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CONTENTS

COMPUTER VISION SYSTEM : APPLICATIONS AND CHALLENGES

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Abstract

The traffic on the roads is increasing day by day. There is dire need of developing an automatic system that can correctly manage and control the traffic on roads. The traffic data of various vehicle types on roads is also important for taking various decisions related to traffic. Installing CCTV camera on the road side and collecting data related to road traffic heaving various category of vehicle is helpful for monitoring vehicles under homogenous and heterogeneous traffic conditions. In this paper, we have studied computer vision system for online and offline videos in India as well as other countries. The paper also discusses the various methods and applications of video based automatic traffic control system. The various challenges faced by the researchers for developing such systems are also discussed.

Index: Computer vision system, methods, Applications and Challenges

INTRODUCTION

Computer vision is an important field of artificial intelligence where decision about a real world scene having high dimensional data is taken. The general steps used in this process are acquiring, processing, analyzing the image(s), and convert it into numerical or symbolic form. It is used to understand the scene electronically and this process is equivalent to the ability of human vision. The numerical or symbolic information of a scene is decided based on the appropriate model constructed with the support of object geometry, image height, color, size of tiers and learning theory.
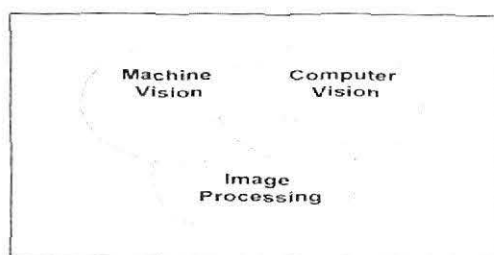
Figure 1: Various types of machine vision system

Figure 1 shows the various alive areas of computer vision system. The scene under consideration is converted into the image(s) or the video(s), comprising of many images, using camera(s) focused from different locations on a scene. The various vision related areas such as scene estimation, vehicle detection, image background and foreground extraction, video tracking, object recognition, object pose estimation and image

segmentation are considered as subareas of computer vision. Similarly, various other fields such as image processing, image analysis and machine vision are also closely related to computer vision. The techniques and applications of various above said areas overlap with each other. Moreover, the techniques used in all these areas are more or less identical. The difference in names only lies on the application where the techniques are applied.

2. VISION SYSTEM FUNDAMENTALS

The computer vision system can work in different situations such as day time or night time under different weather conditions. Vehicle identification system is a computer vision system used to monitor and control the various activities related to the vehicles on road. Some categories of vehicle identification system are briefed as:

2.1 ONLINE AND OFFLINE VEHICLE IDENTIFICATION

The vehicle identification can be viewed as offline vehicle identification and online vehicle identification. In case of offline vehicle identification, video images considered for identification are offline (already acquired) and vehicle identification techniques are applied on the images and their comparison is made with the images already present in the database. In case of online vehicle identification, the camera(s) are installed on the road side and the video images are captured online and their comparison is made with the images already present in the database. The difference in offline and

online video identification system is that the images/videos are captured online in case of former and are already available in system storage in case of later.

2.2 IMAGE PROCESSING AND IMAGE ANALYSIS

Image processing and image analysis both deals with 2D images. In image processing an image is transformed into another form by applying some operations such as color contrast, edge detection, noise removal and geometrical transformations. The image contents are not interpreted in image processing whereas in case of image analysis the interpretation of images is made on the basic of the properties such as color, shape, of the contents they contain.

2.3 VIDEO ANALYSIS / VIDEO INTERPRETATION

Video analysis is a process in which a given video is automatically analyzed to detect and determine the temporal and spatial events. The main factors in video analysis are: motion detection, video tracking, background abstraction and behavior analysis for situation awareness purpose.

Video interpretation is a video telecommunication service that uses devices such as video cameras or videophones to provide sign language or spoken language to interpret a language. This is done through a remote or offsite interpreter to communicate with a person heaving communication barrier. Video interpreter facilitates communication between the participants who are located together at the other site. They communicate by using any communicating device like headphones or microphones.

2.4 HOMOGENOUS / HETEROGENEOUS TRAFFIC

Homogeneous traffic is a hypothetical synchronized flow of traffic of similar vehicles where all vehicles move with the same speed, irrespective of time and maintain the same space gap between them. The conditions under which the traffic move is also called as homogenous traffic conditions. Heterogeneous traffic is unsynchronized and unregulated i.e. there is an irregular movement of all type of vehicles.

3. VEHICLE IDENTIFICATION AND CLASSIFICATION SYSTEM (VICS)

A VICS system for identification and classification of moving vehicles on the road side videos is of great importance today. In India the traffic related information is gathered manually. One of the easy ways to exchange information related to traffic between different computers is by using network which is helpful for making many

kind of decisions related to traffic management. The new advancement in a VICS system is helpful for traffic control as well as collecting statics data related to the vehicles that is helpful in making many decisions. A number of vehicle identification and classification systems have been developed by various prominent authors but are not 100% accurate. Figure 2 and 3 demonstrates the homogenous and heterogeneous traffic respectively.

Figure 2: Homogenous traffic

Figure 3: Heterogeneous traffic

In VICS system, vehicle identification and classification can be done in two ways online and offline. In offline system, the identification and classification of vehicles is done on already videos related to the traffic whereas in online system, the images are captured by CCTV camera installed on the road side and the system identifies and classifies the vehicles directly from that video.

Vehicle identification and classification system (VICS) is an intelligent vehicle recognition system used to manage traffic on roads. There is dire need of monitoring and controlling traffic on road using efficient and effective cost effective method. In VICS the images of the vehicles are captured using a video camera installed on road side. In VICS the decision may be made on the basis of a single camera on one side or

multiple cameras installed at different locations at particular angle depending upon the requirement and level of sophistication of the system. The video are converted into shots and frames, then features extraction and classification method are applied to identify and classify the vehicle.
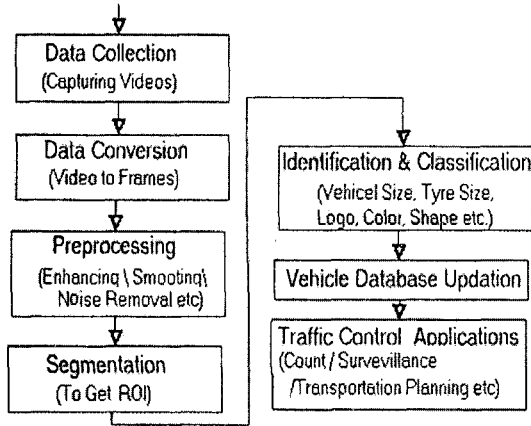
Figure 4: General steps in Video based identification and classification system (VICS).

In VICS, the traffic data extractor using CCTV is used to identify, classify and track each object moving through the scene to check its speed, path, vehicle class and counting. It analyses the video sequence in order to locate an object passed through a view of the camera(s), track it until it leave the obscured scene. The object parameter extractor analyses an object as soon as it is available, capture it and compare it with the objects already stored in computer system database. It identifies, classifies and tracks that object and all its parameters are saved in a result list. Vehicle identification and classification system consists of various phases such as image collection, pre-processing, normalization, segmentation, feature extraction and representations, recognition and post-processing [1, 2, 3]. The Figure 4 illustrates the different phases of online vehicle identification and classification system.

The noise and distortions in collected images are reduced with pre-processing and normalization [3]. Feature extraction is essential for efficient data representation and for further processing. Also, high recognition performance could be achieved by selecting suitable feature extraction method. Features can be on the basis of shape, size, color, size of tires, logo etc [3].

Segmentation is a phase of vehicle recognition in which data is represented in pixels and colors so that nature of each pixel or color can be studied individually. The four types of methods that are used for identification are region based, contour based, shape based, color based, neural network based methods, syntactical or structural methods and template matching. These methods have their own advantages and disadvantages [10]. For classification purpose, earlier more emphasis was given on statistical and neural network techniques but later the trends of using efficient technique such as SVM [12] was introduced. In its initial phase, this technique was used in object identification and then applied to vehicle classification. Post-processing refers to the procedure of correcting misclassified results by applying image knowledge. All the possible outcomes of an individual image are studied in terms of boundary, size, color and shape of the vehicle. The performance of various online and offline systems are detailed in Table 1.

Table 1: Summarization of Studies on Vehicle Identification and Classification Vehicles.

Method	Identification/ Classification	Country	Sucess rate	References
SVM to identify PPlive, Pstream, UUsee, QQlive d spot	Classification Online video	China	93.5%	Liu et.al (2004)
Window based online learning algorithm	Identification Offline videos	Canada	80%	Vinod Nair et.al (2005)
Algorithm based on Width to Height Ratio (WHR)and Base to abdomen Ratio (BAR)	Identification	Malaysia	93%	zara Mohammed et, al (2005)
Bayesian Network	Classification and identification online	California USA	88%	Brendan .M et al (2006)
online boosting algorithm	Classifier and detection Ofline and offline	USA	97%	Wen-Chung et.al (2010)
MBF and IPHOG	Classification Online Videos	London U.K	99.78%	Zezhi Chen et al (2011)
virtual detection loops	Counting and classification online	USA	97.4%.	Shuguang Li et.al (2013)
thermal energy in traffic flow surveillance	Thermal Vehicle detection	Japan	91.2%	Sanjivani Shantaiya et.al(2014)
Fine-grained Recognition algorithms and SVM Only for cars	Classifier Offline videos	China	97.38%	Jin Zhan et.al(2014)
Algorithms k Gaussian distribution models	In both	USA	86.6%	A. Jazayeri et.al (2015)

Scale invariant feature transform (SIFT)	onlineBoth for identification and classification of vehicle and pedestrians	China	91.4%	Xuehua Song et. al
TRAZER	Vehicle Recognitation	India	89.5%	Dlagnekov and Belongie, 2005
Neural Network/Blob tracking	Classification offfine	Guwahati	88.25%	C. Mallikarjuna et.al(2009)
Quantized wavelet features method SVM	Identification and classification online	Wardha, India	90%	Daigavane P.M et al (2011)
Random forest algorithm.	Detection online	New Delhi India	93.94 %	Swapan Kumar et.al(2012)
adaptive background modeling and classification is achieved by novel	Classification online	New Delhi India	94.8%.	Swapan Kumar et.al(2012)
	Identification and classification Online	Mumbai	82.8%	Pradeep Kumar et.al(2013)
Decision tree Algorithms	Classification Online	Ahmadabad India	85%	Sanjivani Shantaiya et.al(2014)
SVM RBF	In both	Vijayawada India	90.00%	R.S Vaddi et.al(2015)
Traffic Analyzer and Enumerator— TRAZER.	Vehicle detection offline	Guwahati	95%	C. Mallikarjuna et.al(2015)
knowledge-guided boundary algorithms	template matching for automatic vehicle identification	Uttar Pradesh India	95%	RAJIV KUMAR et.al

4. APPLICATIONS OF COMPUTER VISION SYSTEM

The various applications of image/video based image analysis, recognition and understanding are [28]:Video Surveillance, Traffic Management System, Vision based intelligent Transport System, Intersection Control, Incident Detection, Vehicle Classification, Monitoring, Revenue collection, Historical Traffic information, Congestion Map and travel time estimate, Public Transport information, Individual Vehicle Management, Accident Handling, Conventional Driver Assistance system, Traffic Surveillance, Transportation Planning and Intelligent Traffic Guidance system, Vehicle counting, License plate localization, and Electronic Toll collection system. Some of the applications are briefly explained as:

A) Public Transformation Information System: Public transportation information system is a computer based system that gives information about the public transport vehicles such as buses, trucks, ambulance etc. The system consists of the number of hardware and software components installed on-board vehicles that communicate with central server with wireless system *i.e.* using GPS. The system can provide real time tracking of the vehicles location and status, vehicles time table performance and reliability reports, vehicles arrival information at a particular location, and traffic light priority for late running vehicles.

B) Intelligent Transportation Systems (ITS) are the Intelligent system which provide various innovative services for the transportation. The Information communication technology is used in intelligent transport system to control traffic management, preparing data base about the traffic, mobility management as well as interface with other mode of transportation.

C) Electronic Toll Collection ETS is a system by which toll is collected automatically. By this device, the vehicle which passes through the toll, is recorded and stored in the database and Likewise charged .The amount is debited from the account of registered customer. To pay the toll fee the vehicle need not stop. For this process to be effective it is essential that first of all vehicles is registered with the concerned authorities. This collection system of toll was developed by William Vickey, a Nobel Laureate. To test the effectiveness of this system, a system was put in place. Fixed transponders were fixed at the underside of the vehicles and the readers were fixed under the surface of the highway. Leading countries like Norway, Portugal, America started using this technology .Norway found it so convenient that now there are 25 toll roads there. Electronic fee collection system has been successful .Portugal was the first country in the world which applied single universal system of toll thorough out its length and breadth. It has now been used in parking lots and gas stations. America not to be left behind has implemented it in most of its states. Licenses plate recognition is a technology which recognizes the number plates of the vehicles. It has varied uses. Most importantly it is used in complex security system. For example it makes the work much simpler in parking lots where is so much time and labour is required to recognize the number plates. It has its drawbacks on which research is going on, inclement weather conditions like heavy rain, fog, mist darkness, velocity of the car its angle, illumination conditions, distance of the car, plate rotation and conservation, the number of vehicles at a place are areas which still need to be worked upon. Researcher are working upon such a system which is fool proof and which works well in all weather conditions and does not have other problems associated with it.

D) License plate localization: License plate recognition system is used to read the number plates of the vehicles. Various methods have been proposed to read the number plates. This application can work in differ areas like complex security systems and parking admission to urban traffic control. Various problems such as fog, rain, shadows, irregular illumination conditions, variable

distances, cars' velocity, scene's angle on frame, plate rotation, color distribution and conservation, number of vehicles in the scene etc are faced by the researchers for the devolvement such systems. These effects make plate recognition much more complex and difficult than the traditional pattern recognition systems. The main goal of this research is to develop a new intelligent plate recognition system that can work in all situations.

E) Intersection Control: Intersection control: who does not wish to have green light all the way to his destination, we all pray for green light when going out for work. It has been found especially useful for heavy goods vehicles which are difficult to maneuver. Many trials have been carried out and have met success. These fuel guzzling vehicles can save a lot of fuel, help in preserving environment, save travel time if prior information is provided to these vehicle owners. A test was carried out at Helmond in velker lands. Where vehicles were stopped at traffic light controlled intersection. The results were amazing, it was found that instead of 13% of time the drivers spent 6% fuel consumption got lowered. There was 13% reduction in fuel consumption. There was average increase of H.G.V. the vehicles which are on emergency duty like fire engines, save even better results. There was improvement of 8% in their speed. This dream may result in realty with improving technology and research.

F) Historical Traffic Information System- Historical traffic information system keeps the record of history of various vehicles on the records. The system generally works on the basis of GPS based devices attached to each vehicle that keeps real time record of the vehicles on roads irrespective of their locations. In additions, it also uses digital map to aware the location of the vehicles on road. The system is helpful for business and Govt organisations.

5. CHALLENGES

While designing system based on image/video based image analysis, recognition and side traffic Management, a number of challenges is faced by these researcher understanding are [28] and some of these are as:

- In western countries like USA, lane marking system is implemented. Vehicles are allowed to move in specific rows according to speed limit and vehicle type, etc. but in India in most of the cases traffic system is non-lane based.

- Road conditions are more varied and traffic is unstructured, there is lack of discipline and overloaded vehicle movement is quite natural.

- In India, vehicles are parked frequently by the sides of the roads. There is no separate System for vehicle parking management.

- Roads are not only occupied with vehicles, so many obstacles on roads create disturbance to the traffic. Pedestrians do not have separate ways for their movement in most of the cases. Shapes of the vehicles have a key role in recognition; there is high intra-class variance among Indian vehicles. It creates the chances for miss recognition.

- Within same vehicle class there are large variety and models. These look different in size and appearance. It is generally observed in Indian vehicles like cars and Truck's.

- Vehicles detection using optical sensor is very challenging due to huge difference within class Variability in vehicles appearance.

- Hundred of vehicle images and billion of classes like two wheelers, Three wheelers, low motor vehicles, heavy motor vehicles.

6. CONCLUSIONS

The majority of work has been carried out for offline videos in foreign countries such as China, Canada, Malaysia, California USA, London U.K, and Japan. The interesting part of such research is that there is proper lane system and rule and regulations on roads. The vehicles are moving according to their speed. The various vehicle management systems developed do not give 100% accuracy. A vision system works in various stages like Pre-processing, Feature extraction and classification techniques Work done in Indian context is purely for mixed traffic conditions. They are lacking in within same vehicle class there are large varieties and models. Shapes of the vehicles have a key role in recognition, there is high intra-class variance among Indian vehicles Roads are not only occupied with vehicles, so many obstacles on roads create disturbance to the traffic.

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DESIGN AND SYNTHESIS OF NEW LANOSTEROL/TRIAZOLE CONJUGATES

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Abstract

Fungi are eukaryotic organisms, whose cellular functions resemble those of plants and animals. Hence, selectivity plays a crucial role in the quest for safe and effective chemotherapeutic drugs. For the first time sterol carrier proteins (SCP) are suggested to be one of the key targets for the development of safe and effective antifungal therapeutics. With this hypothesis novel lanosterol/fluconazole conjugates were designed and their regioselective synthesis was achieved in very high yield via Cu(I) catalyzed intermolecular 1,3-dipolar cycloaddition. As a control lanosterol/1,2,4-triazole conjugate was also synthesized.

Key words: medicinal chemistry, antifungal agents, steroids, conjugation, [2+3] cycloaddition.

INTRODUCTION

In recent years fungal infections have emerged as a major cause of disease and mortality [1-3]. Antifungals play an important role in human medicine, agriculture and veterinary medicine. Among several antifungal agents, azoles are emerging groups of antifungal agents which act by inhibiting the isozyme cytochrome P-450, responsible for C-14 demethylation of lanosterol 1 (Figure 1) in ergosterol 2 biosynthesis in the fungal cell membrane.
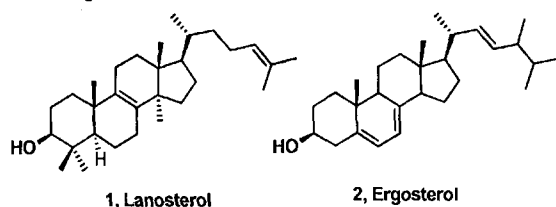

Figure 1 Lanosterol and Ergostrol

Although there are number of classes of agents that are being used to treat invasive mycoses, the targets are heavily aimed on the fungal membrane sterol, ergosterol, and its biosynthesis [4]. Antifungal resistance is increasing day by day and there is always a need to develop new antifungal agents that can work well against pathogens by different mode of action. In order to seek improved azole antifungal agents, fluconazole/lanosterol conjugates 3 and 4 (Figure 2) were designed based on our hypothesis that unusual hybrid molecules can have improved physical, biological and medicinal properties [5].
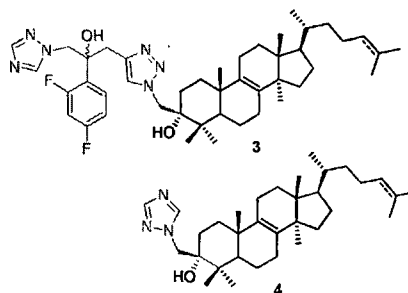
Figure 2 Novel lanosterol-triazole conjugates.

Biosynthetic pathways of protozoa, fungi and plants are carried out by Δ24(25) sterol methyl transferase and several side chain derivatives of Lanosterol are known to inhibit this enzyme. So, Lanosteryl steroid unit was specifically selected for this purpose [6]. These molecules presumably alter the viscosity of the lipid core of the plasma membrane of fungal cells by interfering in the ergosterol biosynthesis [7]. The integrity of fungal membranes are shown to be disrupted by azole antifungals in a similar way by inhibiting the cytochrome P450 linked monooxygenase component of lanosterol C14-demethylase, blocking the biosynthesis of ergosterol, a key membrane component [8]. Lanosterol 1 represents a key branching point in the biosynthesis of ergosterol 2. Biosynthesis of ergosterol from Lanosterol

is a multistep pathway which is catalysed by various membrane-bound enzymes. In most of the fungi, the first step involves methylation at C-24 and followed by sequential demethylations at C-14 and C-4 [9]. The C-24 methylation is unique to the ergosterol biosynthesis. In fungi, biosynthesis of ergosterol takes place in Endoplasmic Reticulum (ER) during which the intermediate lanosterol gets transported to Mitochondria (Figure 3). In Mitochondria, lanosterol gets transformed to 24-methylene-24,25-dihydrolanosterol and transported back to ER for further biosynthetic transformations [7a].

Figure 3 Transfer of sterols within ER to mitochondria during ergosterol biosynthesis.

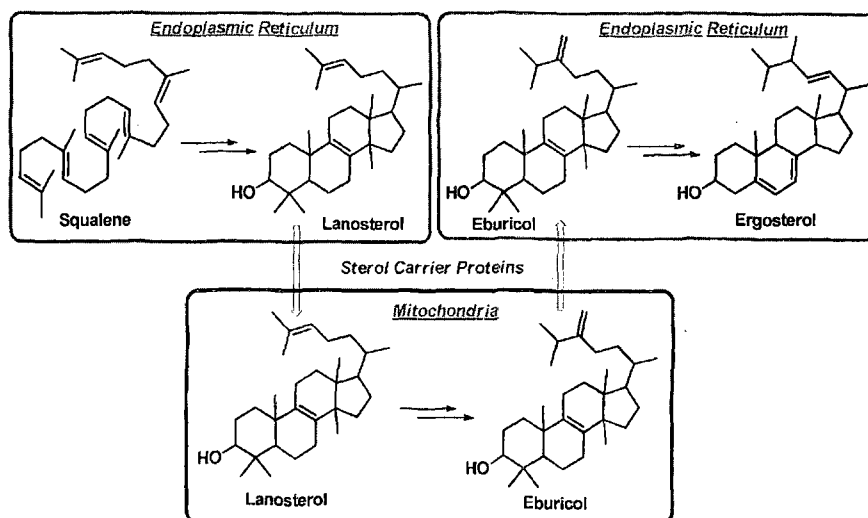A non-catalytic carrier protein (s) (sterol carrier protein) does this job of transfer of sterols within ER to mitochondria [10]. It is clear from this discussion that the sterols having lanosterol like backbone can best fit in to the active site pocket of these sterol carrier proteins and the molecules derived from these sterols may be very specific and more active as antifungal agents. Based on these assumptions, we designed novel lanosterol-azole conjugates **3** and **4** (Figure 2). Being modified azole derivatives, these molecules were predicted to inhibit cytochrome P-450 dependent C-14 demethylation of lanosterol. On the other hand the altered lanosterol backbone may be involved in binding to fungal sterol carrier proteins altering their role as well as for targeting the whole drug at the appropriate site of action.

Bioconjugation is an important emerging area for researchers in every discipline of science. It opens a new area to synthesize highly biologically diverse molecules by covalent coupling of two or more bioactive molecules with the combined properties of its individual components [11]. In continuation of our work on bile acids [5,12], we designed Lanosterol/Flucanazole bioconjugates **3** and **4** (Figure 2) linked together with

1,2,3-triazole, which may be considered as an isostere of one of the 1,2,4-triazole component of fluconazole. Capability of hydrogen bonding and stability towards metabolic degradation of 1,2,3-triazole units makes them important candidate as a bridging unit which can be favorable for solubility and binding biomolecular targets[13]. 1,2,3-Triazole moiety does not have natural occurrence, although the unnatural molecules containing 1,2,3-triazole unit show significant biological activities [14,15].

1,3-Dipolar cycloaddition of organic azides and terminal acetylene has been a method of choice for the synthesis of 1,2,3-triazoles [16]. In our approach to achieve the synthesis of these new molecules, we performed Huisgen (click) reaction [17] to couple terminal alkyne of flucanazole **8** [5c] and terminal azide of 24,25-dihydrolanosterol **12**, in the presence of Cu(I) catalyst to synthesize lanosterol-fluconazole conjugate **3**. Accordingly, we synthesized 2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)pent-4-yn-2-ol **8** by propargylation of the corresponding ketone **7** [18] using propargyl bromide and zinc dust (Scheme 1).
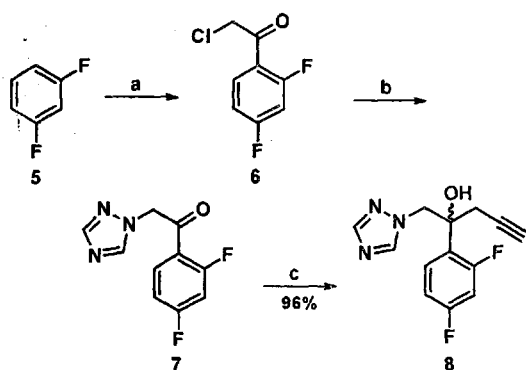
Scheme 1. Reagents and conditions (a) AlCl₃, 1,2-dichloroethane, chloroacetyl chloride, 25 °C, 7 h; (b) 1,2,4-triazole, NaHCO₃, toluene, reflux, 4 h (55 % in two steps); (c) Zn, propargyl bromide, DMF/THF, 25 °C, 5 h.

Figure 4 ORTEP [21] views of compounds 7 and 8.

The racemic compound 8 comprises an alkyne component. In the ^{1}H NMR spectrum of 8 [5c,5d], the acetylenic proton was analysed as triplet at δ 2.06 ppm and the β methylene was appeared as doublet of doublet at δ 2.87 ppm. In the ^{13}C NMR spectrum it showed intricacies of various ^{19}F-^{13}C coupling as in fluconazole molecule [19]. IR spectrum confirms the presence of acetylenic group wherein the absorption because of acetylenic group was observed at 3307 cm^{-1}. The crystal structures [20] of difluoro compounds 7 and 8 (Figure 4) were resolved, to study the two-dimensional arrangement of these molecules.

The synthesis of azido lanosterol unit 12 was attempted from the commercially available (60/40) mixture of lanosterol and dihydrolanosterol 9 (Scheme 2). In the first step C-3 hydroxyl functionality of 9 was oxidized to lanostanone 10 using CrO₃/H₂SO₄ (50 % yield). Improved yield for the oxidation of 9 was obtained using pyridinium fluorochromate (PFC, 74 %). Lanostanone 10 was then stereoselectively converted to spiro-3(R)-oxirane 11 by reaction with trimethyl sulfoxonium iodide (TMSOI). The regioselective epoxide ring opening of oxirane 11 using NaN₃ and catalytic amount of LiClO₄ furnished the required β-azido alcohol 12 in 84 % yield.
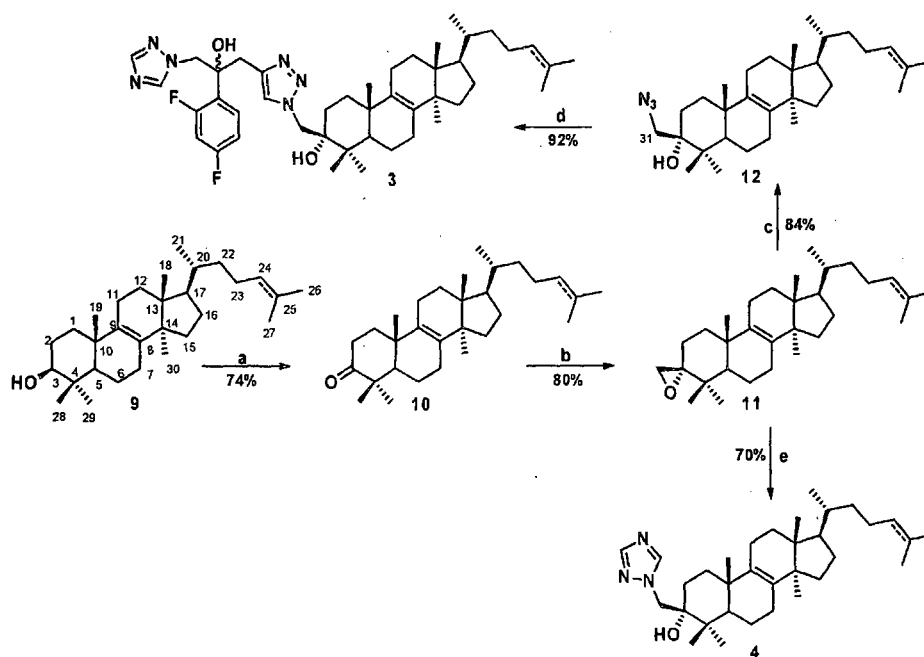
Scheme 2. Reagents and conditions: (a) CrO₃, H₂SO₄, H₂O, 10 °C, 10 min. or PFC, CH₂Cl₂, 25 °C, 2.5 h; (b) TMSOI, NaH, DMSO/THF, 100 °C, 5h; (c) NaN₃, LiClO₄, DMF, 60 °C, 4h; (d) 8, CuSO₄-5H₂O (5 mol %), sodium ascorbate (40 mol %), DMF/H₂O (9:1), microwave, 5 min; (e) 1,2,4-Triazole, DMF, 100 °C, 4h.

The absolute structure of β-azido alcohol **12** was established by single crystal X-ray analysis (Figure 5), which indirectly confirmed the equatorial addition of methylene by oxosulfonium ylide.

12
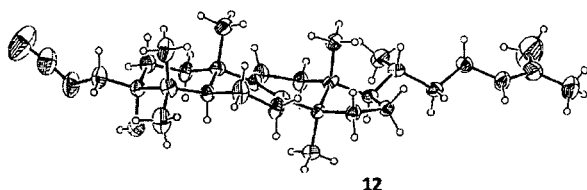
Figure 5 ORTEP view of compounds **12**.

IR of this compound showed absorption due to azido group at 2102 cm^{-1} and in ^1H NMR spectrum, resonances corresponding to C-31-methylene protons were observed as two separate doublets at δ 3.21 ppm (C-31H_a) and δ 3.63 ppm (C-31-H_b)(H_aH_b geminal coupling J = 12 Hz).

Among the various possible reaction conditions for cycloaddition reaction between alkyne **8** and azide **12**, microwave assisted Cu(I) catalyzed reaction was found to be the most appropriate [22]. Compound **8** was allowed to react with azide **12** under microwave conditions in DMF/H_2O using catalytic amount of Cu(I) to give dihydrolanosterol-fluconazole conjugate **3** as a diastereomeric mixture in 89 % yield (Scheme 2). In the ^1H NMR spectrum of compound **3**, resonances corresponding to C-31-methylene protons were identified as two separate peaks at δ 3.12 and 3.52 ppm. The expected six aromatic peaks were observed at 6.66-6.77 (m, 2H), 7.30-7.40 (m, 2H), 7.83 (s, 1H), 8.16-8.25 (m, 1H). In addition, mass spectrum showed molecular ion peak at 745.5 (M+H). To investigate the exact effect of fluconazole moiety in conjugate **3**, lanosterol-triazole conjugate **4** was also synthesized by the regioselective opening of epoxide ring in compound **11** using 1,2,4-triazole (Scheme 2). Synthesis of this compound **4** was confirmed by ^1H NMR spectroscopy in which C-31 methylene protons were appeared as two separate doublets at δ 4.19 ppm (C-31H_a) and δ 4.38 ppm (C-31-H_b)(H_aH_b geminal coupling J = 12 Hz). The two aromatic protons of 1,2,4-triazole functionality were appeared at 7.94 and 8.19 δ ppm.

The synthesized lanosterol-azole conjugates **3, 4** and the other intermediates were subjected for the biological evaluation to study their antifungal activity against different fungal strains such as *Candida albicans, Candida parapsilosis, Cryptococcus neoformans, Sporothrix schenckii, Trichophyton mentagrophytes* and *Aspergillus fumigatus*. However, these molecules did not

show any inhibition upto 50µg/mL concentration. It was interesting to observe a complete loss of antifungal activity of conjugate **3**, as the similar cholic acid analogue was found to be active against fungi [5b,5c]. Isolation and structural investigation of sterol carrier proteins (SCP) in fungi and study of it's *in vitro* inhibition are the further goals of this project.

In conclusion, for the first time sterol carrier protein (SCP) is suggested to be a key targets for the development of safe and effective antifungal therepeutics. Novel lanosterol-fluconazole conjugates were designed and their regioselective synthesis was achieved in very high yield *via* Cu(I) catalyzed intermolecular 1,3-dipolar cycloaddition.

1-(2,4-Diflurophenyl)-2-(1H-1,2,4-triazole-1-yl)ethanone (7) and 2-(2,4-diflurophenyl)-1-(1H-1,2,4-triazole-1-yl)pent-4-yn-2-ol (8).

The intermediates **7** and **8** were synthesized from difluoro benzene **5** using literature procedures [5,19].

Lanostanone (10). Compound **9** (0.426 g, 1.0 mmol) in acetone (20 mL) was stirred with Jones Reagent (1 mL) at 5-10 ^0C for 5 min. Methanol (5 mL) was added after 5 min, the solvent was evaporated and the crude solid material was dissolved in EtOAc/H_2O (5:1) mixture (100 mL). The organic layer was washed with cold H_2O (2x10 mL), 10 % NaHCO$_3$ (2x10 mL), brine (2x10 mL) and dried over Na$_2$SO$_4$. Solvent was evaporated under reduced pressure to afford crude product. Purification by column chromatography on silica gel (5 %, EtOAc/PE) afforded compound **10** (0.212 g, 50 %) as a white crystalline solid. Improved yields for the oxidation of **9** were obtained using pyridinium fluorochromate (PFC). Compound **9** (5.0 g, 11.7 mmol) was dissolved in dry DCM (80 mL), and PFC (4.6 g, 23.5 mmol) was added and the resulted suspension was stirred at 25 ^0C for 2.5 h. The reaction mixture was filtered through celite and further diluted with 200 mL of DCM. The organic layer was washed with cold H_2O (2x15 mL), 10% NaHCO$_3$ (2x10 mL), brine (2x15 mL) and dried over Na$_2$SO$_4$. Solvent was evaporated under reduced pressure to afford crude product. Purification by column chromatography on silica gel (2%, EtOAc/PE) afforded compound **10** (3.68 g, 74%) as a white crystalline solid. IR v_{max} (Nujol) 1711 cm^{-1}. With the similar reaction conditions 24,25-dihydrolanostanone was synthesized from 24,25-dihydrolanosterol in 78 % yield. The spectroscopic data is consistent with that reported in the literature.

Epoxide (11): TMSOI (0.33 g, 1.5 mmol) and NaH (0.036 g, 1.5 mmol) were dissolved in DMSO (6 mL) and heated at 50 °C for 1h. To this hot reaction mixture lanostanone **10** (0.424 g, 1.0 mmol) in THF (4 mL) was

added drop wise for the period of 10 min. The resulted reaction mixture was heated at 70 °C for 7 hrs. Crushed ice (10 g) was added and the compound was extracted with Et_2O (3x50 mL). The organic layer was washed with cold water (2x20 mL), brine (20 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to give 0.39 g of crude product. Purification by column chromatography on silica gel (1 % EtOAc/Hexane), furnished compound **11** (0.35 g, 80 %) as gummy solid. ^1H NMR ($CDCl_3$, 300 MHz) δ 0.71 (d, J = 4.36 Hz, 3H), 0.84-0.90 (m, 15H), 0.99 (s, 3H), 1.08 (s, 3H), 2.42 (d, J = 4.41 Hz, 1H), 2.82 (d, J = 4.41 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.7, 18.6, 18.7, 19.1, 21.0, 21.7, 22.5, 22.7, 22.8, 24.1, 24.2, 26.3, 27.9, 28.0, 28.2, 29.7, 30.8, 31.0, 33.8, 36.4, 36.5, 36.7, 37.3, 39.5, 44.4, 49.0, 49.8, 50.1, 50.4, 63.3, 134.3, 134.4; MS (LCMS) m/z 439.39 $[M+H]^+$.

Azidoalcohol (12): The oxirane **11** (0.219 g, 0.5 mmol) was reacted with NaN_3 (0.390 g, 6 mmol) in the presence of catalytic amount of $LiClO_4$ (0.020 g, 10%) in DMF (10 mL) at 100 °C for the period of 24 hrs. Crushed ice (10 g) was added and the compound was extracted with Et_2O (3x50 mL). The organic layer was washed with cold water (2x20 mL), brine (20 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to give 0.24 g of crude product. Purification by column chromatography on silica gel (0.5 % EtOAc/PE), furnished azide **12** (0.17 g, 71 %) as white solid. IR v_{max} (Nujol) 3576, 2103 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.69 (s, 3H), 0.83 (s, 3H), 0.88 (s, 3H), 0.92 (d, J = 6.75 Hz, 3H), 0.97 (s, 3H), 0.98 (s, 3H), 1.60 (s, 3H), 1.68 (s, 3H), 3.21 (d, J = 12.0 Hz, 1H), 3.62 (d, J = 12.0 Hz, 1H), 5.10 (t, J = 7.0 Hz, 0.7H); MS (LCMS) m/z 504.39 $[M+Na]^+$.

Lanosterol-fluconazole Conjugate (3): The alkyne **8** (0.132 g, 0.5 mmol) and the azide **12** (0.289 g, 0.6 mmol) were dissolved in DMF/H_2O 4:1 (5 mL). To this solution, $CuSO_4·5H_2O$ (0.05 equiv) and sodium ascorbate (0.40 equiv) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 5 min at 415 W. The reaction mixture was cooled, ice was added, and it was then extracted with Et_2O. The extract was washed with water and brine. Solvent was evaporated under reduced pressure and crude product was purified by column chromatography on silica gel using EtOAc to obtain lanosterol-fluconazole conjugate **3** (0.343 g, 92 %) linked with 1,4-disubstituted 1,2,3-triazole. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.68 (s, 3H), 0.86 (s, 3H), 0.87 (s, 3H), 0.89 (s, 3H), 0.94 (s, 9H), 1.60 (s, 3H), 1.68 (s, 3H), 3.11 (m, 1H), 3.52 (m, 1H), 4.26-4.38 (m, 2H), 4.60-4.70 (m, 2H), 5.10 (t, J = 6.5 Hz, 0.6H), 6.64-6.67 (m, 2H), 7.30-7.41 (m, 2H), 7.83 (s, 1H), 8.20 (d, J = 16 Hz, 1H); MS (LCMS) m/z 767.48 $[M+Na]^+$.

Lanosterol-triazole conjugate (4): The oxirane **11** (0.27g, 0.62 mmol) and 1,2,4-triazole (0.086g, 1.24 mmol) were dissolved in DMF (10 mL) and were heated at 100 °C for 36 hrs. Crushed ice (10 g) was added and the compound was extracted with Et_2O (3x50 mL). The organic layer was washed with cold water (2x20 mL), brine (20 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to furnish 0.30 g of crude product. Purification by column chromatography on silica gel (1.5 % EtOAc/PE), afforded lanosterol-triazole conjugate **4** (0.22 g, 70 %) as white solid. IR v_{max} (Nujol) 3391, 1618, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.68 (s, 3H), 0.85, 0.88, 0.98, 1.03, 1.60, 1.68 (multiple singlets, 21-H), 4.20 (d, J = 14.2 Hz, 1H), 4.38 (d, J = 14.2 Hz, 1H), 5.10 (m, 0.5H), 7.94 (bs, 1H), 8.19 (bs, 1H); MS (LCMS) m/z 530.41 $[M+Na]^+$.

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(20) Single crystals of all the compounds **7**, **8** and **12** obtained from ethyl acetate–petroleum ether mixture. X-ray intensity data were collected on a Bruker SMART APEX CCD diffractometer at room temperature. All the data were corrected for Lorentzian, polarisation and absorption effects using Bruker's SAINT and SADABS programs. SHELX-97 (Sheldrick, G. M. SHELX-97 program for crystal structure solution and refinement; University of Gottingen: Germany, 1997) was used for structure solution and full matrix least squares refinement on F2. Hydrogen atoms were included in the refinement as per the riding model. Crystallographic data for the structural analysis of **7**, **8**, and **12** have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 854836, 854837, and 854838, respectively. Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union road, Cambridge CB2 1EZ, UK (fax: C44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk) or via www.ccdc.cam.ac.uk/conts/retrieving.html.

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TYPE 2 DIABETES MELLITUS: PREVALENCE AND DIFFERENCES AMONG PRE- AND POSTMENOPAUSAL WOMEN

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Abstract

Type 2 Diabetes mellitus is growing at a very fast pace and is about to gain the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease as per data available. Women when in their reproductive phase are considered in the safer zone as compared to the men of their age. Once they attain menopause the chances of developing type 2 diabetes comes at par with men. Overweight or obesity further aggravates the situation and increases the chances of developing the disease. On dividing the women in different BMI ranges, it was observed that the number of diabetic women increases as we move from lower to higher BMI groups preferably among postmenopausal women. It is assumed that the redistribution of body fat occurring preferably due to fall in estradiol levels among postmenopausal women could have been the reason behind the higher prevalence of type 2 diabetes as compared to their premenopausal counterparts.

Key Words: Type 2 diabetes, Obesity, premenopausal women, Postmenopausal women

INTRODUCTION

Diabetes has attained the status of chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Prior to 1999, the fasting plasma glucose cut point for diabetes was considered as 140 mg/dl, instead of the current 126 mg/dl (WHO, 1999). The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. In 2012 diabetes was the direct cause of 1.5 million deaths and high blood glucose was the cause of another 2.2 million deaths (WHO, 2016).

Between 2010 and 2030, it is expected that there would be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw et al., 2010). As per International Diabetes Federation, worldwide the number of diabetic people is predicted to rise from 415 million at present to 642 million by the end of 2040 (http://www.diabetesatlas.org/, retrieved on November 22nd, 2016 at 16.40). Being overweight or obese increase the chances of developing the type 2 diabetes. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease (Joshi and Parikh, 2007; Kumar et al., 2013). In 2000, India topped the world with the highest number of people with diabetes mellitus (31.7 million)

followed by China (20.8 million) with the United States (17.7 million) in second and third place, respectively (Wild et al., 2004).

Women are at high risk for diabetes if they are overweight. They appear to be at particular risk as the gender advantage for coronary heart disease (CHD) is compensated by an increased incidence of obesity and diabetes. Obesity and type 2 diabetes are particularly injurious to women's health. Women who are obese are especially susceptible to type 2 diabetes and diabetic women have disproportionally higher relative risk of CHD than diabetic men. Women further face the challenges with the advancing age especially after transition from pre- to postmenopausal phase due to decline followed by termination of ovarian estrogen production and continuation of androgen production. Reports show that postmenopausal estrogen therapy reduces fasting plasma glucose levels (Kanya et al., 2003). A BMI > 30 kg/m^2 (obese subjects), led to increase the blood pressure, triglycerides, and fasting glucose levels, in addition to being associated with low HDL-C levels compared to a normal BMI (non-obese subjects). This data indicates that "obese" individuals have more cardiovascular risk factors (Bagnoli et al., 2014).

The present study was aimed to assess the prevalence of diabetes among women and then compare it between pre-menopausal and post-menopausal women to see the effect of their

menopausal status and obesity. It was further planned to assess the prevalence of diabetes in different BMI ranges like BMI<18.5, 18.5-22.9, 23-24.9 and ≥25 among both pre- as well as postmenopausal women. To observe diabetes in different BMI ranges make this part of the work as unique.

MATERIAL AND METHOD

The data for present study was collected from 595 women working in different educational institutes and hospitals [330 premenopausal (women with regular menstrual cycles) and 265 postmenopausal women (those who had reached natural menopause and had their last menstrual bleeding at least one year previously as per guidelines given by WHO, 1996)]. Women suffering from any other ailment or on some specific medication were not considered for the study.

A thorough review of literature was done after the study was approved by the Ethical Review Committee of Guru Nanak Dev University, Amritsar. The nutritional status of the subjects was assessed by calculating Body Mass Index (BMI). BMI is most commonly recommended and widely used for classifying overweight/ obesity and underweight in adults for epidemiological studies. WHO (1998) also advised the use of BMI for the estimation of the prevalence of obesity within population and the risks associated with it because this variable correlates strongly with total body fat content or fat mass, sufficiently independent of height in young and middle aged adults (Frayn, 2005). The anthropometric measurements like weight and height were taken on each subject using standardized methodology given by Weiner and Lourie, 1981. BMI was calculated from measured values of height and weight in each subject with the following formula:

BMI = Weight (kg) /Height (m) ^{2}

Both pre- and postmenopausal subjects were divided into four groups each as, underweight, normal, overweight and obese according to cut-off values of BMI given by WHO (2000) for Asian populations as per details given below:

Body Mass Index (BMI) kg/m^2	Nutritional Status
< 18.5	Underweight
18.5-22.9	Normal
23.0-24.9	Overweight
≥25	Obese

Assessment of Type 2 Diabetes Mellitus

Diabetes was diagnosed either by previous history of diabetes or if the fasting blood glucose was ≥126 mg/dl as per criterion given by WHO (1999).

Normal (mg/dl)	Diabetic (mg/dl)
< 126	≥126

STATISTICAL ANALYSIS

Data was managed on a questionnaire proforma designed for this purpose and then managed on an excel spread sheet. Data analyses were performed using SPSS 16.0 (Statistical Package for Social Sciences, SPSS Inc. Chicago, Ill) for Windows. The results of continues variables were presented as mean± standard deviation or percentage. The statistical significance for intergroup differences was analyzed by the Chi-square test.

RESULTS

The average ages of pre- and postmenopausal women were observed as 42.42± 5.99 and 52.50± 4.20 years, respectively (data not shown). *Table 1* and *Figure 1* depict the prevalence of Type 2 diabetes mellitus (T2 DM) among the pre- and postmenopausal women. In the pooled data, 11.26% (67) women were observed as diabetic and 88.74% (528) as non-diabetic. When assessed separately, it was noticed that among premenopausal women, 8.48% (28) subjects were diabetic whereas 91.51% (302) as non-diabetic. Similarly among postmenopausal women, the proportion of diabetic and non- diabetic subjects was observed as 14.72% (39) and 85.28% (226), respectively. It is evident from the table that the prevalence of diabetic subjects was 6.24% higher among postmenopausal women as compared to premenopausal women and difference between the groups was statistically significant (χ^2= 5.18, df=1, p<0.05).

Table 1: Percent prevalence of Diabetes among Pre- (Pre-M) and Postmenopausal Women (Post-M) according to WHO (1999) criteria

Category	Diabetic	Non-Diabetic	χ^2
Pre-M (330)	8.48 (28)	91.51 (302)	
Post-M (265)	14.72 (39)	85.28 (226)	5.18 df=1, P<0.05
Total (595)	11.26 (67)	88.74 (528)	

(Digits in parenthesis are the number of subjects)
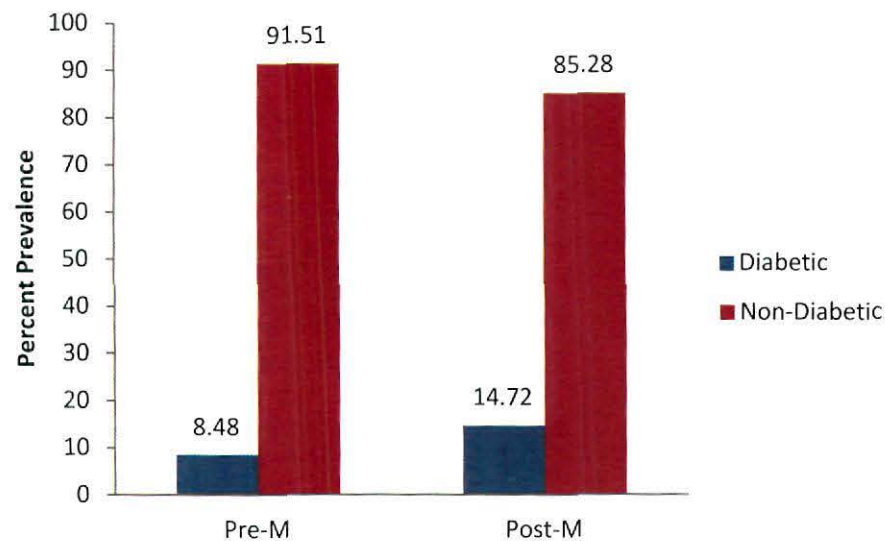
Fig.1 Comparison between pre- (Pre-M) and postmenopausal (Post-M) women for percent prevalence of diabetes

Table 2 and *Figure 2* show the prevalence of T2 DM among pre- and postmenopausal women according to different BMI categories. Among premenopausal subjects having BMI≥25 kg/m², 9.05% of the subjects were diabetic whereas 10.00% of women found diabetic were having BMI in the array of 23.0-24.9 kg/m². Among women with BMI ranging from 18.5-22.9 kg/m², the prevalence of diabetes was observed as 5.76% whereas all premenopausal women with BMI<18.5 kg/m² were normal. It was observed that the prevalence of T2 DM was the highest among premenopausal women having BMI in the array of 23.0- to 24.9 kg/m².

Among postmenopausal women also, none of the subjects having BMI<18.5 kg/m² was diabetic whereas 8.33% of postmenopausal women found diabetic were having BMI in the series of 18.5-22.9 kg/m². The percent prevalence of diabetic subjects having BMI in the range of 23.0-24.9 kg/m² and ≥25 kg/m² was noticed as 10.25 and 16.58%, respectively. It is evident from this table that the prevalence of diabetes increased with increase in the BMI among postmenopausal subjects as compared to premenopausal women and postmenopausal subjects with BMI≥25 kg/m² had much higher prevalence of diabetes as compared to their premenopausal counterparts.

Table 2: Percent prevalence of Type 2 Diabetes Mellitus among Pre- (Pre-M) and Postmenopausal (Post-M) women on the basis of BMI (kg/m^2)

Pre-M (330) BMI categories (kg/m^2)				Post-M (265) BMI categories (kg/m^2)			
<18.5 N=6	18.5-22.9 N=52	23.0-24.9 N=40	≥25 N=232	<18.5 N=3	18.5-22.9 N=24	23.0-24.9 N=39	≥25 N=199
-	5.76 (3)	10.00 (4)	9.05 (21)	-	8.33 (2)	10.25 (4)	16.58 (33)

(Digits in parenthesis are the number of subjects)
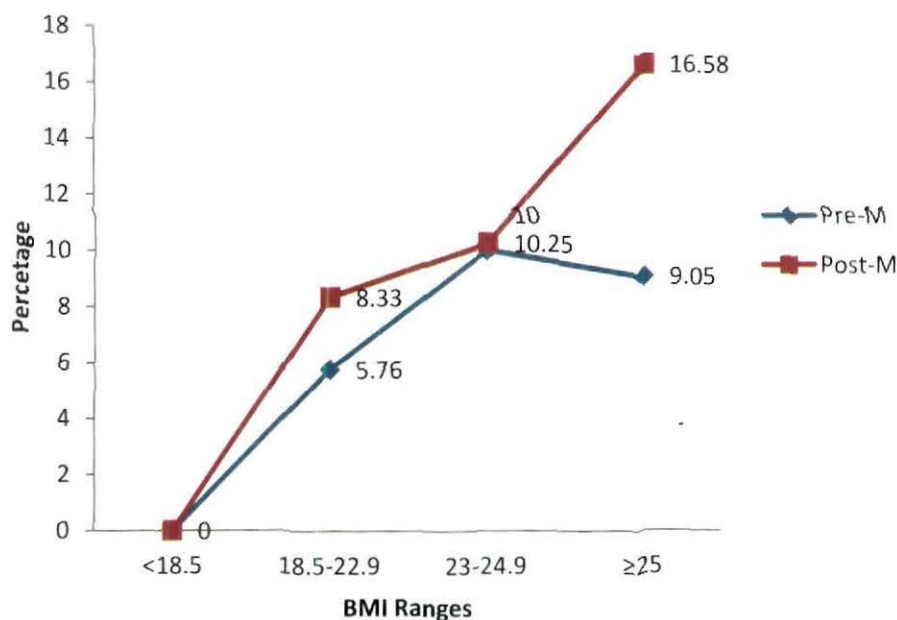
Fig. 2 Percent prevalence of diabetes among pre- (Pre-M) and postmenopausal (Post-M) women on the basis of BMI

DISCUSSION

The prevalence of diabetes in the present study was observed as 11.26%. The present study further demonstrates that there is significantly higher prevalence of diabetes (p<0.05) among postmenopausal women as compared to premenopausal women. Two things are reflected from these findings; diabetes increases as we move from lower to higher BMI and BMI increases after menopause among women. Our previous findings coupled with many from the literature also support that obesity increases in postmenopausal women as compared to pre-menopausal women (Khokhar et al., 2010 a). If the BMI of a person is over 30, the chances are already up to 10 times more likely to get diabetes.

In the span of ten-year period, those with a BMI of over 35 become up to 80 times more likely to develop the condition than someone with a BMI of less than 22 kg/m^2 (http://www.diabetes.org.nz/news_and_events/ world_news/obesity_increases_diabetes_risk_by_80_ times, retrieved on November 23rd, 2016 at 17.30). Overweight or obese people have added pressure on their body's ability to use insulin to properly control blood sugar levels, and are therefore more likely to develop diabetes (http://www.obesity.org/content/ weight-diabetes, retrieved on November 23rd, 2016 at 18.00).

Literature reflects that being overweight or obese, a person is at greater risk of developing type 2 diabetes particularly if excess weight is around your abdomen. Studies suggest that abdominal fat leads fat cells to

release 'pro-inflammatory' chemicals, which further make the body less sensitive to the insulin that is produced by disrupting the function of insulin responsive cells and also affects their ability to respond to insulin. It is learnt that visceral adipocytes release an excess amount of free fatty acids (FFAs) and are very resistant to the antilipolytic effect of insulin (Despres, 2006; Gastaldelli, 2008). Obesity is also thought to trigger changes to the body's metabolism. These changes make adipose tissue to release fat molecules into the blood, which might affect insulin responsive cells and lead to reduced insulin sensitivity (http://www.diabetes.co.uk/diabetes-and-obesity.html, retrieved on November 24th, 2016 at 19.00). A report published in *Science* proposes that overeating pressurizes the membranous network of endoplasmic reticulum (ER). When the ER has more nutrients to process than it can handle, it sends out an alarm signal directing the cell to dampen down the insulin receptors on the cell surface. This leads to insulin resistance and to persistently high concentrations of the sugar glucose in the blood which is one of the sure signs of diabetes (http://www.medicinenet.com/script/main/art.asp?articlekey=39840, retrieved on November 26th, 2016 at 19.31). Due to redistribution of body fat in postmenopausal women, more fat accumulates in abdominal region. This aggravates the situation as compared to premenopausal women. As explained earlier also, higher level of visceral adiposity during menopausal transition is associated with increasing insulin resistance, elevated FFA levels, as well as decreased adiponectin levels (Brown et al., 2008).

A fall in ovarian hormones at the menopause leads to diverse functional and hormonal disturbances among women. Increase in body weight and a decrease in basal metabolism further leads to greater weight gain among them (Khokhar et al., 2010 b; Sutton-Tyrrell et al., 2010; NAMS, 2012; Gravena et al., 2013). Studies conducted by the staff of Mayo Clinic suggest that the hormones estrogen and progesterone affect the cells' response to insulin. Changes in hormone levels at menopause can trigger fluctuations in the blood sugar level. Weight gain during the menopausal transition and after menopause increase the need for insulin (http://www.mayoclinic.org/diseases-conditions/diabetes/in-depth/diabetes/ART-20044312, retrieved on June 22nd, 2017). To study the beneficial role for endogenous estradiol in glucose homeostasis in women, a prospective cohort study with a follow-up of 11 years concluded that early menopause leads to more prolonged estradiol deficiency which is further associated with a greater risk of T2D (Brand et al., 2013).

The present study concludes that obesity increases the chances of type 2 diabetes and increase in BMI increases its prevalence. Women also become obese due to transition from pre-to postmenopausal phase due to decline in the level of circulating female hormone estradiole. Another factor could have been the age. Postmenopausal women are older than premenopausal ones and estradiole level decreases with age (Orentreich et al., 1994). Higher age, higher BMI coupled with decreased levels of estradiol further increase the prevalence of diabetes among postmenopausal women.

LIMITATION OF STUDY

The main limitation of the study is that this is a cross sectional study and men counterpart has not been included in it for comparison.

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AN INTROSPECTION OF AGILE METHODOLOGIES VIS-A-VIS SOFTWARE QUALITY IMPROVEMENT

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Abstract

Software development is a key element in the contemporary era of technology. The limitations of the traditional approach gave rise to a need for a methodology which could replace the flaws of traditional approach with the strengths of the flexible approach. This developed in the form of agile practices. These practices are adaptive and people-oriented in nature as compared to traditional approaches which are more process-oriented and sequential in nature. The software development using agile approaches authorises and gives opportunities to the project developers to deliver the software product quickly and correctly by including the customers in the development process and continuously adapting to their changing requirements. But the confusion arises at the time of selecting a methodology as per the requirements. Since there are diverse and multiple agile methodologies prevailing in software engineering, choosing the appropriate one is indeed a challenging quest.

The present paper is an attempt to analyse the varied agile methodologies in order to improve the software quality through performance analysis in a given set of circumstances.

Keywords: Agile, Scrum, Software development, Software quality, Sprint.

INTRODUCTION

Agile Software Development methodologies are gaining popularity among various software development organizations as they are customer oriented and productive. The limitations of the traditional approach gave rise to a need for a methodology which could replace the flaws of traditional approach with the strengths of the flexible approach. The agile practices are adaptable to changes and involve customers in the development process. The inflexible traditional approaches were unable to compete with these lightweight software development methods which could well understand and cope with the customer's demands. Agile software development processes helped the developers to complete the project in a very short frame of time leading to their popularity. Many studies and surveys are being conducted on agile methods and the software development has entered a new era. Since there are diverse and multiple agile methodologies prevailing in software engineering, choosing the appropriate one is indeed a challenging quest. The agile processes have started invading the software development industry to provide good quality software in minimal time.

AGILE METHODOLOGY

The Agile methodology is a recent development framework, which is used as a solution to the limitations of the traditional approaches in software engineering. For developing good quality softwares, various other problems emerged. These problems are:

Changing needs of the customer: The user needs are dynamic so they do not have clear vision about the requirement specification in the early stages. Only when application reaches them, they realize their true needs from the software.

Customer involvement: As software projects do not involve the customers beyond the requirement specification stage it increases the chances of project failure.

Deadlines and budgets: Tight deadline and low budget along with the correct high end software leads to competitions between the developers.

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Miscommunications: Due to different ways of expression by the customers and understanding by the developers of the software requirement specifications a lot of misunderstanding of the customers need is created.

Traditional approaches follow a fixed design procedure as compared to agile methodologies which follow a flexible process. The project developers begin with a simple software design and there is no planned next course of action. The developers work on short modules. Usually the time duration consists of weekly or fortnightly sprints. After each sprint, the priorities in the software to be developed and the sprints are examined and evaluated before actually being reviewed by the customers. It helps in identifying the errors and the feedback of the user is also taken before initiating the next sprint. Agile development concept was proposed in 2001 and Agile Manifesto was published wherein the twelve principles were documented. The primary principle was to give utmost importance to customer satisfaction by quickly delivering the software and accepting the changing needs of the user at any stage of the development process. The other principles involve reducing the delivery time of the software, co-ordination among the users and the software developing team during the development of the project and to trust, support and provide a congenial environment to the project team in developing the software. According to the agile manifesto the most appropriate way of sharing information is the direct face to face communication between the developing team and the user. As per the manifesto, the major parameter for measuring the performance is the working software. It also states that agility can be increased by continuously giving importance to the technological perspective and through effective project design. To conclude, it says that being simple is necessary and the developing team should show how to become more efficient at regular intervals and then should adapt to changes as per the needs.

FRAMEWORK FOR AGILE PRACTICES

Enhancement of success rate of software development is, to a great extent, based upon using an appropriate methodology so as to conform to the specific characteristics of the software. In order to deliver a project within budget, within deadlines and according to the needs of the user, the selection of an appropriate

methodology plays an important role in the development of the project.

Agile methodologies constitutes a group of approaches which are more flexible in nature ie they are adaptable to the changing needs of the user even at later stages and involves user in the development process. Some of the existing agile practices are:

Scrum Methodology: One of the important practices from the agile group of methodologies is Scrum methodology. It is a method that contains many features of the iterative, incremental and traditional methodologies. The major focus in scrum is to create the software flexibly in the continuously changing environment by focusing on the functioning of the members of the development team. A potential set of functionality is generated after completing each iteration in scrum. The primary objective in the scrum method is to use the concept of sprint. A sprint consists of thirty days duration along with a group of specified objectives. The process of scrum begins with a planning stage. In this phase a backlog list is created. This list provides details pertaining to the functionality of various releases of the software taking into consideration the risk associated with each release.

Extreme Programming (XP): Another important and commonly used practice from the group of agile methodologies is the extreme programming. It involves a group of different agile approaches. These approaches when taken together, forms another combined effective software approach. The process of extreme programming initiates with a planning phase. The feasibility of the software from different perspectives, the hard work needed and the timeline for the first release is examined and evaluated by the developing team in the planning stage. After this the other important characteristics of the software which need to be developed are defined by the user in the form of stories.

Feature-Driven Development (FDD): The Feature-Driven Development methodology is also an important agile practice. This approach differs from other practices in a sense that it lays emphasis on planning and the open design. The primary focus in the development process is on the characteristics of the system. Therefore it requires various other supporting methodologies for developing a software.

Dynamic System Development Methodology (DSDM): Another common practice from the agile

group is the dynamic system development methodology. It was developed in 1990s in the United Kingdom. This approach differs from the other approaches in the sense that the main idea is reversed in this as compared to other agile approaches. In DSDM instead of fixing the functional requirements and then adjusting the resources to achieve those requirements, the resources and schedules are fixed and then the functional requirements are adjusted according to the fixed resources.

Adaptive Software Development Methodology (ASD): Apart from the above mentioned methodologies, another approach is ASD. The adaptive software development comprises of three phases which are speculate, collaborate and learn. These phases lead to efficient planning, re-evaluation and inspection i.e. review. In the first phase i.e. speculation phase whatever we need to achieve after completing each pass, is defined. The significance of team work by sharing the information in between the development team is emphasized during the second i.e. collaboration phase. After this, the next is the third i.e. learning phase which is carried out after each pass. This is done to enhance and improve the developer's expertise as well as the quality.

COMPARISON OF AGILE TECHNOLOGIES

The comparison of agile methodologies with their respective features and the identified weaknesses is summarized below in table 1:

Agile Method	Special Characteristics	Identified Weakness
Scrum	Small, independent and self-organizing development teams, thirty days sprint	No details are provided with the system and acceptance testing during the release sprint of thirty days
Extreme Programming	User oriented, small teams. Effort needed and release timeline is examined and evaluated	The overall view is not much focussed and emphasized
Feature-Driven Development	The emphasis is on planning and the upfront design	Not a complete methodology as it emphasises only on design therefore requires other supporting approaches
Dynamic System Development Methodology	Resources and schedules are fixed and then the functional requirements are adjusted accordingly, quick application development	Not much clarity on actual use of methodology as only the members have access to white papers related t its use
Adaptive Software Development Methodology	Enhances and improves the developer's expertise as well as the quality, iterative development	Focus is more on the concepts rather than the practical implementation

Table 1: Comparison of Agile Technologies

BENEFITS OF AGILE IN CONTRAST TO TRADITIONAL METHODOLOGIES

The traditional methodologies consist of a sequence of varied activities like software requirements analyses and definition, design, implementation, testing and maintenance. For this, the needs need to be defined and documented at the initiation of the development. Traditional methodologies are conventional in their approach as they are unable to handle the frequently changing needs of the users. In response to traditional methodologies, concept of agile approaches has emerged which leads to obtaining software in a shorter period of time, using less resources as the software development is flexible to make last moment changes, straightforward and cooperative. Due to existence of multiple agile practices, there arises a need to analyse as to which methodology shall be suitable for the given requirements of the user. The use of agile practices for software development helps the team to accept the changes given by the customers at each stage and also helps the developers to reduce the risk in the initial stage of development of the project. The customers also get strong sense of ownership as they are able to see the development in the project throughout the project and the customer is able to give the feedback at each stage of development. The early adaption of feedback result the system · meets the customers'

requirements. The developers get chance to learn from mistakes at each stage of development which reduces the time lag and enhances the quality of the developed software. In a software if the time required for marketing is a matter of great concern, then being agile can result in early delivery of the working software. The agile practices are more user-focused.

PRECINCTS OF USING AGILE METHODOLOGY

The agile methodologies require lot of customer involvement while developing a software, so it may pose difficulties for such customers who do not have enough time for such kind of participation. The situation may worsen if the development team is not located at the same physical location as agile practices supports close working associations for easily handling the projects. Moreover as agile approaches support time bound delivery so there is a possibility that few targets may not get completed within the prescribed time limit. The repetitive behaviour of agile development may also affect the overall quality of the system, as there is not much focus on understanding of the developed system as a whole at the initial phases of software development. It becomes more relevant in complex systems with huge implementations or with softwares which involve high level of integration. In agile approaches, the focus is only on the code which may lead to memory loss, as documentation done is not substantial. The software designed using agile will not provide a reusable version as they build problem specific system rather than general one leading to decrease in the quality and performance of the software.

CONCLUSION

Lack of proper project development experience, technical incompetence of software developers, dearth of proper design, inadequate methods of implementation has changed the software development scenario in the contemporary era of technology. The iterative nature and user involvement in agile methodology is responsible for its increased use and acceptance in software development industry. Hence, the novel techniques for software development are the need of the hour to control the complexity of the large software systems so as to deliver the software well in time and within budget. Though agility brings quality but it has its own shortcomings. A careful decision has

to be made for each project as in some cases traditional methods would score over agile methodology. Negligence to choose an apt agile method may also lead to software failure or completion of software ahead of budget and the timeline.

Also Scrum which supports the management role in software development is a good option. But a re-evaluation of other agile approaches like extreme programming (XP), feature driven development (FDD), lean software development (LSD) and others may widen the scope of agile driven software development. Therefore the agile processes have started invading the software development industry to provide good quality software in minimal time.

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"NAGOYA PROTOCOL AND STRATEGIC PLAN FOR BIODIVERSITY 2011-2020: INTERNATIONAL AND NATIONAL SCENARIO WITH SPECIAL REFERENCE TO INDIA"[1]

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Abstract

Biodiversity is a wide term and refers to the infinite variety of life forms and is the *sine qua non* for the resilience of ecosystems and life forms and their ability to prevent and to recover from disasters and adverse conditions. Furthermore, biological diversity plays an important role in the spiritual and cultural life of human societies.[2] At the international level, the Convention on Biological Diversity (CBD), 1992 adopted for biodiversity conservation with 196 members.[3] It is important to note that at its tenth meeting (October 2010, Nagoya, Japan) there was move from policy-making to implementation and the CBD COP adopted: The Nagoya Protocol on Access to Genetic Resources and The CBD Strategic Plan for the period 2011-2020, including the Aichi biodiversity targets.

It is commendable to note that in eleventh COP held in India in 2012, developed countries agreed to double funding to support efforts in developing states towards meeting the internationally-agreed Biodiversity Targets, and the main goals of the Strategic Plan for Biodiversity 2011-2020.[4] In January 2013 Convention on Biological Diversity (CBD) Executive Secretary Braulio Ferreira de Souza Dias apprised UN Secretary-General Ban Ki-moon on the major outcomes of the eleventh meeting of the Conference of the Parties (COP 11) and highlighted the issue of the mainstreaming of biodiversity in the sustainable development agenda of the UN, through system-wide implementation of the Strategic Plan for Biodiversity 2011-2020 and the Aichi Targets.[5] India was one of the first few countries to enact legislation i.e., the Biological Diversity Act in 2002 and notified the Rules in 2004, to strictly implement BDC.[6] However, a careful examination and critical analysis shows that, more than anything else, need of the hour is strict implementation of the 2011-2020 Strategic Plan and progress achieved towards the Aichi Biodiversity Targets. Further, with globalization and increasing influence of Intellectual Property Rights, there is also an urgent need to develop appropriate national and International guidelines for strictly implementing the provisions of ABS and thereby preventing misappropriation of traditional knowledge as well as conservation of bioresources for the future generations. This paper is a humble attempt to examine these issues from a theoretical perspective.

Methodology: It is theoretical study based on international and national instruments, reports, article and the internet.

Keywords: Biodiversity, ecosystems, Convention on Biological Diversity (CBD), 1992, Biosafety.

INTRODUCTION

Wildlife protection is one of the oldest subjects of international environmental law and initially it involved the protection of particular species or groups of species. However, only in last decade of twentieth century emphasis was laid on conservation of biodiversity as there were various threats to it both at national and international levels. Significantly, Biodiversity is a wide term and refers to the infinite variety of life forms; genetic diversity (variation of genes within individual species), species diversity (variety of species in flora and fauna), and ecosystem diversity (variety of ecosystems, such as rainforests, coral reefs and deserts), that exist on our planet. This biological diversity is the *sine qua non* for the resilience of ecosystems and life forms and their ability to prevent and to recover from disasters and adverse conditions. Furthermore, biological diversity plays an important role in the spiritual and cultural life of human societies.[7]

The major step in this regard by the UNs was the adoption of Convention on Biological Diversity (CBD), 1992 which provides a universal framework for achieving biodiversity conservation and mutual benefits for developed and developing countries, their economies and their ecosystems.[8]

It is heartening to note that the CBD, 1992 is a widely accepted global treaty on biodiversity and sustainable development.[9] Till date the Conference of the Parties (COP) has held 11 ordinary meetings and one extraordinary meeting in 2000 where the Cartagena Protocol on Biosafety (CPB) or Biosafety Protocol,[10] was adopted. Significantly, the main drawback of this Convention is that it is process-oriented instrument and does not include specific targets in its objectives, its success depends to a large extent on the willingness of the Contracting parties to pursue the objectives of the Convention, and on their cooperation for the

conservation and the sustainable use of biological resources, and the sharing of the benefits arising out of the utilization of the genetic resources. It is important to note that till its 9th meeting there was emphasis on policy making, however, at its tenth meeting (October 2010, Nagoya, Japan) there was move from policy-making to implementation and the CBD COP adopted:

a.   the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization, which sets out rules and procedures for implementing the Convention's third objective;

b.   the CBD Strategic Plan for the period 2011-2020, including the Aichi biodiversity targets; and

c.   a decision on activities and indicators for the implementation of the Resource Mobilization Strategy.

Significantly, the General Assembly[11] declared 22 May, the date of the adoption of CBD, as the International Day for Biological Diversity to spread awareness for the implementation of the Convention. Theme of 2017 is: "Biodiversity and Sustainable Tourism" as diversity in species, ecosystems and landscapes attracts tourism and promotes economic growth and the tourist sector can help reduce threats to key wildlife populations, and can maintain or increase biodiversity, through tourism revenue. [12]   The year 2010 was declared as International Year of Biodiversity and the United Nations General Assembly declared the period from 2011 to 2020 as the United Nations Decade on Biodiversity [13] and is intended to support the implementation of the 2011-2020 Strategic Plan for Biodiversity that was adopted by Decision X/2 of the 10th Conference of the Parties to the Convention on Biological Diversity (COP10) in October in Nagoya.[14] Let's briefly analyse the protocol and plan:

1.   Nagoya Protocol and Strategic Plan for Biodiversity 2011–2020: International Scenario

2.1   Nagoya Protocol

Being a supplementary international agreement to CBD, the Nagoya Protocol applies to genetic resources that are covered by the CBD, and to the benefits arising from their utilization. It also covers traditional knowledge (TK) associated with genetic resources.

It is important because it will create greater legal certainty and transparency for both providers and users of genetic resources and thus would enhance the contribution of biodiversity to development and human well-being. It will establish more predictable conditions for access to genetic resources; and will help to ensure benefit-sharing when genetic resources leave the contracting party providing the genetic resources. By helping to ensure benefit-sharing, the Nagoya Protocol creates incentives to conserve and sustainably use genetic resources.[15]

It is important to note that the Nagoya Protocol sets out three core obligations[16] for its contracting Parties to take measures in relation to access to genetic resources, benefit-sharing and compliance. Let's briefly analyse these obligations.

1.1.1.   Access obligations

It is heartening to mention that in order to prevent misuse of genetic resources the Protocol has provided that contracting States must take various domestic-level access measures to access genetic resources.[17] Certainly such obligations would lead to sustainable use of the genetic resources. Significantly, most of the contracting parties have started performing their access obligations.

2.1.2.   Benefit-sharing obligations

Such obligations involve Domestic-level benefit-sharing measures which provide for the fair and equitable sharing of benefits arising from the utilization of genetic resources with the contracting party providing genetic resources. Utilization includes research and development on the genetic or biochemical composition of genetic resources, as well as subsequent applications and commercialization. Sharing is subject to mutually agreed terms. Benefits may be monetary or non-monetary such as royalties and the sharing of research results.[18] It is important to mention that by ensuring benefit sharing, the Protocol is creating incentive to conserve and sustainably use genetic resources and indirectly enhancing contribution of biodiversity to development of well being.

2.1.3.   Compliance obligations

It is important to mention that just enacting law, rules and regulation regarding genetic resources is not sufficient accordingly compliance obligations are also mentioned under the Nagoya Protocol.[19] Therefore, the state parties are required to formulate laws, rules, regulations, procedure, policies and progammes for successfully fulfilling their obligations.

2.2   Strategic Plan for Biodiversity 2011–2020

In 2010 Parties to the CBD adopted the Strategic Plan for Biodiversity 2011–2020[20], a ten-year framework for action by all countries and stakeholders to safeguard biodiversity and the benefits it provides to people. The purpose of the said Plan is to promote effective implementation of the Convention through a strategic

approach, comprising a shared vision, a mission, and strategic goals and targets also known as "the Aichi Biodiversity Targets" that inspires broad-based action by all Parties and stakeholders. The Strategic Plan also provides a flexible framework for the establishment of national and regional targets and for enhancing coherence in the implementation of the provisions of the Convention and the decisions of the Conference of the Parties.[21] Significantly, this plan mainly reflects how a country intends to fulfill the objectives of the CBD and the concrete actions it intends to take.

As part of the Strategic Plan 20 ambitious targets, known as the Aichi Biodiversity Targets, were adopted. To achieve these targets, Governments are required to establish national targets in tune with the Aichi Biodiversity Targets. It is important to note that the development of national targets and their incorporation into updated National Biodiversity Strategies and Action Plans (NBSAPs) is a key process in fulfilling the Aichi Biodiversity Targets. Further, these targets are mentioned under following five goals:

i.   Strategic Goal A: This goal is to address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society. However, main targets under this goal are that by 2020: people must be aware of the values of biodiversity and the steps they can take to conserve and use it sustainably; biodiversity values must be integrated into national and local development and poverty reduction strategies; incentives(including subsidies) which are harmful to biodiversity must be eliminated, and positive incentives for the conservation and sustainable use of biodiversity must be developed in tune with the CBD; Governments, business and stakeholders at all levels must take steps to achieve or have implemented plans for sustainable production and consumption and have kept the impacts of use of natural resources well within safe ecological limits.

ii.   Strategic Goal B: This goal is to reduce the direct pressures on biodiversity and promote sustainable use. However, main targets under this goal are, by 2020; the rate of loss of all natural habitats, including forests, must be at least halved if not brought to zero; to avoid overfishing and aquatic animals and plants must be managed and harvested sustainably, and legally, recovery plans and measures are in place for all depleted species; areas under agriculture, aquaculture and forestry must be managed sustainably, ensuring conservation of biodiversity; pollution, must be brought to levels which are not detrimental to ecosystem function and biodiversity; invasive alien species and pathways must be identified, and measures must be taken to prevent their introduction and establishment; and by 2015, the

multiple anthropogenic pressures on coral reefs, and other vulnerable ecosystems affected by climate change or ocean acidification must be minimized, so as to maintain their integrity and functioning.

iii.   Strategic Goal C: This goal involves improving the status of biodiversity by safeguarding ecosystems, species and genetic diversity. However, main targets under this goal are, by 2020: at least 17 per cent of terrestrial and inland water, and 10 per cent of coastal and marine areas, must be conserved; the extinction of known threatened species must be prevented and their conservation status, especially of those in decline must be improved and sustained; the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, must be maintained, and strategies must be developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity.

iv.   Strategic Goal D: This goal is to enhance the benefits to all from biodiversity and ecosystem services. However, main targets under this goal are, by 2020; ecosystems that provide essential services, including services related to water, and contribute to health, livelihoods and well-being must be restored, considering the needs of women, indigenous and local communities, and the poor and vulnerable; ecosystem resilience and the contribution of biodiversity to carbon stocks must be enhanced, and efforts must be made for climate change mitigation and adaptation and to combat desertification; and by 2015, the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization must be in force and operational, consistent with national legislation.

v.   Strategic Goal E: This goal is to enhance implementation through participatory planning, knowledge management and capacity building. However, main targets under this goal are: by 2015 each Party must have developed, adopted as a policy instrument, and has commenced implementing an effective, participatory and updated national biodiversity strategy and action plan; by 2020, the traditional knowledge, innovations and practices of indigenous and local communities relevant for the conservation and sustainable use of biodiversity, and their customary use of biological resources, are respected, full and effective participation of indigenous and local communities, at all relevant levels for effective implementation of CBD; knowledge, the science and technologies relating to biodiversity must be improved, widely shared, transferred, and applied; by 2020, at the latest, the mobilization of financial resources for effectively implementing the Strategic Plan for Biodiversity 2011-2020 from all sources. This

target will be subject to changes contingent to resource needs assessments to be developed and reported by Parties.

Significantly, efforts were made by all contracting parties to achieve their goals. Lets briefly analyse the efforts made by India *in* this regard.

3.   Nagoya protocol and Strategic Plan for Biodiversity 2011–2020: National Scenario

India

India is one of the 12 mega biodiversity countries of the world and one among the 192 signatories to the Convention on Biological Diversity (CBD) at Earth Summit in Rio de Janeiro in 1992. India became a Party to the Convention in 1994. Significantly, in India the conservation and sustainable use of biodiversity, based on local knowledge systems and practices, are also mentioned in the Constitution of India.[22] Further, to comply with its obligation under the CBD, the Government of India has enacted Biological Diversity Act, 2002 (BDA). Significantly, the Act provides a framework for access to biological resources and sharing the benefits arising out of such access and use. The Act also includes in its ambit the transfer of research results and application for intellectual property rights (IPRs) relating to Indian biological resources.

The Act is to be implemented through a three-tiered institutional structure: National Biodiversity Authority (NBA)[23], State Biodiversity Boards (SBBs)[24], Biodiversity Management Committees (BMCs) at the local level. When the foreigners, non-resident Indians, body corporate, association or organization that is either not incorporated in India or incorporated in India with non-Indian participation in its share capital or management use biological resources and associated knowledge occurring in India for commercial or research purposes or for the purposes of bio-survey or bio-utilisation they are required to take the approval of the National Biodiversity Authority.[25]

3.1. Fifth report submitted by India in 2014 under CBD, 1992[26]

It is heartening to note that 5th report under the CBD, 1992 was submitted by India well in time i.e, 31.03.2014. This report is submitted in three parts. In Part I, an update on biodiversity status, trends and threats in India and implications for human well-being have been given.   Here India has mentioned: Biodiversity profile of India[27]; Status and trends in terrestrial and aquatic ecosystems;[28] Species population trends;[29] Status and conservation of threatened plants species and their habitats; Threats to biodiversity such as threat assessment in

biogeographic zones of India & Habitat fragmentation at landscape level; and   Legal framework and conservation jurisprudence.

In Part II of the said report India has talked about Updating National Biodiversity Action Plan such as Background; National Biodiversity Targets (Process of updating National Biodiversity Action Plan; Indicators for National Biodiversity Targets; Agencies that will be responsible for monitoring progress in •achieving National Biodiversity Targets; Monitoring and reporting frequency; Implementation of National Biodiversity Action Plan); Assessment of funding support for biodiversity conservation in India such as   Financial overview of schemes of Ministry of Environment and Forest (MoEF) i.e., (MoEF's core funding for biodiversity conservation during 2013-2014; MoEF's non-core funding for biodiversity conservation during 2013-2014);   Outlays at the State level (Peripheral funding: Biodiversity related programmes of allied Ministries/Departments); Total estimated funding for biodiversity conservation during 2013-14 and National Biodiversity Targets and Millennium Development Goals.

Significantly, Part III of the report deals with India's progress towards Aichi Biodiversity Targets and contributions to the relevant 2015 targets of the Millennium Development Goals. Conservation of biodiversity at the national level requires inputs from several Ministries/Departments at the Central and State Governments' levels which shows mainstreaming of biodiversity concerns in development planning processes. It is important to note that the policies, programmes and projects of roughly 23 Ministries/Departments of the GOI, are directly or indirectly related to biodiversity conservation and hence important for achieving progress towards Aichi Biodiversity Targets. Let's briefly analyse the Part III of the said Report especially the efforts made for achieving Aichi Biodiversity Goals and Targets:

3.2.1 Strategic Goal A: Address the underlying causes of biodiversity loss by Mainstreaming biodiversity across government and society- Aichi Biodiversity

Target 1: Important actions taken by the GoI for achieving target I i.e., spreading awareness about the value of biodiversity through TV, radio, press. Significantly, environment concepts and issues are incorporated in the curricula at school and college level; The NGO, World -Wide Fund for Nature (WWF) India, dealing with nature conservation, environmental protection and development-related issues is doing commendable job to promote harmony between human beings and nature for more than four decades; The Centre for Media Studies (CMS) started various initiatives and one of them is the CMS VATAVARAN-

Environment and Wildlife Film Festival and Forum supported by MoEF and presently it has reached out to over 200,000 people since 2007[30]; The Biological Diversity Act, 2002, requires the local level Biodiversity Management Committees (BMCs) to prepare People's Biodiversity Registers (PBRs) in consultation with local people. PBRs contain comprehensive information on availability and knowledge of local biological resources; and *Citizen Science for conservation* is a movement in which volunteers help professional scientists to collect and analyse data while also promoting biodiversity awareness and conservation. '

Target 2: It is commendable to note that in India, there are a number of legal, policy and administrative measures supported by several programmes which contribute to achievement of Aichi Biodiversity Target 2. However, important measures are: India enacted the *Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) in 2005* which focused on land and water resources and includes water harvesting and conservation; soil conservation and protection; irrigation provisioning and improvement; renovation of traditional water bodies; land development and drought proofing; afforestation; horticulture development; and pastureland development.

The 'green jobs' created under the scheme are contributing to replenish the deplete the water table and afforestation; *The National Mission on Medicinal Plants*, under the Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy (AYUSH), is implementing a centrally sponsored scheme, for cultivation of medicinal plants on private lands for establishment of nurseries to supply quality planting materials; Under *The National Rural Livelihoods Mission (NRLM)* a special programme, *Mahila Kisan Sashaktikaran Pariyojana (MKSP)* was launched in 2010-2011 for empowering rural women in agriculture by making systematic investments to enhance their participation and productivity. Further, it also focuses on allied activities such as animal husbandry, collection non-timber forest produce and fisheries; MoEF in collaboration with United Nations Development Programme (UNDP) has instituted *'Biodiversity Governance Awards'*. Any institution that works towards biodiversity management is eligible for the award and winner is entitled for cash prize of INR 100,000 and runners up INR 50,000. The first such awards were given during COP-11, in October 2012 where UN under secretary general Rebeca Grynspan and Smt. Jayanthi Natarajan, minister of environment and forests, Government Of India presented the award in the category of protected area to periyar tiger reserve.[31]

Target 3: India has taken following actions for achieving this target i.e., elimination of subsidies harmful to biodiversity: India has taken significant measures to promote positive incentives that encourage activities beneficial to biodiversity such as a 'green bonus' or providing State funding allocations based on growing stock of forests, ratio of dense forest cover to total forest cover, and total carbon stock; Organic farming is being promoted across the country under the *National Project on Organic Farming (NPOF)*[32]; The Government of India is taking steps to encourage balanced fertilizer use so as to maintain soil biodiversity.; the Government scheme the *Rajiv Gandhi Gramin Vidyutikaran Yojana* has been facilitating rural electrification, thus providing an alternative to kerosene. Further, *Direct Benefit Transfer scheme* from 1st January 2013 is to be implemented for the direct transfer of subsidies for fertilizers, cooking gas, kerosene directly to farmers.

Target 4: Significantly, Government of India has started various policies in this regard such as: National *Forest Policy, 1988*[33], National *Conservation Strategy and Policy Statement for Environment and Sustainable Development, 1992*[34], National *Policy and Macrolevel Action Strategy on Biodiversity, 1999*[35], National *Agriculture Policy, 2000*[36]; National *Seeds Policy, 2002*[37]; National *Tourism Policy, 2002.*[38]

Apart from this various steps are being taken to ensure an integrated approach with participation of various stakeholders to protect the environment, some of which are: Federation of Indian Chambers of Commerce and Industry (FICCI) and *Earth Watch Institute India* are working to promote the concept of 'citizen science' for conservation and protection of urban water bodies; The GoI has enacted the *National Action Plan for Climate Change (NAPCC)*, and *the National Mission on Micro Irrigation in 2010* with the objective to enhance the efficiency of water usage, increase crop productivity and enhance farmers' incomes; A very positive step is that various corporates are also getting engaged in promoting biodiversity conservation activities under Corporate Social responsibility (CSR). The GoI has enacted the Companies Act 2013 which requires that every company with a net worth of INR 500 crore or more or turnover of INR 1000 crore or more or a net profit of INR 5 crore or more during any financial year must spend two percent of the average net profits made by the company during every block of 3 years for CSR activities. Significantly, ensuring environmental sustainability has been identified as one of the nine activities to be covered under CSR activities.[39]

3.2.2. Strategic Goal B: Reduce the direct pressures on biodiversity and promote Sustainable use- Aichi Biodiversity Targets 5 to 10

Target 5: India has adopted various measures to deal with critical issues like deforestation and degradation of the forests: The National Forest Policy (1988) aims at maintaining a minimum of 33% of the country's geographical area under forests and tree cover; *The National Afforestation and Eco-Development Board (NAEB)* provides support to Forest Development Agencies through the National Afforestation Programme; *Reducing Emissions from Deforestation and Forest Degradation* (REDD) is a global endeavour to create an incentive for developing countries to protect, manage better and save their forest resources and, thus contribute to the global fight against climate change and India is successfully participating in it;[40] In February 2013, MoEF launched the National Programme on Conservation of Aquatic Ecosystem (NPCAE) scheme for conservation of lakes and wetlands and *Green India Mission (GIM)* is one of the eight missions under *the National Action Plan for Climate Change (NAPCC)* for increased forest/tree cover on 5 m ha of forest/non- forest lands and improved quality of forest cover on another 5 m ha of non-forest/ forest lands.

Target 6: India has a vast Exclusive Economic Zone (EEZ), representing different types of ecosystems that range from those in the Open Ocean or shelf regions to the intertidal or sub-tidal ecosystems, mangroves, corals, and estuaries. Fisheries in the coastal zone determine the livelihood of a large number of fishermen, who are economically more vulnerable to changes in the environment. In this regard various initiatives are taken;[41] *Botanical Survey of India (BSI)*, another leading organization of MoEF, is involved in exploration, inventorying and documentation of floral diversity in protected areas;[42] *The National Centre for Sustainable Coastal Management (NCSCM)*, an autonomous body under the MoEF supports integrated management of the coastal and marine hazard risk management by enhancing knowledge and research[43]; Further, *The National Institute of Ocean Technology (NIOT)* is established with the main aim of developing reliable indigenous technology to solve the various engineering problems associated with harvesting of non-living and living resources in the Indian EEZ.[44]

Target 7: It is important to mention that to sustain and manage its forests, agricultural lands and fisheries India has taken various measures and some important are: *The National Agriculture Policy (NAP) 2007* essential for sustained increases in the productivity, profitability and stability of major farming systems by creating an economic stake in conservation, to strengthen the bio-security of crops, farm animals, fish and forest trees;[45] The Coastal Aquaculture Authority (CAA) was established under the Coastal Aquaculture Authority Act, 2005 to regulate coastal aquaculture activities;[46] and The National Fisheries Development Board (NFDB) set up to realize the untapped potential of the fisheries sector, in fish culture, processing and marketing of fish, and to apply modern tools of research and development to optimizing the production and productivity in fisheries.[47]

Target 8: In India various sources of pollution which pose threats to biodiversity are from; improper disposal of municipal solid wastes, improper/inadequate sewerage disposal, excessive use of chemical pesticides and hazardous chemicals. Various initiatives in this regard are: The Central Pollution Control Board (CPCB) established by MoEF has been playing a key role in control of pollution in the country; Pilot studies were conducted for urban areas by the *Centre for Spatial Environmental Planning* created at the CPCB under the World Bank funded Environmental Management Capacity Building Project. *The Ecocity programme* has been conceptualized for improving the environment and achieving sustainable development; Water quality is being monitored through the Water Quality Monitoring network at rivers, lakes, ponds, tanks and groundwater locations; Noise has been notified as a pollutant under the Air (Prevention and Control of Pollution) Act, 1981. CPCB is making efforts towards implementation of Noise (Regulation and Control) Rules, 2000 and establishment of Noise Monitoring Network.

Target 9: It is important to note that addressing the problem of Invasive Alien Species is urgent because the threat is increasing due to global trade, transport, and tourism with several social, economic and environmental impacts. India has an estimated 18,000 plants, 30 mammals, 4 birds, 300 freshwater fishes and 1100 arthropods that are invasive and it has made various efforts in this regard. [48]

Although so far there is no exclusive legislation or policy in India in this regard, but the NBAP 2008 in its objectives has mentioned ways for the regulation of introduction of invasive alien species and their management. India has established 'Forest Invasive Species Cell' in the Indian Council of Forest Research and Education, Dehradun to establish database on forest invasive species in India. Further, the 12th Five Year Plan (2012-2017) has emphasised the need for a national invasive species monitoring system to track the introduction and spread of invasive species and advised that such a system should be linked to the State Forest Departments, and field staff should be trained to collect information on invasive species.

Target 10: India's second national communication to the United Nation Framework Convention on Climate Change (UNFCCC), submitted in 2012 has emphasised the need to generate resources for conducting research on the effects of climate change on marine ecosystems, vulnerability assessment,

coastal zone engagement, sustainable habitat development etc. In this regard various initiatives are: The 12th Five Year Plan (2012-2017) aims to integrate low carbon growth in the current climate change initiatives so that by 2020 emissions can be reduced by 25-35% relative to 2005 levels (Planning Commission 2011). It is important to note that he important achievements: are completion of aerial photography of 70,000 km of the coastal zone; plantation of over 9000 ha of mangrove; offering protection to more than 100,000 sea turtles, of which more than 10,000 hatched and were released to the sea; first successful regeneration of corals on the mainland coast; and conversion of 20 villages into 'solar villages'.[49]

3.2.3 Strategic Goal C: To improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity – Aichi Biodiversity Targets 11 to 13

Target 11: Important initiatives for Conservation planning has been taken up in India on the basis of the Biogeographic Classification of India which are: India's has various scientifically managed Protected Areas (PAs) i.e., as of 26 March January 2014, there are 6902 PAs covering 166,851 km or 5.07% of the country's geographical area. [50] So far, 18 Marine Protected Area (MPAs) have been identified in peninsular India, and more than 100 MPAs have been designated in the islands; Other important conservation approaches include Community Conservation Areas (CCAs), sacred groves, natural world heritage sites, biodiversity heritage sites, Biosphere Reserves, KBAs, Alliance for Zero Extinction (AZE) sites, Ramsar sites, Key Biodiversity Areas (IBAs), Medicinal Plant Conservation Areas (MPCAs) and Ecologically Sensitive Areas (ESAs); Species-specific projects[51] are under implementation across the PAs as well as in areas outside the PA network. India has initiated a process of identifying Transboundary Protected Areas (TBPAs). Twenty one PAs were identified as occurring along or adjacent to the Indian border in mountains, forests, deserts and coastal regions. In order to further cooperation for TBPA management, India has started periodic meetings at the park level, especially with Nepal and Bhutan; Lastly The Indian Army and Indo-Tibetan Border Police have played an important role in preparing a road map to protect the Black-necked crane and its habitat in the high altitude wetlands in Ladakh, which are the only known breeding grounds for Black-necked crane in India.

Target 12: India has taken significant steps to reduce/minimize the decline in population of key threatened species and enhance their conservation status. Significantly important actions are; Species recovery plans for 16 terrestrial and 7 marine species (Dugong, four species of Sea turtle, Irrawaddy dolphin

and Whale shark) are being prepared. Some of these are at various stages of implementation with the active support of scientific institutions, civil society organisations and scientists. The conservation measures are taken for some rare, endangered and threatened species. Recovery plan for species viz. Edible-nest swiftlet, Nilgiri tahr, etc., are under preparation. Gir National Park (GNP) and Gir Wildlife Sanctuary (GWS) are making efforts for reversing the declining population of Asiatic lion. [52]

Target 13: Plant and animal genetic diversity is vulnerable to 'genetic erosion', the loss of individual alleles/genes and of combinations of alleles/genes, such as those found in locally adapted landraces. According to the Food and Agriculture Organization (FAO), replacement of local varieties by modern varieties resulting in reduction of the sheer number of cultivars is the main cause of genetic erosion. India conserves all the diversity she has. India has started various projects such as a World Bank-aided National Agricultural Innovation Project in 2010[53] initiated by Indian Council of Agriculture Research (ICAR) and the National Project for Cattle and Buffalo Breeding (NPCBB) has focused on development and conservation of indigenous breeds.[54]

3.2.4 Strategic Goal D: Enhance the benefits to all from biodiversity and ecosystem services- Aichi Biodiversity Targets 14 to16

Target 14: In order to harmonize the development efforts with conservation of environment and ecology, important initiatives taken by India are: The National Rural Livelihoods Mission was established by the GoI to implement the new strategy of poverty alleviation for the rural poor so that they can cross the threshold of poverty and become productive agents;[55] Further, The Bharat Rural Livelihoods Foundation (BRLF) fosters and facilitates civil society action in partnership with the Government for transforming the livelihoods and lives of people in areas such as the Central Indian adivasi belt for empowerment of the Adivasis; and The National Mission for Sustaining Himalayan Ecosystem (NMSHE) under NAPCC was launched in February 2014, with a budget outlay of INR 550 crore during the 12th Five Year Plan period (2012-2017) to develop a sustainable national capacity to continuously assess the health status of the Himalayan Ecosystem.

Target 15: In India the major process of land degradation is soil erosion (due to water and wind erosion), which contributes to over 71% of the land degradation. Soil erosion due to water alone contributes to about 61.7% and that by wind erosion to 10.24%. To solve this problem of land degradation various initiatives are: 22 major programmes are being implemented in the country, including the Green India

Mission, which will help increase the quality and extent of forest cover in 10 mha of land; The Eco Task Force (ETF), is working in controlling ecological degradation through plantation and soil conservation works; lastly, The eight national missions[56], which form the core of the NAPCC represent multi-pronged long-term and integrated strategies for achieving key goals in the context of climate change. The MoEF has requested each State Government to prepare a State Action Plan.[57]

Target 16; As India has played a leading role in the negotiations for Nagoya Protocol on ABS, and as the President of CoP-11 to the CBD, it has been working with the CBD Secretariat and other Parties to expedite ratifications of this important international agreement. Aichi Biodiversity Target 16 could thus become the first Aichi Biodiversity Target to be achieved.

3.2.5 Strategic Goal E: Enhance implementation through participatory planning, knowledge management and capacity building- Aichi Biodiversity Targets 17 to 20

Target 17: Regarding developing and effectively implementing National Biodiversity Strategies and plan of actions important initiatives taken by India are: enactment of the Biological Diversity Act in 2002, Section 36 of which empowers the Central Government to develop National Biodiversity Action Plan (NBAP). It is important to note that this plan is based upon the main principle in the National Environment Policy (NEP) that human beings are at the centre of concerns of sustainable development and they are entitled to a healthy and productive life in harmony with nature. The NBAP which has been developed in consultation with various stakeholders, attempts to identify threats and constraints in biodiversity conservation.

Target 18: India is rich in traditional knowledge associated with biological resources. This traditional knowledge is both coded[58] and non-coded.[59] There is a separate Department in the Government, AYUSH that deals with developing and propagating officially recognized ancient and holistic systems namely, Ayurveda, Yoga, Naturopathy, Siddha, Unani and Homeopathy which have been making significant contributions towards the health care of the people. Various attempts have been made for protection of traditional knowledge in the country: 'The Wealth of India' series, is an encyclopedia series on India's raw material resources of plants, animals and minerals. It consists of eleven volumes and two supplements; BMCs have been setup under Biological Diversity Act for conservation, sustainable use and documentation of biodiversity and chronicling traditional knowledge. So far 32,210 BMCs have been constituted by local bodies in 23 States;[60] The Biological Diversity Act provides for

establishment of People's Biodiversity Register (PBRs) by the BMCs for documenting traditional knowledge relating to biodiversity. Significantly, a total of 1901 PBRs have been registered in 14 States; The Patent Act, 1970, provides traditional knowledge as non patentable invention; A commendable step in this regard is the Traditional Knowledge Digital Library (TKDL) database is a value added digital database developed by the GoI[61] to prevent misappropriation of traditional knowledge at international patent office's so that cases of bio-piracy can be prevented. India has already signed TKDL Access Agreement with various countries.[62] It is important to note that such agreement have resulted in preventing misappropriation of Indian traditional knowledge. For example, due to such agreements there is cancellation of grant of two patents, withdrawal of 75 patent application amendments/modifications of claims in 32 applications, and rejection/cancellation of two applications.

Target 19: Activities for improving financial, human, scientific, technical, and technological capacity to implement the Convention on Biological Diversity are being undertaken through various policies, plans and programmes such as forest, agriculture, horticulture, irrigation, science and technology, and various specialized national and State level institutions, that deal with biodiversity issues. Important efforts are: Various ministries in India such as MoEF, MoST, MoA, MoHRD etc., are promoting research and development in multidisciplinary aspects of environment protection and biodiversity conservation. India has established a large number of research and training institutions[63] in the field of biodiversity which have comprehensive programmes and activities relating to conservation of biodiversity; It is important to note that the MoEF has set up nine Centres of Excellences[64] for strengthening awareness, research and training in priority areas of environmental science and management; Further, the National Knowledge Commission (NKC) was constituted in 2005 as a high-level advisory body to the Prime Minister of India to guide policy and direct reforms, focusing on certain key areas such as education, science and technology, agriculture, industry, e-governance etc. Easy access to knowledge, creation and preservation of knowledge systems, dissemination of knowledge and better knowledge services are core concerns of the commission; FRHLT has developed the raw drug repository, which specializes in collection of plant raw drugs used in Indian systems of medicine.

Target 20: India's core funding for biodiversity (under the MoEF's biodiversity-related programmes) is INR 15,643.4 million; the net non-core funding is *INR* 2598

million. This amounts to a total of core and non-core funding by MoEF of *INR* 18,241.4 million for the year 2013-2014. The overall funding for biodiversity conservation amounts to *INR* 92044.5 million (USD 1482.68 million) for 2013-2014 from 77 schemes of 23 Ministries/ Departments.

4.   Conclusion and Suggestions

A critical analysis shows that at the international level conservation of biodiversity became very important in the last decade of 20th century when CBD 1992 was adopted and presently there are 196 members. All the states are making sincere efforts to protect the biodiversity. Till its 9th meeting there was emphasis on policy making, however, at its tenth meeting (October 2010, Nagoya, Japan) there was move from policy-making to implementation and the CBD COP adopted Nagoya Protocol and Strategic Plan of Action 2011-2020. The **Secretariat for the Convention on Biological Diversity** is financed from contributions made by Parties and non-Parties to the three Trust Funds[65] established by the Conference of the Parties.

India, a mega diverse country with only 2.4% of the world's land area, accounts for 7-8% of all recorded species in the world. It has a variety of ecological habitats including forests, grasslands, wetlands, desert, coastal and marine ecosystems. India signed the Nagoya Protocol on 11th May 2011, and ratified it on 9th October 2012. Though conservation and sustainable use of biodiversity, is mentioned in the Constitution of India (Article 48A and Article 51(g) and India has enacted Biological Diversity Act 2002 for implementing the Convention on Biodiversity, 1992. Significantly, Nagoya Protocol is implemented by India through this Act. It is heartening to note that India has made sincere efforts in fulfilling her commitments towards conservation of biodiversity, its sustainable use and the fair and equitable sharing of benefits arising from the use of biodiversity and natural resources. India considers conservation of biodiversity as a national priority and recognizes its crucial linkages with the livelihoods and well-being of millions of her people, particularly the poor and vulnerable.

It is important to note that a multi-tier mechanism has been developed under the National Biological Diversity Act, 2002 for implementation of the NBAP, which includes the National Biodiversity Authority (NBA), State Biodiversity Boards (SBBs) and Biodiversity Management Committees (BMCs). India has taken some actions recently to achieve the 2020 Aichi Biodiversity Targets. Significantly, various monitoring programs have been put in place in several ecosystems as well as for particular species. Significantly, India has spent approximately *INR* 9200 crores or USD1483 million in 2013–2014 on activities that have a direct as well as indirect impact on conservation of biodiversity.

Apart from this Act, the Government of India has started various support mechanisms such as legislation, funding, capacity-building, coordination, mainstreaming, etc. for national implementation. The main strategies and policies related to biodiversity include the Biodiversity Act (2002), National Wildlife Action Plan (2002-2016), National Environmental Policy (2006), NBAP (2008) and NAP for Climate Change (2008).

India's National Biodiversity Action Plan (NBAP), 2008, was developed prior to CBD's Strategic Plan for Biodiversity 2011–2020 and the Aichi Biodiversity Targets. Therefore, to make the NBAP in tune with the Aichi Biodiversity Targets, the NBAP was updated during preparation of the fifth National Report under the CBD, 1992 wherein India has developed her own National Biodiversity Targets through an extensive consultative process.

Despite various challenges to biodiversity conservation faced by the country, India is making progress towards achieving the goals and biodiversity targets, both Aichi and National, for effective biodiversity conservation. In the third meeting of the Intergovernmental Committee for the Nagoya Protocol (ICNP) held in Republic of Korea in February 2014, sincere efforts made by India in this regard were appreciated.[66]

It is commendable to note that the Fifth National Report under CBD submitted by India well in time i.e., 31.04.2014, mainly focuses on the status of and trends in biodiversity, threats to biodiversity and implications for human well-being.

It has been observed that Indian law on biodiversity is in tune with CBD, 1992 but strict implementation is urgently required. Also, there is also an urgent need to develop appropriate national and International guidelines for strictly implementing the provisions of ABS and thereby preventing misappropriation of traditional knowledge as well as conservation of bioresources for the future generations. All said and done, natural calamity in a country is bearable and manageable because other countries always come for its rescue but threat to biodiversity is unbearable and unmanageable as every country all over the globe will be affected and no entity would be there to help. Therefore, it is right time for us to make joint efforts for conservation of biodiversity before it becomes too late. Undoubtedly, we are moving in the right direction; still we have a long way to go. Therefore, important suggestions are;

i.   There should be international cooperation for implementation of Nagoya protocol, strategic goals and Aichi Biodiversity Targets.

ii. Sincere efforts should be made by the states to enact law in tune with the CBD, 1992 and for implementation of Nagoya protocol and the Strategic plan of Action.

iii. The enforcement machinery under the CBD, 1992 should recognize the sincere efforts of the member states for implementing the Nagoya protocol, strategic goals and Aichi Biodiversity Targets.

iv. Various programmes and policies should be formulated by the states to recognize the efforts by every entity especially corporations under the Corporate Social responsibility for implementing the Nagoya protocol strategic goals and Aichi Biodiversity Targets. Further, giving certificates, awards or incentives to the corporations would make them more inclined towards CSR.

v.   Further, UN agencies should also recognize efforts of corporations under the Corporate Social responsibility for implementing the Nagoya protocol strategic goals and Aichi Biodiversity Targets.

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[1]   Updated version of a paper accepted for presentation in the 2014 IASIA *Annual Conference on Good governance: the Position of Students, Scholars and Practitioners Port Elizabeth, South Africa*, 30 June to 4 July, 2014.

[2]   Available at http://legal.un.org/avl/ha/cpbcbd/cpbcbd.html accessed on 08.01.2017.

[3]   Ibid.

[4]   Available at https://www.cbd.int/doc/press/2012/pr-2012-10-20-cop-11-en.pdf accessed on 15.01.2014

[5]   Available at http://www.cbd.int/doc/press/2013/pr-2013-02-07-newyork-en.pdf accessed on 02.02.2014.

[6]   Available at http://envfor.nic.in/downloads/public-information/India_Fourth_National_Report-FINAL.pdf accessed on 12.02.2017.

[7]   Available at http://legal.un.org/avl/ha/cpbcbd/cpbcbd.html, accessed on 12.04.2017.

[8]   The conceptual design of the CBD started with an analysis of "technical, legal, economic and financial matters relating to the conservation, accessibility and use of [genetic] resources" by the Secretariat of the International Union for Conservation of Nature (IUCN) in implementation of the World Conservation Strategy, which had been launched in 1980 by IUCN in cooperation with the United Nations Environment Program (UNEP), the World Wildlife Fund (WWF), the Food and Agriculture Organization (FAO) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) (IUCN General Assembly resolution 15/10, Christchurch, New Zealand, 1981). When a first draft for a convention on in situ conservation of flora and fauna was circulated by the IUCN to Governments and non-governmental organizations, UNEP and a number of States became interested in the idea of developing a universal biodiversity convention. Available at http://legal.un.org/avl/ha/cpbcbd/cpbcbd.html accessed on 13.02.2017.

[9]   As of 2016, the Convention has 196 parties, which includes 195 states and the European Union. All UN member states except United States and some Non-UN member states have ratified the treaty. Non-member states that have ratified are the Cook Islands, Niue, and the State of Palestine available at https://www.cbd.int/information/parties.shtml accessed on 01.06.2017.

[10] And entered into force on 11 September 2003.

[11] by its resolution 55/201 of 20 December 2000

[12] http://www.un.org/en/events/biodiversityday/ accessed on 01.06.2017.

[13] Resolution 65/161.

[14] Available at https://www.cbd.int/sp/, accessed on 04.01.2014.

[15] Available at http://www.cbd.int/abs/about/#objective, accessed on 01.01.2017.

[16] *Ibid.*

[17] Create legal certainty, clarity and transparency for both provider and users of genetic resources; Provide fair and non-arbitrary rules and procedures; Establish clear rules and procedures for prior informed consent and mutually agreed terms; Provide for issuance of a permit or equivalent when access is granted; Create conditions to promote and encourage research contributing to biodiversity conservation and sustainable use; Pay due regard to cases of present or imminent emergencies that threaten human, animal or plant health; and Consider the importance of genetic resources for food and agriculture for food security *Supra note 15.*

[18]   *Ibid.*

[19]   Take measures providing that genetic resources utilized within their jurisdiction have been accessed in accordance with prior informed consent, and that mutually agreed terms have been established, as required by another contracting party; Cooperate in cases of alleged violation of another contracting party's requirements; Encourage contractual provisions on dispute resolution in mutually agreed terms; Ensure an opportunity is available to seek recourse under their legal systems when disputes arise from mutually agreed terms; Take measures regarding access to justice and Take measures to monitor the utilization of genetic resources after they leave a country including by designating effective checkpoints at any stage of the value-chain: research, development, innovation, pre-commercialization or commercialization. *Supra note 15.*

[20]   Available at https://www.cbd.int/undb/media/factsheets/undb-factsheet-sp-en.pdf, accessed on 15.01.2014.

[21]   Available at http://www.cbd.int/decision/COP/default.shtml?id=12268 accessed on 15.01.2017.

[22]   Article 48A and Article 51(g) of Indian Constitution.

[23]   For details visit http://www.nbaindia.org/. accessed on 15.04.2017

[24]   So far 25 States have established the SBBs.

[25]   Available at http://www.nbaindia.org/accessed on 30.04.2017.

[26]   Available at http://www.cbd.int/doc/world/in/in-nr-05-en.pdf, accessed on 15.04.2017.

[27]   Such as Biogeographic classification and biodiversity characterization; Biodiversity hotspots; Importance of biodiversity and its implications for human well-being; Faunal and floral diversity; Fungi and lichen diversity; Marine biodiversity; Endemism; Domesticated biodiversity.

[28]   Such as Forests; Wetlands Mangroves, corals and sea grasses; Medicinal Plant Conservation Areas; Air and water quality trends.

[29]   Such as Flagship species; Status and population trends of key terrestrial and aquatic/ marine wild animal Species; Aquatic biodiversity

[30]   Available at www.cmsindia.org, www.cmsvatavaran.org accessed on 15.04.2017.

[31]   Available at http://www.in.undp.org/content/india/en/home/ourwork/environmentandenergy/nominations-open-for-the-india-biodiversity-awards-2014-/ accessed on 15.04.2017.

[32]   Available at www.ncof.dacnet.nic.in, accessed on 15.04.2017.

[33]   It main objective is to maintain environmental and ecological balance.

[34]   It provides the basis for integration and internalization of environmental considerations in the policies and programmes of different sectors.

[35]   It outlines a series of macro-level statements of policies, gaps and strategies neededfor conservation and sustainable use of biodiversity.

[36]   It is mainly concerned with Indian agriculture, rural infrastructure and value addition.

[37]   It covers areas such as seed varietal development production, quality assurance, seed distribution and marketing, infrastructure facilities, etc.

[38]   It lays emphasis on ecotourism for helping in eliminating poverty ensuring employment, preserving cultural heritage and improving overall environment.

[39]   Section 135 of the Indian Company Act, 2013 read with Schedule VII annexed to The Companies Act 2013 available at http://mca.gov.in/Ministry/pdf/The_Companies_Bill_2012.pdf accessed on 13.01.2014. Also see http://cconpo.icai.org/wp-content/uploads/2012/06/Salient-Features-of-Companies-Bill-2012-on-Corporate-Social-Responsibility.pdf accessed on 28.02.2017.

[40]   Available at http://www.moef.nic.in/sites/default/files/ProceedingsoftheREDDWorksho18-4-2012.pdf).

[41]   Available at www.zsi.gov.in. accessed on 01.02.2017.

[42]   Available at www.bsi.gov.in. accessed on 12.02.2017.

[43]   Available at www.ncscm.org. accessed on 15.03.2017.

[44]   Available at http://www.niot.res.in/ accessed on 15.04.2017.

[45]   Available at http://www.indg.in/agriculture/ruralemploymentschemes/ national-policy-for- farmers-2007 accessed on 15.03.2017.

[46]   Available at http://caa.gov.in/; http://dahd.nic.in/dahd/coastal-aquaculture-authoritycaa.aspx accessed on 15.02.2014.

47   Available at http://nfdb.ap.nic.in/; http://dahd.nic.in/dahd/division/fisheries/national-fisheries-development-board-nfdb.aspx accessed on 11.04.2017.

48   Anil et. al., 2002.

49   For detail visit http://wwwwds.worldbank.org/external/default/WDSContentServer/WDSP/SAR/2013/05/24/90224b081be924f/1_0/Rendered/PDF/India000Integr0Report000Sequence006.pdf accessed on 15.04.2014.

50   his is a significant increase of 1.72% and a net 2 2 increase of 56,694 km since 1988, when there were 54 National Parks covering 21 ,003 km and 373 Sanctuaries covering 2 2 88,649 km , giving a combined coverage of 109,652 km or 3.34% of the country's geographical area.

51   For example Project Tiger, Project Elephant and Project Snow Leopard.

52   There are four species of bustards in India: Great Indian Bustard (GIB), the Lesser florican, the Bengal florican and the Houbara bustard.

53   Available at http://www.naip.icar.org.in/index.html accessed on 12.03.2017.

54   Available at http://www.dahd.nic.in/dahd/schemes/animal-husbandary/f-no-48-72009-ts-sheep/nationalprojectfor-cattle-buffalo-breeding.aspx. accessed on 12.03.2017.

55   Available at http://rural.nic.in/sites/downloads/latest/NRLM%20Procurement%20Manual.pdf. accessed on 11.04.2017.

56   (1) Solar Energy Mission, (2) National Mission for Enhanced Energy Efficiency, (3) National Mission on Sustainable Habitat, (4) National Water Mission, (5) National Mission for Sustaining the Himalayan Ecosystem, (6) Green India Mission (7) National Mission for Sustainable Agriculture and (8) National Mission on Strategic Knowledge for Climate Change;

57   Available at http://moef.nic.in/downloads/publicinformation/Black%20Carbon%20Research %20Initiative.pdf. accessed on 10.04.2017.

58   As in the texts of Indian systems of medicine such as Ayurveda, Unani and Siddha.

59   Which exists in the oral undocumented traditions.

60   Available at nbaindia.org accessed on 02.04.2017.

61   for (i) preservation of traditional knowledge; (ii) prevention of misappropriation of traditional knowledge by breaking the language and format barriers of traditional knowledge systems, and providing access of these knowledge systems to patent examiner (s) in five international languages i.e., English, German, French, Spanish and Japanese, for establishing the prior art; and (iii) creation of linkages with modern science to initiate active research projects for new drug discovery and development, based on the time tested traditional knowledge systems leading to more affordable health care for the poor.

(i) European Patent Office February 2009, (ii) United State Patent & Trademark Office (November 2009), (iii) Canadian Intellectual Property Office (September 2010), (iv) German Patent Office (October 2009), (v) United Kingdom Patent & Trademark Office(February 2010) and (vi) Intellectual Property, Australia(January 2011)

63   Government organizations: ZSI, BSI, FSI, IIFM, FRI, ICFRE, WII, G.B. Pant Institute of Himalayan Environment and Development, Centre for Ecological Sciences (CES), IISc, ICAR, CSIR, NBRI, NIO, CIMAP, NEERI, ISRO, NRSC, Indian Council for Social Science Research (ICSSR) etc.

Non Governmental Organizations: BNHS, WWF, WTI, Wildlife Protection Society of India (WPSI), Centre for Wildlife Studies, (CWS), ATREE, International Society of Naturalists (INSONA), The Energy Research Institute (TERI), Centre for Science and Environment (CSE), MSSRF, Environment Protection Training and Research Institute (EPTRI), etc.

64   CEE, Ahmedabad; CPREEC, Chennai; CES, Bangalore; CME, Dhanbad; SACON, Coimbatore; CEMDE, Delhi; TBGRI, Thiruvananthapuram; MSE, Chennai; FRLHT, Bengaluru

65   The General Trust Fund for the Convention on Biological Diversity (BY Trust Fund), which is the major source of funding for the Secretariat and is funded from the assessed contributions of Parties to the CBD, based on the United Nations scales of assessment; The Special Voluntary Trust Fund (BE Trust Fund) for additional voluntary contributions in support of approved activities of the Convention on Biological Diversity; and The Special Voluntary Trust Fund (BZ Trust Fund) for Facilitating Participation of Parties in the Convention Process. Available at http://www.cbd.int/convention/parties/contributions.shtml accessed on 30.04.2014.

66   Available at http://www.cbd.int/doc/world/in/in-nr-05-en.pdf, accessed on 22.04.2017.

MASS PROPAGATION OF A FLORICULTURALLY AND MEDICINALLY IMPORTANT EPIPHYTIC ORCHID *DENDROBIUM AMOENUM* WALL. EX LINDL. THROUGH ASYMBIOTIC SEED CULTURE: A STUDY *IN VITRO*

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Abstract

Dendrobium amoenum Wall. ex Lindl., a floriculturally and medicinally important epiphytic orchid species of tropical to sub-tropical climates is distributed along the Himalayas from Garhwal eastwards to Sikkim at an altitude of 900-1600 m. The present paper reports the *in vitro* propagation of *Dendrobium amoenum* through asymbiotic seed culture with a view to developing an efficient protocol for its mass propagation. The germination potential of its seeds was tested on agar gelled VSE (Vigna seedling extract) and M (Mitra *et al.*, 1976) medium supplemented with growth additives (IAA, IBA, GA$_3$, 2,4-D, NAA, KN at 1 mgl^{-1} each; P, CH, YE at 2 gl^{-1} each and AC (2 gl^{-1}). *In vitro* raised seedlings with 2-3 leaves and 1-2 roots were gradually hardened and successfully transferred to community pots in the green house. Based on present observations, different optimal nutritional combinations are suggested for early onset of seed germination (M), protocorm multiplication (M+IBA+KN), early differentiation and seedling formation (M+YE), in the species.

Key Words: *Dendrobium amoenum, in vitro*, asymbiotic, immature seeds, protocorms

INTRODUCTION

ORCHID SEEDS are very distinctive as compared to the seeds of other flowering plants as these are microscopic, dust like and are produced in large numbers within a capsule ('pod') but lack storage tissue. Despite such simple organization, they exhibit a great deal of diversity in size, form, and structure (Pathak *et al.*, 2011a; Vij *et al.*, 1992). These seeds are non-endospermic and require a fungal stimulus for germination in nature (Bernard, 1904), since they lack appropriate metabolic machinery (glyoxysomes) for conversion of their lipidaceous food reserves into more utilizable forms (Harrison, 1977; Poole and Sheehan, 1982; Vij and Sharma, 1983). Though a large number of orchid species from diverse habits and habitats have been successfully germinated asymbiotically *in vitro* (Arditti *et al.*, 1982a,b; Baker *et al.*, 2014; Chen *et al.*, 2015; Pathak *et al.*, 1992, 2001, 2011b; Piri *et al.*, 2013; Santos *et al.*, 2016; Verma *et al.*, 2013; Vij and Pathak, 1988), data is still meagre in terms of the orchid family.

Dendrobium amoenum Wall. ex Lindl., a floriculturally and medicinally important epiphytic orchid species of tropical to sub-tropical climates is distributed along the Himalayas from Garhwal eastwards to Sikkim at an altitude of 900-1600 m; it blooms during May-June. The fresh paste of its pseudobulbs and leaves is applied to cure burnt skin and dislocated bones (Vaidya *et al.*, 2002). Its natural populations are, however, getting rarer due to depletion of its habitats as a consequence of clearing of forests for agricultural and other developmental purposes. The present paper reports the *in vitro* propagation of *Dendrobium amoenum* through asymbiotic seed culture with a view to developing an efficient protocol for its mass propagation.

MATERIALS AND METHODS

Plant Material

Immature seeds of *Dendrobium amoenum* were used as explants

Surface Sterilization of Capsules

The green undehisced capsules were scrubbed with 'Teepol' and repeatedly washed with running tap water for 5-10min. The capsules were subsequently washed

with distilled water and surface sterilized with 0.1% $HgCl_2$ solution for 5-8 min followed by a treatment with streptomycin (0.03%, 8 min) and Bavistin (0.01%, 8 min) prior to washing thoroughly with sterile distilled water so as to remove any traces of the sterilizing agents. Further, capsules were split opened with a sterilized blade to scoop out the immature seeds.

Nutrient Media and Incubation Conditions

The germination potential of these seeds was initially assessed on agar (1.4%) gelled VSE (Vigna seedling extract) and the undefined medium (Vigna Seedling Extract: VSE) was prepared by 100, 150, and 200gm of 5 days old *Vigna sinensis* seedlings in half litre distilled water for 20 minutes. The filtrate was supplemented with 2% sucrose and its volume raised to 1 litre by adding distilled water prior to its gelling with agar (1.4%). Subsequently, the seeds were inoculated on agar (0.9%) gelled M media (Mitra *et al.*, 1976) with and without growth additives [Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), Naphthalene acetic acid (NAA), Gibberelic acid (GA_3), 2,4-Dichlorophenoxyacetic acid (2,4-D), Kinetin (KN), at $1mgl^{-1}$ each either alone or in combination; Peptone (P), Casein hydrolysate (CH), Yeast extract (YE), Activated charcoal (AC) at 2 gl^{-1} each)]. The pH of the nutrient medium was adjusted at 5.6 with 1N NaOH or HCl before autoclaving at 121°C at 1.1 Kg cm^{-2} for 20 minutes.

Acclimatization

In vitro raised seedlings with 2-3 leaves and 1-2 roots were gradually hardened by sequentially removing the growth additives, vitamins, sucrose and minor salts from the nutrient matrix at 15 days interval. The seedlings were gently removed from the culture vessels with the help of a long forceps, washed thoroughly with lukewarm water, in order to remove any other agar sticking to these and were potted in community pots (6 cm diameter), filled with a potting mixture of charcoal, pine bark, and brick pieces (1:1:1) with topping of *Sphagnum* moss, and hardened for 6-8 wks in the green house.

Statistical Analysis

One way analysis of variance was performed with respect to each response (average ± standard error against each additive as mention in Table 1). As ANOVA results showed the non significant difference of additives at 5% level of significance, various groups of additives showing identical/similar response were formed statistically. To this end, Tukey Test was performed at 5% level with respect to each response.

RESULTS

The seeds though showed germination response on agar gelled *Vigna* seedling extract (VSE) medium, rhizogenesis and subsequent growth of the seedlings were variously affected depending upon the concentration of VSE used. At $100gl^{-1}$ of VSE, seeds showed germination within 3 weeks and seedlings were obtained in 31 weeks. Its higher concentration $(150gl^{-1})$ promoted seed callusing. Seedlings with multiple shoots were obtained at its further higher concentration (200gl⁻1). VSE in general, supported good germination in *Dendrobium amoenum* and proved beneficial in promoting healthy seedling growth. VSE was earlier used to raise healthy seedling growth in *D. mirbelianum* and *D. schulleri* (Soerohaldoko, 1980). Incidentally, most of the species so far tested in VSE belong to the genus *Dendrobium* and their variable response seems to be related to their differential nutrient requirements and/or the quality of VSE. In this connection, it is worthwhile to mention that VSE was presently prepared from *Vigna sinensis* seedlings, whereas it was earlier obtained from *V. radiata* seedlings in case of *D. mirbelianum* and *D. schulleri*.

Presently, the immature seeds procured from unripened capsules were inoculated on an agar gelled M medium with and without different PGRs /or an organic supplement P, YE and CH with an aim to assess the efficacy of different additives on the onset of germination, protocorm multiplication, differentiation and subsequent seedling development (Figs. 1-8). In the basal medium, the embryonal mass developed into chlorophyllous spherules with absorbing hairs in 3.00±0.81 wks; protocorms formed in 7.00±0.81 wks multiplied (Fig.4), first leaf and root primordia were differentiated in 12.00±0.81 and 18.00±0.81 wks respectively, and seedlings were formed in 26.00±0.81 wks old cultures (Fig.6). Incorporation of AC in the medium though delayed spherule formation, proved ineffective during protocorm formation, differentiation and seedling formation. Presence of IAA in the nutrient pool, delayed onset of germination and leaf differentiation. Incorporation of AC in IAA enriched medium advanced rhizogenesis and seedlings with long roots were observed (Fig.8). IAA has proved earlier inhibitory in *Vanda* (Goh, 1971); promotory in *Phalaenopsis* (Ernst, 1967) and ineffective in *Platanthera bifolia* (Hadley, 1970). Presence of IBA in the nutrient pool though delayed spherule formation, advanced root formation incorporation of AC in the nutrient pool proved ineffective. Perusal of literature similarly indicates discordant effects of IBA during germination and seedling growth in orchids. It proved ineffective in several species (Kano, 1965) and accounted for stunted shoot growth in *Pachystoma senile* (Vij *et al.*, 1985), but its role in promoting germination in *Cypripedium* and *Vanilla planifolia* (Hegarty, 1955), protocorm growth in *Dendrobium*

Table.1. *In vitro* asymbiotic germination response of immature seeds of *Dendrobium amoenum* on M medium with or without growth additives.

Medium	Time taken in wks for development of				
	Spherule	protocorms	1st leaf	1st root	seedling
M	3.00 ± 0.81^{a}	7.00 ± 0.81^{abc}	12.00 ± 0.81^{cd}	18.00 ± 0.81^{def}	26.00 ± 0.81^{ef}
M+AC	6.25 ± 0.95^{cde}	9.00 ± 1.41^{cd}	13.00 ± 0.81^{def}	19.50 ± 1.29^{efg}	25.00 ± 0.81^{cde}
M+IAA	6.75 ± 0.95^{de}	9.00 ± 0.81^{cd}	14.75 ± 0.95^{ef}	20.00 ± 0.81^{fgh}	24.75 ± 1.25^{cde}
M+IAA+AC	7.50 ± 1.29^{e}	9.00 ± 0.81^{cd}	12.50 ± 1.29^{def}	16.00 ± 0.81^{bcd}	24.50 ± 1.29^{cde}
M+IBA	6.50 ± 1.29^{cde}	8.50 ± 1.29^{bcd}	11.75 ± 0.95^{abc}	14.50 ± 1.29^{abc}	24.00 ± 2.16^{cde}
M+IBA+AC	6.50 ± 1.29^{cde}	7.33 ± 0.57^{abc}	11.50 ± 1.29^{bcd}	16.50 ± 1.29^{bcde}	25.00 ± 0.81^{cde}
M+GA$_3$	6.50 ± 1.29^{cde}	7.75 ± 0.95^{abcd}	14.75 ± 0.95^{ef}	22.50 ± 1.29^{h}	28.75 ± 0.95^{f}
M+GA$_3$+AC	7.00 ± 0.81^{de}	10.50 ± 1.29^{d}	15.00 ± 0.81^{f}	21.50 ± 1.29^{gh}	25.75 ± 0.95^{def}
M+2,4-D	5.75 ± 0.95^{abcd}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M+2,4-D+AC	5.75 ± 0.95^{abcde}	10.50 ± 1.29^{d}	12.50 ± 1.29	18.50 ± 1.29^{defg}	24.00 ± 1.41^{cde}
M+NAA	5.50 ± 1.29^{abcde}	6.75 ± 0.95^{abc}	$10.75\pm0.95a^{bcd}$	14.50 ± 1.29^{abc}	22.50 ± 1.29^{bcd}
M+NAA+AC	4.75 ± 0.95^{abcde}	6.75 ± 0.95^{abc}	11.00 ± 0.81^{bcd}	14.00 ± 0.81^{ab}	21.75 ± 0.95^{bc}
M+KN	5.00 ± 0.70^{abcde}	9.60 ± 0.89^{cd}	11.80 ± 1.09^{bcd}	18.60 ± 0.89^{defg}	22.40 ± 1.14^{bcd}
M+KN+AC	5.75 ± 0.95^{abcde}	9.25 ± 1.70^{cd}	12.75 ± 0.95^{def}	17.50 ± 1.29^{cdef}	22.50 ± 1.29^{bcd}
M+IAA+KN	5.25 ± 1.70^{abcde}	7.75 ± 0.95^{abcd}	11.50 ± 1.29^{bcd}	16.25 ± 1.70^{bcd}	21.75 ± 0.95^{bc}
M+IAA+KN+AC	5.25 ± 1.70^{abcde}	7.75 ± 0.95^{abcd}	11.50 ± 1.29^{bcd}	16.25 ± 1.70^{bcd}	21.75 ± 0.95^{bc}
M+IBA+KN	3.75 ± 0.95^{abc}	7.75 ± 0.95^{abcd}	10.75 ± 0.95^{abcd}	14.00 ± 0.81^{ab}	22.50 ± 1.29^{bcd}
M+IBA+KN+AC	5.00 ± 0.81^{abcde}	8.00 ± 1.00^{abcd}	9.00 ± 1.00^{ab}	12.00 ± 1.00^{a}	22.00 ± 1.00^{bc}
M+2,4-D+KN	5.50 ± 1.29^{abcde}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M+2,4-D+KN+AC	5.00 ± 0.81^{abcde}	7.75 ± 0.95^{abcd}	11.50 ± 0.57^{bcd}	18.50 ± 0.57^{defg}	23.50 ± 1.29^{cde}
M+NAA+KN	6.75 ± 0.95^{de}	7.25 ± 1.25^{abc}	12.75 ± 0.95^{def}	18.00 ± 0.81^{def}	23.75 ± 0.50^{cde}
M+NAA+KN+AC	6.00 ± 2.16^{bcde}	9.25 ± 1.70^{cd}	12.25 ± 0.50^{de}	$18.25\pm1.70d^{ef}$	23.75 ± 2.62^{cde}
M+P	3.75 ± 0.95^{abc}	7.75 ± 0.95^{abcd}	13.00 ± 1.41^{def}	16.25 ± 1.70^{bcd}	20.00 ± 0.81^{b}
M+P+AC	3.75 ± 0.95^{abc}	7.25 ± 1.70^{abc}	12.50 ± 1.73^{def}	14.75 ± 0.95^{abc}	19.75 ± 0.95^{b}
M+CH	3.25 ± 0.95^{ab}	5.00 ± 1.29^{a}	9.50 ± 0.57^{abc}	16.75 ± 0.95^{bcde}	22.00 ± 2.16^{bc}
M+CH+AC	3.25 ± 0.95^{ab}	5.00 ± 1.29^{a}	9.50 ± 0.57^{abc}	16.75 ± 0.95^{bcde}	22.00 ± 2.16^{bc}
M+YE	3.00 ± 0.81^{a}	5.75 ± 0.95^{ab}	8.25 ± 0.50^{a}	11.75 ± 1.25^{a}	15.75 ± 1.25^{a}
M+YE+AC	3.00 ± 0.81^{a}	5.75 ± 0.95^{ab}	8.25 ± 0.50^{a}	11.75 ± 1.25^{a}	15.75 ± 1.25^{a}

Entries in column nos. 2 to 6 are mean± S.E; same alphabetical letter in the superscript denotes that the corresponding means are in the same group using Tukey test at 5%.
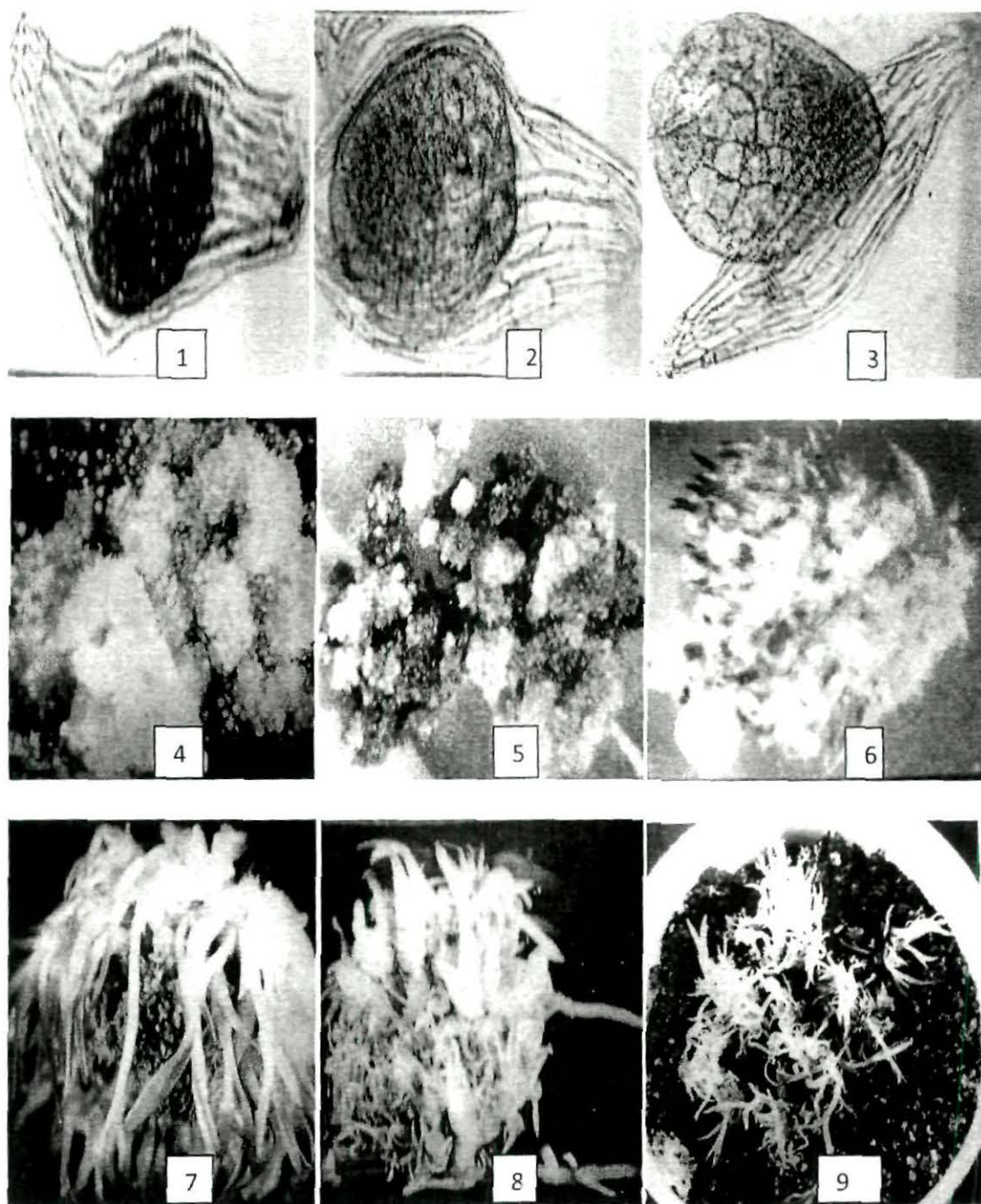
Figs. 1-9. Mass propagation of a floriculturally and medicinally important epiphytic orchid *Dendrobium amoenum* through *in vitro* asymbiotic seed culture: 1, Seed at the time of inoculation (M); 2-3, Apical and lateral rupturing of the seed coats (M); 4, Chlorophyllous protocorms (M); 5, Seed callusing [M+2,4-D (1 mgl^{-1})]; 6, Differentiation of leaves (M); 7, Seedlings with healthy leaves [M+YE(1mgl^{-1})]; 8, Seedlings with long roots [M+IAA (1 mgl^{-1})+AC (2 mgl^{-1})]; 9. Seedlings transferred to a community pot.

(Pages, 1971) and protocorm multiplication in *Eulophia dabia* (Sharma and Vij, 1986) is well documented. GA$_3$ in the nutrient medium proved ineffective during protocorm formation, delayed leaf and root differentiation. Elongated protocorms and seedlings were obtained in this combination. Chlorophyll development was impaired and seed callusing was observed in the nutrient medium containing 2,4-D (Fig.5).The use of AC in the above combination, however, proved beneficial in inducing differentiation. Seedlings complete with 2-3 leaves and 1-2 roots were obtained in 24.00±1.41 weeks. Similar results were obtained when 2,4-D was incorporated with KN. However, presence of AC remained ineffective. Presence of 2,4-D in the nutrient medium accounted for reduced germination frequency in *Vanda* Miss Joaquim (Goh, 1971) and *Coelogyne punctulata* (Sharma and Tandon, 1986); suppressed chlorophyll development in *Cymbidium eburneum*, *Dendrobium moschatum*, *Rhynchostylis retusa*, *Thunia alba* and *Vanda parviflora* (Sood, 1984); impaired protocorm growth and development in *Cymbidium* (Fonnesbech, 1972) and *Thunia alba* (Sood, 1984) and seed callusing in *Vanda* Miss Joaquim (Goh, 1971) and *Cymbidium aloifolium* (Bopaiah and Jorapur, 1986). Presence of NAA advanced rhizogenesis and seedling formation while some seeds showed callusing in AC enriched medium. According to Sood (1984), NAA was useful at 0.5-2.5 mgl^{-1} for *Pachystoma senile* germination whereas its higher doses proved lethal. The growth of root and shoot was promoted at lower concentration and inhibited at higher concentration of NAA in *Cymbidium* (Fonnesbech, 1972), *Cattleya* and *Dendrobium* (Vajrabhaya and Vajrabhaya, 1976) cultures. Earlier, Straus and Reisinger (1976) also recommended the use of NAA at low concentration for better results. GA$_3$ accounted for impaired germination in *Cattleya* (Hirsh, 1959), *Bletilla*, *Brassolaeliocattleya* and *Dendrobium* (Kano, 1965), chlorophyll development and protocorm differentiation in *Cattleya*, *Cymbidium*, *Cypripedium* and *Odontoglossum* (Humphreys, 1958). It, however, proved useful for the development of buds and protocorms (Kusumoto, 1978), enhanced growth of leaf and shoot in terrestrial European species (Harbeck, 1963) and *Cymbidium* (Bose and Mukherjee, 1976). Small sized chlorophyllous spherules were obtained in KN supplemented nutrient medium. KN favoured germination in *Cypripedium calceolus* reginae (Harvais, 1982) and *Coelogyne punctulata* (Sharma and Tandon, 1986); protocorm multiplication in *Eria fragrans* (Vij and Mahant, 1988); protocorm growth in *Platanthera bifolia* (Hadley, 1970) and seedling growth in *Orchis purpurella* (Hadley and Harvais, 1968). Incorporation of IAA and KN with and without AC showed similar results as protocorms were formed in 7.75±0.95 wks and differentiation of first root and first leaf primordia took place in 11.50±1.29 and 16.25±1.70 wks respectively.

Complete seedlings were obtained in 21.75±0.95 wks. Presence of IBA and KN together with and without AC enriched nutrient pool induced multiple protocorm formation (Fig. 4) though some seeds callused.

Presence of peptone in the nutrient medium though proved ineffective during onset of germination, protocorm formation, differentiation, advanced seedling formation. Organic supplements like CH with and without AC proved beneficial in advancing leaf differentiation while incorporation of YE not only proved beneficial in healthy leaf formation (Fig. 7),but also advanced rhizogenesis and seedling formation. Perusal of literature reveals that incorporation of P in the medium favoured germination, protocorm multiplication, and supported better seedling growth in *Cattleya*, *Dendrobium* and *Vanda* (Morel, 1974); *Cymbidium macrorhizon* (Vij and Pathak, 1988); *Dactylorrhiza maculata* (Van waes and Debergh, 1986); *Goodyera biflora* (Pathak *et al.*, 1992); and *Rhynchostylis* (Vij *et al.*, 1997). Benign effect of CH was demonstrated during germination and seedling growth in *Dactylorrhiza purpurella* (Harvais, 1972); *Aerides multiflora*, *Rhynchostylis retusa*, *Saccolabium calceolare*, and *Vanda testacea* (Vij *et al.*, 1981); and *Eria spicata*, *Pholidota articulata*, and *Satyrium nepalense* (Pathak, 1989). Combination containing CH promoted protocorm formation, their growth, and subsequent differentiation in *Eulophia dabia* (Sharma and Vij, 1986); it however, detrimentally affected the subsequent seedling growth in *Spathoglottis plicata* (Cheenaveeraiah and Patil, 1975). Presently AC in the nutrient medium either proved beneficial in advancing the morphogenetic event or inducing differentiation in the cultures in some selected nutritional combinations. It has been reported earlier to promote germination, protocorm multiplication and healthy growth of seedlings in a large number of orchid species (Pathak *et al.*, 2001; Vij and Pathak, 1988; Werckmeister, 1970). Seedlings were hardened and potted in community pots (6 cm diameter), filled with a potting mixture of charcoal, pine bark, and brick pieces (1:1:1) with topping of *Sphagnum* moss, and these were hardened for 6-8 wks in the green house (Fig. 9).

Based on the present observations, the optimal nutritional combinations for early onset of seed germination (M), protocorm multiplication (M+IBA+KN), early differentiation and seedling formation (M+YE) are suggested. The present paper successfully reports the development of an efficient protocol for mass propagation of *Dendrobium amoenum* through *in vitro* asymbiotic seed culture.

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ASSESSMENT OF QUALITY AND HYDRO CHEMICAL CHARACTERIZATION IN GROUNDWATER OF MOGA DISTRICT, PUNJAB, INDIA

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Abstract

Quality of water refers to number of physical, chemical and biological characteristics. Groundwater samples from Moga district, southwest Punjab were measured for physicochemical parameters as well as major ion chemistry to evaluate the groundwater suitability for drinking and irrigation purposes and to present the current hydrochemical status of groundwater of this district. Temporal variations were analyzed by comparing the 60 groundwater samples during pre- and post-monsoon using various methods as per APHA (2005). It was found that the major physico-chemical parameters of groundwater were within the permissible limits. But certain parameters such as total dissolved solids, total Hardness, electrical conductivity, sulfate, fluoride, nitrate and magnesium content were above the WHO permissible limits at some places in Moga District. Though the present assessment of groundwater quality in the study area indicates that many samples exceed the desirable limit as per BIS (2012) and WHO (2011). Many samples which have exceeded limit are from Baghapurana Block of the Moga District. Detailed analysis of groundwater was done with respect to irrigational suitability as per USSL and Wilcox diagram was used to understand the combined effect of EC and Na%. This study is significant as the groundwater resources are limited and the quality and quantity of groundwater are deteriorating with time due to anthropogenic inputs.

Keywords: - Groundwater, Moga, Monsoon, Water quality, Fertilizer.

INTRODUCTION

Water plays important role in several metabolic, physiological and other activities in human body as well as in other living beings (Mittal and Arora, 2014).Groundwater is well thought-out to be the most vital natural and fresh resource on earth which is used for drinking and irrigation purposes. Groundwater is about 20% of the world resource of fresh water and widely used by industries, irrigation and for domestic purposes (Usha et al., 2011). Southwest Punjab is facing problems like water-logging, salt water encroachment, groundwater pollution and salinity, which are the consequences of intensive irrigation and agricultural practices (Kochhar et al., 2007; Sharma et al., 2016a, 2016b, 2016c). Water shortage has become an increasingly serious problem, especially in the arid and semi-arid regions of India, where the average annual precipitation is less than 500 mm (Keesari et al. 2014). Groundwater contamination is generally irreversible i.e. once it is contaminated; it is difficult to restore the original water quality of the aquifer (Singh et al., 2013). Groundwater quality studies become unavoidable since its poor quality may badly affect its users (Prasanna et al., 2010). The quality of groundwater depends upon overall proportional amount of different chemical constituents present in groundwater (Ghosh et al., 2011). Chopra and Krishan 2014 and Singh et al. (2011) have reported that about 47 % of the groundwater is unfit for drinking and agricultural purposes due to high salinity. According to World Health Organization safe drinking water is the basic necessity for the development, health and wellbeing of mankind (WHO, 2003). Today Groundwater has become much vulnerable to contamination due to population explosion and industrialization. Kakar and Bhatnagar (1981) reported the percolation of industrial effluents in the soil contaminated the groundwater in Ludhiana. Groundwater can be contaminated through varieties of chemical substances released at or near the soil surface from point and non point sources (Gautam et al, 2013). Some substances found naturally in rocks or soils such as iron, manganese, arsenic, chlorides, fluorides, sulfates or radio nuclides can become dissolved in groundwater (Chilton, 1996).

The state of Punjab is one such state where groundwater level as well as quality is deteriorating very

fast and only 25 blocks out of 141 falls in the safe category, but most of the blocks in this category too have saline water. About 94% of the total sown area in Punjab is irrigated, out of which 61.6% is irrigated by tube wells and 38.4% by canals. Various activities like intense agriculture, power generation, chemical and other industries are potential sources of groundwater contamination. The type and extent of chemical contamination of the groundwater largely depend on the anthropogenic activities mainly by acid rains, fertilizers, industrial waste, garbage and domestic waste (Kaushik and Kaushik, 2006). The most important type of groundwater contamination in Moga District is due to excessive use of fertilizers and pesticides which causes contamination in groundwater. The wide range of contamination sources is one of many factors contributing to the complexity of groundwater quality and its assessment (Agarwal, 2005). The main objectives of the study are to determine the different physico-chemical parameters of water samples collected from different sites of the study area and to assess the groundwater quality and hydro chemical characterization in groundwater in Moga District.

STUDY AREA DESCRIPTION

The state of Punjab is located in the northwestern part of India. Punjab having geographical area of about 50,362 Sq. Km is predominantly an agricultural state where 80% of population is engaged in agriculture and has highest % age of irrigated land compared to total cultivable land in India. Excessive extraction of groundwater has resulted in decline of water table in more than 80% of the State area having fresh/fit quality of groundwater in North-Western, Central and South-Eastern part of the State which has been reported in many studies (Krishan *et. al.,* 2013). Moga district is located at 30°8'N to 75°17'E (Fig.1). It has an average elevation of 217 meters (711ft). The study area is occupied by Indo-Gangetic alluvial plain belonging to Quaternary age (GSI 1976). The area as a whole is almost flat with a gentle slope towards the Western and Northwesterly direction. Tube wells are more important in comparison to canals as far as percentage of area irrigated by these two methods is taken into consideration. There were 65,558 shallow and deep tube wells owned by farmers (CGWB 2007). The district is traversed by network of canal. The

main canals in the area which feed the various distributaries, minors, sub-minors & field channels (water courses) are Sirhind feeder and Sidhwan Branch and Abohar canal which partly irrigate southern part of the district and leaves Moga to irrigate parts of Bathinda, Muktsar and Ferozepur districts.

SAMPLING AND MEASUREMENT

A total of 60 water samples were collected from canals, existing hand pumps, bore wells, tube wells tapping different depths from 100-450 feet and covering different parts of the district during both pre-monsoon (30) and post-monsoon (30) periods. The location map of the groundwater sampling sites in the study area is shown in Fig.1. Samples were collected from the five blocks in the Moga district. The blocks include Nihal Singh wala, Baghapurana, Moga 1, Moga 2 and Dharmkot. Groundwater samples were collected by running the tubewells for about 10-15 minutes to ensure that standing water in the pipes have been taken out and normal running water has started coming out. The water samples were collected in 2 litre PET (poly ethylene terephthalate) bottles after thoroughly rinsing with the same water. Temperature of the water was noted with the help of an ordinary thermometer at the site itself. The samples were filtered and stored in polyethylene bottles that were initially washed with nitric acid and rinsed thoroughly with distilled water. Another set was collected and acidified to pH = 2 by adding ultrapure nitric acid for cation measurements. Physical parameters like pH, conductivity and temperature were measured in situ using field kit, whereas chemical analysis was carried out in the laboratory. Analysis were done for the major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, F^-, Cl^-, HCO_3^-, CO_3^{2-}) using APHA (2005) method. Ca^{2+}, Mg^{2+}, HCO_3^-, CO_3^{2-} were analyzed by titration method, Na^+ and K^+ by flame photometer and NO_3^- and SO_4^{2-} by U.V. Spectrophotometer and F^- was estimated by SPADNS method(spectrophotometer).The basic purpose of multivariate analysis was to generate groups of correlated elements from the initial data set. The statistical software Minitab17, MapInfo 6.5 and Rockworks 15 were employed for the calculations and data presentations.
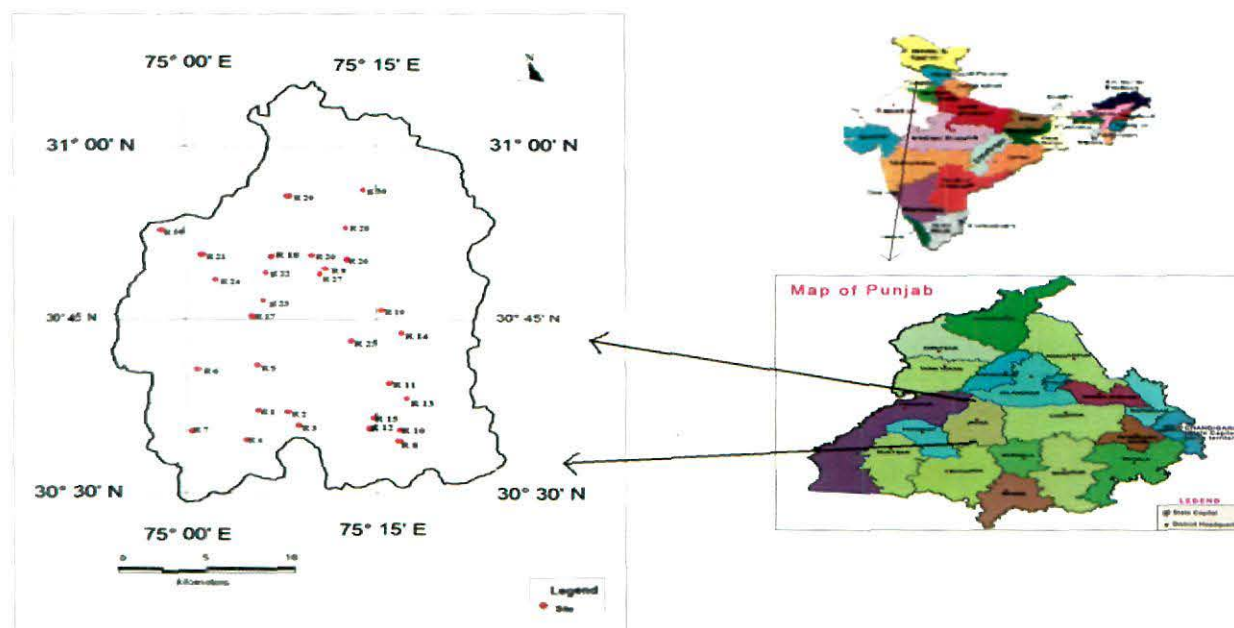
Figure 1: Location of the Moga district and the sample locations

RESULTS AND DISCUSSIONS

The hydrochemical data of pre- and post-monsoon seasons are summarized in Table 1. Fig. 2 shows graph showing physico-chemical parameters of groundwater samples during pre-monsoon and post-monsoon period. The accuracy of the chemical ion data was calculated using charge balance equation given below and the charge balance error (CBE) of most of the samples was within the accepted limits of ±5 % (Hounslow 1995). The brief details of quality parameters are as under:-

$$CBE \% = \frac{meq\ (cations) - meq(anions)}{meq\ (cations) + meq\ (anions)} \times 100$$

Table 1 Statistics of groundwater chemistry of both seasons.

S.	Parameter	Pre-monsoon				Post-Monsoon			
		Range		Mean	Std. dev.	Range		Mean	Std. dev.
		Min.	Max.			Min.	Max.		
1.	pH	7.1	8.8	7.87	0.35	7	8.9	7.69	0.39
2.	EC (µS/cm)	623	2650	1400.46	518.63	460	1934	1024.7	373.80
3.	TDS(mg/l)	404	1451	879.3	300.09	295	1231	668.5	244.23
4.	TH (mg/L)	84	340	214.26	69.75	96	750	230.26	139.87
5.	Ca^{2+} (mg/L)	15.9	149	70.33	34.211	33.64	87.84	56.309	14.505
6.	Mg^{2+} (mg/L)	4.5	92	44.64	23.56	10.24	58.56	31.88	12.21
7.	Na^+ (mg/L)	11.34	89.1	50.84	22.60	8.8	97.5	49.58	25.48
8.	K^+ (mg/L)	1.7	63.1	13.69	13.25	2.7	42.3	22.90	11.33
9.	Cl^- (mg/L)	9.94	350.55	91.37	88.28	4.97	139.16	53.58	30.89
10.	HCO_3^- (mg/L)	60	390	194.87	73.92	20	330	189.33	78.56
11.	NO_3^- (mg/L)	0	143.95	45.38	42.65	8.88	74	43.53	13.82
12.	SO_4^{2-} (mg/L)	23.98	299	163.33	68.49	26.66	240.7	104.21	56.506
13.	PO_4^- (mg/L)	0.02	0.30	0.11	0.074	0.02	0.905	0.157	0.204
14.	F^- (mg/L)	0	6.1	1.26	1.37	0	0.331	0.16	0.091

EC electrical conductivity, TDS total dissolved salts, TH total hardness, all the values are in mg/L except pH, EC (µS/cm).   Min. minimum, Max. maximum, Std. Dev. standard deviation.

Geochemical interpretation of the hydro chemical data inferred that Na^+, Mg^{2+} are major cations followed by Ca^{2+}, K^+ while in the case of anions $SO_4^{2-} \geq NO_3^- \geq F^- \geq PO_4^-$. The pH values of the groundwater samples in Moga area ranges from 7.1 to 8.9 with a mean value 7.87 indicating slightly alkaline in nature but suitable for drinking and industrial purposes (Table 2). The fluctuations in electrical conductivity correlated positively with the total dissolved solids which are the common indicators of polluted waters (Jayalakshmi, 2011). EC values varied between 623 µS/cm to 2650 µS/cm. Highest value of EC µS/cm has been recorded in the sample taken from Ajit Singhwala from Nihal Singh wala Block i.e. 2650 µS/cm. High EC in water samples could be due to leaching or dissolution of the aquifer material or mixing of saline sources or a combination of these processes (Hem 1991; Hounslow 1995).The total dissolved solids measures the total concentration of all mineral constituents dissolved in water and is related to the problem such as excessive hardness. The TDS of the samples collected varied from 404 to 1451 mg/L with an average value of 879.3 mg/L. 86.66% samples exceed the desirable limit of 500 mg/l indicating presence of slightly concentration of salts and related to the problem such as hardness and are conditionally unfit for human consumption. Total hardness (TH) is caused by various dissolved salts of Ca^{2+} and Mg^{2+} and is often a critical parameter used for assessing drinking water suitability. Consumption of water with high TH may raise the risk of calcification of arteries, urinary concretions, diseases of kidney or bladder or stomach disorder. TH was determined by the following equation (Todd 1980) and expressed as mg/L of $CaCO_3$:

$$TH = 2.497 \, {}^*Ca^{2+} + 4.115 \, {}^* Mg^{2+}$$

where Mg^{2+}, Ca^{2+} and TH concentrations are expressed in mg/L. The TH levels in groundwater of this district were in the range of 84-750 mg/L. Water classification based on TH (Durfor and Becker 1964) is given in Table 3. Majority of the samples fall in very hard category for both the seasons. Chloride in drinking water is not generally harmful to human being until present in higher in higher concentration. It is the most abundant anion in the human body and contributes significantly along with its associated cations to the osmotic activity of the extra cellular fluid (DNHW, 1975). The chloride ion in the groundwater of the study area varied between 9.94 mg/l and 350.55 mg/l with mean value 91.37 mg/l. Most of the water samples are within the desirable limit of BIS. Thus, ground water in the area is fit for drinking purposes as far as chloride content is concerned. The amount of calcium in the groundwater of the study area ranged from 33.64 mg/l to 149 mg/l. Highest value of calcium is recorded in the sample taken from village Ransih Khurd. The concentration of magnesium varied between 4.5 mg/l to 58.56 mg/l with mean value 31.88 mg/l. Majority of water samples exceed the permissible limit as per BIS (2012) and causes unpleasant taste to water and are laxative and its salts acts as catharties and diuretics (Montgomery, 1985). Thus, the water from these locations is unfit for domestic purposes.

The application of excessive nitrogenous fertilizer to the agricultural fields cause leaching from the root zone by water percolating through the soil profile and ultimately accumulates in the groundwater. Excessive concentrations of nitrate in drinking water cause methemoglobinemia in small baby. Nitrate values of groundwater in the study area varies from 0 mg/l to 143.95 mg/l which is above the desirable limit. Thus, the water is unfit for consumption. BIS (2012) & WHO (2011) have not given any guideline limit for sodium and potassium in drinking water. Sodium concentration ranged between 11.34 mg/l to 97.5 mg/l with mean value 49.58 mg/l. The common source of Na^+ in this region is application of fertilizers. Potassium concentration ranged between 1.7 mg/l to 63.1 mg/l with mean value 22.90 mg/l. The principle natural sources for Sulphate include rock weathering, input from volcanoes and input from biological or biochemical process and anthropogenic activities like runoff, rainfall or dry fallout. The concentration of Sulphate varies from 23.98 mg/l to 299 mg/l with mean value 160 mg/l. All the water samples are within the acceptable limit which depicts that water is good for drinking and domestic purposes. Phosphorus additions to water bodies even in small amount can produce accelerated growth of algae and aquatic vegetation, thereby causing eutrophication of the aqueous system (Handa, 1990) the application of phosphatic fertilizers has increased the abundance of phosphate in the groundwater. The concentration of phosphate content in the groundwater sample varies from 0.02 mg/l to 0.905 mg/l with mean value 0.157 mg/l. The concentration of phosphate in the groundwater is very low which depicts fit for domestic consumption. Fluoride concentration in groundwater samples collected ranged from 0 to 6.1 mg/l. Higher values of fluoride more than permissible limit, have adverse impact on health of the population as it leads to decaying of bones and teeth. Highest value of Fluoride is recorded in the sample taken from village Rode i.e. (6.1) mg/l.
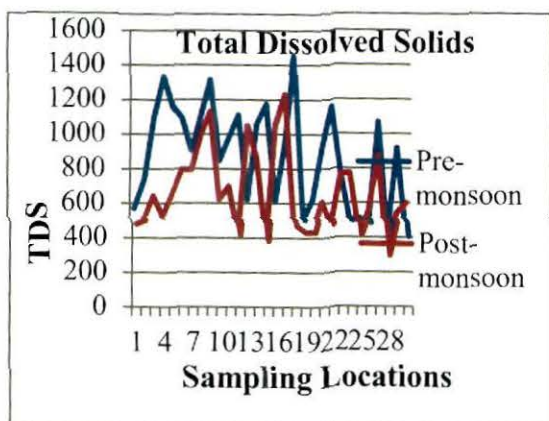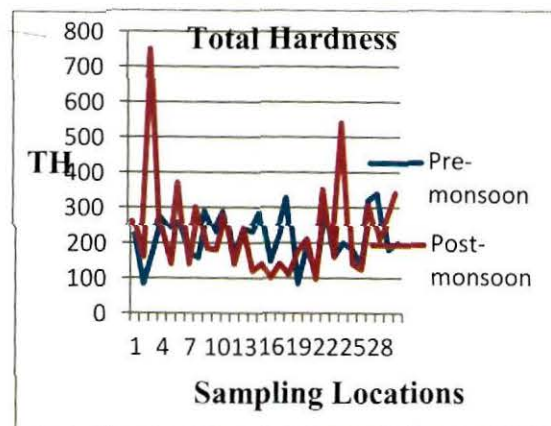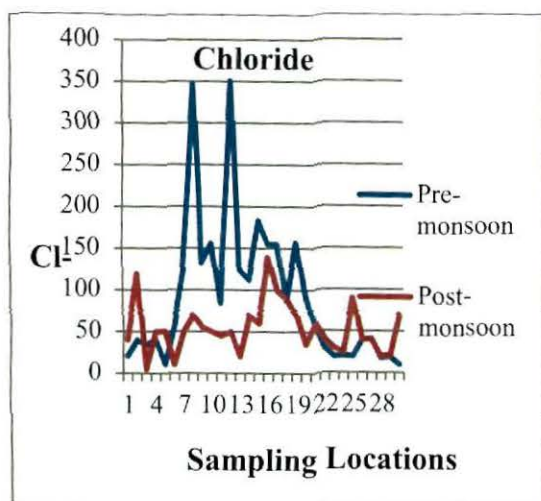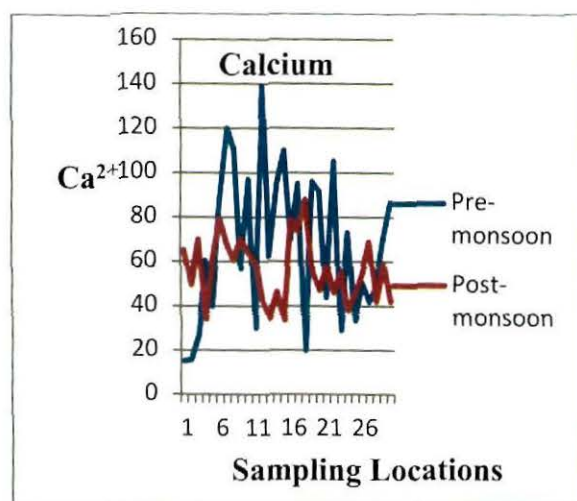
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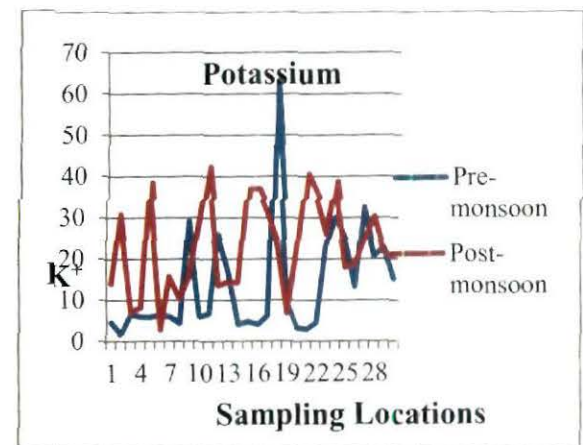

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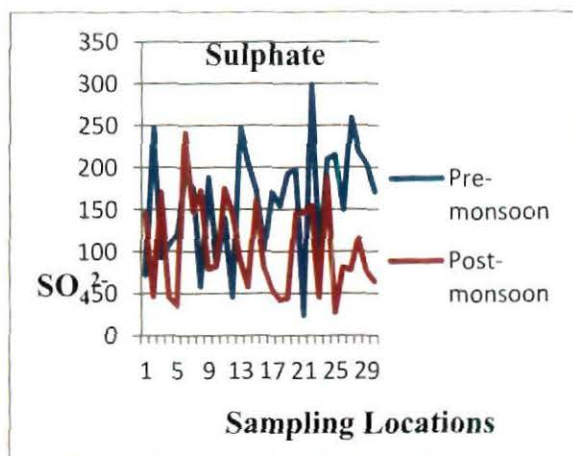
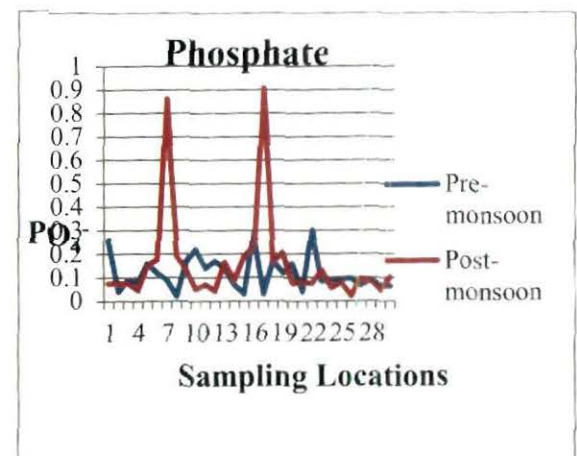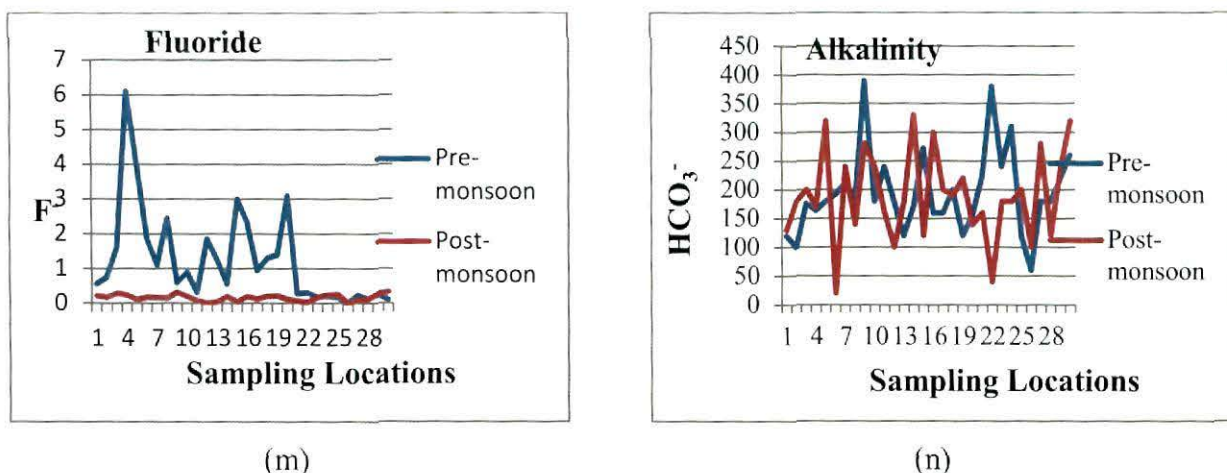(m)   (n)
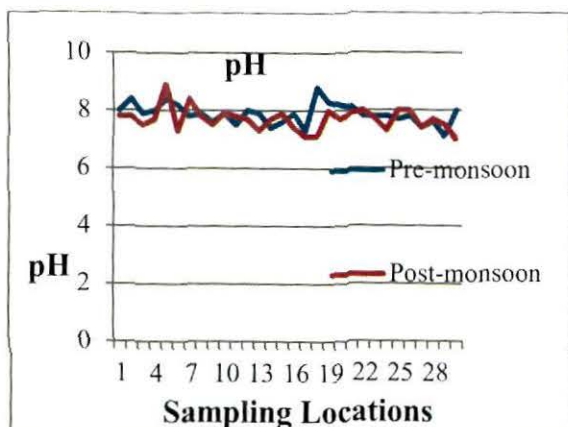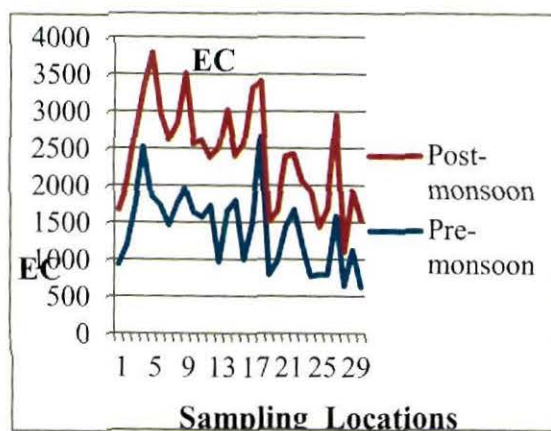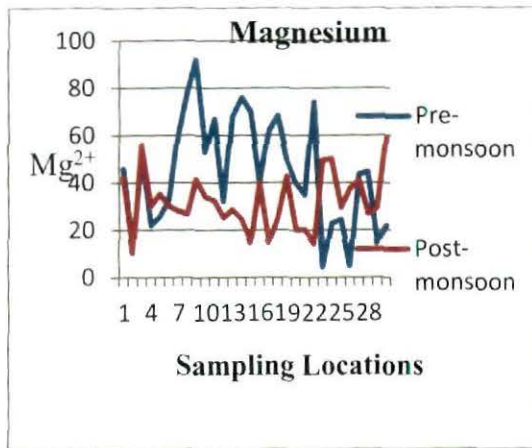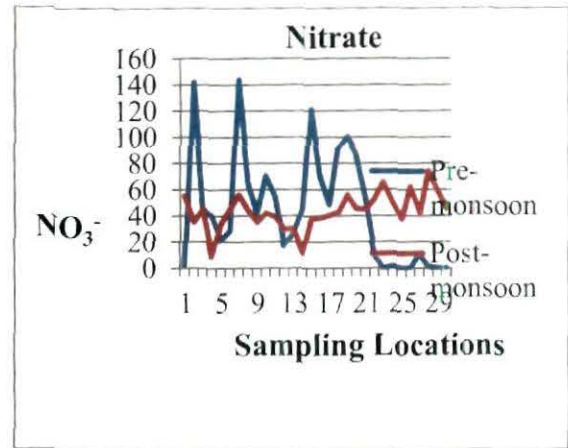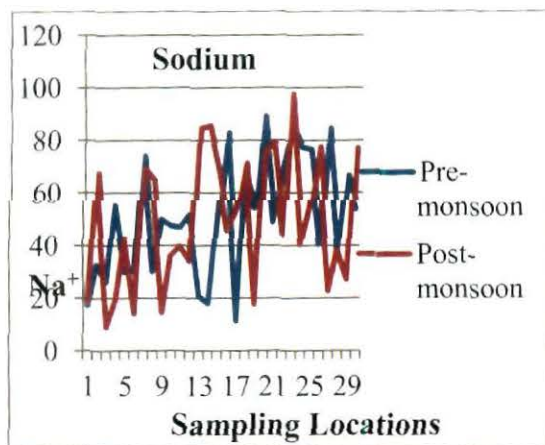
Figure 2: Graph showing physico-chemical parameters of groundwater samples during pre-monsoon and post-monsoon period, Moga District, (a) pH, (b) EC, (c) TDS, (d) TH, (e) Cl^-, (f) Ca^{2+}, (g) Mg^{2+}, (h) NO_3^-, (i) Na^+, (j) K^+, (k) SO_4^{2-}, (l) PO_4^-, (m) F^-, and (n) HCO_3^-.

Table 2 Percentage of water samples exceeding limits for drinking purpose based on BIS (2012)   and WHO (2011)

Parameter	BIS (2012)		WHO (2011)	% Samples exceeding BIS (2012)		% Samples exceeding WHO (2011)	
	DL	PL	Guideline value	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
pH	6.5–8.5	No relaxation	6.5–8.5	3.33	3.33	3.33	3.33
Total hardness (as CaCO3) (mg/L)	200	600	500	56.66	46.66	-	6.66
Total dissolved solids (TDS) mg/L	500	2000	1000	86.66	70	40	13.33
Calcium (Ca^{2+}) (mg/L)	75	200	300	-	3.33	-	-
Magnesium (Mg^{2+}) (mg/L)	30	100	100	63.33	36.66	-	6.66
Chloride (Cl) (mg/L)	250	1000	250	6.66	-	6.66	-
Sulfate ($SO4^{2-}$) (mg/L)	200	400	250	23.33	-	10	-
Nitrate ($NO3^-$) (mg/L)	45	No relaxation	50	46.66	-	43.33	-
Fluoride (F^-) (mg/L)	1.0	1.5	1.5	43.33	-	30	-
Alkalinity (as CaCO3) (mg/L)	200	600	–	-	-	-	-

DL desirable limit, MPL maximum permissible limit

Table 3 Suitability of groundwater for drinking based on TDS and TH

Water class		% of samples in pre-monsoon	% of samples in post -monsoon
TDS (mg/L)			
≤500	Desirable for drinking	–	30
500–1000	Permissible for drinking	33.34	56.67
1000–3000	Useful for irrigation	66.66	13.33
≥3000	Unfit for drinking and irrigation	–	
TH (mg CaCO₃/L)			
≤ 60	Soft	–	–
60–120	Moderately hard	10	13.33
121–181	Hard	23.33	33.34
≥180	Very hard	66.67	53.33

Table 4 Suitability of groundwater for irrigation

| Water class		% of samples in pre-monsoon	% of samples in post-monsoon
EC (µS/cm)			
≤250	Excellent	-	-
250–750	Good	6.66	26.67
750–2000	Permissible	86.67	73.33
2000–3000	Doubtful	6.67	-
≥3000	Unsuitable	-	-
Alkalinity hazard (SAR)			
≤10	Excellent	100	100
10–18	Good	-	-
18–26	Doubtful	-	-
≥ 26	Unsuitable	-	-
Percent sodium			
≤20	Excellent	10	16.67
20–40	Good	50	36.67
40–60	Permissible	36.66	36.66
60–80	Doubtful	3.34	10
≥ 80	Unsafe	-	-
Residual sodium carbonate			
≤1.25	Good	96.66	90
1.25–2.5	Doubtful	3.34	3.33
≥2.5	Unsuitable	-	6.67
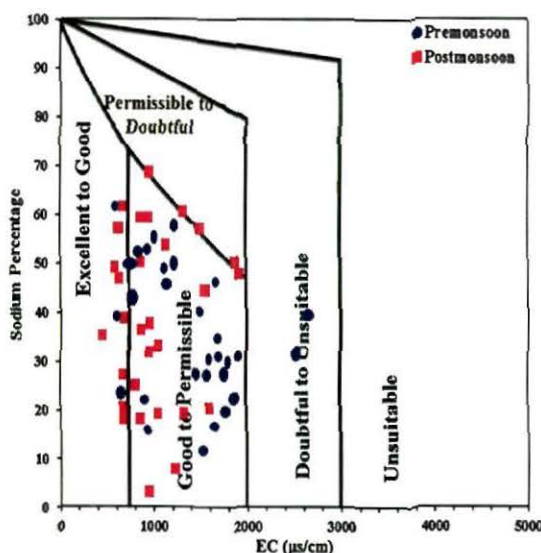
Figure 3: Wilcox diagram of groundwater samples of Moga district

Suitability of groundwater for irrigation has been discussed in Table 4. USSL diagram (SAR versus EC plot) and Wilcox diagram (Na% versus EC plot) were also applied to evaluate the irrigation suitability of groundwater. The suitability of groundwater for irrigation based on EC is classified into four groups (Richards 1954) and the percentages are given in Table 4 and Water samples in pre-monsoon range from Good to Doubtful class and Good to Permissible class during post-monsoon as per EC (μS/cm). SAR (Alkalinity hazard) was found under Excellent class for both pre-monsoon and post-monsoon period. Percent sodium was found to be under Excellent to Doubtful category during both seasons. Residual sodium carbonate was found from Good to Doubtful class during pre-monsoon and post-monsoon period.

United States Salinity Laboratory diagram depicts a detailed analysis of groundwater with respect to irrigational suitability (USSL 1954). As per this classification, lows salinity water ($\leq$ 250 μS/cm) can be used in all types of soils (Fig. 4). Based on USSL classification the groundwater of the study area can be grouped into four zones, C1S1, C2S1, C3S1 and C3S2. Overall samples indicated medium to high salinity and low alkalinity water, which can be used for irrigation in almost all types of soils with little danger of exchangeable sodium. High concentration of Na^+ in irrigation water affects the soil permeability and the texture. This effect is monitored by sodium/alkali hazard, which is expressed as the SAR. This ratio is computed from the relative proportion of Na^+ concentration to Ca^{2+} and Mg^{2+} concentrations in a given water sample. The SAR values for each water sample were calculated using the following equation (Richards 1954):

$$SAR = \frac{Na+}{\dfrac{\sqrt{Ca^{2+} + Mg^{2+}}}{2}}$$

where the concentrations are reported in equivalents per million. SAR values varied from 0 to 4, and majority of the samples (89%) fall in low alkali hazard class (Table 4). Sodium combines with inorganic carbon (HCO_3^- and CO_3^{2-}) to form alkaline soils and combines with Cl^- to form saline soils. Both these soils are not favorable for plant growth. This effect is commonly indicated by Na% (Wilcox 1948) and expressed in epm (milliequivalents per liter). It is calculated using following equation:-

$$Na\% = \frac{(Na^+ + K^+) * 100}{Ca^{2+} + Mg^{2+} + Na^+ + K^+}$$

High Na% in water coupled with high EC decreases the osmotic activity of plants and thus, limits the absorption of water and nutrients from the soil. Wilcox diagram is commonly used to understand the combined effect of EC and Na% (Wilcox 1955). During pre-monsoon, 10% of the groundwater samples (Na%) falls under Excellent category, 50% falls under good class, 36.66% falls under Permissible class and 3.34% comes under the Doubtful type. During Post-monsoon 16.67% samples fall under Excellent category, 36.67% falls under good category, 36.66% falls under Permissible category and 10% falls under Doubtful Category. Most of the groundwater samples fall in excellent to good and good to permissible categories during both seasons while 6% samples fall in doubtful to unsuitable category during Pre-monsoon. The poor groundwater quality during pre-monsoon can be explained by the presence of high salt content containing mainly Na^+, HCO_3^-, SO_4^{2-} and Cl^- ions.
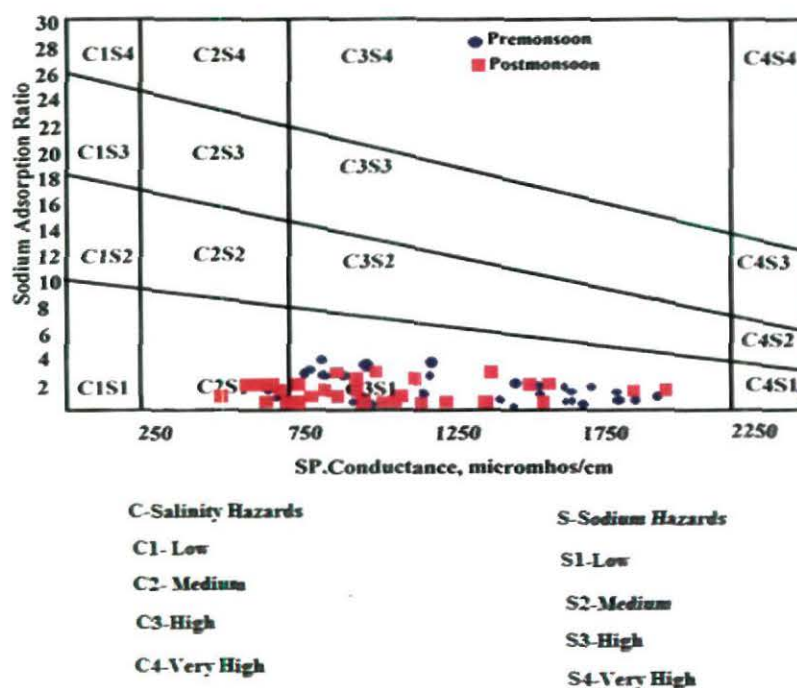
Figure 4: USSL diagram of groundwater samples of Moga district

CONCLUSION

The groundwater samples collected from Moga District were appraised for their chemical composition and suitability for drinking purpose. The effect of monsoon on local groundwater system was studied by comparing the chemistry of pre-monsoon and post-monsoon samples. Results indicate that among different hydro chemical parameters (pH, Total hardness, TDS, Mg^{2+}, Cl^-, SO_4^{2-}, NO_3^-, F^-) were found to be above drinking water permissible limits in most of the samples, whereas contamination by other parameters was marginal. Leaching of salts in the unsaturated zone and contribution of soluble fertilizers play a significant role in contaminating the aquifers of Moga district. Therefore groundwater quality is not suitable for drinking purposes and there is need of some treatment techniques to remove these contaminations from groundwater in study area. Economic rewards and penalties are required for management of groundwater as these can potentially help provide signal to users about economic/opportunity cost of water. This can be achieved by implementing price based and quantity based instruments. To ensure the long term sustainability of the water resources of the area care should be taken to avoid further contamination and degradation of aquifer system and overexploitation of groundwater from deeper aquifer. The government must come out with a legislation to rationalize the use of chemicals in farming operations. The best suitable remedial method recommended by (USEPA, 2003) is the use of Anion Exchange and Reverse Osmosis to treat contaminated water. It is recommended to prefer canal water based drinking supply schemes in southwest Punjab.

ACKNOWLEDGEMENTS

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Buskrik, E.R., K.L. Anderson, and J. Brozek. 1956. Unilateral activity and bone and muscle development in the forearm. Res. Quart., 27 : 127-131.
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