaIO_4$).

The third chapter describes the halocyclization reaction of 3-allyl-β-lactams for the synthesis of 3-spiro-β-lactams. The reaction using 3-allyl-3-phenylthio-β-lactams failed to give any cyclized product. However this reaction using 3-allyl-3-benzylthio-β-lactams afforded desired spiro-β-lactams with both bromine and iodine. The halospiro-β-lactams were subjected to dehalogenation reactions.

The fourth chapter describes Lewis acid mediated isomerization of halogen at C-3 of 3α-chloro-3-phenylthio-β-lactams. Reaction using both $TiCl_4$ and $TiBr_4$ gave epimerized products.

(8) **Name of the Candidate : Neetu Gupta** **Supervisor : B.M. Deb**

QUANTUM MECHANICAL STUDIES ON NONLINEAR OSCILLATORS AND MANY ELECTRON SYSTEM

The study of quantum anharmonic oscillators is a subject of persistent interest because of its usefulness in developing models in diverse phenomena in physical, chemical and biological sciences. Major emphasis has been laid on the calculation of energy eigenvalues and on the establishment of connection between their classical and quantum dynamics. However, the main obstacle in seeking the relevant information is the non-availability of analytical solutions to most of the problems. Accordingly several approximations and numerical techniques have been formulated. The present thesis is concerned with calculation of ground- and excited-state energy eigenvalues of one- dimensional anharmonic, double- and multiple-well oscillators by numerically solving the time-dependent Schrödinger equation (TDSE) in imaginary time and to study the quantum dynamics of two-dimensional nonlinear oscillators under intense laser fields by numerically solving the TDSE in real time. The quantum dynamics of helium atom under strong magnetic fields is also studied in the thesis.

Chapter 1 presents an overview of nonlinear oscillators and quantum chaos. It also includes discussion on methodology that is based on quantum fluid density functional theory (QFDFT) and leads to a generalized nonlinear Schrödinger equation (GNLSE); which is solved to study the dynamics of helium atom under strong magnetic fields.

Chapter 2 presents various applications of anharmonic and double-well (DW) oscillators and brief review of the earlier quantum mechanical studies. It also presents the methodology which is reminiscent of diffusion-quantum Monte Carlo method and is based on evolving the TDSE in imaginary time coupled with the minimization of energy expectation value, maintaining the orthogonality constraint between states. It reports energy eigenvalues of ground and first three excited states of various anharmonic and double-well oscillators. Anharmonic oscillators with a single anharmonic term and with mixed anharmonicities are studied. Various aspects of deep DW potentials such as pseudodegeneracy, effect of asymmetry parameter on pseudodegeneracy and probability density plots are also discussed.

Chapter 3 reports our time-dependent quantum mechanical studies on multiple-well oscillators. Energy-eigenvalues of ground and first three excited states of three-, four-, five-well oscillators are obtained by numerically solving the TDSE in imaginary time. Pseudodegeneracy in deep three-well potentials, effect of asymmetry parameter on pseudodegeneracy and probability density plots are also discussed in this chapter.

Chapter 4 of the thesis presents the study of quantum dynamics of classically chaotic Henon-Heiles (HH) oscillator under intense laser fields with an objective to understand the complicated phenomenon of quantum chaos. With rapid advances in the development of ultrafast lasers, the study of laser-matter interactions has emerged as an important area of research for both experimentalists and theoreticians. The study of coupled oscillators under intense laser fields is important in view of the fact that one-dimensional oscillators have shown relevance in understanding interesting nonlinear phenomena like high order harmonic generation, above threshold ionization and stabilization under super intense laser fields, etc., that are characteristics of atoms/molecules. We have studied the quantum dynamics of an electron moving under the HH oscillator potential in presence of external time-dependent (TD) laser fields of varying intensities with regard to occurrence of quantum chaos. The TD Schrödinger equation is solved numerically and the system is allowed to generate its own wavepacket. Two kinds of sensitivities, namely, sensitivity to the initial quantum state and to the Hamiltonian [2], are examined. An electron in an atom/molecule requires a threshold intensity of the laser electric field to reach the continuum, likewise, the threshold intensity of the laser field for an electron moving in the oscillator potential to reach the continuum is identified and in this region quantum chaos has been diagnosed through a combination of various "signatures" such as the autocorrelation function, quantum "phase space" volume, "phase space" trajectory, distance function, [3] overlap integral, etc. The similarity between the HH potential and atoms/molecules in intense laser fields is also analyzed.

Chapter 5 of the thesis studies the quantum dynamics of nonlinearly coupled oscillators, namely, coupled quartic and coupled double-well oscillators under intense laser fields. Though the classically chaotic coupled quartic oscillator is one of the most widely studied models of quantum chaos, the previous studies were time-independent and required the calculation of accurate eigenenergies and eigenfunctions of highly excited states. The present thesis employs the model of an electron moving in nonlinearly coupled oscillators under intense laser field to study quantum chaos. Following the approach described in chapter 4, several "signatures" of quantum chaos have been examined. The probability density distributions, power spectra and high harmonic generation spectra are also examined in this chapter.

Finally, chapter 6 of the thesis reports the study of helium atom under strong magnetic fields. The behaviour of matter exposed to strong magnetic fields is a subject of increasing interest. This is motivated by the ongoing astrophysical discovery of strong-field objects like white dwarfs (10^2-10^5T) and neutron stars (10^7-10^9T). The competition of the diamagnetic and coulomb binding forces causes a rich variety of complex properties both with respect to the structure as well as dynamics of the considered systems. The GNLSE is employed to study the quantum dynamics of helium atom under strong magnetic fields and the possible signatures of quantum chaos are interpreted.

(9) **Name of the Candidate : Mahmood Shakir Magtoof** **Supervisors : S.S Bari and S.D. Sharma**

SYNTHETIC INVESTIGATION IN AZETIDIN-2-ONES AND OTHER BIOLOGICALLY ACTIVE HETEROCYCLES

The work presented in the dissertation relates to the synthesis of azetidin-2-ones and other biologically active

heterocycles. The study is preceded by an introductory part which gives a brief review of the history and chemistry of β-lactams and related heto\erocycles. The experimental section, followed by references, has been arranged after the discussion of the present work which in turn has been further divided into four chapters.

The **first chapter** describes a new synthetic approach for the preparation of azetidin-2, 3-diones involving a radical initiated rearrangement.

The **second chapter** deals with the preparation of N-unsubstituted β-lactams by CAN oxidation of 1-(4'-diethylamino) phenyl azetidin-2-ones.

The **third chapter** deals with the preparation of β-lactams using chiral bases in the key step for the synthesis of chiral phenylthio/benzylthioazetidin-2-ones.

The **fourth chapter** deals with the preparation of heterocyclic compounds such as 1,3,4-oxadiazole, 1,2,4-triazole some new triazolo-triazole derivatives and 1,2,4-triazin-6-ones.

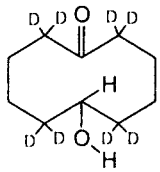
(10) **Name of the Candidate : Preeti Walia** **Supervisors : Tejvir Singh, P. Venugopalan and H. Singh**

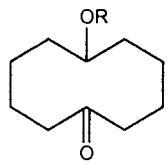
CONTROL OF REACTIVITY IN CYCLODECANE DERIVATIVE: SYNTHESIS AND CRYSTALLOGRAPHY

The overall subject matter of these underscores the significance of reaction medium in controlling the transannular reactivity in cyclodecane derivative. Detailed studies on the ten membered carbocycles 6-hydroxycyclodecanone has been undertaken to investigate general feature of problem;

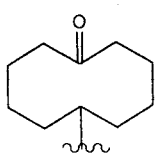
6-hydroxycyclodecanone exist exclusively in the conformation Boat-Chair-boat (BCB) in solid state, however in solution it shows an equilibrium with its hemiacetal.

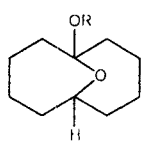
Deuteration studies on 6-hydroxycyclodecanone suggest that it exists as self-redox system in water. The incorporation of deuterium isotopes at positions alpha to carbonyl as well as hydroxyl group formed the basis for the above spececulation. This reaction has been exploited to obtain various 6-keto ether.





R= tBDMS, Me, Et, triphenylemethyle, allyl, benzyle and substitute benzyle.





Similarly ether from hemiacetal were also obtained. The above ethers have been characterized by mp, IR, ^{1}H NMR, ^{13}C NMR and MS. In certain cases single crystal X-ray diffraction studies have also been carried out. Besides the above studies photochemical reactions of 6-hydroxycyclodecanone and brominations of (E) and (Z)-6-cyclodecanone were investigated.

DEPARTMENT OF MATHEMATICS

Name of the Candidate : Sukhvinder Kaur **Supervisor : Harinder Singh**

SOME PROBLEMS OF COUPLED AND GENERALIZED THERMOELASTICITY

The temperature changes produce stresses in continuous media. The source of stress is heat, hence the stresses generated by temperature field is called thermal stresses. If the material of the body in which they originates is elastic, these stresses are said to be thermoelastic. Today engineers and scientists to solve practical problems in structural and material designs are using thermoelastic stress analysis. The aim of this thesis work is to study variation of temperature and variation of thermal stresses in a homogeneous isotropic elastic material considering some problems of coupled and generalized thermoelasticity.

Chapter 1, presents the theory of thermoelasticity, basic concepts related to it and their importance.

In chapter 2, considering importance of moving stress and temperature pulses in high speed machining in this chapter we have studied the effects of temperature and stress (pressure) pulses traveling along across the surface of a thermo-elastic half-space. We consider a homogeneous, isotropic elastic semi space $y \geq 0$, which initially has temperature T_0 and is in stress free state. We study the development of temperature and stresses assuming a two-dimensional temperature fields in the context of generalized theory of thermoelasticity. The variation in temperature field and stress field occurs owing to the action of external loadings (due to heating or cooling). Appropriate initial conditions and boundary conditions are introduced. The solutions are obtained for the variation in temperature field and stress field using method of Power series. Stainless steel is the material considered for the numerical calculations and results are shown through contour maps.

In chapter 3, we have studied the effects of both temperature and stress (pressure) pulses traveling along across the surface of a plate in the context of generalized theory of thermoelasticity. A homogeneous, isotropic elastic plate of thickness "H" which initially is at temperature T_0, is considered in this problem. The variation in temperature field and stress field are obtained after introducing appropriate initial conditions and boundary conditions. For numerical calculations stainless steel is taken as the material and results are shown through contour maps.

When a thermo-elastic body is suddenly heated or cooled from out side, heat flow arises resulting in change in the temperature distribution in the body and development of stress field. In chapter 4, we have studied the development of these fields in each layer of a multi-layered plate using coupled thermo-elastic theory. Each layer of the medium is assumed to be of isotropic elastic thermally conducting homogeneous material. Perfect mechanical bonding at the interface between the different layers is assumed. The method of Laplace transform is used to give the solutions in each layer of the plate, under the boundary conditions at the outer surfaces and interfaces between layers. The numerical computations are carried out for one-, two- and three layered plates to display the variation of the stress and temperature fields. For numerical calculations stainless steel and copper is taken as the material and results are shown graphically.

The investigation of stress concentration around holes and notches of arbitrary shape in a given elastic medium is very important for modern engineering. Considering the importance of high stress concentration, temperature and thermal stresses in a hexagon region with an elliptic hole is presented in chapter 5. We have studied temperature and thermal stresses in hexagon region with a elliptic hole using elliptic co-ordinates. For numerical calculations stainless steel is taken as the material and results are shown graphically.

DEPARTMENT OF MICROBIOLOGY

(1) **Name of the Candidate : Vanashree Yadav** **Supervisors : Sanjay Chhibber, Saroj Sharma and Kusum Harjai**

IMMUNOPROTECTIVE POTENTIAL OF *KLEBSIELLA PNEUMONIAE* O-POLYSACCHARIDE CHOLERA TOXIN B SUBUNIT CONJUGATE AGAINST EXPERIMENTAL LOBAR PNEUMONIA

Respiratory tract infections are the most common potentially severe infections treated by health care practitioners

in both developing as well as developed countries. One form of these infections is pneumonia which because of its high morbidity and mortality rate is considered to be serious infection. Among gram negative bacteria *Klebsiella pneumoniae* is one of the most frequently isolated bacterial pathogen from cases of nosocomial and community acquired pneumonias. Pneumonia caused by this organism is especially dangerous because it has a rapidly progressive clinical course which is often complicated by multilobular involvement and lung abscesses. The mortality rates have been shown to exceed to 50% even in the treated cases and survivors may be left with substantial pulmonary scarring. The main population at risk of developing Klebsiella induced pneumonia includes neonates, elderly, alcoholics and immunocompromised hosts. An increase in the population at risk of developing infection with this opportunistic pathogen coupled with the emergence of extended spectrum beta lactamase (ESBL) producing multidrug resistant strains has promopted the scientists to look for immunological means to combat these infections. The subject has great importance in the present day context.

In the present study a conjugate vaccine was made by employing polysaccharide moiety of the lipopolysaccharide antigen of *Klebsiella pneumoniae* O1 serotype and recombinant cholera toxin B(rcTB)subunit. Conjugate vaccines offer an elegant solution to a long standing hurdle in the production of subunit polysaccharide based vaccines. The use of rCTB is preferred due to its efficacy, safety stability and purity. This has an added advantage as it can be produced in large amounts at relatively low cost. The conjugate was tested in a lobar pneumonia model in mice. The model was established by giving intranasal instillation. The course of experimental pneumonia was studied in terms of induction, establishment and resolution of infection. Experimental pneumonia was accompanied by an influsion of inflammatory cells (macrophages and neutrophils) along with compartmentalized time dependent elevation of TNF-α, MIP – 2 and nitric oxide as observed in bronchio alveolar lavage fluid (BALF).

Lipopolysaccharide antigen of *K. pneumoniae* B 5055 was extracted and purified by gel filtration and ultracentrifugation. The pure material was found to be toxic, pyrogenic as well as induced second degree Schwartzman reaction in rabbits. It was found to be immunoprotective when administered by intramuscular route but with intranasal administration of antigen, protection was only observed at lower dose. On the contrary O-polysaccharide separated from LPS by mild acid hydrolysis, purified by column chromatography did not show any protection when given through intramuscular or intranasal route. In addition O-PS did not show any biological activity in normal or sensitized animals. When conjugated to rCTB subunit, the O-PS provided protection against challenge. Mild bronchopneumonia observed on day 1 post infection, progressed to well developed lobar pneumonia by day 3 post infection and signs of resolving pneumonia were observed on day 7 post infection in experimental animals which was not observed with OPS-rCTB mixture. The conjugate was made by carbodimide condensation reaction using adipic acid dihydrazide as a spacer molecule. Yield of coupled material was 43.5%, protein-polysaccharide ratio was 1.8 and it was nonpyrogenic, nontoxic and did not induce any schwarzman reaction. Conjugate was found to be immunoprotective when administered by intramuscular as well as intranasal route. Innate immune response studied in conjugate immunized animals showed enhanced bacterial clearance from lungs associated with increased influ of PMNS and alveolar macrophages with enhanced phagocytic activity. Accelerated production of proinflammatory cytokines TNF-α and MIP2 was detected in bronchoalveolar lavage fluid (BALF) as well as in lung homogenate supernatant (HS) of immunized animals. Intranasal immunization with conjugate evoked a good systemic and mucosal immune response as high titers of IgG and IgA were detected in serum and BALF of immunized animals. However, Intramuscular immunization with conjugate induced a weak mucosal immune response. The enhanced immune response provided protection against heterologus strains of Klebsiella pneumoniae as well. But the protection was less than that observed with homologous strain.

Conclusion: Based on the results of this study, it is concluded that immunization with K. pneumaniae O-polysaccharide rCTB subunit conjugate provides protection against experimental lobar pneumonia in mice. This polysaccharide conjugate vaccine falls in the category of new generation vaccines and confers protection through activation of nonspecific as well as specific defence mechanisms. The study establishes the efficacy of intranasal route of immunization over the parenteral immunization. It also confirms the mucosal adjuvanticity of rCTB subunit for *K.pneumoniae* O-polysaccharide antigen.

(2) **Name of the Candidate : Vandana Toky** **Supervisors : Sanjay Chhibber and Saroj Sharma**

PROTECTIVE POTENTIAL OF ANTIBIOTIC INDUCED OUTER MEMBRANE PROTEINS OF *KLEBSIELLA PNEUMONIAE* CONJUGATED TO POLYSACCHARIDE MOIETY OF LIPOPOLYSACCHARIDE ANTIGEN IN MURINE SEPSIS MODEL

In spite of available advances in supportive care and potent antimicrobial treatment, sepsis continues to be a leading cause of death among hospitalized patients. Bacterial infections are the most common cause of septic shock whereas fungi, viruses and protozoa are less commonly responsible for the syndrome. The most common causative gram negative organisms associated with sepsis include *Klebsiella pneumoniae*, E. coli, pseudomonas and entero bacter species. Among these, *Klebsiella pneumoniae* continues to be a nighmare for neonatologists, microbiologists and hospital administrators. In the management of sepsis, proper and timely choice of empiric antibiotic therapy is of utmost importance. These antibiotics bring about structural changes in bacteria which augment their susceptibility to humoral and cellular defences. Apart from morphological changes exposure of bacteria to antibiotic unmask/induce expression of new outer membrane protein (OMP) epitopes. Besides this, release of endtoxin (lipopolysaccharide) following antibiotic exposure has also been reported. The rate at which endotoxin is released from the organisms vary with class of an antimicrobial agent. In the present study an attempt was made to protect the animals using conjugates of newer OMP induced/expressed under antibiotic stress with the polysaccharide moiety of LPS in a mouse fibrin thrombin clot model of bacterial sepsis with *Klebsiella pneum*aniae ATCC 43816. Three antibiotics namely ceftazidime, ofloxacin and amikacin belonging to three broad groups of antibiotics were used. No, alteration in the OMP profile of bacteria grown in presence of amikacin was observed. However, proteins isolated from bacteria following growth in presence of ceftazidine and ofloxacin showed expression of new OMPS of high molecular weight (more than 97 KDa) as well as protein with low molecular weight (22 KDa, 30 KDa and 40 KDa). Their immunogencity as well as surface location was confirmed using immunoblotting with respective antisera. The low molecular weight proteins of 40 KDa (PR-CD) and 30 KDa (PR-OF) were expected from the OMP of organisms grown in presence of ceftazidine and oftoxacin. These were purified by gel elution. The purified proteins showed negligible amants of carbohydrate, KDO and nucleic acids. LPS was alos extracted purified and O-polysaccharide was fractionated and resolved on sephadex G-50 column. Two different conjugates were prepared by conjugating PR-CD (40 KDa) and PR-OF (30KDa) with O-PS moiety of LPS antigen by carbodimide method. The conjugates were fractionated on sephadex G-100 column and total yield of coupling for OPS-PR-CD and OPS-PR-OF conjugates was 45.2% and 44.0% respectively. Purified conjugates showed maximum polysaccharide/protein ratio of 1.58 and 1.9 for OPS-PR-CD and OPS-PR-OF respectively. The lethality was assessed in normal and sensitized mice along with pyrogenicity and Schwartzman reaction. Both the conjugates were devoid of any such biological activity. Immunization with both conjugates evoked IgG response as determined by ELISA. These were equally efficient in enhancing the phagocytosis and killing of bacterial by peritoneal macrophages. Following active immunization both the conjugate provided significantly higher protection in terms of percentage survival. There was significant decline in bacteriological load in blood and organs like liver, spleen and kidney. A positive correlation between decrease in viable counts and pathology in the organs was observed. Mild inflammanty changes were detected in animals immunized with conjugates in contrast to severe inflammation in control group. Similarly TNF-α levels were significantly higher in the blood of conjugate immunized protected group as compared to control group. An active immunization strategy may be of limited use in sepsis due to the insufficient time for the antibody response to develop since time lapse in the on set of septic shock is very short. Also most of the cases of sepsis occur in immnocompromised patients admitted in ICU's who may not respond well to active vaccination. Therefore, protective efficacy of passive immunotherapy was tested in the animal model. Simultaneous administration of antisera raised against either of the conjugate along with respective antibiotic resulted in 100% protection in mice following challenge with *K.pneumoniae* There was decline in bacterial counts in blood as well as organs and this correlated well with pathology observed in different groups.

Conclusion: The increasing problem of treating bacterial sepsis has focused the attention of many scientists to look for adjunct therapies. In this direction the results of this study showed that proteins expressed under antibiotic stress when conjugated to polysaccharide moiety of K.pneumoniae make meaningful antigen. The antisera raised against relatively low molecular weight OMPs, when conjugated to O-PS showed better resolution

of infection following its use in conjuction with antibiotics. This strategy would be meaningful as this approach has potential to affect bacteria as well as endotoxin component released in presence of antibiotic.

DEPARTMENT OF PHYSICS

Name of the candidate : Vineet Sharma **Name of the supervisor : S.K. Tripathi**

THERMALLY INDUCED CHANGES ON THE ELECTRICAL PROPERTIES OFAMORPHOUS Se-Te GLASSY ALLOYS

Amorphous materials are interesting from technological and scientific point of view having a vast spectrum of applications in diverse fields. Amorphous chalcogenide system of Selenium-Tellurium has been studied in the present thesis. The detailed investigation of the effect of prolonged thermal annealing treatment and the addition of third element on the electrical properties in the thin films of binary alloy of a-$Se_{85}Te_{15}$ has been carried out. The dark conductivity (σ_d), steady state photoconductivity (σ_{ph}) with temperature, light intensity and the transient photoconductivity at different temperatures, intensities have been carried out. The effect of thermal annealing and the addition of third element in binary $Se_{85}Te_{15}$ glassy alloy thin films on the electrical properties have been studied.

The glassy alloys have been prepared by melt-quenching technique and their thin films by thermal evaporation technique. XRD technique has been employed to characterise amorphous nature of the thin films. The stainless steel sample holder for various electrical measurements have been designed and fabricated.

The electrical measurements have been made on the thin film of binary a-$Se_{85}Te_{15}$ alloy. The σ_d and σ_{ph} measurements have been carried out in vacuum. Temperature dependence of σ_d and σ_{ph} have been found to be thermally activated with single activation energies and to increase with decrease in respective activation energies on subjecting the thin film to prolonged thermal annealing treatment. The intensity (F) dependence of σ_{ph} has been observed to obey the power law showing square root dependence on F irrespective of the time of the thermal annealing treatment (t_a) indicating bimolecular nature of recombination. The decay of photocurrent (I_{ph}) has been observed to be non-exponential in nature at all annealing time observations for transient photoconductivity measurements. A maximum in the rise curve of the I_{ph} has been observed for the initial t_a which changes its behaviour to a steady increase of I_{ph} in the rise curve for prolonged t_a. The decay of I_{ph} has been found to be very fast for initial t_a and become slow with the presence of a persistent I_{ph} for later t_a.

The effect of thermal annealing on the electrical properties of thin films of a-$Se_{85-x}Te_{15}M_x$ alloys (x = 0 and 10 at. % & M = In, Sn and Sb) near the glass transition temperatures (T_g) has been studied. The electrical properties change on subjecting these thin films to progressive thermal annealing treatment. Results for only four of the various annealing time intervals have been discussed in the present analysis. The σ_d and σ_{ph} have been observed to increase with the decrease in respective activation energies after thermal annealing. The photosensitivity (σ_{ph}/σ_d) decreased sharply after thermal annealing treatment. Intensity dependence of σ_{ph} has been observed to show square root dependence irrespective of the t_a. The transient photoconductivity measurements have been carried out at different temperatures and intensities for all the four glassy alloy thin films. A maximum in the rise of I_{ph} at all temperatures and higher intensities has been observed in case of all the glassy alloys for the initial t_a (0 and 30 min), which is most prominent in case of pure a-$Se_{85}Te_{15}$ and a-$Se_{75}Te_{15}Sn_{10}$ thin films. The maximum has been observed to be absent for prolonged t_a (8130 min) and I_{ph} increased steadily with illumination of the thin film at all temperatures. Persistent I_{ph} has been observed during the decay of I_{ph} at later stages of annealing. The σ_{ph}/σ_d has been observed to decrease with increase in temperature for all glassy alloys. The value of σ_{ph}/σ_d after annealing (930 and 8130 min) has been observed to be very low as compared to those for the initial t_a (0 and 30 min). The effect of addition of third element in the binary a-$Se_{85}Te_{15}$ thin film has been observed to affect the σ_{ph}/σ_d. The σ_{ph}/σ_d of the binary a-$Se_{85}Te_{15}$ has been found to be much more than other glassy alloys. The lower value of σ_{ph}/σ_d of a-$Se_{75}Te_{15}M_{10}$ thin films with M = In, Sn and Sb as additive elements may be due to their lower electron affinities as compared to Se or Te.

The effect of addition of third element on the electrical properties of $Se_{85}Te_{15}$ glassy alloy has also been studied. The changes in the electrical properties with variation in concentration of third element in a-$Se_{85-x}Te_{15}M_x$ alloys (M = In, Sn and Sb ; x = 0, 2, 4, 6 and 10) have been investigated. The σ_d and σ_{ph} have been observed to increase with decrease in the respective activation energies from x = 0 at. % to x = 10 at. % in case of addition of In additive. A maximum in σ_d and σ_{ph} at x = 6 at. % has been observed in case of Sn additive and a maximum in σ_d and σ_{ph} at x = 4 at. % of Sb addition has been observed. The temperature dependence of σ_{ph} has been found to show a maximum at x = 2 and x = 4 at. % incase of In additive. The maximum has been observed at all concentrations of Sn, but more prominent being at x = 6 and x = 10 at. %. No maximum has been observed incase for the temperature dependence of σ_{ph} on Sb addition. Intensity has been observed to show square root dependence with σ_{ph} for the thin films of all glassy alloys added with In, Sn and Sb additives, indicating a bimolecular recombination on irradiation of the films with light. The σ_{ph}/σ_d has been observed to decrease with increase in temperature on the incorporation of additives irrespective of their at. % in a-$Se_{85-x}Te_{15}M_x$ thin films. The value of σ_{ph}/σ_d of a-$Se_{85}Te_{15}$ thin film has been observed to decrease sharply on the addition of third element. The charge carrier concentration has been observed to increase as the concentration of In increased in a-$Se_{85-x}Te_{15}In_x$ thin films from x = 0 to x = 10 at. % and showed a maximum at x = 6 at. % of Sn and at x = 4 at. % of Sb additive in a-$Se_{85-x}Te_{15}Sn_x$ and a-$Se_{85-x}Te_{15}Sb_x$ glassy alloys thin films respectively.

UNIVERSITY INSTITUTE OF PHARMACEUTICAL SCIENCES

(1) Name of the Candidate : Devinder Singh **Supervisor : Kanwaljit Chopra**

PHARMACOLOGICAL INTERVENTIONS OF EXPERIMENTAL RENAL FAILURE

Renal failure is a life-threatening illness whose mortality has remained high inspite of the introduction of hemodialysis 25 years ago. This syndrome is reported in approximately 5 per cent of all hospital admissions and 30 percent of intensive care unit patients. To the nephrologist, it remains an extremely frustating disease due to availability of very limited therapeutic options and poor understanding of its basic pathophysiology. Renal oxidative stress plays an important part in etiology of renal failure.

This study was designed to delineate the precise role and contribution of Oxygen Reactive Species in renal dysfunction mediated by ischemia & iron overload and to explore the mechanisms of renal ischemic preconditioning.

1. To establish the role of oxidative stress in ischemic, myoglobinuric and ferric nitrilotriacetate-induced renal damage in rats.
2. To study the effect of various pharmacological agents with antioxidant potential, like trimetazidine and carvedilol in ischemic, myoglobinuric and ferric nitrilotriacetate-induced acute renal failure (ARF).
3. To investigate the effect of certain bioflavonoids, including, quercetin, catechin and naringin on ischemic, myoglobinuric and Fe-NTA-induced ARF.
4. To establish the phenomenon of endogenous renoprotection by renal ischemic preconditioning and to elucidate the mechanisms involved in mediating this effect.
5. To establish the phenomenon of intra-organ preconditioning "remote organ preconditioning" by brief renal ischemia and reperfusion.
6. To study the effect of chemical preconditioning by using cyclosporine A as preconditioning agent.

The salient findings of our studies reveal that:

1. Reactive oxygen species play a pivotal role in renal ischemia/reperfusion injury as is evident from marked increase in lipid peroxidation, significant decline in antioxidant enzyme levels and resultant deterioration in renal function and morphology. Trimetazidine, carvedilol, quercetin, catechin and naringin ameliorated the renal oxidative stress and demonstrated a marked renoprotective activity.

2. Iron-induced oxidative stress contributes to the renal dysfunction as is evident in the glycerol-induced myoglobinuric ARF and ferric nitrilotriacetate-induced oxidative renal damage. The pharmacological modalities used in this study counterbalanced the deleterious effect of glycerol and ferric nitrilotriacetate-induced oxidative renal injury to a great extent and they may find application in the prevention and treatment of oxidant-induced renal injury.
3. Three repetitive cycles of 2 minutes renal ischemia separated by 5 minutes reperfusion period precondition the rat kidney and ameliorates the functional disturbances, altered morphology and renal oxidative stress which are observed after prolonged ischemia and reperfusion. The beneficial effects of ischemic preconditioning in the kidney are speculated to be mediated by K^+_{ATP} channels and PKC.

(2) Name of the Candidate : M. Anjaneyulu **Supervisor : Kanwaljit Chopra**

PHARMACOLOGICAL INVESTIGATIONS ON EXPERIMENTAL DIABETIC NEUROPATHY AND NEPHROPATHY

Although the Diabetic Control and Complications Trial have identified hyperglycemia as a risk factor for development of diabetic complications (The DCCT Research Group, 1993), there is a no consensus regarding the pathogenic link between hyperglycemia and complications. There are a number of equally tenable hypothesis on the origin of complications, including but not limited to, the Maillard, or advanced glycation end product (AGE) hypothesis (Brownlee, 1988), the aldose reductase hypothesis, oxidative stress (Baynes, 1991; Baynes and Thorpe, 1997), reductive stress (pseudohypoxia) (Williamson et al., 1993), true hypoxia (Cameron and Cotter, 1994), carbonyl stress altered lipoprotein metabolism, increased protein kinase C activity (Ishii et al., 1998), and altered growth factor or cytokine activities (Sharma et al., 1997). The various hypotheses overlap and intersect with one another: AGE formation and altered polyol pathway activity which may lead to oxidative stress; oxidative stress may accelerate AGE formation, reductive stress may lead to activation of protein kinase C, AGEs may induce oxidative stress and growth factor expression, and so on. The long list is a strong indication of the uncertainties in our understanding of the pathogenesis of diabetic complications.

With this background, the present work was undertaken to study various pathological mechanisms underlying the development of two major microvascular complications of diabetes i.e., diabetic nephropathy and neuropathy. The studies were also directed to identify potential pharmacological interventions for prevention as well as management of these devastating complications. Thus the present study was designed to meet the following objectives:

- ✓ Role of oxidative stress in pathology of diabetic nephropathy was investigated and various antioxidants were evaluated for their renoprotective effect in STZ-induced diabetic nephropathy in rats.
- ✓ The role of a selective AT_1 receptor blocker, Irbesartan and ACEI, Captopril, was elucidated in diabetic nephropathy.
- ✓ Possible role of a calcium channel blocker, Diltiazem, was investigated in diabetic nephropathy.
- ✓ Effect of modulation of PKC activity was investigated in diabetic nephropathy using a PKC inhibitor, GF109203X.
- ✓ An antioxidant flavonoid, quercetin, was investigated in diabetic neuropathic pain in mice and rats. An attempt was also made to investigate the possible modulation of effect of morphine by quercetin in diabetic mice.
- ✓ Role of neurotransmitter, serotonin and its interaction with other neurotransmitters in the attenuation of diabetic neuropathy was evaluated in diabetic mice.
- ✓ Role of glutamate, dopamine and GABAergic neurotransmission in mediating diabetic neuropathic pain in mice was investigated.

- ✓ Possible role of a downstream signalling mediator, PKC, was evaluated in development of hyperalgesia and allodynia in diabetic rats and mice.
- ✓ Effect of NDGA, a lipoxygenase inhibitor and an antioxidant, was investigated in neuropathic pain in diabetic rats and mice.
- ✓ Effect of chronic treatment with insulin, vitamin E and carvedilol in attenuation of thermal hyperalgesia and allodynia was investigated in diabetic rats.

The salient findings of our studies reveal that:

- ✓ Oxidative stress plays a pivotal role in the development of diabetic nephropathy and point to the possible anti-oxidative mechanism being responsible for the nephroprotective action of quercetin, NDGA and carvedilol.
- ✓ Our results also pointed out that the amelioration of hyperglycemia with once-daily insulin administration alone resulted in partial amelioration of diabetic nephropathy whereas insulin plus vitamin E completely corrected the oxidative stress, renal dysfunction and renal morphology.
- ✓ Oxidative stress/nitrosative stress is increased in the diabetic kidney and AT1 receptor blockade and ACE inhibition along with calcium channel blockage can prevent these changes. The results also suggest that in STZ-induced diabetic rats, the protective action of irbesartan and captopril might be mediated, at least in part, by its effect on tissue oxidant/antioxidant status.
- ✓ Our results also emphasize that role of Protein kinase C in the pathophysiology of diabetic nephropathy and point towards the potential use of antioxidants and PKC inhibitors as a complementary therapy for the prevention/treatment of diabetic nephropathy.
- ✓ The preliminary results in mice indicated an antinociceptive activity of quercetin, a natural flavonoid, probably through the modulation of opioidergic mechanism. These results in diabetic rats also emphasize the role of oxidative stress in the development of significant hyperalgesia and cold allodynia in diabetic rats and points towards the potential of quercetin as a complementary therapy for the prevention/treatment of diabetic neuropathic pain. Also the concomitant intake of quercetin along with morphine may be useful in the diabetic neuropathy.
- ✓ Fluoxetine-induced antinociception primarily involves serotonin pathway modulation through 5-HT(1) and 5-HT(2) receptors, but not through 5-HT(3) receptors, in the chronic pain associated with streptozotocin-induced diabetic neuropathy. Further, the potentiation of the antinociceptive effect of fluoxetine by pindolol indicates the usefulness of a combination of an antidepressant and a 5-HT(1A/1B) receptor antagonist in the treatment of diabetic neuropathic pain in humans.
- ✓ The concomitant administration of low dose NMDA antagonists along with NO inhibitors may be useful in the diabetic neuropathy with reduced toxicity.
- ✓ Results strongly suggest that D_2, but not D_1 dopamine receptors in the dorsolateral striatum are involved in modulation of persistent nociception in diabetic mice.
- ✓ There is evidence that $GABA_A$ and $GABA_B$ receptor-selective agonists reverse thermal hyperalgesia in STZ-induced diabetic mice. Our data supports the hypothesis that like chronic nerve constriction injury neuropathic pain, the diabetic neuropathic pain also may result from the loss of GABA receptor-mediated inhibition of somatosensory pathway.
- ✓ Lastly, our results emphasize the role of oxidative stress and PKC in the development of significant hyperalgesia and cold allodynia in diabetic animals. GF109203X is a candidate for further study in treatment of diabetic neuropathy and points towards the potential concomitant use of antioxidants and PKC inhibitors as a complementary therapy for the prevention/treatment of diabetic neuropathic pain.

DEPARTMENT OF ZOOLOGY

(1) Name of the Candidate : Deepak Wadhawan **Supervisor : Dr. V.K.Walia**

TAXONOMIC STUDIES ON THE SUPERFAMILY GELECHIOIDEA (LEPIDOPTERA) OF HARYANA AND RAJASTHAN

Work on superfamily Gelechioidea of order Lepidoptera was pursued under All India Coordinator Project on Microlepidoptera sponsored by the ministry of Environment and Forests. In spite of great economic importance as pests of food and cash crops of the moths of this superfamily, very little efforts have been made to uniformly assess its faunal diversity in India. This is evident from the previous studies largely restricted to north-eastern and southern states only. In view of highly variable topography, vegetation and environmental conditions of Rajasthan and Haryana (including Chandigarh) and only 48 species of this superfamily having been reported so far, this part of India was, therefore, selected for the present work.

Relentless surveys were made between February and October during 2000-2004 to bring to light the unexplored fauna of this area. As a result, 85 species of 49 genera under 12 subfamilies belonging to 6 families, namely Gelechiidae, Oecophoridae, Lecthoceridae, Cosmopterigidae, Blastobasidae and Scythrididae were collected. New as well as already recorded species have been described in detail by including structure of male and female genitalia. Some of the significant features which make this work unique in its own way are as follows:

- Collection of 79 and 21 species from Haryana and Rajasthan respectively as against only 37 and 11 species recorded earlier.
- Peak season of prevalence of the concerned fauna both in terms of the number of species and specimens is a couple of months succeeding termination of winters and active monsoon period.
- Identification of 44 species and 3 genera as new, in addition, to proposed new combinations for 5 species. Also, 29 species have been reported from the covered area for the first time. Furthermore, 5 species and 3 genera are new records from India.
- Variable percentage of specimens of 8 out of 85 studied species exhibited the phenomenon of intra-individual and intra-specific variations in wing venation irrespective of their sex, month or place of collection.
- Kalesar and Chandigarh revealed maximum richness in biodiversity of gelechioid fauna, yielding as many as 56 and 52 species respectively.
- Out of 1194 collected specimens, females constitute 40% as against 60% of the males.
- Individually at the specific level, if the males of *Eupselia isacta* Meyrick dominated the females to the highest of 10:1 ratio, the females of *Cosmopterix manipularis* Meyrick exceeded that of males to the tune of 7:1 too.

(2) Name of the Candidate : Neetu **Supervisor : S. Chaudhary**

CYTOGENETIC AND MOLECULAR INVESTIGATIONS ON SOME MOSQUITOES

In mosquitoes, the presence of species complexes of closely related species in many of the anopheline taxa has impeded studies on their accurate taxonomic status. In addition to the dependence on morphological characters, it has been realized that there is an urgent need for accurate identification and genetic characterization of vector species, which is crucial as time and money may be wasted in studying the species of no medical importance. In addition to the taxonomic role of chromosomes, the ability of multilocus genetic analysis based on allozyme electrophoresis, is also an important approach for recognizing species of doubtful or overlapping taxonomic characters. In the recent years, the use of polymerase chain reaction (PCR) assays has shown sufficient promise for rapid and accurate identification of cryptic species in the genus *Anopheles*. Motivated by these developments in the area of molecular taxonomy, the present topic of research work entitled **"Cytogenetic and molecular investigations on some mosquitoes"** was undertaken. In this programme of research, the investigations were

designed with the aim of applying polytene chromosome studies for the initial identification of the selected species along with two more sophisticated techniques of allozyme analysis and genomic diagnostics with Random amplified polymorphic DNA- Polymerase chain reaction (RAPD-PCR) for recording the inter and intraspecific variations in the populations of *Anopheles stephensi* type form, *An. fluviatilis* T and *An. culicifacies* A, B and C and *An. subpictus*.

For allozyme studies, the individual adult specimens were homogenized in a suitable buffer and then subjected to polyacrylamide gel electrophoresis for visualizing variations in the following enzyme systems: Esterase (EST), Glucose-6-phosphate dehydrogenase (G-6-PD), Phosphoglucomutase (PGM) and Phosphoglucose isomerase (PGI). All the four enzymes assayed, were found to be controlled by seven loci, out of which six were polymorphic and one was monomorphic as it did not show any electrophoretic variation. Out of the seven loci examined, no diagnostic locus was detected for any of the species under investigation. The genetic characterization of the species was carried out on the basis of seven parameters and the results were analyzed by using POPGENE computer program. The allelic frequency data revealed that frequencies were not uniform for any given locus or species. The mean observed number of alleles per locus was 2.285 ± 0.755 while the percentage of polymorphic loci ranged from 28.57 to 57.14 for different species. The mean observed and expected levels of heterozygosity due to gene diversity for all the loci were 0.170 ± 0.136 and 0.354 ± 0.240 respectively. A significant deviation of genotype frequencies from Hardy-Weinberg equilibrium was observed by employing the Chi-square (χ^2) test. Further, the negative values of inbreeding coefficient (F_{IS}) revealed that all the deviations from equilibrium were due to the excess of heterozygotes especially in the field populations of *An. subpictus* A and *An. culicifacies* A. The non-random mating among the species, as measured by the coefficient F_{ST} ranged from 0 to 0.881 with mean value of 0.661, which indicated higher levels of genetic differentiation among the present species. When Nei's unbiased measures were used to calculate the genetic identity (I) and genetic distance (D), it was found that the genetic homology was highest between An. *culicifacies* B and C and lowest between *An. fluviatilis* T and *An. subpictus* A. A dendrogram of genetic relatedness was then generated by using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) in which *An. stephensi* type form, *An. subpictus* A and *An. culicifacies* A were found to cluster in one group while *An. fluviatilis* T, *An. culicifacies* B and *An. culicifacies* C in another group. Due to limitations in drawing exact genotypic kinship on the basis of allozyme data, it was logical to employ the technique of RAPD-PCR. For this, three random oligonucleotide primers with the following base pair composition viz: 10JB-5'-ACCGCGAAGG-3', 11JB- 5'-GTCCCGACGA-3' and 12JB- 5'-TGATCCCTGG-3', were used. The *in vitro* amplification of genomic DNA was carried out by suitable programming of thermocycler for a total of 30 cycles after which the amplified products were analyzed by agarose gel electrophoresis. The base pair lengths of the amplified DNA fragments were calculated by using standard 100 bp DNA ladder (gene ruler) and Quantity One software. The data thus obtained was analyzed in relation to those DNA fragments, which were "unique" and "conserved" among all the individuals of a given species. Out of three primers, only 12JB was able to amplify conserved fragments from the DNA of all the species covered in the present research work. Accordingly, as many as six bands with base pair length of 200, 411, 985, 1230, 1292 and 1392 could be visualized when examined carefully over UV transilluminator immediately after the completion of the process of amplification. Further, the RAPD diversity and differentiation were quantified by using POPGENE computer program according to which the percentage of polymorphic loci ranged from 14.71% to 44.12% for different species with an average of 29.4 ± 10.7%. The genetic diversity as measured by the Shannon's information index (S) ranged from 0.376 to 0.693 for different species with an average of 0.623 ± 0.102. The coefficient of genetic differentiation (F_{ST}) among them was 1.0, indicating clear differences in their genetic composition. Further, the pairwise genetic distances among the species were calculated by using DARPDistance software which revealed maximum genetic distance between *An. subpictus* A and *An. culicifacies* A and minimum between *An. culicifacies* B and C. The distance matrices, thus obtained, were further used for constructing a tree using Neighbour Joining (NJ) method, in which members of the *An. culicifacies* complex and *An. fluviatilis* T formed one cluster while *An. stephensi* type form and *An. subpictus* A formed another cluster. Among the members of the *An. culicifacies* complex, *An. culicifacies* B and C are more close to each other as compared to A. The data of RAPD-PCR and allozyme diversity and differentiation was then compared for recording the points of genetic significance. It was noticed that more accurate genetic diversity and differentiation could be detected with RAPD markers than with allozyme profiles thus suggesting that mutational rates of the genes or their allelic profiles can be recorded more accurately with RAPD procedure than with allozyme electrophoresis which tends to miss certain nucleotide sequences in the non- coding regions of the genome.

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FORM IV

(See rule 8)

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Professor I.S. Dua, hereby declare that the particulars given above are true to the best of knowledge and belief.

I.S. DUA
Chief Editor

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Buskrik, E.R., K.L. Anderson and J. Brozek. 1956. Unilateral activity and bone and muscle development in the forearm. Res. Quart., 27 : 127-131.

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