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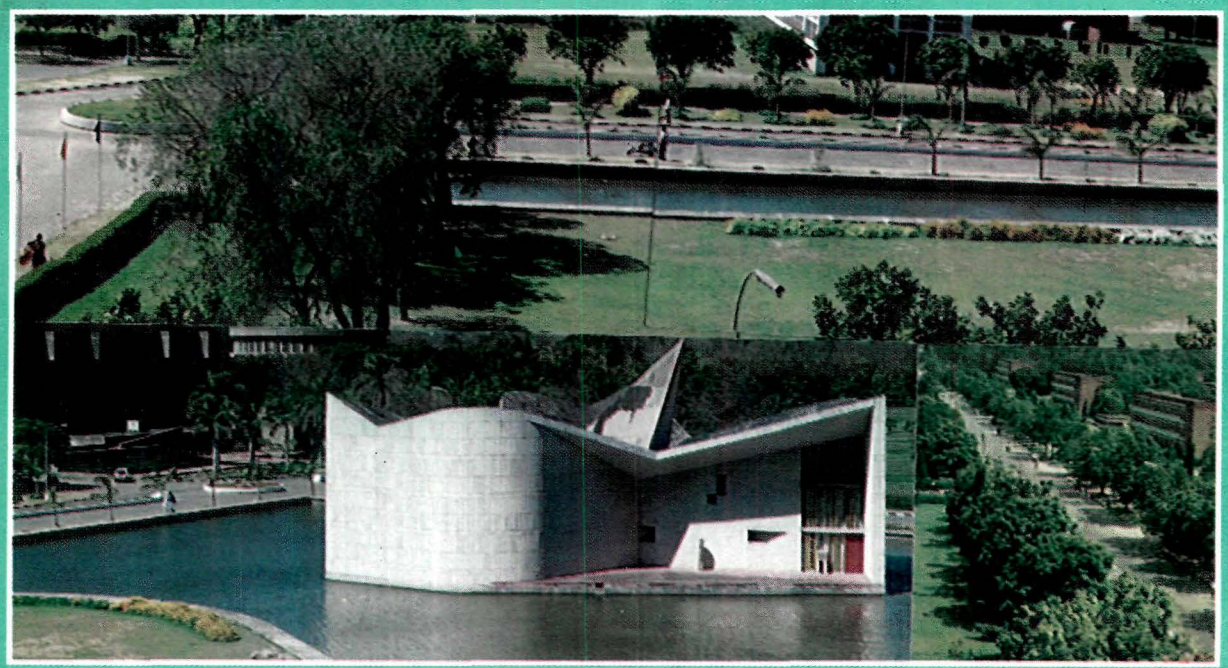

Chief Editor

J.K. GUPTA

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Chief Editor

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STEROIDAL NEUROMUSCULAR BLOCKERS : A REVIEW

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The introduction of neuromuscular blocking agents represents one of the most important advances in anaesthesia and surgery. The 50th anniversary of the first use of tubocurarine in anaesthetic practice by Griffiths and Johanson[65] was celebrated in 1992. During these years research in the area of neuromuscular blocking agents rose dramatically driven mainly by the quest to find ideal muscle relaxants. There has been a considerable increase in the understanding of the mechanisms of action of the agents at the molecular level[3].

ANATOMY OF NEUROMUSCULAR JUNCTION

The concept of the existence of neuromuscular junction was introduced by Claude Bernand[18], who showed that although curare could cause paralysis, it did not effect nerve conduction or prevent the muscle from contracting when directly stimulated. The fundamental anatomy of the frog neuromuscular junction was described by Birks *et al.* in 1960, and serves as a model for other species[21]. The neuromuscular junction consists of three parts: prejunctional nerve ending, the junctional cleft and postjunctional membrane. The nerve terminal contains not only mitochondria and other common subcellular structures, but also numerous vesicles about 70 mm in diameter. These vesicles have been shown to be filled with acetylcholine on the prejunctional membrane, transverse bands can be seen, and these have been called 'active zones' because they are believed to be the sites of acetylcholine release. There are such one thousand active zones at each nerve ending[34].

The junctional cleft is about 60 mm across and contains a basement membrane material that is a mucopolysaccharide. Acetylcholinesterase exists within this basement membrane, although it is particularly concentrated in the folds of the postjunctional membrane[70]. The presynaptically released acetylcholine has to traverse the cleft before it reaches the receptor on the postsynaptic membrane.

The postjunctional membrane is thrown into folds (secondary clefts), with the acetylcholine receptors organized in discrete clusters located on the shoulders of those folds. This means that they are in direct opposition of the active zones of the nerve terminals[44]. There are more than 10,000 receptors/μm^2, each of which is inserted through the phospholipid bilayer of the postsynaptic membrane. The receptors exist as dimmers, and it is likely that there is cooperation between the two components of each receptor pair.

It has been established that the nicotinic acetylcholine receptor is a pentamer of five glycoprotein subunits, which together form a central cation channel[127-128]. Two of the subunits are designated α and are identical. The others are slightly larger and known as β, δ and ε-subunits in the mammalian adult. In the fetus and in various other species, the ε-subunits does not exist, being replaced by the γ-subunits. During the first few weeks of life, the γ-subunits disappear to be replaced by the ε-subunits[101]. The two α-subunits each carry a single recognition site that binds acetylcholine, other agonists, toxins[87] and reversible antagonists, for example

tubocurarine[80,121,146]. Although the two α-subunits have the same amino acid sequence, they reside in different environments. One α-subunits has the β and ε adjacent to it, whereas the other is surrounded by the δ and the ε-subunits. This results in the properties of the two sites being different, and they have been shown not to behave in an identical fashion in their interaction with tubocurarine[114]. Recent immunological studies have shown that the majority of antibodies raised against the acetylcholine receptor in myasthenia gravis are directed against a single region, the "main immunogenic region". This region is located on the extracellular part of α-subunits[17].

The nicotinic acetylcholine receptor has been described as an example of ligand-gated channel because it consists of several subunits that are inserted into a membrane and provide ligand-gated conductance[107]. In contrast, a functional voltage-gated ion channel (e.g. the sodium channel) consists of a single large protein unit[115].

PHYSIOLOGY OF NEUROMUSCULAR JUNCTION

Acetylcholine is the transmitter at the neuromuscular junction. The presynaptic apparatus has the function of acetylcholine synthesis, storage, mobilization, release and recycling[143]. Acetylcholine is formed from the acetylation of choline under the influence of enzyme choline acetyltransferase. This is a soluble enzyme that is manufactured within the cell body. Choline is supplied both from plasma and from the breakdown products of acetylcholine. It enters the nerve terminal under the influence of an active transport system. Acetate is supplied bound to coenzyme A, and acetylcholine synthesis is an energy-dependent process. Then acetylcholine is actively loaded into the vesicles in which it is densely packed. The mechanism of this process has been studied using vesamicol, a drug that inhibits acetylcholine storage by synaptic vesicles[122].

Acetylcholine is present both in the vesicles and free in the axoplasm. It is the acetylcholine within the vesicles which is available for release, and acetylcholine in the cytoplasm being a store. Most of the vesicles are situated at a short distance from the inside of the cell membrane, with the remainder lying very close to the cell membrane behind the regions of the active zones. The former are regarded as being the reserve, while the latter are the immediately available source. It is the size of the immediately available pool that determines the amount of quantal acetylcholine release following stimulation of the motor nerve. The movement of the vesical from reserve to immediately available stores is the process of transmitter mobilization and probably takes place in an energy dependent process involving calcium ions. It has been proposed that phosphorylation of synapsin I in the presence of calcium is involved in the release of a synaptic vesicle from its location on an internal cytoskeleton, thus allowing it to move down towards the release sites[46]. The course of events at the neuromuscular junction leading to transmission of nervous impulses has been earlier described in a review on molecular interactions at the cholinergic receptor in neuromuscular blockade[144].

The process of neuromuscular transmission is extremely fast. The time from the stimulus to the first detection of a postjunctional event (synaptic delay) is as little as 0.2 msec. Within this period, the arrival of the nerve action potential has to trigger the release of acetylcholine, which must then diffuse across the synaptic cleft, combine with and activate the receptors. Transmitter release is effected by the arrival of the action potential, which causes voltage dependent calcium channels in the nerve terminal to open with a resultant rise in the calcium concentration close to the sypnaptic vesicles. The calcium binds to a receptor that is a calcium-binding protein, probably synaptotagnin,[32] and this is related to the activation of a second messenger system[153]. The vesicles fuse with the prejunctional membrane, expelling acetylcholine, and subsequently are directly recycled[8].

The released acetylcholine diffuses across the synaptic cleft to interact with the postjunctional receptor. The acetylcholine receptor is not a receptor in the classical sense, but rather as integral signal transducer. It contains in it a protein agonist moiety, the binding sites for acetylcholine and its agonists and antagonists (receptor function), the ligand gated cation channel (response function), and several types of modulation sites (modulation function).

The postjunctional response starts with the interaction of acetylcholine with the receptor. The binding of the first acetylcholine facilitates the binding of the second. This sequential receptor saturation will induce a chain of conformational transitions, one of which is the active state (a state of high probability of channel opening). This state is long lasting when two molecules of transmitter are bound, but also exists for partially occupied or even unoccupied receptors[91]. In addition, to the two high affinity agonist-binding sites per receptor, other multiple low binding sites have been proposed.

Opening of a channel permits small cations (sodium and calcium particularly) to travel down their concentrations gradients. These concentration gradients, together with the electrical potential across the membrane, results in the main movement being an influx of sodium ions. The mean open time of the channel varies with the activating agonist. The time constant for acetylcholine activation is approximately 3 msec at resting membrane potential of –80 mV and a temperature of 15°C[39].

Each molecule of acetylcholine probably exists long enough to activate a single receptor before it is destroyed by acetylcholinesterase. An increase in the acetylcholine concentration will increase the frequency with which the ion channel opens. Prolonged exposure to the transmitter (receptor saturation), however, may lead to subsequent conformational changes that decrease receptor channel conductivity "desensitization".

The current of sodium ions that flows inwards through the whole motor endplate membrane (endplate current) will change the potential across that membrane sufficiently to depolarize it, producing the end plate potential: when the endplate potential reaches a critical threshold, it will trigger a muscle action potential that subsequently activates the contractile mechanism.

There is a large safety factor in the transmission process, both in the amount of acteylcholine released and in the number of receptors available upon which for it to act. Both are in very much large number than required to produce the critical level of endplate potential necessary to initiate a muscle contraction[120].

NEUROMUSCULAR BLOCKERS

Neuromuscular blockers interrupt transmission of nerve impulses at the skeletal neuromuscular junction[150]. These are used as adjuncts in anaesthesia to achieve adequate muscle relaxation with light anaesthesia. With these drugs the desired muscle relaxation can be obtained without using dangerously high concentrations of anaesthesia. There are electrophysiological differences in their mode of action. They are classified as nondepolarizing (competitive, stabilizing, curariform, antidepolarizing) or as depolarizing agents. The nondepolarising agents block neuromuscular transmission by competing with acetylcholine for receptor sites on the motor end-plate, thus reducing the response of the end-plate to acetylcholine; their action is usually reversed by anticholinesterases. The depolarizing agents interrupt neuromuscular transmission by producing a sustained partial depolarization of the motor end-plate, which renders the tissues incapable of responding to the transmitter, their action is not reversed by anticholinesterases. Generally, the nondepolarizing agents, with a prolonged action, are used in major operations,

while the depolarizing agents, with a much shorter effect are used in minor operations and manipulations.

Savarese and Kitz described the characterstics desirable in an ideal nondepolarizing neuromuscular blocking agent[134]. Such an agent should show a rapid onset of action, rapid dissipation of neuromuscular blockade, lack of cumulative effects, antagonism of the block by a suitable antidote, absence of pharmacological action or toxicity of metabolites, high potency, lack of histamine release, and acceptable cardiovascular effects. A total absence of cardiovascular effects is derived.

However, compounds with mild vagal blocking effect are acceptable as most modern anaesthetic techniques lead to a relative bradycardia and hypotension. Even drugs with mild ganglion blocking effect may have a use, as in hypertensive patients and operations under induced hypotension. It is stated that a mild degree of vagal blocking and/or ganglion blocking action may be advantageous during incubation of trachea, to prevent bradycardia secondary to vagal reflexes, and to prevent hypertension due to stimulus of intubations.

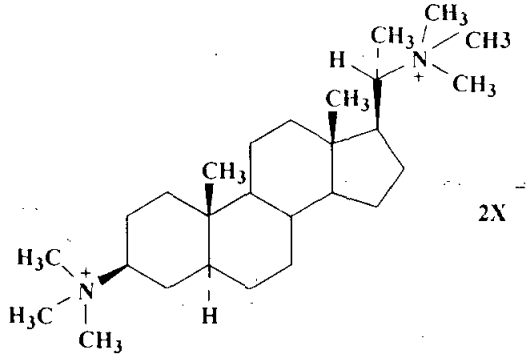
Although nonsteroidal and steroidal neuromuscular blocking agents are available but here only steroidal neuromuscular blockers are described.

AZASTEROIDAL NEUROMUSCULAR BLOCKING AGENTS

The status of clinically used neuromuscular blocking agents in general has been surveyed by Booij[23]. The prominent features are discussed under the heads: Malouetine and other earlier studies; Pancuronium and related investigations; Candocuronium and allied aspects.

MALOUETINE AND RELATED STUDIES

The steroidal nucleus as a supporting moiety for two cationic heads has been considered to be a proposition of good interest. The nucleus is relatively rigid and as such the spatial relationship between two onium centres would be more or less fixed and there would be a limited flexibility through conformational variations. Such compounds would possess appropriate hydrophilic to lipophilic ratio was apparent from the discovery of neuromuscular blocking activity comparable to that of (+) -tubocurarine in the steroidal alkaloid malouetine[73,125] (1) and its C-3 and C-20 configuational isomers[64,72,74]. In these analogues a degree of variation in interonium distance 1.1 to 1.25 nm can be visualised due to free rotation of the side chain, and as such study of the bisonium azasteroids groups directly attached to nucleus was considered worthwhile.



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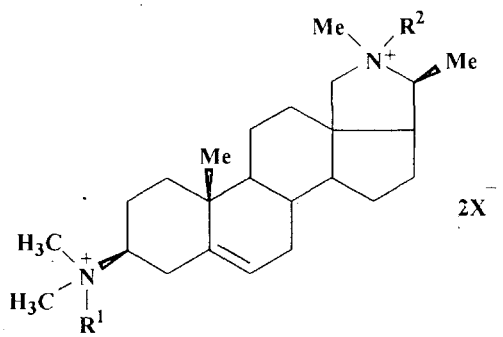
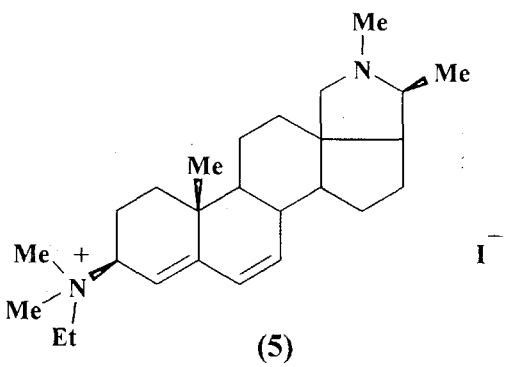


(4)

The related drug, stercuronium iodide (5) is a monoquarternary compound. It is reported to be nondepolarising type, has no histamine release property and has duration of action lying between gallamine and suxamethonium[161]. The onset of action is rapid and the ratio between neuromuscular and ganglion blocking doses is fairly large[98]. The short duration of action was found to be directly related to rapid decline of blood levels as a consequence of hepatic and renal uptake, as shown by whole body autoradiography in rats[67]. There is no biotransformation and drug is excreted unchanged in urine. High degree of cardiac and vagolytic action has led to cessation of use of the drug in clinical practice. It has been suggested that stercuronium (5) has significant affinity for cardiac receptors and the inhibitory muscarinic receptor on sympathetic nerve endings[89].

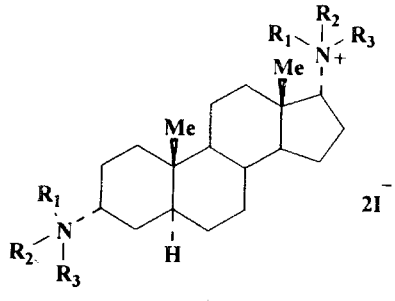


(5)

PANCURONIUM AND RELATED STUDIES

With the development of Pancuronium Bromide (6), a breakthrough was achieved. Pancuronium bromide (Pavulon®)[13,29,47,102,142] (6) got to be the first successful steroidal neuromuscular blocker discovered at Organon Laboratories Limited (U.K.). It is advocated for use in clinical situations where a non depolarising muscle relaxant of medium duration of action is required, due to its high potency with minimal side effects. A brief description may be given about design of pancuronium bromide and ancillary aspects.

A series of 3α,17α -bis (quarternary ammonium)-5α-androstanes (2) were prepared[7], in which the interonium distance (0.92 -1.06 nm) was near the favaourable range and steric hindrance to post junctional binding by β-face angular methyl groups on C-10 and C-13 was excluded. These showed activity, though less than (+)-tubocurarine. On the basis of studies on dipyradinium chloride (3)[14,20,110] and all the eight of its isomers[14,45], tests being done *in vivo* on cat or monkey sciatic nerve tibialis muscle preparation, it was seen that 3β-isomers were in general more potent than the corresponding 3α-compound and there was no general relationship between potency and interonium distances.



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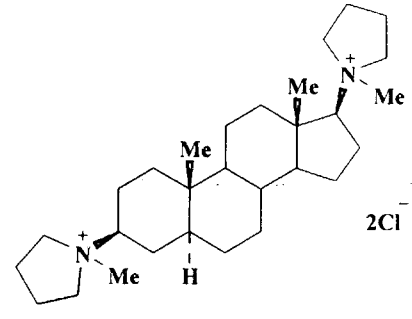
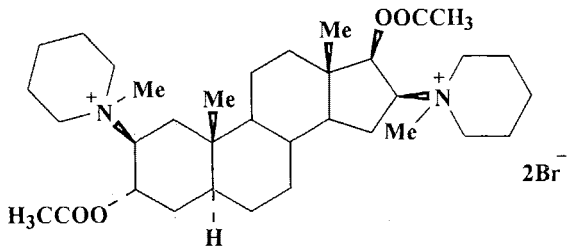


(3)

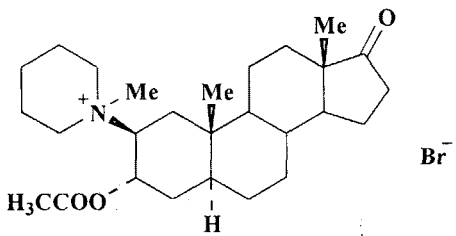
As such Bamford et al.[14], tended to support the adumbration theory of Loewe and Harvey[90], who postulated a one-point attachment theory, where bulk of the molecule, in this case presumably the steroid nucleus, shield the receptor, rather than the suggestion of Cavallito and Gray[36] and Waser[158] that a two-point receptor complex could be formed. The relatively flat steroid nucleus in the 5α-series may be more effective shield than the more folded nucleus in the 5β-series, and therefore it was argued that compound of the 5α-series should be more potent than the corresponding members of the 5β-series, and that indeed was seen to be the case. By testing several monoquarternary androstane derivatives, Bamford *et al.*[15] further supported the one-point attachment theory, and again considered quaternary centre linked to position-3 to be more important than one attached to position-17 in determining potency in the series. The activity of the steroidal quaternary, compounds examined is of non depolarising type.

Most of the quaternary salts (4) derived from the alkaloid conessine[15,33] at Glaxo laboratories (U.K.), showed in the cat short-acting muscle-relaxant properties; shorter block duration, and less cumulation effect. The bisquarternary compounds reported possess the interonium distance of 1.01 nm. Seven of the eight 3-monoquarternery compounds tested are also potent neuromuscular blocking agents; this observation may not be taken as a convincing evidence for one-point attachment since the second nitrogen could get protonated in the system and thus provides the second cationic head N,N'- Dimethyl conessine (4; R1 = R2 = Me) was of comparable potency to (+) - tubocurarine. The rate of recovery was slower in monkey as well as in man[157].



(6)

A programme on synthesis and pharmacological study of 2β-amino-3α- hydroxy-5a-androstanes and derivative[69] and the corresponding 3α-amino- 2β-hydroxy isomers, led to the observation[88] that the corresponding monoquaternary salts possessed neuromuscular blocking activity; the most potent of the series 3α-acetoxy-2β-piperidino-5a-androstan-17 -one methobromide (7) has one sixteenth that of (+)-tubocuraine. The 2 β-piperidino and 3a-acetoxy groups are both considered to be pseudoequational due to the twisted boat conformation of ring A[69]. In this preferred conformation which may be rigid due to steric compression,[132] one may consider it to have ring A substituents in specific molecular conformation akin to the neurotransmitter acetylcholine (8) and thus (7) may be expected to occupy the transmitters site of action and affect neuromuscular transmission.



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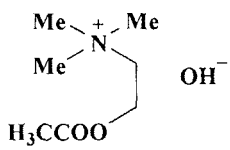


(8)

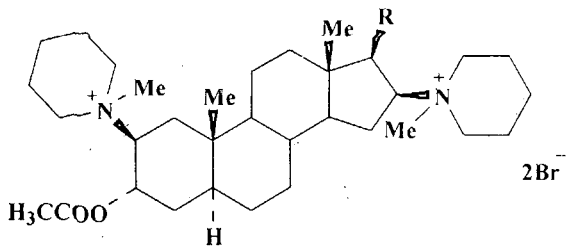
As the monoquaternary analogue (7) had only a low activity, it was thought that a bisquaternary azasteroid may be potent and pancuronium bromide (6) got to be ultimately synthesized[29] and tested[30]. Here also the 16- and 17- substituents are pseudoequatorial.

Structure activity studies[29] on pancuronium bromide and other steroidal neuromuscular blocking agents containing acetylcholine fragments indicated that for high potency it is essential to have nitrogen atoms in the molecule and at least one of these nitrogen atoms should be quarternised. X-ray crystallographic studies[132] reveal the actual interonium distance in the solid state to be 1.108 nm as against 1.06 nm calculated from Drieding models.

Pancuronium out of the series is a drug of interest. It is approximately 5 times more active than (+)-tubocurarine[150]. It has a rapid onset of action. The muscle twitch depression is only marginally increased with successive doses, but the duration of action is significantly extended[78].

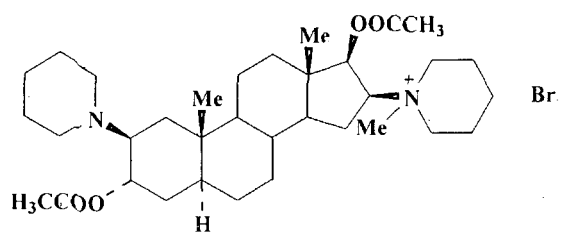
Studies have shown that pancuronium has some cardiovascular side effects, such as moderate increase in heart rate and an increase in cardiac output and blood pressure[38,62,79,140,145]. These effects have been attributed to vagolytic[79], and an indirect[38,49] or a direct[113,168] sympathomimetic effect.

Dacuronium bromide (9), a relative of Pancuronium bromide having 17β-hydroxyl group in place of 17β-acetoxyl was three to four times shorter in duration of action in cats, than pancuronium,[29] but the potency and short time course of action were not borne out in man[55,116,141]. Org 6368 (10)[68] which lacks oxygen function of position-17, was also short acting and lacking in cumulative effects in cats[147] and potency was five time less than that of pancuronium; the potency ratio also being borne out in man[12]. Shorter duration of Org 6368 is mainly attributed to hepatic uptake[4].



(9) R = OH
(10) R = H

Vecuronium Bromide (11) forms a further development along this line. Vecuronium Bromide (Org NC 45; Norcuran®, (11)[24,25,29,48,50,51,94,100,133] has short duration and rapid onset of action and little cumulative effect. It is suggested that quaternary ring D acetylcholine fragment is intrinsically suited to skeletal muscle nicotinic receptors and is relatively unsuited to cardiac muscarinic receptors.



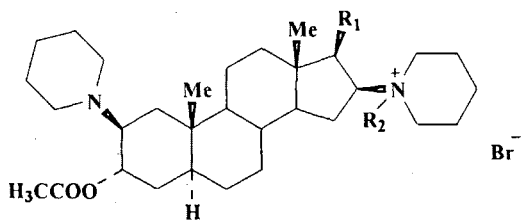
(11)

It is highly selective and possesses a wide margin between neuromuscular and vagal blocking doses. It has low propensity to release histamine and possesses negligible ganglion -blocking activity. The desacetoxy analogues of pancuronium (6) and vecuronium (11) has been examined for their activity in anaesthetised cat[27]. The results showed that the greater neuromuscular potency of pancuronium is lost after removal of one or both of the acetylcholine moieties. The block was faster in onset and shorter in duration.

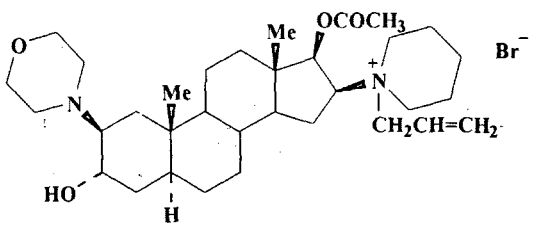
The clinical pharmacology of the vecuronium (11) has been reviewed[152]. The potency of vecuronium appears to be slightly greater than that of pancuronium. It has a more rapid onset and considerably shorter duration of action and faster recovery rate. The usual clinical dose varies from 80 to 100 μg/kg-1. Several clinical studies have been carried out[5,43,53,81]. Vecuronium

is considered to have little or no cumulative effect after repeated administration[52,104]. No adverse cardiovascular side effects are reported[129]. In a recent study it was shown that vecuronium does not influence heart rate, or systolic or diastolic arterial pressure[159,162]. It has been noted that vecuronium may increase the risk of bradyarrhythmias[42,118,131]. This may be explained by probable absence of vagolytic activity of this agent. Vecuronium seems to be free of histaminergic properties[58,59,63,112,118]. In a recent study, it was suggested that due to its intermediate duration of action and apparent lack of clinical neuromuscular effects on new born, vecuronium should be useful muscle relaxant for cesarean section[10].

During studies at Organon, modification in the 17-ester group of vecuronium bromide resulted in the development of two new neuromuscular blocking compounds: Org 9453 (12) and Org 9489 (13). Modifications in the 17 -ester group in the cycloamino structure of the substituents to androstane skeleton of vecuronium bromide (11) led to synthesis of Org-9487 (14), the 16-N-allyl-17 -propionate and Org- 761 7 (15), the 16N-allyl -17 -butyrate analogues of vecuronium (11). In animal studies, these vecuronium analogues were shown to be short-acting compounds with a rapid development of neuromuscular block and short duration of action[28,93] Org 9487 was selected for further development as an alternative to suxamethonium chloride when short lasting relaxation is required[165]. Rocuronium (Org-9426) (16), the 2-morpholino-3-desacetyl, 16-N-allyl pyrrolidino derivative of vecuronium bromide, is a new steroidal neuromuscular blocking drug with a pharmacological profile similar to that of vecuronium with the exception that the onset of action is characterized by a short lag time and rapid initial rate of block development.



		R_1	R_2
(12)	Org-9453	$OCOCH_2CH_2CH_3$	CH_3
(13)	Org-9489	$OCOCH_2CH_3$	CH_3
(14)	Org-9487	$OCOCH_2CH_3$	$CH_2CH{=}CH_2$



(15)

Doses of 500 and 600 μg/kg of rocuronium have not produced cardiovascular effects[56,160]

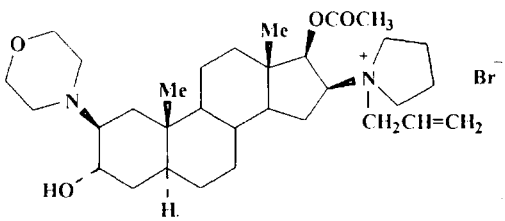
Although, neuromuscular blocking drugs generally are potentiated by volatile, anaesthetic drugs[57,103,105,117,130,155,167] and the time Course of neuromuscular block of rocuronium and its reversibility seem to be only slightly influenced by the anaesthetic technique used[84-85].

Rocuronium lacks in ester group at 3-position and is, therefore, less likely to undergo metabolism in body. No metabolites have thus far been identified in any quantity. Recent work have suggested that the rapid onset of action may be related to reduced plasma protein binding[123] or to an early major presynaptic action[54]. Rocuronium (16) provided intubation conditions similar to those produced by succinylcholine[41,71,124,154,163]. In a study of anaethesied patients Org- 9426 was

found to be 6-8 times less potent than vecuronium and atracurium but produced neuromuscular blockade more rapidly.

Org 9426 is approximately 20% as potent as vecuronium and exhibits a typical nondepolarising neumuscular block[108]. The duration of action is however similar to that of vecuronium in cats, pigs and dogs[35,109]. Studies in humans have suggested an ED_{95} of approximately 250 to 305 μg/kg[40,56,111,160]. The onset of action is relatively rapid with this relaxant, although smaller doses may show an initial rapid effect followed by a slower phase[126]. These authors noted that while the time to 80% block after a dose of 240 μg/kg was 1.9 min, the time to attain a maximum block of 90% was 4-6 min. Doses of 570 to 850 μg/kg are associated with onset of maximal block and good to excellent intubating conditions in about 90 to 110 sec, and a duration of clinical relaxation of between 27 and 45 min[56,86,106]. A dose of 1 mg/kg resulted in maximum block in 1.7 min, a clinical relaxation lasting 53 min and a recovery index of 20 min during halothane anaesthesia[164]. Use of the 'priming' or the divided dose technique did not confer any advantage in terms of the onset of action of Org 9426[56].

At the chemical works of the Gedeon Richter limited (Budapest, Hungary) there was designed Pipecuronium bromide (RGH-1106, Arduan®) (17)[112] an analogue corresponding to pancuronium which has piperazino moieties attached to position 2 and 16 rather than piperidines as in the latter. It is of interest to note that pipecuronium bromide where the interonium distance is much longer than in the other steroids, is more potent neuromuscular blocking agent and shows no vegal blocking effect in man.



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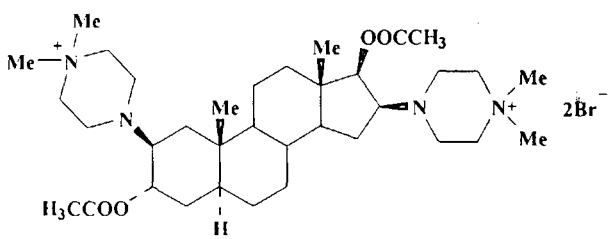


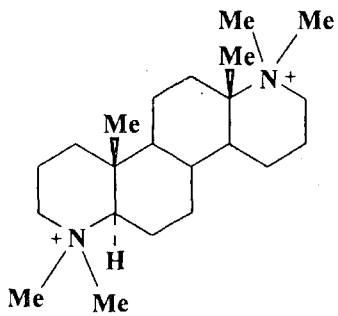
(17)

Pipecuronium is a nondepolarising blocker and in animal experiments[9,77] it has shown activity 2-4 times as that of pancuronium and duration of action is twice as long as that of pancuronium bromide in quiactive doses. It produces no histamine release[11] and is stated to cause no influence on the cardiovascular system.

Safety tests were carried out with pipecuronium[37] followed by clinical studies[6,31,166]. It was shown to be a potent muscle relaxant with medium duration of action. The drug does not cause circulatory side effects[16] even in patients with severe heart disease. In a clinicopharmacological comparison, pipecuronium was found to be 20% more potent than pancuronium[26]. It causes a mild bradycardia in contrast to the heart rate increasing effect of pancuronium. Administration of pipecuronium is accomplished by hemodynamic stability[148]. It may be a clinically useful alternative to pancuronium in whom tachycardia is best avoided. In humans the plasma disease of pipecuronium was found to be higher than that of pancuronium[149].

CANDOCURONIUM AND RELATED STUDIES

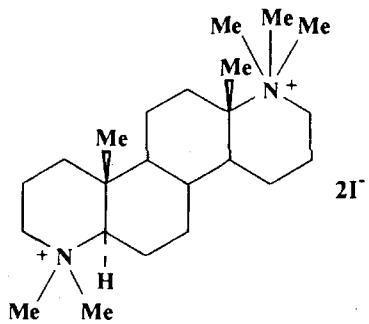
University Institute of Pharmaceutical Sciences, Panjab University, has been actively involved in the synthesis of bisonium steroids as potential neuromuscular blocking agents, with one or both of the cationic systems present as part of the steroid ring skeleton at different interonium distance[138] of which the first designed was 4,17 α-dimethyl-4, 17α-diaza-D- homo-5α-androstane dimethiodide (HS-342) (18). In anaesthetised cat HS- 342 exhibited non-depolarising blocking activity approximately equal to that of (+)-tubocurarine, duration of action one third that of (+)-tubocurine and rapid onset of action[96-97].



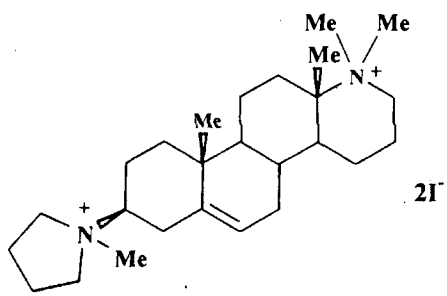
(18)

It also possessed a ganglion blocking activity. The combination of blocking action is probably related to the interonium distance 0.87 nm[119] which falls between optima for these two activities. HS-467 (19) which has interonium distance 0.902 nm[119] was approximately equipotent to (+)-tubocurarine.

In the series, the compound 17a-methyl-3j3-pyrrolidino-17a-aza-D-homo-5-androstene dimethiodide (20)[139] Candocuronium (formerly known as Chandonium iodide after the name of Chandigarh city), proved to be of particular interest. It possesses a powerful non-depolarising neuromuscular blocking activity of short duration and rapid onset being only slightly less active than pancuronium[60-61,66]. It has little or no ganglion blocking activity.



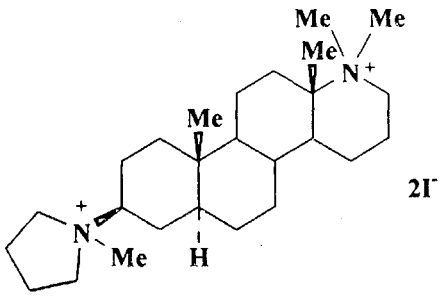
(19)



(20)

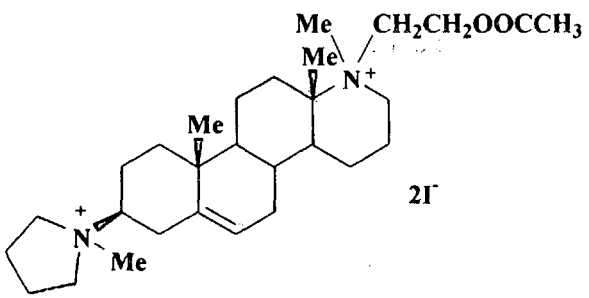
By Drieding model, the N+ ...N+ distance in Candocuronium was found to be 1.02 nm. The X-ray diffraction studies showed the distance to be 1.029 nm.[148] Earlier, the N+ ...N+ distance in 17a-methyl-3β-pyrrolidino-17a- aza-D-homo-5-androstane (HS-309), the tertiary amine corresponding to Candocuronium, was found to be 1.014 nm by X-ray diffraction[92].

Candocuronium was used as prototype for further chemical modifications[135]. The saturated congener dihydrochandonium iodide (HS-692) (21) and the analogues possessing bulkier cationic heads were synthesized.



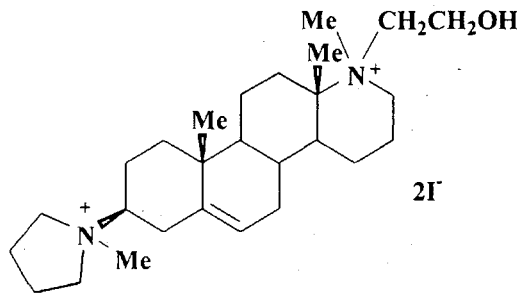
(21)

The saturation of 5,6 double bond in Candocuronium (20) and increase in the onium bulk in 20 or 21 diminishes the potency[151]. Still, dihydrocandocuronium (21) is a compound of interest, having high potency (half of chandonium), short lasting neuromuscular blocking, no ganglion block, and least vagolytic action, in anaesthetized cat.



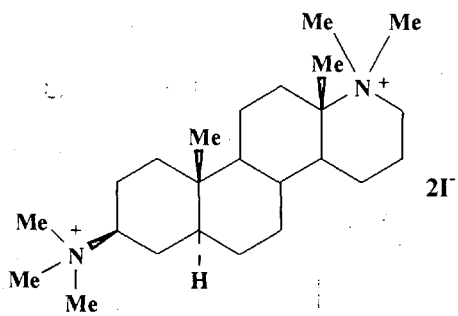
(22)

Synthesis of HS-627 (22) which contains acetylcholine like fragments was carried out[136]. Since pancuronium bromide (6), has bulky quaternary groups and contains acetylcholine like fragments HS-626 (23) and HS-627 (22) were approximately equipotent with Candocuronium as neuromuscular blocking agents in anesthetised cat[95].

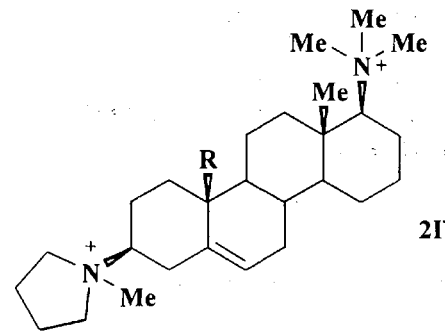


(23)

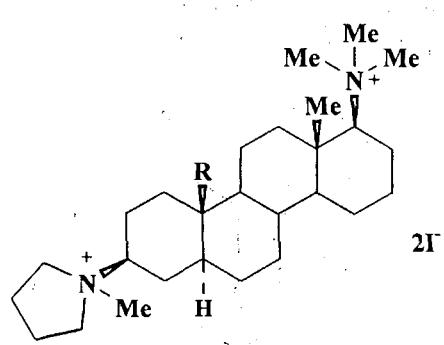
Some other modifications related to Candocuronium which include HS-777 (24), HS-873 (26) and HS 892 (28)[19,137].



(24)

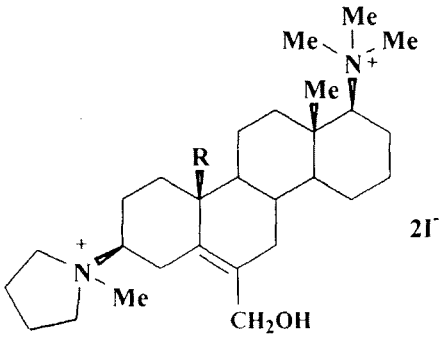


(25) R = H
(26) R = Me

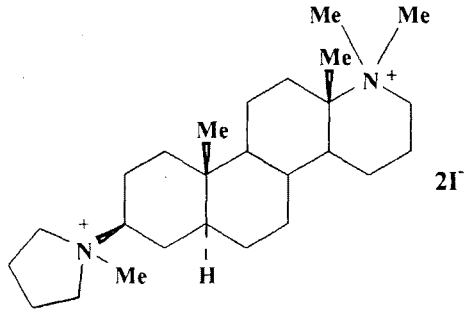


(27)

The Gedeon Richter scientists have designed RGH-4201 (Duador®), (29), 3α-isomer of 21 and showed it to be equipotent with Candocuronium in conscious dog but 2-3 times less active is anaethetised cat[22]. The action is non depolarising, rapid in onset and of short duration. Certain comparison have been made of RGH-4201 (29) with other neuromuscular blocking agents including vecuronium (11).

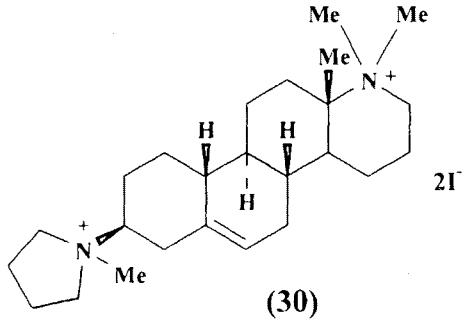


(28)



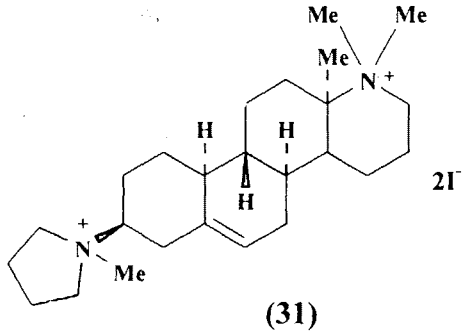
(29)

19-Nor analogues of 30 has been reported by the Organon group[99]. This was found to be 3-4 times less active than candocuronium iodide (20).



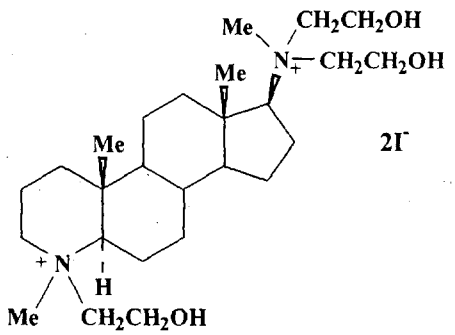
(30)

Interestingly, the enatiomer 31 has virtually the same potency as 30. It appears that effect of complete change in lipophilicity was found by the removal of the 10-methyl group of candocuronium iodide.

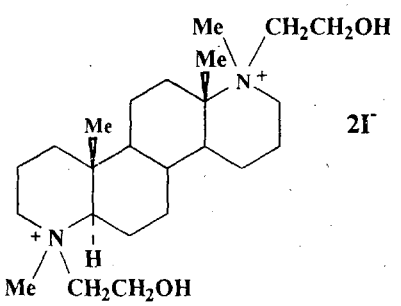


(31)

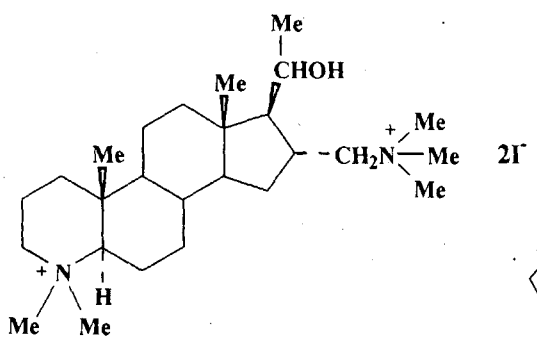
Various analogues (32-42) related to the candocuronium have been synthesized[1,76,82-83] and evaluated for their neuromuscular blocking activity at the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh.



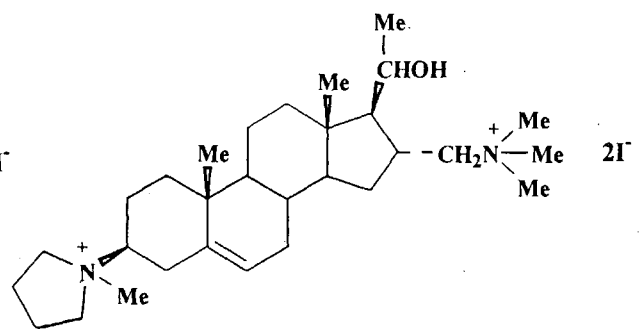
(32)



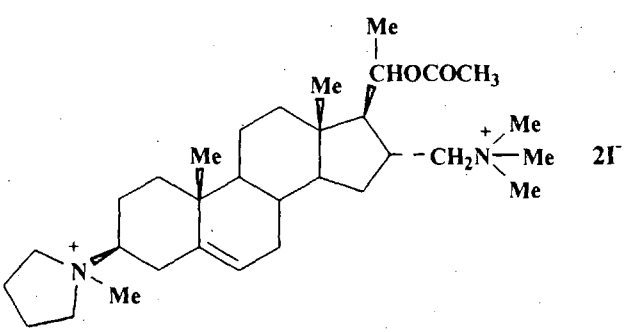
(33)



(34)

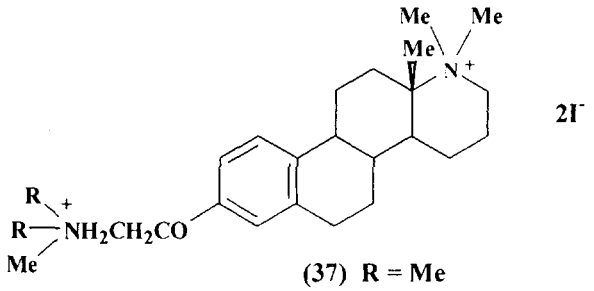


(35)



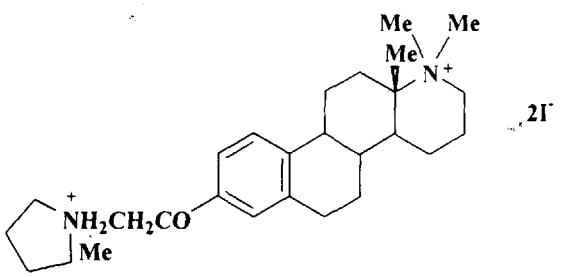
(36)

In compounds 37-42, ring A is aromatic and hence flat and thus affect the conformational perspectives of the molecules. The interonium distances are flexible[82-83].

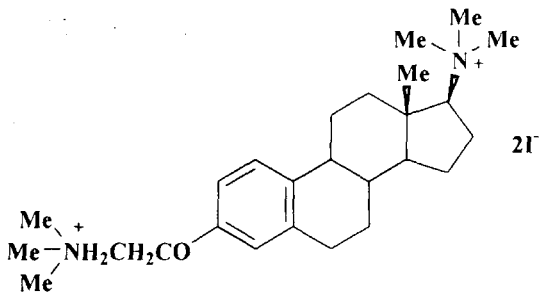


(37) R = Me
(38) R = Et

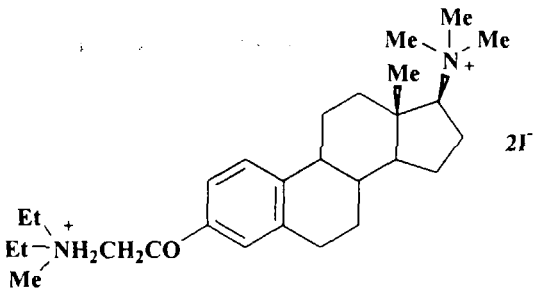
Due to different conformations, which the chain at position-3 may assume in these compounds, interonium distances may vary. All the compounds were found to be active but none proved to be better than candocuronium.



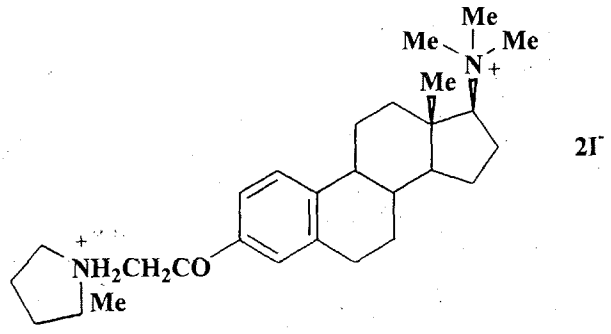
(39)



(40)



(41)



(42)

Although numerous other modifications related to candocuronium have also been carried out at the University Institute of Pharmaceutical Sciences, Panjab University but Candocuronium remains the most promising compound[2,75,156].

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SELENIUM SUPPLEMENTATION DURING ACUTE CADMIUM EXPOSURE : CHANGES IN THE CADMIUM DISTRIBUTION AND REDOX SYSTEM

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Abstract

In the present studies, the mice receiving single exposure of cadmium (Cd) with and without simultaneous selenium [Se] exposure, were analyzed for the cadmium distribution in liver and kidney. Liver accumulated six times higher cadmium as compared to the kidney. This was further substantiated by the biodistribution studies, wherein the maximum retention of ^{109}Cd activity was found in liver. In other organs viz. spleen, heart and blood, the activity gradually decreased between 1 to 48 hours. On the contrary kidney showed a gradual increase in ^{109}Cd activity with the elapse of time.

Selenium supplementation showed a significant recovery in the activity of glutathione peroxidase (GSH-Px) and catalase which were inhibited by the cadmium exposure. Selenium supplementation seemed to prevent the loss of catalase activity but it was still lower than that in the control animals. The total glutathione level was not affected either by cadmium only or during Cd+Se administration. It is possible that the Se supplementation plays a role in providing protection against the cadmium by modulating the redox active enzymes viz glutathione peroxidase.

Key words : Selenium supplementation, cadmium exposure, redox system.

INTRODUCTION

Long term exposure to cadmium leads to a variety of toxic manifestations in the kidney, liver, testes, heart, pancreas, qastro-intestinal tract and the bones in the laboratory animals and the human population[5]. However, liver has been marked as the target during the acute cadmium exposure. It is considered that in liver cadmium gets bound to a newly induced low molecular weight proteins, metallothioniens, which plays a role in the detoxification of a number of essential and non-essential metals[6,20]. Also, liver is efficiently equipped with a capacity to synthesize metallothionien thereby enabling it to trap Cd efficiently.

The rate of protein synthesis is parallel to the production of metallothionien mRNA[4,15] and due to a high rate of transcription it can be found within one hour of the stimulation by the metal[2]. Finally, it is only the free or non-protein bound cadmium that disrupts several intracellular activities thereby causing toxicity.

In addition to metallothionien, there are several antioxidant and glutathione related enzyme to counter the metal toxicity[19]. Selenium has also been found to have the capacity to counter the toxicity of several metals e.g. Hg, Cd, As, in addition to its well established anticarcinogenic role[3,18]. Although the underlying mechanism involved in this process is not clearly established yet. In the present study an attempt has been made to study the protective role of selenium in acute exposure to cadmium.

MATERIALS AND METHODS

For the present study, virgin female 8 weeks old Balb/c mice (Appx. 25g body weight) were procured from the Central Animal House of Panjab University. These were acclimatized in the departmental animal house for three weeks before using them for the experimental work. During

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the coarse of the treatment, animals were fed on standard pellet diet (Hindustan Lever Ltd) and water *ad* libitum.

All the routine chemicals were of Analytical Grade from E Merck and other biochemicals were obtained from Sigma - Aldrich, USA. ^{109}Cd (1.56 mCi/mg) was from NEN,USA.

The animals were treated in the following groups :

I : Control – 0.2ml of 0.9% NaCl, ip.

II : Cadmium treated – 2mg Cd/kg body weight, ip.

III : Se treated -- 0.5mg Se/kg body weight, ip.

IV : Cd+Se treated – both the doses given simultaneously, ip.

Twenty four hours after the administration of the respective doses, the animals were sacrificed by cervical dislocation under the mild anesthesia. The concentration of cadmium in the kidney and liver was determined by atomic absorption spectrophotometery using the wet ashing method as explained in our earlier publication[8].

Kinetics of ^{109}Cd biodistribution was studied in various organs viz blood, liver, kidney, spleen and heart by measuring the radioactivity at following intervals viz. 1,3,6,12,24 and 48 hours after injecting (i.p.) a known amount of activity. Radio activity was measured in the gamma- scintillation counter and was expressed as counts/min/g of tissue wet weight.

For biochemical estimations, the tissue was homogenized (10%) in the cold 20mM Tris-HCl buffer (pH 7.4), using the mechanically driven Teflon fitted Potter-Elvehjham type homogenizer for one minute. The temperature of 4^0C was maintained during the homogenization and the centrifugation at 10.000g for 20 min, to get the post mitochondrial fractions. The biochemical estimations were carried out in the post mitochondrial fractions of the tissue thus obtained. Glutathione peroxidase was estimated following the coupled enzyme method with glutathione reductase, using hydrogen peroxide as the substrate[17]. Total glutathione was quantitated by the flourimeteric method described by Hisin and Hilf[7]. In this procedure glutathione reacts specifically with O-phathaldehide at pH 8.0, resulting in the formation of a highly fluorescent product that is activated at 350nm with an emission peak at 420nm. Catalase was estimated by the method described by Luck[14] in which catalase activity is reflected by the decrease in the absorption of light in the region 230 to 250nm caused by the decomposition of hydrogen peroxide by the catalase. Data was analyzed by calculating mean$\pm$SEM using Student's T test.

RESULTS

Table 1 show the accumulation of cadmium, as determined by atomic absorption spectrophotometer (AAS), in liver and kidney, after the acute administration of cadmium and selenium in the different treatment groups. During the acute exposure, it is the liver which has six times more accumulation of cadmium as compared to that in kidney (Table 1). However, during the co-administration of cadmium and selenium no significant change in the cadmium level was observed in liver or kidney as compared to group II (only cadmium treated).

Table : 1 Cadmium levels as determined by Atomic Absorption Spectrophotometer in kidney and liver of mice 24 hours after the Cd and Se administration.

Organ	Control I	Cadmium treatment II	Selenium treatment III	Cadmium + Selenium treatment IV
KIDNEY	0.41 ±8.02	6.75*** ±0.83	0.47 ±0.0:	5.21*** ±1.04
LIVER	0.74 ±0.06	37.41*** ±4.04	0.56 ±0.10	48.50*** ±4.88

Each value is the Mean±SEM of six observations.
*P<0.05 **P<0.01 ***P<0.001,
(Represents the comparison with group I)

Indicated in the Table 2 is the progressive decline of ^{109}Cd level in various organs after single injection of ^{109}Cd. Here again liver showed the highest percentage of ^{109}Cd accumulation as compared to other organs. Further it was found that the clearance from the liver was faster than that from kidney, where it continued accumulating even after the elapse of 48 hours. In the case of tissue like heart and spleen, redistribution of cadmium was faster.

Table: 2 Kinetics and biodistribution of ^{109}Cd in normal female Balb/c mice

Organs	1hr.	3hr.	6hr.	12hr.	24hr.	48hr.
BLOOD	112.3±8.63 (6.7)	1.63±1.45 (0.1)	1.74±0.04 (0.15)	1.36±0.04 (0.097)	1.4±0.04 (0.097)	1.5±0.07 (0.11)
LIVER	472.3±6.87 (28.2)	322.5±4.75 (20.5)	214.4±6.24 (18.6)	228.6±4.0 (16.4)	246.1±4.79 (17.1)	186.8±5.51 (13.6)
KIDNEY	71.8±1.27 (4.2)	82.7±1.43 (5.3)	62.6±1.77 (5.45)	84.5±6.07 (6.07)	91.3±1.67 (6.35)	86.6±2.11 (6.30)
SPLEEN	89.02±2.05 (5.30)	63.0±2.22 (4.0)	22.5±0.98 (1.96)	20.5±1.3 (1.47)	20.4±1.3 (1.42)	17.1±0.92 (1.24)
HEART	20.6±0.91 (1.23)	18.6±0.67 (1.20)	11.5±0.91 (1.00)	13.1±0.70 (0.94)	14.9±0.82 (1.04)	13.5±0.82 (0.98)
CPM INJECTED	1671	1571	1148.6	1392.2	1436.9	1371.7

Results expressed in 10^3 xCPM/g tissue (wet weight).
Values in brackets are the percent counts of the total counts injected.
Each value is the Mean±SEM of six observations.
Dose of ^{109}Cd [1.5μCi/animal in 0.2ml of saline, ip.]
Tissue was dissolved in 0.5ml of 30% KOH.

Table 3 includes data of the changes in some of the enzymes in liver following the acute cadmium exposure in the different treatment groups. There was no significant change in the total glutathione content in liver in various treatment groups. But a significant reduction in glutathione peroxidase was observed with cadmium administration. However, this activity improved with Se supplementation. The catalase activity in liver was also seen falling with the acute cadmium treatment which also improved significantly following Se supplementation. However, the recovery of the catalase activity was still less as compared to the control values.

Table : 3 Biochemical estimations in the liver of the mice 24 hours after the Cd and Se administration.

ENZYME	Control I	Cadmium Treatment II	Selenium Treatment III	Cadmium + Selenium Treatment IV
Glutathione peroxidase	677.8 ±13.75	630.0** ±5.48	705.8*** ±12.18	881.5^{***c} ±35.3
Glutathione (total) [ng/mg protein]	284.3 ±16.29	253.8 ±9.46	270.82 ±7.87	255.4 ±5.27
Catalase (units/mg protein)	160.2 ±6.96	98.36*** ±4.57	116.9*** ±4.25	108.3*** ±4.91

Units are μ mol/mg protein/minute unless stated otherwise.
Each values is the Mean±SEM of 7-8 observations.
$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, represents the comparisons between control and other groups.
$^{c}P<0.001$, represents comparison between Group II and IV.

DISCUSSION

The consequence of cadmium toxicity although is well established but the results are different during the acute and the chronic cadmium exposures. There are several mechanisms by which biological system handles the onslaught of important mechanisms by which the biological system handles the onslaught of various toxic exposures including that of cadmium. One of the important mechanisms is by binding to the metallothionein, a low molecular weight protein, which is induced within one hour of the stimulation by the metals[2].

Also several workers have proposed the role of Se in modifying the heavy metal toxicity in addition to its metabolic role in regulating the redox status of the living cells[8].

The present study examined the antagonistic role of selenium during the acute cadmium exposures. It clearly indicated that liver was the main site of cadmium accumulation during its acute exposure. The concentration of cadmium in liver was seen to be six times higher than that in kidney (Table I). However, in our previous studies[13] following the chronic exposure of cadmium, kidney was found to accumulate three times more cadmium as compared to liver. This may be attributed to the capacity of liver to synthesize metallothionein after cadmium administration[21] which binds to the metal. However, during the chronic treatment such proteins after degradation might release the metal free and thereby caused its redistribution. In the present study, the

kinetics of biodistribution of ^{109}Cd also showed maximum cadmium accumulation in liver as compared to the other organs viz kidney, spleen, blood and heart. However while comparing to liver and kidney the rate of decline from all these organs is distinctly faster.

Kidney in fact showed a continuous accumulation of ^{109}Cd from 1 to 48 hours after the exposure. Friberg[5] et al and Jonah and Bhattacharya[10] have also reported similar results. The supplementation of Se along with Cd in the present study, did not show any change in cadmium accumulation either in liver or kidney, whereas concomitant administration of Se reduced the cadmium level in both the organs during the chronic administration studies[9,22].

Since the results seem to be different following the acute administration of the elements such as Hg and As. Therefore it is quite possible that there is a specific time required for the Cd and Se to interact and to possibly form the complex. Also, the role of intracellular milieu of enzymes in promoting this complex formation cannot be ruled out. The GSH content in present study was not effected during the acute cadmium exposure. Possibly the acute exposure of both the elements is not effective enough to show the apparent reduction in Cd accumulation caused by the Cd-Se complex formation as shown in the earlier studies[13].

Since Se in an essential trace element and is required in the body to maintain the antioxidant status, it is highly probable that any kind of interaction between Se and Cd would consequently lead to an imbalance in the oxidative stress management. In the present study, a significant reduction in the glutathione peroxidase activity in liver could be explained on the basis of a high affinity binding of Cd and Se, which thereby reduces the bioavailability of Se for the *de novo* synthesis of GSH-Px. The effect of lower bioavailability of Se during the cadmium exposure is also apparent from the ^{75}Se labeling experiment whereby it was noticed that more amount of ^{75}Se was bound to high molecular weight proteins in the presence of cadmium[8].

Catalase, which is specially responsible for the removal of higher rates of H_2O_2 generation[16], forms an integral component of the cellular antioxidant dependent defense mechanism. It functions in a manner similar to that of glutathione peroxidase by registering a significant decrease in the activity during acute Cd exposure which recovers partially on Se supplementation. Decrease in this enzyme activity in liver on Cd and Se treatment could be ascribed either to a fall in the substrate level i.e. hydrogen peroxide[1] or to a reduced synthesis of the enzyme itself as a result of higher intracellular concentration of Cd. Selenium responsive enzyme. GSH-Px also decreases hydrogen peroxide levels[16]. The fall of hydrogen peroxide level may be ascribed to a decreased activity of Zn/Cu superoxide dismutase also. In the present study, no significant alteration in the total glutathione concentration was seen during Cd and Cd+Se exposures. GSH, an intracellular GSH containing tripeptide, is normally responsible for scavenging the various free radical and the heavy metals. Although it is difficult to conclude the reason for such an observation , but it may be speculated that either the dose administered or the time of only 24 hours between the injection and sacrifice was inadequate to reflect the changes in the glutathione levels. It may be stated that the increased consumption of glutathione leads to stimulation of hepatic glutathione turnover. The depletion of glutathione is apparent only if the synthesizing capacity is lowered / not exceeded / limited[11,12].

The experiments reported here have therefore confirmed the toxic nature of cadmium during the acute exposure. Simultaneous selenium exposure, although does not seem to significantly alter the biodistribution of cadmium in the different tissues but the metabolic alterations, especially in the antioxidant and the glutathione related system show the disruption from the normal status

with a partial recovery with Se supplementation. These experiments therefore consolidate the involvement of selenium in detoxifying cadmium.

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PIERID BUTTERFLIES (LEPIDOPTERA) OF BUXA TIGER RESERVE, JALPAIGURI, WEST BENGAL

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Abstract

Pierid butterflies in the Buxa forest are represented by 17 species belonging to 9 genera. A map showing the distribution of the species has been included. The seasonal distribution of the species has also been recorded.

Key words : Pierid butterflies, morphology, distribution, abundance.

INTRODUCTION

Pierid butterflies are known by about two thousand species and include some of the most abundant ones. Many of the pierids are migratory and therefore widely distributed. They are commonly known as whites, jezebels, orange tips, brimstones and sulphurs. Like the swallowtails, the forelegs of the pierids are fully developed in both sexes, but unlike that family, the hindwings are channelled at the inner margin to fit the abdomen.

The pierids encountered in the Buxa Tiger Reserve, Jalpaiguri, West Bengal, as revealed by our survey between 1994 and 1997, include 17 species under 9 genera[5]. A report on the same is given in this communication.

MATERIALS AND METHODS

All the butterflies have been collected, preserved (pinned), labelled for further study and identified with the help of standard taxonomic keys[1,8,7,4,3,9,2]

The examples are now in the collection of Entomology Laboratory, Department of Zoology, University of Calcutta.

RESULTS

1. *Appias albina darada* (C. Felder & R. Felder)

   Pieris darada C. Felder & R. Felder, 1865, Reise Novana, Lep., 2 : 166.

   Common name : The Common albatross.

2. *Appias hippo* (Cramer)

   Papilio hippo Cramer, 1782, Pap. Exot., iii.

3. *Appias indra* (Moore)

   Pieris indra Moore, 1857, Cat. Lep. Mus. E.I.C., i, 74.

   Common name : The Plain Puffin.

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4. *Appias libythea (Fabricius)*

Papilio libythea Fabricius, 1775, Syst. Ent., 471.

Common name : The Striped Albatross.

5. *Appias lyncida* (Cramer)

Appias lyncida Cramer, 1777, Uitl. Kapellen, 2 (11) : 52.

Common name: The Chocolate Albatross.

6. *Catopsilia pomona pomona* (Fabricius)

Papilio pomona Fabricius, 1775, Syst. Ent., 479.

Common name : The Lemon Emigrant.

7. *Catopsilia pyranthe* (Linnaeus)

Papilio pyranthe Linnaeus, 1758, Syst. Nat. ed. x : 469.

Common name : The Mottled Emigrant.

8. *Cepora nerissa* (Fabricius)

Papilio nerissa Fabricius, 1775, Syst. Ent., 471.

Common name : The Common Gull.

9. *Delias acalis pyramus* (Wallace)

Thyca pyramus Wallace, 1867, Trans. Ent. Soc., 347.

Delias acalis pyramus Wallace; Yagishita et al.,1993, Khepera Publ., Tokyo, Japan, 247.

Common name : The Red-Breast Jezebel.

10. *Delias aglaia* (Linnaeus)

Papilio aglaia Linnaeus, 1758, Syst. Nat. ed., x, i, 465.

Common name : The Red-Base Jezebel.

11. *Delias descombesi* (Boisduval)

Pieris descombesi Boisduval, 1836, Spec. Gen. Lep., i, 465.

Common name : The Red-Spot Jezebel.

12. *Delias eucharis* (Drury)

Papilio eucharis Drury, 1773, III, Ex. Ent., ii, 16.

Common name : The Common Jezebel.

13. *Eurema hecabe* (Linnaeus)

Papilio hecabe Linnaeus, 1758, Syst. Nat. ed., x, 470.

Common name : The Great Orange Tip.

14. *Hebomoia glaucippe* (Linnaeus)

Papilio glaucippe Linnaeus, 1758, Syst. Nat. ed. x, 469.

Common name : The Great Orange Tip.

15. *Ixias pyrene* (Linnaeus)

Papilio pyrene Linnaeus, 1764, Mus. Ulr., 241.

Common name : The Yellow Orange Tip.

16. *Leptosia nina* (Fabricius)

Papilio xiphia Fabricius, 1781, Spec. Ins. ii, 43.

Common name : The Psyche.

17. *Pieris canidia* (Sparrman)

Papilio canidia Sparrman, 1768, Amaen, Acad., vii, 504.

Common name : The Indian Cabbage White.

DISCUSSION

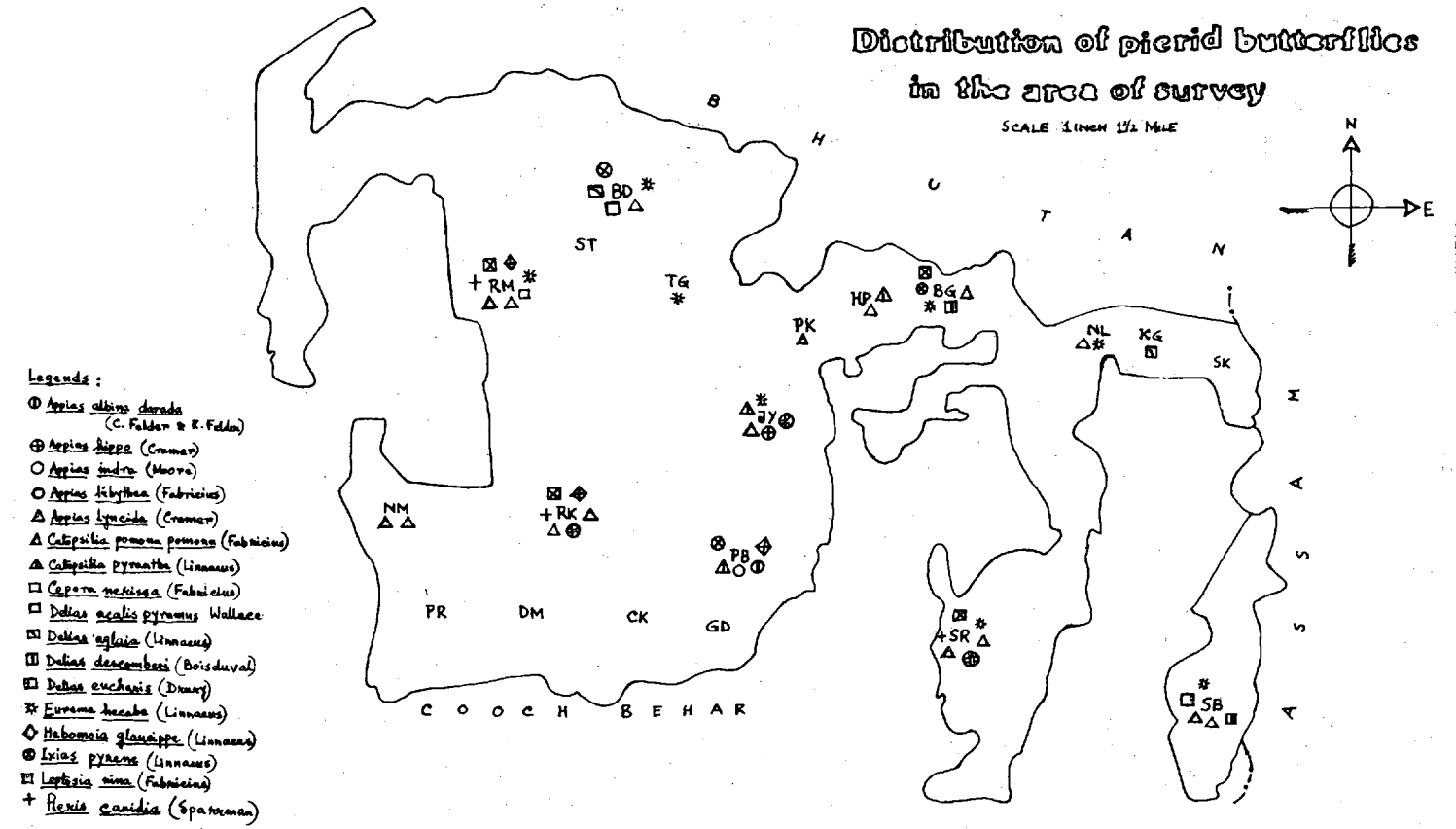
Out of the 9 recorded pierid genera namely, *Appias* Hubner, *Delias* Hubner, *Catopsilia* Hubner, *Cepora* Billberg, *Eurema* Hubner, *Hebomoia* Hubner, *Ixias* Hubner, *Leptosia* Hubner and *Pieris* Schrank, the first three are represented by 5, 4, 2 species respectively

and the remaining genera by 1 species each. The genera like *Cepora* Billberg, *Delias* Hubner, *Hebomoia* Hubner, *Ixias* Hubner, *Leptosia* Hubner and *Pieris* Schrank show rather a limited distribution (vide map). Compared to the nymphalids, the pierids constitute the 2nd most diverse group of butterflies in this forest[6]. Maximum abundance of the pierid butterflies in the forest is during the summer (Table 1).

Table 1 : Showing seasonal distribution of the recorded butterfly species

Name of the Species	Seasonal Occurrence		
	Summer	**Autumn**	**Winter**
1. *Appias albina* darada (C. Felder & R. Felder)	+(WSF)		
2. *Appias hippo* (Cramer)			+(DSF)
3. *Appias indra* (Moore)	+(WSF)		
4. *Appias libythea* (Fabricius)	+(WSF)		
5. *Appias lyncida* (Cramer)	+(WSF)	+(WSF)	+(DSF)
*6. *Catopsilia pomona pomona* (Fabricius)	+		+
7. *Catopsilia pyranthe* (Linnaeus)	+(WSF)		
8. Cepora nerissa (Fabricius)	+(WSF)		
*9. *Delias acalis pyramus* (Wallace)		+	
*10. *Delias aglaia* (Linnaeus)		+	+
*11. *Delias descombesi* (Boisduval)			+
*12. *Delias eucharis* (Drury)			+
13. *Eurema hecabe* (Linnaeus)	+(WSF)	+(DSF)	+(DSF)
14. Hebomoia glaucippe *(Linnaeus)*	+(WSF)		
15. *Ixias pyrene* (Linnaeus)	+(WSF)	+(WSF)	+(DSF)
*16 *Leptosia nina* (Fabricius)	+		+
*17. *Pieris canidia* (Sparrman)	+		

WSF : Wet Season Form
DSF : Dry Season Form
* : Seasonal variation does not exist


Distribution of pierid butterflies
in the area of survey
SCALE 1 INCH 1½ MILE
N
E
B H U T A N
ASSAM
COOCH BEHAR
BD
ST
TG
RM
HP
BG
PK
NL
KG
SK
JY
NM
RK
PB
PR
DM
CK
GD
SR
SB
Legends :
Appias albina darada (C. Felder & R. Felder)
Appias hippo (Cramer)
Appias indra (Moore)
Appias libythea (Fabricius)
Appias lyncida (Cramer)
Catopsilia pomona pomona (Fabricius)
Catopsilia pyranthe (Linnaeus)
Cepora nerissa (Fabricius)
Delias acalis pyramus Wallace
Delias aglaia (Linnaeus)
Delias descombesi (Boisduval)
Delias eucharis (Drury)
Eurema hecabe (Linnaeus)
Hebomoia glaucippe (Linnaeus)
Ixias pyrene (Linnaeus)
Leptosia nina (Fabricius)
Pieris canidia (Sparrman)
BG – Bhutanghat, BD – Buxaduara, CK – Chalsa, DM – Damanpur, GD – Gadadhar, HP – Hatipota, JY – Jayanti, KG – Kumargram, NL – Nimati, NM – Nimati, PB – Panbari, PR – Phasthara, PR – Poro, RM – Raimatang, RK – Rajabhatkhawa, SK – Santosh, ST – Santrabari, SB – South Bhalka, SR – South Raydak, TG – Tashigaon.

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EFFECT OF LEAF EXTRACT OF *AZADIRACHTA INDICA* ON SOME PHOSPHATASES IN *TRICHURIS GLOBULOSA* (NEMATODA)

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Abstract

Effect of ethanolic neem leaf extract (prepared in Soxhlet apparatus) was studied on some phosphatases in *Trichuris globulosa* (v. Linstow). Three enzymes, viz., glucose-6-phosphatase (G- 6-pase), acid phosophatase (ACP) and alkaline phosphatase (ALP) were assayed in their respective buffers and these enzymes showed a typical Michaelis menten substrate saturation kinetics. V_{max} (maximum of the apparent enzyme velocity) and K_m (substrate affinity constant) were calculated directly from the plots. The activity of the enzymes was shown to be effectively inhibited by the *in vitro* addition of the neem extract to the enzyme assay system at a concentration of 0.5%. ACP activity was inhibited in an un-competitive nature as both the Vmax and the Km were lowered i.e., the drug affects the substrate utilisation by the enzyme. ALP activity was inhibited in the same way as ACP i.e., un-competitive where both the V_{max} and the K_m values were lowered. G-6-Pase activity was inhibited in a non-competitive manner in which both the substrate utilization and the enzyme molecules were affected i.e., V_{max} was lowered and K_m remained unchanged.

Key words : *Azadirachta indica*, *Trichuris globulosa*, phosphatases.

INTRODUCTION

Trichuris globulosa is one of the most common nematode parasites in sheep, goat and cattle, causing serious helminthic diseases, thus resulting in considerable loss to our veterinary wealth.

Although a lot of work has been carried out on the taxonomy of this parasite, physiological and biochemical study is limited. Sarwal *et.al.*[12] and Jasra *et.al.*[3], reported that *T.globulosa* possesses a full compliment of enzyme systems of lipid metabolism, glycolysis, Kreb's cycle, phosphatases, transaminases and other NAD and NADP dependent enzymes. These enzyme systems play a significant role in the physiology of the parasite and could be well-exploited in controlling this parasite by blocking their enzyme system.

The synthetic drugs that are used to control these parasites have proved to be very effective during their wide and frequent use. But their widespread use has resulted in emergence of resistant forms of parasites[2,11]. Keeping the above mentioned facts in view, an attempt has been made to study the effect of ethanolic leaf extract of *Azadirachta indica*, commonly called as neem, on some of the glycolytic enzymes and phosphatases of the carbohydrate metabolism in *T.globulosa* under in vitro conditions at different substrate concentrations.

MATERIALS AND METHODS

Live adults of *T.globulosa* were collected from the large intestine of goats (*Capra hircus*). These parasites were thoroughly washed in physiological saline (0.9% NaCl). Worms were then dried on a filter paper and their net wet weight recorded.

A 10% homogenate of the worms was made in 0.25M sucrose, centrifuged at 10,000 rpm in a cold centrifuge for 10 min at 4°C. The supernatant was collected which served as a source of enzyme activity.

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Preparation of Neem leaf extract

Fresh neem leaves were thoroughly cleaned, dried in an oven at a temperature of 35C° for 3-4 days. The weight of these leaves was recorded and these were extracted with ethanol for 24 hrs as described by Takiru *et.al.*[18]. Further concentrations were made by diluting with distilled water.

Preliminary experiments were conducted to find LD_{50} with ethanolic leaf extract. The effect of this plant extract on various enzymes was determined by the direct addition of this drug at a concentration of 0.5% to the enzymes assay systems. The following methods were used for enzyme assays:

Glucose-6-phosphatase (G-6-Pase) was assayed by the method of Swanson[17] using 0.1M Citrate buffer - (pH-6.2); acid phosphatase (ACP) by the method of Natelson[8] using acid phosphatase reagent as a buffer (pH-5.2) and alkaline phosphatase (ALP) was assyaed by Natelson's method[8] using alkaline phosphatase reagent as a buffer(pH-8.5).

Proteins were estimated by modified Sodium Dodecyl Sulphate-Lowry procedure described by Lees and Paxman[4]. Enzyme activities were determined in triplicate and the mean values were plotted according to Lineweaver-Burk (Siegal[15]).

V_{max} (maximum of apparent initial enzyme velocity) and K_m (substrate affinity constant) were determined directly from the plots.

RESULTS

Enzyme activity showed typical Michaelis-Menten substrate saturation kinetics. The enzymes activity increased with increase in substrate concentration followed by a decrease, thereby achieving a steady state kinetics (Figs. 1-3).

Ethanolic neem leaf extract has been shown to affect the activity of these enzymes when added in vitro to the standard enzyme assay system at a LD_{50} concentration of 0.5%.

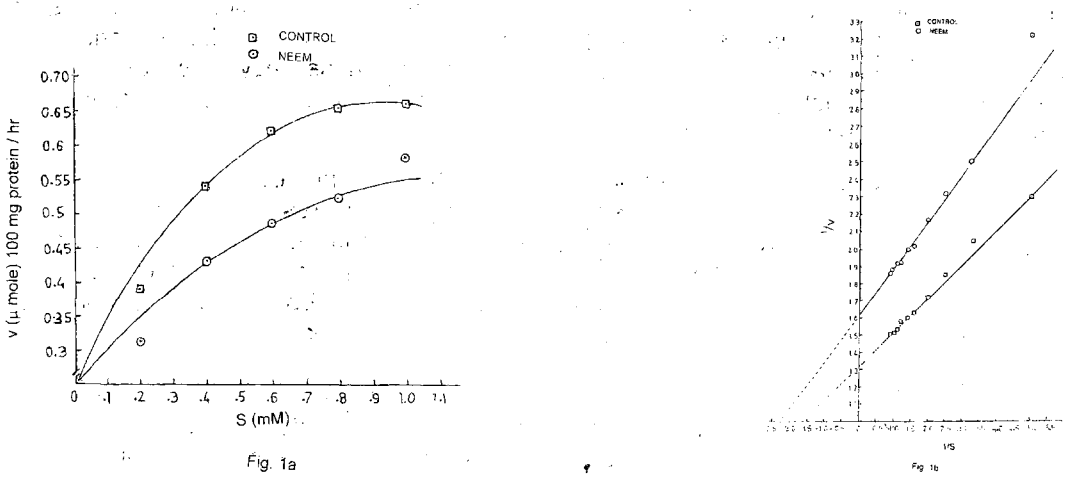

Fig. 1a

Fig.1 (a) Effect of Neem on acid phosphatase in *T. globulosa*.

Fig.1 (b) V_{max} and Km of acid phosphatase in the presence of neem (I/V vs I/S Lineweaver-Burk plot).

ACP activity was inhibited in the presence of the extract. The V_{max} of the enzyme was lowered and also the K_m was considerably affected (Table-1) Fig.1-a suggesting that the inhibition was of uncompetitive nature wherein substrate utilisation by the enzyme is affected.

G-6-Pase activity was also found to be inhibited by the addition of the extract. The V_{max} of the enzyme was considerably lowered while K_m was not altered (Table-1) Fig.-3a. This indicates the non-competitive nature of the inhibition suggesting that the enzyme molecules are inhibited by the extract.

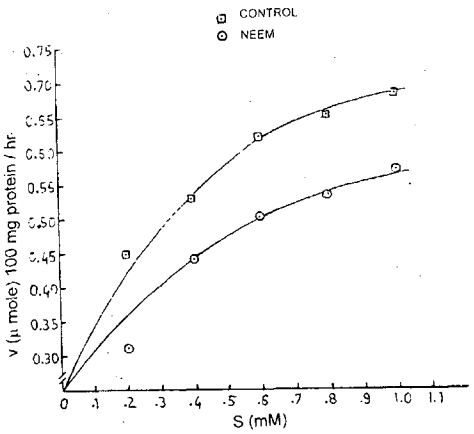

Fig. 2a

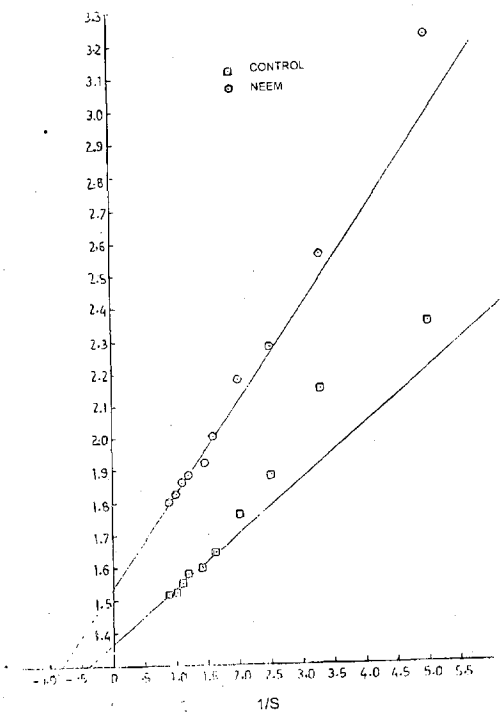

Fig. 2b

Fig.2 (a) Effect of Neem on alkaline phosphatase in *T. globulosa.*

Fig. (b) V_{max} and Km of alkaline phosphatase (I/V vs I/S Lineweaver-Burk plot).

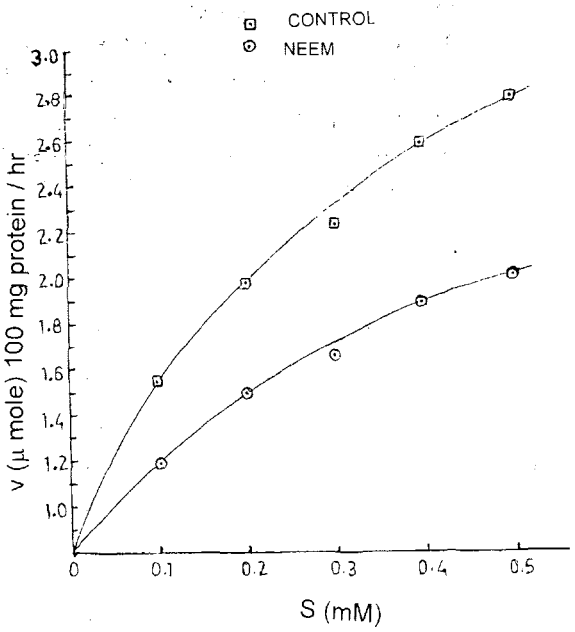

Fig. 3a

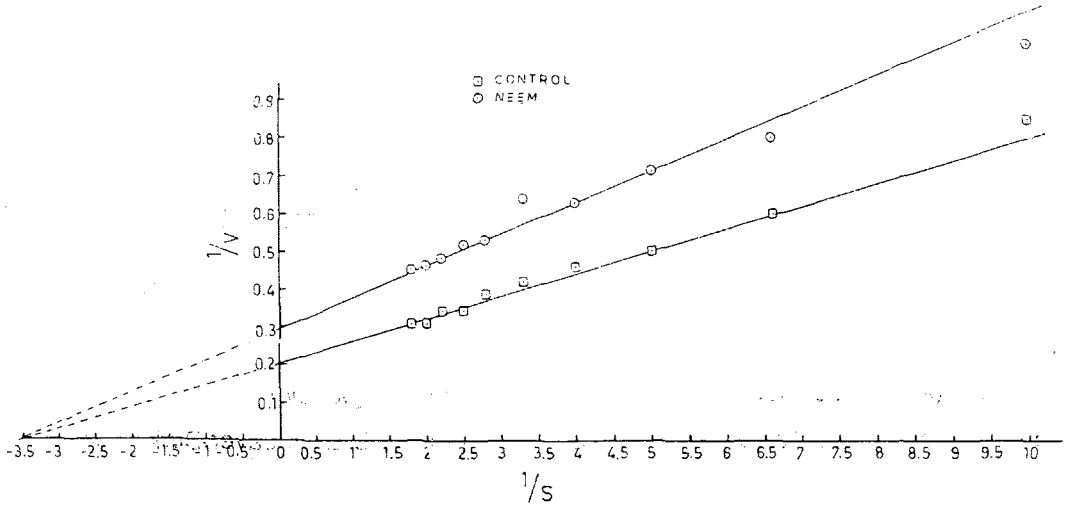

Fig. 3b

Fig.3 (a) Effect of Neem on Glucose-6- phosphatase is *T. globulosa.*

Fig.3 (b) V_{max} and Km of Glucose-6-phosphatase (I/V vs I/S Lineweaver-Burk plot).

Table 1 : *In vitro* effect of neem extract on the enzymes of carbohydrate metabolism in *T. globulosa.*

Enzymes	Control (Km)	Neem (Km)	Control(V_{max})	Neem(V_{max})
Glycolysis: Glucose-6 Phosphatase	0.27	0.27	4.76	3.33
Phosphatases: Acid phosphatase	0.64	0.44	0.75	0.61
Alkaline Phosphatase	2.5	1.25	0.73	0.65

DISCUSSION

From the results of the experiments conducted, it is evident that *T.globulosa* possesses some of the enzymes of carbohydrate metabolism. The inhibition of the above mentioned enzymes may prove fatal for the parasite since the phosphatases i.e. ACP and ALP are believed to be involved directly in the absorption of nutrients and it has also been suggested that their presence may be indicative of active transport[6]. ACP activity in the intestine may be related to extra cellular digestion, absorption, phosphorylation of the transported nutrients, excretion and other relative functions. Pappas and Read[10], Levy and Read[5] and Starling and Fischer[16] suggested that the function of phosphatases is digestive. They act as intrinsic digestive enzymes. G-6-Pase is also an important enzyme in the processes of glycolysis and gluconeogenesis. It helps in hydrolysis of glucose-6-phosphate to inorganic orthophosphate and glucose. Thus, it plays an important role in the digestion and absorption[6,10]

Inhibition of enzymes of carbohydrate metabolism in parasitic helminths may prove to be fatal since they depend for their energy supply, almost entirely, on this metabolism[14]. Since these enzymes play such a crucial role in the survival of these parasites, their inhibition may ultimately lead to a decrease in generation of energy, thus proving fatal for the parasite.

Intensive research on the biological evaluation of neem is being conducted[1,7,13]. However, little work has been done on its effects on enzymes[9]. This is for the first time that an attempt has been made to correlate the enzyme activity with nematicidal activity of neem.

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COTYLOPHORON CHAUHANI (TREMATODA : PARAMPHISTOMIDAE) : SURFACE MORPHOLOGY AS REVEALED BY SEM

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Abstract

Surface topography of *Cotylophoron chauhani* reveals that this amphistome has cobble-stone like structures in the anterior half and only constrictions in the posterior half. Rim of mouth is surrounded by five rows of concentrically arranged rings having sensory papillae and cilia emanating from their pits. Papillae around the acetabulum are button like. Genital sucker is well developed. Cirrus has knob like tip and is finely ridged.

Key words : *Cotylophoron chauhani*, Trematoda, Scanning electron microscopy.

INTRODUCTION

The tegument of the trematodes forms a physiologically important interface with the host environment[8] and an understanding of the nature of this covering layer, therefore, is of fundamental importance. In the recent past, the technology of SEM has been employed to study the ultrastructure and surface features of this interface and its significant role in taxonomy. According to Kuntz *et al.*[13] SEM data may be useful in recognizing small morphological differences between species. It has also been suggested[12] (Hoole and Mitchell, 1981) that the arrangement of papillae on the adult may help to provide a basis for diagnosing closely allied species. The amphistomes can only be identified by studying the histology of the pharynx, acetabulum and genital atrium. The study of SEM will ,therefore, be useful to elucidate the surface topography and reveal the ultrastructural differences. The surface topography of only a few amphistomes has been described. The present paper illustrates the surface topography of *Cotylophoron chauhani* (Gupta and Gupta 1972), an amphistome from the rumen of goats in Chandigarh.

MATERIALS AND METHODS

Live mature worms were collected from the rumen of goats at the Chandigarh slaughter house and identified as per Nasmark[16]. For SEM studies, the specimens were fixed in 9% phosphate buffered glutaraldehyde (16ml of 25% glutaraldehyde in 84 ml. of buffer, pH. 7.4) for 24 h at 4^0C and then washed in buffer. The specimens were post fixed in 1% osmium tetroxide for 1h at 4^0C, rinsed in buffer for 5-10 min and incubated in saturated solution of thiocarbohydrazide (TCH) for 15 min at 25^0C. These were then washed thoroughly in tetroxide for 30-60 min and rinsed in distilled water for over 15 min. Dehydration was done through graded acetone series to pure amyl acetate. The specimens were then dried in critical point dryer (CPD) using liquid CO_2. They were then fixed in stubs, coated with gold in sputter and finally scanned under JEM 1200 EX JEOL.

***Cotylophoron chauhani* Gupta and Gupta 1972.**

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OBSERVATIONS

Body : 5.2-6.3 x 2.2-2.5 mm. (Fig. 1). Dorsoventral diameter 1.22 mm, Acetabulum 1.7 mm. in diameter, in relation to body length 1: 3.35. Paramphistomum type with D.E.C.M.$_1$-23; D.E.C.M$_2$-25, D.I.C.M.-32, V.E.C.M.-18, V.I.C.M.-44 (Fig.2), pharynx : Paramphistomum type, genital atrium : Cotylophoron type. Genital sucker present.

The entire body surface of the worm is thrown into well defined constrictions which surround the whole worm clothing it with many ring-like structures. Owing to the tegumental structure, the body surface is clearly differentiated into distinct parts. In the anterior half of the body, apart form these constrictions, there are swellings present on the body surface which make this particular half a cobble-stone like appearance (Fig.3). The constrictions, having cobble-stone like appearance, are twenty three in number. Whole of the surface surrounding the genital atrium has

these swellings. These terminate posterior to the genital atrium approximately in the mid-body. The tegument towards the posterior half of the body is smooth save for constrictions (Fig.3).

At the anterior extremity, an oral sucker surrounds the mouth (Fig.1). The oral sucker is surrounded by five concentrically arranged rings which in turn have swellings of sensory papillae (Fig.4). The papilla has a small head-like structure showing a coiled pattern (Fig.5). Each papilla has openings at its apical end. It is interesting to note that some of the openings have hair-like structures (cilia) emanating from the pits or openings (Fig. 6).

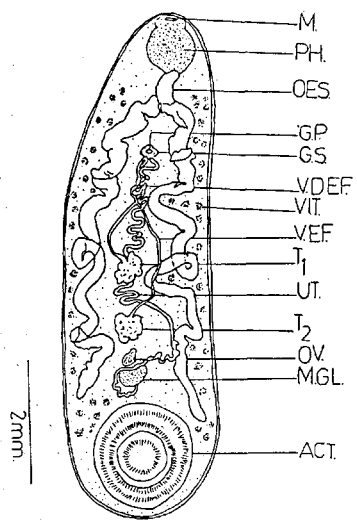

Fig. 1

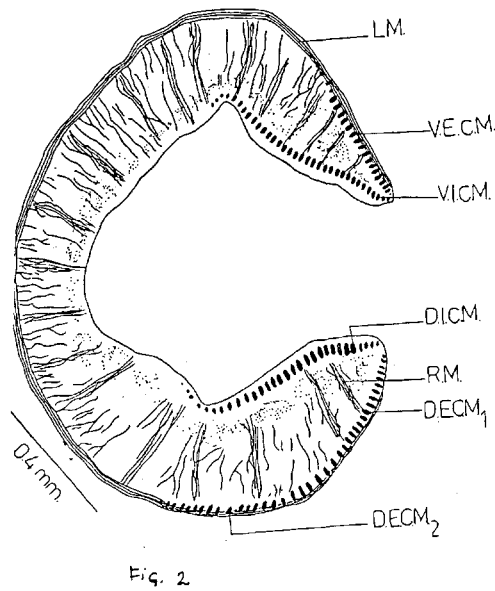

Fig. 2

***Cotylophoron chauhani* : Light and SEM**

Fig. 1 Whole mount, ventral view

Fig. 2 Acetabulum showing arrangement of muscles

Immediately after the mouth and the oral sucker, there is a narrow area which joins the anterior tip of the worm to the rest of the body which is bulky and has constrictions (Fig.3).

The genital atrium shows the presence of a well-developed genital sucker. It has a smooth surface on its periphery and fine wrinkles towards the genital pore. The cirrus is seen everted through the genital pore. The surface of the cirrus has a knob-like, bilobed swelling (Fig.7). The surface surrounding the genital sucker is thrown into cobble-stone like swellings.

The acetabulum is muscular with a wide opening. The latter is highly folded with a wreath-shaped ridge. The surface of the acetabulum is thrown into "gullies" similar to a heavily eroded landscape. The inner rim of the posterior sucker shows the presence of small button-like structures which might be sensory papillae (Fig. 8).

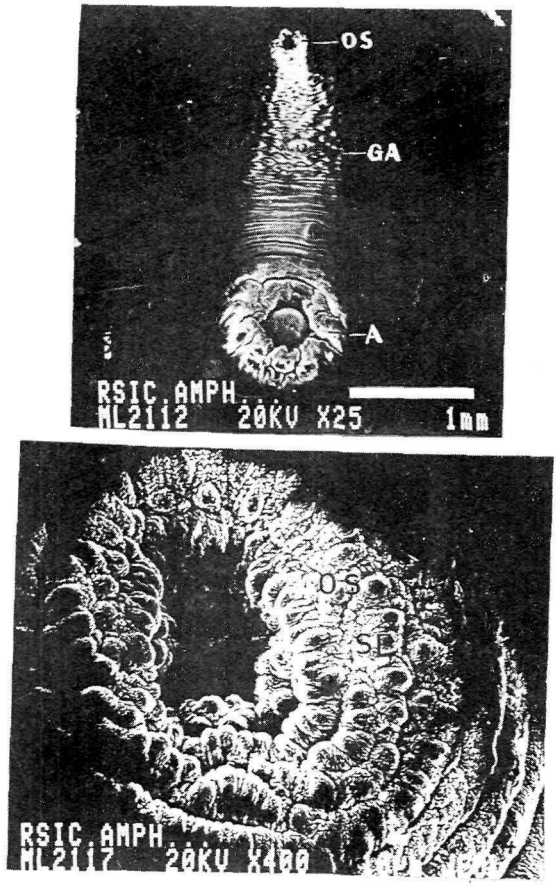

Fig. 3 SEM of whole mount, ventral view

Fig. 4 Anterior end showing sensory papillae

Fig. 5 Sensory papillae, enlarged

Fig. 6 Sensory papillae showing their openings

Fig. 7 Genital atrium showing genital sucker and cirrus

Fig. 8 Acetabulum showing button like sensory papillae

DISCUSSION

SEM studies on *Cotylophoron chauhani* show that the worms of this genus, are characterized by their body being differentiated into two distinct regions- the knobby anterior half and the constricted posterior half. Such a differentiation has not been seen in any other genus of amphistomes scanned so far. The cobble-stone like structures in the anterior-half and the constrictions in the posterior half on the general tegument of this amphistome must be primarily involved in the absorption, since attachment is entirely done by acetabulum and the pharynx. This is further confirmed since tegument lacks spines. The spines have been observed in many trematode species viz. *Fasciola hepatica*[4,5], *Leucochloridium* sp.[1,2], male *Schistosoma haematobium*[11], *S.japonicum*[18], *Urogonimus macrostomus*[3]. *Clonorchis sinensis*[9]. Further, in *Schistosoma* spp. the female has few spines as compared to male because it has not to attach itself to the host's surface.

Surrounding the oral opening are sensory papillae which are arranged in five concentric rings in *C.chauhani*. Several concentric rows of circumoral papillae are present in *Bilatorchis papillogenitalis* and *Orthocoelium indonesiense*[6,7]. Circumoral papillae have also been described by Nollen and Nadakavukaren[17] in *Megalodiscus temperatus*. The opening of these papillae have hair-like structures (cilia) emerging from them. The function of such receptors is still disputable, but due to circumoral location, it appears to be sensory. However, both tangoreception and rheoreception have also been postulated[14,15].

Earlier, the body of the trematodes was thought to be non-ciliated cytoplasmic syncytium. According to SEM studies there are hair-like structures which can be equated to cilia. This, however, can only be confirmed if sections are cut and viewed through Transmission Electron Microscope (TEM) to try and establish the 9+2 structure. The presence of a well-developed genital sucker may be an advantage to the worm specially to facilitate firm attachment during the act of copulation.

The acetabulum is well-developed to help in the anchorage of the worm to its substratum and the button-like papillae present on it may help it to discern its surroundings.

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MITES OF THE FAMILY PHYTOSEIIDAE (ACARI : MESOSTIGMATA) ASSOCIATED WITH CROP PLANTS AT HISAR (HARYANA) INDIA

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Abstract

A study on the mites of family Phytoseiidae associated with fruits and vegetables at Hisar revealed the occurrence of 12 species namely *Amylyseius alstoniae* Gupta, *A. delhiensis* (Narayanan and Kaur), *A. fallacies* (German), *A. finlandicus* (Oudemans), *A. indicus* (Narayanan and Kaur),*A.multidentatus* (Swirski and Shechter), *A. neococcineae* Gupta, *Phytoseius jujuba* Gupta, P. *minutus* Narayanan, Kaur and Ghai, *P. mixtus*,Choudhari, *P. roseus* Gupta and *Typhlodromus homalii* Gupta. Of these, *A. fallacies* and *A. multidentatus* are already known from Haryana state. Mites and their associated plants including three fruit trees and four vegetable crops are given in this paper.

Key words : Taxonomy, acari, redators, phytoseiidae, crop plants.

INTRODUCTION

Phytoseiid mites are the most dominating predatory mites that are reported to feed on destructive mites of the families Tetranychidae Tenuipalpidae and Eriophyidae[6]. In India, the first Phytosiid mite namely *Amblyseius longispinosus* was reported by Evans in 1953 on paddy crop in Coimbatore, Tamil Nadu. To-date over 150 species have been reported[2,3,4,7] from India. However, till recently only one species i.e. *Amblyseius fallacies* was known from Karnal on paddy crop from Haryana[4]. Subsequently, Mathur *et al.* (1994) reported the occurrence of *Amblyseius multidentatus, Phytoseius indicus* and *A. fallacies* from Hisar[5].

Table. 1.Phytoseiid mites and their associated plants in Haryana.

Sr.No.	Name of the Mite	Host Plant	
		Technical Name	Common Name
1.	*Ambliyseius alstoniae*	*Ficus carica*	Anjeer
		Ziziphus jujuba	Ber
2.	*A. delhiensis*	*Zizyphus jujuba*	Ber
		Capsicum putescens	Mirch
3.	*A.fallacies*	*Solanum melongena*	Brinjal
4.	*A.finlandicus*	*Luffa acutangula*	Tori
		Capsicum putescens	Mirch
5.	*A. Indicus*	*Solanum melongena*	Brinjal
6.	*A.multidentatus*	*Abelmoscus esculentus*	Bhindi
		Solanum melongena	Brinjal
7.	*A. neococcineae*	*Abelmoscus esculentus*	Bhindi
8.	*Phytoseius jujuba*	*Zizyphus jujuba*	Ber
9.	*P.minutus*	*Solanum melongena*	Brinjal
10.	*P. mixtus*	*Ficus carica*	Anjeer
		Zizyphus jujuba	Ber
11.	*P. roseus*	*Zizyphus jujuba*	Ber
12.	*Typhlodromus homalii*	*Mangifera indica*	Mango

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KEY TO THE COMMON ANGIOSPERMIC FAMILIES OF NW INDIAN PLAINS

(Based on Bentham and Hooker's System)

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Abstract

The present key to the common angiospermic families of North West Indian plains has been designed to help students to assign the flowering plants of the region to their respective families. The key is of the indented or yoked type and is based mostly on exomorphic characters. The steps to be followed while using the key have been listed. A total of 119 families have been included, 93 from dicotyledons and 26 from monocotyledons. The key is based on Bentham and Hooker's system of classification.

Key words : Angiospermic families, Indian plains, Bentham and Hooker's system.

INTRODUCTION

The present key has been designed to help the students to assign the flowering plants of NW India to their respective families according to the Bentham and Hooker's system of classification. Since the present key is based mostly on single character, it is likely that some other family not included in the key may be identified as belonging to one of the list advanced. Hence, when the family of a plant has been traced, it should be confirmed by comparing the other diagnostic features of the family.

HOW TO USE THE KEY

The key given here is of the indented or yoked type and is dichotomous throughout, i.e. at every stage a choice has to be made between two contrasting alternatives(leads) , which together make up a couplet. To facilitate reference to particular leads , each couplet is numbered and each lead of a couplet is given the same number. In order to determine the family of a plant, proceed along the following steps:

I) Consult the general scheme of key character to the main classes (given under each group) in order to find out the class to which your specimen belongs ;

II) Proceed with the class indicated from the general scheme and confirm it by studying its other characters. Consult the characters given under each class and proceed to find out the sub-class of the class to which your specimen belongs ;

III) Proceed to the sub-class diagnosed and study the various characters given under it to confirm its diagnosis ;

IV) Read the two alternatives of the character(s) under a head and you will either reach the name of a family or a reference to some number. Continue following the number diagnosed and studying the character given under each number till you find the name of the family.

It will sometimes happen that the specimen does not agree with all the characters given in a

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particular lead. When such a situation arises, one must decide as to which of the two leads of the relevant couplet the specimen agrees the most. In general the most reliable diagnostics are placed at the beginning of each lead , so these characters should be observed with particular care.

THE KEY

The primary division of flowering plants into two main classes (excluding the class Gymnospermae of Bentham and Hooker) Dicotyledonae and Monocotyledonae is based upon the number of cotyledons in the embryo; arrangement and structure of vascular bundles of the stem ; venation of leaves; root morphology; and the number of members of each whorl of floral parts of the flower (numerical plan of the flower). Thus, in order to find out whether your specimen belongs to the class Dicotyledonae or the Monocotyledonae, use the following key :

1. Leaves usually net-veined (except *Tragopogon* and *Bupleurum*) , with or without stipules, alternate, opposite or whorled; flowers with parts (sepals, petals , and stamens) in 2s , 4s or 5s, or the parts numerous (except the genus *Argemone*, family **Menispermaceae** and **Lythraceae**) ; cotyledons usually 2 , lateral ; tap root usually persistent, branched ; vascular bundles of the stem open and arranged in a ring.....Class - **Dicotyledonae**

1. Leaves usually with parallel veins (except *Smilax and Dioscorea*),without stipules,opposite only in some aquatic plants; flowers with parts generally in 3s; cotyledon 1; mature root system wholly adventitious; vascular bundles of the stem closed and scattered.....Class - **Monocotyledonae**

Class - Dicotyledonae

The class dicotyledonae is sub-divided into three sub-classes on the basis of character of perianth. Thus, if your specimen belongs to class dicotyledonae, proceed with the following key :

1. Flowers with perianth and this usually in two whorls and distinguishable into calyx and corolla :

2. Petals free (except family **Cucurbitaceae**) .....sub-class -1 : **Polypetalae**

2. Petals united (except some members of family **Ericaceae**).....sub-class -2 : **Gamopetalae**

1. Flowers without perianth (naked flowers) or perianth if present then not distinguishable into calyx and corolla.....sub-class -3 : **Monochlamydeae**

Sub-class -1 : Polypetalae

Flowers with biseriate perianth, the inner whorl of perianth comprising polypetalous perianth, rarely gamopetalous or apetalous by abortion (petals united to some extent in members of family **Cucurbitaceae).**It is sub-divided into three series : **Thalamiflorae**, **Disciflorae**, and **Calyciflorae**. Thus, if your specimen belongs to sub-class **Polypetalae**, proceed with the following key:

1. Flowers hypogynous ; sepals usually distinct and separate, free from the ovary; thalamus not discoid ; ovary superior .....Series-1 : **Thalamiflorae**

1. Flowers hypopgynous, perigynous, or epigynous; sepals distinct or united; thalamus discoid; ovary inferior or superior :

2. Thalamus generally expanded into a disc (in the form of a ring, cushion, or spread

over the base of calyx tube or confluent with the base of the ovary, or broken up into glands) ; ovary superior..... Series-2 : **Disciflorae**

2. Disc mostly absent ; gynoecium often enclosed by the development of the floral axis into a cup (hypanthium) ; ovary often inferior..... Series -3 :**Calyciflorae**

Series -1 : Thalamiflorae

Flowers hypogynous ; sepals usually polysepalous, free from the ovary ; petals in one or more series, rarely inserted on a short or long torus or on a disc; stamens numerous (rarely definite); carpels 1-many, generally apocarpous, sometimes partially fused;ovary superior; embryo minute, endosperm fleshy. Thus, if your specimen keys down to this series, proceed further as follows :

1.Gynoecium apocarpous (sometimes reduced to only one carpel -**Ranales**):

2. Gynoecium of only one carpel :

3. Sepals spurred, petals forming a nectary..... Family : **Ranunculaceae**

3. Sepals not spurred, petals not forming a nectary..... Family : **Berberidaceae**

2. Gynoecium of several carpels :

4. Aquatic herbs:

5. Leaves whorled, with horn-like spines; flowers unisexual ..... Family: **Ceratophyllaceae**

5. Leaves alternate, without horn-like spines; flower bisexual ..... Family :**Nymphaeaceae**

4. Plants generally terrestrial (except *Ranunculus aquatilis*):

6. Flowers 4- or 5-merous..... Family : **Ranunculaceae**

6. Flowers 3 –merous:

7. Plants herbs or woody, erect or climbers; flowers bisexual..... Family : **Magnoliaceae**

7. Plants herbs or woody climbers, flowers unisexual..... Family : **Menispermaceae**

1. Gynoecium syncarpous :

8. Placentation parietal (**Parietales**):

9. Gynoecium either open at the top or with a cup-like depression ..... Family: **Resedaceae**

9. Gynoecium normal:

10. Flowers zygomorphic, pentamerous; anterior petal gibbous or saccate at the base ..... Family : **Violaceae**

the base ..... Family : **Violaceae**

10 .Flowers actinomophic, other than pentamerous :

11. Petals 4, cruciate (arranged like a cross):

12 .Stamens generally 6, tetradynamous..... Family : **Brassicaceae (=Cruciferae)**

12.Stamens usually many, sometimes 4 or 6, if 6 then not tetradynamous ..... Family : **Capparaceae**

11. Petals 2+2 or 3+3 :

13. Sap clear; stamens many (more than twice as many as petals), free; nectaries absent..... Family : **Papaveraceae**

13. Sap not milky or coloured; stamens definite (as many as or twice as many as petals or fewer, usually 4); nectary present..... Family : **Fumariaceae**

8. Placentation other than parietal :

14. Ovules pendulous (**Polygalales**); petals fringed..... Family : **Polygalaceae**

14. Ovules not pendulous :

15. Placentation free central (**Caryophyllales**):

16. Ovary half inferior; capsule dehiscing transversely ..... Family : **Portulacaceae**

16. Ovary superior; fruit opening by longitudinal valves or irregularly :

17. Leaves reduced , scale like; lime-secreting plants; nodes not swollen; flowers with hypogynous discs..... Family : **Tamaricaceae**

17. Leaves normal ; nodes generally swollen; discs absent..... Family : **Caryophyllaceae**

15. Placentation axile (**Guttiferales** and **Malvales**):

18. Leaves opposite or whorled (**Guttiferales**):

19. Stamens free ; leaves not gland-dotted..... Family : **Elatinaceae**

19. Stamens in groups ; leaves gland -dotted..... Family : **Hypericaceae**

18. Leaves generally alternate (**Malvales**):

20.Stamens free or connate at the base, sometimes united into 5 bundles; anthers bithecous:

21. Flowers in cymes; stamens numerous, in five bundles; style 1 ..... Family: **Tiliaceae**

21. Flowers solitary or clustered; stamens 5-10; styles 2-5.....Family: **Sterculiaceae**

20. Stamens united in a tube (monadelphous) ; anthers monothecous:

22. Herbs / woody, often with stellate hairs; epicalyx usually present .....Family : **Malvaceae**

22. Trees, often with swollen trunks, without stellate hairs ; epicalyx absent.....Family : **Bombacaceae**

Series-2 : Disciflorae

Flowers hypogynous; sepals distinct or united, free or adnate to the ovary; thalamus usually expanded into a disc (in the form of a ring, cushion, or spread over the base of calyx tube, or confluent with the base of the ovary, or broken up into glands); stamens usually definite in number and inserted upon or at the outer or inner base of the disc ; carpels 2-many; placentation axile; ovary superior. Thus, if your specimen belongs to the series Disciflorae, then proceed with the following key :

1. Flowers individually conspicuous (**Geraniales**) :

2. Trees or shrubs (rarely herbs and then the gynoecium stalked i.e. gynophore present) :

3. Leaves gland-dotted ; stamens usually polyadelphous.....Family : **Rutaceae**

3. Leaves not gland-dotted ; stamens united at the base into a ring or free ; flowers bisexual.....:Family : **Meliaceae**

2. Herbs (gynophore absent) :

4. Leaves palminerved .....Family : **Geraniaceae**

4. Leaves not palminerved :

5. Plants wild, xerophytic;style single with a terminal stigma .....Family:**Zygophyllaceae**

5. Plants cultivated or escape, mesophytic ; styles always separate (usually 5).....Family : **Linaceae**

1. Flowers individually not conspicuous (**Celastrales and Sapindales**):

6. Stamens opposite the petals (**Celastrales**):

7. Leaves stipulate, stipules falling off or spinose, often with 3-5 prominent basal nerves.....Family : **Rhamnaceae**

7. Leaves exstipulate ; plants climbers with leaf opposed tendrils; fruit a berry.....Family : **Vitaceae**

6. Stamens alternate with the petals (**Sapindales**):

8. Stamens borne inside the disc ; fruit a capsule or samara(except *Sapindus mukorossi*).....Family : **Sapindaceae**

8. Stamens borne on or outside the disc:

9. Leaves stipulate, stipules falling off; stamens 4 or 5; ovary 3-5 locular.....Family : **Celastraceae**

9. Leaves exstipulate ; stamens 5-8 ;ovary unilocular.....Family : **Anacardiaceae**

Series-3 : Calyciflorae

Flowers perigynous, sometimes epigynous; sepals united, rarely free, often adnate to the ovary; petals uniseriate and often adnate to the ovary, rarely free; disc adnate to the base of the calyx, rarely swollen or raised into a torus or gynophore; stamens perigynous, usually inserted on or beneath the outer margin of the disc; gynoecium often enclosed by the development of the floral axis into a cup(hypanthium) ; ovary inferior. Thus if your specimen conforms to the series Calyciflorae then proceed with the following key :

1. Carpel one or if more than one then these free or connate below (except some **Saxifragaceae**), varying from hypogynous to epigynous (**Rosales**) :

2. Leaves with glandular hairs ; plants insectivorous.....Family : **Droseraceae**

2. Leaves without glandular hairs ; plants not insectivorous :

3. Thick fleshy herbs .....Family : **Crassulaceae**

3. Herbs, shrubs or trees but not thick and fleshy :

4. Gynoecium of one carpel , superior ; fruit generally a legume:

5. Corolla actinomorphic, petals valvate in bud, united ; stamens 4 - many; leaves bipinnate, rarely reduced to phyllodes..... Family : **Mimosaceae**

5. Corolla zygomorphic, petals imbricate in bud, petals free ; stamens 10 or fewer ; leaves simply pinnate, of 3 leaflets or simple :

6. Corolla papilionaceous, upper petal exterior, seed usually with an incurved radicle; stamens 10, diadelphous (monadelphous in *Crotalaria*).....Family : **Fabaceae**

6. Petals all alike, upper petal interior, or petal 1 or petals absent; seed usually with a straight radicle; stamens 10, in two whorls of 5 each.....Family : **Caesalpiniaceae**

4. Gynoecium of one or more united carpels, superior or inferior; fruit other than a legume :

7. Leaves usually with stipules; corolla rosaceous.....Family : **Rosaceae**

7. Leaves exstipulate; corolla not rosaceous.....Family : **Saxifragaceae**

1. Carpels as a rule united ; ovary generally inferior (except **Lythraceae** where gynoecium lies at the bottom of calyx cup , and the **Passifloraceae** where it is superior):

8. Styles of the carpels completely united into a single structure (**Myrtales**) :

9. Leaves with translucent aromatic glands.....Family : **Myrtaceae**

9. Leaves not gland-dotted :

10. Ovary present at the bottom of the calyx cup.....Family : **Lythraceae**

10. Ovary enclosed in a distinct floral tube :

11.Woody plants; stamens 8-10; ovary unilocular ; ovules 2-6, pendulous.....Family : **Combretaceae**

11.Usually herbs; stamens 4-8; ovary 1- 5-locular, usually 4-locular; ovules many with axile placentation.....Family : **Onagraceae**

8. Styles as many as carpels or only partially united :

12. Styles of carpels free :

13. Plants neither succulent nor fleshy; placentation axile, ovule solitary in each loculus, pendulous from the apex (**Umbellales**) :

14.Usually herbs without stellate hairs; flowers usually bisexual; petals imbricate in bud ; carpels 2; fruit schizocarpic, splitting into 2 mericarps.....Family : **Apiaceae (=Umbelliferae)**

14.Plants mostly woody, often with stellate hairs; flowers often unisexual;petals valvate in bud ; carpels 2-5; fruit a drupe or a berry .....Family : **Araliaceae**

13. Plants succulent or fleshy; placentation parietal (rarely axile-**Passiflorales** and **Ficoidales**):

15. Leaves often fleshy; flowers usually unisexual; perianth 2-12, usually 4 in 2 pairs in male flowers, both pairs unequal..... Family : **Begoniaceae**

15. Plants succulent; flowers bisexual; sepals and petals distinct, petals numerous :

16. Stem succulent,often very spiny; leaves usually absent or very reduced or falling early; flowers usually solitary; fruit a berry.....Family : **Cactaceae**

16. Stem and leaves succulent, spines usually absent ; leaves usually opposite, often all basal; inflorescence cymose; fruit usually a complex capsule opening when wet and closing when dry / rarely fleshy and indehiscent.....Family : **Aizoaceae**

12. Styles of carpels only partially united (**Passiflorales**):

17. Flowers bisexual ; stamens and carpels often borne on a stalk (androgynophore) ;styles 3-5, united only at the base; ovary superior .....Family : **Passifloraceae**

17. Flowers usually unisexual ; stamens and carpels not borne on a stalk ; style 1 (rarely 3 and free) ; ovary inferior.....Family : **Cucurbitaceae**

Sub-class -2 : Gamopetalae

Flowers with biseriate perianth, petals generally united (only slightly united or even free in some members of family Ericaceae). This sub-class is sub-divided into 3 series : Inferae, Heteromerae, and Bicarpellatae. If your specimen is diagnosed as belonging to the sub-class Gamopetalae, then proceed with the following key :

1. Ovary inferior.....Series- 1 : **Inferae**

1. Ovary superior :

2.Carpels more than two; stamens in two whorls.....Series -2 : **Heteromerae**

2. Carpels two; stamens in one whorl.....Series- 3 : **Bicarpellatae**

Series - 1 : Inferae

Ovary inferior, stamens in one whorl and as many in number as the corolla lobes. Thus if your specimen works out to be belonging to series Inferae, then proceed with the following key :

1. Leaves generally alternate, ovary unilocular (rarely more than 1-celled and if so then with only one ovule) :

2. Flowers borne in capitula (**Asterales**).....Family : **Asteraceae (=Compositae)**

2. Flowers borne singly (**Campanulales**).....Family : **Campanulaceae**

1. Leaves generally opposite or whorled; ovary bilocular (**Rubiales**) :

3. Herbs; flowers involucrate; fruit an achene.....Family : **Dipsacaceae**

3. Herbs, shrubs or trees; flowers not involucrate; fruit not an achene :

4. Leaves simple, stipules conspicuous.....Family : **Rubiaceae**

4. Leaves simple, exstipulate , if pinnate then these stipulate.....Family : **Caprifoliaceae**

Series-2 : Heteromerae

Ovary usually superior ; stamens epipetalous or free from the corolla, opposite or alternate to corolla lobes, as many as the petals. If your specimen belongs to series Heteromerae, then proceed as follows :

1. Plants parasitic on soil fungi.....Family : **Monotropaceae**

1. Plants green, autotrophic :

2. Stamens opposite to corolla lobes, placentation free-central, ovary unilocular (**Primulales**) .....Family : **Primulaceae**

2. Stamens alternate to corolla lobes, placentation axile, ovary multilocular (**Ebenales**):

3. Plants with watery sap; flowers tetramerous.....Family : **Ebenaceae**

3. Plants with milky sap; flowers with 4 - many floral parts.....Family : **Sapotaceae**

Series- 3 : Bicarpellatae

Ovary usually superior ; stamens epipetalous ; leaves opposite ; carpels 2. Thus if your specimen belongs to series Bicarpellatae, then proceed with the following key for family diagnosis :

1. Flowers regular (actinomorphic) :

2. Small herbs ; leaves radical and generally narrow. veins parallel .....Family : **Plantaginaceae**

2. Herbs, shrubs or trees ; leaves not radical , veins not parallel :

3. Leaves opposite or whorled (**Gentianales**) :

4. Stamens reduced to 2 ; placentation axile; ovules attached to the inner angle near the base or apex.....Family : **Oleaceae**

4. Stamens 4 or 5 : placentation parietal ,axile or basal :

5. Carpels mostly free, united in the stigmatic disc; latex and corona present :

6. Pollen grains free; leaves mostly exstipulate
(stipulate in *Tabernaemontana)*.....Family : **Apocynaceae**

6. Pollen grains sticky, forming pollinia; (except *Cryptostegia*) leaves
stipulate.....Family : **Asclepiadaceae**

5. Carpels completely united; corona and latex absent.....Family : **Gentianaceae**

3. Leaves alternate or sub-opposite in the apical regions (**Polemoniales**) :

7. Ovary oblique; placenta swollen with numerous ovules .....Family : **Solanaceae**

7. Ovary not oblique; placenta not swollen; ovules 2 in each loculus :

8. Plants with hispid hairs; fruit a drupe or four nutlets.....Family : **Boraginaceae**

8. Plants generally not hairy (if so, hairs soft);
fruit a capsule .....Family : **Convolvulaceae**

1. Flowers irregular (zygomorphic) :

9. Stem circular; each loculus with more than 2 ovules (**Personales**) :

10. Bracts persistent, often spiny; funicles persistent.....Family : **Acanthaceae**

10. Bracts not conspicuous; funicles not persistent :

11. Seeds winged ; leaves compound.....Family : **Bignoniaceae**

11. Seeds not winged ; leaves generally simple :

12. Disc below the ovary conspicuous.....Family : **Scrophulariaceae**

12. Disc below the ovary absent :

13.Ovary bi- or tetralocular.....Family : **Pedaliaceae**

13.Ovary unilocular.....Family : **Gesneriaceae**

9.Stem generally tetragonous; each loculus with only 2 ovules (**Lamiales)** :

14. Aromatic herbs; ovary at flowering time lobed; style generally
gynobasic, 2-cleft at the apex .....Family: **Lamiaceae (=Labiatae)**

14. Non -aromatic plants; ovary at flowering time not lobed;
style normal, entire at the apex .....Family : **Verbenaceae**

Sub-class- 3 : Monochlamydeae

Flowers without perianth i.e. flowers naked or the perianth if present then not distinguishable into calyx and corolla. Thus, if your specimen is a dicot. and then keys down to this sub-class , proceed with the following key for diagnosis of the family :

1. Plants either parasitic or unisexual:
   2. Plants non-parasitic ; unisexual (**Unisexuales**) or bisexual and naked (**Micrembryae**):
      3. Aromatic herbs or shrubs ; flowers bisexual, inconspicuous; perianth absent.....Family : **Piperaceae**
      3. Herbs, shrubs, or trees, usually non-aromatic ; flowers unisexual; perianth present:
         4. Pistil tricarpellary ; latex present.....Family : **Euphorbiaceae**
         4. Pistil not tricarpellary, if so then latex absent :
            5.Carpels 2-4; ovules many; seeds plumed.....Family : **Salicaceae**
            5. Carpel 1-2 (rarely 3); ovules 1 or 2; seeds not plumed :
               6. Male and female flowers on the same tree ; inflorescence a catkin ; leaves opposite or whorled ,compound or reduced :
                  7. Carpels 2; ovules 2, parietal ; leaves whorled , reduced to scales, non-aromatic; fruit a samara in woody cones.....Family : **Casuarinaceae**
                  7. Carpels 2-3; ovule 1, basal; leaves usually opposite,often pinnately compound , aromatic, gland-dotted; fruit a nut with complex lobed and folded cotyledons.....Family : **Juglandaceae**
               6. Male and female plants separate ; inflorescence various ; leaves simple,alternate :
                  8. Carpel 1; ovary naked ; ovule 1, basal ; plants often with rough or stinging hairs.....Family : **Urticaceae**
                  8. Carpels 2 (often one carpel aborting); ovary enveloped by perianth; ovule 1, apical ; plants often with milky sap, without stinging hair:
                     9. Usually woody plants with milky sap; perianth in male flowers of 2-6 free segments ; fruit usually a syncarp.....Family : **Moraceae**
                     9.Herbs without milky sap; perianth in male flowers of 5 united segments; fruit an achene.....Family : **Cannabaceae**
   2.Plants parasitic (**Achlamydosporeae**) :
      10. Plants stem parasites; perianth often large and showy.....Family : **Loranthaceae**
      10. Plants root parasites; flowers small, not very showy.....Family : **Santalaceae**

1. Plants neither parasitic nor unisexual :

11. Gynoecium of 2 or more united carpels, (**Curvembryae**) :

12. Stipules forming an ochrea.....Family : **Polygonaceae**

12. Ochrea absent :

13. Bracts conspicuous, variously coloured; fruit enclosed in perianth..... Family : **Nyctaginaceae**

13. Bracts not conspicuous :

14. Stamens 5 or fewer:

15. Perianth herbaceous, soft..... Family: **Chenopodiaceae**

15. Perianth scarious, stiff .....Family : **Amaranthaceae**

14. Stamens (including staminodes) 8-10:

16. Gynoecium of 1-5 carpels; style 1..... Family: **Phytolaccaceae**

16. Gynoecium bicarpellary, unilocular; styles 2.....Family**: Illecebraceae**

11. Gynoecium monocarpellary ; stigma capitate (**Daphnales**)or often 3-cleft :

17. Evergreen trees or shrubs, more or less aromatic; leaf buds usually large.....Family : **Lauraceae**

17. Shrubs, non-aromatic; leaf buds normal.....Family : **Thymelaeaceae**

Class : Monocotyledonae

If your specimen matches the characters of this class (given in the starting couplet of the key), then proceed as follows in order to find out the family to which your specimen belongs :

1. Small, floating aquatic plants ; no distinction into stem and leaves.....Family : **Lemnaceae**

1. Plants rooted, rarely floating aquatics ; plant body differentiated into stem and leaves :

2. Inner perianth petaloid ; gynoecium syncarpous :

3. Ovary inferior :

4. Submerged or floating aquatics.....Family: **Hydrocharitaceae**

4. Plants terrestrial:

5. Flowers actinomorphic or weakly zygomorphic ; stamens 6,3 or rarely many, very rarely 5,2 or 1 :

6. Climbing shrub; leaves heart-shaped or very divided , net- veined; flowers unisexual.....Family : **Dioscoreaceae**

6. Plants not climbing ; leaves open-veined; flowers by bisexual:

7. Stamens 3, staminodia absent ; leaves often sharply folded, their base overlapping ; style-branches often divided.....Family : **Iridaceae**

7. Stamens 6 or 3 ; staminodia present ; leaves usually not as above ; style-branches not divided :

8. Flowers in a spike ; leaves fleshy or leathery, thick, spine-tipped .....Family : **Agavaceae**

8. Flowers in umbels or solitary ; leaves usually not fleshy.....Family : **Amaryllidaceae**

5. Flowers strongly zygomorphic or asymmetric ; stamens 5,2 or 1 (very rarely 6) ,when stamen 1, it is united with the style to form a column :

9. Fertile stamens 2 or 1 , united with the style to form a column , pollen usually borne in coherent masses (pollinia) ; leaf veins when visible, all parallel to margins.....Family : **Orchidaceae**

9. Fertile stamens 5 or 1(rarely 6), not united with the style ; pollen granular ; leaf with a distinct midrib more or less parallel to margins, the secondary veins parallel to each other, running at an angle from midrib to margins :

10. Fertile stamens 5 or rarely 6.....Family : **Musaceae**

10. Fertile stamen 1, petal-like ; staminodes 5 :

11. Fertile stamen with a thread-like filament and wider anther with 2 pollen bearing lobes ; not petal-like.....Family : **Zingiberaceae**

11. Fertile stamen in part petal-like and with only one pollen bearing lobe.....Family : **Cannaceae**

3. Ovary superior (**Coronareae**)

12. Aquatic herbs.....Family : **Pontederiaceae**

12. Terrestrial plants :

13. Perianth usually petal-like ; staminodes absent.....Family : ***Liliaceae**

13. Perianth strongly differentiated into calyx-like outer whorl and a corolla-like inner whorl ; staminodes usually present.....Family : **Commelinaceae**

2. Inner perianth either not petaloid or if petaloid then the carpels are free :

14. Perianth small, sepaloid, stiff (**Calycinae**) :

15. Scandant shrubs or trees.....Family : **Arecaceae (=Palmae)**

15. Usually perennial herbs, rarely annuals :

16. Flowers bisexual; fruit (capsule) enclosed in the perianth.....Family : **Juncaceae**

16. Flowers unisexual ; capsule free.....Family : **Eriocaulaceae**

14. Perianth 0 or reduced , if not reduced then with free carpels :

17. Flowers enclosed in dry or chaffy bracts called glumes (**Glumiflorae**):

18. Leaves usually arranged on 3 sides of the stem; stems triangular ; leaf sheaths closed (not split on any side) ; style 1 with 2 or 3 papillose stigmas.....Family : **Cyperaceae**

18. Leaves alternate in 2 ranks on the stem ; stems circular ; leaf sheaths open (split on one side); styles generally 2, feathery.....Family : **Poaceae (=Gramineae)**

17. Flowers not enclosed in glumes :

19. Carpels united

20. Marshy perennials; flowering axis not enclosed in a spathe.....Family : **Typhaceae**

20. Plants terrestrial ; flowering axis enclosed in a spathe:

21. Trees or shrubs with aerial roots and with palm-like habit.....Family : **Pandanaceae**

21. Generally perennial herbs ; leaves radical:

22. Flowers unisexual; spadix prolonged above the flowers and enclosed in a spathe..... Family : **Araceae**

22. Flowers bisexual; spadix neither prolonged nor enclosed in a spathe ..... Family: **Acoraceae**

19. Carpels free (**Apocarpeae**):

23. Flowers generally conspicuous ; both series of perianth generally present and the inner generally coloured:

24. Perianth segments 3-6; stamens 3-6; marshy plant:

25. Flowers bisexual in terminal pyramidal panicles.....Family : **Alismataceae**

25. Flowers unisexual, in globose heads.....Family: **Sparganiaceae**

24. Perianth segments 4; stamens4;

submerged aquatics.....Family: **Potamogetonaceae**

23. Flowers inconspicuous, usually green ; perianth 0, tubular

or 3-4 segments.....Family : **Najadaceae**

*The family **Liliaceae** has now been segregated into several families

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HISTOLOGICAL AND BIOCHEMICAL CHANGES IN SUSCEPTIBLE AND RESISTANT BARLEY LEAVES DURING EARLY STAGES OF INFECTION BY *PYRENOPHORA TERES*

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Abstract

SEM studies revealed direct epidermal invasion of barley by *Pyrenophora teres*. However, there was delay and decline in penetration attempts in case of the resistant cultivar as compared to the susceptible variety. Moreover, the damage to cell membrane systems was less severe and occurred much later in the resistant host. Similarly, the two varieties differed in response to infection by the pathogen, histochemically and biochemically. Higher concentrations of proteins and lipids were recorded in the infected susceptible host at 24 and 48 hpi (hours post inoculation), whereas, greater amounts of carbohydrates were found in the healthy resistant and infected susceptible hosts. Accumulation of phenolic compounds was faster in the resistant variety in response to infection by the pathogen, which increased with progress of infection from 24 to 72 h. Similarly, the infected resistant variety recorded higher lignins and callose. Interestingly, the resistant barley leaves developed numerous papillae (which contain mainly callose) as compared to the barley resistant and healthy/infected susceptible leaves. It is suggested that phenolics, lignins, callose and papillae (containing mainly callose) produced by the resistant barley host, in response to infection by *Pyrenophora teres* at an early stage, play an important role in resisting the growth of the fungus. In the susceptible cultivar these responses being too weak and too late, do not limit the fungal development.

Key words: *Pyrenophora teres*, infection, Barley, biochemical, histological.

INTRODUCTION

Pyrenophora teres is the causal organism of net blotch disease of barley. Both the chemical control of the disease and breeding of resistant varieties generally require a detailed knowledge of the host-pathogen interaction. The host-parasite interaction, which is not fully compatible, involves a degree of resistance, that may occur at any stage of interaction and it may be triggered by several biotic and abiotic factors. An earlier study[19] determined.

some characteristics of the resistance mechanisms suggested that the accumulation of materials might be elicited by a fungal metabolite. The later studies[16,23] focussed on the role of contact phenomena and toxins in the pathogenesis of the disease.

The studies conducted at an early stage of infection seem more crucial as it is at this time when the pathogen comes in contact with the host possibly eliciting various changes. This paper deals with the biochemical, structural and ultrastructural studies conducted using susceptible and resistant varieties of barley cultivars at an interval of 24, 48 and 72 hours post inoculation (hpi) with *P. teres*.

MATERIALS AND METHODS

Healthy fully expanded, uninjured barley leaves (4-5 leaf stage) of susceptible (DL-70) and moderately resistant (DL-170) (obtained from Division of Plant Breeding, Punjab Agricultural University, Ludhiana, India) were used. Twenty-day old monosporic isolates of *P. teres* (obtained

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from Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India) cultured on PDA were used for experimentation. Excised leaves were placed in moist petriplate chambers with both ends between folds of cotton and inoculated with spore suspension drops (0.05 ml of 1 x 10^6 spores/ml). They were then incubated with un-inoculated controls for 24, 48 or 72 h at 28±1°C.

Microscopical Tests

The susceptible and resistant host leaves were cut into small pieces, fixed and cleared in Clarke's fixative[17] and then rehydrated through alcohol series to water before staining. The stained samples were mounted in glycerine for observation using Carl-Zeiss/Nikkon microscopes with in-built light and UV sources. For fluorescent stains epi-illumination was used with appropriate exciter and barrier filters. All observations were based on 100 spore counts from five replicates.

Qualitative histochemical staining

Total Proteins: The tissue samples were stained for the total proteins using Mercuric Bromophenol Blue[10] and Coomassie Brilliant Blue[14].

Total Lipids: Nile Blue sulphate[17], Sudan Black B[18] and Rhodamine B[17] standard methods were used for the histological evaluation of the total lipids.

Lignins: The tissues were examined for histological reactions using Toludine Blue O[15] and Phloroglucinol-HCl[17] for the lignins.

Poly-phenols: Diazotised Sulphanilic acid[17] was used for the staining of tissues for poly-phenols.

Callose: Fluorescent staining reaction of the leaf tissue for callose was observed using Aniline blue[12] at 365 nm exciter filter and 420 nm barrier filters.

Tannins: Exposure to 1% (w/w) Ferric chloride in 0.1N HCl[4] resulted in the staining of the tissues for tannins.

Quantitative estimations

Healthy and infected barley leaves of the two varieties were dried in the oven at 65±1°C to a constant weight. Spores also were dried in a similar manner for the quantitative analysis. Various constituents were analysed from 0.1 g dried tissue in six replicates. The results were reported as mg/100 mg dry weight tissue.

Lipids: Lipids were extracted by placing relevant tissues in a mixture of chloroform-methanol (2:1 v/v) for 24 h. The solvent was decanted, evaporated and dried for one h at 100± 1°C as in the method of Angra-Sharma & Sharma[4].

Total Sugars and Starch: Total Sugars and starch were determined by the method of Dubois *et al.*[13]. The sugars were extracted from the relevant tissues/spores using 80% ethanol. To each 2 ml aliquot 0.05 ml 80% (w/v) phenol solution and 5 ml concentrated H_2SO_4 were added. The reaction mixture was then allowed to stand for 20 min at 25°C and the optical density measured at 490 nm. Glucose was used as standard.

The starch content of the leaf tissue/spores was determined by extracting the residues, left after ethanolic extraction of sugars, with dilute perchloric acid. The extract was processed in the same way as in the case of total sugars.

Phenolic Compounds: Phenolic compounds were extracted using 95% ethanol. One ml of ethanol extract was diluted to twice its volume with distilled water to this was added 1 ml of 0.5 N HCl, 1 ml Arnow"s reagent, 10 ml distilled water and 2 ml NaOH. After 30 sec of addition of the alkali, pink colour developed was estimated at 515 nm[21]. Chlorogenic acid was used as standard.

Proteins: Proteins were extracted from the tissue in 0.1 M phosphate buffer (pH 7.5) using chilled pestle mortar. The slurry was filtered through cheesecloth and the filtrate centrifuged at 2,000 rpm for 15 min. To 1 ml of centrifugate 5 ml of Coomassie Brilliant Blue reagent and 4 ml of buffer were added and the colour developed for 30 min. Spectrophotometric observations were made at 595 nm using BSA as standard[6].

Scanning Electron Microscopy

1. **Fixation:** Leaf tissues cut into small pieces and fixed in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h in cold. This was followed by four washes of 10 min each. The material was post fixed in 2% Osmiun tetraoxide and kept overnight at 4°C. the tissues were then washed again with the cacodylate buffer.
2. **Dehydration:** After fixation the tissues were dehydrated in acetone-water series for 20 min. This was followed by 95% acetone at room temperature for 20 min. The material was then shifted to 100% acetone at room temperature. Three changes were given every 20 min. with absolute acetone.
3. **Mounting of Samples for SEM:** After dehydration the material was mounted on steel stubs with aluminium paint and sputter coated with gold using Hitachi IB[3] ion coater (vacuum was maintained at 0.08 Torr and the ionisation done at 6 mA for 4 min) to get a gold deposition of 200°A on the samples. The coated samples were then scanned on a Hitachi 405 S SEM at 25 kV (operating filament current) and the results photographed on 100 ASA black and white ORWO film[4].

RESULTS

Qualitative Studies:

The observations of histological staining at 24, 48 or 72 h for susceptible and resistant barley leaves infected with *P. teres* are presented in Table 1.

Proteins: Staining with Coomassie blue R 250 and mercuric bromophenol blue indicated presence of proteins (blue) in the susceptible and resistant host tissues, germ tubes, area around infection sites and infection structures (appressoria, infection peg, colonisation hyphae). There were differences, however, in the two tissues with regard to the amount, as reflected by the stain uptake by the tissues. The stain intensity was higher in the susceptible host and the blue colour uptake by the tissue increased as the infection progressed from 24 to 72 h. No marked change in the intensity of blue colour was observed in the resistant host tissue for the three incubation periods studied. It was interesting to note that the fungal spores on the resistant host did not stain positive for proteins at 24 hpi unlike the spores on the susceptible variety. Subsequently, however, with increased incubation period (48 to 72 h) the spores on the resistant variety exhibited presence of proteins.

Table 1: Histological stain reaction of *Pyrenophora teres* on susceptible (S) and resistant (R) barley leaves.

Compo-nent	Stain/Histological test	Time of Incuba-tion	Host tissue		Spores		Germ tubes		Infection Structures and/or infection peg	
			S	R	S	R	S	R	S	R
Proteins	Coomaissie Blue R 250	24 hrs	+	+	-	-	+	+	+	+
		48 hrs	++	+	++	+	++	+	++	+
		72 hrs	+++	+	++	+	++	+	+++	+
	Mercuric Bromophenol Blue	24 hrs	+	+	+	-	+	+	+	+
		48 hrs	++	+	++	+	++	+	++	+
		72 hrs	+++	+	++	+	++	+	+++	+
Lipids	Nile Blue Sulfate	24 hrs	+	+	+	-	+	+	+	+
		48 hrs	+++	+	++	+	++	+	+++	+
		72 hrs	++++	++	+++	+	+++	+	++++	+
	Sudan Black B	24 hrs	+	+	+	+	+	+	+	+
		48 hrs	++	+	++	+	++	+	++	+
		72 hrs	+++	++	++	+	++	+	+++	++
	Rhodamine B	24 hrs	+	+	-	-	-	-	-	-
		48 hrs	+	+	-	-	-	-	-	-
		72 hrs	+	+	-	-	-	-	-	-
Lignin	Toludine Blue O	24 hrs	+	+	+	+	+	+	+	+
		48 hrs	++	++	+	+	+	+	+	+
		72 hrs	++	+++	+	+++	++	+++	++	++++
	Phluroglucinol HCl	24 hrs	-	-	-	-	-	-	-	-
		48 hrs	-	-	-	-	-	-	-	-
		72 hrs	-	-	-	-	-	-	-	-
Phenolics (a) Phenols (b) Tannins	Diazotised Sulfanilic acid	24 hrs	+	++	+	+	+	+	+	++
		48 hrs	++	+++	+	+	+	++	++	+++
		72 hrs	++	++++	+	+	+	++	+	++++
	$FeCl_3$	24 hrs	-	-	-	-	-	-	-	-
		48 hrs	-	-	-	-	-	-	-	-
		72 hrs	-	-	-	-	-	-	-	-
Callose	Analine Blue	24 hrs	+	+	-	-	-	-	+	+
		48 hrs	++	++	-	-	-	-	+	+++
		72 hrs	+++	+++	-	-	-	-	++	++++

+ denotes a positive stain reaction and ++/+++/++++ indicate the higher degree of stain intensity (+ being the lowest and ++++ the highest intensity), similarly - indicates a negative stain reaction. R = Resistance host tissue and S = susceptible host tissue. Observations reported for five replicates.

Lipids: Both the resistant and susceptible hosts indicated presence of lipids and lipoproteins because of blackening of tissues with Sudan Black B. The two tissues contained free fatty acids and phospholipids as well (Blue stain reaction with Nile Blue sulphate). The infecting fungus also exhibited presence of lipids, lipoproteins, free fatty acids, and the phospholipids. Although both the resistant and the susceptible host tissues contained various lipid components yet the

susceptible variety had higher amounts of these constituents as reflected in the higher stain intensity for the incubation periods studied. Total lipids were observed in the resistant and the susceptible host tissues only (white pink fluorescence with Rhodamine B). No fluorescence was observed at the infection site or the fungus.

Lignins: Toludine blue O gave a positive blue green colour for the two types of host tissues, fungal spores, germ tubes, and all the infection structures implying presence of lignins. Comparatively, the resistant host always showed higher amounts of lignins than the susceptible variety. Moreover, no appreciable change in the stain intensity was observed in the susceptible tissue with the progress of infection from 24 to 72 h. Phloroglucinol-HCl, however, gave a negative stain reaction.

Tannins and Phenolics: Tannins were absent as neither the host tissue nor the fungal structures stained with $FeCl_3$.

Yellow to light orange colour with diazotised sulfanilic acid indicated presence of poly-phenols. The stain intensity was higher in the resistant host and the associated infection structures, and increased as the infection progressed to 72 h. No appreciable change in the stain uptake was observed in the susceptible host from 24 to 72 hpi.

Callose: Callose deposition was evident in the cell walls of the resistant and susceptible tissue around the infection sites (Yellow fluorescence with aniline blue) and the infection structures (appressoria, penetration peg; colonisation hyphae). The amount of callose was, however, higher in the resistant tissue, as reflected in the increased stain intensity. And interestingly, as the infection progressed beyond 24 h, the resistant host developed papillae like structures near the infection site, which also contained callose. No such pehnomena could be recorded for the susceptible variety.

Quantitative Studies:

Quantitative estimations for lipids, proteins, carbohydrates and phenolics along with the percent change in the infected resistant and susceptible compared to their respective healthy controls are recorded in Table 2.

The lipid content was much higher in the susceptible variety for both healthy and infected host tissues. The infected susceptible variety recorded an increase in lipids as the infection progressed to 48 h (164.7%). Beyond 48 hpi the lipids declined (23%), as compared to its healthy control. Interestingly, the infected resistant host showed a gradual decline in the lipids with the increase in incubation from 24 to 72 h.

Unlike lipids, higher amounts of proteins were observed in the healthy resistant tissue. However, as the infection progressed, there was a decline in the protein content in the resistant cultivar. Although the infected susceptible variety recorded an initial decline in proteins up to 48 hpi, incubation beyond 48 hpi resulted in increased protein values. It is appreciable that the qualitative and quantitative observations tally well for the lipids and proteins, for the two host varieties.

Comparison of change of the infected resistant and susceptible host tissues revealed interesting differences for total sugars, starch and total carbohydrates. Total sugars, which were higher initially (24 hpi) in the susceptible infected host declined as the infection was allowed to progress till 72 h. The resistant infected variety recorded a gradual decline in total sugars with incubation. Interestingly, the resistant healthy tissue had higher total sugars than the healthy susceptible

variety. Similarly, the starch content was higher in the infected resistant tissue as compared to the healthy susceptible. After infection the starch content, which was higher in the susceptible host, declined with the progress of infection. Total carbohydrates recorded the patterns as observed in case of total sugars for both the varieties.

Table 2: Quantitative estimation of lipids, proteins, total sugars, starch, total arbohydrates and phenolics in leaf tissue of *Pyrenophora teres* infected barley.

Leaf tissue type	Cultivar type	Lipids	Proteins	Total Sugars	Starch	Total Carbohyd rate	Phenolics
		mg/100 mg dry tissue weight					
24 h old healthy	Susceptible	17.00	11.00	1.80	1.20	3.00	12.20
	Resistant	15.00	6.00	5.55	5.88	11.43	5.60
48 h old healthy	Susceptible	17.00	21.90	8.40	1.00	9.40	4.40
	Resistant	8.00	24.60	9.15	7.40	16.55	3.00
72 h old healthy	Susceptible	13.00	5.90	8.70	2.85	11.55	8.10
	Resistant	19.00	25.00	3.30	9.40	12.70	6.50
24 h old infected	Susceptible	52.00 (+205.88)	11.40 (+3.63)	10.50 (+483.33)	7.70 (+541.67)	18.20 (+506.67)	7.80 (-36.07)
	Resistant	26.00 (+73.33)	9.30 (+55.00)	2.70 (-51.35)	4.55 (-22.62)	7.25 (-36.57)	6.65 (+18.75)
48 h old infected	Susceptible	45.0 (+164.71)	10.80 (-73.99)	10.80 (+28.57)	3.30 (+230.00)	14.10 (+50.00)	4.80 (+9.09)
	Resistant	13.00 (+62.50)	6.40 (-73.99)	4.95 (-45.90)	8.20 (+10.81)	13.15 (-20.54)	6.80 (+126.66)
72 h old infected	Susceptible	10.00 (-23.09)	28.80 (+368.14)	4.35 (-50.00)	5.50 (+92.98)	9.85 (-14.72)	2.80 (-65.43)
	Resistant	11.00 (-42.11)	1.50 (-94.00)	3.15 (-4.55)	6.70 (-28.72)	9.85 (-22.44)	5.40 (-16.92)
Extract of *Pyrenophora teres*		10.00	8.80	3.00	1.40	4.40	3.00

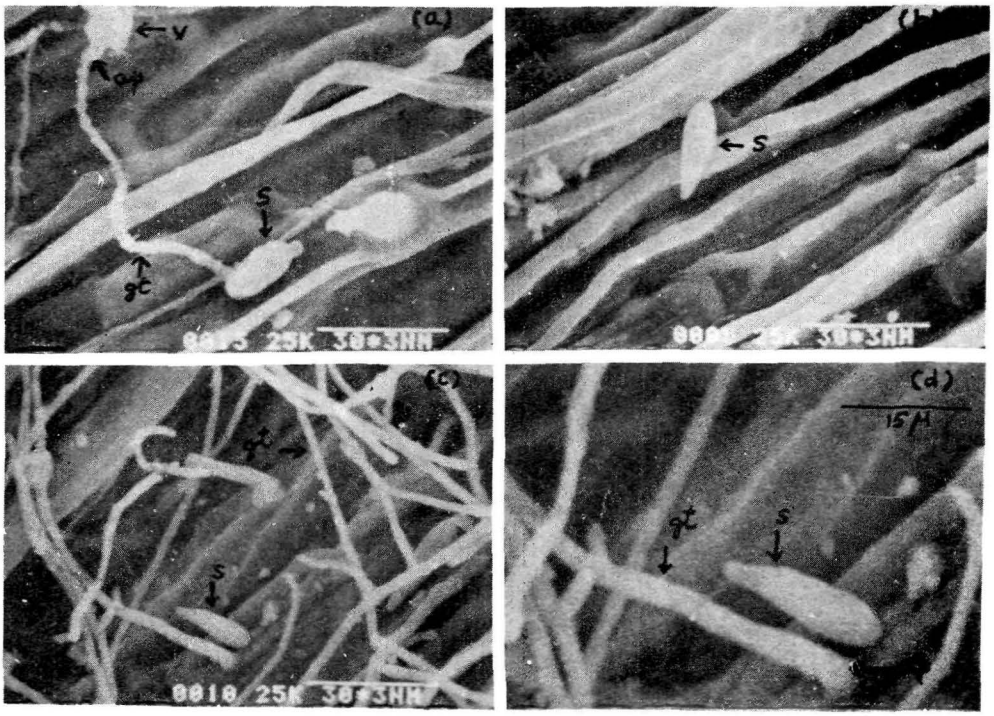
Value in parenthesis indicate the change in the chemical constituent of the infected leaf over the healthy leaf constituent and + or - indicates an increase or decrease. **The results are mean values from five replicates.**

Interestingly, although the phenolic compounds were always higher in the uninfected susceptible host than the healthy resistant variety, infecting the tissue with *P. teres* changed the levels of phenolic compounds. The infection of the resistant host resulted in greater amounts of phenolics produced by it, whereas, the phenolics declined gradually in the infected susceptible host.

Scanning Electron Microscopy:

The comparison of infection stages of *P. teres* on the two hosts, as evident from SEM studies, revealed some differences. The penetration process was mainly direct and was completed by 24 hpi on the susceptible variety (Plate 1a). On the resistant variety 30% spores had not even germinated up to 24 h incubation (Plate 1b). At 48 and 72 hpi almost 90% spores had germinated on the resistant variety but very long germ tubes were observed compared to those on the susceptible variety. Therefore, as lesser number of appressoria were formed on the

resistant variety hence the penetration attempts by the fungus on the host also too were low (Plate 1c and d).



Explanation to Plate I :

SEM of susceptible and resistant barley leaves infected by *Pyrenophora teres*:

a. A conidium showing germination, terminal appressorium and penetration on surface of a susceptible barley leaf (24 hpi, bar length 30 microns)

b. Ungerminated conidium on the resistant leaf surface (24 hpi; bar length 30 micron).

c. Long and branched germ tube on the leaf surface of the resistant cultivar (48 hpi; bar length 30 micron).

d. Enlarged view of two penetration attempts by the fungus on surface of a less compatible host cultivar (48 hpi; bar length of 'c' as 15 micron for this micrograph).
S = conidium, Ap= appressorium, Gt= germ tube, V- vesicle

DISCUSSION

SEM revealed some interesting differences in the response of the susceptible and resistant cultivars of barley towards *P. teres,* which causes net blotch disease. While the susceptible cultivar supported development of the pathogen resulting in the formation of appressoria, infection peg, and colonisation hyphae by 24 hpi, the resistant variety afforded resistance to the germination of the fungal spores by this time. These observations are in accordance with the earlier study[11] wherein formation of similar infection structures on the susceptible barley host was observed.

At 48 hpi, there was a well-oriented growth of germ tubes of *P. teres* on the susceptible variety. By this time, however, only a few spores could germinate on the resistant cultivar and the germ tubes were too long and erratic unlike the susceptible variety. By 72 hpi, the cell wall collapse was total in the susceptible infected host with severe plasmolysis. However, the damage to the cell wall system was much less and occurred later in the resistant barley host. Similar observations were made for *Cochliobolus sativus* infecting susceptible and resistant wheat[9].

Biochemical analysis of various constituents, as influenced by the host-pathogen interaction, showed higher protein content in the susceptible host, which increased as the infection progressed from 24 to 72 h. It was interesting, however, to note that the protein content declined in the resistant variety. The pathogen stress seems to be causing the relevant changes in the protein synthesis of the host, by producing greater amounts of pathogenesis related proteins for infection to progress in the susceptible variety, as in the *Erysiphe graminis*-barley complex[7].

Total sugars, starch and carbohydrates were much higher in the healthy resistant barley leaves, as compared to the susceptible variety. Likewise, high total sugars have been recorded for healthy wheat cultivars resistant to *D. hawaiiensis*[13]. The wheat varieties resistant to *Helminthosporium* blight are known to exude sugars in greater quantities[5]. It may be that higher sugars, as observed in the resistant variety exudates, cause plasmolysis of the germinated spores resulting in fewer/delayed infections as observed for H. *maydis*[24] and *H. carbonum*[3].

The carbohydrates in the susceptible barley host increased only after the infection had progressed beyond 24 h, whereas, the resistant host recorded a decline. Carbohydrates serve as a continuous energy source for the growing pathogen and are known to accumulate at the infection sites in barley susceptible to *D. teres*[2]. The changes in the carbohydrate levels, as observed in this study, may be attributed to the host-pathogen interaction, the host replenishing the sequestered sugars and the pathogen influencing the host's carbohydrate synthesizing system.

Lipids, lipoproteins, free fatty acids and phospho-lipids have been observed in the resistant and susceptible barley hosts. Total lipids were higher in both the healthy and the infected susceptible cultivars. Higher degree of fatty acid unsaturation has been reported for the citrus leaves susceptible to citrus bacterial canker than the resistant ones[20]. The lipid content decreased in the susceptible barley leaves as the infection progressed to 72 h. This decline as recorded in this study compares well with the lipid reduction during pustule eruption in the rust affected *Vicia faba* leaves[8]. Lipid reduction seems to be either because of nutrient depletion or the increasing demand for respiratory energy for the growing pathogen[29].

Phenolics have long been associated with passive and active defence response of plants[25, 27]. Presence of high amounts of poly-phenols at the infection site of the resistant host, observed as early as 24 h, is defence against the infecting pathogen. The phenolics increased with the

increase in infection till 48 h. Similar response for phenolics, on infection, has been recorded in the resistant maize leaves infected with *H. maydis*[4]. Lower phenolic compounds as observed in the susceptible barley host may be due to the reduced matabolic activity of the host, in response to infection, which results in reduced phenolic compounds being synthesised.

Lignins were identified at the infection sites in the two cultivars studied. The infected resistant barley host had greater amounts than the susceptible variety, which are comparable to the leaf rust fungus infecting maize[4] and the leaf rust fungus infecting wheat plants[26]. Lignification of the cell walls is an intrinsic mechanism of the host to resist penetration of the infecting fungus[32], also the host may lignify the fungal hyphae. Lignification response of the host is an active mechanism of resistance, towards the invading pathogen, and involves induction of enzymes in the lignin biosynthesis pathway[26,28].

Strong aniline blue fluorescence at the infection site in case of resistant barley host indicates presence of callose. Similar observations, with respect to callose have been made for leaf rust fungus in wheat, by Southerton and Deverall[30] and *H. maydis* infecting maize[4]. The resistant infected barley host developed papillae, which contained callose deposits. Papilae formation, by host in response to infection, and the increase in size of papillae significantly affording resistance to the fungal attack have been reported[1]. Jorgensen and co-workers[22] observed papillae formation in barley leaves infected with *Rhyncosporium secalis.*

It may, therefore, be concluded that the resistance seems related to the constitutive proteins, lipids, total sugars, and starch and total carbohydrates. Further, on infection the pathogen stress appears to contribute towards changes in these constituents different in the resistant and susceptible cultivars. Higher amounts of lignins, phenolics and callose in the resistant variety, in response to infection, accompanied with papillae formation act as barriers towards development of the fungus. It appears that various chemical constituents produced by the host are a multi-pronged effort of the host to prevent development of the pathogen.

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CURRENT STATUS OF INDIAN BRUCHIDAE

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Abstract

A complete up–to–date list of Indian Bruchidae has been given, with keys to subfamilies and genera and first and subsequent important references for each genus and species. Details of the localities and the host plants have also been included. The report includes 96 species referable to 10 genera under 3 sub families.

Key words : Bruchidae, keys, host plants, localities, India.

INTRODUCTION

Bruchidae is a small family of Coleoptera with about two thousand species known from all over the world. The family belongs to the super family Chrysomeloidea which also includes Cerambycidae and Chrysomelidae and, is characterized by pseudotetramerous tarsi. The members of the family Bruchidae are seed borers and attack their largely wild leguminous hosts in the field and the edible legumes in the stores. The species attacking the green pods of wild legumes, are usually univoltine, host specific and have long developmental period as well as adult life span. These are generally referred to as field bruchids. About a dozen species, however, are associated with stored edible legumes. They are multibrooded, usually have a long range of host seeds and short developmental period as well as adult life span. They are commonly designated as store bruchids.

The Indian Bruchidae remained almost neglected till work on their taxonomy was started in this laboratory during 1964. Before this only twelve species had been reported from this country (Mukerjii and Chatterjii, 1951). Thereafter, sampling of Indian Bruchidae resulted in the collection of as many as 69 species under 9 genera including 46 new species and 16 first records (Arora, 1977, 1979). A few more species have been added to the Indian list of Bruchidae by individual workers (Pajni & Gupta, 1975; Arora & Singal, 1977, 1979; Pajni & Singh, 1977; Singh, 1977, 1985; Singh & Saini, 1978, 1983; Singal, 1980, 1989; Singal & Pajni, 1986, 1990; Tewari & Pajni, 1995).

In order to increase the utility of this publication keys to the studied subfamilies and genera pertaining to Indian bruchid fauna have been included. In addition to first references of the genera and species, information has also been included on the host plants and localities of different species. The type material of most of the species is available in the Entomology section of the Department of Zoology, Panjab University, Chandigarh unless otherwise mentioned for other species.

FAMILY BRUCHIDAE STEPHANS

Bruchidae Stephans, Syst. Cat. Brit Insec. Baldwin & Cradock, London. 80,1829

Head small, broadest at level of eyes, somewhat retracted into prothorax; eyesemarginateshallowlyor deeply in front; antennae II–segmented, usually serrate or pectinate and rarely filiform; prothorax broad or bell–shaped, usually narrowed in front; hind legs generally

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thicker and longer than other two pairs; hind femora flat or channelled below, with carinae bearing a preapical single spine or a row of many spines; tarsi pseudotetramerous, each with 4th tarsomere covered by bilobed 3rd tarsomere.

Key to the Indian Subfamilies of Bruchidae

1. Hind femora very strongly thickened, each provided with a ventral median keel carrying a comb-like row of strong spines; hind tibiae strongly arched, ea ch produced into a sharp tooth at apex; eyes with emargination shallow; elytra with sharply punctate striae reaching apex ..... **Pachymerinae**

   Hind femora moderately thickened, ventrally flat, or channelled with one or both edges carinate, carinac with or without preapical spines, but never with a comb-like row of spines; hind tibiae straight or only slightly curved; eyes with emargination moderate to deep; some striae on elytra not reaching apex **2**

2. Hind tibiae without movable spurs; head with a strong constriction behind eyes; antennae serrate to pectinate; scutellum quadrangular ..... **Bruchinae**

   Hind tibiae with a pair of long apical movable spurs; head without a constriction behind eyes; antennae filiform; scutellum triangular ..... **Amblycerinae**

SUBFAMILY – PACHYMERINAE BRIDWELL

Pachymerinae Bridwell, Proc. Entomol. Soc. Wash., 31 : 141–160, 1929

Key to Genera of Subfamily Pachymerinae

Head elongate; temples strongly produced behind eyes; eyes finely facetted, emarginate for half of their length; pronotum with lateral margins depressed, its hind margin semi–circularly produced between elytra, unisinuate; scutellum minute; elytra flattened, with humeral callosities prominent, not much narrowed apically, not covering base of pygidium; parameres completely fused, forming a boat–shaped structure; ventral valve absent ..... ***Caryopemon***

Head short; temples not produced; eyes coarsely facetted, hardly emarginate in front, strongly projecting; pronotum flattened, not produced semi–circularly between elytra; scutellum large; elytra convex, eongate, narrowed and deflected apically, covering base of pygidium; parameres fused throughout except free tips; ventral valve present..... ***Caryedon***

GENUS - *CARYOPEMON* JEKEL

Caryopemon Jekel, Ins. Saunders, 1:25, 1855.

Caryopemon hieroglyphicus Jekel

Caryopemon hieroglyphicus Jekel, Ins. Saunders, I : 205. 1855.

Caryopemon hieroglyphicus : Singal, Ph.D, Thesis, Panjab University, Chandigarh, 1980.

Host : Not known

Locality : Shillong (Megh.)

GENUS – *CARYEDON* SCHONHERR

Caryedon **Schonherr**, Tab. Synopt. Fam.Curculion.,Isis-von oken, 2:1134,1823.

Caryedon acaciae (Gyll.)

Bruchus acaciae Gyllenhal, in Schonh., Gen. Curc., I: 97, 1833.

Pachymerus acaciae : Hoffmann, Faune de France, 44: 95, 1945.

Caryedon acaciae : Arora, Oriental Insects, Suppl. 7: 104 - 105, 1977.

Host : *Acacia auriculiformis* Cunn.

Locality : Ambala to Pipli Road (Har.), Delhi to Agra Road, Kathgodam & Lucknow (U.P.), Agra to Bharatpur Road (Raj.), Hazaribagh (Bih.).

Caryedon arori Singh & Saini

Caryedon arori Singh & Saini, J.ent. Res., 2(2): 131 - 132, 1978.

Host : *Cassia occidentalis* L.

Locality : Patiala (Pb.)

Type material in Zoology Museum, Punjabi University, Patiala, Punjab, India.

Caryedon bauhinidus Singal

Caryedon bauhinidus Singal, Entomon, 14 (1 & 2) : 127–132, 1989.

Host : *Bauhinia* sp.

Locality : Okha port (Guj.)

Type material in Entomology Deptt., CCS HAU, Hisar, Haryana, India (Singal's Collection)

Caryedon crineus Arora

Caryedon crineus Arora, Oriental Insects, Suppl. 7: 103 - 104, 1977.

Host : *Cassia occidentalis* L., *Cassia* sp., *Bauhinia variegata* L., *Acacia catechu* Willd.

Locality : Solan to Salogra Road (H.P.), Gurdaspur (Pb.), Jammu & Srinagar (J & K), Allahabad, Nanital & Meerut (U.P.), Baroda (Guj.), Aurangabad (Mah.), Madras & Coimbatore (T.N.), Mysore & Bangalore (Kar.), Bhubaneshwar, Cuttack & Bhanjanagar (Orisa), Burdwan, Malda & Behrampore (W. Ben.), Shillong (Megh.), Panjim (G.).

Caryedon indus (Motschoulsky)

Caryedon indus Motschoulsky, Etudes ent., 7 : 98, 1858.

Pachymerus ceylonicus Pic, Melanges L'Echange Linnenne, 5 : 25, 1924

Caryedon languidus Mukerjii & Chatterjee, Indian J. Ent., 13(1) : 1–29, 1951.

Pachymerus Indus : Vazirani, Jour. Bomb. nat. Hist. Soc., 72(3) : 752, 1974.

Caryedon Indus : Decelle, Ent. Scand. Suppl., 4 : 190, 1973–75

Caryedon Indus : Singal, Entomon 14 (1&2) : 127–132, 1989.

Host : Grass underneath *cassia fistua.*

Locality : Ranchi (Bih.)

Caryedon languidus (Gyll.)

Bruchus languidus Gyllenhal, in Schonh., Gen. Curc., 5 : 129, 1839.

Pachymerus languidus : Pic, Coleopterorum Catalogus, 55 : 8, 1913.

Caryedon languidus : Mukherji, Ramdas Menon and Chatterji, Proc. R. Ent. Soc. London, 26 : 103, 1957.

Caryedon languidus : Arora, Oriental Insects, Suppl. 7 : 101–102, 1977.

Host : *Cassia occidentalis* L., *Cassia tora* L., *Cassia auriculata* L.

Locality : Chandigarh (U.T.), Delhi (U.T.), Sundarnagar and Nahan (H.P.), Hardwar and Kathgodam (U.P.), Mount Abu (Raj.), Siliguri (W. Ben.), Simlipal forest (Ori.), Mysore, Brindawan and Bangalore (Kar.)

Caryedon lineatonota Arora

Caryedon lineatonota Arora, Oriental Insects, Suppl. 7 : 100 - 101, 1977.

Host : *Cassia tora* L., *Cassia* occidentalis L.

Locality : Andaman islands

Caryedon montanus Arora

Caryedon montanus Arora, Oriental Insects, Suppl.7 : 99 - 100, 1977.

Host : *Acacia pennata* Willd., *Cassia* sp.

Locality : Kalka to Kasauli hills, Mandi, Palampur & Baijnath (H.P.), Rishikesh, Nainital & Ranikhet (U.P.).

Caryedon opacus Arora

Caryedon opacus Arora, Oriental Insects, Suppl. 7: 103 - 104, 1977.

Host : *Acacia catechu* Willd., *Acacia farnesiana* Willd., *Acacia modesta* Wall.

Locality : Jammu (J&K), Pathankot (Pb.), Chandigarh (U.T.), Mandi (H.P.), Dehradun, Kanpur & Varanasi (U.P.), Hathikund & Ambala (Har.).

Caryedon patialensis Singh & Saini

Caryedon patialensis Singh & Saini, J.ent. Res., 2(2) : 132 - 134, 1978.

Host : *Cassia tora* L.

Locality : Patiala (Pb.)

Type material in Zoology Museum, Punjabi University, Patiala, Punjab, India.

Caryedon prosopidis Arora

Caryedon prosopidis Arora, Oriental Insects, Suppl. 7: 105, 114, 1977.

Host : *Prosopis juliflora* (Sw.)

Locality : Chandigarh (U.T.), Delhi (U.T.), Una (H.P.), Hissar (Har.), Ahmedabad & Porbander (Guj.), Coimbatore and Madurai (T.N.), Trivandrum (Ker.), Hyderabad (A.P.).

Caryedon punjabensis Pajni and Singh

Caryedon punjabensis Pajni and Singh, Oriental Insects, 11(2): 225-228,1977.

Host : *Cassia tora* L.

Locality : Chandigarh (U.T.), Patiala, Amritsar & Gurdaspur (Pb.), Ambala (Har.), Jammu & Udhampur (J&K).

Caryedon serratus (Oliver)

Pachymerus serratus Oliver, Encycl. Meth., 5: 199, 1790

Caryedon serratus : Arora, Oriental Insects, Suppl. 7: 99, 1977.

Host : *Tamarindus indica,* L., *Cassia fistula* L., *Acacia nilotica* Del., *Albizzia lebbek* Benth, *Cassia tora* L., *Pongamia pinnata* Pierre ex Prain, *Delonix regia* Rafin

Locality : Widely spread throughout India.

Caryedon tejpurensis Singal

Caryedon tejpurensis Singal, Entomon, 14 (1&2) : 127–138, 1989.

Host : *Cassia* sp.

Locality : Charduar forest Tejpur (Ass.)

Type material in Entomology Deptt., HAU, Hissar, Haryana, India (Singal's Collection)

SUBFAMILY – BRUCHINAE PIC

Bruchinae Pic, Coleopterorum catalogues Part 55. Bruchidae : 6, 1913

Key to the Indian Genera of Subfamily Bruchinae

1. Pronotum broad, toothed on lateral margins near middle; hind femur channelled below with a preapical tooth on outer margin; middle tibiae of male provided with a tooth or plate

preapically; antennae not serrate; saccus in male with thick pubescence only ..... ***Bruchus***

Pronotum narrowed anteriorly, with sides straight or convex, without lateral teeth; hind femur flat below or channelled with a tooth on inner margin, or same sometimes accompanied by a similar tooth on outer margin; middle tibiae without any pre-apical processes; antennae serrate or pectinate; saccus in male without thick pubescence but may bear plates, tubercles or both **2**

2. Base of pronotum with a pair of calli in middle; hind femur channelled below, with a preapical tooth on both outer and inner carinae; parameres totally free; saccus usually with a pair of dented plates; multivoltine species mostly attacking stored edible seeds..... ***Callosobruchus***

Base of pronotum without calli; hind femur without any teeth on outer edge; parameres variously fused at base; univoltine or bivoltine species attacking seeds of wild plants **3**

3. Hind femur without or with a minute preapical tooth on inner edge below, saccus variously clothed. **4**

Hind femur with a large preapical tooth on inner edge followed by one to three smaller teeth apically; saccus naked terminally, without denticles. ..... ***Specularius***

4. Hind femur sulcate below without a tooth on inner carina..... ***Sulcobruchus***

Hind femur flat, when sulcate with a tooth on inner carina.....5

5. Pronotum conical, strongly narrowed in front; hind femur flat, not canaliculate below. ..... ***Conicobruchus***

Pronotum subconical, not strongly narrowed in front; hind femur canaliculate below, with a preapical tooth on inner carina . . . . . ***Bruchidius***

GENUS – *BRUCHUS* LINNAEUS

Bruchus Linnaeus, Syst. Nat. 1(2) Ed. 12 : 604, 1767

Bruchus lentis Froel.

Bruchus lentis Froelich, Naturf., 28 : 57, 1799

Bruchus lentis : Arora, Oriental Insects, Suppl. 7 : 19–22, 1977

Host : Stored seeds of *Lens culinaris* Medik

Locality : Widely spread throughout India, attacks green pods in the field.

Bruchus pisorum L.

Bruchus pisorum Linnaeus, Systema Nature, I Ed. 10 : 356, 1758.

Bruchus salicis Scopoli, Entomologia Carniolical Vindobonae : 22, 1763.

Bruchus pisi Linnaeus, Systema Nature, 1(2) Ed. 12, Holmiae : 604, 1767.

Bruchus cyrciger Geoffroy, in Fourcroy : Entomologia, Parisiensis Sive Catalogus Insectorum 1 : 112, 1785.

Bruchus lunaris Rey, Famille de Bruchides, Echanges, 9 : 3, 1893.

Bruchus pisorum : Arora, Oriental insects, Suppl. 7 : 18-20, 1977.

Host : Stored *Pisum sativum* L., *Lathyrus odoratus* L.

Locality : Widely spread throughout India, attacks green pods in the field.

GENUS - *CALLOSOBRUCHUS* PIC

Bruchus (Callorobruchus) Pic, Rev. d'Ent., 22 : 6, 1902.

Callosobruchs analis (Fab.)

Bruchus analis Fabricius, Species Insectorum, 1, Hamburgi, 1:75, 1781.

***Callosobruchus analis*:** Southgate, Bull. Ent. Res., 48: 79, 1958.

***Callosobruchus* analis:** Arora, Oriental Insects, Suppl. 7: 27, 32-33, 1977.

Host : *Vigna radiata* (Roxb.), *V. aconitifolius* (Jaq.), *V. mungo* (L.), *V, sinensis* (L.) Savi ex Hassk., *Cicer arietinum* L., *Glycine max* Merr., *Cajanus cajan* (L.) Millsp., *Dolichos lablab* L.

Locality : Widely spread throughout India.

Callosobruchus anjaliae Singal & Pajni

Callosobruchus anjaliae Singal & Pajni, Bull. Entomologique De Pologne, 59:761- 782, 1990.

Host : *Desmodium* sp.

Locality : Izatnagar (U.P.)

Type material in Entomology Deptt., CCS HAU, Hisar, Haryana, India (Singal's Collection)

Callosobruchus antenniferus Singal & Pajni

Callosobruchus antenniferus Singal & Pajni, Bull. Entomologique De Pologne, 59: 761 - 782, 1990.

Host : Not known

Transitory Host : Grass

Locality : National Park Hazaribagh (Bih.)

Type material in Entomology Deptt., CCS HAU, Hisar, Haryana, India. (Singal's Collection).

Callosobruchus cajanis Arora

Callosobruchus cajanis Arora,Oriental Insects, Suppl. 7:24, 1997.

Host : *Cajanus cajan* (L.) Millsp.

Locality : Pusa area Delhi

Callosobruchus chinensis (L.)

Curculio chinenis Linnaeus, Systema Nature, 1 Ed. 10: 386, 1758.

Curculio scutellaris Schonh., Gen. Curc., 1:33, 1833

Pachymerus chinensis : Schilsky, Die Kafer Europas, 41 : 99, 1905

Callosobruchus chinesis : Hoffman, Faune de France, 44: 87, 1945.

***Callosobruchus chinesis*:** Arora, Oriental Insects, Suppl. 7: 23-24, 1977.

Host : *Cicer arietinum* L., *Lens culinaris* Medik, *Vigna mungo* (L.) *V. radiata* (Roxb.), *V. aconitifolius* (Jaq.), *V. sinensis* (L.) Savi ex Hassk., *Dolichos biflorus* L., *D. lablab* L. *Cajanus cajan* (L.) Millsp., *Pisum sativum L., Cassia tora* L., *Cassia sp., Acacia modesta* Wall.

Locality : Widely spread throughout India.

Callosobruchus dolichosi (Gyll.)

Bruchus dolichosi Gyllenhal, in Schonh. Gen. Curc., 5, 1839.

Callosobruchus dolichosi : Singal & Pajni, Bull. Entomologique De Pologne, 59: 761 - 782, 1990.

Host : Not known

Locality : Bondla forest and Panjim (G.) Kalinga forest, Bhanjanagar, Ghatgaon forest, Khoenjhar and Simlipal hills (Ori.).

Callosobruchus indica Pajni and Gupta

Callosobruchus indica Pajni and Gupta, Oriental Insects, 9 (4): 447 - 49, 1975.

Host : Not known

Transitory host : *Dahlia* sp. flower, Grass, *Trifolium alex–andrianum* Bory & Chaub.

Locality : Chandigarh (U.T), Ambala (Har.), Bangalore (Kar.).

Callosobruchus maculatus (Fab.)

Bruchus maculatus Fabricius, Systema Entomologia, Flensburgi et Lipsiae: 65, 1775.

Bruchus vicinus Dej., in Schonh., Gen. Curc., 1: 36, 1833.

Bruchus ambiguus Chev., in Schonh., Gen. Curc., 5 : 11, 1839.

Bruchus sinuatus Fahr., in Schonh., Gen., Curc., 5:8, 1839

Callosobruchus maculatus : Hoffman, Faune de France, 44:89, 1945.

Callosobrchus quadrimaculatus Shomar, Bull. Soc. Ent. Egypte,47:182, 1963.

Callosobruchus maculatus	:	Arora, Oriental Insects, Suppl.7: 25-26, 1977.
Host	:	*Vigna radiata* (Roxb.), *V. aconitifolius* (Jaq.), *V. mungo* (L.), *V. sinensis* (L.) Savi ex Hassk., *Cajanus cajan* (L.) Millsp., *Cicer arietinum* L., *Pisum sativum* L., *Trifolium alexandrianum* Bory & Chaub.
Locality	:	Widely spread throughout India.

Callosobruchus multinigrus Singal & Pajni

Callosobruchus multinigrus		Singal & Pajni, Bull. Entomologique De Pologne. 59 : 761 - 782, 1990.
Host	:	Not Known
Transitory host	:	Grass
Locality	:	Hamirpur, Palampur and Mandi (H.P.), Kalka (Har.)

Type material in Etomology Deptt. CCS HAU, Hisar, Haryana, India (Singal's Collection).

Callosobruchus nigripennis (Allard)

Bruchus nigripennis Allard, Ann. Soc. Ent. Belg., 39 : 226, 1895

Bruchus (Callosobrucus) nigripennis : Pic, Junk, Col. Cat., 55 : 37, 1913.

Callosobruchus nigripennis : Singal & Pajni, Bull. Entomologique De Pologne, 59: 761 - 782, 1990.

Host	:	*Indigofera* sp.
Locality	:	Chandigarh (U.T.), Dharwar (Kar.), Poona (Mah), Bandla forest and Panjim (G.).

Callosobruchus phaseoli (Gyll.)

Bruchus phaseoli Gyllenhal, in Schonh., Gen. Curc., 1:37, 1833

Callosobruchus phaseoli	:	Singal & Pajni, Bull. Entomologique De Pologne, 59 : 761 – 782, 1990.
Host	:	Stored *Dolichos lablab* L.
Locality	:	Kanpur (U.P.)

Callosobruchus sasangirensis Singal & Pajni

Callosobruchus sasangirensis Singal & Pajni, Bull, Entomologique De Pologne,

59 : 761 – 782, 1990.

Host : Not Known

Transitory host : Grass

Locality : Sasangir forest (Guj.)

Type material in Entomology Deptt., CCS HAU, Hisar, Haryana, India, (Singal's Collection).

Callosobruchus stoutus Singal & Pajni

Callosobruchus stoutus Singal & Pajni, Bull, Entomologique De Pologne, 59 : 761-782, 1990.

Host : Not known

Tranistory host : Grass

Locality : Poona (Mah.), Coimbatore (T.N.)

Type material in Entomology Deptt., CCS HAU, Hisar, Haryana, India, (Singal's Collection).

Callosobruchus sukhanensis Singal & Pajni

Callosobruchus sukhanensis Singal & Pajni, Bull, Entomologique De Pologne, 59: 761 - 782, 1990.

Host : Not Known

Transitory host : Grass

Locality : Chandigarh (U.T.)

Type material in Entomology Deptt., CCS HAU, Hisar, Haryana, India, (Singal's Collection).

Callosobruchus theobromae (L.)

Bruchus theobromae Linnaeus, Syst. Nat., Ed.12 : 605, 1767.

Callosobruchus theobromae : Arora, Oriental Insects, Suppl. 7 : 25, 1977.

Host : *Glycine max* Merr. *Cajanus cajan (L.)* (green pods)

Locality : Widely spread throughout India

GENUS - *SPECULARIUS* BRIDWELL

Specularius Bridwell, J. Washington Acad. Sci., 28: 71, 1938.

Specularius bridwelli Arora

Specularius bridwelli Arora, Oriental Insects, Suppl. 7: 37-39, 1977.

Host : *Rhynchosia minima* (L.) DC., *Rhynchosia* sp., *Cassia* sp.

Locality : Delhi (U.T.), Chandigarh (U.T.), Nainital & Pantnagar (U.P.), Coimbatore (T.N.), Mysore (Kar.) Siliguri (W. Ben.), Dwarka & Baroda (Guj).

Specularius maindroni (Pic)

Bruchus (Callosobruchus) maindroni Pic, Melanges, 11: 6, 1914.

Specularius maindroni : Arora, Oriental Insects, Suppl. 7:38-41, 1977.

Host : *Vigna vexillata* Benth, *Indigofera* sp.

Locality : Gonda, Brindaban, Ravatpur & Kanpur (U.P.), Madurai & Vellore (T.N.), Mysore & Hassan (Kar.), Bhopal (M.P.), Ragunath forest, Junagarh, Gir forest, Sasangir & Rajkot (Guj.), Burdwan (W.Ben.), Jodhpur & Mount Abu (Raj.)

GENUS - *SULCOBRUCHUS* CHUJO

Sulcobruchus Chujo, Trans. Nat. Hist. Soc. Formosa, 27: 189-201, 1937.

Sulcobruchus kingsolveri Arora

Sulcobruchus kingsolveri Arora, Oriental Insects, Suppl.7:86-87, 1977.

Host : *Albizzia* sp; *Dalbergia latifolia* Roxb.

Locality : Nahan (H.P.), Ramnagar (U.P.), Bangalore (Kar.), Ootacamund & Madras (T.N.), Bhubaneswar (Ori.).

GENUS - *CONICOBRUCHUS* DECELLE

Conicobruchus Decelle, Rev. Zool, Bot. Afr.,4: 181, 1951.

Conicobruchus albopubens (Pic)

Bruchidius albopubens Pic, Melanges Exot. Moulins, 57:26, 1931

Conicobruchus albopubens : Arora, Oriental Insects, Suppl. 7: 34-36, 1977.

Conicobruchus albopubens : Singal & Pajni, Goebios new Reports, 5 (2) : 97-100, 1986.

Host : *Cyamopsis psoralioides* DC. (green pods), *Crotolaria cosmosa* Baker.

Locality : Moradabad, Dehradun, Pilibhit & Pantnagar (U.P.), Chandigarh (U.T.), Bikaner (Raj.), Okha (Guj.), Nagpur (Mah.), Coimbatore (T.N.), Mysore & Bangalore (Kar.). Burdwan (W. Ben.), Hazaribagh (Bih.)

Conicobruchus caerulens (Champ.)

Bruchus caerulens Champion. Ent. Mon. Mag., 55 : 244–245, 1919.

Coincobruchus caerulens : Singal & Pajni, Geobios new Reports, 5(2): 97-100, 1986.

Host : *Desmodium nutans* Wall.

Locality : Chamba (H.P.)

Conicobruchus impubens (Pic)

Bruchus impubens Pic, Echange, 45: 24, 1929.

Conicobruchus decellei Arora & Singal,Oriental Insects, 11(2): 229 - 231,1977.

Conicobruchus impubens : Singal & Pajni, Geobios new Reports, 5(2):97-100, 1986.

Host : Not known

Transitory host : Grass, *Foeniculum vulgare* (Miller), *Linum usitatissimum* L., *Holarrhena* sp.

Locality : Chandigarh (U.T.), Ambala (Har.)

Conicobruchus indicus (Pic)

Bruchus indicus Pic , Echange, 25: 118, 1909

Conicobruchus indicus : Arora, Oriental Insects, Suppl. 7: 33-35, 1977.

Conicobruchus indicus : Singal & Pajni, Goebios new Reports, 5 (2): 97 -100, 1986.

Host : *Crotolaria juncea* L., *Glycine max* Merr., *Crotolaria medicaginea* DC. (green pods in the field)

Locality : Kansal & Chandigarh (U.T.), Hissar (Har.), Allahabad (U.P.), Jaipur (Raj.).

Conicobruchus kashmiricus (Pic)

Bruchus kashmiricus Pic, Echange, 45:4, 1929.

Conicobruchus alpina Arora and Singal, Oriental Insects, 13 (1-2): 55 - 59, 1979.

Conicobruchus kashmiricus : Singal & Pajni, Geobios new Reports, 5(2): 97-100, 1986.

Host : Not known

Transitory host : Grass

Locality : Udhampur (J&K)

GENUS– *BRUCHIDIUS* SCHILSKY

Bruchidius Schilsky, Kaf. Eur., 41:8, 1905.

Bruchidius albizziae Arora

Bruchidius albizziae Arora, Oriental Insects, Suppl. 7: 55–56. 1977.

Host : *Albizzia lebbek* Benth, *Albizzia procera* (Roxb.) Benth, *Acacia catechu* Willd., *Cassia* sp.

Locality : Rajpura (Pb.), Chandigarh (U.T.), Ambala (Har.), Dehradun (U.P.), Jammu & Udhampur (J&K), Bikaner & Mount Abu (Raj.) Hessan forest (Kar.), Madras & Vellore (T.N.), Mormugoa (G.), Nalgonda (A.P.)

Bruchidius amarae Arora

Bruchidius amarae Arora, US PL 480 Project (1974–79), Final Tech. Rept. : 30–32, 1980

Host : *Albizzia amara* (Roxb.)

Locality : Bangalore (Kar.), Trichur forest (Ker.)

Bruchidius andrewesi (Pic)

Bruchus andrewesi Pic, Ann. Mag. Nat. Hist., 10(9): 330, 1932.

Bruchidius andrewesi : Arora, Oriental Insects, Suppl. 7:50, 1977.

Host : *Acacia auriculiformis* Cunn.

Locality : Ambala to Pipli Road (Har.), Delhi to Agra Road (U.P.), Agra to Bharatpur Road, Jaipur and Udaipur (Raj.)

Bruchidius angustifrons Schilsky

Bruchidius angustifrons Schlilsky, Kauf. Eur., 41:52. 1905.

Bruchidius angustifrons : Arora, Oriental Insects, Suppl. 7:47, 1977.

Host : *Sesbania sesban* (L.) Merr. var. *bicolor* (W.A.) P.W. Ander

Locality : Chandigarh (UT.), Pinjore, Kalka & Faridabad (Har.), Delhi (U.T.), Moradabad, Meerut & Dehradun (U.P.), Udaipur (Raj.), Bombay (Mah.), Coimbatore, Madurai & Vellore (T.N.), Hessan forest (Kar.), Burdwan (W. Ben.), Junagarh (Guj.)

Bruchidius annusurindrii Arora

Bruchidius annusurindrii Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 61–63, 1980.

Host : Not Known

Transitory host : *Sesbania* sp. flowers, *Lantana* sp. flowers, Grass.

Locality : Anantpur & Hyderabad (A.P.)

Bruchidius aureus Arora

Bruchidius aureus Arora, Oriental Insects, Suppl. 7: 60–61, 1977.

Host : *Albizzia lebbek* Benth

Locality : Widely spread throughout India

Bruchidius bangalorensis Arora

Bruchidius bangalorensis Arora, US PL 480 Project (1974–79), Final Tech, Rept.:32–34, 1980.

Host : *Acacia* sp.

Locality : Bangalore (Kar.)

Bruchidius blemeris Arora

Bruchidius blemeris Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 22–24, 1980.

Host : Not known

Transitory host : Grass, *Lantana indica* Wall. flowers

Locality : Dehradun (U.P.), Chandigarh (U.T.), Chhatbir Zoo & Nangal (Pb.), Jammu & Udhampur (J & K), Rangpa & Gangtok (Sik.), Udaipur (Raj.), Jwalamukhi and Kangra (H.P.), Nalgonda, Warangal, Khamam, Vijaywada, Anakapalli, Kakinda, Anantpur, Eluru & Hyderabad (A.P.), Hasimara (W.Ben.)

Bruchidius brunnetashii Arora

Bruchidius brunnetashii Arora, US PL 480 Projcet (1974–79), Final Tech. Rept.: 36–37, 1980.

Host : Not known

Transitory host : Grass,

Locality : Madurai Forest (T.N.)

Bruchidius brunneus Tewari & Pajni

Bruchidius brunneus Tewari & Pajni, Res. Bull. Panjab Univ., 45(1–4): 41–42, 1995.

Host : Not known

Transitory host : Grass

Locality : Bombay (Mah.)

Bruchidius cassiae Arora

Bruchidius cassiae Arora, Oriental Insects, Suppl. 7:46, 1977.

Host : *Cassia tora* L., *Cassia* sp.

Locality : Widely spread throughout India

Bruchidius compositus Arora

Bruchidius compositus Arora, Oriental Insects, Suppl. 7: 43–44, 1977.

Host : Not known

Transitory host : *Launaea nudicaulis* Hook. flowers

Locality : Chandigarh (U.T.)

Bruchidius desmodei Arora

Bruchidius desmodei Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 42–44, 1980.

Host : *Desmodium pulchellum* Benth

Locality : Simlipal hills Jaishipur (Ori.), Kangra (H.P.),

Coimbatore (T.N.)

Bruchidius dimorphous Arora

Bruchidius dimorphous Arora, Oriental Insects, Suppl. 7:53–54, 1977.

Host : *Acacia pennata* Willd., *Acacia* sp.

Locality : Kalka hills, Sundarnagar & Mandi(HP), Rishikesh (U.P.), Bangalore (Kar.)

Bruchidius dorsivalvis Arora

Bruchidius dorsivalvis Arora, US PL 480 Project (1974–79), Final Tech. Rept.:44–46, 1980.

Host : Not known

Transitory host : Grass

Locality : Burdwan (W.Ben.), Patna(Bih.), Poona (Mah.), Kinnersamiganj (A.P.)

Bruchidius endotubercularis Arora

Bruchidius endotubercularis Arora, US PL 480 Project (1974–79), Final Tech.Rept.:24–26, 1980.

Host : *Albizzia* sp.

Locality : Hessan forest (Kar.), Madurai(T.N.)

Bruchidius eupatoricus Arora

Bruchidius eupatoricus Arora, US PL 480 Project (1974–79), Final Tech. Rept.:34–36, 1980.

Host : Not known

Transitory host : *Eupatorium* sp. flowers, Grass

Locality : Hessan forest (Kar.), Ahmedabad & Junagarh (Guj.), Bombay & Poona (Mah.)

Bruchidius flapoparamerica Arora

Bruchidius flapoparamerica Arora, US PL 480 Project (1974–79), Final.Tech. Rept. : 50–52, 1980.

Host : Not known

Transitory host : Grass

Locality : Ahmedabad & Sasangir forest (Guj.), Poona (Mah.), Bhopal (M.P.)

Bruchidius flavovirens Arora

Bruchidius flavovirens Arora, Oriental Insects, Suppl. 7: 56, 1977.

Host : *Albizzia procera* (Roxb.) Benth

Locality : Palampur, Baijnath, Solan to Salogra Road & Chamba (H.P.), Ranchi (Bih), Siliguri (W.Ben.) Gauhati (Ass.)

Bruchidius indigoferae Singh & Saini

Bruchidius indigoferae Singh & Saini, J. ent. Res., 2(2): 129–130, 1978.

Host : *Indigofera tentoria* L.

Locality : Patiala (Pb.)

Type material in Zoology Museum, Punjabi University, Patiala, Punjab, India

Bruchidius Kashmirensis Arora

Bruchidius Kashmirensis Arora US PL 480 Project (1974–79), Final Tech. Rept.: 48–50, 1980.

Host : Not known

Transitory host : Grass

Locality : Chashmashahi, Kukarnag, Harvan, Kangan, Srinagar, Sopore, Bhadarwah, Udhampur and Garhi (J & K).

Bruchidius lineolatus Arora

Bruchidius lineolatus Arora, Oriental Insects, Suppl. 7: 57–58, 1977.

Host : *Albizzia procera* (Roxb.) Benth

Locality : Palampur, Sundernagar, Baijnath Ahuju, Mandi, Chamba Ghat (H.P.).

Bruchidius maculipygus (Champ.)

Bruchus maculipygus Champion, Ent. Mon. Mag., 55:244–45, 1919.

Bruchidius maculipygus : Arora, Oriental Insects, Suppl. 7: 57, 1977.

Host : *Acacia pennata* Willd., *Acacia intsia,* Willd., *Acacia* sp.

Locality : Kalka hills, Sunderngar & Mandi (H.P.), Rishikesh (U.P.), Poona and Khandala (Mah.), Bangalore (Kar.), Coimbatore (T.N.), Hazaribagh (Bih.)

Bruchidius manglorensis Tewari & Pajni

Bruchidius manglorensis Tewari & Pajni, Res. Bull. Panjab Univ., 45(1–4): 39–41, 1995

Host : Not known

Transitory host : Grass

Locality : Mangalore (Kar.)

Bruchidius mathaii Arora

Bruchidius mathaii Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 28–29,1980.

Host : *Albizzia* sp.

Locality : Trichur (Ker.)

***Bruchidius meibomiaca* Arora**

Bruchidius meibomiaca Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 52–54,1980.

Host : Not known

Transitory host : *Meibomia umbellate* Kuntz.

Locality : Mangalore (Kar.)

Bruchidius microminutus Arora

Bruchidius microminutus Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 59–61, 1980.

Host : Not known

Transitory host : Grass

Locality : Nalgonda, Khammam, Anantpur, Hyderabad (A.P.)

Bruchidius mimosae Arora

Bruchidius mimosae Arora, Oriental Insects, Suppl. 7: 54–55, 1977.

Host : *Mimosa rubicaulis* Lamk.

Locality : Loharu (Pb.), Kangra, Ranital, Solan & Dera Gopipur (H.P.), Ramnagar, Dehradun &Garampani(U.P.), Chandigarh (U.T.), Ramgarh Cantt. and Ranchi (Bih.), Madurai (T.N.) Hessan forest (Kar.), Poona (Mah.) and Baripada (Ori.)

Bruchidius minutus (Fab.)

Bruchus minutus Fabricius, Systema Eleutheratorum, 2:401, 1801.

Bruchidius minutus : Arora, Oriental Insects, Suppl. 7: 44–45, 1977.

Host : *Acacia modesta* Wall.

Locality : Chandigarh (U.T.), Bharatpur (Raj.) Bangalore (Kar.), Morugoa (G.), Jhansi (U.P.), Bhopal (M.P.), Sasangir forest (Guj.), Pune (Mah.)

Bruchidius multilineolatus Arora

Bruchidius multilineolatus Arora, Oriental Insects, Suppl. 7: 58–59, 1977.

Host : *Albizzia lebbek* Benth

Locality : Widely spread throughout India

Bruchidius mussoriensis Arora

Bruchidius mussoriensis Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 54–56, 1980.

Host : Not known

Transitory host : Grass, *Trifolium alexandrianum* Bory & Chaub.

Locality : Dehradun, Rajpur & Mussoorie (U.P.), Gwalior (M.P.)

Bruchidius nangalensis Arora

Bruchidius nangalensis Arora, US PL 480 Project (1974–77), Final Tech. Rept.: 57–58,1980.

Host : Not known

Transitory host : Grass

Locality : Nangal (Pb.), Kasauli (H.P.)

Bruchidius nigricans Arora

Bruchidius nigricans Arora, Oriental Insects, Suppl. 7: 50–51, 1977.

Host : Not known

Transitory host : Grass

Locality : Panjab University, Chandigarh

Bruchidius obscurus Arora

Bruchidius obscurus Arora, Oriental Insects, Suppl. 7: 48–49, 1977.

Host : Not known

Transitory host : *Capris decidua* (Forsk.), Grass, *Lantana camara* L., *Eupatorium* sp. *Cassia tora* L., *Trifolium alexandrianum* Bory & Chaub.

Locality : Dehradun forest (U.P.), Chandigarh (U.T.), Jammu (J & K), Palampur (H.P.), Ajmer (Raj), Mormugoa (G.), Bangalore (Kar.), Trichur (Ker.), Gwalior(M.P.).

Bruchidius orientale Arora

Bruchidius orientale Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 20–21, 1980.

Host : Not known

Transitory host : Grass

Locality : Imphal (Mani.), Gauhati (Ass.), Chandigarh (U.T.) Hamirpur & Sundernagar (H.P.)

Bruchidius orissiensis Arora

Bruchidius orissiensis Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 40–42, 1980.

Host : Not known

Transitory host : Grass

Locality : Simlipal hills Jaishipur (Ori.)

Bruchidius pygomaculatus Arora

Bruchidius pygomaculatus Arora, Oriental Insects, Suppl. 7: 62, 1977.

Host : *Albizzia lebbek* Benth, *Albizzia procera* (Roxb.) Benth, *Acacia modesta* Wall., *Acacia catechu* Willd., *Cassia tora* L.

Locality : Widely spread throughout India

Bruchidius pygoquadrimaculatus Singh & Saini

Bruchidius pygoquadrimaculatus Singh & Saini, Entomon, 8(4): 385–387, 1983.

Host : *Albizzia* sp.

Locality : Patiala (Pb.)

Type material in Zoology Museum, Punjabi University, Patiala, Punjab, India.

Bruchidius punctoterminalis Arora

Bruchidius punctoterminalis Arora,

US PL 480 Project (1974–79), Final Tech. Rept.: 46–48, 1980.

Host : Not known

Transitory host : Grass

Locality : Batote (J & K)

Bruchidius sahlbergi Schilsky

Bruchidius sahlbergi Schilsky, Kauf Eur., 41:94, 1905.

Bruchidius sahlbergi : Arora , Oriental Insects , Suppl. 7:49–50, 1977.

Host : *Acacia farnesiana* Willd.

Locality : Chandigarh (U.T.), Ambala to Pipli Road (Har.), Dehradun (U.P.), Imphal (Mani.), Baripada (Ori.), Ramgah Cantt. & Ranchi (Bih.)

Bruchidius saundersi (Jek.)

Bruchus saundersi Jekel, Ins. Saund., 1:6, 1855

Bruchidius saundersi : Arora, Oriental Insects, Suppl. 7: 51–52, 1977.

Host : *Albizzia lebbek* Benth, *Albizzia* sp.

Locality : Widely spread throughout India

Bruchidius schilskyi Arora

Bruchidius schilskyi Arora, Oriental Insects, Suppl. 7: 61–62, 1977.

Host : *Albizzia lebbek* Benth, *Acacia modesta* Wall., *Acacia catechu* Willd.

Locality : Pathankot hills, Gurdaspur and Amritsar (Pb.), Dehradun, Rishikesh, Hardwar & Pilibhit (U.P.)

Bruchidius siwalikus Arora

Bruchidius siwalikus Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 38–40, 1980.

Host : Not known

Transitory host : Grass

Locality : Palampur, Mandi & Sundernagar (H.P.), Simlipal hills forest (Ori.), Ranchi and Ramgarh (Bih.),Junagarh (Guj.) Poona, Parbhani & Nagpur (Mah.), Jabalpur (M.P.) , Dehradun, Kanpur & Agra (U.P.)

Bruchidius solanensis Arora

Bruchidius solanensis Arora, Oriental Insects, Suppl. 7: 52–53, 1977.

Host : *Albizzia*sp.

Locality : Solan to Salogra Road (H.P.)

Bruchidius tephrosiae Arora

Bruchidius tephrosiae Arora, Oriental Insects, Suppl. 7: 47–48, 1977.

Host : *Tephrosia purpurea* (L.) Pers., *Tephrosia* sp.

Locality : Widely spread throughout India

Bruchidius urbanus (Sharp)

Bruchus urbanus Sharp, Ann. Mag. Nat Hist., 17(5): 35, 1886.

Bruchidius urbanus : Arora, Oriental Insects, Suppl. 7: 59–60, 1977.

Host : *Albizzia procera* (Roxb.) Benth

Locality : Palampur, Baijnath, Ahuju, Sundernagar, Mandi & Chamba Ghat (H.P.), Tejpur & Jorhat (Ass.), Nalgonda & Hyderabad (A.P.), Siliguri (W.Ben.)

Bruchidius variegata Arora

Bruchidius variegata Arora, US PL 480 Project (1974–79), Final Tech. Rept.: 26–27, 1980.

Host : *Albizzia* sp.

Locality : Bangalore, Mysore, Hessan forest (Kar.), Trichur (Ker.)

Bruchidius vulgaris Arora

Bruchidius vulgaris Arora, Oriental Insects, Suppl. 7:45, 1977.

Host : *Alysicarpus monilifer* DC., *Lathyrus hirsuta* L. seeds.

Transitory host : Flowers of some ornamental plants, weeds and shrubs.

Locality : Widely spread throughout India.

SUBFAMILY – AMBLYCERINAE BRIDWELL

Amblycerinae Bridwell, Proc. Entomol. Soc. Wash., 34 : 103, 1932

Key to the Indian Genera of Subfamily Amblycerinae

Intercoxal process of the prosternum acuminate, completely separating hind coxae. Elytra not widened caudad from humeri, tenth elytral stria complete, reaching apex. Exophallic valve helmet-shaped. Parameral tips elongated. ..... ***Spermophagus.***

Intercoxal process short, triangular, separating coxae only for half of their length. Elytra gradually widened caudad from humeri, tenth elytral stria not extending beyond middle of elytra. Exophalic valve conical. Parameral tips short. ..... ***Zabrotes***

GENUS – *SPERMOPHAGUS* SCHONHERR

Spermophagus Schonherr, Gen. Curc., 1: 102, 1833.

Spermophagus abdominalis (Fab.)

Bruchus abdominalis Fabricius, 1781, Species Insectorum I. Hamburgi, 1: 75

Spermophagus abdominalis : Cheverolet, Ann. Soc. ent. Bull. Fr., 7(5) : 135, 1877.

Spermophagus abdominalis : Singal, Ph.D Thesis, Panjab University, Chandigarh, 1980.

Host : Not known

Transitory host : *Ipomoea hederacea* Facq.

Locality : Coorg (Kar.)

Spermophagus aeneipennis Pic.

Spermophagus aeneipennis Pic, Me'langes exotico. ent., 26: 9, 1917.

Spermophagus aeneipennis : Singal, Ph.D Thesis, Punjab University, Chandigarh, 1980.

Host : Not Known

Transitory host : *Chrysanthemum* sp., *Abelmoschus moschatus* Medic

Locality : Trivandrum (Ker.), Hyderabad (A.P.), Vellore (T.N.), Bhubaneswar (Ori.).

Spermophagus albofasciatus Gyll.

Spermophagus albofasciatus Gyllenhal, in Schonh, Gen. Curc., I : 110, 1833.

Spermophagus albofasciatus : Arora, Oriental Insects, Suppl. 7: 90 - 91, 1977.

Host : Not Known

Transitory host : *Hibiscus canabinus* L., *Crotolaria occulata* R.Grah., *Carthamus tinctorius* L. flowers, *Argemone* sp. flowers, *Trifolium alexandrianum* Bory & Chaub. flowers, grass.

Locality : Sirhind, Hoshiarpur & Kharar (Pb.), Chandigarh (U.T), Kulu, Palampur & Mandi (H.P.), Saharanpur, Pilibhit, Nainital & Dehradun (U.P.), Jammu, Srinagar & Udhampur (J&K), Ranchi, Ramgarh & Patna (Bih.).

***Spermophagus albosparsus* Gyll.**

Spermophagus albosparsus Gyllenhal, in Schonh., Gen. Curc., I : 110, 1833.

Spermophagus albosparsus Arora, Oriental Insects, Suppl. 7 : 88 - 89, 1977.

Host : *Not Known*

Transitory host : *Abelmoschus esculentus* L., *Sonchus oleraceus* L. flowers, *Roystonea regia* (Kunth) Cook flowers, *Convolvulus* sp. flowers, *Althaea rosea* Cav. flowers *Foeniculum* vulgare (Miller) flowers, *Ziziphus* sp. flowers.

Locality : Sirhind, Kharar & Amritsar (Pb.), Ambala (Har.) Moradabad, Saharanpur & Nanital (U.P.), Jammu & Udhampur (J&K), Palampur (H.P.), Nagpur & Parbhani (Mah.), Trivandrum (Ker.), Ootacamund & Coimbatore (T.N.), Siliguri (W. Ben.), Johrat, Dibrugarh & Tejpur (Ass.).

Spermophagus latescenta Arora

Spermophagus latescenta Arora, Oriental Insects, Suppl. 7: 89-90, 1977.

Host : *Acacia modesta* Wall. pods

Transitory host : *Pyrus communis* L. Leaves, Tagetes erecta L. flowers, *Cosmos bipinnatus* Cav. flowers, *Foeniculum* vulgare (Miller) flowers, *Rosa damascena* Mill. flowers, *Launaea nudicaulis* Hook flowers, *Trifolium alexandrianum* Bory & Chaub. flowers, *Cymopsis psoralioides* DC. flowers, *Ziziphus* sp. flowers, *Hibiscus canabinus* L., *Papaver somniferum* L.

Locality : Chandigarh (U.T.), Amloh (Pb.) Bharatpur, Jodhpur & Mount Abu (Raj.), Allahabad, Bareilly & Varanasi (U.P.), Jammu (J&K), Gwalior & Jabalpur (M.P.), Ahmedabad & Baroda (Guj.), Chatrapur (Ori.), Siliguri & Darjeeling (W. Ben.), Hazaribagh & Patna (Bih.), Solan, Palampur & Mandi (H.P.).

Spermophagus notatipennis Pic

Spermophagus notatipennis Pic, Ann. Mag. nat. Hist., 9 (10): 332, 1932

Spermophagus notatipennis : Singal, Ph.D Thesis, Panjab University, Chandigarh, India, 1980.

Host : Not Known

Transitory host : *Tagetes sp., Petunia alba* Hort. ex Ferguson & Ottley

flowers, *Calotropis* sp. flowers, *Helianthus annuus* L. flowers, *Foeniculum vulgare* (Miller) flowers.

Locality : Chandigarh (U.T.), Hessan (Kar.), Ootacamund (T.N.),Darjeeling (W.Ben.), Ramgarh & Hazaribagh (Bih.).

Type material in Entomology Deptt., HAU, Hissar, Haryana, India (Singal's Collection)

Spermophagus tessellatus Motschoulsky

Spermophagus tessellatus Motschoulsky, Et. Ent., 7: 97, 1858.

Spermophagus tessellatus : Arora, Oriental Insects, Suppl. 7: 92-93, 1977.

Host : Not Known

Transitory Host : *Pyrus communis* L. leaves, *Foeniculum* vulgare (Miller) flowers, *Tagetes erecta* L. flowers, *Rosa damascena* Mill. flowers, *Vernonia anthelmintica* Willd. flowers, *Petunia violacea* Lindl. flowers, *Ipomoea* sp. flowers, *Iberis amara* L. flowers.

Locality : Chandigarh (U.T.), Amloh (Pb.), Ambala (Har.), Dehradun, Rishikesh, Pantnagar & Nainital (U.P.), Solan & Simla (H.P.), Sasangir & Porbander (Guj,) Bhubaneswar (Ori.), Mormagaon (G.).

GENUS - *ZABROTES* HORN

Zabrotes Horn, Trans. Amer. Ent. Soc., 12:156, 1885.

Zabrotes subfasciatus (Boh.)

Spermophagus subfasciatus Bohemann, in Schonh., Gen. Curc., 1 : 3, 1833.

Spermophagus musculus Bohemann, in Schonh., Gen. Curc. I :112, 1833.

Zabrotes subfasciatrus : Lukianovich and Terminasian, Zool, Inst, Nauk, SSSR, No.67: 199, 1957.

Zabrotes subfasciatus : Arora, Oriental Insects, Suppl. 7:92, 97-98, 1977.

Host : *Phaseolus vulgaris* L., *Vigna sinensis* (L.) Savi ex Hassk.

Locality : Widely spread throughout India

Abbreviated list of Indian states

Abbreviation		***Name of the state***
A.P.	–	Andhra Pradesh
ASS	–	Assam
Bih.	–	Bihar
G.	–	Goa

Guj.	–	Gujarat
H.P.	–	Himachal Pradesh
Har.	–	Haryana
J&K	–	Jammu and Kashmir
Kar.	–	Karnataka
Ker.	–	Kerala
M.P.	–	Madhya Pradesh
Mah.	–	Maharashtra
Mani.	–	Manipur
Megh.	–	Meghalaya
Ori.	–	Orissa
Pb.	–	Punjab
Raj.	–	Rajasthan
Sik.	–	Sikkim
T.N.	–	Tamil Naidu
U.P.	–	Uttar Pradesh
U.T.	–	Union Territory
W. Ben.	–	West Bengal

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HENSELIZATION OF G-DOMAINS

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This note is concerned with a property that is inherited by Henselization. The main result shows that if a normal G-domains R has its Henselization ${}^{h}R$, is also a G-domains. For our purposes ,we use the method of Henselization introduced by Greco [1]. We give sufficient details to make the exposition self contained. In our discussions R will denote a commutative ring with identity and **a** an ideal contained in the J(R) radical of R.

Definition 1. An integral domain R will be called a G-domain if its quotient field K is finitely generated as a ring over R. It can be readily seen that if R is a G-domain then K=R[1/u] for some non zero element u of R. This u is contained in the intersection of all the (minimal) non-zero prime ideals of R.

As examples of G-domains we give the ring Q_p of p-adic integers and any discrete rank 1 valuation ring. The following is an example of a G-domain which is not a valuation ring.

Let K = Q(X) be the rational function field in one variable over the rational number field. For a fixed prime number p, the rational number r/s with (r,s)=1 is called p-integral if (p,s)=1 and p-divisible if p divides r. A polynomial f(X) in Q[X] is said to be primitive for p, if all its cofficients are p-integral while not all are p-divisible. In Q(X) the ring R generated by (i) all p-integers of Q, (ii) $p^{2n+1} \cdot X^{n(2n+1)}$ for n=0,1,2,....and (iii) the elements 1/f(X) where f(X) is a p-primitive polynomial, is a G-domain with K=R[1/p].

Definition 2. A monic polynomial $f(X)= \sum_{i=0}^{n} a_i X^i$ in R[X] is called an N-polynomial with respect to the ideal **a** (contained in the radical of R) if a_0 belongs to **a** and a_1 is a unit modulo **a**. By a simple N-extension (S,**b**) of the pair (R, **a**) we mean a ring $S= M^{-1}.R[x]$ where M=1+(**a**,x).R[x] where R[x]=R[X]/(f(X)),where f(X) is an N-polynomial over R with respect to **a** and **b** is the ideal generated by **a** in S.

We first prove the following :-

Lemma 1. Let R be a normal G-domain and **a** in Rad.R.If f(X)is a monic irreducible N-polynomial over R with respect to **a** then the simple N-extension (S,**b**) arising out of f(X),is also a G-domain.

Proof: As R is a G-domain,there exists a u in R such that K=R[1/u].We show that R[x]=R[X]/(f(X)) is a G-domain.If g(X) in R[X] is such that g(x)≠0, then as f(X) is irreducible, we have (f(X),g(X))=1 over K. Therefore ,there exist polynomials p(X) and q(X) in K[X] such that p(X).g(X)+q(X).f(X)=1,so that p(x).g(x)=1. Hence 1/g(x)=p(x) in K[x]=R[1/u].[x]=R[x].[1/u].Thus K[x]= K(x), the field of qotients of R[x] is generated by 1/u,thereby showing that R[x] is a G-domain. In the above lemma normality of R is essential

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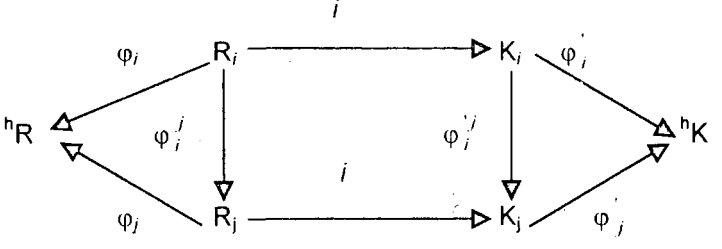
because it can well happen that a polynomial that is irreducible over R may be reducible over the integral closure as the following example shows:

Example: Let k be a field and X,Y be two indeterminants. Set $A=k[X,Y]/(X^2+XY+Y^3)=k[x,y]$ and R be the localization of A at (x,y).Then R is a local integral domain with k(x,y) as its field of quotients .Now x/y does not belong to R while the polynomial $f(t)=t^2+t+y$ is an N-polynomial which is irreducible over R. But it has a root t=x/y in the integral closure of R in k(x,y).

In [1] S.Greco obtains the Henselization of R at **a** as a direct limit of simple N-extensions.For this he takes all the N-polynomials.In case the Henselization turns out to be a domain then we can get it as a direct limit of simple N-extensions obtained from all irreducible N-polynomials.

Theorem: Let R be a normal G-domain and R be the Henselization of R at **a** (contained in the radical of R).If R is a domain, then it is a G-domain.

Proof: From our assumption ,we have R is a domain and by construction ${}^hR=\mathrm{Lim}_{i\in I}\ R_i$,where R_i are N-extentions. Therefore each R_i turns out to be a domain. From lemma 1,we find that if (R, **a**) is a G-domain, then so is $(R_i, \mathbf{a}_i)$ for each i. If φ_i is a homomorphism of R_i in ${}^hR$, and K_i is the field of quotients of R_i, then $K_i = R_i[1/u]$. Let ${}^hK=\lim K_i$. We claim that ${}^hK$ the field of quotients of ${}^hR$ is generated by a single element over ${}^hR$. For i<j, consider the following:



Here φ'_i restricted to R_i is φ_i and φ'^{j}_i restricted to R_i is φ^j_i. We have $\varphi^j_i(u_i)=u_j$ and i is the monomorphism of R_i in K_i for each i. As the direct limit of fields is a field, ${}^hK=\lim_{i\to\infty} K_i$ is a field and likewise ${}^hR$ is an integral domain. If we set $u=\varphi_i(u_i)$ then we contend that ${}^hR[1/u] = {}^hK$-the quotient field of ${}^hR$. Let $x\neq 0$ in ${}^hR$. Then there exist a t in I such that $\varphi_t(x_t)=x$ with $x_t\neq 0$ in R_t. As R_t is a G-domain, $1/x_t \in R_t[1/u_t]$, so that $a_t u_t^{-n} = x_t^{-1}$ for some a_t in R_t and $\varphi_t(1/x_t)= 1/\varphi_t(x_t) = 1/x$, that is $1/x=\varphi_t(1/x_t)=\varphi_t(a_t u_t^{-n})$ in ${}^hR[1/u]$. Thus 1/x is in ${}^hR[1/u]$. Consequently ${}^hR$ is also a G-domain.

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A REPORT ON BEE- FLIES (BOMBYLIIDAE : DIPTERA) FROM ARUNACHAL PRADESH, INDIA.

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Abstract

The present study deals with 8 species of bee-flies (Bombyliidae : Diptera) belonging to 5 genera under 2 subfamilies of which 4 species belonging to 4 genera of the subfamily Exoprosopinae are reported for the first time from the state of Arunachal Pradesh.

Key words : Bombyliidae, Arunachal Pradesh, Anthracinae, Exoprosopinae

INTRODUCTION

The bombyliids, or **bee-flies** are a group of moderate to large sized colourful flies, bearing dense pubescence, usually with long projecting proboscis and darkly marbled wings. The adults are of great economic importance as they act as pollinators. The bombyliid fauna of Arunachal Pradesh is still far from being well known. Though 19 species under 12 genera are reported from North- East India , only 4 species under 3 genera are reported[1] by name from the NEFA (old name of Arunachal Pradesh). Though the area is climatologically not the ideal place for bombyliids to thrive, it harbours a small but quite diverse population of these bee-flies. In this present study 8 species of bombyliids belonging to 5 genera under 2 subfamilies are reported of which 4 species under 4 genera of the subfamily Exoprosopinae is being recorded for the first time from the State . The present communication is based on the National Collection of Zoological Survey of India, Kolkata.

MATERIALS AND METHODS

Arunachal Pradesh is a part of the Eastern Himalayan range located between 26°28' to 29° 30' N latitudes and 91° 30' to 97° 30' E longitudes. The state is bound by Bhutan on the West, China in the North and North-East, Myanmar on the east and the Indian states of Nagaland and Assam in the East and South-East. In the north-eastern region of India, Arunachal Pradesh occupies the largest area (83,743 sq km) and consists of the sub- mountain and mountainous ranges sloping down to the plains of Assam. The climate varies sharply with changes in topographical and altitudinal aspects.The collections have been mostly made from different altitudes and districts of Arunachal Pradesh.

Subfamily ANTHRACINAE

1. *Anthrax distigma* Wiedemann

1828. *Anthrax distigma* Wiedemann , *Aussereurop. zweifl. Insekt.* , 1:309.

Material examined : 7♂,6♀,Subansiri div,Tamen (host: cucurbits), 475m.,20.v.1966, coll.A.N.T.Joseph & pty.; 2 ♀, Elephant flat, 400m., 22.iii.73, coll. S.K.Tandon & pty.

Distribution :Arunachal Pradesh, Bihar, Karnataka, Kerala, Meghalaya, Sikkim, Tamil Nadu, Uttaranchal, West Bengal; Bangladesh, Jawa, Myanmar, Phillipines, Sri Lanka , Sulawesi.

Remarks : Moderate sized flies, often silvery or shining black, wings nearly clear.

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Subfamily EXOPROSOPINAE

2. *Villa aperta* (Walker)

1852. *Anthrax aperta Walker, Insecta Saundersiana,* 1:180.

Material examined : 1♂., Dirong Dzong, Kameng div.(host: tomato flowers),1604 m., 28.vii.1961,coll. S. Biswas.

Distribution: Arunachal Pradesh, Himachal Pradesh, Meghalaya and Uttaranchal; Myanmar.

Remarks : Large sized flies, body black; covered with blackish-brown hairs, sides of thorax and abdomen white, and wings clear. This is the first record from this state.

3. *Villa paniscus* (Rossi)

1790. *Bibio paniscus* Rossi, *Fauna Etrusca* ,2:256.

Material examined : 1♂, Dirong Dzong, Kameng div.(host: dahlia and marigold flowers),1.v.1966, coll. A.N.T.Joseph & pty.

Distribution : Arunachal Pradesh, Uttararanchal; Europe.

Remarks : Body black, covered with twany pubescence and black scales and with pale grey iridescent wing.

4. *Litorhyncus lar* (Fabricius)

1781. *Bibio lar* Fabricius ,*Spec.Insect.* 2: 414.

Material examined : 1♂, Dirong Dzong, Kameng div.(host: chilly and brinjal plants),1.v.1966, coll. A.N.T. Joseph & pty.

Distribution: Arunachal Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Maharashtra, Tamil Nadu, Uttaranchal; Sri Lanka; Australia.

Remarks : Large sized flies, body black, sides of thorax and abdomen with dull orange brown pubescence and dark brown pictured wing. Recorded for the first time from here.

5. *Exoprosopa (Exoprosopa) insulata* (Walker)

1852. *Anthrax insulata* Walker , *Insecta Saundersiana,* 1:72.

Material examined : 1♀, Dirong Dzong, Kameng div.(host: tomato and chilly flowers),1.v.1966, coll .A.N.T.Joseph & pty.

Distribution: Arunachal Pradesh, Bihar, Himachal Pradesh, Madhya Pradesh, Meghalaya, Karnataka, Tamil Nadu, Uttaranchal, West Bengal; Myanmar; Nepal, Sri Lanka.

Remarks : Moderate sized flies, body brownish yellow with shining black abdomen and spotted wings. This is the first record from the State

6. *Ligyra aurantiaca* (Guerin- Meneville)

1844. *Anthrax aurantiaca* Guerin –Meneville. *Icon ,du regne anim, Dipt.*, 3 : 539.

Material examined : 1♀, Elephant flat (host: flowers of unknown wild creeper), 400m. 22.iii.73, coll.S.K. Tandon & pty.

Distribution: Arunachal Pradesh, Bihar, West Bengal

Remarks : Moderate sized flies, body black with bright yellow pubescence, lower abdomen with patch of silvery white scales, wings dark brown with violet iridescence. This is also a first report from this State as well as from North-East India.

7. *Ligyra oenomaus* (Rondani)

1875.*Hyperalonia oenomaus* Rondani, *Annali Mus.Civ Stor.nat.Giacomo Doria* ,7 :453.

Material examined : 1♀, Tipi, Kameng div.(host: wild orchid flowers), 3.v.1966. coll.A.N.T. Joseph & pty.

Distribution:Arunachal Pradesh, Assam, Sikkim and West Bengal;Borneo, Malaysia and Phillipines

Remarks : Large sized flies, body black, covered with orange- yellow pubescence, abdomen with yellowish white spots, clear lightly pictured wing..

8. *Ligyra tantalus* (Fabricius)

1794. *Anthrax tantalus* Fabricius , *Ent.Syst. 4:260.*

Material examined : 1♀,Tipi, Kameng div.(host: wild orchid flowers), 3.v.1966, coll. A.N.T. Joseph & pty.

Distribution: Arunachal Pradesh, Assam, Uttaranchal, West Bengal; Indonesia, Malaysia, Taiwan,Thailand, Philippines, Sulawesi.

Remarks : Large sized flies, thorax black with bright orange hairs, abdomen black with median ventral white stripes, dark brown violet tinged wing.

DISCUSSION

The State of Arunachal Pradesh physiographically is just not diverse but unique in the sense that it encompasses not only the flood plains and the foot hills but also the Greater Himalayan ranges. Moreover "the state lies in a transitional ecological province between the Indian, Indo-Malayan, and sino-Myanmarese biogeographic realms, leading to a mingling of biological elements besides its own."

All the 8 species reported from the State are essentially Oriental in distribution, with an exception of 2 species which have spread themselves as far as the Palaearctic region (*V.paniscus*) and the Australian region (*L. lar*). However all the species show a distinct Himalayan bias always occuring in the Himalayan ranges (East, West and central). Of them the lone species (S. family: Anthracinae) *Anthrax distigma* is extremely widespread in India extend to Myanmar and Philippines in the East and to Sri Lanka and Jawa in the South. *Villa paniscus* and *Villa aperta* are restricted in their distribution in between north western and north eastern Himalayan slopes. Among the 7 species of the subfamily Exoprosopinae, *Litorynchus lar* is certainly widespread in the Indian subcontinent, though it exhibits a noticeable discontinuity in the Peninsular India. *Exoprosopa insulata* is almost uniform in its distribution all over India, from the Himalayas to the southern tip and extends its range upto Sri Lanka. Out of the three species of *Ligyra* ,*L. oenomaus* and *L. tantulus* are restricted in their distribution in Himalayan region with an exception of *L.tantalus* which is also reported from Assam; whereas *Ligyra aurantiaca* is distributed in the eastern part of India and found mostly in the high altitudes plains and low- altitudes foothills.

Considering the distribution pattern of all the species within the Indian subcontinent it can be inferred that the species of bombyliids discussed so far probably have a preference to the high altitude of Himalayas as they are by and far consistently found in the Himalayan ranges and foot hills. The uneven zoogeographical features of the wet and hilly Arunachal Pradesh

contribute to a great extent to the diversity of the bombyliid fauna as found in the State. But this zoogeographical analysis, however remains tentative due to poor collection of bombyliid fauna from this area. So, it may still need a thorough and intensive exploration for a fuller knowledge on the species diversity of the family Bombyliidae.

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ALLOZYME VARIATIONS IN SOME POPULATIONS OF ORIENTAL SPRUCE *(Picea Orientalis* (L.) LINK) IN TURKEY

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Abstract

The genetic variation in 12 natural populations of oriental spruce (*Picea orientalis (L.)* Link) was investigated at enzyme level using the horizontal starch gel electrophoresis. The seed samples were collected from 12 stands and their megagametophytes used in isoenzymes analysis of leucine amino peptidase (LAP) and glutamate oxalaceteta transaminase (GOT or ATT) enzymes. It was demonstrated that the allozyme variants in the LAP enzyme system was coded by a total of six structural genes at the two loci and GOT enzyme system was coded by a total of eight structural genes at the three loci. This study showed that some populations of oriental spruce have variations and important genetic differences.

Key words : Isoenzyme, Population differentiation, Alleles, Gene loci, *Picea orientalis.*

INTRODUCTION

Oriental spruce *(Picea orientalis* (L.) Link.) is one of the forty species of spruce in the World. It is widespread on the Eastern Black Sea region and its backward section or inner part of the Eastern Black Sea region. The mixed and pure stands of oriental spruce begin just west of the Melet river and extend along the north slopes of Giresun and Eastern Black Sea Mountains spreading as far as the southern section of Caucasican Mountains in the Georgia[12].

It is generally recognized that forest tree species, especially conifers, are characterized by a considerable variation, both across their native range and from tree to tree within the stands. This potential reflects a mechanism of the adaptive strategy specific for these typically long-lived organisms, which mostly respond with a wide genetic variation to environments heterogeneous in site and time[2]. Information on the distribution of allelic variation in natural populations can be obtained with the aid of electrophoretic analysis of isoenzymes. This is because enzymes are composed of polypeptides synthesized by the action of one or more structural genes.

Isozyme analysis can be used to estimate the genetic structure of the population and to characterize genetic variability independent of the selected phenotype[14]. Many experiments in various coniferous tree species have been conducted to find gene frequencies and genetic distance[6, 7, 9]. It is known from isoenzyme analysis that *Picea orientalis* (L.) Link. exhibits high genetic variability in its natural distribution[13]. However, to the best of our knowledge, there are no studies on enzyme system of oriental spruce on population basis.

This paper describes the use of isozyme analysis to examine genetic variation in populations. These populations were assessed to determine true provenance and to establish new seed sources.

MATERIALS AND METHODS

Wind-pollinated seeds were collected from 12 populations of *Picea orientalis (L.) Link.* in Turkey (Figure 1 and Table 1). Three of these populations were selected from seed orchards of oriental

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spruce. Samples were made of bulked seeds using 20 trees per provenance for all populations. The allozyme analysis was carried out on the endosperm. About 80 endosperms were examined for bulk provenance collections. For inferring individual genotypes in populations, seeds from single trees were assayed by examining 4 endosperms for each tree.

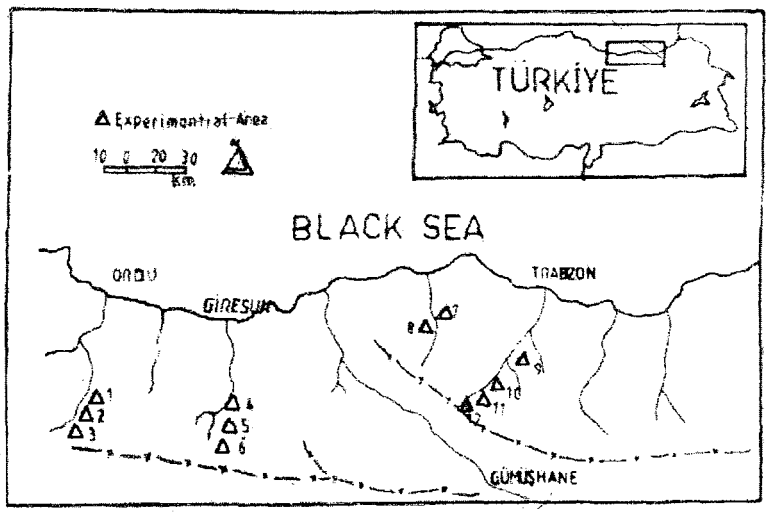

Fig. 1. Locations of the studied populations

Table 1. Specifications of populations studied.

Pop. No	Populations	Altitude (m)	Latitude (N)	Longitude (E)
1	Ordu – Çambasi	1050 - 1350	40° 43' 20"	37° 57' 15"
2	Ordu – Çambasi	1350 - 1650	40° 42' 39"	37° 55' 38"
3	Ordu – Çambasi	1650 - 1950	40° 38' 58"	37° 56' 16"
4	Gireşun - Dereli	1050 - 1350	40° 35' 50"	38° 27' 15"
5	Giresun - Dereli	1350 - 1650	40° 34' 48"	38° 27' 00"
6	Giresun - Dereli	1650 - 1950	40° 33' 45"	38° 26' 15"
7	Trabzon - Karadağ	1350 - 1650	40° 56' 37"	39° 24' 00"
8	Trabzon – Karadağ	1650 - 1950	40° 55' 38"	39° 22' 55"
9	Trabzon – Maçka	750 - 1050	40° 48' 55"	39° 38' 50"
10	Trabzon – Maçka	1050 - 1350	40° 41' 45"	39° 28' 00"
11	Trabzon – Maçka	1350 - 1650	40° 40' 30"	39° 25' 45"
12	Trabzon – Maçka	1650 - 1950	40° 40' 16"	39° 25' 30"

Horizontal electrophoresis on starch gel was utilized for the Leucine Amino Peptidase (LAP E.C. 3.4.11.1.) and the Glutamate Oxalacetate Transaminase (GOT or AAT E.C.2.6.1.1.) enzyme systems. Staining, gel and electrode buffer systems were performed according to Ashton and Braden[1].

The cones were air-dried and the seeds extracted by hand. Megagametophytes from 4 to 5 seeds were analysed per tree and each enzyme. The megagametophyte was dissected from the seed and crushed in homogenization buffer (pH=3.7, 100 ml. distilled H_20+970 mg Tris-HCl). The homogenates were absorbed onto Whatman No 1 filter paper wicks and placed separately in starch gel for electrophoresis[5, 8].

Electrophoretic technique and stain preparation were modified from Conkle et Al[5]. After the gels had moved about 5 or 8 cm, they were sliced horizontally into 2-3 mm thick gel slices, and then transferred to individual staining trays. The stain was prepared fresh on the day of the run. The staining solution was poured over the sliced gel and the gel was incubated at 37°C for 1 hour in darkness. When the bands on the gel were well resolved, the solution was removed and interpreted.

Where several zones of activity were observed for a single enzyme, capital letter following the enzyme abbreviations was used; the first letter designated the most anodal zone. Within a zone of activity the lowest number designated the fastest allele.

Data Analysis

Allozyme frequency data were used to calculate, on a population basis single locus measure of genetic diversity. Several different statistics were calculated, including per cent polymorphic loci (P), average number of alleles per locus (A), expected heterozygosity (*He*), and genetic distance (d_o).

The average number of alleles per locus was calculated by averaging over all polymorphic and monomorphic enzyme systems. A locus was considered polymorphic when at least two allozymes occur, and the frequency of the least common alleles was greater than 1.0 % P., it was calculated as:

$$\% P = \frac{\text{Number of polymorphic loci}}{\text{Total number of loci}}$$

The expected proportion of heterozygosities (*He*) at each locus was calculated according to Nei[10] as,

$$He = 1 - \sum_{i=1}^{k} X_{i^2}$$

where, X_i is the frequency of the *i*'th allele summed over k alleles.

Allozyme frequency data were used to calculate, on a population basis, single locus measures of genetic distance. Genetic distance was calculated as[7],

$$d_0 = 0.5 \sum_{i=1}^{k} |X_i - Y_i|$$

where, X_i and Y_i are the frequencies of the i'th allele in the X and Y populations, respectively and k is the number of the alleles present at a locus. d_O is then averaged over all loci using the arithmetic mean.

RESULTS AND DISCUSSION

Genetic Structure

Both LAP and GOT enzyme systems representing a number of 5 loci were resolved with sufficient consistency and clarity. The isoenzyme banding patterns of LAP and GOT enzymes systems and the allele frequencies with expected heterozygosity at each locus is shown in Table 2.

Leucine Amino Peptidase (LAP)

Gel stained for LAP had two zones of activitiy, LAP-A and LAP-B. LAP-A zone showed two single banded and LAP-B zone showed three single banded and one double banded variants, which were segregated as alleles at one locus. Generally, the frequency of A at LAP-A1, the frequency of B2 at LAP-B was very high in all populations. Genetic control of LAP loci by 2 independent loci has been reported for several other conifer species[3,11].

Table 2. Allele frequencies and expected heterozygosity for 5 polymorphic loci in 12 populations of *Picea orientalis L. Link.*

Locus	Allel	POPULATIONS											
		1	2	3	4	5	6	7	8	9	10	11	12
LAP	A1	0,900	0,900	1,000	0,950	0,900	0,800	0,900	0,850	1,000	1,000	1,000	0,900
A	A2	0,100	0,100	0,000	0,05	0,100	0,200	0,100	0,150	0,000	0,000	0,000	0,100
He		**0,180**	**0,180**	**0,000**	**0,095**	**0,180**	**0,320**	**0,180**	**0,225**	**0,000**	**0,000**	**0,000**	**0,180**
	B1	0,100	0,200	0,050	0,000	0,000	0,000	0,000	0,050	0,000	0,000	0,200	0,050
LAP	B2	0,600	0,600	0,750	0,250	0,750	0,800	0,850	0,800	0,450	0,850	0,500	0,650
B	B3	0,000	0,200	0,000	0,150	0,000	0,000	0,000	0,000	0,000	0,100	0,000	0,050
	B4	0,300	0,000	0,200	0,600	0,275	0,200	0,150	0,150	0,550	0,050	0,300	0,250
He		**0,540**	**0,560**	**0,395**	**0,555**	**0,379**	**0,320**	**0,255**	**0,335**	**0,495**	**0,265**	**0,620**	**0,510**
GOT	A1	0,200	0,100	0,150	0,050	0,100	0,000	0,050	0,000	0,100	0,000	0,150	0,100
A	A2	0,800	0,900	0,700	0,750	0,900	1,000	0,950	0,850	0,850	0,900	0,850	0,850
	A3	0,000	0,000	0,150	0,200	0,000	0,000	0,000	0,150	0,050	0,100	0,000	0,050
He		**0,320**	**0,180**	**0,465**	**0,395**	**0,180**	**0,000**	**0,095**	**0,255**	**0,265**	**0,180**	**0,255**	**0,265**
GOT	B2	0,000	0,050	0,000	0,000	0,150	0,000	0,050	0,000	0,150	0,000	0,150	0,150
B	B3	1,000	0,800	1,000	0,950	0,850	1,00	0,800	0,850	0,800	1,000	0,750	0,800
	B5	0,000	0,150	0,000	0,050	0,000	0,000	0,150	0,150	0,050	0,000	0,100	0,050
He		**0,000**	**0,335**	**0,000**	**0,095**	**0,255**	**0,000**	**0,335**	**0,255**	**0,335**	**0,000**	**0,405**	**0,335**
GOT	C2	0,650	0,850	0,750	0,200	0,200	0,000	0,900	0,600	0,500	0,200	0,400	0,500
C	C4	0,350	0,150	0,250	0,800	0,800	1,000	0,100	0,400	0,500	0,800	0,600	0,500
He		**0,455**	**0,255**	**0,375**	**0,320**	**0,320**	**0,000**	**0,180**	**0,480**	**0,500**	**0,320**	**0,480**	**0,500**

Glutamate Oxalacetate Transaminase (GOT)

There were 3 zones of activity on gels stained for this enzyme (GOT-A, GOT-B and GOT-C). GOT-A and GOT-B were polymorphic with three single-banded variants which were segregated as alleles at each one locus. The slowest zone (GOT-C) showed two single-banded variants, which were segregated as alleles at one locus. In other conifers[4] three loci genetic control has been suggested when three zones of activity are present on zymograms.

Generally, at GOT-A the frequency of A2, and at GOT-B the frequency of B3 were very high in all populations. At GOT-C, C2 was more frequent than C4 in some populations (population numbers 1, 2, 3, 7, 8). However, C4 was more frequent than C2 in some populations (population numbers 4, 5, 6, 10, 11). There are highly significant allele frequency differences among populations.

Genetic Diversity

The mean expected heterozygosity per population ranged from 0.1280 (population number 6) to 0.4630 (population number 4) with a mean of 0.2800. The average percentage of polymorphic loci was 80.00%, with a maximum of 100% (population numbers 2, 4, 5, 7, 8, 11, 12) and a minimum of 40.00% (population number 6). The average number of alleles per locus was calculated as 2,15 and ranged from 1,400 (population number 6) to 2,400 (population number: 2, 4, 9).

Genetic Differentiation

Genetic relationships between populations were determined by computing measures of genetic distance, proposed by Gregorius[7] and are given in Table 3.

Table 3. Mean genetic distance among the studied populations

Pop. No	1	2	3	4	5	6	7	8	9	10	11
2	0.159	-									
3	0.104	0.196	-								
4	0.242	0.329	0.267	-							
5	0.148	0.234	0.194	0.190	-						
6	0.217	0.317	0.229	0.259	0.119	-					
7	0.163	0.117	0.150	0.338	0.098	0.229	-				
8	0.129	0.175	0.125	0.275	0.161	0.175	0.109	-			
9	0.163	0.246	0.188	0.163	0.161	0.279	0.225	0.196	-		
10	0.221	0.259	0.167	0.196	0.123	0.138	0.217	0.175	0.225	-	
11	0.142	0.200	0188	0.225	0.140	0.259	0.229	0.196	0.096	0.213	-
12	0.100	0.159	0.150	0.225	0.092	0.204	0.150	0.109	0.100	0.188	0.109

The values of genetic distance based on the LAP and GOT enzymes system in all samples range between 0.092 and 0.338. The maximum value was found between population numbers 4 and 7 (d_o=0.338) and the minimum between population numbers 5 and 12. Mean genetic distance of all the populations was found as 0.186 LAP and GOT enzyme systems. This average value is rather higher than other coniferous tree species. According to the literature, the average genetic distance is 0.065 for *Picea abies* (L.) Karst. in Italian populations[6], 0.035 for *Picea abies* (L.)

Karst. in European populations[9], 0.003 to 0.012 for *Picea abies* (L.) Karst. in natural Latvian populations[7], and 0.1759 for *Picea orientalis* (L.) Link[13].

It could be concluded that the genetic diversity for natural populations of *Picea orientalis* (L.) Link in Turkey is very high when compared to other species indicated in this research.

Natural populations of some plant species have been found to be genetically subdivided into smaller subpopulations. Studies using isozyme techniques have shown that populations of some tree species are similarly genetically sub-structured. The results of this study show that isozyme analysis could be safely used to assess the genetic structure and to certify the seed source of *Picea orientalis* L. Link.

ACKNOWLEDGEMENTS

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THE EFFECT OF SOME FACTORS ON THE GROWTH OF CAPER SHRUB (*Capparis ovata* Desf.) NATURALLY DISTRIBUTED ON THE FOREST ROAD SLOPES IN ARTVIN DISTRICT OF TURKEY

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Abstract

Forest roads are the most significant infrastructual establishments in forestry operations. Plants on the slope not only keep soil particles together by their roots but also decrease surface erosion by their upper parts. In this study, *Capparis ovata* species growing naturally on the road slopes were examined along Artvin-Coruh watershed of Turkey to determine the relationship between slope and species existence. The species were distributed more on the southern slopes than on the northern slopes. Maximum coverage was observed on full-slopes at 280 m and the least on 100% slopes at 350 m. The plants in the group of 0-25% sloped areas (3.242 m^2) had the largest area covered. Plant coverage area was found to be higher in the 3rd altitude group (400 m.<). The relationship between aspect and plant coverage was not significant. In conclusion, *Capparis ovata* occupies less area on high slope groups. While in areas of low altitude with sunny exposures (i.e., south and south east slopes) it has the highest coverage in percent.

Key words : Caper shrub, *capparis ovata*, forest road slopes.

INTRODUCTION

Forest roads are the most significant infrastructure for forestry operations, especially for transportation of raw materials, personnel and equipment, apart from providing recreational necessities to the villagers. In other words, these provide economic, social and even cultural benefits[5].

Side slopes, made during forest road constructions, initially lose their stability by the effects of natural factors such as water, wind and frost. Depending on soil type, slope and water permeability, finger erosion, wind erosion, landslide and stone rolling occur. Stabilising studies on high sloped areas with inorganic materials often cost more than those of organic materials. Further, the appearance is also neither satisfactory nor preferred. It is stated that stabilising these kinds of areas with vegetation is cheaper, lasting, needs less care, and generally the best and the safest[3].

For soil erosion control purposes, grasses and covering plants together are used in many areas which have favourable effects for soil erosion on stabilising side slopes. Having dense and good root structure, grasses have the ability of stabilising soil up to 20 to 25 mm depth. This effect is 40-60% for the grasses which extend their roots to 25 mm, and 60-80% for the grasses which extend their roots up to 50 mm soil depth. These areas are later supported with shrubs and trees[4].

Acar (1997) mentioned that plant species on cut-slopes generally come from those present on the upper part of the slope, but full-slopes were occupied by pioneer plants that take advantage of the medium created by new full-slopes[1].

The most important step on taking biopreventive measures for checking erosion on forest road

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slopes is the selection of suitable stabilising plant taking climatic and slope conditions into consideration. The plant species developing tap root hold excess water and prevent landslides. The species having thick stems have also preventive effects against stone rolling. In order to get effective protection on the forest road slopes, problems should be determined correctly and the required plant species should be chosen accordingly.

Capparis ovata, a prostrate shrub and *C. spinosa* found in most arid zones of Mediterranean countries are called "capers"[9,10]. These were known long ago for their medicinal qualities. They have a deep root system and short underground stem from which the branches grow. *C. ovata* can achieve 1 m in height and occupy an area of 15 m^2, with a canopy made up by four to six radial branches from which many secondary stems grow. The caper shrub is a winter deciduous species, which by mid April produces new branches and levels[9]

The capers characteristics include a plant which is adapted to poor soils, where water and nutrients are major limiting factors[10]. Native plants grow spontaneously in cracks and crevices of rocks and stone walls. Plants grow well in nutrient poor sharply-drained gravely soils. Mature plants develop large extensive root systems that penetrate deeply into the earth. Capers are more often seen hanging, draped and spiralling as they scramble over soil and rocks. Caper shrub is resistant to drought and its roots reach 40 meters down[7].

The purpose of this study is to find out the adaptation and stabilisation effects of caper shrubs at different altitudes and aspects on forest road slopes in Artvin region.

MATERIALS AND METHODS

In this study, the distribution, coverage and extend of *Capparis ovata* on forest road slopes were examined in Artvin region (Figure 1 and 2). Measurements were made on forty nine samples taken randomly from structurally different sample plots (Table 1). These sample plots in the area were selected from three elevation zones (200-300 m., 300-400 m. and over 400 m.) and four aspect groups, including various slope categories as well. Each sample plots on the slopes was considered 10 m. x 3 m. (30 m^2). In these sample plots, each species coverage was calculated following standard procedures and then cover values of sample plots were found[1,2].

For the species which are uniform and compact growth form as well as having conspicuous centre of main stem, crown area (CO, cm^2) were measured according to two perpendicular radii (r1, r2) (Figure 3, 3a):

$$CO = \pi [(r1 + r2)/4)]^2 \qquad (1)$$

For the untidy spreading species, the coordinates (X, Y) of species crown were established horizontally (X) and vertically (Y) on the different points with 10-15 cm intervals (Figure 3b). The formula is given as;

$$CO = \sum \{(Yn\text{-}1\text{-}Yn\text{-}2) + (Yn\text{-}3\text{-}Yn\text{-}4)/2]^* [(Xn\text{-}1\text{-}Xn\text{-}3) + (Xn\text{-}2\text{-}Xn\text{-}4)/2] \qquad (2)$$

The areas covered with caper shrub were classified according to the slope, aspect and altitudes and measurements taken. Statistical evaluations were conducted using "Statgraphics" programme, exploring the significance levels performed by analysis of variance (ANOVA).

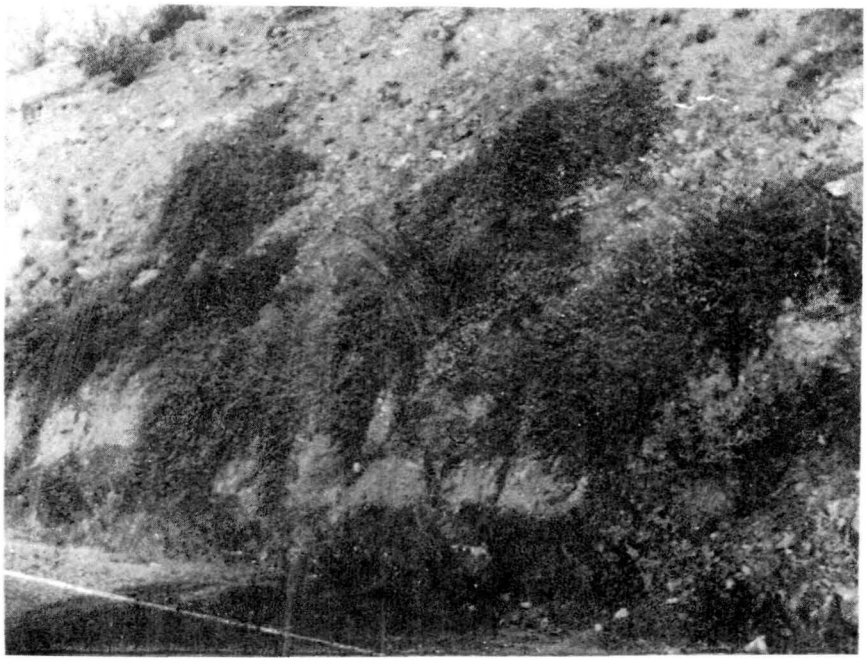


Fig.1. View of caper shrub plants on cut-slope.

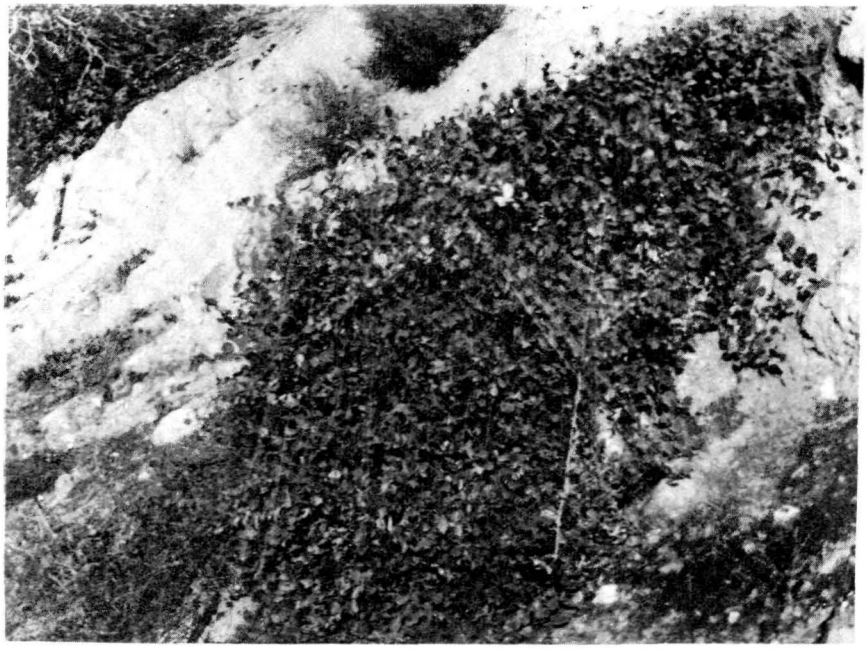


Fig. 2. Another view of caper shrub on the road.

Table 1 : Measured values of *Capparis ovata* in sample plots

Plot No.	Kind of road	Aspect	Elevatio (m)	Slope (%)	Slope type	Number of samples	Total area (m^2)	Mean area (m^2)
1	Valley	South	300	80	Cut	5	3.57	0.71
2	Valley	South	300	70	Fill	4	3.85	0.96
3	Valley	South	300	70	Fill	4	3.92	0.98
4	Valley	South	310	95	Cut	5	7.14	1.43
5	Valley	South	280	95	Cut	2	4.90	2.45
6	Valley	South	280	90	Fill	3	3.91	1.30
7	Slope	Northeast	310	90	Fill	3	2.01	0.67
8	Slope	Northeast	290	40	Fill	2	1.91	0.95
9	Slope	Northeast	280	0	Fill	2	7.57	3.78
10	Slope	Northeast	280	0	Fill	1	3.41	3.41
11	Valley	South	500	70	Cut	2	6.49	3.24
12	Valley	Southeast	350	100	Cut	2	1.12	0.56
13	Valley	West	420	0	Fill	2	5.23	2.61
14	Valley	West	450	50	Fill	5	10.63	1.93
15	Valley	Southeast	450	80	Cut	3	7.22	2.41
16	Valley	Southeast	460	80	Cut	2	2.86	1.43
17	Valley	South	505	40	Cut	2	3.99	2.00

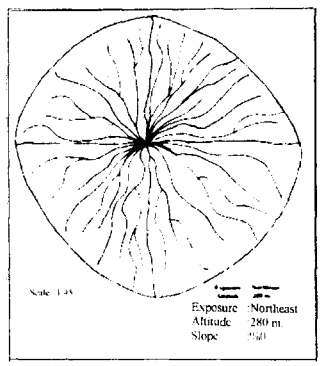
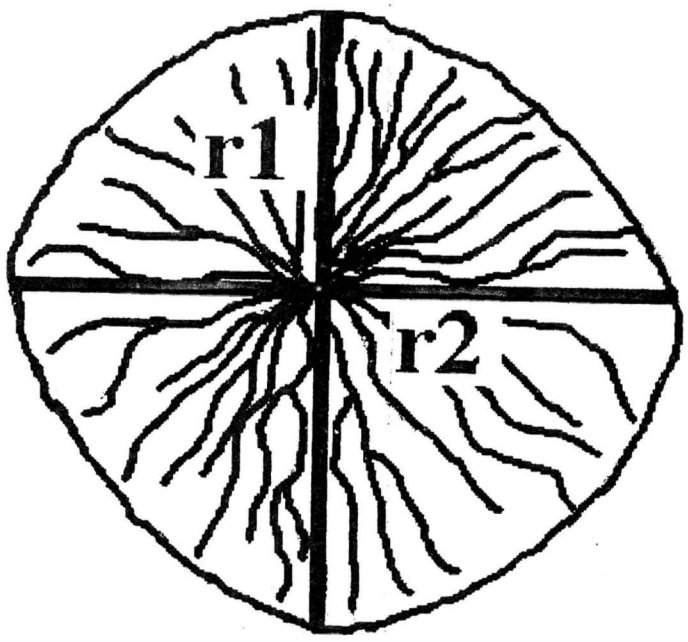
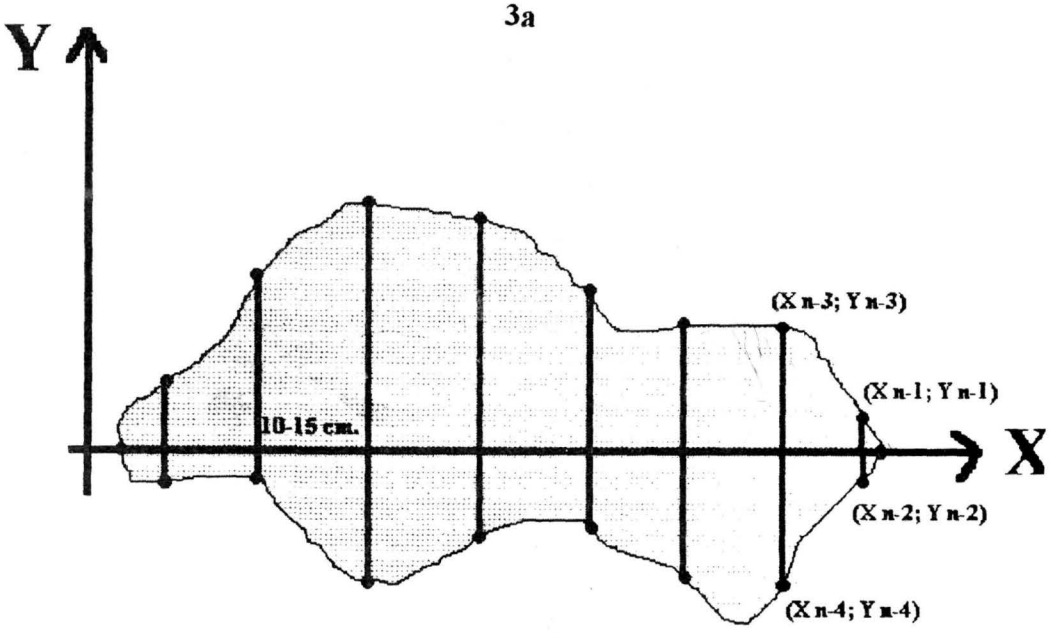


Fig. 3. *C. ovata* drawing in scale.



3a



3b

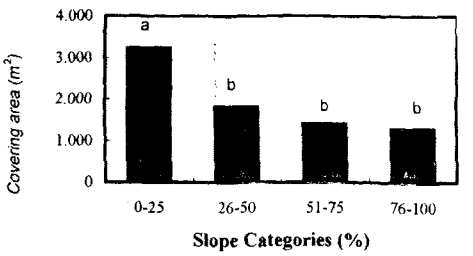
Fig. 3. Measuring of *C. Ovata* in sample plots (3a; Uniform species, 3b: Spreading species)

The areas covered with caper shrub were classified according to the slope, aspect and altitudes and measurements taken. Statistical evaluations were conducted using "Statgraphics" programme, exploring the significance levels performed by analysis of variance (ANOVA).

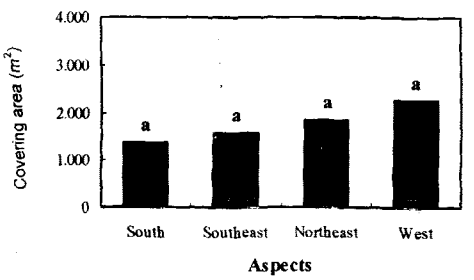
RESULTS AND DISCUSSION

The values of caper shrub on side slopes at different altitudes are given in Table 1. The values later were evaluated and compared to some environmental factors, to determine the relationships between them. In all plots, minimum coverage area was found to be 0.56 and the maximum 3.78 square meter (Table 1).

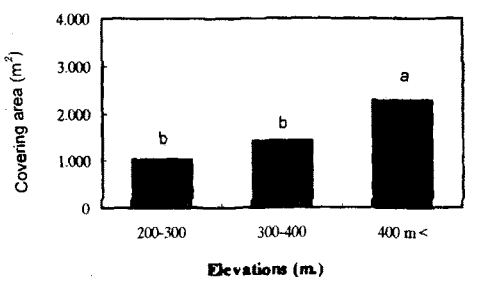
According to the results of the analysis of variance, a relationship between coverage area of the plant and slope was found clearly. The relationship was at 99% significance level (Fig.4a). To evaluate which slope groups were significantly different from each other, Duncan multiple range test was used. Results indicated that the plants in the group of 0-25% sloped areas had a better growth than others. With an increase in slope, plant coverage area decreased. Gradual decrease in soil depth may account for this.



4a



4b



4c

Fig. 4. Analysis of variance showing the relationship of caper coverage areas according to slope category (Fig.4a), aspect (Fig.4b) and elevation (Fig.4c) parameters (Values in bars with the same letter are significantly different from each other $p \leq 0.05$).

The analysis of variance revealed that the relationship between aspect and plant coverage was not significant at 99% significance level (Figure 4b). Therefore, Duncan test was not performed. It was observed that the plant species mostly spread on south facing areas than on north facing areas.

The analysis of coverage area and altitude data revealed that there was a significant relationship at α=0.01 significance level (Figure 4c). Plant coverage area was found to be higher in the 3rd altitude group (400 m.<). With increasing altitude, optimal distribution area of the plant species became nearer and this explained why this species found most in the 3rd altitude level. Caper shrub is optimally distributed at 300-1000 meters in this region.

The study results indicated that the differences between coverage areas and the inclinations of slopes are found to be directly proportional. This is explained by lesser soil depth with increasing slope.

Pugnaire and Esteban (1991) stated that *Capparis ovata* grows well on infertile lands, arid zones and unsuitable environmental conditions and its root system goes deep[9]. Ozdemir and Ozturk (1996) mentioned that the soils where *Capparis spinosa* and *Capparis ovata* naturally distributed were formed from limestone, and were rich in sandy-loam organic materials[8]. In addition, a study on *Capparis decidua* showed that this species is best when used for stabilising the dunes and wind erosion. The ratios of carbon, nitrogen and phosphor in the soil are also increased[6].

Recently, some indigenous plant species for revegetation in degraded areas of this region have received increasing attention. For this purposes, selected grasses and groundcover species were examined along roadside slopes and other areas for planting[2]. In this paper, *Capparis ovata* was observed in natural habitats regarding its growing status. It can be concluded caper shrub can be safely and effectively used on the areas in which this shrub makes its optimum distribution to stabilise the cut and full slopes. Caper can also be safely and successfully used to stabilise watersheds of dams that are susceptible to erosion. Caper shrub is preferred on these kinds of areas because it develops roots which go deeply, is long-lived and prevents surface runoff. In addition, it may bring some extra income for local people in the regions and make contribution to socio-economic structure.

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LIFE HISTORY OF *HYOPHILA INVOLUTA* (HOOK.) JAEG.

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Abstract

The studies on the life history of *Hyophila involuta* have been carried out. Plant are dioecious, form loose sods, are about 15-20 mm long and sparsely branched. The development of stem and leaf is usual and stem anatomy is simple with central strand, cortex and epidermis. However, some cells of central strand and cortex show collenchyma like thickenings. Nerve anatomy shows 'deuter' and 'steroidal' cells but no 'begleiter' cells.

Gemmae borne in clusters in the axils of leaves and stem tip serve as important means of vegetative reproduction. Archegonial development follows usual pattern and sometimes shows an isthmus-like constriction in the massive stalk.

The sporogonium development follows usual pattern and capsule is gymnostomous. The spores are spherical smooth surfaced, 6 to 15 µm in diameter and may germinate at one, two or three points simultaneously. Besides leafy buds, gemmae or tubers may also develop on protonema. The formation of few celled structures in germinating spore reminds one of such a condition in *Andreaea* and some liverworts. Absence of peristome and non-functional stomata in apophysis appear to be of secondary nature.

Key words : Life history, *hyophila involuta.*

INTRODUCTION

The studies of Bryophytes of India, particularly of Rajasthan did not get the attention they deserved. A programme on the study of Bryophytes of Rajasthan and adjoining areas had been undertaken. This included taxonomic, morphological and experimental studies of mosses and the present investigations form a part of the programme.

MATERIALS AND METHODS

The plants were collected from Mount Abu (Rajasthan) from near Bharatpur House, on way to Kodra Dam from the sides of hillocks during the months of April to September which is the normal growing period. One lot of the material was fixed in F.A.A. on the spot for abrupt killing and the other lot was brought to the laboratory in polythene bags in which a cotton swab soaked in formaline was put to avoid decay. Both the lots were then washed with water to remove all debris and fixed in F.A.A. The studies were carried out by usual microtome sectioning and whole mount method. For spore germination mature capsules were collected and stored in paper packets.

Spore germination experiments were carried out with Knop's and Beneck's nutrient media and their pH was adjusted to 5.8 before autoclaving. One lot of petriplates were constantly illuminated with 820 Lux light by two tube lights (40 W each) and a bulb (100 W) from a distance of 16 inches and the other lot was placed in the room in natural light.

OBSERVATIONS

External morphology of plant The plants form loose sods, are dioecious, each plant being straight about 15 to 20 mm long, sparsely branched (Fig. 1). The lower 3/4 of stem is

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microphyllous with radiculose base and upper with gradually larger leaves aggregated at the tip. The stem tip bears gemmae for vegetative propagation.

Mature Stem

Mature stem has a central strand and a cortical region (Figs. 2, 3). The central strand is made up of the thin-walled, narrow, vertically elongated cells showing slight thickenings at the corners like 'collenchyma' cells in transverse section. The cortical cells are relatively broader showing some inter-cellular spaces. Some of the cells in this region also show thickenings at the corner in T.S. Although the internal structure of stem of *Hyophila involuta* is broadly similar to that of *Funaria hygrometrica*[2] and other members of bryidae, the thickenings observed in the cells in *Hyophila* are not seen in other mosses.

The stem grows by means of pyramidal four-sided apical cell with three flat lateral cutting surfaces which cut off segments on its three faces regularly. The segments are cut off in angle position of apical cell resulting in the shifting of the position of the flat faces (Fig. 4) which in turn is responsible for spiral arrangement of leaves. Each segment divides transversely and later periclinally forming inner and outer cells. Inner cells contribute to central tissue and outer to the leaves, cortical region of the stem and lateral branches (Fig. 5). Thus the growth pattern of gametophyte of this species is similar to that in mosses in general[2,6].

The Structure of Mature Leaf

The mature leaf is lanceolate with a distinctly narrow base (Fig. 6). It is about 1 to 3 mm long and 0.5 to 1 mm wide with acute apex, upper margin (about 1/3) serrate and lower smooth. Teeth are 1 to 3 celled, very small, gradually diminishing in size towards base. There is no border in the leaf. Nerve is percurrent to slightly excurrent, straight, obliquely placed at the extreme tip in mature leaves.

In cross section the leaf shows a thick midrib and single layered lamina on either side (Fig. 7). The midrib is much projecting on the lower side and flat to slightly convex towards upper side. In the lamina, cells are larger, thin-walled and papillate on the upper surface. The cells of three to four rows at the margin of leaf are less papillate. In the midrib region the cells of the upper and lower epidermis are thick-walled and almost quadrate as seen in C.S. Almost along the middle of the nerve, there is curved plate of large, polygonal thick-walled cells. These cells are referred as 'deuter cells'[9] (Fig. 7). Between the upper epidermis and the deuter layer and the lower epidermis and the deuter, there are steroidal bands comprising highly thick-walled cells — the steroids or steroidal cells.

In association with leaves, 2-4 cells high, uniseriate hairs are also developed in axillary position (Fig. 8).

Development of Leaf

As is typical of all mosses the leaf grows with the help of an apical cell with two cutting faces. It cuts off two series of segments alternately and initially forms a single cell thick plate of cells about six to seven cells in length and about five cells wide (Figs. 8, 9).

Hereafter, the median cells give rise to the midrib and those towards the margin from the wing part of leaf (Fig. 8). The cells of the wing region divide only anticlinally to give rise to single cell thick wing of the leaf. The cells forming the midrib divide periclinally forming four tiers or layers of cells. Out of these tiers, the lower most forms the lower epidermis, the second from below gives

rise to lower band of steroidal cells, the third one forms the deuter layer. The cells of the upper most tier divide once more periclinally with the upper daughter cells giving rise to upper epidermis while the lower ones form the upper band of steroidal cells.

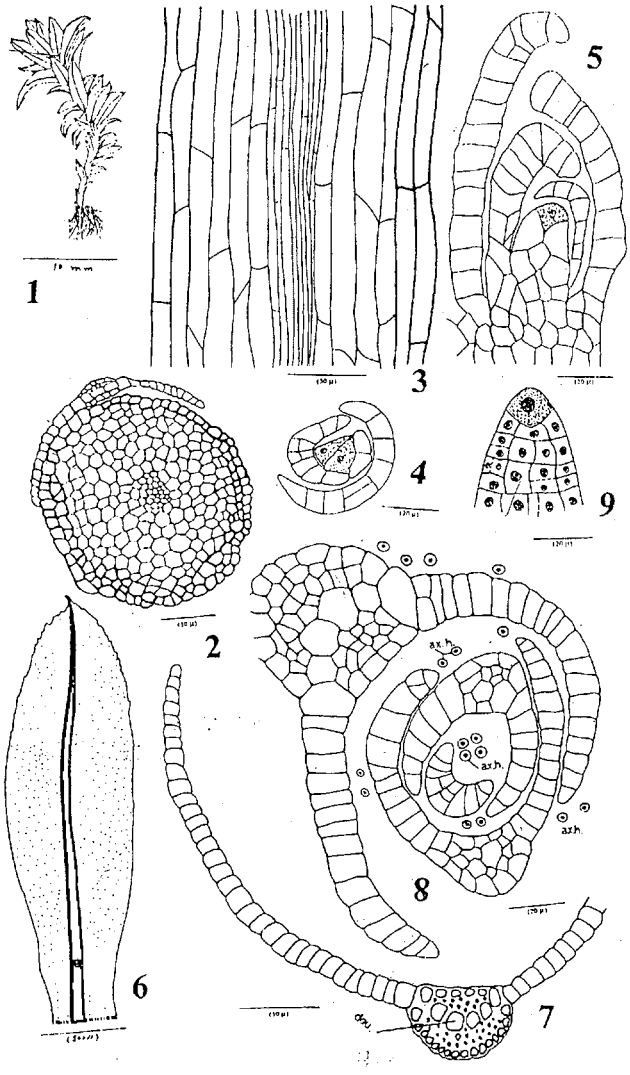

Plate I

Fig. 1, a complete plant. Fig. 2, T.S. of mature stem showing collenchyma like thickenings in some of the cortical cells. Fig. 3, L.S. mature stem. Fig. 4, T.S. stem tip showing apical cell and its segments. Fig. 5, L.S. stem tip showing apical dome with apical cell, its segments and young leaves. Fig. 6, leaf as seen from adaxial side. Fig. 7, T.S. mature leaf. Fig. 8, T.S. tip of the plant showing leaves of various ages and axillary hairs. Fig. 9, surface section of a young leaf showing an apical cell.

REPRODUCTION

Vegetative

Apart from fragmentation, gemmae are an efficient means of vegetative propagation in this species. They are borne in clusters in the axils of leaves and on the stem tip enclosed by terminal tuft of leaves. The gemma has a short or long, branched or unbranched stalk (Fig. 10). When branched each branch bears a gemma at its tip. The multicellular body of the gemma may be irregular, discoid or cylindrical in shape (Figs. 11-13).

Sexual Reproduction

Sexual reproduction is oogamous and the plants are dioecious. Unfortunately male plants at proper stages of development could not be collected and the development of male sex

organs could not be followed. However, the structures of mature organs indicate that the development is of usual normal type.

Development of Archegonium

Development of archegonium follows the usual pattern[2,5] (Fig. 14-18). Archegonium is initiated from any surface cell at the tip. It becomes protuberant and functions as archegonial initial, which divides by one or more transverse walls forming a filament of two to four cells (Fig. 14). In the terminal cell of the filament, two obliquely vertical, intersecting walls are laid and an apical cell with two cutting faces is formed (Fig. 15). This apical cell may cut off one or two segments and soon becomes three-sided by laying down of another obliquely vertical wall. At this stage, there are three peripheral cells enclosing an axial cell in a young developing archegonium at its tip. The axial cell undergoes asymmetric division forming an upper primary cover cell and a lower central cell (Fig.16). Further divisions of the central cell contribute to the neck canal cells (partly), venter canal cell and egg (Figs. 17-18). The primary cover cell functions as an apical cell for some time and contributes to the upper part of the neck of archegonium (both neck cells and neck canal cells) and ultimately divides anticlinally to form a lid or cover at the top of the archegonial neck. The peripheral cells contribute to the jacket of the venter and lower part of the neck of archegonium.

Mature archegonium

It has a long and massive stalk, a distinct swollen venter and long narrow neck (Fig. 18). The stalk is made up of uniform cells like that in most mosses. Jacket in venter is two to three cells thick and about 15 to 20 cells in perimeter. Jacket in neck region is mostly six cells in perimeter but some times at places it may be seven cells also. Occasionally an isthmus like construction is observed between venter and stalk (Fig. 21).

Fertilization results in the formation of zygote in the venter of archegonium (Fig. 19).

Development of Sporophyte

The zygote divides by transverse wall forming an upper epibasal and a lower hypobasal cell (Fig. 20). An apical cell with two cutting faces is established by two oblique intersecting divisions in each of these cells (Figs. 21, 22). The activity of the apical cell of hypobasal region results into the formation of short acuminate foot, which pierces into the stem tip and absorbs nourishment. The apical cell cuts off two series of segments on its two faces regularly. Further division of segments are not very regular. A segment may first divide by median wall (Fig. 23) and daughter

cells undergo quadrate type of divisions as is the case in the segments of epibasal region. A few more divisions in these cells result in the formation of foot composed entirely of thin-walled parenchymatous cells (Figs. 24, 25). A fully developed foot shows differentiation into a central region of narrower thin walled cells with scant contents and peripheral region of relatively larger cells with dense contents. It is felt that these surface cells or peripheral cells serve as absorptive tissue and absorb nourishment from the gametophyte by direct contact.

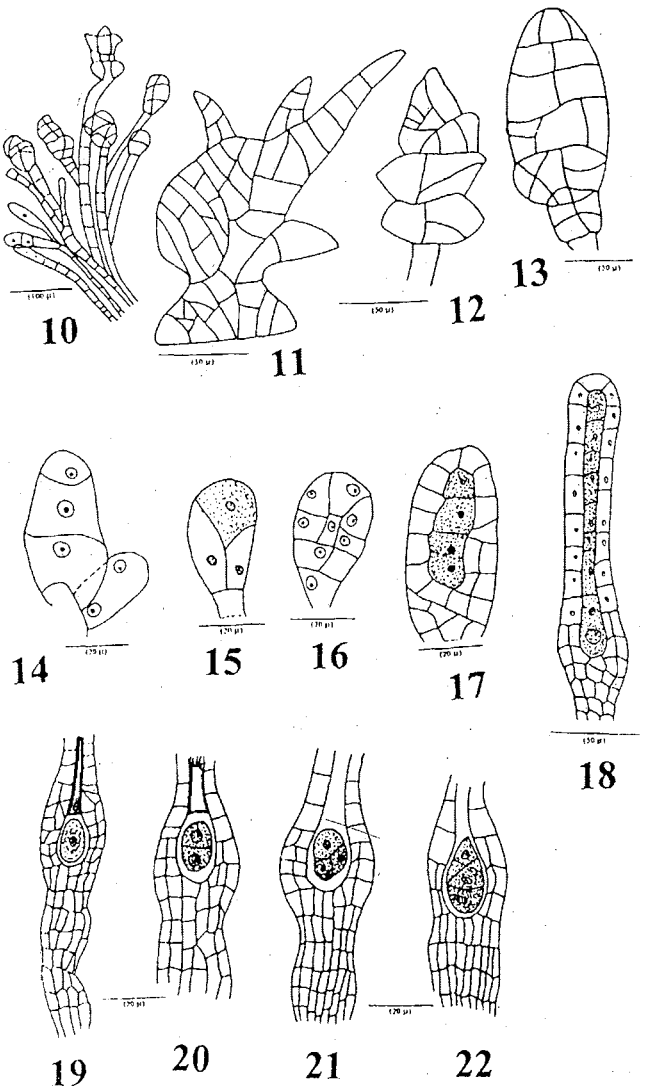

Plate II

Fig. 10, a cluster of gemmae with long branched stalks. Figs. 11-13, different forms of gemmae. Figs. 14-18, various stages of development of archegonium. Fig. 19, oospore in the cavity of venter of archegonium. Fig. 20, oospore divided into epibasal and hypobasal cell. Figs. 21, 22, young embryos in the venter of archegonium showing lower and upper apical cell respectively.

The activity of apical cell of the epibasal region results into the formation of seta and capsule. This apical cell cuts off two series of segments on its two faces alternately in a regular manner. The division of these segments follow a very regular order and almost with mathematical precision. Each segment divides by a median vertical wall to form two cells resulting into four cells arranged in a quadrate manner (Fig. 26). Each of these cells now divides either periclinally or by quadrate type of divisions to form two embryonic zones - amphithecium and endothecium (Figs. 27, 28).

The amphithecial cells then divide periclinally to form an inner layer of cells, which may be designated as Ist amphithecial layer and outer layer of cells. Cells of the outer layer then divide anticlinally to form 16 cells. Some times there may be an additional anticilinal division in one or more of the cells of outer amphithecial layer so that the number of cells in the outer most ring may be more than sixteen (Fig. 29). The cells of outer amphithecial layer divide once again periclinally and then anticlinally in systematic way to form 2 to 3 layered amphithecial region. The four endothecial cells also divide a few times in a regular order. These cells once again divide by quadrate type of divisions mostly, but occasionally they may divide periclinally also. These divisions are then followed by more divisions in other planes.

Seta

Up to the time, the capsule is not differentiated, the products of the upper apical cell (i.e. amphithecial and endothecial cells) contribute to the formation of seta (Fig. 30).

The structure of mature seta is comparable to that of stem except that there are no thickenings at the corners of the cells in the central region and cortical region. The central strand is made up of thin-walled, elongated cells, while cortex is having broad, isodiametric parenchymatous cells. In the older part of seta a layer or two of cortical cells inside the epidermis become thick-walled and is referred to as hypodermis. This region provides mechanical strength. After the required length of seta is formed the cells at the tip region mature to give rise to capsule.

Development of Capsule

The sporogonium now stops growing in length and tip swells, internal tissue differentiation takes place and three parts of the capsule i.e. operculum, theca and apophysis are formed.

Operculum

Operculum is the distal most part of capsule. It is conical, long beaked, about ½ of the length of capsule, slightly curved and with a distinct ring at the base (Fig. 31).

In this region the cells of the amphithecium and endothecium cease to divide and simply increase in size. The outer and radial walls of the outer most layer of cells become heavily thickened forming stiff and hard epidermal layer of operculum (Fig. 32). All the cells inner to epidermis both of amphithecium and endothecium remain thin-walled parenchymatous. These parenchymatous cells shrivel and break down at maturity.

Peristome

Peristome teeth are absent. A ring of thin walled papillate cells is present at the rim of capsule (Fig. 33).

Body or Theca

It is one-half of the length of the capsule and is 1.5 to 2.5 mm long and about 0.5 mm wide.

Transverse and longitudinal sections show that there is a central columella, surrounded by a spore sac and an air space with trabeculae. The archesporium appears to be arising from the outer-most layer of endothecium and becomes 2-3 cells thick (Fig. 34). The rest of the endothecium contributes to the columella and inner wall of the spore sac. The outer wall of the spore sac, the air space and jacket of the capsule develop from amphithecium. The outermost layer of cells of the jacket constitutes the epidermis. Outer and radial walls of epidermal cells are heavily thickened and the capsule thus has smooth surface.

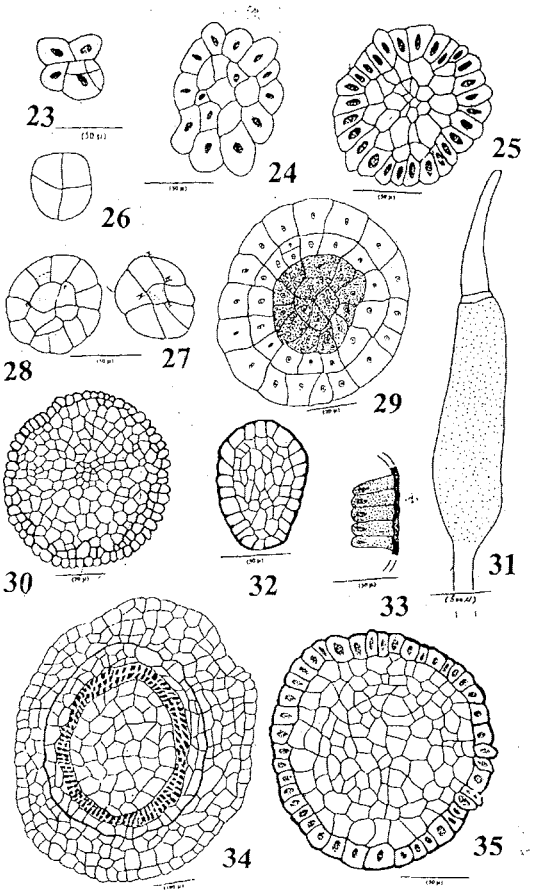

Plate III

Figs. 23-25, transverse sections of foot region of the sporogonium showing sequence of further development of segments formed by apical cell. Figs. 26-29, transverse sections of the apical region of sporogonium showing regular divisions in amphithecial and endothecial regions. Fig. 30, T.S. of mature seta. Fig. 31, complete capsule showing different regions (w.m.). Fig. 32, transverse section of opercular region of capsule showing thickened hard epidermal cells and parenchymatous inner cells. Fig. 33, thin-walled papillate cells present at the rim of capsule. Fig. 34, T.S. body region of young capsule showing 2 to 3 cells thick archesporial tissue. Fig. 35, T.S. of capsule through apophysis region showing stomata.

Apophysis

The basal region of the capsule, representing the apophysis is not clearly demarcated from the body. The epidermis in the apophysis region is provided with stomata (Fig. 35). The guard cells of stomata are slightly papillate, but there is no stomatal aperture. The substomatal cavity has also not been seen. This shows that the stomata are non-functional. Inside the epidermis all the cells are thin-walled parenchymatous.

The act of fertilisation provides stimulus for the development of sporogonium and also to the cells of jacket of venter of the archegonium which become meristematic and form protective covering called calyptra around the young developing sporogonium. The calyptra is 4 to 6 cells thick and its cells are thick- walled, more so in the outer layers.

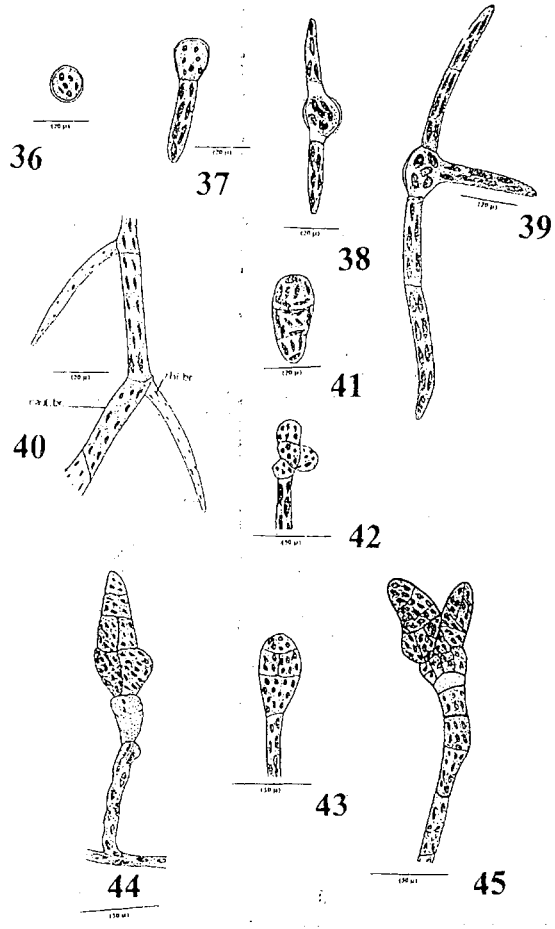

Plate IV

Figs. 36-45 (w.m.); Fig. 36, spore. Figs. 37-39, germinating spores showing germination from one, two and three points respectively. Fig. 40, part of protonema showing caulonemal and rhizoidal branches. Figs. 41-45, different forms and shapes of gemmae developed on protonema. Fig.45, a germinating gemma.

Spore and its germination

The spores are spherical, smooth surfaced, measuring 6 to 15 µm in diameter with a two-layered spore coat (Fig. 36).

Germination of spores is broadly on the usual lines and spore may germinate from one, two or three points (Figs. 37 - 39). The germination starts in one to three days' time and a fully developed protonema is formed in about 8 to 11 days. The protonema has usual three types of branches; (i) The chloronemal branches, (ii) caulonemal branches and (iii) rhizoidal branches (Fig. 40). The important feature which needs to be emphasised is that when the spores are germinating in full Knop's and full Benecke's nutrient solution, occasionally the spore divides a few times to give rise to 'gemma-like' or 'palmella-like' structure and from the cells of this multicellular mass, filaments are then given out (Figs. 41-43). Secondly the protonema besides forming leafy buds on its branches also gives rise to gemmae or tubers of various forms and shapes (Figs. 44, 45). This is particularly so when the cultures are relatively in drier medium and temperature is around 20°C to 21°C. The details of gemmae with regard to their form, shape and development are similar to what has already been described in *Semibarbula orientalis*[8].

In laboratory cultures gemmae germinated after 50 to 60 days in Benecke's liquid medium. Leafy buds are formed on the protonema as usual.

DISCUSSION

Hyophila involuta (Hook). Jaeg. belongs to subfamily Barbuloidae of family Pottiaceae which has six subfamilies, the other subfamilies being Cinclidotoidae, Pottioidae, Trichostomoidae, Eucladioidae and Leptodontoidae.

During the spore germination in *Hyophila involuta* quite often the spore divides a few times to form a few-celled mass, before the filaments are given out. This happens in conditions of more nutrition (i.e. in full Knop's or full Benecke's solution) as is observed in *Timmiella dimunata* as well[7]. The formation of a mass of cells before the filament is formed is normal feature in *Andreaea* among the mosses and among the liverworts in such members, where spore germination is precocious. Whether this feature has some phylogenetic value or is simply the result of nutritional conditions, remains to be seen. It is felt that studies on spore germination of different members of different moss groups, in different media, may throw more light on this point.

In the drier condition of medium, the protonema develops many gemmae or tubers of various forms and shapes. Such gemmae develop in *Timmella dimunata*[7] as well, in similar condition, however, the gemmae are more numerous in *Hyophila involuta*. Andrews and Redfearn[1] reported the formation of gemmae in *H. tortula* due to changed environment. Chopra and Rawat[3] interpreted the production of gemmae on the protonema of *Bryum klinggraeffii* as an inherent potentiality of gemmiferous mosses. It would be worthwhile to study this point further to decide, whether it is the inherent tendency or the effect of environment or both, which is responsible for the production of gemmae on protenama.

The leaf is ovato-oblong to lanceolate in outline without a distinct leaf base. The nerve anatomy shows deuter layer and steroidal bands, but there are no begleiter cells. It will be interesting to study the anatomy of nerve in other members of different subfamilies, to find out if it can be used as one of the diagnostic features.

The developmental pattern of the stem is broadly the same as described for other mosses. However, in *Hyophila involuta* in the mature stem, some cells in the central and inner cortical region are 'collenchyma' like in nature with slight thickenings at the corners as seen in transverse section. Stem anatomy of some of the larger and robust mosses, like members of family Polytrichaceae and Dawsoniaceae is much complicated and shows regions, like hydrome cylinder, leptome, leptome mantle, etc.[5,9] In most other mosses, the stem shows a central strand of narrow thin-walled parenchymatous cells and a cortex of larger thin-walled cells[2,4,6]. A few layers of cortical cells next to the epidermis may become thick-walled depending upon the age of axis. Even in members of Polytrichaceae, like *Atrichum*[4] and *Oligotrichum*[6] where the plants are small and delicate, stem anatomy is like any other moss e.g. *Funaria*[2]. The presence of 'collenchyma' like cells in the stem of *Hyophila involuta* is thus quite interesting. It appears to be an intermediate stage between highly developed and simple type of anatomy. The archegonial development is on the same lines as in most mosses. The segments of the apical cell, contributing to the stalk follow different patterns (e.g. antheridial and sporogonial pattern) for the formation of central and peripheral cells. This lends support to the idea of homology of male and female sex organs and the homology between sex organs and sporogonium. Stalk of the archegonium is long and massive and there is slight constriction between the stalk and the venter. However, there are no thick-walled cells in the stalk, unlike that observed in *Timmiella dimunata*[7].

Sporogonial development is normal. The mature capsule, however, is gymnostomous. The absence of peristome appears to be a secondarily achieved character. It is supported by the presence of non-functional stomata in the apophysis region of the capsule.

CONCLUSION

The formation of gemmae during spore germination in varying environments appears to be an inherent property in gemmiferous mosses as suggested by Chopra and Rareat[3] also. Anatomy of leaf and the presence of some collenchyma like cells in the cortex and central strand of stem are characters which are intermediate between the highly developed and simple mosses and may have some phylogenetic value.

The concept of homology between sex organs and that between sex organs and sporogonium is further strengthened.

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Abbreviations used

ax. h., axillary hair; caul. br. caulonemal branch; deu., deuter cells; Rhi. br., Rhizoidal branch.

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CHEMICAL CONSTITUENTS OF SOME MOSSES OF MAHABALESHWAR

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Abstract

An analysis of the chemical constituents of *Diaphanodon procumbens* (C. Muell.) Ren. et Card., *Meteoriopsis ancisteroides* (Ren. et. Card.) Broth. and *Pinnatella alopecuroides* (Hook.) Fleisch. has revealed the relative concentration of various elements as $Cu^{++} > Ni^{++}$, Fe^{++} > than K^{+} and Na^{+} in the plant materials. Since these taxa are epiphytic. the up take of inorganic elements is from atmospheric dust and leachates dissolved in the rainwater which flows over these taxa.

Key words : Mosses, Mahabaleshwar, *Diaphanodon procumbens Meteoriopsis ancisteroides Pinnatella alopecuroide.*

INTRODUCTION

Mosses have gained a considerable importance due to their applied value in medicine, agriculture, and pollution monitoring[1]. Their chemical constituents, particularly organic, have been the main focus of attention of several investigators in different parts of the world. Their inorganic constituents, however, have not received the deserved attention as evident from only a limited study in that direction[5-10].

The present study is taken up with a view to determine the elemental content of mosses collected from Mahabaleshwar (a hill station of Maharashtra), located in Western ghats.

MATERIALS AND METHODS

Collections were made from several localities - Sunset Point, Arthur's Seat, Old Mahabaleshwar, Lingmala, from Mahabaleshwar 17°56' N and 74°40'E). The average altitude of this site is 1350m. (4500 ft. approx.) and annual rainfall is 6250 mm.

PREPARATION OF SAMPLES

The plant material was collected alongwith the substratum. Substratum attached to the plant material was removed by washing with distilled water and collected separately. The plant material was dried in folds of blotting sheets and then kept in shade for natural drying. The known amount of each sample was then kept in muffle furnace at 450 - 470°C to make the ash. The ash was dissolved in a mixture of HCL and HNO_3 (3:1) and diluted with distilled water to make the final volume to 500 ml. This served as the stock solution for the estimation of various elements. The instrument used was Uv-2000 spectrophotometer to analyse Cu^{++}, Ni^{++}, Fe^{++}, PO^{-3}_4. For the estimation of Na^{+} and K^{+}, flame photometer was used.

Estimation of Copper (Cu^{++}).

5 ml. of liquor Ammonia and 5 ml of solution diethyldithio carbonate was added to the stock

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solution of 50 ml. The O.D. was taken at 430 nm on uv 2000 spectrohotometer. The concentration in ppm of the element was calculated using standard graph.

Estimation of Nickel (Ni^{++})

25 ml. of 1 molar HCl and 5 ml of Bromine water were added to the stock solution of 50 ml. 10 ml. of conc. NH_4OH was carefully added in the cooled solution and there after to this 10 ml. of Dimethylglyoxime was added. The O.D. was taken at 445 nm. The concentration in ppm was calculated using standard graph.

Estimation of Iron (Fe^{++})

2 ml of conc. HCl and 1 ml of hydroxylamine hydrochloride was added to the stock solution of 50 ml. The solution was boiled to reduce the volume to half. 10 ml of ammonium acetate buffer and 1 ml of Phenanthroline solution was added to the cooled solution. After 10 minutes, O.D. was taken at 510 nm. The concentration in ppm was calculated using standard graph.

Estimation of (PO^{-3}_4)

5 ml of sodium molybdate solution and 2 ml of hydrazine sulphate was added to the stock solution of 50 ml. Then the flask containing solution was kept in water bath for 10 minutes and after cooling the solution, O.D. was taken at 830 nm. The concentration was calculated in ppm using standard graph.

OBSERVATIONS AND RESULTS

The results obtained from the present study are given in Table-1 and the information available in the literature is summarised in Table -2.

Table-1: Chemical constituents in plant material (plant) and substratum(Sub.)

Name of the taxon	Elemental Concentration in ppm												
	Cu^{++}		Ni^{++}		Na^{+}		K^{+}		Fe^{++}		PO^{-3}_4		
	Plant	Sub	Plant	Sub	Plant	Sub	Plant	Sub	Plant	Sub	Plant	Sub	Sub
Diaphanodon procumbens (C.Muell.) Ren.et.Card.	13	26	1.2	2	100	250	300	600	620	1400	93	180	180
Meteoriopsis reclinata (C.Muell.) Fleisch.	39.2	81.4	0.8	1.6	60	100	530	1009	421	600	104	150	150
Meteoriopsis anicistrodes (Ren. et.Card.)Broth.	23	45.3	1.39	2.00	105	207	505	890	739	1700.7	152	296	296
Pinnatella alopecuroides (Hook.)Fleisch	15.82	29.7	0.97	1.9	220	421	540	945	316.4	619	166	280	280

Table-2 : Comparative data about the elements investigated by previous workers and in present investigation.

Contents	Huneck(in Schuster 1983) Musci (Mosses)		Sharma (1987) Jungemaniales(leafy liverworts)		Present investigation (Mosses)	
	Plant (Conc.in ppm)	Sub (conc.in ppm)	Plant (Conc.in pm)	Sub (Conc.in pm)	Plant (Conc.in ppm)	Sub (Conc.in ppm)
Cu^{++}	5.211	Not detected	4.3-62.5	4.0-48	13.-39.2	26-81.4
Fe^{++}	78-2500	"	480-13230	960-26220	316.4-739	600-1700.7
Ni^{++}	Not detected	"	Not detected	Not detected	.8-1.39	1.6-2.0

As evident from Table 1, the relative concentration of trace elements in *Diphanodon procumbens* (C. Muell) Ren. et. Card. is $Cu^{++} > Ni^{++}$ and the concentration of Fe^{++} is more than that of K^+ and Na^+. Concentration of PO^{-3}_4 is 93 ppm in the plant material and 180 ppm in the supporting substratum.

The relative concentration of trace elements in *Meteoriopsis reclinata* (C. Muell.) Fleisch. is $Cu^{++} > Ni^{++}$ and the concentration of K^+ is more that of Fe^{++} and Na^+. Concentration of PO^{-3}_4 is 104 ppm in the plant material and 150 ppm in the supporting substratum.

The relative concentration of trace elements in *Meteoriopsis ancistrodes* (Ren. et Card.) Broth. is $Cu^{++} > Ni^{++}$ and the concentration of Fe^{++} is more than that of K^+ and Na^+. The concentration of PO^{-3}_4 is 152 ppm in the plant material and 296 ppm in the supporting substratum.

In *Pinnatella alopecuroides* (Hook). Fleisch., the relative concentration of trace elements is $Cu^{++} > Ni^{++}$ and the concentration of K^+ is more than that of Na^+ and Fe^{++} and concentration of PO^{-3}_4 is 166 ppm in the plant material and 280 ppm in the supporting substratum.

Since all the investigated taxa are epiphytes, it is logical to conclude that their mineral content is drawn from atomspheric dust and leachates dissolved in rain water which flows over these epiphytic taxa.

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PALYNOLOGICAL STUDIES ON SOME MOSSES OF WESTERN GHAT UNDER SEM

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Abstract

Palynological study, using SEM, is made in five moss taxa. Of these taxa, *Entodon prorepens* (Mitt.) Jaeg. has non-aperturate spores. In the remaining 4 taxa i.e. *Hymenostylium recurvirostre* (Hedw.) Dix., *Octoblepharum albidum* Hedw., *Hyophila involuta* (Hook.) Brid. and *Macromitrium sulcatum* (Hook.) Brid., the spores are aperturate. Wide differences in the ornamentation of the sporoderm (tuberclate, granulate, tubercles mixed with granules) are also observed in the five taxa.

Key words : Palynological studies, mosses, Western ghat, SEM.

INTRODUCTION

The palynological study of mosses has not received its deserved attention in our country. A few studies that were made earlier, essentially pertained to the spores of liverworts alone[1,6,8]. In mosses, SEM and TEM studies were made by Olesen and Mogensen[5] with promising results. The present authors (Menon, 1992 unpublished thesis; Dabhade and Menon[2]) have made extensive studies on the spores of mosses of Western Ghats. Presently, the results of our study on five moss taxa is reported here.

MATERIALS AND METHODS

The fruiting material with ripe spores were collected from Mahableshwar. It was brought to Mumbai, purified in the laboratory to ensure freedom from mixtures with other taxa. The spores were studied by the same technique which is outlined earlier[2]. The photomicrographs were taken at the magnification indicated in the figures. The vouchers are deposited in the Herbarium of the Department of Botany, College of Arts and Science, Thane, Maharashtra, India.

OBSERVATIONS AND RESULTS

***Octoblepharum albidum* Hedw.** (Microphotograph No.1)

This epiphytic moss, easily recognized by its whitish-green tufts and characterised by oblong-lingulate leaves, broad nerve showing single-layered triangular chlorocysts (in T.S.), was collected from Mahabaleshwar .It was found growing in the hollow cavities of tree trunks.

Palynological characteristics:

Under L.M. : Spores spherical to oval, 15-18μm in diameter, yellowish brown.

Under SEM : Spores with apertural depression showing scanty ornamentation Sporoderm tuberculate with tubercles of various size and loosely placed.

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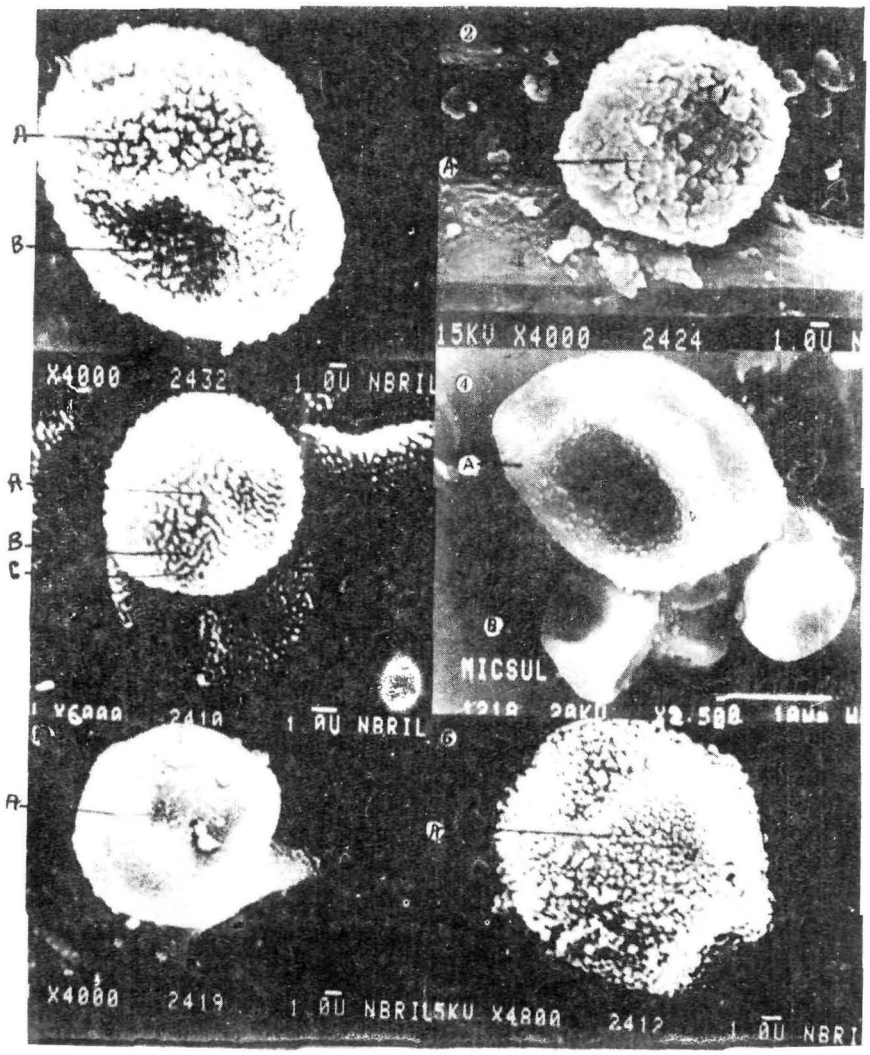

Explanation of Microphotographs No.1-6 of Spores of Mosses under SEM.

1. *Octoblepharum albidum* Hedw. (X4000)
   A - Varied size tubercles; B - Apertural depression
2. *Hymenostylium recurverostre* (Hedw.) Dix. (X4000)
   A - Apertural depression with dense tubercles on spore
3. *Hyophila involuta* (Hook.) Brid. (X6000)
   A - Distinct tubercles; B - Fused tubercles; C - Striations
4. *Macromitrium sulcatum* (Hook) Brid. (X2500)
   A - Macrospore with distinct tubercles (Megagrain); B - Microspore (micrograin)
5. *Macromitrium sulcatum* (Hook.) Brid. (X4000)
   A - Macrospore i.e. Megagrain showing tubercle to centre and periphery.
6. *Entodon prorepens* (Mitt) Jaeg. (X4800)
   A - Non-aperturate, sporoderm with irregular tubercles intermixed with granules.

***Hymenostylium recurvirostre* (Hedw.) Dix.** (Microphotograph No.2)

This taxon prefers calcareous substrata. It was found growing in rust-brown tufts at Arthur Seat Point, Mahableswar. The opaque, densely papillose leaf cells, and systylious condition of the operculum help easy distinction.

Palynological characteristics:

Under L.M. : Spores spherical to oval, 9-12 μm in diameter, brownish orange.

Under SEM : Spores aperturate. Sporoderm beset with densely packed and fused tubercles.

***Hyophila involuta* (Hook) Brid.** (Microphotograph No.3)

This taxon, growing luxuriantly at Malcom Peth Bazar, Mahabaleshwar, is found to occur in shade and relatively exposed localities both at low and high altitude. Once familiar, it is readily identified by the ligulate or spathulate leaves, mamillose leaf cells and gymnostomous capsules.

Palynological characteristics:

Under L.M. : Spores spherical to oval, 7-10 μm in diameter, yellowish brown.

Under SEM : Spores with fewer tubercles at apertural region, tubercles loosely scattered, wart-like, forming striations at certain places.

***Macromitrium sulcatum* (Hook.) Brid.** (Microphotograph No. 4 & 5)

This corticolous taxon was found growing luxuriantly on the branches of trees in Pratap Singh Garden, Mahabaleshwar. It has creeping stem with erect branches and sulcate capsules.

Palynological Characteristics:

Under L.M. : Anisosporous, spores spherical, 9μm in diameter, brownish yellow.

Under SEM : Heterosporous, microspores 9μm; macrospores 17μm - 23 μm in diameter. Apertural depression present. Sporoderm granular with few large tubercles.

***Entodon prorepens* (Mitt.) Jaeg.** (Microphotograph No.6)

The double-nerved ovate-oblong to lanceolate leaves with acute apex, oval to linear smooth upper laminal cells, the basal ones elongate with narrow curved lumens with single large tubercular papillae distinct alar cells; erect and distinctly sulcate capsules containing markedly unequal spores characterize this taxon.

Palynological observations:

Under L.M. : Spores spherical to oval, 14 μm - 20 μm in diameter, yellowish brown in colour.

Under SEM : Spores non-aperturate. Sporoderm tuberculate with intermixed small granules.

Palynological parameters viz. aperture type, size and the architectural designing of the sporoderm provide valuable clue for taxonomic and phylogenetic purpose. The apertural

region in bryophytes is known to be proximal as observed in the tetrad stage[3-4]. Apertural depression was observed in *Macromitrium sulcatum* (Hook.) Brid., *Hymenostylium recurvirostre* (Hedw.) Dix., *Hyophila involuta* (Hook.) Brid., and *Octoblepharum albidum* Hedw. Heterospory - presence of microspores and macrospores is observed in *Macromitrium sulcatum* (Hook.) Brid. Phylogenetically, ornamentation which serves to provide increased protection of the protoplasm of the spore, is considered as primitive. It would, therefore, appear that ornamentation formed of excrescences is primitive and those formed of depressions are advanced[3-4]. Dense excrescences observed in *Hymenostylium recurverostre* (Hedw.) Dix., *Hyophila involuta* (Hook.) Brid., *Octoblepharum albidum* Hedw. *Entodon prorepens* (Mitt.) Jaeg. are considered as primitive while depression-type with granular ornamentation observed in *Macromitrium sulcatum* (Hook.) Brid. is considered an advanced feature. Additionally, heterospory in *M. sulcatum* (Hook.) Brid. is also regarded as a derived feature.

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CHECKLIST OF AQUATIC AND SEMI AQUATIC BUGS (HEMIPTERA: INSECTA) OF CHANDIGARH

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Abstract

Enumeration of 30 species belonging to 17 genera spread over 9 families of aquatic and semiaquatic bugs from Chandigarh alongwith key to families is included in this paper.

Key words : Aquatic bugs, Chandigarh.

INTRODUCTION

The aquatic and semi aquatic groups of insects are over all indicators of long term environmental conditions and constitute integral components of almost all freshwater communities[3-5,7]. Due to their poor dispersal capability these insects also serve as Zoogeographical indicators[2]. Their role in biological control of mosquitoes need hardly be emphasised. Inventorisation of aquatic insects becomes imperative to understand the functional aspects of community structure in any aquatic ecosystem, which further provides information on energy flow within the respective ecosystem[1].

A total of 3558 described species of aquatic and semiaquatic bugs are known from the world, of which 7.5% (269 species) occur in India, occupying diverse ecological niches ranging from salt water pools to mountain lakes, from hot springs to torrential streams, rivers and oceans[6].

The present study embodies the result of collections of aquatic and semi-aquatic hemipterans made from various habitats such as, Sukhna lake, Nepli, Kansal and adjoining areas, temporary and permanent pools and puddles, Sukhna Choe and Patiala Rao around Chandigarh. A list of 30 species belonging to 17 genera spread over 9 families is presented here alongwith a key to families.

List of aquatic and semi-aquatic hemipterans recorded from Chandigarh

Suborder : Heteroptera

Infraorder : Gerromorpha

Family : Gerridae

Subfamily : Gerrinae

1. *Aquarius adelaidis* (Dohrn)
2. *Neogerris parvula* (Stal)
3. *Limnogonus (Limnogonus) fossarum fossarum* (Fabricius)
4. *Limnogonus (Limnogonus) nitidus* (Mayr)

Subfamily : Halobatinae

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5. *Metrocoris communis* (Distant)

Subfamily : Trepobatinae

6. *Naboandelus signatus* Distant

Family : Veliidae
Subfamily : Microveliinae

7. *Microvelia (Microvelia) diluta* Distant

8. *Microvelia (Microvelia) douglasi* Scott

Family : Mesoveliidae

Subfamily : Mesoveliinae

9. *Mesovelia vittigera* Horvath

Family : Hydrometridae

Subfamily : Hydrometrinae

10. *Hydrometra greeni* Kirkaldy

Infra Order : Nepomorpha

Family : Notonectidae

Subfamily : Anisopinae

11. *Anisops barbatus* Brooks

12. *Anisops campbelli* Brooks

13. *Anisops cavifrons* Brooks

14. *Anisops exiguus* Horvath

15. *Anisops nigrolineatus* Lundblad

16. *Anisops sardeus* Herrich – Shaffer

17. *Anisops waltairensis* Brooks

Subfamily : Notonectinae

Tribe : Notonectini

18. *Enithares lineatipes* Horvath

Family : Nepidae

Subfamily : Ranatrinae

Tribe : Ranatrini

19. *Ranatra elongata* Fabricius

20. *Ranatra filiformis* Fabricius

Subfamily : Nepinae

Tribe : Nepini

21. *Laccotrephes griseus* (Guerin-Meneville)

22. *Laccotrephes ruber* (Linnaeus)

Family : Belostomatidae

Subfamily : Belostomatinae

23. *Diplonychus annulatus* (Fabricius)

24. *Diplonychus molestus* (Dufour)

25. *Diplonychus rusticus* (Fabricius)

Family : Belostomatidae

Subfamily : Lethocerinae

26. *Lethocerus indicus* (Lepeletiler & Serville)

Family : Pleidae

27. *Paraplea frontalis* (Fieber)

Family : Corixidae

Subfamily : Corixinae

Tribe : Agraptocorixini

28. *Agraptocorixa hyalinipennis hyalinipennis* (Fabricius)

Subfamily : Micronectinae

29. *Micronecta quadristrigata* Breddin

30. *Micronecta scutellaris scutellaris* (Stal)

Key to the families of aquatic and semi aquatic hemiptera of Chandigarh

1. Antenna conspicuous and visible in dorsal view; inserted in front of eyes, and longer than head lives on the surface of water, walking or skating; wingless &winged form..... **2**

-- Antenna hidden, not visible in dorsal view and hidden; very much shorter than head; lives in submerged water; always winged forms..... **5**

2. Claws of atleast front tarsi inserted before its tip..... **3**

- Claws of all legs inserted at the tip..... **4**

3. Hind femora long, passing end of abdomen; middle legs inserted closer to hind legs than to front legs; head with out a median groove or a line..... **Gerridae**

Key to families.....of Chandigarh (Adopted and modified from Thirumalai[6],1999.)

-- Hind femora short, not passing end of abdomen; middle legs inserted almost midway between hind and front legs; head with a median groove or a line..... **Veliidae**

4. Stick like insect, body long, head as long as entire thorax; antenna and legs thread like, always winged form, simple ovipositor and not saw- like..... **Hydrometridae**

-- Small, slender, body short, head shorter than thorax; antenna and legs not thread like, saw- like ovipositor, winged and wingless forms..... **Mesoveliidae**

5. Rostrum short, triangular, not distinctly segmented; front tarsi single segmented, scoop-like, fringed with setae..... **Corixidae**

-- Rostrum long, cylindrical, distinctly segmented; fore tarsi one or more segmented, simple without setae..... **6**

6. Oblong, oval or elongate forms with out abdominal appendages, swims upside down..... **7**

-- Flattened forms with or with out abdominal appendages, swims normally with back towards the water surface..... **8**

7. Elongate forms with long ore-like hind legs; hind tibia and tarsi ciliated, abdomen with a mid ventral keel..... **Notonectidae**

-- Oval forms, all legs simple and alike, hind tibia and dorsi not ciliated, abdominal ventral with out keel ..... **Pleidae**

8. Tip of the abdomen with a long and slender siphon..... **Nepidae**

-- Tip of the abdomen with out a distinctive pair of flat retractile strap-like appendage; size ranges from 15 - 100 mm..... **Belostomatidae**

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EFFECT OF DIFFERENT CONCENTRATIONS OF *LANTANA CAMARA Linn.* EXTRACT ON SPORE GERMINATION OF *PHYSCOMITRIUM JAPONICUM* (Hedw.) Mitt. IN HALF KNOP'S LIQUID CULTURE MEDIUM AND DOUBLE DISTILLED WATER

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Abstract

Leaf extract of *Lantana camara* inhibits maximum spore germination followed by stem and root extracts. Allelochemicals are present in increasing order in root, stem and leaves, respectively. Extract diluted with double distilled water was more inhibitory than the extract diluted by Half Knop's liquid culture medium. Spore germination is directly proportional to time period (no. of days) and inversely proportional to the concentration of allelochemicals in the extract.

Key words : Allelopathy, inhibition, spore germination, *Lantana camara.*

INTRODUCTION

According to Rice[5], allelopathy includes both inhibitory and stimulatory biochemical interactions between plants including fungi and microorganisms. Rice[4] reviewed studies on allelopathy among terrestrial plants. Recently, Narwal and Tauro[3] complied all the information available on field observations, methodology used in investigating allelopathy and its role in pest management. Leaching has been considered a principal process by which plant metabolites are released in the soil[8]. A variety of chemicals may be released into the surroundings during the process of leaching. They contain water soluble inorganic and organic compounds. Allelopathy of most plants has been carried out for their action on seed germination and seedling growth. Keeping this in mind, the present study highlights the effect of allelochemicals present in different plant parts of *Lantana camara* on *Physcomitrium japonicum.* Bryophytes are sensitive to even small variations in the environment and serve as good experimental material. Moreover, these plants are also interesting in terms of their physiology and ecology since they are less independent of their environment than the flowering plants. There is much to be learnt about their relationship with the environment.

L. Camara is a wild shrub found growing in various parts of Indian subcontinent.

MATERIALS AND METHODS

Fresh root, stem and leaves of *Lantana camara* were dried in an oven at 80°C for 24 hours and chopped into small pieces. Ten grams of each dried plant parts were soaked in 100 ml distilled water for 24 hours and filtered. Clear filtrate i.e., extract was diluted with Half Knop's medium (HKM) and double distilled water (DDW). Thus, the required percentage of extract + HKM, and extract + DDW was prepared, which was autoclaved at 15 lb per square inch for 15 – 20 minutes.

Undehisced capsules of *Physcomitrium japonicum* were surface sterlised with 2% of calcium hypochlorite for 1-3 minutes. Approx. 25 – 30 spores were spread in 0.01 ml of spore suspension on petri dishes containing Whatman's filter paper no 1 moistened with different concentrations of *Lantana* extract (root, stem & leaf) in HKM and DDW separately. Thus 2,4,6,8,10,12, 15 and 18%

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of these extracts were taken. Pure HKM and DDW were used as controls. These petri dishes were kept in different conditions of day, night and diffused light. The spore-inoculated petri dishes were also kept in BOD incubator in fluorescent tube light for proper climatic conditions at 22 ±2°C.

OBSERVATIONS

The extract of *L. camara* markedly decreased the percentage of spore germination with increase in its concentration in Half Knop's medium as well as in double distilled water. It is observed from Table 3 and 4 that the percentage of spore germination was higher in Half Knop's liquid culture medium than that in double distilled water. This may be due to presence of nutrients in the medium. Leaf extract of *L. Camara* maximally inhibits spore germination, followed by stem and root extracts in the medium and in double distilled water. Inhibition increased with increase in the concentration of *L. camara* leaf, stem and root extracts, respectively. Further, it is evident from Table 1 and 2 that with increasing number of days (15, 25 & 35 days) the percentage of germination increased. This may be due to fall in concentration and quality of extract in the samples and some of the allelochemicals might not be as effective as they were in the initial stages.

Table 1. Effect of *Lantana Camara* root, stem & leaf extracts on spore germination in *Physcomitrium japonicum* on 15th, 25th & 35th day in HALF Knop's liquid culture medium.

Concentration in %	15th day			25th day			35th day		
	LRE	LSE	LLE	LRE	LSE	LLE	LRE	LSE	LLE
Control	66.5	66.5	66.5	68.44	68.44	68.44	70.11	70.11	70.11
2%	56.77	50.94	50.00	57.69	52.33	51.63	58.52	54.55	53.34
4%	44.45	41.35	40.53	46.33	46.42	41.00	47.93	47.78	45.66
6%	34.26	35.21	26.46	36.42	36.42	29.66	38.91	37.5	32.04
8%	24.86	24.45	19.41	26.36	26.05	21.22	28.81	28.01	23.00
10%	13.40	11.76	6.05	15.92	15.16	8.09	18.44	17.64	11.00
12%	8.5	5.06	2.47	10.11	9.50	0	14.12	12.33	0
15%	2.47	0.96	0	6.14	2.22	0	10.51	0	0
18%	0.81	0	0	0	0	0	0	0	0

LRE : *Lantana* Root Extract; LSE : *Lantana* Stem Extract; LLE : *Lantana* Leaf Extract

Table 2. Effect of *Lantana Camara* root, stem & leaf extracts on spore germination in *Physcomitrium japonicum* on 15th, 25th & 35th day in double distilled water.

Concentration in %	15th day			25th day			35th day		
	LRE	LSE	LLE	LRE	LSE	LLE	LRE	LSE	LLE
Control	61	61	61	63.58	63.58	63.58	64.11	64.11	64.11
2%	54	51.11	49.28	55	52.97	51.17	57.18	52.84	53.28
4%	42.83	39.57	39	45.62	45.08	39.1	47.88	47	45.61
6%	33.28	34	25.51	36.36	36.14	28	38.5	37.18	30.42
8%	23.21	22.63	19	25.66	24.38	20.53	28.67	26.36	21.64
10%	12.5	11.38	3.44	15.66	14.74	5.66	17.96	17.51	8.23
12%	6.84	2.22	0	9.43	4.44	0	13.28	9.43	0
15%	1.12	1.5	0	4.12	0	0	8.52	0	0
18%	0	0	0	0	0	0	0	0	0

LRE : *Lantana* Root Extract; LSE : *Lantana* Stem Extract; LLE : *Lantana* Leaf Extract

RESULTS AND DISCUSSION

Spore is the first cell of gametophytic generation and the method of asexual reproduction. It is responsible for the distribution and evolution of species too.

It is evident from the data shown in Table 1 and 2 that the spore germination percentage is directly proportional to the time period (number of days) and inversely proportional to the concentration of allelochemicals present in the various extracts of *Lantana* (Table 3 & 4). It is also concluded that the extract diluted with Half Knop's liquid medium enhanced spore germination as compared to the extracts diluted with double distilled water.

Inhibition of growth of one plant by another through the release of allelochemicals is well known. Suresh and Vinaya Rai[6], Bansal[1] and Lasanework and Michelson[2] confirmed loss in the yield of field crops due to the influence of allelochemicals. Several chemicals belonging to the categories of aminoacids, sugars, phenols, terpenes, terpenoids and organic acids have been reported in leachates from a number of plant species.

Results in Table 3 and 4 show that the inhibition of spore germination of *P. japonicum* is minimum due to root extract and maximum due to leaf extract diluted with Half Knop's liquid culture medium and double distilled water. Further, the spore germination is always more in extracts diluted with HKM (Half Knop's liquid culture medium). The results obtained show that the various extracts are inhibitory for the spore germination due to the presence of allelochemicals. Supplementing the extract with Half Knop's liquid culture medium increase the spore germination which may be due to the presence of various nutrients in it as well as some antagonistic effects also. Further, with the increase in time period decrease in the effect of inhibitory principles was observed which may be probably due to decrease in the activity of active principles involved in

allelopathic effects. The spread of *Lantana* is serious threat for the existing bryoflora of the subcontinent and measures should be taken well in time to check its further spread.

Table 3. Mean values of Effect of *Lantana Camara* Root, Stem and Leaf extract on germination of *Physcomitrium japonicum* spores at 15th, 25th & 35th day in Half Knop's medium.

Concentration in %	Mean Values (%)		
	LRE	LSE	LLE
Control	68.35	68.35	68.35
2%	57.66	52.61	51.69
4%	46.24	45.18	42.40
6%	36.53	36.38	29.39
8%	26.68	26.17	21.21
10%	15.92	14.85	8.38
12%	10.91	8.96	0.82
15%	6.37	3.43	0.00
18%	0.27	0.00	0.00

LRE : *Lantana* Root Extract; LSE : *Lantana* Stem Extract; LLE : *Lantana* Leaf Extract

Table 4. Mean values of Effect of *Lantana Camara* Root, Stem and Leaf extract on *Physcomitrium japonicum* spores germination at 15th, 25th & 35th day in double distilled water.

Concentration in %	Mean Values (%)		
	LRE	LSE	LLE
Control	62.90	62.90	62.90
2%	55.39	52.31	51.24
4%	45.44	43.88	41.24
6%	36.05	35.77	27.98
8%	25.85	24.46	20.39
10%	15.37	14.54	5.78
12%	9.85	5.36	0.00
15%	4.59	0.50	0.00
18%	0.00	0.00	0.00

LRE : *Lantana* Root Extract; LSE : *Lantana* Stem Extract; LLE : *Lantana* Leaf Extract

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ABSTRACTS

OF

Ph.D. THESES

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SOME CONTRIBUTIONS TO MULTIPLE COMPARISONS AND RANKING AND SELECTION PROCEDURES

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A motivation to the present work is that most of the multiple comparisons and ranking and selection procedures proposed by several researchers are applicable for the complete samples from various populations. But many situations arise where the experimenter cannot measure the data below and above some limits or the data contain outliers, which may call for taking adverse effect decisions. In such situations the experimenter does not want to use the information provided by the extreme observations or he is constrained to work with censored data to draw statistical inferences. Motivated by such practical situations, various procedures discussed in this dissertation are useful in the case of censored data.

The work in this dissertation, presented in five chapters, continues the studies due to:

(i) McDonald (1977), Patel and Wyckoff (1990) to the exponential probability model under censored data (chapter II);

(ii) Mishra (1986a) for the simultaneous selection of extreme populations in terms of scale parameters for the general location-scale probability model when the data are censored with applications to two-parameter exponential populations (chapter III);

(iii) Lam (1986) to select good populations in terms of scale parameters using censored data (chapter IV). Applications of the procedures have been demonstrated by taking uniform and exponential probability models.

(iv) Bohrer (1979), Bohrer et al. (1981) and Liu (1997) to propose a multiple three decision procedure for comparing several exponential populations with a control (chapter V). The motivation was to compute critical points when the treatments and control populations were unsymmetric.

(v) Hayter (1990) and Gill and Dhawan (1997, 1999) by taking censored samples from the k members of location-scale family for testing homogeneity of scale parameters against simple ordered alternative and to obtain simultaneous one sided confidence intervals for the ratios of ordered scale parameters (chapter VI). Applications to uniform and exponential families are demonstrated with tables of critical constants.

In Chapter II of this dissertation, the data from ith source/ population/ process is assumed to follow exponential distribution indexed by location parameter $\mu_i \in R$ and scale parameter $\theta_i \in R_+$, $i = 1,\ldots,k$. The population associated with the smallest scale parameter $\theta_{[1]}$ is called the "best'. For selecting a subset which contains the 'best' population with probability at least P^* (a pre-specified constant in the interval (0,1)) a class of selection procedures based on sample quasi-range, appropriate in situations when the data are contaminated by outliers or data are censored, is proposed. Tables for the implementation of some members of the proposed class are provided.

Constriction of a set of 100 P^*% simultaneous confidence intervals for the ratios $\frac{\theta_{[1]}}{\theta_i}$, i =1,...,k is demonstrated by using a member of the proposed class. Properties, and comparisons with the existing procedures of the members of proposed class, are discussed.

In Chapter III, we consider a general location-scale probability model with cdf $F((x-\mu_i)/\theta_i)$ for the data from the ith population/ treatment, $i = 1, \ldots, k$. Define populations associated with $\theta_{[1]}$ and $\theta_{[k]}$ as the 'best' and the 'worst' respectively. A class of selection procedures for simultaneous selection of two subsets, one containing the population associated with parameter $\theta_{[1]}$ and the other containing the population associated with the parameter $\theta_{[k]}$, with probability at least a pre-specified value $P^*\left(\frac{1}{k(k-1)} < P^* < 1\right)$, based on sample quasi-range, is proposed. $100P^*$% simultaneous confidence intervals for $\left(\frac{\theta_{[i]}}{\theta_{[1]}}, i = 2,\ldots,k; \frac{\theta_{[k]}}{\theta_{[i]}}, i = 1,\ldots k-1\right)$ are also discussed. Application of these procedures for two-parameter exponential probability model is considered and necessary tables for the implementation of some members of the proposed class are provided. Comparisons of two members of the proposed class with the existing procedure are also discussed for the slippage parametric configuration.

In Chapter IV, we again restrict to a general location-scale probability model with cdf $F((x-\mu_i)/\theta_i)$ for the data from the ith population/ treatment, $i = 1, \ldots, k$. Let the set G of good populations be defined as $G = \{i : \theta_i \le \delta_1 \theta_{[1]}$ and the set B of the bad populations as $B = \{i : \theta_i \ge \delta_2 \theta_{[1]}$, where $\delta_2 > \delta_1 > 1$. We propose a class of selection procedures, most appropriate for censored data as well as for the data containing outliers, to select a subset $S \subseteq \{1,\ldots,k\}$ such that $P[G \subseteq S] \ge P^*$ (a pre-specified value), $\frac{1}{k} < P^* < 1$. Simultaneous confidence intervals for the ratios $\frac{\theta_{[1]}}{\theta_i}, i = 1,\ldots,k$ and $\frac{\theta_{[i]}}{\theta_{[j]}}, i \ne j,\ i, j = 1,\ldots,k$, that can be derived with the help of the proposed procedures are also discussed. Applications to exponential and uniform probability models are considered and necessary tables for the selection constants are provided. A class of selection procedures is also proposed so that the probability of omitting a good population or selecting a bad population is less than or equal to $1-P^*$.

In Chapter V we consider $(k + 1)$ $(k \ge 1)$ experimental populations such that ith population is characterized by the location parameter μ_i and real parameter θ_i, $i = 0,1,\ldots,k$. In terms of location parameter, population π_i is termed as better (worse) than control $\mu_i - \mu_0 > 0$ $(\mu_i - \mu_0 > 0)$. Similarly, in terms of scale parameter, if $\theta_i / \theta_0 < 1$ $(\theta_i / \theta_0 > 1)$ then population π_i is considered as better (worse) than control. A multiple three-decision procedure, based on equal sample sizes say n

from the treatment populations and a sample of size n_0 (possibly different from n) from the control population, for two-sided comparisons of treatments better than the control and the treatments worse than the control is proposed. This procedure, for a given α $(0 < \alpha < 1)$, ensures the probability of no misclassification to be at least $1-\alpha$ irrespective of the configuration of $\underline{\mu} = (\mu_0, \mu_1, ..., \mu_k)'$. The constants necessary for the implementation of the proposed procedure have been provided. Simulation study is carried out for the validity of the critical points and it was found that the simulated values of the probability of no misclassification, using the critical constants, are in agreement with the nominal level $1 - \alpha$. The scale version of the proposed procedures has been discussed with application to normal probability model.

In Chapter VI, again restricting to k members of location-scale family (as considered in Chapters II & III), we propose a class of tests, based on ratios of sample quasi-ranges, to test the null hypothesis $H_0: \theta_1 = \ldots = \theta_k$ against the simple ordered alternative $H_1: \theta_1 \leq \ldots \leq \theta_k$ with at least one strict inequality. For a fixed non-negative integer r, $r = 0, 1, \ldots, [n/2] - 1$, a member of our class of tests is based on the test statistic $W_r = \max_{1 \leq i < j \leq k} (T_{jr} / T_{ir})$, where $T_{ir} = X_{i:n-r} - X_{i:r+1}$ is the quasi-range using sample of size n from ith population, $i = 1, \ldots, k$ and $[x]$ represents the greatest integer less than or equal to x. Here $X_{i:r+1}$ and $X_{i:n-r}$ represent $(r+1)^{th}$ and $(n - r)^{th}$ order statistics in the i^{th} sample, $i = 1, \ldots, k$. The null hypothesis is rejected iff the statistic W_r exceeds a suitable critical point. Applications of the test procedure have been demonstrated by taking exponential and uniform probability models with relevant tables of critical constants. The Three-decision procedure and construction of simultaneous confidence intervals for the ordered pair-wise ratios of the scale parameters is illustrated. Comparison of some members of our class with the test of Gill and Dhawan (1999), in terms of simulated power, is discussed under exponential probability model.

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INTER-RELATIONSHIP OF MAGNESIUM AND ANTIOXIDANT POTENTIAL IN DIABETES MELLITUS

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The present study was planned with an objective to establish 'Interrelationship between Magnesium and Antioxidant Potential in Diabetes Mellitus'.

Experimental Studies

Magnesium deficiency led to a significant decrease in the body weights of magnesium deficient rats as compared to the control rats, suggesting that magnesium is indispensable for growth.

Significant decrease in serum magnesium was observed in the magnesium deficient rats in the second week whereas in diabetic rats, decreased levels of magnesium in serum were visible only by fourth week. RBC magnesium levels were not decreased until fourth week in both the groups. Urine magnesium excretion was drastically reduced in the magnesium deficient rats whereas in diabetic rats, urinary magnesium excretion was increased almost three times of control rats.

Tissue magnesium decreased the liver, heart, and muscles of magnesium deficient rats. In diabetic rats, magnesium levels were decreased only in muscle, confirming magnesium deficiency in diabetes.

Magnesium deficiency in rats led to significant increase in the blood glucose and plasma insulin levels thereby suggesting that magnesium plays an important role in glucose homeostasis.

A significant decrease occurred in vitamin C, and E, uric acid, total thiols in plasma and RBC GSH in magnesium deficient as well as diabetic rats. Plasma MDA and nitrite levels were substantially increased thereby suggesting that magnesium deficiency is a state of increased oxidative stress. Antioxidant enzymes (SOD and GST) activities were also diminished in some but not all the organs in magnesium deficient and diabetic rats suggesting that both magnesium deficiency as well as diabetes is state of increased oxidative stress.

Magnesium deficiency as well as experimental diabetes caused a significant increase in the triglycerides and concomitant decrease in HDL-Cholesterol levels in plasma thus showing dyslipidemia in these rats.

Ionic alterations were also observed in the magnesium deficient rats by the presence of hypercalcemia and hypokalemia. ATPase and Ca^{2+} ATPase in RBCs and NA^{+} K^{+} ATPase and Ca^{2+} ATPase in some but not all organs of the experimental rats.

Supplementation Studies

Supplementation of magnesium in the magnesium deficient as well as diabetic rats caused significant recovery in the body weights as compared to control rats.

Serum and RBC magnesium level were also restored to normal levels in the supplemented groups. Urine magnesium levels were increased in the magnesium deficient rats.

Magnesium supplementation led to marginal but significant decrease in the blood glucose and plasma insulin levels. The blood glucose levels could not be restored to normal levels suggesting some irreversible alterations in the glucose homeostasis by magnesium deficiency.

Magnesium supplementation almost restored the vitamin C levels in magnesium deficient and diabetic rats and significantly increased thiol levels. MDA levels were also decreased significantly in both the groups.

Magnesium supplementation caused decrease in plasma triglyceride levels in magnesium deficient and diabetic rats. A significant increase in ATPase activity (Na^+ K^+ ATPase and Ca^{2+} ATPase) was observed in RBC and some tissues of magnesium deficient and diabetic rats.

Parameters of oxidative stress were found to be significantly disturbed as shown by decreased levels of vitamins C & E, total thiols, uric acid and RBC GSH and increased MDA levels. TRAPc was also decreased in the diabetic patients.

The results of this study show that magnesium deficiency causes accelerated oxidative stress and is also associated with dyslipidemia and ionic alterations. The study also indicates that mechanism responsible for the oxidative stress and ionic alterations in diabetes may be partly mediated through magnesium deficiency. The study demonstrates that magnesium deficiency produces a deleterious effect on glucose handling, which seems to be further aggravated by hyperglycemia. It seems very important to point out that magnesium deficiency and hyperglycemia aggravate each other in a true pathogenic vicious cycle. The implication of magnesium as an important factor in glucose metabolism might at least partially explain its postulated role in late diabetic complications. Repletion of magnesium was associated with restoration of antioxidant levels, decreased oxidative stress, correction of altered ionic homeostasis and impaired ATPase activity further supporting the viewpoint of the study. The potential role of magnesium as therapeutic agent has not been well appreciated in the past.

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AGE RELATED EFFECTS OF ETHANOL ON THE EXPRESSION OF INTESTINAL FUNCTIONS IN RATS

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Ethanol consumption during pregnancy has been described to be etiologically associated with multiple birth defects in offsprings, termed as the 'Fetal Alcohol Syndrome' (FAS). In the present study, rat model of chronic alcoholism was used. Ethanol at a dose of 2g/kg/day was administered orally prior to and during gestation and the effects on the intestine of developing rats were assessed.

In utero, ethanol exposure significantly depressed the intestinal length and weight of pups and DNA and RNA contents, which persisted during postnatal development (4 weeks of study) compared to controls.

Intestinal ADH activity was significantly low at day 20 and 30 of postnatal development in the experimental groups compared to controls.

The activities of brush border lactase and sucrase were appreciably low in prenatally ethanol exposed rats during the postnatal development compared to the controls. But alkaline phosphatase activity was enhanced.

Western blot analysis of AP exhibited differential expression of the various isoforms of the enzyme (92 kDa and 66 kDa respectively), during postnatal development in rat intestine.

Prenatally ethanol exposed pups, showed a significant decrease in Na^+ dependent glucose uptake compared to controls. Also Na^+ - dependent ^{14}C-glycine and ^{3}H-leucine uptake rates revealed a considerable decline at day 4 and 8 of postnatal development in rats prenatally exposed to ethanol. The Na^+ - independent amino acid transport rates were not affected by ethanol exposure in utero. The mRNA levels encoding Na' dependent D-glucose transporter (SGLT 1) revealed no visible change in the expression of SGLT 1 in control and experimental groups during postnatal development. The oligonucleotide probe hybridized to 4.5 Kb and 2.8 Kb transcripts in both the control and ethanol exposed rat intenstine.

Light microscopy studies revealed hypertrophy of the liver cells (hepatocytes), increased vacuolation alongwith few pycnotic nuclei (necrosis of cells). Intercellular walls were damaged and confluence of cells was observed in 8 and 30 days old liver sections of ethanol exposed rats compared to controls. Histological examination of intestine from 8 and 30 days old rats from ethanol fed mothers showed necrosis of the epithelial layer with severe disruption of the villi with respect to control.

From these findings, it is thus concluded that prenatal ethanol exposure leads to developmental changes of intestinal functions in rats. Although the molecular mechanism responsible for these observations is unknown but it is apparent that changes in the development of (a) brush border

enzymes (b) transport systems for glucose and aminoacids (c) IgG receptor (d) glycosylation profile of MVM and (e) intestinal morphology were observed upto 3-4 weeks of postnatal age. This may suggest that in utero ethanol exposure influences the intestinal activities later in the life of the offsprings.

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EXPRESSION OF IRON REGULATED OUTER MEMBRANE PROTEINS IN ESCHERICHIA COLI AND THEIR POTENTIAL FOR PREVENTIVE INTERVENTION IN URINARY TRACT INFECTIONS

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The present investigation was carried out to study the expression of iron regulated outer membrane proteins in uropathogenic organisms and their role in preventive intervention against urinary tract infections. The salient findings of the study are given below.

Thirty-three *Escherichia coli* strains were isolated from the urine samples of patients with symptoms of acute episode of urinary tract infection and were serotyped. The mail serotypes encountered, were 017 (21.2%), 06 (18.1%), 08(6%), 09 (6%), and 0117 (6%). The growth kinetics of E. coli strains in urine and M9 medium containing different concentrations of Dipyridyl was determined. The yield of bacterial cells grown in urine was comparatively lesser than, in chemically defined medium (M9) and medium containing dipyridyl (M9 + dipyridyl). In the presence of dipyridyl, growth was concentration dependent. High concentration of dipyridyl was found to be toxic as many of the strains failed to grow at this cocentration (250\iμM) and other strains showed poor growth at 200μM. Hence 150 μM concentration of dipyridyl was selected for bacterial growth in iron deplete medium.

The iron mopping mechanism of these isolates were studied in terms of siderophore production. All E. coli isolates produced enterochelin. But, aerobactin production was observed in only 54.5% uroisolates of E. coli. Iron regulated outer membrane protein profile of uroisolates of E. coli. Iron regulated outer membrane protein profile of uroisolates of E. coli (22) grown in M9 medium (2.4μM Fe), M9 medium containing 150μM dipyridyl (undetectable Fe) and urine (1.9μM Fe) showed that : Different strains of E. coli expressed different IROMPs in the molecular weight range of 70 to 95 kDa in iron deplete M9 medium. Out of 22 strains of E. coli, 15 strains were found to express three or more IROMPs under iron limiting conditions and 7 strains expressed two or less IROMPs. In urine grown cells, 18 out of 22 strains of E. coli expressed three or more IROMPs in the molecular weight range of 70 kDa to 95 kDa and only 4 strains showed 2 or less IROMPs. On comparing the expression of IROMPs of organisms grown in urine with that grown in iron deplete M9 medium, it was observed that IROMPs in the two media were in the molecular weight range of 70 to 95 kDa. However, expression varied from strain to strain in the two media. Four IROMPs (80.85.90.95 kDa) of U33 strain were purified by eluting the proteins from SDS-PAGE gel. To resolve and isolate closely spaced IROMPs on SDS-PAGE gel, extended electrophoresis by running the gel for period of only 4 hrs at 50v helped to reduce the time of 16-18 hrs taken in earlier studies.

The IROMPs of U33 strain were found to be immunogenic, when checked by immunobloting. Dose dependent increase in anti IROMP antibody level was observed in immunized animals as checked by ELISA. Heterologous cross reactivity of IROMPs was checked with the antisera

obtained from animals immunized separately with whole cells of E. coli uroisolates (10 strains) grown under iron deplete conditions. The antisera raised against E. coli, grown both in iron deplee medium and urine (5/10 strains) reacted with all the four (95.90.85 & 80kDa) IROMPs of U33 while remaining strains (5/10) reacted only with three proteins (90, 85 and 80kDa).

Potential of anti IROMP antibodies for passive protection was evaluated. Mice were immunized with 50µl of Hyper immune serum (HIS) by 1route and challenged by ascending route at different time intervals. Anti IROMP antibodies could be seen in urine after 2 hrs of immunization with HIS. Urine culture positivity was minimum (50%) in mice challenged after 4 hrs of passive immunization. With increasing time interval from 0 to 4 hrs, between injections of antisera and challenge, there was an increase in protection (40 to 80%) followed by decrease in protection at 6 hrs and 24 hrs. Maximum renal damage occurred when infection was given immediately after passive immunization i.e. without protection.

To check the direct effect of hyper immune serum (anti-IROMPs antibodies) in preventing renal colonization by homologous E. coli (U33) strain, the bacteria 10^9 cfu/ml) mixed with HIS were given by intravesical route. The results showed that after 48 hrs of passive immunization, only 16% of animals showed positive urine culture compared to 88% in infected control group. Intervesical immunization of animals with 100µl of HIS resulted in 83% protection against ascending UTI.

In addition protective potential of iron regulated outer membrane proteins (80, 85, 90 & 95 kDa) of E. coli uroisolates (U33) against ascending pyelonephritis was evaluated in terms of kidney tissue damage and renal bacterial load in actively immunized animals. Two does, 100µg each of crude preparation of IROMPs provided 100% protection against intraurethral bacterial challenge as the kidneys were found to be sterile and no damage was observed histologically. Maximum protection was observed with 100µg dose of purified IROMPs, which decreased with the decrease in concentration of IROMPs.

In order to see the role of different IROMPs in affording protection, separately experiment with two set of proteins i.e., 95kDa and 90kDa as well as 85kDa and 80kDa were carried out. Immunization of mice with 80 & 85 kDa IROMPs resulted in protection of 62.5% of animals in comparison to 50% in mice immunized with 90 & 95kDa. Significant increase in immunoglobulin IgG anti-IROMPs antibody level was seen in mice immunized with different doses of purified IROMPs. In presence of immune (anti IROMP antibodies) sera, increased uptake of E. coli by normal macrophages was observed accompanied by iIncreased intracellular killing of E. coli by macrophages from immunized animals. Significantly higher cellular immune response (DTH response) elucidated by mouse foot pad swelling was observed in immunized infected group, which was higher than in control infected group. Two fold increase in cytokines (IFN-Y, IL-2 & IL-4) was seen in the immunized infected group when compared to infected control animals.

The study brings out development of both humoral as well as cell mediated immune response following immunization with all the four proteins (IROMPs) together as well as when in given in groups of two. These IROMPs can prove to be potential antigens for protection against UTI.

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STUDY OF PHOTON-ATOM INTERACTION PROCESSES AND ANALYTICAL APPLICATIONS USING EDXRF TECHNIQUE

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The present work reports the experimental study of photon-atom interactions and subsequent processes in the X-ray energy region using energy dispersive fluorescence (EDXRF) technique. Annular and point source geometry in reflection and transmission mode have been used in the present work. Photons of energies 22.1 keV and 88.03 keV, and 59.54 keV emitted from annular radioactive sources of ^{109}Cd and ^{241}Am, respectively, have been used in the direct excitation mode. The ^{241}Am source was also used as a primary exciter in the secondary excitation mode along with Rb, Mo, Sn and Dy secondary exciters. The photons emitted from the target were detected using a Si(Li)/HPGe detector coupled to a PC based multichannel analyzer through a spectroscopy amplifier.

In the present work, the measurements of elastic scattering differential cross-sections for the 22.1 keV photons at 133° by C, S, Ge, As, Se, Rb, Ag, Te, Ba, Sm, Ho, Tm, Yb, Hg and Tl elements, and for the 88.03-keV photons at 125° by Zn, Mo, Sn, Ba, Gd, Ho, Yb, Ta, W, Pt, Au, Hg, Tl, Pb, Bi, Th and U elements have been done. In the case of measurements at 22.1 keV photon energy, emphasis was on the Ag element, where the elastic scattered photon yield was deduced from the observed 22.1 keV peak by subtracting the contribution of the fluorescent Ag-Kα X-rays produced by the 88.03 keV γ-ray and internal bremsstrahlung photons from the ^{109}Cd radioisotope. In the case of measurements at 88.03 keV photon energy, emphasis was on Pb and Bi elements having K shell binding energy very close to the incident photon energy. The measured elastic scattering differential cross-sections have been compared with those obtained in the independent-particle approximation through calculations of the modified-relativistic form factors (MF's), a combination of the MF's and angle-independent 'anomalous' scattering factors (ASF's), and the relativistic second-order S-matrix.

The inelastic scattering cross-sections have been measured for the 22.1 keV photons at 133° by Be, C, Mg, Al, S, Ti, Fe, Cu, Zn, As, Se, Y, Zr, Ag, In, Sn, Te, Gd, Dy, Ho and Tm elements, and for the 88.03 keV photons at 131° by Be, C, Mg, Al, S, Ti, Fe, Cu, Zn, Se, Y, Mo, Ag, In, Sn, Te, Gd, Dy, Ho, Tm and Bi elements. In these measurements, emphasis was on the elements having K shell binding energy close to the incident photon energy. These measurements were performed under vacuum (~10^{-2} Torr). The measured inelastic scattering cross-sections have been compared with those calculated using the relativistic Klein-Nishina cross-section and the non-relativistic Hartree-Fock incoherent scattering functions. The measured cross-sections are found to be in good agreement with the calculated values for all the elements under investigation except for Y and Zr elements at 22.1 keV incident photons, where the measured cross-sections are ~25% lower than the calculated ones. In the case of 88.03 keV incident photon, measured cross-sections are found to be in good agreement with the calculated values for all the elements under investigation except for Gd, Dy, Ho, and Tm, where these values are lower by ~20%, but for Bi (B_K = 90.526 keV), it is higher by 16%; B_K is the K shell binding energy of the element.

The L X-ray production (XRP) differential cross-sections have been measured for Pb at 22.6 and 59.5 keV ($B_{L1}<E_{inc.}<B_K$) in the angular range 50°-130°, for Th and U at the 25.8 and 46.9 keV, and for Th at

59.54 keV incident photon energies ($B_{L1}<E_{inc.}<B_K$) at an angle of 130°. The L XRP differential cross-sections have also been measured in Pb, Th and U following selective ionization of the L_3 sub-shell at the 13.6 keV/ 17.8 keV incident photon energy ($B_{L3}<E_{inc.}<B_{L2}$) in the angular range 90°-160°. The present measurements establish that the emission of L_3 sub-shell X-rays, in the case of Pb, Th and U following selective photoionization of L_3 sub-shell and photoionization of L_i (i =1,2,3) sub-shells, is isotropic within experimental error.

Integral L XRP cross-sections in Pb, Th and U at different incident photon energies, deduced assuming isotropic emission of the L X-rays, are found to be in good agreement with those evaluated using the most reliable theoretical values of L_i(i=1,2,3) sub-shell photoionization cross-sections, fluorescence yields, X-ray emission rates and Coster-Kronig transition probabilities.

The L_1-L_3 Coster-Kronig yield, f_{13}, for Pb, Th and U has been determined using measured intensities of the Lα X-rays emitted following selective ionization of the L_3 sub-shell by the Rb/Mo K X-rays ($B_{L3} < E_{inc.} < B_{L2}$) and of those emitted following ionization of the L_i (i = 1,2,3) sub-shells by the 59.54 keV γ-rays ($B_{L1} < E_{inc.} < B_k$); $B_{K/Li}$ being the K shell / L_i sub-shell ionization threshold of the target element. The present measured f_{13} value for Pb agrees well with the semi-empirical value and is 15% lower than the value based on the relativistic Dirac-Hartree-Slater (RDHS) model. The present measured f_{13} value for both Th and U agree well with the value based on the RDHS model and is ~12% higher than the semi-empirical values compiled by Krause.

In addition, the L_i(i= 1,2,3) sub-shell fluorescence yields (ω_i) for Pb, Th and U have been deduced using the measured X-ray production cross-sections at the incident photon energies ranging 13-60 keV and the theoretical photoionzation cross-sections. The measured ω_i yields have been compared with the available semi-empirical values and the theoretical values based on the RDHS calculations.

In the last part of the thesis, archaeological pottery samples from Harappan, Black-Slipped, Sunga, Kushana and Late-Medieval periods were analyzed using the Energy Dispersive X-ray Fluorescence (EDXRF) technique employing the fundamental parameter approach. The 17.8 keV photons, from Mo secondary exciter in conjunction with X-ray tube, were used for exciting the samples. Ten elements, namely, K, Ca, Ti, Mn, Fe, Cu, Zn, Rb, Sr and Y have been quantified. The measured concentration values for the elements in the pottery samples of different periods do not vary significantly. Thus first indications from the EDXRF results are that the pottery of different periods was made from same type of soil, most probably of local origin.

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PHENOMENOLOGICAL FERMION YUKAWA COUPLINGS AND THEIR IMPLICATIONS FOR LOW ENERGY PHENOMENA

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Yukawa couplings or the fermion mass matrices are "*completely arbitrary*" in the Standard Model. Texture specific mass matrices have been phenomenologically formulated keeping in mind features such as hermiticity and hierarchy etc.. In particular, we have formulated all possible non-trivial hermitian texture 6 zero, texture 5 zero and texture 4 zero (52 in all) quark mass matrices, diagonalized them exactly and constructed the corresponding exact V_{CKM} matrices. Interestingly, all the texture 6 zero as well as most of the texture 5 zero and texture 4 zero quark mass matrices are ruled out by the considerations of the low energy data (LED) consisting of quark masses and the magnitudes of the V_{CKM} elements. In the case of texture 4 zero matrices, it is the modified Fritzsch type mass matrices, with non-zero (2,2) element in M_U and M_D, given as

$$M_U = \begin{pmatrix} 0 & A_U & 0 \\ A^*_U & D_U & B_U \\ 0 & B^*_U & C_U \end{pmatrix}, \quad M_D = \begin{pmatrix} 0 & A_D & 0 \\ A^*_D & D_D & B_D \\ 0 & B^*_D & C_D \end{pmatrix},$$

which accommodate the present LED. For example, the corresponding V_{CKM}

$$\begin{pmatrix} 0.9732 - 0.9777 & 0.2100 - 0.2300 & 0.0013 - 0.0060 \\ 0.2097 - 0.2299 & 0.9722 - 0.9771 & 0.0360 - 0.0440 \\ 0.051 - 0.0144 & 0.0344 - 0.0440 & 0.9990 - 0.9993 \end{pmatrix},$$

has an excellent overlap with the PDG V_{CKM}. The texture 5 zero matrices, obtained from the above matrices with D_D=0, are also not ruled out unambiguously by the above mentioned LED.

Before examining the CP violating features of the quark mass matrices, related to the phases of the complex elements, we have carried out a detailed analysis of the CKM phenomenology with a view to evaluate CP violating phase δ, V_{td} and angles α, β and γ of the unitarity triangle by incorporating the constraints due to unitarity, K^0 - K^0 mixing and B^0 - B^0 mixing independently of each other as well as collectively. Our findings, which are in agreement with the contemporary analyses as well as with the latest BaBar (sin 2β=0.59 $\pm$ 0.14 $\pm$ 0.05) and Belle (sin 2β=0.99 $\pm$ 0.14 $\pm$ 0.06) results, are given in the Table below.

	Unitarity based results	CKM fit	Using texture 5 zero matrices	Using texture 4 zero matrices
δ	$50^\circ \pm 20^\circ$	37° - 70°	18° - 130°	22° - 163°
	$130^\circ \pm 20^\circ$			
α	20° - 139°	80° - 124°	44° - 141°	15° - 158°
β	6.5° - 31°	15° - 31°	11° - 27°	8° - 43°
γ	$50^\circ \pm 20^\circ$	37° - 70°	28° - 121°	11° – 157°
	$130^\circ \pm 20^\circ$			

Interestingly, when a corresponding analysis of texture 5 zero and texture 4 zero matrices is carried out, it looks that texture 5 zero matrices are ruled out by the Belle measurements, whereas texture 4 zero mass matrices look to be in agreement with BaBar and Belle measurements as well as with the CKM fits, as is evident from the table. Also, using texture 4 zero mass matrices, an evaluation of the parameter J leads to the range $J=(1.9\text{-}5.6)\times10^{-5}$ in agreement with the unitarity based analysis.

Motivated by the success of texture 4 zero matrices in explaining CKM phenomenology, we have considered similar texture for the lepton mass matrices. A detailed analysis satisfying the constraints on mixing angles and mass square differences due to the solar and the atmospheric neutrinos, not only gives limits on the neutrino masses, but also provides useful constraints on s_{13} For example, with the full scanning of input parameters, we obtain

$$m_1 \sim 10^{-3} - 10^{-2}\ \text{eV} \quad m_2 \sim 10^{-2}\ \text{eV} \quad m_3 \sim 0.055\ \text{eV} \quad \text{LMA},$$
$$m_1 \sim 10^{-8} - 10^{-5}\ \text{eV} \quad m_2 \sim 10^{-3}\ \text{eV} \quad m_3 \sim 0.055\ \text{eV} \quad \text{SMA},$$
$$m_1 \sim 10^{-5} - 10^{-4}\ \text{eV} \quad m_2 \sim 10^{-4}\ \text{eV} \quad m_3 \sim 0.055\ \text{eV} \quad \text{LOW},$$
$$m_1 \sim 10^{-5} - 10^{-3}\ \text{eV} \quad m_2 \sim 10^{-4}\ \text{eV} \quad m_3 \sim 0.055\ \text{eV} \quad \text{VO}.$$

The corresponding s_{13} ranges are

$$s_{13} \sim 0 - 0.20 \quad LMA,$$
$$s_{13} = 0.038 - 0.094 \quad SMA,$$
$$s_{13} = 0.028 - 0.099 \quad LOW,$$
$$s_{13} = 0.035 - 0.098 \quad VO,$$

implying that if s_{13} is found to be outside the range (0.01-0.1), then SMA, LOW and VO solutions look to be ruled out for texture 4 zero mass matrices.

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AN EXPERIMENTAL STUDY OF NEUTRAL AND CHARGED PARTICLE FLUCTUATIONS IN Pb-Pb COLLISIONS AT 158A GeV.

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We have primarily focused on studying the event-by-event fluctuations in the neutral pion fraction, f, thereby exploring the formation of Disoriented chiral condensate (DCC) in Pb-Pb collisions at 158 AGeV. Investigations are also made for fluctuations in the pseudorapidity and azimuthal angle distributions of charged particles as well as photons to extract events exhibiting the non-statistical fluctuations which may be due to the formation of Quark-Gluon Plasma.

The data on Pb+Pb collisions at 158 AGeV, from the WA98 experiment has been used. Charged particles were measured with the Silicon Pad Multiplicity Detector (SPMD) covering pseudorapidity region, $2.35 < \eta < 3.75$ whereas photons were measured with PhotonsMultiplicity Detector (PMD), covering pseudorapidity region $2.8 < \eta < 4.4$. The transverse energy measured by the Mid-rapidity Calorimeter (MIRAC) was used to characterize the centrality of the collisions.

The fluctuations in pseudorapidity and the azimuthal distributions of particles (photons as well as charged particles) are studied on an event-by-event basis. Basically we have used the local particle density and the power spectrum technique. An attempt has been made to investigate the jet type fluctuations in the limited η - ϕ zones as well as ring type fluctuations confined to a particular pseudorapidity bin having full azimuthal coverage. The fluctuations in the local particle density are in agreement with the VENUS+GREAT results which includes detector effects whereas the data show more fluctuations as compared to mixed events. Some events have been detected which have shown large fluctuations in the number of photons in the limited η - ϕ zones. This needs further investigation.

The power spectrum method has been used to filter events exhibitng large fluctuations. The threshold limit has been determined by the sum of mean power spectrum and four times of its corresponding dispersion. This threshold was set from the sample of mixed events which are devoid of any non-statistical fluctuations. One dimensional power spectrum has been calculated for both the species of particles (N_r and N_{ch}) in pseudorapidity as well as in azimuth. Some events have been filtered which have shown recognizable peaks beyond the set threshold value. The percentage of the filtered events are more in data as compared to mixed and V+G events. Thus, we have events which exhibit large non-statistical fluctuations in pseudorapidity and azimuthal distributions of charge particles as well as of photons though the probability is quite small.

An extensive study has been made in this thesis regarding the DCC search. DCC formation can produce a spectacular event structure with some region of the event dominated by charged pions and other by neutral pions. We have developed non-statistical fluctuations in the neutral pion fraction, f, on an even-by-event basis. It is worth mentioning that this is for the first time event-by-event search for DCC has been performed at the microscopic level. All the earlier attempts to

search DCC, have looked for the global effect. As DCC is expected to be formed in the not-so-central events we have analysed all the minimum bias (≈ 294 K) events. Experimental results have been compared with those generated using the VENUS 4.12 which were also processed through GEANT 3.15 to take care of the detector effects. Events with $f > 0.55$ are found to be more in data as compared to those seen in the V+G sample. Further, the statistical significance of results is obtained by comparing the results with those of mixed events. Two different sets of mixed events (I and II) are generated by different methods. There is no hidden non-statistical fluctuation embedded in the generated mixed event samples. So it can serve as proper base to compare the results. It is seen that data has more events as compared to mixed events (I and II) though their probability is very small.

To establish the genuineness of these events we have passed these events through some critical tests. These events have passed these tests. The events with anomalously high values of neutral pion fraction f are found to (i) increase with decreasing centrality of the collision, (ii) decrease with increase in the domain size.

Search is also made for the events having anomalous f values in the pseudorapidity bins with complete azimuthal coverage to investigate ring type DCC formation. The results of data are observed to be consistent with those of mixed events. Therefore, there is no possibility of ring type DCC formation.

The top 15-80% of the events are analysed for photon excess globally. Some events have been observed which have large number of photons as compred to charged particles in the overlap region of the PMD and the SPMD. Detectors were found to be working well during these filtered events. No irregular behaviour is noticed in the immediate preceding and succeeding events w.r.t. these events. We have found ≈(0.67±0.04)% events with E_T = 50 - 100 GeV and (0.16 ±0.03)% events with E_T = 100 - 150 GeV, having neutral pion fraction, $f > 0.70$.

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DETERMINATION OF THE TOP QUARK MASS USING NEURAL NETWORKS

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Particle physics is the study of fundamental constituents of matter and their interactions. However, which particles are regarded as fundamental has changed with time as physicists knowledge has improved. Modern theory - called the *Standard Model* (SM) has been extremely successful so far. It attempts to explain all the phenomena of particle physics in terms of properties and interactions of a small number of particles of three distinct types. The first two are called leptons and quarks and are spin 1/2 fermion, the third is a set of spin 1 bosons called gauge bosons which act as `force carriers' in the theory. Therefore, one can say that the SM of particle physics is a description of nature at very small distance scales, typically scales smaller than that of an atomic nucleus (10^{-15} m). In the SM, these particles are all assumed to be elementary *i.e.* they are treated as point particles, without internal structure or excited states. Both the quarks and leptons are grouped into three generations of two particles each. The corresponding particles in each generation have similar properties, except for their masses, which increase with each successive generation. All normal matter (protons, neutrons and electrons) is composed of particles from the first generation. Particles in higher generations can be produced in high energy interactions, but they are unstable and ultimately decay into first generation particles or photons.

The top quark is the heaviest elementary particle. It was discovered at the Tevatron in 1995 by the DØ and CDF collaborations. The top quark mass is one of the most important parameter of the SM, as together with the mass of *W* boson (m_W) it constraints on the mass of the SM Higgs boson. Therefore, it is very important to get any improvement in the precision of the top quark mass measurements. The Higgs boson, the *W* boson and the top quark contribute via radiative corrections to observables already measured at LEP and SLC so that the measured observables together with the measured values of the *W* and the top mass restrict the allowed mass range of the Higgs boson. Recent experimental constraints on the Higgs boson mass are consistent with the Standard Model. In future, when the *W* boson mass should be measured to an accuracy of 40 MeV, a precision of about 1 GeV in the top quark mass would yield a prediction in the Higgs boson mass of $\delta m_H / m_H \leq 40\%$. We have been motivated to use different methods to determine the top quark mass which are relatively simple. The aim of this thesis is to determine the mass of this particle using different ``non-conventional" methods especially using Neural Networks. We have also tried to determine the top quark mass using method of four vectors and the method of eμ invariant mass.

For the analysis, HEP data collected at FNAL (Fermi National Accelerator Laboratory) with DØ detector at center-of-mass energy ($\sqrt{s}$) 1.8 TeV for Run I (1992-1996) has been used. The Fermilab Tevatron collides beams of protons and antiprotons are counter-rotating in a four-mile

ring of magnets located at the FNAL in Batavia, Illinois. Until the LHC at CERN, where $\sqrt{s}$ = 14 TeV is built, this is the largest center-of-mass energy available. The Tevatron is among the more recent machines in a long line of accelerators which have contributed tremendously to the development of particle physics. The accelerator is designed such that these collisions occur at two points, each of which is surrounded by a massive apparatus designed to measure the results of the collisions. One of these, the DØ detector, provided the data used in this analysis. The DØ detector is upgraded to center-of-mass energy of 2.0 TeV in Run II. As a result, the Run II data would be quite large; about 100 times the Run I data. To exploit the full use of high statistics, the main task is to reduce systematic errors so that we can benefit from the reduced statistical errors. We need to explore different methods to cross-check the results and also use different methods which may yield smaller systematic errors by using as many as decay modes as possible. We have tried to achieve this goal by studying the top quark mass using/developing three different methods namely neural networks method, method of four vectors and the method of $e\mu$ invariant mass in one of the channel via which top quark can decay. These methods will be useful for analyses of DØ Run II data as well as for LHC ($\sqrt{s}$ = 14 TeV) where high statistics will be available. We present the results of top quark mass study based on three non-conventional methods using $t\bar{t} \rightarrow W^{+}bW^{-}\,\bar{b} \rightarrow (e/\mu)\nu\,(e/\mu)\nu$ decay in $p\bar{p}$ collisions at $\sqrt{s}$ = 1.8 TeV. The analysis assumes Standard Model coupling and branching ratios and is based on an integrated luminosity of 108.3 ± 5.7 pb^{-1} accumulated by DØ detector during the Fermilab Tevatron Collider Run I (1992-96). These methods would be quite useful in the determination of the top quark mass for DØ Run II where high statistics would be available. Using neural network analysis, the top quark mass obtained is m_t = 177.9 GeV. The systematical uncertainty in m_t comes out to be 2.17 GeV and statistical uncertainty in the measurement of m_t is about 56 - 59 GeV. In DØ Run II because of the introduction of a central magnetic field, a significant improvement in the muon momentum measurement is anticipated. Taking this fact into an account, the uncertainty reduces to ~40 - 42 GeV. Number of top events from $e\mu$ channel are expected to be about ten times more than the number in Run I which will further reduce the present uncertainty approximately to 6.3 - 6.5 GeV. By using the method of four vectors, systematic uncertainty is 1.52 GeV and statistical uncertainty is ~45 - 49 GeV for three $e\mu$ events which would reduce to ~ 7.5 - 8.9 GeV in DØ Run II and will lead to the precision in the top quark mass. Using the method of $e\mu$ invariant mass, the expected systematic error will translate by a factor of 5.

In addition to the study of the top quark mass, besides studying the different non-conventional methods to determine the top quark mass, I have contributed in the R & D work for the fabrication of pixel scintillation counters along with the group at Panjab University (PU). Our group and Delhi University group were assigned to build about 120 Pixel Scintillation Counters. These counters were supposed to be used in forward muon detectors in the muon system. The dimensions of the final counter as well as the material to be used were finalized at Fermilab and passed on to us for the final fabrication of these counters. The total set of drawings were designed and developed in the department during the tenure of my work and got approved from Fermilab, USA before final fabrication was carried out in the department. Five prototype pixel counters were fabricated which were to be used in the forward muon detector in the muon system of DØ upgrade for Run II. Mechanical fabrication work was done at the department as well as in the Central Tool Room Ludhiana (CTRL). The counters have been checked, tested and their performance was found to be extremely satisfactory.

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STUDIES ON INHIBITORY EFFECT OF CURCUMIN AND ITS ANALOGUES AND SULPHORAPHENE ON COOKED FOOD MUTAGENS

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Recently, much attention has been focused on the role of diet in the aetiology of cancer. Diet is a complex mixture of chemical entities and may contain substances that cause cancer as well as agents that can inhibit or modulate the development of neoplasia. Human beings are exposed to a unique class of dietary mutagens/ carcinogens generated, while cooking non vegetarian foods like beef, chicken and fish. Several studies published in recent years show a correlation between intake of fried meat and development of cancer and it has been hypothesized that various kinds of mutagenic heterocyclic amines are formed by cooking these protein rich foods at high temperatures. There is considerable evidence to indicate that man is exposed to heterocyclic amines through diet and is susceptible to the carcinogenic effects of these highly potent mutagens and reported rodent and non-human primate carcinogens. Also presently, there is enough evidence to show that chemical mutagenesis and carcinogenesis can be inhibited by a large number of naturally occurring compounds of plant origin. These inhibitors are minor constituents of some commonly consumed vegetables, fruits, beverages and spices and the evaluation of antimutagenic potential of the chemical constituents of plants has been of recent interest especially because the data can be extrapolated to human cancer prevention.

In view of these observations, for the present study, curcumin which is present in daily dietary food item turmeric-*Curcuma longa* L., Zingiberaceae; and its various analogues; and two isothiocyanates namely sulphoraphene: a constituent of common vegetable radish (*Raphanus sativus* L., Cruciferae); and sulphoraphane: a major constituent of broccoli (*Brassica oleracea italica* L., Cruciferae) were selected for studying their antimutagenic action against seven cooked food mutagens (heterocyclic amines): 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1) using Ames *Salmonella*/reversion assay in the presence of Aroclor1254-induced rat liver S9 homogenate. Choice of these agents was based on a comprehensive literature survey.

The various antimutagens included in the present study were curcumin, its natural analogues demethoxycurcumin and bisdemethoxycurcumin; and structurally related compounds such as tetrahydrocurcumin, dibenzoylmethane, dibenzoylpropane, vanillin, ferulic acid, isoferulic acid and caffeic acid and two isothiocyanates namely sulphoraphene and sulphoraphane.

Analysis of results of these studies indicated that all the three natural curcuminoids (**curcumin, demethoxycurcumin and bisdemethoxycurcumin**) were highly effective in antagonizing the S9-mediated mutagenic effects of all the tested heterocyclic amines in a dose-dependent

manner. In general, curcumin and demethoxycurcumin showed stronger inhibitory effects as compared to those of bisdemethoxycurcumin in both frameshift mutation sensitive TA98 and base pair mutation sensitive TA100 strains of *S. typhimurium* Both curcumin and demethoxycurcumin were found to exhibit greater antimutagenic effects in TA100 than in TA98 against all tested cooked food mutagens. **Tetrahydrocurcumin**, a colourless, synthetic derivative, prepared by hydrogenation of curcumin, was found to be ineffective against all the cooked food mutagens at the tested dose levels. **Dibenzoylpropane**, although lacking the β-diketone configuration indicated to be essential for biological activity of curcumin, was very effective in suppressing the mutagenicity induced by all the tested mutagens. Interestingly, **dibenzoylmethane** possessing central β-diketone moiety was found to be the most active amongst the compounds investigated. **Vanillin, ferulic acid and isoferulic acid** showed very good inhibitory activity against all the tested mutagens except Trp-P-2. **Caffeic acid**, which lacks a methoxy group on the benzene ring did not exhibit any antimutagenic effect against the tested heterocyclic amines except Glu-P-1. The important structural requirements responsible for high antimutagenic potential of curcumin against cooked food heterocyclic amines were found to be: unsaturation in the side chain, methoxy group on the benzene ring and central β–diketone moiety in the curcumin molecule.

Sulphoraphane from broccoli and **sulphoraphene** isolated from a widely consumed vegetable-radish showed potent antimutagenic effects against food-derived heterocyclic amines induced base substitution mutagenesis. Sulphoraphene, possessing a double bond in the alkyl chain was a stronger antimutagen as compared to sulphoraphane. These inhibitory effects are proposed to be due to the suppression of metabolic activation of cytochrome P450 enzymes.

From the results of present investigations it may be concluded that both curcuminoids and isothiocyanates represent a class of valuable phytonutrients with potent antimutagenic effects against cooked food-derived heterocyclic amine mutagens. Such an antimutagenic effect of curcuminoids and sulphoraphane and sulphoraphene against heterocyclic amines has not been reported earlier. From these findings **it may be suggested that consumption of turmeric in food as an additive or as an ingredient in food preparations and substantial intake of cruciferous vegetables like broccoli, cabbage, cauliflower and radish, may contribute to potential health benefits as cancer chemopreventive agents against heterocyclic amine induced carcinogenesis.** Moreover, consumption of fresh vegetables, fruits and certain beverages that contain ferulic acid, isoferulic acid, caffeic acid and vanillin may also be helpful in chemoprevention of cancer, as these have also shown antimutagenic potential in our studies. However, these findings also suggest a need for further biochemical, enzymatic and *in vivo* investigations in animal models as well as in humans, to establish the anticarcinogenic effect of these agents in man.

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SYNTHESIS AND BIOLOGICAL SIGNIFICANCE OF HETEROSTEROIDS OF POTENTIAL MEDICINAL INTEREST

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There continues to be a considerable interest in modified steroids. The synthesis of the nucleus, the study of reaction mechanisms and stereochemistry along with the investigation of their biological potency pose a challenge to the medicinal chemists to obtain lead compounds. Heterosteroids are steroid molecules containing heteroatoms and may be divided into nuclear and extra nuclear heterosteroids and can be natural and synthetic. The heterosteroids have a broad range of activity. In this work, the synthesis, study and biological activities of some heterosteroids in androstane and estrane series related to neuromuscular blocking, steroid hormone biosynthesis inhibitory and antineoplastic activity has been undertaken.

In case of the neuromuscular blockers, several bisquaternary ammonium compounds such as pancuronium bromide, rocuronium bromide and chandonium iodide have been found to possess nondepolarising neuromuscular blocking activity in a number of pharmacological assays. In these cases, the optimal interonium distance (1.0-1.2 nm) has been maintained. However, a number of new neuromuscular blocking agents, e.g., atracurium besylate, mivacurium chloride and pipecuronium bromide do not fall within these parameters. The reason for these exceptions is uncertain. We thought it worthwhile to prepare bisquaternary ammonium compounds with extended interonium distance and increased lipophilicity to investigate the effect of such modifications on the neuromuscular blocking activity. This was done by introduction of various functionalities at position 16 of steroid nucleus. Similarly, the knowledge of mechanism of aromatisation has led to successful design and development of modified androgens as potential aromatase inhibitors. Steroidal aromatase inhibitors that have been developed to date are the analogues of androstenedione nucleus and incorporate chemical substituents at varying positions on the steroid.

The following classes of compounds were synthesized: Bis- and monoquaternary ammonium steroids bearing *N, N*- dimethylamino-benzylidene moiety; bis- and monoquaternary ammonium compounds prepared from 16-(4-pyridylmethylene)-17-oxo-5-androsten-3β-ol; bisquaternary ammonium compounds in estrone series bearing *N, N*-dimethylaminobenzylidene moiety; 16-arylidene derivatives of estrone methyl ether; 16-arylidene derivatives of estrone and oxime derivatives of androstane.

The structures of these compounds have been established with spectral and elemental analyses. The compounds have also been tested for their neuromuscular activity. Some of the compounds have shown potential biological activity and the detailed studies are being carried out. Similarly, for the antineoplastic activity, the compounds were tested at the National Cancer Institute, Bethesda, U.S.A. The compounds have shown antineoplastic activity but it is statistically insignificant for further studies. Similarly, the estrogen derivatives were tested for their estrogenic/

antiestrogenic activity at the Institut Jules Bordet, Belgium. All the compounds have shown weak estrogenic activity.

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STUDIES ON SEED METABOLISM AND GROWTH PARAMETERS OF BAMBOO SEEDS IN RELATION TO EXOGENOUS PLANT GROWTH REGULATORS

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In the present investigation, the seeds of two species of bamboos *viz. Dendrocalamus strictus* and *Dendrocalamus membranaceus*, with different viabilities and varying ageing period were studied for the age-induced biochemical, physiological and growth parameters. The response of these seeds to exogenous applications of plant growth regulators was also noted. Under physiological studies, various germination parameters *viz.* germination percentage, vigour index and seed vigour were studied to determine age-induced reduction in these parameters at an interval of six months and their possible enhancement upon exogenous application of plant growth regulators (gibberellic acid, indole-3-butyric acid, 1,2,4-acid and resorcinol). Biochemical studies included the estimation of enzyme activities *viz.* α-amylase, ß-amylase, peroxidase and glutamate dehydrogenase with seed ageing of six months, with and without the exogenous application of plant growth regulators. The study on growth parameters was carried out on the seedlings raised from seeds of both species at an ageing interval of six months with and without the foliar application of PGRs (gibberellic acid, indole-3-butyric acid, 1,2,4-acid, resorcinol and a combination treatment of IBA+GA_3).

A. Physiological studies

D. strictus seeds had germinability of 30% at 5 months of age which was reduced to 20% at 11 months of age and then to 10% at 17 months of age. At the initial stage of ageing, the PGR treatments were not effective in increasing the various germination parameters but with further aging of 6 months the seeds became responsive to only some concentrations of the PGR treatments.

With aging of 17 months, the seeds still retained viability of 10% and at this stage. 1,2,4-acid (15 ppm) was the most effective treatment in increasing all the germination parameters significantly. In aged seeds, phenols and GA_3 (30 ppm) remained to be the most effective treatments in increasing growth significantly.

D. membranaceus seeds showed high germinability of 40% at 24 months of ageing which was reduced to zero with further seed ageing of six months. Like in *D. strictus*, in *D. membranaceus* seeds also, the exogenous application of all the PGR concentrations was not effective at initial stage of ageing. Only the lower concentrations were promotory whereas the higher PGR concentrations were inhibitory. Further ageing in *D. membranaceus* seeds led to their complete loss of viability. All the PGR treatments at this stage were effective except for R (10ppm) in enhancing various germination parameters.

B. Biochemical studies

A decline in the α-amylase activity was observed with seed ageing of 6 months in both *D. strictus* and *D. membranaceus* seeds. In *D. strictus* seeds, R (20 ppm) was the most effective treatment in increasing the enzyme activity but with further seed ageing of 6 months, IBA (20 ppm) became

the most effective treatment. In *D. membranaceus* seeds, IBA (20 ppm) increased the activity effectively after 48 hrs of germination whereas after 6 months of further ageing, GA_3 (40 ppm) effectively increased the activity after 96 hrs of germination. In both the species, the PGR treatments were not very effective in enhancing α-amylase activity. But with seed ageing of 6 months, the seeds became responsive to the exogenous application of PGRs indicating depletion of endogenous hormones with ageing.

ß-amylase activity also decreased with ageing in *D. strictus* seeds. IBA (20 ppm) treatment proved to be the best treatment in giving maximum enzyme activity and germination percentage both in aged seeds. The *D. membranaceus* seeds on the other hand, R (20 ppm) was the best treatment in giving maximum enzyme activity in aged seeds. ß-amylase activity appeared to be an indicator of germinability in *D. strictus* seeds but not in *D. membranaceus.*

In both the species, a considerable decline in peroxidase activity was seen with seed ageing. Only in *D. strictus*, the PGR treatments were effective in increasing the enzyme activity after 6 months ageing.

Glutamate dehydrogenase activity also declined with ageing in seeds of both the species. In *D. strictus* seeds, none of the PGR treatments were effective in increasing the enzyme activity in aged seeds while in *D. membranaceus* seeds, the PGR treatments were effective only after ageing.

C. Growth parameters

In both the species, growth parameters showed varying response to PGR treatments probably due to varying time of ageing and the genotypic difference in species.

In *D. strictus* seedlings, the seedling height was increased to the maximum by the treatment with R (50 ppm) followed by IBA (25 ppm) +GA_3 (25 ppm) , IBA (50 ppm) and 1,2,4-acid (50 ppm) treatments whereas GA_3 (50 ppm) treatment decreased the seedling height. The treatment with IBA (50 ppm) and R (50 ppm) led to the early initiation of secondary shoots as well as produced the highest secondary shoot number while the height of the secondary shoots was increased to the maximum by combination treatment IBA (25 ppm) + GA_3 (25 ppm). Again combination treatment of IBA (25 ppm) + GA_3 (25 ppm) produced highest number of leaves followed by R (50 ppm) and IBA (50 ppm). Thus, resorcinol (*i.e.* diphenol) and combination treatment of IBA + GA_3 proved to be the most effective treatment in increasing these growth parameters (supported by Setia *et al.*, 1991).

In *D. strictus* seeds, the various parameters of productivity were increased to the maximum by treatment with IBA (50 ppm) whereas R (50 ppm) produced the maximum seedling height.

D. strictus seedlings raised from 11 months aged seeds showed different response to PGR treatments than in those raised from 5 months aged seeds. With ageing of six months, different PGR treatments enhanced different productivity parameters to different degree. IBA (25 ppm) + GA_3 (25 ppm) becoming effective in increasing more productivity parameters and by a greater degree as compared to seedlings raised from 5 months aged seeds.

In *D. membranaceus* seedlings (raised from 24 months aged seedlings), R (50 ppm) proved to be the best treatment for all the parameters studied. Seedlings raised from 30 months aged seeds also followed the same trend but the effectiveness of the treatments increased. Growth and productivity performance of the seedlings raised from 30 months aged seeds was comparatively lower than the seedlings raised from 24 months aged seeds.

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THERMODYNAMIC AND RELATED STUDIES OF MIXTURES OF SOLVENTS AND SURFACTANT IN SOLVENTS

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Excess molar volumes V_m^E and ultrasonic velocities u of the liquid mixtures of pyrrolidin-2-one (PY) with halogenated alkanol or aromatic hydrocarbons or nitriles or surfactants and hexamethylphosphortriamide with halogenated alkanol have been determined over the whole concentration range of the mole fraction x where x is the

mole fraction of PY or HMPA.

In the mixtures of PY + halogenated alkanol, V_m^E values are negative in the mixture of PY + TFE throughout the composition range with a tendency to become positive at large x whereas PY + TCE, and + HFP present an S-shaped behaviour of V_m^E, being negative at low x and positive at high x. On replacing PY with HMPA, similar variation in V_m^E is observed for HMPA with halogenated alkanol except for HMPA + TCE where the values of V_m^E are positive throughout the whole mole fraction range. On comparing the results of PY and HMPA with each other, it looks like that the expected interaction of the type P=O---H-O in HMPA + halogenated alkanol is stronger as compared to the interaction of the type C=O----H-O in PY + halogenated alkanol. This may be related to the fact that P=O group is more polar than C=O group. The stronger interaction leads to a greater breakdown of the hydrogen bonded structure of halogenated compounds and thus producing more positive V_m^E in HMPA mixtures than in pyrrolidin-2-one mixtures.

The values of V_m^E are found to be negative in the mixtures of PY with each of aromatic hydrocarbons and increase in the order o-xylene < ethylbenzene < m-xylene < p-xylene. It may be inferred that negative contributions to V_m^E are due to the accommodation of PY in aromatic hydrocarbons and the dipole-induced dipole interaction between the (-NH) group of pyrrolidin-2-one and the π electron on the benzene ring of an aromatic hydrocarbon.

The mixtures of PY + nitriles also show negative deviations for all the mixtures and vary in the order valeronitrile < benzonitrile < butyronitrile < propionitrile < acetonitrile < acrylonitrile. The structural effects seem to dominate throughout the composition range as compared to physical and chemical effects. In the case of mixture of PY with monoethylene glycol, the measured V_m^E is positive throughout the composition range with very small negative deviation at large x. The chemical and physical contributions seem to dominate in this system throughout the composition range except at very high mole fractions of PY. The V_m^E is negative for the other three mixtures:

PY + di-, + tri-, and + tetraethylene glycol and decreases in the order tetra- < tri- < di- indicating that structural effects dominate the chemical and physical contributions.

Negative deviations are also observed for PY + surfactants *i.e.* Tritons and vary in the order TX-45 < TX-114 < TX-100 < TX-405. Since the differences between molar volumes and free volumes of the real components is quite large which indicates that structural effects are dominating. For Tritons the number of oxyethylene groups increase in the sequence TX-45 < TX-114 < TX-100 < TX-405. The increasing negative excess molar volumes with the increase in number of oxyethylene groups in PY + Triton mixtures indicate that PY may become encaged in the structure of Triton thus reducing the amount of free volume.

Prigogine–Flory–Patterson theory has been utilized for the quantitative estimation of different contributions *viz.* interactional, free volume and P^* effect to V_m^E. In the mixtures of PY + halogenated alkanol, the interactional term is postive whereas free volume and P^* effect are negative. Both the interactional and P^* are important in magnitude in these mixtures but the overall magnitude of V_m^E is decided by the third contributions *i.e.* free volume term. In case of HMPA + halogenated alkanol, free volume contribution is almost zero for TFE system but its contribution in HFP is much more than TCE mixture. The third contribution P^* is very small negative in TFE system but quite large negative in HFP system. Interactional term which is large in fluoro systems plays a dominating role. When the interactional term is larger than the sum of the other two contributions the calculated V_m^E values are all positive as in case of TFE. Free volume and P^* contributions also play an important role in predicting the sign and magnitude of V_m^E. Contrary to fluoro systems, in the mixture HMPA + TCE the dominating role is played by P^* effect, the magnitude of interactional and free volume term being same but opposite in sign. It seems that at lower concentration of HMPA, structural effects overtakes whereas interactional term is dominant at higher concentrations of HMPA.

In PY + aromatic hydrocarbons, the free volume contribution is negative and decreases in the order o-xylene < m-xylene < p-xylene < ethylbenzene. P^* effect is also negative and follows the order o-xylene < ethylbenzene < m-xylene < p-xylene. The interactional contribution is small as compared to the other two contributions. It is negligible for p-xylene and small negative for m-xylene whereas its magnitude is small positive for o-xylene and ethylbenzene. The results clearly indicate that both the free volume term and the P^* effect are responsible for the observed negative V_m^E variation. The contribution of interactional parameter is insignificant as compared to the free volume and P^* effect. The overall magnitude is decided by the free volume term for pyrrolidin-2-one and nitriles. In comparison to the other two contributions *i.e.* interaction and P^* effect, its effect is less in benzonitrile and highly significant in acetonitrile. The magnitude of the free volume contribution along with the sign and magnitude of the internal energy term are very important in producing the behaviour. The P^* contribution plays a significant role in explaning the observed V_m^E.

The interactional contribution term is positive and large for PY + monoethylene glycol but is negative for all the other mixtures: PY + di-, + tri-, and + tetraethylene glycol and increase in the order of mono- > tetra- > tri- > di-. The free volume contribution is small negative for tetra- and negligible for the other three glycols in PY. Similarly, the P^* effect is small positive in the case of tetra- and decrease in the order tetra- > tri- > di- > mono-, showing no contribution in the case of PY + mono-. It may be concluded that the interactional term is playing a dominant role in predicting the sign of V_m^E but the free volume and P^* factor play an insignificant role except for PY + tetra- where P^* factor is also contributing to some extent.

For PY + TX-45 mixture, the negative V_m^E is mainly due to the significant negative P^*. For the rest of the three mixtures *i.e.* TX-114, TX-100 and TX-405, with increase in oxyethylene groups in Tritons, the interactional term contribution increases nearly four fold. Thus making the V_m^E more negative in TX-405, inspite of the positive contribution due to P^*. One may conclude that the net V_m^E in these mixtures is the result of balance between the three contributions. Except TX-45 mixture, the interactional contribution is responsible for the large negative V_m^E values in these mixtures.

The measured ultrasonic velocity of mixtures of PY with other components shows a increase from lower value of other component to a higher value of PY over the whole mole fraction range except for PY + monoethylene glycol where there is decrease in the value of u with increase in x. For PY + TX-405, it also shows decrease and then increase at high x *i.e.* a minima occur at high mole fraction of PY. The u data has been utilized to estimate the deviation in ultrasonic velocity u^D, molar isentropic compressibility K_S and its excess counterpart K_S^E in order to understand the behaviour in these mixtures.

For PY + halogenated alkanol, negative deviations in K_S^E and positive in u^D vary in the sequence HFP > TFE > TCE. In the case of HMPA + halogenated alkanol, K_S^E is negative for HFP, positive for TFE and S-shaped in TCE being small negative at lower mole fraction but slightly positive at very high mole fraction. The trends in u^D show exactly the opposite. A comparison of K_S^E variation with V_m^E for PY or HMPA + halogenated alkanol reveals that the two behaves differently. This may be attributed to the fact that interstitial accommodation is also facilitated by the increase of pressure, thus the trends in K_S^E are different than those of V_m^E.

Negative K_S^E values are also obtained in PY + aromatic hydrocarbon or nitriles while u^D shows positive variation. The trends in K_S^E are same as those for V_m^E but in the case of nitriles the order is different. This may be explained on the basis of denser packing of the molecules which results in an increase in the ultrasonic velocity and a decrease in the compressibility in the solution. This process continues until a concentration is reached at which all cavities are filled.

In the system of PY + glycols, u^D increases in the order di- > tetra- > tri- > mono- whereas reverse trend is being observed in K_S^E *i.e.* mono- > tri > tetra- > di- K_S^E values are negative for PY + TX-45, + TX-114 and + TX-100 and positive for PY + TX-405 mixture. The results may be correlated with the number of oxyethylene groups present in the system *i.e.* as the number of oxyethylene group increases, compressibility decreases. Deviations seem to be larger at higher mole fractions of PY. A comparison of K_S^E with V_m^E shows that the two varies in a opposite manner.

In order to corroborate the observations obtained from above mentioned parameters, partial and apparent molar quantities have also been estimated. In PY + halogenated alkanol, the negative $\overline{V}_1^{E,o}$ and $\overline{K}_{S,1}^{E,o}$ and the opposite $\overline{V}_2^{E,o}$ and $\overline{K}_{S,2}^{E,o}$ except for the negative values of $\overline{K}_{S,2}^{E,o}$ for TFE and HFP clearly indicate that, in dilute PY region, structural effects are dominating whereas destruction of associated structures is important in dilute halogenated alkanol region.

Similar observations have been obtained in case of HMPA + halogenated alkanol. Partial excess molar quantities are negative for PY + aromatic hydrocarbons or nitriles thus indicating the fitting of PY into the cavities of other component. The structural effects seem to dominate the overall behaviour in these systems.

In the case of mono- system, the partial excess molar volumes curves show a distinct maxima in dilute region. The partial excess molar compressibilities are also positive thus indicating positive contribution due to breaking of self-associated pure components. For other three mixtures: PY + di-, + tri-, and + tetraethylene glycol partial excess molar quantities are mostly negative with a minima in $\overline{V}_1^E$ dilute region of PY + tetra-. Negative partial molar values indicate the dominance of structural effect over the non-specific interaction.

The excess partial molar quantities are also negative for PY + Tritons systems in both the dilute PY and Triton region except for the values of $\overline{K}_{S,i}^E$ of TX-405 which are highly positive. Results indicate that fitting of PY into the Triton cavities is responsible for the negative excess partial molar quantities. In the case of PY + TX-405, the observed positive values of $\overline{K}_{S,i}^E$ indicate that contribution to disruption of self-associated PY on mixing with Tritons is significant.

Apparent molar volume and compressibility values have been used to estimate the partial molar volumes $\overline{V}_2^o$ and compressibilities $\overline{K}_{S,2}^o$ at infinite dilutions graphically and further to calculate the change in volume □V and compressibility □K at infinite dilutions. The partial molar volumes at infinite dilutions for PY + another component are found to be close to the molar volumes in all the mixtures except in PY + valeronitrile, + benzonitrile and HMPA + halogenated alkanol whereas partial molar isentropic compressibilities at infinite dilution are slightly larger than their corresponding molar isentropic compressibilities in these systems except for PY + ethylbenzene, PY + acrylonitrile, + acetonitrile, + propionitrile, butyronitrile, + valeronitrile and PY + glycols. In case of PY + Tritons, the partial molar volumes at infinite dilution of Tritons are found to be higher as compared to their corresponding molar volumes. Differences are observed on comparing the limiting $\overline{K}_{S,2}^o$ values of Tritons with the corresponding molar isentropic compressibility $K_{S,2}$ for

TX-45, TX-100 and TX-405, except for TX-114. Large differences are also observed in $\overline{K}^{o}_{S,2}$ and $K_{S,2}$ in case of HMPA + halogenated alkanol.

Close agreement in $\overline{K}^{o}_{S,2}$ and $K_{S,2}$ indicates that after dissolution the molecular volume of the solute molecules is practically unaffected and that the molecules are in a force field similar to that present in the pure liquid state. Deviations observed in mixtures of the measured systems imply that after dissolution the molecular volume is affected and this disagreement for the given mixtures can be analyzed in terms of structural and geometrical contributions.

The experimental slope of the infinite dilution molar volumes and isentropic compressibilities calculated by a linear extraoplation using the least-squares fit in terms of parameter S_v and S_k provides the information about solute-solute interactions arising from dilute concentration effects. In case of PY + nitrile both S_v and S_k parameters are negative for all the mixtures implying the absence of strong interactions. On the other hand in PY + glycols, S_v and S_k values are positive for PY + mono- and negative for PY + di-, + tri-, and + tetra- indicating that relatively strong interactions are operating in former system compared to latter systems. For PY + Tritons, S_v values are negative in all the mixtures and vary in the order TX-45 < TX-114 < TX-100 < TX-405. Less negative values in TX-45 and large negative in TX-405 indicate that the interaction between unlike molecules increases as the number of oxyethylene groups increase in the system.

Thermo-acoustical parameters such as free volume V_f, specific acoustic impedence Z, solvation number S_n, relative association R_A, molecular association M_A, Rao's constant R, Wada's constant W, intermolecular free length L_f, van der Waals constant b, molecular radius r, geometrical volume B, molar surface area Y and available volume V_a of a binary mixture have also been calculated. The curves of various parameters *viz.* Z, S_n, R, W, L_f and b do not show any sudden variation with the increase in the mole fraction thus indicating the absence of any complex formation in the studied systems The behaviour of R_A and M_A over the whole composition range nearly match the results of other studies

An attempt was made to estimate ultrasonic velocities at different concentration using theoretical and empirical equations *i.e.* Flory's theory, Jacobson's free length theory, Schaaff's collision theory, Junjie's empirical relation and Nomoto's relation. A comparison of computed u values with experimental ones in term of percentage errors for all the mixtures reveals that the Free length theory is unable to predict the ultrasonic velocities of the mixtures accurately, the error range being 7.96...63%. Flory's theory and Schaaffs collision factor theory show an error of 0.2... 12% and 0.19...8.46% respectively in comparison to Junjie and Nomoto empirical equations which show lower % error in the range 0.43...4.96% and 0.2...4% respectively. To summarize, Nomoto's empirical equation and Junjie's relation fit the data reasonably well, with the former providing a better fit.

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SYNTHESIS OF 2-AZETIDINONES AND OTHER HETEROCYCLES AS POTENTIAL ANTIBACTERIAL AGENTS

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The synthesis of heterocyclic compounds have received a great attention in academic and industrial areas with particular mention to pharmaceutical industries. Heterocyclic compounds have been utilized as pharmaceutical agents to combat a variety of diseases. The importance of heterocyclic compounds among medicinal agents has continued to stimulate further research in this direction. The physico-chemical & biological properties of this class of drugs have been scrutinized like no others, yet considerable research efforts are still being made to achieve further progress. The desired aim can be achieved only by the introduction of more & more drugs.

The role of ß-lactams in antibacterial chemotherapy need no introduction. The pharmaceutical importance of penicillins, cephalosporins, carbapenems, thienamycin, nocardidins & mono-bactams have added further impetus to the study of this class of heterocyclic systems.

Keeping this in mind, the synthesis of new ß-lactams & other heterocyclic compounds through very simple methods and mild reaction compounds have been achieved and a number of these compounds have been tested for antibacterial activity. Several new ß-lactams & N-acyloxazolidines were synthesized from substituted ethanol imines & phenoxy acetic acid using benzenesulfonylchloride as acid activating reagent. N-unsubstituted ß-lactams had also been synthesised using a very simple method from N-(2-hydroxyethyl ß-lactams.

In view of the recent number of publications on the modification of the ß-lactam ring by alternation of groups present at N-1, C-3 & C-4 we become interested to synthesize some new N-substituted ß-lactams i.e., N-(3-hydroxypropyl) ß-lactams which have not been studied earlier. Various N-(3-hydroxypropyl) ß-lactams & N-acetylated tetrahydro -1,3-oxazines were synthesized through the intermediacy of N-(3-hydroxypropyl) imines. N-acylated dihydro-1,3-benzoxazine has been synthesized by annulation of phenyl-2-carbinol imine with phenoxyacetic acid. The synthesis of N-3-(aminopropyl) ß-lactam and N-acyl tetrahydropyrimidine have also been attempted.

The approach towards chiral ß-lactams using Staudinger reaction condition involve three different strategies. Firstly, ß-lactams can be obtained by the annelation of chiral ketenes with chiral imines derived from chiral amines & achiral aldehydes. Secondly, chiral imines derived from chiral aldehyde & achiral amine may be employed for the chiral ß-lactam synthesis. Thirdly, chiral ketenes can be used to annelate with achiral imines to generates optically active ß-lactams. Our efforts towards the synthesis of some new chiral ß-lactams utilizes the second approach i.e. annelation of chiral imines derived from achiral aliphatic amines and chiral aldehyde with achiral ketene. We decided to use 2,3-isopropylidene-D-glyceraldehyde and various aliphatic amines for the imine formation.

The Pictet-Spengler reaction is applied for the synthesis of dibenzoquinolizines and tetrahydro-ß-carbolines. The pharmacological properties of 1,3-disubstituted 1,2,3,4-tetrahydrop- ß-carbolines is quite well known. They have been shown to inhibit monoamino oxidase And bind with nanomolecular affinity to serotonin receptors. In the course of our work directed towards the construction of ß-lactam rings we became interested to synthesize tryptophan-derived imines. We attempted the reaction of L-tryptophan methyl ester hydrochloride and various aldehydes in an aprotic solvent – DCM in presence of triethylamine at rt, but to our surprise the products isolated in all cases were 1,3-disubstituted tetrahydro-ß-carbolines instead of expected imines. This method has also been successful in accomplishing the diastereoselective synthesis of tetrahydro ß-carboline using homochiral D-glyceraldehyde acetonide.

Methods for the synthesis of 2-substituted benzothiazoles have been extensively studied because compounds containing this hetereocyclic nucleus are of industrial as well as biological interest. They are used as fluorescent brightening agents for textiles and plastics, additives in textile dyeing, lasers and biological stains. The wide applications of benzothiazole prompted us to reinvestigate its synthesis starting from o-aminobenzene thiol and various aldehydes.

The benzimidazole nucleus is found in a variety of naturally occurring compounds such as vitamin B_{12} and its derivatives. Several benzimidazoles are commercially available as pharmaceuticals vetarinary products and fungicides. We also became interested to synthesize these biologically active heterocycles under mild reaction conditions.

R & S-glyceraldehyde acetonides have been utilised as versatile synthons for the synthesis of optically active natural products. They have also been used for the synthesis of many chiral ß-lactams. Thus, the synthesis of chiral benzofused heterocycles was undertaken as a part of our research work. We have synthesized some new chiral benzo-fused heterocycles starting from various ortho disubstituted benzenes and R-glyceraldehyde acetonide.

Finally the activities of the synthesized compounds were evaluated by nutrient-agar plate diffusion method. The main aim of these investigations is to study the change in the activity with the variation in structure of the molecule and thereby establishing a correlation between the structure of compounds and their antibacterial properties. The 2-hydroxy propyl ß-lactams were found to be active against some of the bacteria. The 4,4-disubstituted ß-lactams were found to be moderately active. N-acylated tetrahydro-1,3-oxazines showed some activity against a few bacteria and the tetrahydro-8-carboline was also found to be active against a few bacteria. The chiral benzothiazoline and benzimidazole derivatives were found to be high active against a number of bacteria.

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RATIONALLY DESIGNED CHIRAL AMINES AND THEIR USES IN ORGANIC SYNTHESIS

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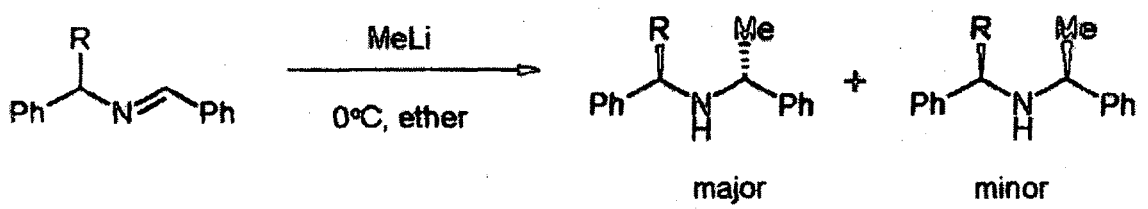
Nucleophilic addition to Schiff bases of chiral amines: origin of diastereoselectivity in Schiff bases of chiral amines without additional heteroatom.

The optically active amines are components of many naturally occurring and biologically active compounds. The increasing importance of enantiomerically enriched amines derives from their extensive use as auxiliaries, resolving agents and intermediates in the synthesis of both natural and unnatural compounds. Of the many strategies available for the asymmetric synthesis of amines, one of the most attractive strategy is the asymmetric 1,2-addition of nucleophiles to the carbon-nitrogen double bond. This method can achieve at the same time the formation of optically active amines and the construction of carbon skeleton of the amines. A variety of asymmetric alkylations of imines have been explored and this mainly involves either enantioselective alkylations of achiral imines in the presence of various external chiral ligands or nucleophilic addition of organometallic regents to aldimine derived from chiral amines.

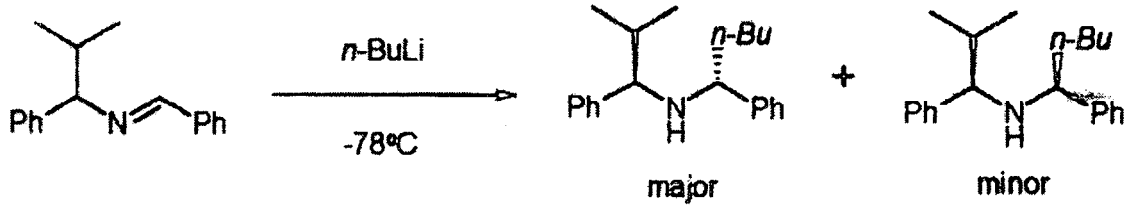
Ab initio calculations were performed with GUASSIAN 94 program packages. The theoretically observed diastereoselectivity appears to be sterically controlled and the major diastereomer formation occurs when the nucleophile approaches from the side of the phenyl group. If this really holds true then increasing the size of alkyl group should increase diastereoselectivity in these reactions because then the attack from the side of the alkyl group will be further inhibited.

In order to check the validity of the theoretical work and subsequent proposals, various primary amines with varying steric bulk of alkyl group were prepared starting from the corresponding ketones. Racemic chiral Schiff bases were treated with methyllithium at 0°C to give the corresponding secondary amines as a mixture of two diastereomers. Diastereoselectivity increases with increasing the size of the alkyl group in the amine part of Schiff base, from methyl to ethyl to *iso*-propyl as predicted according to theoretical calculations. Therefore, steric factors of the alkyl group at immediate vicinity of the resident chiral center are important. This is what is expected when steric factors are important to control diastereoselectivity.



No.	R	Diastereomeric Ratio	Yield(%)
1	Me	70:30	63
2	Et	86:14	52
3	*i*-Pr	92.08	51
4	*n*-Bu	82:18	65
5	*i*-Bu	78:22	59

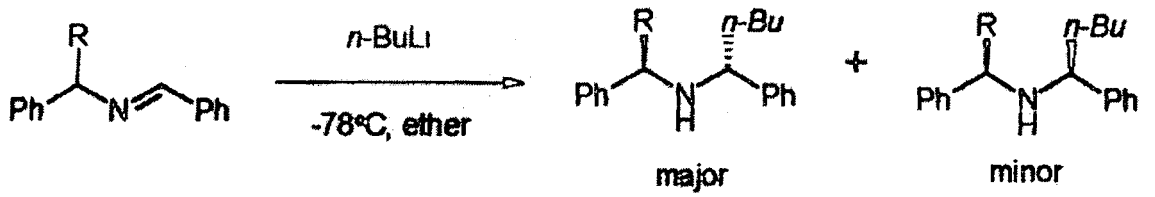
The effect of the solvent on diastereoselectivity and reactivity was also investigated. Addition of *n*-butyllithium to *N*-benzylidene-2-methyl-1-phenyl propylamine in various solvents was carried out at -78°C. The highest diastereoselectivity was observed in ether or *n*-hexane:ether (1:1) mixture. The reaction was fast (<1h) in DME but the diastereoselectivity was low. This could be due to reduced aggregation of *n*-butyllithium, due to complexing ability of the solvent, which in turn may have reduced the effective steric bulk of the nucleophile. Lower diastereoselectivity was also observed in THF and the rate of reaction was in between that of ether and DME. In *n*-hexane and toluene, no reaction was observed even after 24h during which the temperature was also raised to -40°C. These investigations suggest that ether is the solvent of choice, which inadvertently we chose for our investigations.



No.	Solvent	Diastereomeric Ration	Yield(%)
1	Ether	97:03	82
2	THF	75:25	72
3	DME	67:33	81
4	*n*-Hexane	No Reaction	
5	*n*-Hexane:Ether(1:1)	97:03	61
6	*Toluene*	No Reaction	

Other alkyl lithium i.e., *n*-BuLi was also used as a nucleophile. Addition of *n*-butyllithium to Schiff bases was carried out in ether. This addition was accomplished at -78°C. Similar trend was observed as in case of methyllithium addition. Highest diastereoselectivity (97.03) was observed

when R is *iso*-propyl group. The higher diastereoselectivity could be due to bulkier nature of *n*-BuLi aggregate or lowering of the temperature or both.



No.	R	Diastereomeric Ratio	Yield(%)
1	Me	73:27	79
2	Et	73:27	76
3	*i*-Pr	97:03	82

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SYNTHESIS, CHARACTERIZATION AND REACTIVITY OF α-ARYL AND α, α-DIARYL-α-FLUORO ACETOPHENONES

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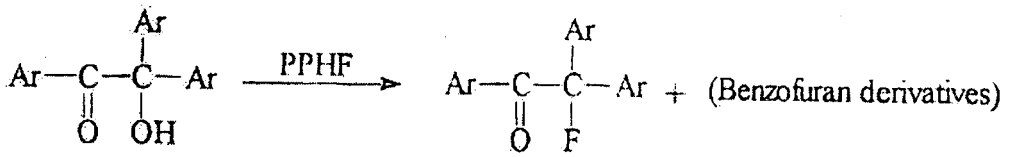
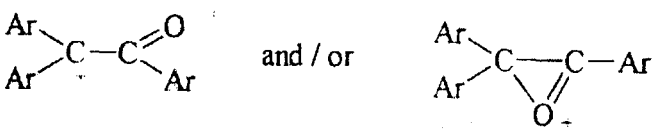
The thesis describes the synthesis, charaterisation and reactivity of α-aryl and α, α-diaryl-α-fluoro acetophenones (1,2,2-triaryl-2-fluoro ethanones) which have been achieved by the treatment of 1,2,2-triaryl-2-hydroxy ethanones with pyridinium poly (hydrogen fluoride). These include: 2-fluoro-1,2,2-triphenyl ethanone **(147)**, 2-fluoro-2-(1-naphthyl)-1,2-diphenyl ethanone **(148)**, 2-fluoro-1-(2-methylphenyl)-2,2-diphenyl ethanone **(149)**, 2-fluoro-1-(2-ethylphenyl)-2,2-diphenyl ethanone **(150)**, 2-fluoro-2-(2-methylphenyl)-1,2-diphenyl ethanone **(151)**, 2-fluoro-2-(2-ethylphenyl)-1,2-diphenyl ethanone **(152)** and 2-fluoro-1,2-bis(4-methylphenyl)-2-phenyl ethanone **(153)**. During the synthesis of α-fluoroketones **148** and **152**, the formation of 3-(1-naphthyl)-2-phenyl benzofuran **(154)** and 3-(2-ethylphenyl)-2-phenyl benzofuran **(155)** respectively have also been observed.



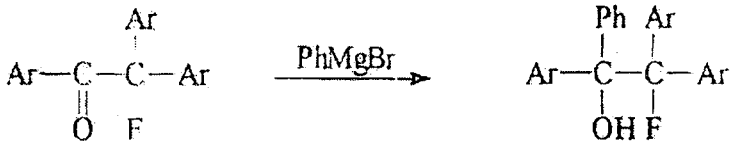
Both type of products (α-fluoroketones and/or benzofuran derivatives) may form through the intermediacy of relatively unstable carbenium ion and/or bridged oxiranyl ion. Although the initial formation of an electron deficient carbocation is verified by reduction studied, the intervention of bridged oxiranyl ion accommodates the retention of configuration and optical activity in the



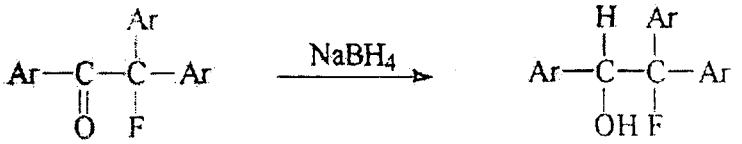
fluorodehydroxylated product of S(-)-2-acetoxy-1,1,2-triphenyl ethanol **(156a)**. The presence of methoxy group in α-hydroxyketone **127** however stabilises the positive charge on carbonyl oxygen atom which in turn abstracts the hydride ion from the solvent to give a ketone **(158)**. Some comments are also offered to understand the deffering results obtained in diphenyl methyl alcohols bearing EWG on aryl ring or cationic centre. The α-fluoroketones are characterised on the basis of their melting point, infrared, ultraviolet, $^{1}H^{13}C$, ^{19}F NMR, mass spectral data. In

certain cases single crystal X-ray diffraction studies have also been carried out. A critical examination of mass spectra of α-fluoroketones revealed that the molecular ion (M^+) is absent except in **147** (0.2%), **148** (5%) and **152** (0.8%). The main path of fragmentation showed that the aryl peak (Ar-C≡O^+) is the base peak in all α-fluoroketones except **148** which shows $Ar_2C{=}F^+$ as base peak. Unlike α-hydroxyketones, α-fluoroketones do not undergo rearrangement in mass spectrometer.

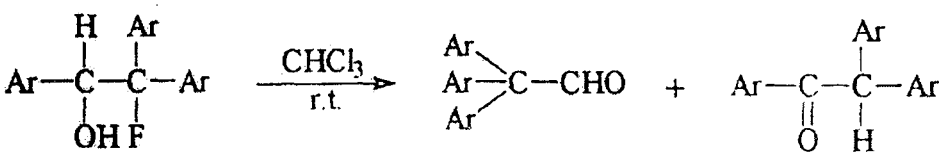
The nucleophilic cleavage of C-F bond in α-fluoroketones does not occur with reagents such as water, methanol, sodium methoxide and Grignard reagent. However, phenyl magnesium bromide reacts with carbonyl group to give tetraaryl fluorohydrin.



All fluoroketones undergo the reduction with sodium borohydride to give a mixture of fluorohydrins. Although most of these not been purified. However, the fluorohydrin **167** was



studied by single crystal X-ray crystallography. An interesting rearrangement has been observed when the solution of a fluorohydrin in chloroform was kept at room temperature. It showed the formation of triaryl ethanal and ketone.



A number of mechanisms have been presented for the above reaction. Keeping the single crystal X-ray structure of **167** in view, and the theoretically calculated total energy and distances (F---H) in model compound, 1-fluoro-2-hydroxy ethane **(176)**, a concerted mechanism has been proposed.

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EFFECT OF SOME DOPANTS ON THE KINETICS OF FORMATION AND HYDRATION OF TRICALCIUM SILICA PHASE

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Clinker is manufactured by firing a mixture of calcareous and argillaceous materials in appropriate proportions at 1450°C. Calcareous materials mostly used are limestone, chalk, sea shells etc., and argillaceous materials used are clay and sand stone. OPC clinker comprises of major phases as tricalcium silicate (C_3S), dicalcium silicate (C_2S), tricalcium aluminate (C_3A) and tetracalcium aluminoferrite (C_4AF). Out of these, tricalcium silicate and dicalcium silicate together amounts to about 80% part of the clinker. A good clinker contains about 50-60% C_3S.

In the recent past, there has been a trend to manufacture high strength cements such as 43 and 53 grade, which are made from clinker having high C_3S (>50%). Tricalcium silicate is the main phase responsible for high strength in clinker. In the clinker, the role of tricalcium silicate phase is influenced by the accompanying constituents such as C_2S, C_3A, C_4AF, alkalies, free lime, free magnesia and minor constituents e.g. Mg, Sr, Ba, Ti, Cr and Mn etc. These minor impurities play a vital role in modifying the properties of clinker because they get doped in various phases of the clinker as per their saturation solubility in individual phase. Besides acting as mineralizers, these influence the structure of the constituent phases of clinker.

In the manufacture of commercial clinker, the time of retention and the temperature of firing have a strong bearing on the production and quality of clinker. Both these factors i.e. the retention time and the temperature of firing are influenced by the presence of dopants. As the ultimate performance of cement is the result of structural components of clinker, it is very essential to study the role of these components, especially the tricalcium silicate. In clinker, the study of this phase is very complicated because of the influence of the accompanying constituents e.g. C_2S, C_3A, C_4AF, alkalies etc. Hence it is more appropriate to investigate the activity of this phase as an individual phase in presence of varying amounts of dopants.

Minor constituents e.g. Mg, Sr, Ba, Ti, Cr and Mn are very common in raw materials used for the manufacture of clinker. This study was undertaken to investigate the impact of the elements Mg, Sr, Ba, Ti, Cr, and Mn on the formation and hydration of tricalcium silicate phase. Tricalcium silicate phase was made at 1450°C by firing a mixture of calcium carbonate, quartz and the respective dopant in the appropriate proportions. Following investigations were carried:

i. Effect of dopant on the lime silica reaction at 1450°C,

ii. Investigation of the effect of dopant concentration from 0.5 to 5% on the solubilities of the dopants in tricalcium silicate phase at 1450°C,

iii. Study of the impact of respective dopant on the crystal size of C_3S,

iv. Study of the impact of different dopants on the formation and stabilization of various polymorphs of C_3S at 1450°C,

v. Investigation of the formation of various compounds in the 3CaO:1 SiO_2:x Dopant system at 1450°C,

vi. Study of the distribution of the respective dopant in C_3S and the other accompanying phases (C_2S, interstitial),

vii. Study of the rate and degree of hydration of C_3S doped with various concentrations of the respective dopant from one hour to 28 days.

The techniques used for the study are chemical analysis, XRD, Microscopy (Optical and Scanning electron microscope), Energy Dispersive Analysis of X-rays (EDAX) and thermal analysis (TGA).

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MOLECULAR DESIGNING OF NOVEL CONDUCTING POLYMERS WITH LOW BAND-GAPS

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In our present work we have focussed on the molecular designing of novel conjugated polymers with low band gaps. Our strategy of investigation was donor-acceptor polymerization. We have investigated the electronic structures and conduction properties of some novel donor-acceptor polymers, using the ab-initio Hartree Fock Crystal Orbital method. Donor-Acceptor polymers can be viewed as consisting of a backbone of sp^2- hybridized carbon atoms analogous to that of trans-cisoid polyacetylene (cis-PA) but stabilized alternately by different bridging groups X and Y which are covalently bonded to form heterocycles. Different X and Y groups tried by us include X= S, O, NH and Y= >C=O, >C=C $(CN)_2$. All the polymers studied have been predicted to have band gap values less that 2 eV with some predicted to have band gaps even less than 1 eV. It is also observed that the band gap is smaller for Y=>C=C $(CN)_2$ as compared to Y=>C=O for a given X=(S, O, NH). The highest occupied crystal orbital (HOCO) and the lowest unoccupied crystal orbital (LUCO) have π- nature in all the polymers studied here. All the six donor-acceptor polymers on the basis of their geometry and π- bond order values, are predicted to have benzenoid like electronic structures and their small band gap values can be shown to arise from the strong bonding interaction between the LUCO of the conjugated skeleton containing only X and the LUMO of the electron accepting group Y terminated by hydrogen atoms. It is also seen that for each electron donating group X, polymers with Y=>C=C$(CN)_2$ have the largest values of both I.P and E.A which shows that polymers containing Y=>C=C$(CN)_2$ are expected to be the best candidates for reductive (n-) doping while those with Y=>C=O the best for oxidative (p-) doping. The band gap values are predicted to decrease in the order PFUCN < PFUCO < PPYCN < PPYCO < PCNTh < PCDT. PFUCN is thus predicted to have the smallest band gap and PCDT the largest. It is also analyzed that the polymers derived from pyrrole (X=NH) have the lowest value of both I.P and E.A, while those derived from furan (X=O) have the largest value of E.A and the ones from sulphur (X=S) have the highest value of I.P.

We have also studied the electronic structures and conduction properties of some copolymers of donor-acceptor polymers using the simple negative factor counting (NFC) method on the basis of Dean's negative eigenvalue theorem. The trends in the electronic structures and conduction properties of the copolymers $(A_mB_n)_x$ are studied as a function of the (i) block sizes m and n and (ii) arrangement of the units (periodic or random) in the copolymer chain. We have investigated two pairs each of Type I and Type II- staggered superlattices in this thesis. $(A)_x$ PCDT and $(B)_x$ PCNTh forms the first pair and $(A)_x$ PFUCO and $(B)_x$ PFUCN the second pair of Type II-staggered superlattices. $(A)_x$ PFUCO and $(B)_x$ PCDT forms the first pair and $(A)_x$ PPYCN and $(B)_x$ PCDT the second pair of Type I superlattices. Our results show that for both types of superlattices turning the electronic properties intermediate between those of two homopolymers is easier by synthesizing periodic copolymers. Random copolymerization on the other hand, is expected to lead relatively faster to the saturated electronic properties characteristic of the lower

band gap component and largely independent of the larger band gap component. For both the types, increasing the block sizes m and n for a given composition seems to be the best method to make the copolymer chain a better conductor of electricity both intrinsically and extrinsically. In the case of Type I superlattices higher percentage of smaller band gap component makes a copolymer more p-dopantphilic besides increasing its intrinsic conductivity while increasing the amount of the larger band gap component makes it more insulating and less dopantphilic. The trends are reversed in the case of Type II- staggered superlattices. To have a copolymer with prospects for both p- and n- doping, as well as better intrinsic conductivity, increasing the block sizes m and n of the two components A and B for a given composition is the best solution. These results are important guidelines for designing of novel copolymers based on donor-acceptor polymers.

The density of states (DOS) curves corresponding to the aperiodic distribution reveal broader regions of allowed energy states with fewer gaps in between. The result is that the band gap for random distribution is less than that for the corresponding periodic distribution. The energy positions (peaks) for aperiodic distribution are scattered over a much wider range, whereas narrower peaks are observed in periodic distribution. The saturation in the band gap value is reached much faster in aperiodic distribution than in the periodic distribution. Tuning the electronic properties to a certain value intermediate between those of two polymers is easier by synthesizing periodic copolymers while achieving an electronic property characteristic of the low band gap component is possible by preparing aperiodic copolymers. In the last chapter we have done a comparative study of the effect of incorporation of vinylene linkages on the electronic structures of heterocyclic polymers and donor-acceptor polymers. Both periodic and random distributions of vinylene units have been studied. The results show that the incorporation of vinylene units makes these polymers better conductors of electricity both intrinsically as well as extrinsically.

It is important to mention here that the results reported in this work have been obtained on the basis of the ab-initio Hartree Fock Crystal Orbital method using minimal basis set and the NFC method based on Dean's negative eigenvalue theorem. Ab-initio band structure results using minimal basis set, though reproducing the trend, are known to overestimate the band gap values by a factor 4-5 and thus, the same overestimation is carried over to the band gap values of the copolymers. Though by using better basis sets and by taking into consideration electron correlation effects, the calculated band gap values will become closer to the experimental results, nevertheless, the results reported in this work with regard to designing of novel donor-acceptor polymers, copolymers of donor-acceptor polymers and incorporation of vinylene linkages in different polymers, provide important guidelines to the experimentalists for their synthesis.

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ON SOME PROBLEMS IN HENSELIAN VALUED FIELDS

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Let v be a Krull valuation of a field K with residue field k_v and value group G_v. Let $\bar{v}$ be a fixed prolongation of v to an algebraic closure $\overline{K}$ of K. The theory of extensions of v to a simple transcendental extension $K(x)$ of K originated from the work of MacLane. In 1936 Maclane gave an iterative method of describing all valuations of the rational function field $K(x)$ which extend v, in case v is discrete. Those prolongations w *of* v *to* $K(x)$ for which the residue field of w is a transcendental extension of the residue field of v generated a lot of interest (referred to as residually transcendental prolongations of v). In 1967 Nagata used these prolongations in an attempt to solve Zariski's Cancellation problem and conjectured that the residue field k_w of such a prolongation w of v to $K(x)$ is a simple transcendental extension of a finite extension of k_v. In 1983, Jack Ohm proved Nagata's Conjecture. In 1990, Alexandru, Popescu and Zaharescu gave a description of all these extensions of v to $K(x)$ by means of **"minimal pairs"**. A pair (α, δ) belonging to $\overline{K} \times \ni$ is said to be minimal (with respect to K and $\bar{v}$) if whenever β belonging to $\overline{K}$ satisfied $\bar{v}(\alpha - \beta) \geq \delta$, then $[K(\alpha) : K] : \leq [K(\beta) : K]$. Clearly when $\alpha \in K$, then (α, δ) is a minimal for which each $\delta \in \ni$. It is immediate from the definition that a pair (α, δ) belonging to $(\overline{K} \setminus K) \times \ni$ is minimal if and only if δ is strictly greater than each element of the set $M(\alpha, K)$ defined by

$$M(\alpha, K) = \{\bar{v}(\alpha - \beta) : \beta \in \overline{K}, [K(\beta) : K] < [K(\alpha) : K]\}.$$

This led to the invariant $\delta_k(\alpha)$ defined for those $\alpha \in \overline{K} \setminus K$ for which $M(\alpha, K)$ has an upper bound in $\ni$, by

$$\delta_k(\alpha) = sup\{\bar{v}(\alpha - \beta) | \beta \in, \overline{K} [K(\beta) : K] < [K(\alpha) : K]\}.$$

$\delta_k(\alpha)$ is called the **main invariant** of α.

In 1965, Popescu and Zaharescue proved that if (K, v) is a complete discrete rank 1 valued field, then $\delta_k(\alpha) \in M(\alpha, K)$ for each $\alpha \in \overline{K} \setminus K$ [J. Number Theory 52 (1995) 98 -118]. They also proved that $\delta_k(\alpha)$ satisfies a fundamental principle which is similar to the well known Krasner's Lemma. In 1999, Khanduja and Saha proved that $\delta_k(\alpha)$ satisfies this principle when (K, v) is a henselian valued field of any rank [Mathematika 46 (1999), 84-92]. However unlike in the complete discrete rank 1 case, there are instances when $\delta_k(\alpha) \in \ni$ but fails to belong to $M(\alpha, K)$. This has led us to consider the following problem.

How can we characterize those henselian valued fields (K, v) for which to each $\alpha \in \overline{K} \setminus K$, there corresponds $\beta \in \overline{K}$ satisfying $[K(\beta) : K] < [K(\alpha) : K]$ and $\delta_k(\alpha) = \bar{v}(\alpha - \beta)$?.

We have solved this problem by proving

THEOREM. *Let v be a henselian valuation of any rank of a field* K and $(\overline{K}, \bar{v})$ *be as above. The following two statements are equivalent.*

(i) *To each $\alpha \in \overline{K} \setminus K$, there corresponds $\beta \in \overline{K}$ with $[K(\beta) : K] < [K(\alpha) : K]$ such that $\delta_k(\alpha) = \bar{v}(\alpha - \beta)$*

(ii) *For each $\theta \in \overline{K}$, $K(\theta)/K$ is a defectless extension with respect to the valuation obtained by restricting $\bar{v}$.*

The paper containing the proof of this theorem has appeared in [Proc. Edinburgh Math. Soc. 45 (2002) 219 -227].

The theorem stated above shows that when *(K, v)* is a henselian valued field all whose simple extensions are defectless, then to each $\theta \in \overline{K} \setminus K$, one can associate $\alpha \in \overline{K}$ of smallest degree over *K* such that $v(\theta - \alpha) = \delta_k(\theta)$. Such a pair (θ, α) of elements of $\overline{K}$ *with* $\deg\theta > \deg\alpha$ is called a **distinguished pair** (more precisely *(K, v)*-**distinguished pair).** A chain $\theta = \theta_0, \theta_1, \ldots, \theta_m$ of elements of $\overline{K}$ will be referred to as a **complete distingiuished chain** for θ if (θ_i, θ_{i+1}) is a distinguished pair for $0 \leq i \leq m - 1$ *and* $\theta_m \in K$.

In 1995, Popescu and Zaharescu proved the existence of a complete distinguished chain for each θ belonging to $\overline{K} \setminus K$ in case *(K, v)* is a complete discrete rank 1 valued field. Taking *(K, v)* to be a henselian valued field of any rank, we have characterized those elements $\theta \in \overline{K} \setminus K$ for which there exists a complete distinguished chain. It has been shown that complete distinguished chains for an elements θ in $\overline{K} \setminus K$ give rise to several invariants associated with θ, which are the same for all *K*-conjugates of θ.

We have also proved a theorem giving several characterizations of tame fields which has been accepted for publication in "Valuation theory and its applications, Vol.II", Fields Institute Communication Series (2003).

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3. 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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