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तत् त्वं पूषन् अपावृणु सत्यधर्माय दृष्टये

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A Letter to the Editor

Scenario of *Drosophila* research in India

Drosophila is a genus of flies of the family Drosophilidae (class: Insecta and order: Diptera). At global level 1579 species have been described[1,2] and estimated to have several thousands[3]. So far 135 species of *Drosophila* have been reported from India which includes both new species and new record from India [4]. Rich species diversity has been reported from Hawaiian Islands where more than 500 species occur and picture-winged species provide unique opportunity to study evolution [5]. In India most extensively investigated species from evolutionary point of view is *Drosophila ananassae,* a cosmopolitan and domestic species endowed with many genetic peculiarities[6].

Drosophila melanogaster was described for the first time by Meigen in 1830 and was used for experimental studies by Carpenter in 1905. Morgan was first to use *D. melanogaster* for genetical studies in 1909 and made important contribution in genetics e.g. theory of linkage and sex-lined inheritance. First spontaneous visible mutation (white eye) was detected in D. *melanogaster* by Morgan. Since then it has been extensively used at global level for studies of genetics, development, population biology, evolution and behaviour. In recent years, it is also being used for studies in the area of molecular biology. Due to the development of molecular techniques, DNA sequencing of genome of *D. melanogaster* and few other species has also been done. Thus it has proved to be one of the premier organisms for studies in biology and serves as model organism for such studies. *Drosophila* has certain advantages because of which it is used as a model organism for laboratory studies: cosmopolitan distribution, short generation time, easy handling, small size, easy rearing, high fertility, clear morphology, small number of chromosomes and presence of polytene chromosomes. Apart from these advantages, it is neither a vector nor a pest.

Although *D. melanogaster* is most extensively studied species, a number of other species have also been used for different kinds of studies: *D. simulans, D. mauritiana, D. ananassae, D. bipectinata, D. malerkotliana, D. pseudoobscura, D. persimilis, D. robusta, D. subobscura, D. nasuta* etc. Earlier studies employing *Drosophila,* were mainly concerned with linkage and crossing-over, gene mapping, mutations, sex-determination, chromosome maps, polytene chromosomes, chromosome replication, dosage compensation, puffing activity, chromosomal aberrations, population genetics, evolution, role of natural selection and genetic drift, behaviour, taxonomy, phylogeny, hybridization, sexual isolation, ecology etc. and in recent years *Drosophila* has also been found useful for studies in the area of molecular biology.

As far as my knowledge goes, *Drosophila* research in India began in 1920s at Kolkata with particular reference to taxonomy. Bezzi (see Sturtevant[7]) was the first person in this field who reported *D. repleta* from Kolkata . Bruneti [8] for the first time described a new species, *D. prashadi* from Kolkata. As pointed out by Ray-Chaudhuri and Mukherjee [9], the description of this species is not adequate and refers mostly generic characters. Duda [10] reported *D. bipectinata* from Darjeeling (see Prashad and Paika [11]). Sturtevant [12] reported four species of *Drosophila* from Chennai: *D. melanogaster, D. ananassae, D. montium and D. tristipennis.* Ray-Chaudhuri and Mukherjee [9] reported two new species, *D. emulata* and *D. brunettii* from Kolkata. These two

species closely resemble *D. melanogaster* and *D. bipectinata* [4]. Thus all the three new species of *Drosophila* described from India are considered as invalid species [4]. Later on, *Drosophila* taxonomy research was carried out at Punjab University, Banaras Hindu University and Mysore University which resulted in the discovery of a large number of new species as well as new records from India (see Gupta [4]). Research in *Drosophila* genetics in India was initiated by Prof S P Ray-Chaudhuri at Zoology Department, Calcutta University in 1940s after obtaining Ph D under the supervision of Nobel Laureate Prof H .J. Muller from Edinburgh[13]. When Prof Ray-Chaudhuri moved to Zoology Department, Banaras Hindu University in 1960, he continued his research on *Drosophila* with particular reference to population genetics, crossing-over, mutagenesis, cytogenetics and taxonomy. The work on genetics of *D. ananassae* which he initiated at Calcutta University, was continued at Banaras Hindu University. During that period, research on *Drosophila* was also initiated at several institutions in India such as I V R I, Izatnagar, I A R I, New Delhi, Mysore University, T I F R, Mumbai and I I Sc, Bangalore. Presently, there are a large number of researchers employing *Drosophila* in their research working in different institutions of the country and focusing their research in various areas such as evolutionary genetics, behaviour, molecular genetics, ecological genetics, speciation, molecular biology, dosage compensation, biochemical genetics, cytogenetics, cell biology, developmental biology etc. *Drosophila* researchers in India have published a large number of papers and have made significant contributions in their respective areas. *Drosophila* research is being pursued at different universities and institutes in different places in India such as Varanasi, Kanpur, Kolkata, Rohtak, New Delhi, Mysore, Bangalore, Mumbai, Pune, Hyderabad, Jammu, Goa,, Lucknow, Bhopal, Mohali, Noida, Mangalore, Jhansi, Agra etc.

Michan , Sortibran, Rodriguez-Arnaiz and Ayala [14] published a bibliometric analysis of global *Drosophila* research from 1900 to 2008 in *Drosophila* Information Service (USA). Based on the data obtained from Pub Med and Science Citation Index, they investigated the scientific productivity of *Drosophila* research among researchers, countries, institutions, journals, and subject areas. A total of 36,486 documents were obtained from Pub Med and from Science Citation Index 48,981 documents were obtained. Their study included 4,600 institutions and 45,415 researcher names. Most prolific are 34 researchers with more that 100 research papers published by each researcher. In the list of most prolific *Drosophila* researchers at the global level, there is only one name (B. N. Singh) from India at 24th position. The ten countries with the highest number of publications are: USA, France, England, Japan, Germany, Canada, Spain, Switzerland, USSR and Australia. Maximum publications are from USA (21,508) but India does not come in this list. There are 501 different institutions such as universities, institutes, departments and corporations. The most productive institutions are the Russian Academy of Sciences, the C N R S, France, and Harvard University, USA. No institution from India comes in the list of 50 institutions shown by Michan et al. [14]. Presently, about 75 researchers in India are using *Drosophila* (as per list prepared by Prof S C Lakhotia of Banaras Hindu University, personal communication). *Drosophila* is a very good model organism for studies in biology and more researchers should use this dipteran insect endowed with many advantages which will enhance the scientific productivity of *Drosophila* research in India because the scenario of *Drosophila* research in India encompassing about eight decades shows that we are lagging

behind from many countries of the world.

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Section A : Earth Sciences

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Geometry of mesoscopic folds in the vicinity of Disang and Piphima thrusts in Kohima district, Nagaland

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Abstract

The schuppen belt of Nagaland and Kohima synclinorium are multiply deformed regions in the NE India. The study of structures in the vicinity of Disang Thrust and Piphima Thrusts of the Schuppen belt reveals at least four phases of tectonic deformations. Based in the geometric analyses in the folded rock layers, the present study reveals that the majority of the folds of the study area are developed due to flexure slip mechanism and modified due to later deformations.

Introduction

In Northeast India the Assam – Arakan Orogenic province consists of three segments namely the Naga Hills, Chin Hills and Arakan Yoma segments. Two orogenic belts namely, the Himalayan belt to the north and the Assam – Arakan belt to the southeast mark a zone of plate convergence (Acharyya, 1991). A north-south convergence of India and Tibet has resulted in a straight collision while northwest-southeast convergence of the Indo-Sinian plate culminated into an oblique collision (Dewey et al., 1989; Burchfiel, 1993; Uddin and Lundburg, 1998a & b, Naik, 1998). In the Assam-Arakan belt, suturing of plates extended progressively southwestwards like a zipper as the two continental plates converged obliquely with a pole in the Naga Hills region (Biswas and Agarwal, 1990). The Naga Hills, reaching a height of about 3840 meters, is quite narrow (average width of orogen=250 km) and lie on the border of India (Nagaland & Manipur states) and Myanmar. Most of the earlier workers have related the stratigraphic inconsistencies and along-strike changes in the crustal nature with those of the varied and complicated nature of tectonic regimes in the region. Despite the fact that the Nagaland and the adjoining areas in the vicinity of Assam plains hold good potentialities for petroleum and natural gas, the region could not receive due attention of the geological community; may be because of its remoteness and disturbed socio- political conditions. The pioneering works of Evans (1932, 1964) and Mathur & Evans (1964) and some works done by GSI, ONGC, Oil India Limited and the state Directorate of Geology and Mining are the only bases for any type of geological studies in the region. The lithostratigraphy, structure and regional correlations have been attempted by workers like Bhandari et al., (1973), Dasgupta (1977), Banerjee (1979), Sinha and Chatterjee (1982), Rao (1983), Murthy (1983), Gangu and Khar (1985), and Naik et al., (1991), Acharyya (1986, 1991, 2007), Naik et al., (1991) and Nandy (1983, 2000).

Study Area

Geologically, the present area of investigation forms parts of the Schuppen Belt and Kohima Synclinorium. The Schuppen belt with its over thrust masses of varying width (10 – 40 km); extends all along the western margin of Nagaland state for about 200 kilometer trending NE-SW. Evans (1964), described the Schuppen belt as consisting of eight or more over thrust slices of Tertiary sediments that have over ridden each other from east to west.

The area under study falls between latitudes 25°43′35″ N and 25°47′29″ N, and longitudes 93°55′00″ E and 94°02′10″ (Fig.1) and covers nearly 85 sq. kms including areas lying near Kiruphema, Piphima & Pherima villages. It is located at a distance of 20 kms northwest of Kohima -the capital town of Nagaland state. The average height of the area is 1045 meters above mean sea level. As a whole, the Tertiary rocks in the study area present an immature geomorphology (Chakrabarti and Banerjee, 1988).

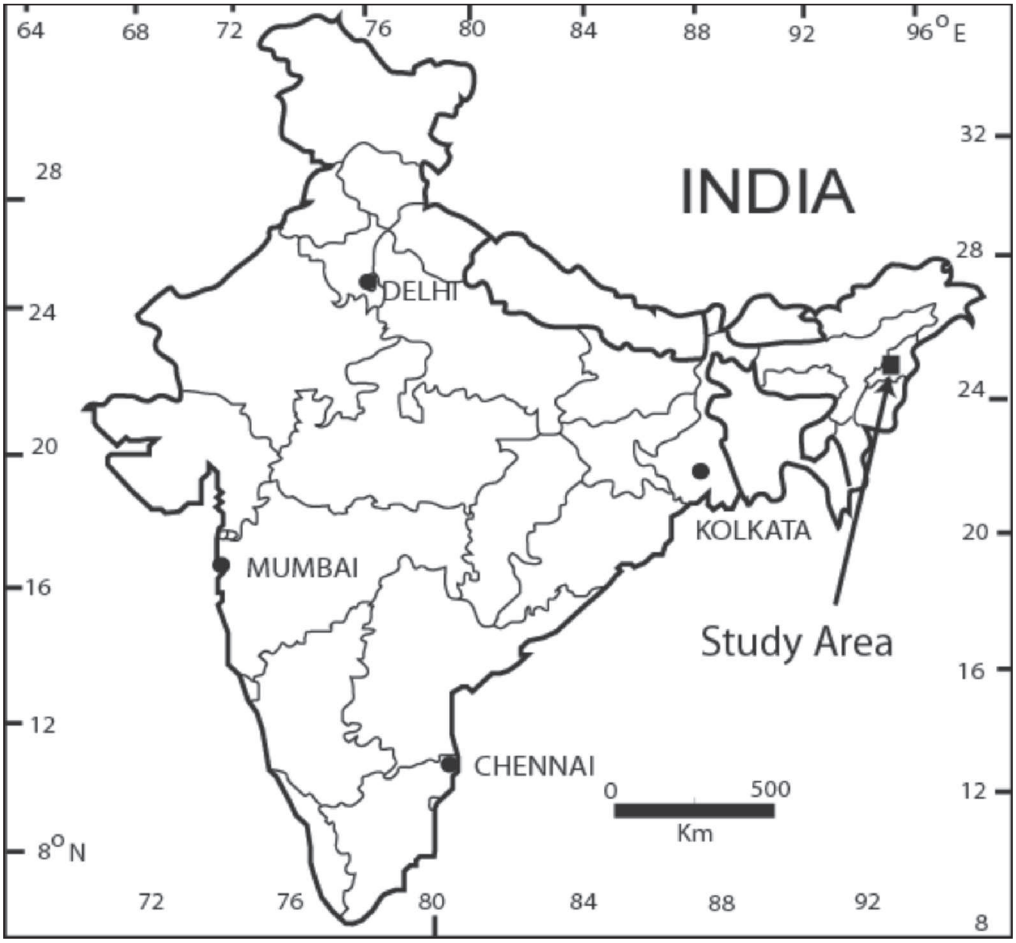

Fig.1 Location of the study area

Geological Setting

The rocks of the present study area belong to Disang Group, Barail Group and the transition sediments in between, named as Disang Barail Transition Sediments (DBTS) (Fig.2). The lithology of these geological formations have been studied in detail by different workers including Evans (1932, 1964); Mathur and Evans (1964); and Rao (1983) and Nakhro (2009). The dominant geological units in the present study area are briefly described below:

Disang – Barail Transitional Sequences (DBTS): The Disang Group occupies a tract between the Naga Ophiolite Complex and the Disang thrust in Nagaland and Manipur. Disang Group comprises dark to black carbonaceous, ferruginous, concretionary shales with a few interbeds of siltstones and sandstones. The lower most contact of this group is not exposed. It passes conformably upwards into the Barail Group in regions other than Schuppen belt. In present area of study, a part of the Disang Group is not exposed in its characteristic forms as described elsewhere, rather is shows the mixed characters of Disang and overlying Barail Group rocks. Therefore in the present work, those rocks in the study area which have mixed characteristics of Disang and Barail sequences have been described as Disang- Barail- Transition Sequences (DBTS) and their characteristics have been described separately. These beds have shown large variation in their orientation which may vary between 08º-74º towards SW & SE because of high degree of deformation.

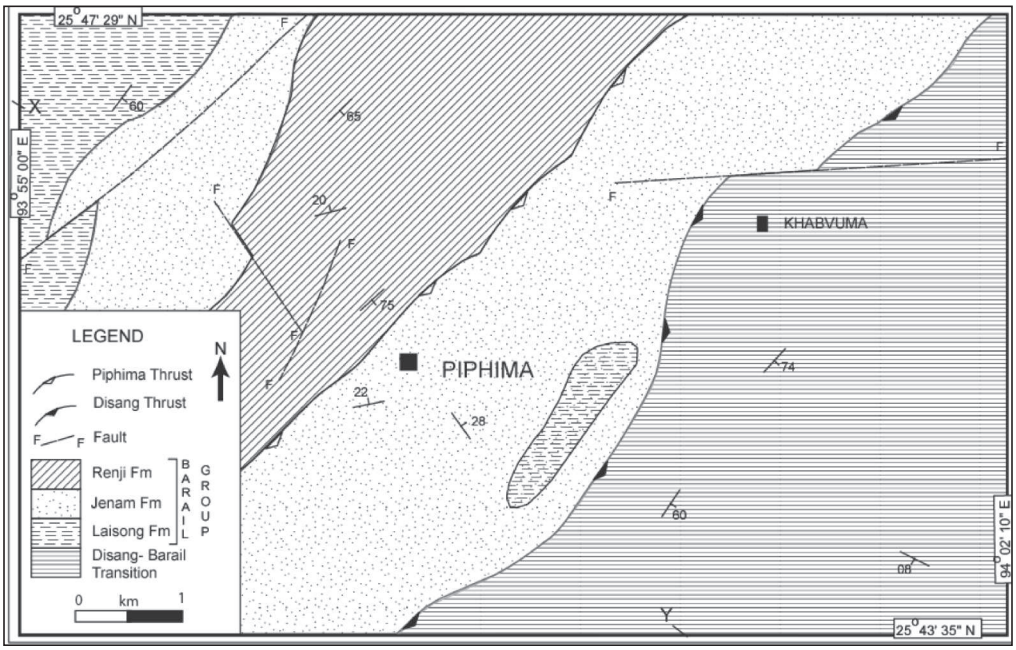

Fig.2 Geological Map of the study area, Kohima district, Nagaland

Barail Group: The Barail Group of rocks possesses a basic character of being arenaceous with the consistent development of carbonaceous facies. These occur as sub-parallel thrust slices in

the western part of the area beginning with the Disang-Haflong Thrust.The part of the study area lying in the Schuppen Belt is characterized by Laisong, Jenam and Renji Formations of the Barail Group. Due to thrusted nature of lower contacts, the thickness of different Barail strips is found to be variable. The general dip of beds varies between 20º - 75º towards NW & SE respectively except for the locations having high degree of deformations. The geological map of the study area (Fig. 2) shows the distribution of various litho-units comprising Disang-Barail Transition and Barail Group. A brief account of the geology is given in Table 1.

Table 1. Geological succession of study area

		Renji Formation
Oligocene----------	Barail Group----------	Jenum Formation
		Lisong Formation
----------Tectonic Contact – Disang Thrust		
Upper Cretaceous to Eocene	Disang- Barail Transition Sediments(DBTS)	
	Disang Group	

Folds

The study area falls in the Schuppen belt of Nagaland which comprises of a number of thrust sheets, however our study is concentrated on the folds found in the vicinity of two thrusts namely Piphima thrust and Disang thrusts (Fig.2). The Disang thrust makes the tectonic contact of Schuppen belt with the Kohima synclinorium, while the Piphima thrust is nearest thrust to this tectonic contact in the schuppen zone, and thus these two together make a thrust slice in the schuppen belt. Mesoscopic structures in one thrust slice may or may not be same as in other slices of the belt. The study area is structurally very complex and exhibits multiple deformations. These deformations are reflected in the rocks of the study area in the form of folds, faults, thrust, joints and other planar and linear features at varying scales. The structures which can be observed on the hand specimen size to size of an outcrop on the hills are known as mesoscopic structures. Folds are important mesoscopic structures which are formed due to ductile deformation in the rocks and thus they record the most intense phases of deformation. Therefore In present work the detailed study and analysis of mesoscopic folds have been taken up.

The mesoscopic folds observed in the area belong to isoclinal (Fig.3C), tight (Fig.3D), close (Fig.3A), open (Fig 3D) and chevron types. Often, these folds do not exhibit their ideal form possibly due to the affect of later deformational episodes i.e. refolding. The chevron folds and kink bands have been developed characteristically in the vicinity of fault zone in the thinly foliated rocks such as shale of DBTS. These folds, which are developed on some prominent S-surfaces (S_1 or S_2), have also shown superposition of one style over another. At times, presence of thin incompetent layers between the competent ones has prompted development of drag folds especially in the vicinity of fault zone. The observations on superposition of folds in the present area lead to the genetic interpretations of fold types whereby isoclinal and tight isoclinal folds can be assigned to the first phase of folding (F_1), the tight and close folds to the second (F_2) and the open folds to the third generation (F_3). The sharp hinged chevron folds or kink bands and drag

folds may be assigned to the fourth generation (F_4) of folding (Nakhro 2009).

Nevertheless, one should bear in mind that all the above folds (F_1, F_2, F_3 & F_4) are liable to change their shape and style as well in their multilayered structure due to successive tectonism of the later phases. The variation in the fold style may also relate to compositional variations among layers. For example, the thicker quartz rich sandstone layers in a F_2 fold may show an open fold style, while the thinly bedded shale shows the close style .

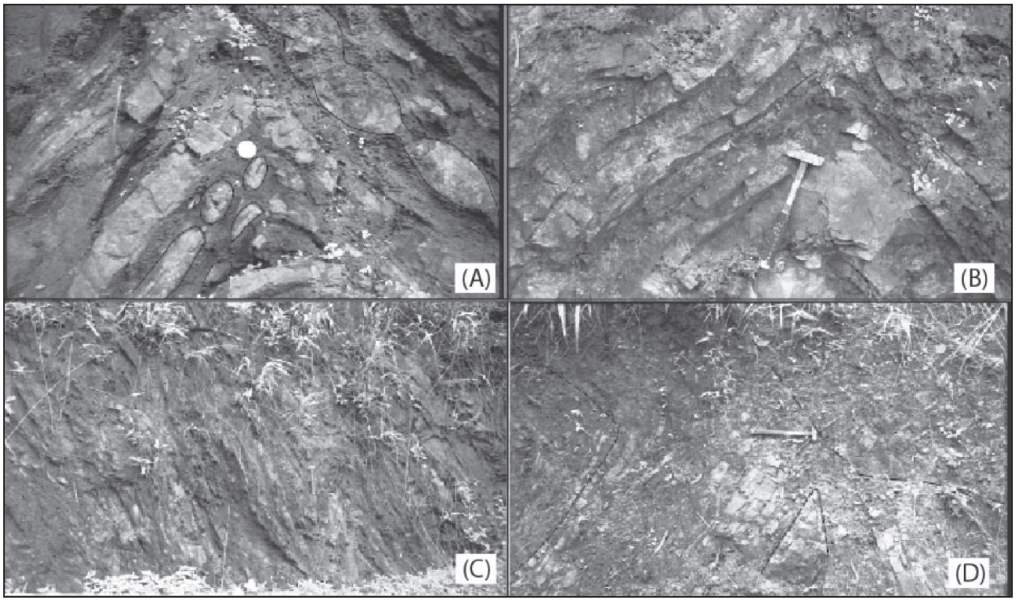

Fig.3 (A) Close folds in the Renji Formation (B) Open fold in Renji Formation (C) Isoclinal fold in thinly bedded Jenam Formation (d) Open recumbent and tight fold in Lisong Formation

Fold Profile Geometry

The development of a geometrical classification of the folded layer is based on dip isogon pattern, variation of the relative orthogonal and axial surface-parallel thicknesses on the profile section (Hudelston, 1973; Ramsay and Huber, 1987). Such a classification plays an important role in the study of fold morphology and in elucidating the principal folding mechanism (Ramsay, 1967). It is also possible to study the changes in the shape of different folded layers on the fold profile. The shape of any one layer in the folded structure depends on the relationship of bounding surfaces of the layer and in particular, the relative rates of change of the inclination of these bounding surfaces. Therefore, study of several profile sections of the fold will not only give a better idea about fold morphology in three dimensions, but also in understanding the possible mechanism involved in its evolution. The geometrical classification of fold morphology is also significant in terms of strain (Hobbs, 1971).

For the geometrical classification of the folds of study area, tracings of the fold profiles have been used. The fold profiles were obtained either directly from the field, field photographs

or hand specimen folds. Care was taken in each case so that the sections (Fig 4) under analysis, as far as possible, were perpendicular to the fold hinge.

Thickness Measurements

The thickness parameters, which involves measurement of data on the orthogonal distance (called as orthogonal thickness (`t') and along the axial surface (`T') between the tangents drawn at equal dip angle (α) on the fold profile, were introduce by Ramsay (1967). He (1967) utilised the ratios t'_α (= t_α/t_o) and T'_α (=T_α/T_o) and the dip angle α for graphically classifying the folds (cf. Ramsay 1967; Ramsay and Huber, 1987).

In order to geometrically classify the folds of the area 3 multilayered folds comprising of four to five layers (Fig.4) have been chosen. The thickness parameters on the profile section of these folds have been measured along the dip isogons as described by Ramsay and Huber (1987). The orthogonal thickness ratios t'_α (= t_α/t_o) were calculated from those data. The t'_α value against change of angle (α) has been plotted for each fold to represent the variation in geometry of each layer of folds with change in α values. Thus, the t'_α vs α plots (Fig.4) which have been joined by a free hand curve describe the change in geometry of the individual folded layer.

The t'_α vs α plots of folds of the area (Fig.4) suggest that majority of the layers are not restricted to any particular class of Ramsay and Huber (1983) and they show change in their geometry from one class to another. The plots of these folds (Fig. 4) reveal that most of the folds of study area belong to class 1C geometry. However fold geometries belonging to 1A, 1B, 2 & 3 are also present.

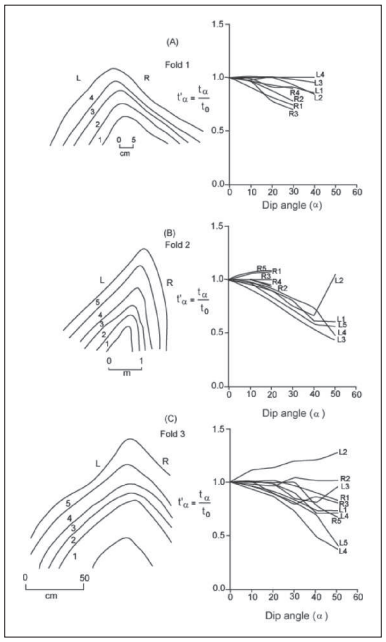

Fig.4 Fold profile traces and their orthogonal thickness parameters plots.

Variation in Multilayer Fold Geometry

In order to understand the variation in the fold profile geometry in a multilayer sequence of fold, the classification scheme of Srivastava and Gairola (1999) has been applied on the folds of the study area. The folds on which the scheme has been applied are shown in Fig. 4 and their plotting in $\sigma_n(t'_\alpha)$ vs α diagrams is depicted in Fig.5. $\sigma_n(t'_\alpha)$ represents the population standard deviation of the orthogonal thickness parameter t'_α for n number of layers in a multilayered sequence of fold and α represents the dip angle for the isogons on which the thickness measurements for t'_α were done. In this diagrams, the variation in fold geometry have been shown in quarter wave sectors. The L and R (Fig. 5) attached with fold number represents the left and right quarter wave sector of the fold numbered 1, 2 or 3 (Fig.4). From the plots, it is evident that no fold of the study area is analogous or isodeviatoric in true sense. The folds show variations in their geometry from one class to another which may range from sub-analogous to non-analogous fold.

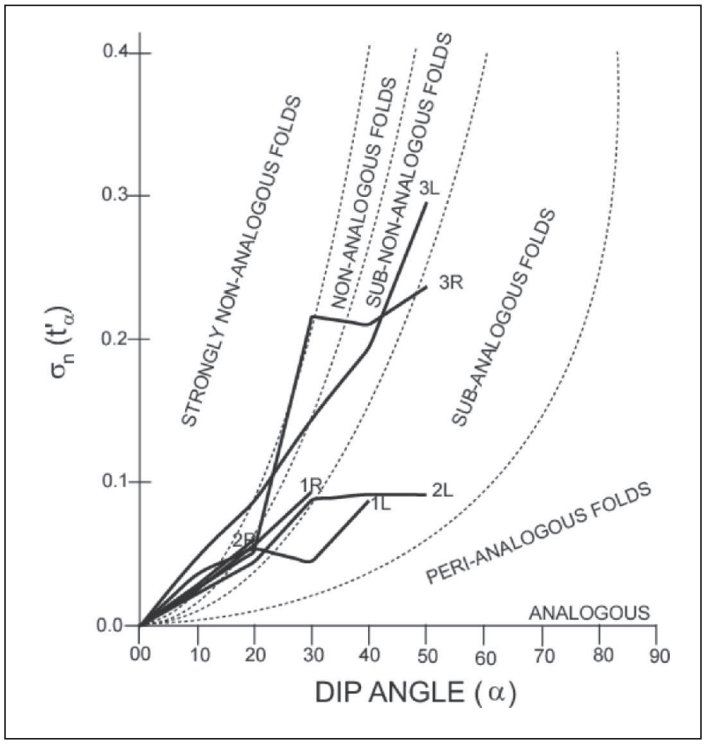

Fig. 5 Plotting of multilayered folds in Srivastava and Gairola (1999) diagram.

SUMMARY AND CONCLUSIONS

The Palaeogene sequences of the study area comprise parts of the Fold – Thrust Belt stretching NE – SW along western margin of a collision orogen in Naga Hills. The Naga Hills constitute northern extension of the Indo-Burman Ranges (IBR) which is considered to be an ideal setting for understanding the geodynamic processes involved in the evolution of the

northeast Indian crustal block through late Mesozoic - Cenozoic period. The present investigation is an attempt to understand the deformational phases of the Palaeogene sequences developed in parts of the Kohima synclinorium and the schuppen belt of Nagaland. Structurally, the study area is made up of very complex structures on mesoscopic and macroscopic scales. For the purpose of systematic study, mesoscopic structures were studied as planar fabrics, linear fabrics and folds. The detailed geometric analysis of the mesoscopic folds is done in the present work in order to understand the possible kinematics and mechanism of the fold development in this geologically complex region of NE India.

The detailed study of these structures reveals that the region has suffered at least four phases of the deposition namely D_1, D_2, D_3 and D_4 (Nakharo, 2009). These deformational phases have brought about intricate patterns of distribution of rocks types of the region in the form of folding, faulting and thrusting of complex nature. The D_1 and D_2 deformational phases were mainly of ductile nature while the D_3 and D_4 have been mainly of brittle nature which has resulted in many fractures, faults and imbricate thrusting in the Schuppen Belt of Nagaland.

The geometrical analysis of the folds of the study area has been done which reveals that most of the folds of the area belong to class 1C geometry. However fold geometries belonging to 1A, 1B, 2 & 3 are also present. The geometry of the folded layer is an important criterion for study of fold morphology and also in describing the principal folding mechanisms involved in the development of the folds. According to Ramsay and Huber (1983), the folds of class 1A and 3 indicate differential compression in their evolution while class 1B and 1C suggest a flexure-slip mechanism, and class 2 suggests a slip mechanism in the fold formation. Therefore a flexure slip mechanism is the most dominant mechanism involved in the development of folds of these rocks occurring in the study area. The later deformations have modified the geometry of these folds and therefore they have shown much variation in the Srivatava and Gairola plots for multilayered folds.

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Metamorphism in Chaukhutia Area and possible linkages of Almora Nappe with the Higher Himalayan Metamorphics

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Abstract

The Almora Group is considered an equivalent of the Munsiari Formation of the Higher Himalaya and metamorphics comprising Almora Nappe, classified as the Almora Group have been largely believed to be low grade metamorphics. One of the grounds for distinguishing the Munsiari Formation from the Vaikrita Group is the lower grade metamorphism of the former. We present findings that the grade of metamorphism in the Almora Group is far higher than presumed by earlier workers and comparable to the Vaikrita Group but perhaps a direct correlation of the Almora Group of rocks either with the Munsiari Group-- as believed by many or with the Vaikrita Group does not appear reasonable.

Introduction

The Lesser Himalayan Nappes, including the Almora Nappe, are the detached patches of a once continuous thrust sheet equivalent to the Main Central Thrust of Higher Himalaya, which moved southwards from the Higher Himalaya to cover almost the whole of the Lesser Himalaya sometimes during the early Eocene. Later, erosion of the uplifting Himalaya led to the present detached disposition of Lesser Himalayan nappes and klippes in Uttarakhand and elsewhere in the Himalaya. Although the broad tectonic scenario is largely agreed upon by the geologists but many finer but crucial details of correlation of the Lesser Himalayan nappes with their root zone are still debated. Heim and Gansser(1939) and Gansser (1964) identified nappes of the Lesser Himalaya as tectonically transported from the Higher Himalaya. The Almora Nappe was correlated with the Munsiari Formation by Valdiya (1980) and he also distinguished the underlying Munsiari Formation from the Overlying Vaikrita Group by the Main Central(=Vaikrita) Thrust in the Higher Himalaya. Valdiya (1980) and Valdiya and Goel (1983) suggested upper amphibolite facies conditions for the Vaikrita Group and low grade metamorphism --greenschist facies conditions for the Munsiari Formation with very local attainment of epidote amphibolite facies conditions. Valdiya and Goel (1983) inferred peak metamorphic temperatures around 450°C and pressures around 4kbar for the Munsiari Formation rocks.

The area of interest is located in the Almora district of Uttarakhand comprising a part (East longitudes 79°15'56" to 79°27'49" and North latitudes 29°45'19" and 30°00' 00") of the Almora Nappe that extends Far East into Nepal. The paper addresses metamorphic conditions of part of the Almora Nappe and discusses latter's correlation with its possible root zone in the

Higher Himalaya.

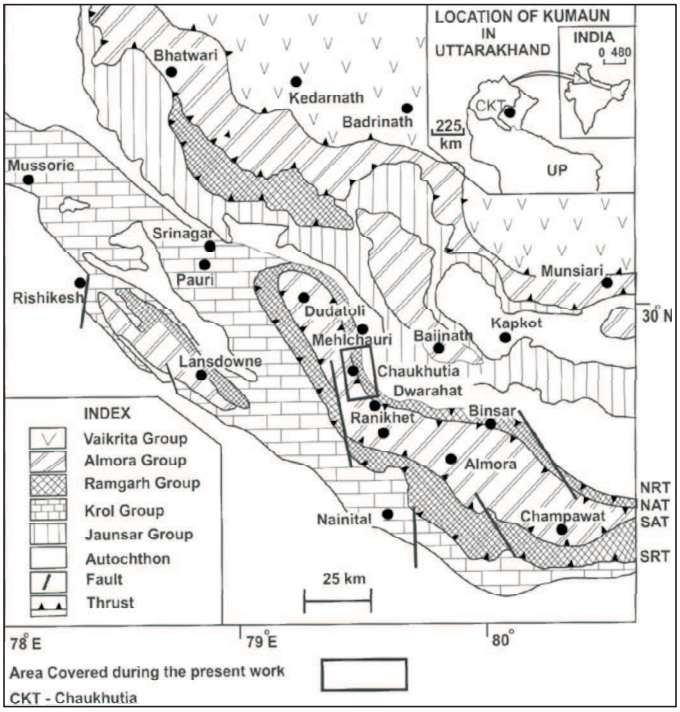

Fig.1 Generalized geological map of part of the Kumaun Himalaya (modified after Joshi, 1999)

Structural Set Up and Lithology

Almora Nappe is the name given to the composite nappe comprising the lower Ramgarh Nappe and the tectonically overlying Almora Nappe (Heim and Gansser, 1939, Gansser, 1964, Valdiya, 1980). The composite Almora Nappe is tectonically delimited from the underlying low grade metasedimentaries of the Lesser Himalaya by the Ramgarh Thrust with its northern exposure known as the North Ramgarh Thrust (NRT) and its southern exposure termed as the South Ramgarh Thrust (SRT) (Joshi, 1999). The top most part of this tectonic ensemble is the Almora Nappe senu stricto constituted by the Almora Group of rocks tectonically overlying the Ramgarh Group of rocks with the North Almora Thrust (NAT) in the North and the South Almora thrust (SAT) in the south as the two exposures of the Almora Thrust (Joshi, 1999). Most of the Ramgarh Group and the lower most part of the Almora Group of rocks are sheared with the degree of shearing decreasing up section, and this sheared ensemble responsible for bringing the Almora Nappe from the Higher Himalaya to its present location has been collectively termed as the Basal Shear Zone of the Almora Nappe by Joshi (1999). The focus of present study are the metamorphics of the Almora Group comprising parts of the Almora Nappe immediately lying south of the North Almora Thrust in the Chaukhutia area.

Bedding (S_o) marked by the lithological colour banding trends E-W to WNW-ESE with southerly dips between 10-35degrees.Four generations of folds identified by Joshi (1999) and

Joshi and Tiwari (2000, 2009) for the Almora area are also common in the Chaukhutia area. These are the isoclinal F_1 folds the tight F_2 folds, (both F_1 and F_2 plunging NE), the open F_3 folds (with sub vertical axial planes in other parts of the nappe but not observed in the area) and the F_4 folds (plunging ~15° southeast) generally occurring as broad open warps.

The metamorphic rocks belong to Saryu Formation of the Almora Group (of Valdiya, 1980) are exposed between Bhatkote and Chauna village in the Chaukhutia-Naugaon sector. The rock types are schists, gneisses and quartzites. The brownish grey and whitish grey schists are fine to coarse grained comprising quartz, muscovite, biotite and garnet. These schists are interlayered with dirty white and brownish-grey hard and compact fine to medium grained quartzites. Quartz and mica can be identified in hand specimens. The schists grade into whitish-grey and dirty white gneisses, some of which are weakly foliated, comprise medium grained minerals while others are composed of medium to coarse-grained minerals. Quartz, feldspar, mica and garnet can be identified with unaided eye.

Petrography

Major mineral constituents of schists are quartz, micas (both muscovite and biotite), chlorites, garnets, kyanites and plagioclase feldspars and the minor minerals include zoisite, sphene and magnetite. Tourmaline and zircon are also present in minor amount. Retrograded chlorite as well as sericite is present. Two generations of quartz, mica, biotite and chlorite are present. The dominant foliation is defined by the larger second generation micas which are at places cross cut by a third generation of small mica flakes. However, three types of garnets have been identified in the schists of the Saryu Formation. The first generation garnets are relict garnets that at places are stretched parallel to the foliation and contain quartz, magnetite and tourmaline inclusions. Garnet-I is pre-kinematic to the major deformation associated with the regional metamorphism affecting the area. Garnet –II are helicitic garnets containing quartz, magnetite and rare plagioclase inclusions. These garnets are syn-kinematic to the major deformation accompanying regional metamorphism. The euhedral inclusion free Garnet-III is post-kinematic to the major deformation. Garnet-III also commonly occurs as overgrowth on the syntectonic garnet cores (Fig.2).

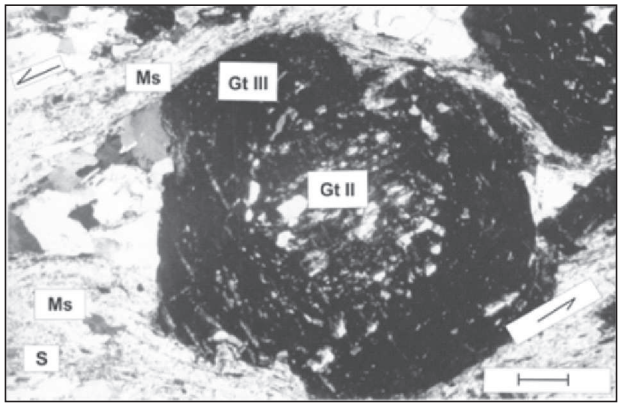

Fig.2 Synkinematic Garnet -I core with helicitic trails and the idioblastic Garnet -II rim

Saryu Formation garnets are characterized by synkinematic cores with either helicitic or snowball inclusion trails or inclusion free idioblastic garnet rim. The helicitic trails developed in response to the deformation concomitant with the regional metamorphism probably during the F1 and F2 folding. The last stage of garnet growth responsible for the fresh euhedral garnets or euhedral overgrowths on the existing garnets outlasted formation of S2 (F2) during the D_2-deformation.

Medium grained kyanites are associated with biotite, muscovite and quartz in the thin sections. Two generations of kyanite, viz. kyanite-I and kyanite-II occurring at different orientations are seen. Owing to its small modal content the kyanites could not be identified in field and have also been missed by all the earlier workers (Lal, 1959, 1969; Mishra, 1971). Plagioclase feldspars are common with the An content reaching ~ 11%.

Minerals Assemblages

1. Muscovite-biotite-plagioclase-K-feldspar-quartz
2. Muscovite-chlorite-biotite-plagioclase-quartz-tourmaline-magnetite.
3. muscovite-biotite-kyanite-garnet-plagioclase-quartz-magnetite-tourmaline
4. Muscovite-biotite-kyanite-garnet-plagioclase-quartz±carbonates-magnetite-tourmaline.
5. Muscovite-biotite-kyanite-garnet-plagioclase-quartz±zoisite-sphene-zircon.

Medium to coarse-grained gneisses of the Saryu Formation comprise quartz, muscovite, biotite, kyanite, sillimanite and K-feldspar. Garnet is present in some gneisses but is not common. Two varieties of quartz are observed in the gneisses and the older quartz invariably shows a reaction boundary whenever it is in contact with muscovite suggesting the reaction Muscovite+ quartz = K-feldspar+sillimanite+H_2O (Fig.3) Four types of muscovite and three types of biotite have been distinguished in these gneisses

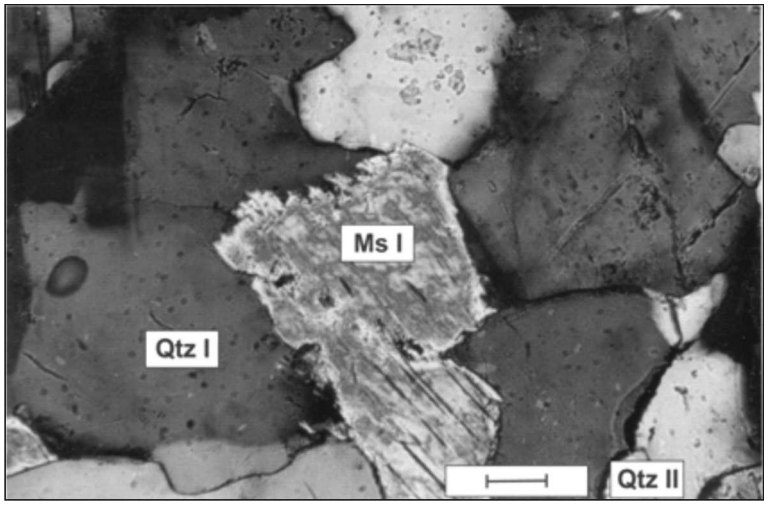

Fig. 3 Reaction boundary between muscovite-I and quartz-I suggesting the reaction Muscovite+ quartz = K-feldspar+sillimanite+H_2O

Similar to the schists two types of garnets are also observed in gneisses of the Saryu Formation of the Almora Group. Medium grained kyanite grains associated with quartz, muscovite and plagioclase also persist in the gneisses. However, randomly oriented sillimanite needles occur in association with K-feldspar, plagioclase and quartz. Plagioclases are also similar to the schists albeit with higher An content.

Mineral Assemblages

1. Muscovite-biotite-garnet-kyanite-sillimanite-plagioclase-K-feldspar-quartz.
2. Kyanite-sillimanite-garnet-biotite-muscovite-plagioclase-K-feldspar ± myrmekite ± tourmaline.
3. Sillimanite-K-feldspar-biotite-muscovite-plagioclase-quartz ± apatite.

METAMORPHIC ZONES

Four metamorphic zones have been demarcated in the area on the basis of discontinuous mineral reactions identified by petrographic studies. These are the biotite zone, garnet zone, kyanite zone and the sillimanite zone. The biotite zone encompasses phyllites, phyllitic schists, mica-schists and quartzites near the southwestern end of the area. Biotite zone is followed by the garnet zone and the garnet zone is repeated thrice along the Chaukhutia – Naugaon transect (Fig. 4). The biotite zone and the garnet zone collectively belong to the green schist facies of regional metamorphism. The biotite zone and the garnet zone are mylonitized in the proximity of the North Almora Thrust (Fig. 4). Kyanite zone comprises kyanite-garnet-mica schists that are not affected by mylonitization. The mineral assemblages of this zone are products of regional metamorphism representing kyanite – zone of amphibolites facies. The kyanite zone is repeated twice in the area (see Fig. 4). The sillimanite zone covers the sillimanite – garnet gneisses that have totally escaped the shearing related to the North Almora Thrust. On the basis of the mineral assemblages the sillimanite zone has been placed in the upper amphibolites facies of regional metamorphism. The reaction isograds between the various zones have been identified on the basis of the following reactions:

Zone –I: Biotite - Zone

…….Garnet – Biotite Isograd ……

Chlorite + muscovite + quartz = garnet + biotite + H_2O ……..(1)

(Thompson and Norton, 1968)

Zone –II: Garnet - Zone

……. Kyanite – Biotite Isograd …….

Almandine garnet + chlorite = kyanite + annite (biotite) ……(2)

(Spear and Selverstone, 1983)

Zone –III: Kyanite – Zone

……Sillimanite – K-feldspar Isograd……

Muscovite + albite+ quartz = K-feldspar + sillimanite + melt …….(3)

(Thompson and Tracy, 1979)

Zone –IV: Sillimanite - Zone

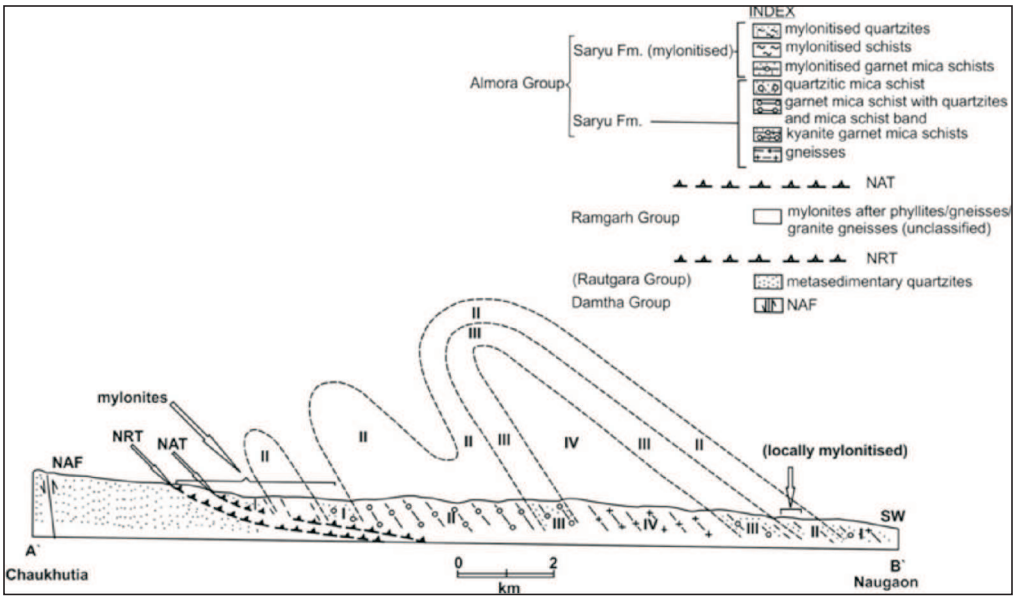

Fig. 4 Geological section showing the interpreted disposition of metamorphic zones deduced from the field and petrographic studies

The repetition of metamorphic zones observed along the geological section (Fig. 4) raises serious questions about the inverted metamorphism for this part of the Almora Nappe vis a vis the inverted metamorphic sequence of the Higher Himalaya---the root zone of the nappe. Most of the earlier workers (viz. Vannay and Grassemann, 1998; Vannay et al., 1999 and many others) have interpreted inverted metamorphism in almost all the sectors of Lesser- and Greater Himalaya. However, in the present area it is clear that the reaction isograds are folded and the limited apparent Inverted Metamorphic Sequence at the base of Almora Nappe is a consequence of a post metamorphic regional folding.

Physical Conditions of Metamorphism

On the basis of petrographic observations and metamorphic reactions a petrogenetic grid has been prepared for the area (Fig. 5). The broad P-T path the rocks have followed has been deduced on the basis of metamorphic reaction identified petrographically and is shown by arrows in the diagram. At lower grades the reactions (1) and (2) viz. the reactions responsible for the formation of garnet-biotite and kyanite-biotite (annite) assemblages have been crossed to the higher temperature side but as kyanite -plagioclase is still a stable association obviously the assemblages are on the lower pressure side of the reaction (4). The gneisses in the area have formed by the reaction (3) viz. muscovite + albite+ quartz = K-feldspar + sillimanite + melt. The petrographic evidence for this reaction is present in the unstable muscovite-plagioclase assemblage (Fig. 3) and in the occurrence of randomly oriented sillimanite needles within the K-feldspar. The regressive path of the arrow has been drawn on the basis of development of the last generation fresh tiny muscovite flakes when the aluminosilicate and K-feldspar have reacted

with the water still present in the system and the reaction (3) proceeded backwards. Joshi and Tiwari (2009) have suggested peak metamorphic conditions in excess of 700°C at slightly less than 8kbar on the basis of detailed geothermobarometric calculations and petrographic studies form the adjacent eastern part of the Almora Nappe in the Chhara- Someshwar transect.

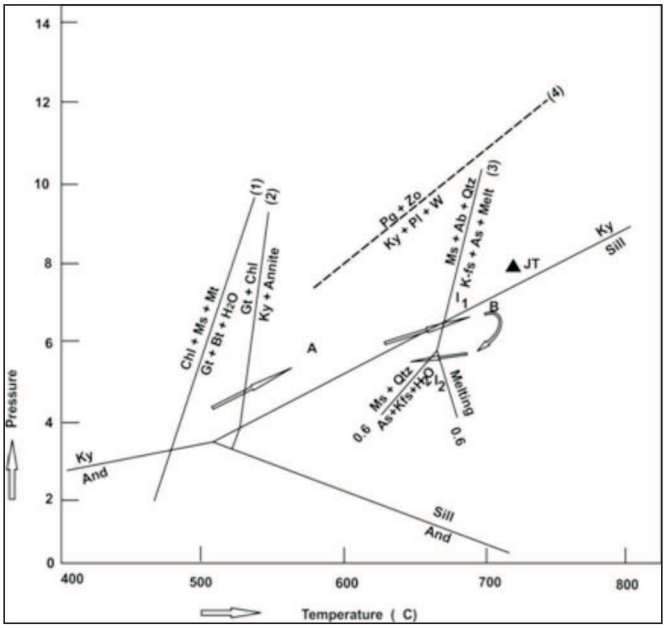

Fig. 5 P-t path for the metamorphic rocks of the Chaukhutia area deuced by petrographhic studies. The filled in triangle JT shows the peak metamorphic cionditions deduced by detailed geothermobarometry by Joshi and Tiwari(2009) form the adjacent Chhara-Someshwar transect.

Discussion and Conclusions

Central Crystallines (=Higher Himalayan Metamorphics, HHC) are largely accepted by most of the workers as the root zone of crystalline thrust sheets scattered all over the Lesser Himalayan sedimentaries with a thrust at their base which is equivalent to the Main Central Thrust (Fuchs, 1975,1981;Valdiya, 1976, 1978,Joshi and Gairola, 1980, Srikantia, 1988). Valdiya (1980) correlated the Almora Group rocks largely comprising the Almora Nappe with the Munsiari Formation of the Higher Himalaya. However, Valdiya and Goel (1983) suggested largely greenschist facies conditions for the Munsiari Formation only locally reaching epidote amphibolite facies conditions. On the basis of the mineral assemblages they concluded that the Munsiari Formation indicates comparatively lower P-T conditions, with temperatures reaching ~ 450° C at pressures around 4 kbar. However, the presence of kyanite and sillimanite in the rocks of the Chaukhutia area along with the almandine garnets clearly shows that the rocks comprising this part of the Almora Nappe have been subjected to much higher pressure- temperature conditions than those suggested for the Munsiari Formation. In contradistinction to the P-T estimates by Valdiya and Goel the present work suggests far higher temperatures of around 700°C

around at least 6.5kbar pressures on the basis of petrographic observations and deduced mineralogical reactions. Joshi and Tiwari (2009) also calculated temperatures in excess of 700°C at pressures around 8kbar from the adjacent Almora area which are close to the physical conditions of metamorphism deduced for the present area (Fig. 5). Thus it is clear that the upper amphibolite facies Almora Group of rocks comprising Almora Nappe cannot be equated with the green schist facies metamorphics of the Munsiari Formation of the Higher Himalaya on the basis of the grade of metamorphism. Moreover, the repetition of the metamorphic isograds in the metamorphics of the Almora Group of rocks poses further problems for equating it with the Higher Himalayan metamorphics characterized by Inverted Metamorphic Sequence. In view of these two serious problems it appears very difficult to correlate the Almora Group of rocks with any of the exposed sequences of the Higher Himalaya.

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Characterization of Deonar Porcellanites from Sidhi district, Madhya Pradesh

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Abstract

The pyroclastic and volcanogenic deposit of Deonar Porcellanite Formation of Semri Group (Vindhyan Supergroup) represents the records of violent explosive felsic volcanism during late Mesoproterozoic. Five lithounits have been identified in the porcellanites exposed along Amiliya-Bahari section in Sidhi district. Based on the field observations, petrography and other features, three phases of volcanic eruption and sedimentation have been recognized, suggesting a mixed provenance for Deonar Porcellanite.

Introduction

Deonar Porcellanite of Semri Group, Vindhyan Supergroup extends as a linear belt from Palamau district of Bihar in the east and Sidhi district of Madhya Pradesh in west. Deonar Porcellanites form laterally discontinuous small hillocks as strike-parallel chain of ridges for several tens of kilometers. Individual mounds range from 50 to l00m in height and 300m to one kilometer in width. Deonar Porcellanite comprises thinly bedded, fine grained siliceous sediments, felsic volcanic tuffs, tuffaceous shales and sandstones, forming conspicuous outcrops, along the banks and river bed of the Son river in Sidhi district (Fig, 1).

Law (1954) carried out detailed geological investigations in Sidhi district and concluded the porcellanite to be of pyroclastic origin, occurring with siliciclastics like shales, sandstones etc. Ghosh (1971) attributes extensive lateral continuity of these beds is the result of turbidity current based on his study in Sidhi district. Soni et al. (1987) noted the presence of a mineralized fractured zone within the pyroclastic rocks near Sihawal in Sidhi district. They also considered the occurrence of large sized volcanic bombs and lapilli near Rampur as an indication of the proximity of another focus. Rasmussen et al. (2002) and Ray et al. (2002) published precise U-Pb zircon ages of 1628$\pm$8 and 1631$\pm$5 for Deonar Porcellanite. The present paper aims at recognition and characterization of lithounits in Deonar Porcellanites of Lower Vindhyans. The description is based on observations along the classical Amiliya-Bahari section in Sidhi district (Fig, 1).

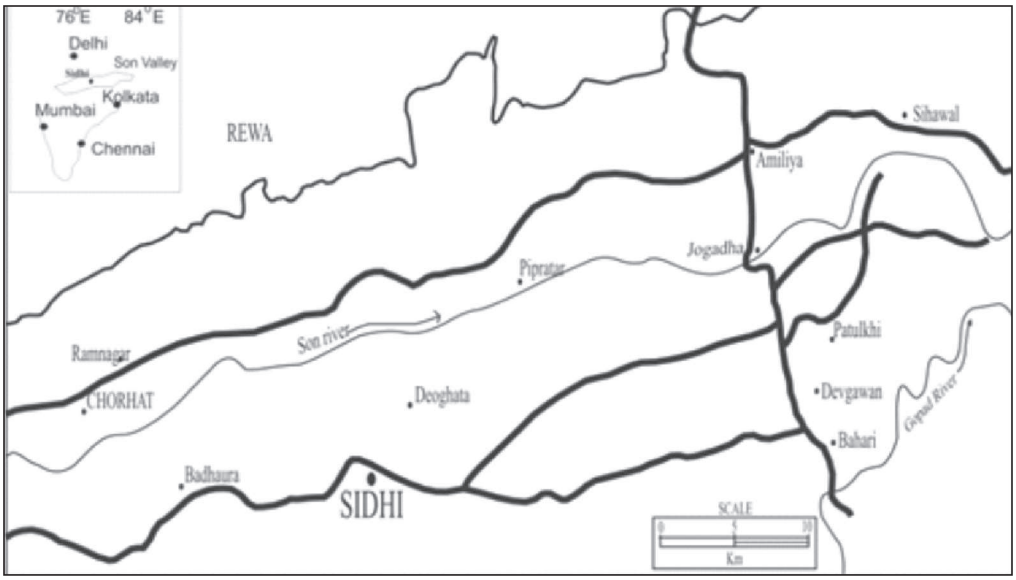

Fig. 1: Map showing location of the study area

Geological Setup

The stratigraphic succession around Sidhi district in M.P. comprises (i) the basement Sidhi granite (ii) Mahakoshal Group and (iii) Vindhyan Supergroup (Table 1). Vindhyan Supergroup has been divided into Lower and Upper Vindhyan, the former comprises Semri Group and the latter includes Kaimur and Rewa Groups. Upper Vindhyan is represented by clastic sediments whereas the Semri Group comprises mainly biochemical sediments with the volcanogenic activities.

The Semri Group unconformably rests over the metamorphosed Mahakoshal Group overlying the basement Sidhi Granite. Sidhi area exposes the oldest rock units of tonalite trondjhemite series formed during proto-continental stage (3.7-2.9 Ga) representing the ancient crust (Roy and Bandyopadhyay, 1990). The evolution of the granitic basement equivalent to Chhotanagpur Granitic Gneissic Complex resulted in the development of magmatic suites represented by migmatites, rhyodacite, alkaline intrusive and highly felspathised gneisses. Mahakoshal Group consists of phyllites, shales, slates with mafic intrusive present as sills and dykes (Sarkar et al., 1998). Preexisting rifted basins led to the deposition of Mahakoshal Group (2.6 to 1.9 Ga), which is classified as Greenstone Belt. The pre-Vindhyan basement embodies the whole tectono-magmatic-sedimentary cycle, which commenced with deep fracturing and emplacement of ultrabasic lavas near the surface, followed by sedimentation and basic volcanic (Yedekar, 1990; Nair et al. 1995; Roy and Devarajan, 2000).

Table 1: Stratigraphic succession around Sidhi district, Madhya Pradesh (after Ramakrishnan and Vaidyanadhan, 2008)

Upper Vindhyan	Rewa Group Kaimur Group
	Unconformity
Lower Vindhyan/ Semri Group	Rohtas Limestone Basuhari Sandstone Bargawan Limestone Olive Shale **Deonar Porcellanite** Deoland Sandstone
	Unconformity
	Mahakoshal Group
	Unconformity
	Sidhi Granite

Deonar Porcellanite

It consists of silicified pyroclastic rocks that are the records of a violent explosive felsic volcanic activity during the deposition of Lower Vindhyans at 1630 Ma (Rasmussen et al. 2002; Ray et al. 2002). Deonar Porcellanites are composed of thick stacks of pyroclastics and volcaniclastics conformably overlying the hard compact sandstones of Deoland Formation. Porcellanite is overlain by Olive Shale along a conformable contact. These rocks are massive but often show laterally persistent flow layers. Apparently in most instances the porcellanites dominantly exhibits vitroclastic texture and subordinate particulate texture. However there are considerable variations in internal character along and across strike, mainly reflected in the form of presence or absence of very well developed plane parallel banding (mm-scale) and some deformation features.

The porcellanite is dominantly fine-grained and are mineralogically similar, consisting of quartz and feldspar phenocrysts set in a recrystallized mosaic of quartz and feldspar with widely varying amount of devitrified groundmass, sericite, carbonates and Fe-oxides. Petrographic observations attest the pyroclastic origin of the Deonar Porcellanite, indicated by the presence of abundant pumice fragments, delicate glass shards, rhyolitic fragments, deeply embayed quartz grains and cryptocrystalline groundmass. On the basis of welding intensity of ignimbrites, Branney et al. (1992) have further extended the classification into: extremely high-grade ignimbrites (lava-like ignimbrites), high-grade ignimbrite (rheomorphic ignimbrite), moderate-grade ignimbrites that have both welded and non welded zones, and low-grade ignimbrites with no welding and distinct particulate nature.

Porcellanite comprises welded and unwelded ignimbrite as implied by thin section study (Mishra and Sen, 2008). Welded ignimbrite exhibits pumice fragments with tapering ends, welded to each other along the swirling margins by dark brown ferruginous material, referred to as "eutaxitic texture" (Bull and McPhic, 2007). Whereas, unwelded ignimbrite signifies loosely welded volcanic ash beds. They are composed of fragments of angular quartz, broken glass, K-feldspar (Fig. 2), muscovite, biotite, lithic fragments and rarely plagioclase, lying in vitric and cryptocrystalline groundmass.

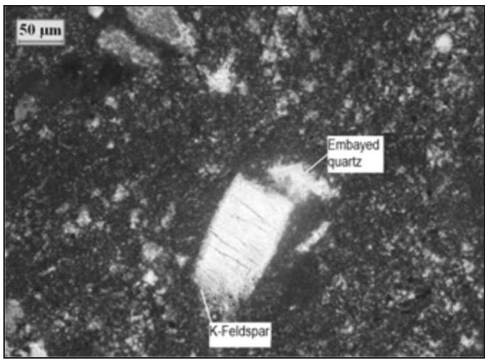

Fig. 2: Fractured and clouded K-Feldspar and embayed quartz in cryptocrystalline groundmass (between cross nicols)

Recognition of lithounits in Deonar Porcellanites

Deonar Porcellanite is dominantly of banded nature with alternating pale and dark bands. The massive varieties are of variegated colors- opaline green, buff, grey, grayish black etc. They also include fine grained tuff, pumice and lapilli fall deposits, volcanic breccias and bombs etc. The tuffaceous layers have been divided into lithounits based on colour, texture, grain size and sedimentary features. On the basis of field observations along Amiliya-Bahari section (Fig. 1), five lithounits namely, Banded Porcellanite, Brownish green unwelded tuff, Opaline green welded tuff, Opaline and coarse grained buff coloured tuff and Opaline and coarse grained grey tuff have been identified and shown in the vertical profile section (Fig.3).

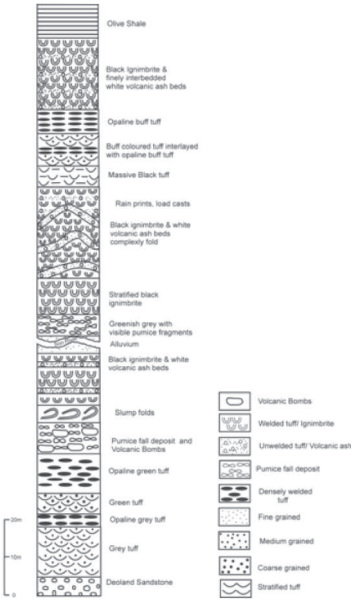

Fig. 3: Vertical profile along Amiliya–Bahari section

i) Banded Porcellanite consists of laterally persistent dark and pale bands of varying thickness ranging from hairline thickness to >30cm. The unwelded volcanic ash beds are of pale colour whereas the welded tuff is represented by dark colour. The lamination in dark and pale banded porcellanite is parallel to the strike of the area. This is the most commonly occurring lithounit of Deonar Porcellanite. Occasionally, the banded porcellanites show elliptical and flattened volcanic bombs of > 50 cm (Fig. 4) occurring parallel to the lamination, within pale coloured bands. Apart from banding, porcellanites often exhibit sedimentary features namely cross lamination, graded bedding, convolute structure, load cast and rain print. Porcellanite shows rhomboidal and columnar joint pattern. Deonar Porcellanite occurs as highly folded into innumerable sharp anticlines and synclines along Amiliya-Bahari section. Banded porcellanite also exhibit soft sediment deformation, possibly due to seismicity during explosive volcanism.

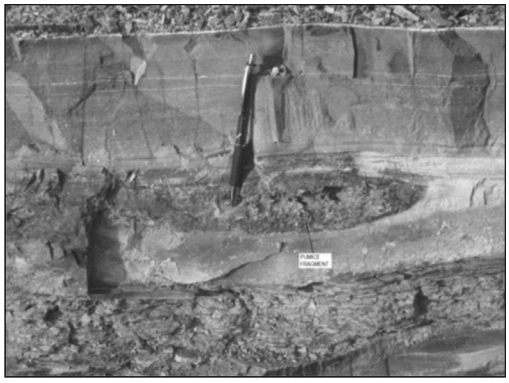

Fig. 4: Elliptical volcanic bomb filled by scoracious material enclosed by pale coloured band of banded porcellanite

ii) Brownish green unwelded tuff is coarse grained with floating elliptical shaped fragments of lapilli. The fragments are not uniformly distributed floating in the matrix of sandy nature. Some beds have more concentration of lapilli fragments (Fig. 5). They are thickly bedded (>200cm) with intervening thin laminations (<8cm). The sedimentary structures encountered are current bedding and load cast. This lithounit is exposed along northern bank of river Son at Jogadha.

Fig. 5: Brownish green tuff showing cross bedding. The arrow on the right side indicates visible lapilli fragments aligned parallel to the bedding.

iii) Opaline green welded tuff is very fine grained, transluscent and medium bedded variety of porcellanites. The fresh surface shows vitreous luster, conchoidal fracture and typical green colour, whereas their weathered off white surface shows waxy luster. This lithounit is well represented at Devgawan.

iv) Opaline and coarse grained buff coloured tuff are exposed at the northern bank of river Son. Opaline buff coloured variety is very fine grained, welded, translucent and thinly bedded variety of porcellanites. The fresh surface shows vitreous lustre and conchoidal fracture, whereas their weathered surface shows waxy luster. Buff colored, coarse grained, thickly bedded tuff (~2.5m) is particulate in nature. There are intervening layers of medium grained (~1m) and fine grained (12-24cm) tuff. Fine grained variety is more siliceous with conchoidal fracture.

v) Opaline and coarse grained grey tuff is very fine grained, thinly bedded, with waxy luster and conchoidal fracture. Grey coloured welded, opaline tuff occurs with green opaline tuff. Coarse grained, massively bedded variety of dark grey tuff is exposed with opaline tuff.

Discussion and Conclusion

Deonar Porcellanite is the expression of explosive felsic volcanic activity in the Lower Vindhyans at the late Mesoproterozoic. Porcellanite based on the field observations in the study area are moderate-grade ignimbrites comprising both welded and non welded zones, and low-grade ignimbrites with no welding and distinct particulate nature. The variation in welding characters and gradation of eutaxitic flow-laminated rheomorphic tuffs from the more particulate flow deposits strongly suggests that the porcellanites in study area are pyroclastic in origin. Discriminating between welded and unwelded tuff is important in understanding the depositional environment and eruptive style (Fisher and Schmincke, 1984; Gifkins et al., 2005). Generation of welding textures requires high temperature during or shortly after deposition, and are thought to be more common with subaerial deposits. However, welding in pyroclastic flow deposits is possible under shallow submarine condition also (Smith, 1960; Fisher and Schmincke, 1984). The explosive eruptions take place when a mixture of pyroclasts and gas are discharged into the atmosphere.

Three distinct phases of volcanic eruption and sedimentation can possibly be deciphered in Deonar Porcellanites, based on lithologic association, grain size, welding and sedimentary structures. The active phases of eruption started under water with ejection of welded ignimbrites and pumice-fall tuff represented by dark bands of banded porcellanites. The active phases were intervened by spells of quiescence depositing ash fall/ dust indicated by unwelded pale bands of banded porcellanitic tuff. The periods of active volcanism and quiescence was also intervened by the sedimentation, evident from low-grade ignimbrites with no welding and their distinct particulate nature. This observation is based on coarse grained granular, green, buff and grey varieties of tuff and associated sedimentary features. This suggests a substantial detrital input together with component that of volcanic origin; mixed origin. These are also associated with their complementary opaline varieties, suggesting them to be medium grade ignimbrite. Deonar Porcellanites probably represents the amalgamation of episodes of active volcanism, periods of quiescence (ash/dust fall) and normal sedimentation without any marked hiatus.

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Journal of Scientific Research

Section B : Life Sciences

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तत् त्वं पूषन् अपावृणु सत्यधर्माय दृष्टये

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Melatonin in modulation of reproductive functions in the female Indian pygmy field mouse, *Mus terricolor*

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Abstract

The role of melatonin in control of seasonal reproduction is well established and it is known to have pro/antigonadotrophic effect in different species of small rodents. The Indian pygmy field mouse, *Mus terricolor* is a tropical, wild, nocturnal, short day breeder. Till date no report exists regarding the effects of melatonin on reproductive functions of this rodent, therefore, the present experiment was designed to study the melatonin-induced changes on the reproductive functions of female *M. terricolor.* Our study describes the effects of melatonin injections (25µg/100gm body weight) on the ovarian and uterine activity of *M. terricolor* during the reproductively active phase/RAP of its breeding cycle. The results suggest an antigonadotrophic action of melatonin in this rodent as deflected by decreased ovary and uterine weight, degenerative changes in the histology of the ovary and uterus and reduced plasma estradiol and progesterone. In case of short day breeders melatonin is known to act as a progonadotrophic hormone. But, exogeneous melatonin administration at a dose of 25µg/100gm body weight during RAP suppressed the reproductive functions of this tiny rodent suggesting an antigonadotrophic property of melatonin at a higher threshold level. The results suggest a pivotal role of melatonin in adaptive strategy for the regulation of reproduction in seasonal breeders.

Keywords: Melatonin, Reproduction, *Mus terricolor,* Tropical rodent, Seasonal breeder

INTRODUCTION

Photoperiod is regarded to be the most reliable cue to time reproductive activity (Rosa and Bryant, 2003; Muteka *et al.*, 2006). The destination for the information about the day length is the pineal gland where melatonin is secreted. Pineal gland translates neural stimuli mediating photoperiod into hormonal stimuli melatonin.The daily duration and the level of melatonin secreted in the pineal regulates the secretion of gonadotropins /gonadal steroids and consequently reproduction in seasonal breeders (Heideman and Bronson, 1990; Frungieri *et al.,* 2005; Nakao *et al*., 2008; Prendergast *et al.,* 2009).The role of pineal and melatonin has been well studied and reported in animals that breed during long day (hamsters: Hoffman and Reiter, 1965; voles: Farrar and Clarke, 1976; ferrets: Herbert *et al.,* 1978; white-footed mice: Glass and Lynch, 1981; Sp/Indian palm squirrel: Haldar and Saxena, 1990; Bisnupuri and Haldar, 2001; Yadav and

Haldar, 2009) and short day (sheep: Lincoln, 1979; Bittman *et al*., 1983; deer: Plotka *et al*., 1984). Turek *et al.* (1975) Turek and Pappas (1980) Petterborg (1986) and Maitra and Ray (2000) reported that melatonin when administered exogenously suppresses gonadal activity, and the effects are dose dependent.

The Several studies have reported the effects of melatonin on reproductive physiology of female animals. Wurtman (1963) for the first time reported in the that melatonin injections delay the spontaneous vaginal opening and cause highly significant reduction in ovarian and uterine weight and incidence of vaginal estrus. Many workers have reported disruption of ovarian cyclicity, reduction in weight of reproductive tract, gonadotropins and gonadal steroids after melatonin treatment (Bridges *et al*., 1976; Tamarkin *et al.,* 1976; Reiter *et al.,* 1980; Richardson *et al.,* 1981; Vriend *et al.,* 1987). Melatonin receptor has been localized in ovary (Clemens *et al.,* 2001) and uterus (Zhao *et al*., 2002), suggesting direct action of melatonin on these organs.

Mus terricolor, commonly known as Indian pygmy field mouse, is a small tropical wild rodent, found throughout South East Asia (Aplin *et al.,* 2003). *M. terricolor*, an important pest of rice and wheat fields, make burrows in the earthen dykes rose for holding water in the cultivated fields (Singh *et al*., 2009). It has an earth colored body and a grey belly and tail slightly longer in proportion to the head and body length (Sharma, 1996; Aplin *et al*., 2003; Singh *et al*., 2009; Basu *et al.,* 2012; Basu and Singaravel, 2012). Singh *et al.* (2009) mentioned seasonal availability and speculated on seasonal reproduction, but did not give any evidence in favor of such theory. *M. terricolor* presents two peaks of annual gonadal cycle, one from October to January, and a second brief phase in April that is dependent on food availability (Data in communication). The evolutionary history and cytogenetics of M.terricolor have been explored in depth **(**Sharma, 1996; Singh *et al*., 2009). The circadian sensitivity of this nocturnal rodent has been studied by Basu *et al.* (2012) and Basu and Singaravel, (2012). Till date no reports exist regarding the effect of melatonin administration on reproduction of small wild rodent. There fore, a the aim of the present study is to explore the effects of exogenous melatonin administration on the ovary and uterus of female *M. terricolor* during reproductively active phase, i.e., in the month of December.

MATERIALS AND METHODS

Maintenance of animals

All the experiments were conducted in accordance with Institutional practice and within the framework of revised Animal (Specific Procedure) Act of 2007 of Govt. of India on animal welfare. Experiment was performed during reproductively active phase of the animal. The mice were collected from the fields of vicinity of Varanasi (Lat. 25º, 18' N; Long. 83º, 1'E) India, following the methods as described earlier (Bardhan and Sharma, 2000; Singh *et al.,* 2009; Basu *et al.,* 2012; Basu and Singaravel, 2012).

After 2 weeks of acclimatization to laboratory conditions healthy young adult non-pregnant female mice of average weight 11$\pm$1 g were randomly selected from the collected rodents and divided into two groups having six female mice in each. They were kept in commercial polypropylene cages during experiments and were maintained in a well-ventilated room exposed to ambient conditions (27 $\pm$ 2ºC, with gentle ventilation). Mice were fed with commercial food pellets along with wheat, paddy/rice and water *ad libitum*.

Experimental groups

Our animal model is a keystone species as it serves as an important part of food web being food of different predatory and endangered birds such as white owl, etc. So in order to avoid any disbalance in the biodiversity by capturing more animals for our experimental purpose we restricted our study during reproductively active phase of the animal when the effects of melatonin can be well exhibited and studied.

First group (n=6) treated with normal (0.9%) ethanolic saline served as control, second group was given intra-peritoneal melatonin injection (25 µg/100 g body wt. Johnston and Zucker, 1980; Maitra and Ray, 2000; Ahmad and Haldar, 2010) for 15 days during evening hours (17:00-18:00 hrs, one hour before sunset).

Melatonin was purchased from Sigma-Aldrich Chemicals, St. Louis, MO, USA. Melatonin was first dissolved in few drops of ethanol and then diluted with normal saline upto the desired concentration and injected.

Sample collection

Twenty four hour after the last injection, the mice were weighed and sacrificed by over-anesthetization during night time between 8:00 pm and 10:00 pm. Trunk blood was collected directly from heart in heparinized tubes and plasma was collected then kept at -80ºC till the ELISA for estradiol, progesterone (Biotron Diagnostics Inc. Hemet, California, USA) and melatonin (Uscn Life Science Inc. USA) was performed. Ovaries and uter in females were dissected out on ice, blotted free from blood, cleaned from extra tissue, and weighed on an electronic balance (Denver Instruments, Gottingen, Germany).Ovary and uterine horn of left side were fixed in Bouin's fluid for histology while that of right side were kept for biochemical estimations of ovarian cholesterol and uterine protein.

Histology

After fixation in Bouin's fluid, ovaries and uteri were processed for routine histological procedure. Some 6-µm sections were deparaf finized, stained using Ehrlich's hematoxylin and Eosin. The stained sections of the tissues were observed under microscope (Leica MPV-3, Germany) and documented.

Biochemical Estimations

Ovarian cholesterol was estimated using commercial kit and manufacturer's protocol (Bio Lab Diagnostics, India). According to the manufacturer's protocol, the provided reagents were added in three set of test tubes i.e. Blank, Standard and Test samples. 1 ml of cholesterol reagent was added to all the three sets. 20µl of distilled water, standard reagent and test samples (homogenate) was added to Blank, Standard and Test samples respectively. After mixing well, all the test tubes were incubated at 37°C for 10 minutes and read at 510nm on Spectrophotometer (UV-200-RS, mrc, Israel). The protein content of the uterus was quantified using the Bradford method (1976).

Hormonal analysis

The plasma contents of estradiol, progesterone and melatonin in respective groups were

estimated using ELISA kit according to manufacturer's instruction.

ELISA for melatonin

The assay for melatonin was performed according to the manufacturer's instruction given on the kit (Uscn Life Science Inc. USA). The intra- and inter- assay variation was <10 and <12% respectively. The sensitivity was 4.68 pg/mL and recovery percentage was between 90-115. All reagents, samples and standards were prepared according to standard protocols. 50µl standard and sample were added to the respective wells followed by 50 µl of Detection reagent A, followed by gentle shaking and incubation for one hour at 37° C, followed by aspiration and washing, thrice. 100 µl of Detection reagent B was subsequently added and the mixture was incubated at 37° C for 30 minutes, followed by washing and addition of 90 µl of substrate solution and incubation at 37° C for 15-25 minutes. Finally, 50 µl of stop solution was added and read at 450 nm.

ELISA for Estradiol and Progesterone

The ELISA kits for estimation of plasma estradiol and progesterone were purchased from Biotron Diagnostics Inc. Hemet, California, USA. According to the manufacturer's instruction, 25 µl of standard, control and samples were added in each well of ELISA plate followed by 100 µl of enzyme conjugate solution. The wells were then incubated with mild shaking at room temperature for two hours. The wells were then aspirated and washed three times with wash solution. Then, 100 µl of the TMB chromogenic solution (substrate) was added to each well and plate was incubated at room temperature for 30 minutes in dark. Finally, 100 µl of stop solution was added in each well and absorbance was recorded at 450 nm. Intra and inter assay variations were less than 5% and 14% respectively. The assay was carried out in triplicate.

Statistical analysis

Statistical analysis of the data was performed with one-way ANOVA followed by Student Newman–Keul's multiple range tests. The differences were considered statistically significant when $P \leq 0.05$.

RESULTS

Body weight

No significant difference was observed between saline-treated control group and melatonin-treated group clearly supporting that selected dose for experiment was physiological and that the animals were maintained properly in healthy condition as in nature with sufficient food and water (Fig. 1).

Weight of reproductive organs

There was a significant reduction in the relative weight of ovary and uterus of melatonin-treated group as compared with control (Fig. 2 and 3 respectively).

Biochemical estimations

We observed a significant increase in the content of ovarian cholesterol in melatonin-treated group as compared with the control group (Fig. 4) while the content of protein was significantly reduced in melatonin-treated group as compared with the control group (Fig. 5).

Hormonal analysis

Significantly decreased peripheral plasma estradiol and progesterone level was noted in

melatonin-treated group when compared with the control group (Fig. 6 and 7 respectively). Peripheral melatonin level was increased in melatonin-treated group as compared with control group (Fig. 8).

Histological observations

The transverse section of uterus of vehicle-treated females showed a normal endometrial histology (Fig. 9). It had a well-developed endometrium, narrow lumen and a large number of proliferated endometrial glands while the endometrium of melatonin-treated females had atrophied condition and hence the lumen was wide with less number of endometrial glands without any proliferation (Fig. 10).

The ovaries of the saline treated female *M. terricolor* showed several corpora lutea and antral follicles (Fig. 11) while melatonin-treated mice showed extensive degenerative changes in the ovary. The sections showed primodial, primary and secondary follicles. In melatonin-treated mice ovary several pyknotic nuclei were present in granulosa cell layer and no signs of ovulation, were observed, i.e., corpora lutea were completely absent (Fig. 12).

DISCUSSION

Melatonin modulates the reproductive-physiology of seasonally breeding mammals (Tamarkin *et al.,* 1976; Yadav and Haldar, 2009; Reiter, 2009; Ahmad and Haldar, 2010). The earlier reports suggest melatonin is neither progonadotrohic nor antigonadotrophic *per se*, but, it is the changing duration of the "nocturnal melatonin surge" that conveys the information about the time of the year to the animals for the hypothalmo-pituitary-gonadal axis to act accordingly and fine-tune their reproductive activity according to season a new aspect of neuroendocrine regulation of reproduction in seasonally breeding rodents.

Our study describes for the first time the effects of melatonin injections on the ovarian and uterine activity of a wild, tropical, nocturnal, short day breeding rodent *M. terricolor* during the reproductively active phase of its annual reproductive cycle. In the present study the antigonadotrophic action of melatonin in female *Mus terricolor* is depicted by a significant reduction in ovarian and uterine weight, suppressed steroidogenesis as evident by increased ovarian cholesterol (ovarian cholesterol could be a marker of low steroidogenesis as it is one of the major components required for the process of steroidogenesis) and reduced plasma estradiol as well as progesterone which further led to altered histological conditions of ovary and uterus.
Wurtman (1963) in rats, Vaughan *et al.* (1976) in mice, Young Lai (1978) in hamsters, Margollis and Lynch (1981) in *Peromyscus leucopus* ; Reiter *et al.* (1980) in rats, and Tamura *et al.* (1998) in rabbits; reported significant reduction in weight of reproductive organs and steroidogenesis after melatonin administration. The reduced steroidogenesis might be due to the action of melatonin on multiple sites, i.e., hypothalamus (reducing GnRH) and pituitary (reducing FSH and LH) thereby ovarian synthesis of E_2 (Vriend *et al.,* 1987; Chan *et al.,* 1995).

The classical target for melatonin action is hypothalamic-pituitary axis. Fraschini *et al*. (1968) was the first one to report that the antigonadal action of melatonin is due to its achon on hypothalamus. Later several workers reported the involvement of hypothalamus in melatonin action. Lang *et al*., (1983) found decreased GnRH level due to melatonin action. Kao and Weisz (1977), Petterborg and Paull/(1984), and Glass and Knotts, (1987) observed modulation of

storage and secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus by melatonin *in vitro* or *in vivo*. Different mechanisms have been proposed that explain melatonin's inhibitory effect on GnRH. The pulsatile pattern of GnRH release, which results in the intermittent release of gonadotrophic hormones from the pituitary, has a critical importance for reproductive functions but the factors responsible for this release pattern are not known. Calcium is a second messenger involved in GnRH release. The increase in Ca^{2+} resulting from melatonin treatment may impair pulsatile GnRH release, which may cause inactivation in gonadotrophic cells in pituitary. Melatonin may act directly by affecting the hypothalamic functions by inhibitory regulation of gonadotropin releasing hormone neurons (Roy *et al*., 2001). Melatonin may also be involved in the control of GnRH gene expression and secretion (Roy and Belsham, 2002).Thus by inhibiting GnRH secretion and by altering its pulsatile secretion melatonin might have inhibitory effect on release of gonadotropins.

Martin and Klein, (1976) reported the first direct melatonin action on the pituitary. Melatonin inhibited the GnRH-induced LH release by neonatal rat anterior pituitary cells *in vitro* (Martin and Klein, 1976) or hamster pituitary *in vivo* (Wun *et al.,* 1986). Other reports suggest that daily properly timed melatonin injections to females inhibit reproduction by reducing the ability of pituitary to secrete the gonadotropins (FSH and LH) thereby reduce the circulating gonadotrophin level (Tamarkin *et al*., 1976; Voordouw *et al.*, 1992). The measurement of LH and FSH is required to support above statement but LH and FSH measurement of this wild rodent can not be done due to lack of specific antibody.

Further, intraperitoneal evening injections of melatonin led to a significant increase in ovarian cholesterol (a key component in steroidogenesis) and significantly reduced the plasma estradiol and plasma progesterone. Our data gets support from previous reports (Voordouw *et al.,* 1992 ; Chan *et al.,*1995) . Vriend *et al.,* (1987) reported that melatonin injections disrupted the normal pattern of gonadotropin secretion and resulted in atrophy of uterus and vagina. These changes were accompanied with depressed serum and pituitary prolactin and depressed level of estradiol. Chan *et al.,* (1995) reported an increased incidence of follicular atresia in the groups treated with melatonin (MEL), methoxytryptamine (MTA) and methoxytryptiphol (MTP). Treatment with melatonin (MEL), methoxytryptamine (MTA) and methoxytryptiphol (MTP) also resulted in lower plasma levels of estradiol-17β and progesterone. Voordouw *et al.,* (1992) observed a significant decrease in LH, estradiol and progesterone in women following melatonin administration either alone or in combination with a synthetic progestin, consequently this combination was shown to be an effective oral contraceptive. In mammals melatonin modulates physiological functions through activation of at least two pharmacological and molecularly distinct receptors, the MT1 and MT2 (Masana and Dubcovich, 2001). Cohen *et al.,* (1978) first reported [3H] melatonin binding sites in cytoplasmic fractions of hamster, rat and human ovaries. Later Yie *et al.,* (1995), and Clemens *et al.,* (2001) detected melatonin binding sites in ovarian tissue using 2-[^{125}I] Iodomelatonin. Brzezinski *et al.,* (1987), and Ronnberg *et al.,* (1990) found melatonin in ovarian follicular fluid suggesting a direct effect of this hormone in ovarian function. Woo *et al.,* (2001) also suggested a direct role of melatonin in regulating ovarian function. The above findings suggest a direct action of melatonin on ovary and thereby on the mechanism for regulating the steroidogenesis. This in turn might have reduced the estrogen level

in the circulation of our animal model.

In addition to alteration in ovarian function melatonin equally controls uterine functions. Zhao *et al.,* (2002) showed the presence of melatonin receptors in the rat uterine endometrium suggesting that melatonin may act directly on the MT1 receptors in the antimesometrial stromal cells to inhibit their proliferation. This action is supposed to be mediated through a pertussis toxin sensitive adenylate cyclase coupled G (i) protein. In the present study melatonin injections altered the histology of uterus and decreased the protein content in the uterus, probably the presence of melatonin receptors in the uterus might be acting directly and regulating the endometrial vascular permeability and decidualization.

Our animal model *M.terricolor* breeds during the winter. In tropical countries like India days are short in winter and peripheral melatonin remains high. In our experiment when we exogenously administered melatonin at a dose of 25µg/100gm body weight, it showed an antigonadotrophic effect and suppressed the reproductive functions of this rodent. Progonadotrophic action of melatonin is known in short day breeders. During short photoperiod (winter) induced melatonin level maintains a threshold concentration in *vivo* that could not suppress the reproduction. But, when melatonin was given exogenously during above condition, it increased/disturbed the threshold level and hence an antigonadotrophic action of melatonin has been reflected. It can thus be inferred that it is the hypothalamo-hypophyseal region which measures the threshold sensitivity for melatonin in this small tropical rodent and thus maintain its adaptive strategies for reproduction. Available reports suggest that direct melatonin action may occur in more than one reproductive tissue, it can act from the level of hypothalamus to the levels of reproductive tract as the receptors for melatonin have been located in the SCN in the brain, pars tuberalis in the pituitary and reproductive tissues (Pang *et al.,* 1998). Thus it can be inferred that melatonin may act at the level of hypothalamus, pituitary, gonads and reproductive tract in this rodent to control its reproduction. Further, the experiments are in progress to show the presence and expression of melatonin receptors on above mentioned neuroendocrine axis.

ACKNOWLEDGEMENT

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FIGURE LEGENDS

Fig. 1 Histogram representing effect of exogenous melatonin injections on body weight of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05,**P < 0.01.

Fig. 2 Histogram representing effect of exogenous melatonin injections on relative ovary weight of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05, **P < 0.01.

Fig. 3 Histogram representing effect of exogenous melatonin injections on relative uterus weight of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05, **P < 0.01.

Fig. 4 Histogram representing effect of exogenous melatonin injections on ovarian cholesterol of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05, **P < 0.01.

Fig. 5 Histogram representing effect of exogenous melatonin injections on uterine protein of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05, **P < 0.01.

Fig. 6 Histogram representing effect of exogenous melatonin injections on plasma estradiol of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05, **P < 0.01.

Fig. 7 Histogram representing effect of exogenous melatonin injections on plasma progesterone of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05, **P < 0.01.

Fig. 8 Histogram representing effect of exogenous melatonin injections on plasma melatonin of *M.terricolor*. Values are expressed as mean, vertical bar on histograms represents ± SEM, N = 6. Significance of difference; *P < 0.05, **P < 0.01.

Fig. 9 Transverse section of the uterus (stained with haematoxylin-eosin) of *M. terricolor* . following saline injection. Note the proliferated endometrial glands. Figure shown in 40X magnification.

Fig. 10 Transverse section of the uterus (stained with haematoxylin-eosin) of *M. terricolor* showing histological changes following administration of melatonin. Note the non-proliferated endometrial glands. Figure shown in 40X magnification.

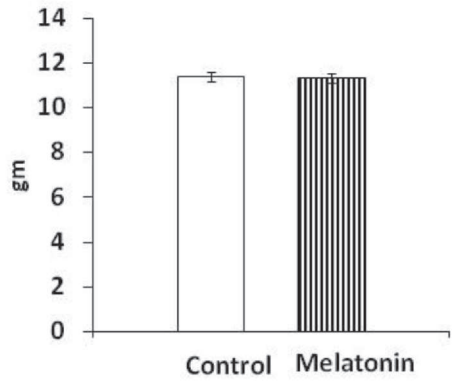
Fig. 11 A and B Transverse sections of the ovaries (stained with haematoxylin-eosin) of *M. terricolor* following saline injection. Figures shown in 10X and 40X magnification.

Fig. 12 Transverse section of the ovary (stained with haematoxylin-eosin) of *M. terricolor* showing histological changes following administration of melatonin.

(A) Section of the ovary. Figure shown in 10X magnification.

(B) Granulosae cells (GCs) showing pyknotic nuclei (Py). Figure shown in 40X magnification.

(C) Figure 12 (B) in enlarged view Figure shown in 100X magnification.



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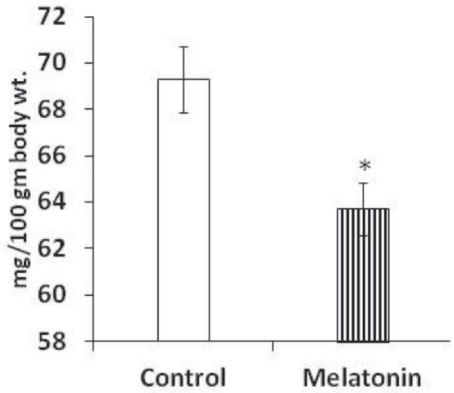
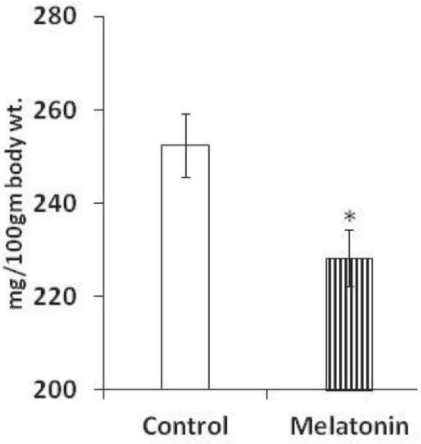


Fig.2



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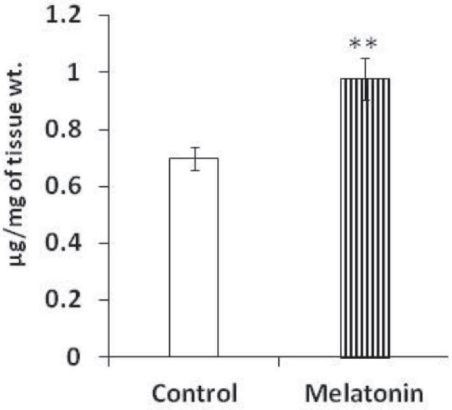

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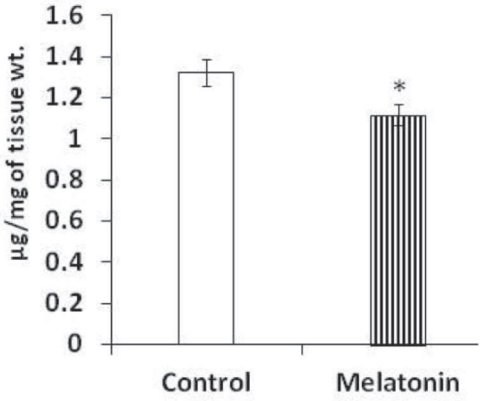
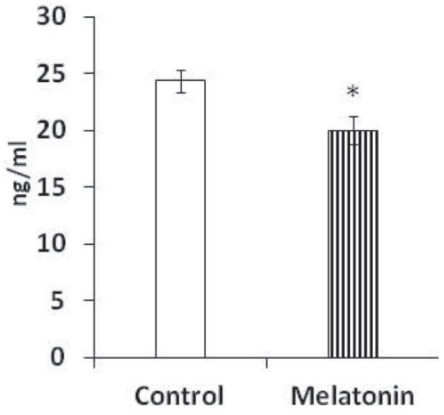


Fig.5



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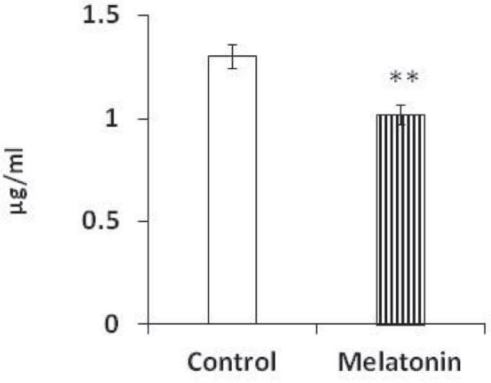


Fig.7

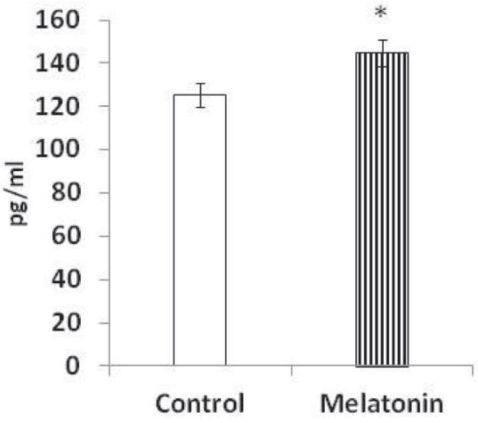


Fig.8

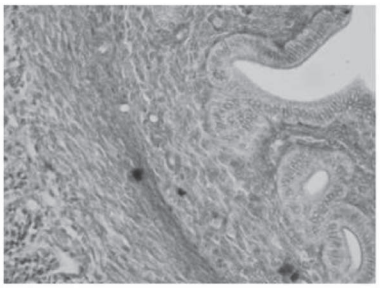


Fig.9

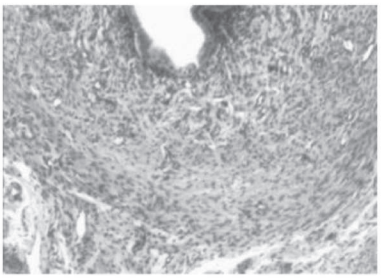


Fig.10

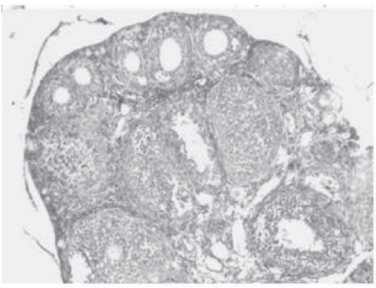
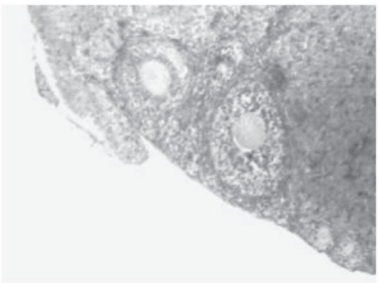


Fig.11A



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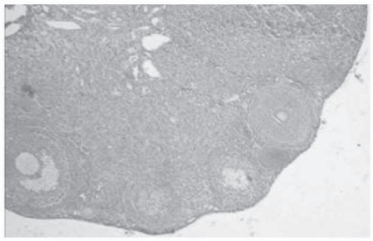

Fig.12A

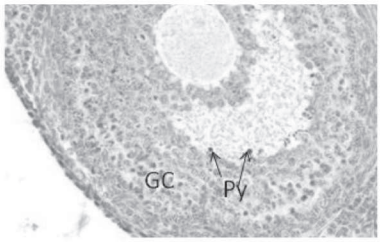

Fig.12B

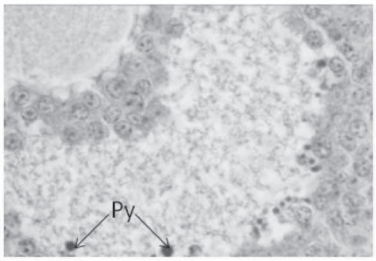

Fig.12C

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Complications associated with Orthodontic Micro-implants - A Review

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Abstract

Orthodontic micro-implant is increasingly gaining popularity in clinical orthodontics to affect skeletal anchorage. The mode of anchorage facilitated by these implant systems has a unique characteristic owing to their temporary use, which results in a temporary, though absolute anchorage. The foregoing properties together with the recently achieved simple application of these screws have increased their popularity, establishing them as a necessary treatment option in complex cases that would have otherwise been impossible to treat. The aim of this comprehensive review is to present and discuss the complications associated with Orthodontic micro implants used to obtain a temporary but absolute/ skeletal anchorage for orthodontic applications.

Anchorage control is one of the most important aspects of orthodontic treatment. According to the glossary of the American Association of Orthodontics (2006), it is defined as the resistance to activation force. To enhance orthodontic anchorage, micro implants have been introduced to reduce reciprocal orthodontic tooth movement (Park et al, 2001). It transfers the anchorage required from the tooth directly to the bone, which results zero loss of anchorage. This technique has enabled many difficult orthodontic movements such as en masse dental retraction, dental intrusion, and rotations of the occlusal plane to be incorporated into the orthodontic treatment plan (Sung et al, 2006). Microimplants have been used to convert cases that previously required orthognathic surgery into cases treatable by orthodontic treatment alone with microimplant support.

Traditionally, orthodontists have used teeth, intraoral appliances, and extraoral appliances to control anchorage, thus minimizing the movement of certain teeth while carrying out the desired movement of other teeth. However, because of Newton's third law—that is, for every action there is an equal and opposite reaction—the ability to completely control all aspects of tooth movement is limited. For example, orthodontists often have inadequate mechanical systems with which to control anchorage, which leads to a loss of anchorage in the reactive unit and thus incomplete correction of intraarch and interarch alignment problems. Moreover, in an attempt to overcome these limitations, clinicians often incorporate bulky acrylic appliances or extraoral appliances, which when combined with the ever challenging problem of uncooperative patients, are often a futile attempt at best.

A temporary anchorage device (TAD) is a device that is temporarily fixed to bone for the purpose of enhancing orthodontic anchorage by supporting the teeth of the reactive unit or by

obviating the need for the reactive unit altogether and which is subsequently removed after use. Temporary anchorage devices can be located transosteally, subperiosteally, or endosteally and they can be fixed to bone mechanically (cortically stabilized) or biochemically (osseointegrated). It should also be pointed out that dental implants placed for the ultimate purpose of supporting prosthesis, regardless of the fact that they may be used for orthodontic anchorage, are not considered TADs because they are not removed after orthodontic treatment. An important note, however, is that the initial incorporation of dental implants into orthodontic treatment made possible infinite anchorage, which has been defined in terms of implants as showing no movement (zero anchorage loss) as a consequence of reaction forces.(Cope, 2005)

Orthodontic micro-implant s have proven to be a useful addition to the orthodontist's armamentarium for control of skeletal anchorage in less compliant or noncompliant patients, but the risks involved with Orthodontic micro-implant s placement must be clearly understood by both the clinician and the patient. Complications can arise during Orthodontic micro-implant s placement and after orthodontic loading in regard to stability and patient safety. A thorough understanding of proper placement technique, bone density and landscape, peri-implant soft-tissue, regional anatomical structures, and patient home care are imperative for optimal patient safety and Orthodontic micro-implant s success.

Risks and complications of Orthodontic micro-implants can be classified under following heading as given by Neal D. Kravitz (2007)

1. **COMPLICATIONS DURING INSERTION**
   a. Trauma to the periodontal ligament or the dental root
   b. Orthodontic micro-implant slippage
   c. Nerve involvement
   d. Air subcutaneous emphysema
   e. Nasal and maxillary sinus perforation
   f. Orthodontic micro-implant bending, fracture, and torsional stress
2. **COMPLICATIONS UNDER ORTHODONTIC LOADING**
   a. Stationary anchorage failure
   b. Orthodontic micro-implant migration
3. **SOFT-TISSUE COMPLICATIONS**
   a. Aphthous ulceration
   b. Soft-tissue coverage of the Orthodontic micro-implant head and auxiliary
   c. Soft tissue inflammation, infection, and peri-implantitis
4. **COMPLICATIONS DURING REMOVAL**
   a. Orthodontic micro-implant fracture
   b. Partial osseointegration

COMPLICATIONS DURING INSERTION

Trauma to the periodontal ligament or the dental root

Interradicular placement of orthodontic Orthodontic micro-implant ss risks trauma to the periodontal ligament or the dental root. Dental roots damaged by orthodontic Orthodontic

micro-implant have demonstrated complete repair of tooth and periodontium in 12 to 18 weeks after removal of the Orthodontic micro-implants

In the maxillary buccal region, the greatest amount of interradicular bone is between the second premolar and the first molar, 5 to 8 mm from the alveolar crest. In the mandibular buccal region, the greatest amount of interradicular bone is either between the second premolar and the first molar, or between the first molar and the second molar, approximately 11 mm from the alveolar crest.

During interradicular placement in the posterior region, there is a tendency for the clinician to change the angle of insertion by inadvertently pulling the hand-driver toward their body, increasing the risk of root contact. To avoid this, the clinician may consider using a finger-wrench or work the hand-driver slightly away from their body with each turn. If the Orthodontic micro-implant begins to approximate the periodontal ligament, the patient will experience increased sensation under topical anesthesia. If root contact occurs, the Orthodontic micro-implant may either stop or begin to require greater insertion strength. If trauma is suspected, the clinician should unscrew the Orthodontic micro-implant or 3 turns and evaluate it radiographically.

Orthodontic micro-implant slippage

The clinician might fail to fully engage cortical bone during placement and in advertently slide the Orthodontic micro-implant under the mucosal tissue along the periosteum. Highrisk regions for Orthodontic micro-implant slippage include sloped bony planes in alveolar mucosa such as the zygomatic buttress, the retromolar pad, the buccal cortical shelf, and the maxillary buccal exostosis if present. Slippage in the retromolar pad can lead to the greatest risk of iatrogenic harm if the Orthodontic micro-implant moves lingually in the submandibular or lateral pharyngeal space near the lingual and inferior alveolar branch nerves. In the retromolar region, serious consideration should be given to flap exposure for direct visualization and a predrilled pilot hole, even for self-drilling Orthodontic micro-implants. Orthodontic micro-implant slippage can occur in dentoalveolar regions of attached gingiva if the angle of insertion is too steep. Placement of Orthodontic micro-implant less than 30° from the occlusal plane, typically to avoid root contact in the maxilla or to gain cortical anchorage in the mandible, can increase the risk of slippage. To avoid this, the clinician can initially engage bone with the Orthodontic micro-implant at a more obtuse angle before reducing the angle of insertion after the second or third turn.

Nerve involvement

Nerve injury can occur during placement of Orthodontic micro-implant in the maxillary palatal slope, the mandibular buccal dentoalveolus, and the retromolar region. Most minor nerve injuries not involving complete tears are transient, with full correction in 6 months. Long-standing sensory aberrations might require pharmacotherapy (corticosteroids), microneurosurgery, grafting, or laser therapy. Placement of Orthodontic micro-implant in the maxillary palatal slope risks injury to the greater palatine nerve exiting the greater palatine foramen. The greater palatine foramen is located laterally to the third molar or between the second and third molars. Orthodontic micro-implant inserted in the palatal slope should be placed medial to the nerve and mesial to the second molar.

Placement of the Orthodontic micro-implant in the mandibular buccal dentoalveolus risks injury to the inferior alveolar nerve in the mandibular canal. The mandibular canal travels forward in an S-shaped curve moving from buccal to lingual to buccal. The inferior alveolar nerve occupies its most buccal position within the body of the mandible at the distal root of the second molar and the apex of the second premolar, before exiting from the mental foramen. Orthodontic micro-implant inserted near the mandibular second molar and the second premolar is at greatest risk for accidental damage to the inferior alveolar nerve. Greater caution is needed in adult patients who might have a more occlusal position of the mandibular canal due to resorption of the alveolar ridge. Placement of Orthodontic micro-implant in the retromolar pad risks injury to the long buccal nerve and the lingual nerve. To avoid nerve involvement and slippage, we recommend that the retromolar Orthodontic micro-implant should be no longer than 8 mm and placed in the buccal retromolar region below the anterior ramus.

Air subcutaneous emphysema

Air subcutaneous emphysema is the condition in which air penetrates the skin or submucosa, resulting in soft-tissue distention. Subcutaneous emphysema can occur during routine operative dental procedures if air from the high-speed or air- water syringe travels under the gingival tissues. The main symptom of air subcutaneous emphysema is immediate mucosal swelling with or without crepitus (crackling). The clinician should be alert for subcutaneous emphysema during Orthodontic micro-implant placement through the loose alveolar tissue of the retromolar, mandibular posterior buccal, and the maxillary zygomatic regions. If a purchase point or pilot hole is to be drilled through the mucosa, the clinician should use slow speed under low rotary pressure.

In case of subcutaneous emphysema, the clinician should immediately discontinue the procedure and take periapical and panoramic radiographs to determine the extent of the condition. The patient should not be dismissed until the swelling begins to regress and an infection can be ruled out. Upon dismissal, the patient should be instructed to apply light pressure with an ice pack for the first 24 hours (Table). The clinician could prescribe a mild analgesic, an antibacterial rinse, such as chlorhexidine, and an antibiotic prophylaxis for a week. In most cases of subcutaneous emphysema, careful observation for further problems or infection is adequate and swelling and symptoms generally subside in 3 to 10 days

Price Protocol for Soft Tissue Swelling (Bleakley, 2007)

Protection	To the injured area
Rest	Avoid heavy mastication
Ice	Apply ice pack to the injury 20 minutes on and 20 minutes off every few hours on first day.
Compression	Apply compression with ice pack to minimize swelling
Elevation	Lie down, but keep injured area elevated

Nasal and maxillary sinus perforation

Perforation of the nasal sinus and the maxillary sinuses can occur during Orthodontic micro-implant placement in the maxillary incisal, maxillary posterior dentoalveolar, and zygomatic regions. A posterior atrophic maxilla is a major risk factor for sinus perforation. The

sinus floor is deepest in the first molar region and can extend to fill a large part of the alveolar process in posterior edentulous spaces. If the maxillary sinus has been perforated, the small diameter of the Orthodontic micro-implant does not warrant its immediate removal. Orthodontic therapy should continue, and the patient should be monitored for potential development of sinusitis and mucocele. For Orthodontic micro-implant placed in pneumatized, edentulous regions of the maxilla, or placed higher in the posterior maxilla when intrusive forces are desired, the clinician should consider angulating the Orthodontic micro-implant perpendicular to the alveolar ridge to avoid damage to the sinus

Orthodontic micro-implant bending, fracture, and torsional stress

Increased torsional stress during placement can lead to implant bending or fracture, or produce small cracks in the peri-implant bone, that affect Orthodontic micro-implant stability. Self-drilling Orthodontic micro-implant should be inserted slowly, with minimal pressure, to assure maximum Orthodontic micro-implants bone contact. A purchase point or a pilot hole is recommended in regions of dense cortical bone, even for self-drilling Orthodontic micro-implants. During Orthodontic micro-implant placement in dense cortical bone, the clinician should consider periodically derotating the Orthodontic micro-implant 1 or 2 turns to reduce the stresses on the Orthodontic micro-implant and the bone. The clinician should stop inserting the Orthodontic micro-implant as soon as the smooth neck of its shaft has reached the periosteum. When removing the hand-driver from the Orthodontic micro-implant head, the clinician should gently separate the hand driver handle from its shaft and then gently remove the shaft from the Orthodontic micro-implant head

COMPLICATIONS UNDER ORTHODONTIC LOADING

Stationary anchorage failure[2] is often a result of low bone density due to inadequate cortical thickness. Bone density is classified into 4 groups (D1, D2, D3, and D4) based on Hounsfield units (HU)—an x-ray attenuation unit used in computed tomography scan interpretation to characterize the density of a substance. D1 (>1250 HU) is dense cortical bone primarily found in the anterior mandible and the maxillary midpalatal area. D2 (850-1250 HU) is thick (2 mm), porous cortical bone with coarse trabeculae primarily found in the anterior maxilla and the posterior mandible. D3 (350–850 HU) is thin (1 mm), porous cortical bone with fine trabeculae primarily found in the posterior maxilla with some in the posterior mandible. D4 (150–350 HU) is fine trabecular bone primarily found in the posterior maxilla and the tuberosity region Sevimay et al reported that osseointegrated dental implants placed in D1 and D2 bone showed lower stresses at the implant-bone interface. D1-D3 bone is optimal for self-drilling Orthodontic micro-implants.

Classification of Bone Density

Bone Density	***Description***	***Tactile Analog***	***Location***
D1	Dense Cortical	Oak	Anterior Mandible Maxillary Midpalatal
D2	Porous Cortical/ Coarse Trabecular	White Pine/ Spruce	Anterior Maxilla Posterior Mandible
D3	Porous Cortical/ Fine Trabecular	Balsa Wood	Posterior Maxilla Posterior Mandible Zygoma
D4	Fine Trabecular	Styrofoam	Posterior Maxilla Tuberosity

Placement of Orthodontic micro-implant in D1 and D2 bone might provide greater stationary anchorage under orthodontic loading. Placement of Orthodontic micro-implant in D4 bone is not recommended due to the reported high failure rate. In general, stationary anchorage failure is greater in the maxilla, with the exception of the midpalatal region, due to the greater trabeculae and lower bone density Peri- implant soft-tissue type, health, and thickness can affect stationary anchorage of the Orthodontic micro-implants. Orthodontic micro-implant placed in nonkeratinized alveolar tissues have greater failure rates than those in attached tissues.

Primary stability refers to the movement, or the lack thereof, of Orthodontic micro-implant upon initial placement. A lack of primary stability almost routinely leads to overt Orthodontic micro-implant mobility, with subsequent failure. Recent evidence suggests that the majority of primary Orthodontic micro-implant stability comes from cortical bone, with lesser stability coming from medullary bone. Upon placement, Orthodontic micro-implant should have at least 0.5 mm to 0.75 mm of available bone stock around its circumference. Because most Orthodontic micro-implant are intended to be placed and loaded at the same visit, the Orthodontic micro-implant must have adequate cortical bone purchase and exhibit no mobility.

Another reason for inadequate primary stability is an overdrilled pilot hole. This problem is more likely in areas of thin cortical bone. The main reason for hole over enlargement is the clinician's inability to hold the handpiece stable and perpendicular to the bone surface during drilling. Any lateral movement during drilling will over enlarge the pilot hole. Excessive trauma during implant surgery is considered an important cause of implant failure. During a pilot-hole osteotomy, most of the energy not used in the cutting process is transformed into heat. Heat production leading to a temperature rise above 47°C for more than 1 minute negatively affects living bone and compromises its regeneration. Complications in this area are best avoided by using drill-free screws.

Orthodontic micro-implant can remain clinically stable but not absolutely stationary under orthodontic loading. Unlike an endosseous dental implant that osseointegrates, orthodontic Orthodontic micro-implant achieve stability primarily through mechanical retention and can be displaced within the bone. Liou et al reported that orthodontic Orthodontic micro-implant loaded with 400 g of force for 9 months extruded and tipped –1.0 to 1.5 mm in 7 of 16 patients. To account for potential migration, the clinician should allow a 2-mm safety clearance between the Orthodontic micro-implant and any anatomical structures.

SOFT-TISSUE COMPLICATIONS

Aphthous ulceration

Minor aphthous ulcerations, or canker sores, can develop around the Orthodontic micro-implant shaft or on the adjacent buccal mucosa in contact with the Orthodontic micro-implant head. Placement of a healing abutment, a wax pellet, or a large elastic separator over the Orthodontic micro-implant head, with daily use of chlorhexidine (0.12%) or Povidine Iodine (3-5 %), typically prevents ulceration and improves patient comfort. The occurrence of an aphthous ulceration does not appear to be a direct risk factor for Orthodontic micro-implant stability, but its presence might forewarn of greater soft tissue inflammation.

Soft-tissue coverage of the Orthodontic micro-implant head and auxiliary

Orthodontic micro-implant placed in alveolar mucosa, particularly in the mandible, might become covered by soft tissue. The bunching and rubbing of loose alveolar tissue can lead to coverage of both the Orthodontic micro-implant head and its attachments (ie, coil spring, elastic chain) within a day after placement. Soft-tissue coverage might be a risk factor for Orthodontic micro-implant stability, as well as a clinical concern for the patient, who might think that the Orthodontic micro-implant has fallen out. Orthodontic micro-implant attachments (elastic chain, coil spring) that rest on tissues will likely become covered by tissue. The soft-tissue overlaying the Orthodontic micro-implant is relatively thin and can be exposed with light finger pressure, typically without an incision or local anesthetic. Soft-tissue overgrowth can be minimized by placement of a healing abutment cap, a wax pellet, or an elastic separator. In addition to its antibacterial properties that minimize tissue inflammation, chlorhexidine slows down epithelialization and might reduce the likelihood of soft-tissue overgrowth. The authors suggest partial insertion with a longer Orthodontic micro-implant (10 mm) in regions of loose alveolar mucosa, leaving 2 or 3 threads of the shaft exposed to minimize the possibility of soft-tissue coverage.

Soft tissue inflammation, infection, and peri-implantitis

Healthy peri-implant tissue plays an important role as a biologic barrier to bacteria. Tissue inflammation, minor infection, and peri-implantitis can occur after Orthodontic micro-implant placement. Inflammation of the periimplant soft tissue has been associated with a 30% increase in failure rate. The clinician should be forewarned of soft-tissue irritation if the soft tissues begin twisting around the Orthodontic micro-implant shaft during placement. Some clinicians advocate a 2-week soft-tissue healing period for Orthodontic micro-implant placed in the alveolar mucosa before orthodontic loading.

COMPLICATIONS DURING REMOVAL

Orthodontic micro-implant fracture

The Orthodontic micro-implant head could fracture from the neck of the shaft during removal. The authors recommend a minimum diameter of 1.6 mm for self-drilling Orthodontic micro-implant that are 8 mm or longer placed in dense cortical bone. The proper placement technique can minimize the risk of Orthodontic micro-implant fracture during its removal. If the Orthodontic micro-implant fractures flush with the bone, the shaft might need to be removed with a trephine.

Partial osseointegration

Although orthodontic Orthodontic micro-implant achieve stationary anchorage primarily through mechanical retention, they can achieve partial osseointegration after 3 weeks, increasing the difficulty of their removal. The Orthodontic micro-implant typically can be removed without complications a few days after the first attempt of removal.

Conclusion

The concept of temporary anchorage devices is a relatively new application of more established clinical methodologies. Although the clinician can look to the literature for many answers, much is unknown and will only be answered by well-designed prospective basic science and clinical trials. The future development of temporary anchorage devices for orthodontic

anchorage will establish a more complete understanding of the biology and biomechanics associated with both osseointegrated and nonintegrated TADs.

As, to achieve absolute orthodontic anchorage has been one of the dreams of most orthodontic clinicians, and using the micro-implants has given as the most effective, powerful way to get the anchorage we want to achieve. Therefore, application of micro-implant will make the treatment procedure easier, and so there is no doubt that it is one of the most useful methods to provide absolute orthodontic anchorage but with caution.

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Oral Lichen Planus- of treatment Modalities

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Abstract

Lichen planus (LP) is one of the most common mucocutaneous disease that manifests itself in the oral cavity and is of worldwide distribution. Its management continues to challenge even the most experienced oral physician. Interplay of host, lifestyle and environmental factors has been implicated in the etiopathogenesis of LP. It is believed that LP is caused by cell mediated immunity initiated by endogenous or exogenous factor. Oral discomfort is the cause of concern in patients with oral lichen planus (OLP). Symptoms can vary from mucosal sensitivity to continuous debilitating pain. As large no. of agents have been used to treat OLP but still search is going on for complete solution for this disease. Oral health care professionals can play a vital role in identifying patients with OLP and should provide appropriate preventive and therapeutic measures that will help to preserve a person's health function and quality of life. The purpose of this review is to discuss various treatment modalities available, which will assist the clinician to manage the OLP patients.

Keywords: - Oral lichen planus; oral mucous membrane.

Introduction

Different conditions of local and systemic origin are manifested in the oral cavity; many of them with controverted and or multifactorial etiology.[1]

Oral Lichen Planus (OLP) is a chronic inflammatory condition that affects the oral mucous membranes with a variety of clinical presentations.[2] The oral eruptions usually have a distinct clinical morphology and characteristic distribution but may also present a wide array of patterns and forms.[3] The cause of the disease is unknown, but the possibility that immunologic factors are involved has been considered.[4] Speculated cofactors in causation such as stress, diabetes, hepatitis C, trauma and hypersensitivity to drugs and metals have varying degree of support.[5]

Oral lichen planus (OLP) can be defined as a common chronic immunologic inflammatory mucocutaneous disorder that varies in appearance from keratotic (reticular or plaque like) to erythematous and ulcerative lesions.[5]

Although the disease was described over 100 years ago, the etiology of lichen planus still remains a mystery involving a possible interaction between genetic, environmental and life style factors.[6] It is still unclear whether OLP represents a single disease or manifestation of

several closely related conditions.[9] The importance of this disease related to its degree of frequency of occurrence and its occasional painful nature.[10] The possible malignant transformation risk still remains controversial.[9]

The disease poses challenge for both the dentist as well as patient. As it is a chronic disease, complete remission are either non existent or infrequent especially in patients with erosive disease. Unpredictable and frequent exacerbations are common and in rare instances continuous pain can be disabling.[11]

Currently, there is no cure for OLP. A large number of agents studied for this disease reflect the inadequacy of any one agent to control the symptoms in all patients. A more thorough understanding of etiology, pathogenesis, clinical presentation and possible malignant transformation may lead to treatment protocol that are accepted uniformly and effective universally in each form of OLP.[11]

Review of Treatment modalities of OLP

To date, no cure for OLP or its dermal counterpart exists.[13, 14] The aim of current OLP therapy are to eliminate mucosal erythema and ulceration, alleviate symptoms and reduce risk of oral cancer.[15, 16]

A large number of agents used in management of the disease reflects the inadequacy of any agent to control symptoms in all patients and indicative of continuing search for solution.[3,12]

General Considerations

Oral Hygiene Maintenance

Due to difficulty in maintaining oral hygiene, accumulation of dental plaque and calculus takes place and may influence the course of LP. Kovesi G, Banoczy J (1973)[13] and Holmstrup P, Schiotz AW, Westergarrd J (1990)[17] found significant improvement in symptoms of OLP after adequate plaque control.

Other Factors

Mechanical trauma of dental procedure, friction from sharp cusps, rough dental restoration and poorly fitting dental prosthesis can be exacerbating factors of OLP and should receive attention.[18]

Various treatment modalities have been designed to improve management of symptomatic oral LP and they are ;

1) Corticosteroids

They are the main stay of treatment of OLP because of their activity in dampering cell mediated immune activity and can be administered topically, intralesionally or systemically.[16]

a) Topical Corticosteroids

They are the most commonly used agents for the treatment of lichen planus and may be applied as ointments, pastes, lozenges, mouth washes or through inhalers with special adaptors.[19]

Different agents like Hydrocortisone hemisuccinates, Betamethasone valerate, Triamcinolone acetonide, Fluocinolone acetonide, Fluocinonide and Clobetasol propionate have been used.[11,16, 20, 21, 22, 23]

In recent years fluorinated and so called "super potent" corticosteroids (Fluocinolone acetonide, Fluocinonide) due to their high anti-inflammatory property have become popular for oral vesiculo erosive diseases including OLP.[11]

Thongprasom K, Luangjarmekorn L, Sererat T, Taweesap W (1992)[23] conducted a study to find out relative efficacy of fluocinolone acetonide compared with triamcinolone acetonide in treatment of OLP. Fluocinolone was found to be effective in a majority of cases without serious side effects and was better than triamcinolone.

b) Intralesional corticosteroid

Intralesional injection of steroids have been found effective and can reduce the symptoms in OLP patients.[76] Eisen D[11] reported that Sleeper (1967) supplemented therapy with intralesional triamcinolone acetonide suspension. 7 patients received 5-7 mg of intralesional acetonide. All patients experienced relief of symptoms within 2 weeks, 3 showed complete healing of lesion and 4 showed dramatic clinical improvement.

Zagarelli DJ (1983)[81] used both topical and weekly intralesional corticosteroid in 7 patients suffering from OLP. After 3 weeks 5 patients were graded as having 100% clinical improvement. A remission of several months was noted in most cases and recurrence was milder than original disease.

c) Systemic Corticosteroid

Systemic corticosteroids are of great value when there has been an acute exacerbation of symptoms and are often used in combination with topical corticosteroids.[18] Both methyl prednisolone and prednisone have been employed and used in high doses of 1.5 – 2 mg/kg/day.[18]

Vincent SD, Fotos PG, Baker KA, Williams TP (1990)[20] stated in their article that if systemic prednisolone is deemed necessary it is advisable to gain disease control by administering relatively high doses for upto 7 days. Prednisolone doses of 40 mg given upon arising as a single dose for 5-7 consecutive days seldom result in appreciable pituitary-adrenal suppression. No tapering is necessary at the end of "prednisolone burst" and alternate day morning therapy was initiated if systemic therapy was considered a necessity.

Carbone M, Goss E, Carrozzo M, Castellano L, Conrotto L, Broccoletti A et al (2003)[25] in their comparative study on systemic and topical corticosteroid treatment of OLP with long term follow up found that there was no significant difference in improvement of both signs and symptoms and in disease free period between 2 groups. They suggested that topical high potency corticosteroid should be mainstay treatment for most OLP patients and systemic corticosteroid should be used when topical approaches have failed.

2) Antifungals

Candida Albicans is present in about 37% of OLP lesions and symptoms of OLP may be exacerbated by candidal overgrowth or infection.[18] Various antifungal agents like Griseofulvin, Nystatin, Ketoconazole and Clotrimazole have been used to reduce the symptoms

Aufdemorte, DeVillez and Gieseker (1983)[26] in their case report reported 3 cases of severe erosive LP that were treated with 500 mg BD griseofulvin and showed remarkable

response, however the response interval varied with patients.

However Bagan *et al* (1985)[27] and Naylor GD (1990)[85] found that griseofulvin had little or no effect on pain, pigmentation changes, disappearance of lesion and did not protect patient from future recurrence.

Vincent *et al* (1990)[20] reported that out of 25 cases of OLP suffering from secondary candidiasis, 19 were treated with ketoconazole 200 mg for 14 days, 2 with nystatin ointment and 4 were treated with clotrimazole oral troches. 21 patients reported resolution of their discomfort and showed clinical evidence of remission after 2-6 weeks, 2 had partial remission and 2 continued to have oral symptoms.

3) Cyclosporin

Due to its property of suppressing T cell cytokine production it may be useful in treatment of OLP.[18]

Voute *et al* (1994)[29] conducted an open trial in which cyclosporine A in an adhesive base was used for treatment of recalcitrant OLP. Out of 9 patients included in the study 4 showed partial response but none of patients had complete remission.

Harpenau *et al* (1995)[31] conducted a study on 24 OLP patients to examine the efficacy of low dose cyclosporine 500 mg/day in treatment. All experimental sites exhibited enhanced healing and decreased pain score as compared to control sites over a period of 4 weeks.

Jungell and, Malmstrom (1996)[31] conducted a study on 7 patients with long standing atrophic or erosive LP who were treated for 4 weeks with cyclosporine A as mouth wash (1 mg solution containing 100 mg per ml cyclosporine). At the end of 3 months of treatment no improvement was noticed.

4) Retinoids

The retinoids have anti-inflammatory properties, perhaps through their interactions with arachidonic acid cascade; they stimulate macrophage activation and antibody dependent cell mediated cytotoxicity. Retinoids may also reduce the CD4 lymphocyte infiltrate and increases the macrophages in OLP thus accelerating the healing process. For this reason synthetic and natural analogues (retinoids) may be useful in treatment of OLP.[89]

Camisa and Allen (1986)[33] conducted a study to evaluate the effectiveness of isotretinoin 10-60 mg/day in treatment of symptomatic oral erosive LP for 8 weeks in 6 patients. Improvement was seen in 5 (83%) patients at completion of therapy but no patient was completely cured and within 2 months on stopping isotretinoin 4 patients had relapses.

Baudet-Pommel *et al* (1991)[34] conducted a study on 25 patients to compare different proportions of inflammatory cell subpopulation before and after treatment with 2 types of aromatic retinoids, topical tretinoin and systemic etretinate as compared to untreated controls. The results suggested that progression under two retinoids was similar to spontaneous evolution. However retinoid treatment resulted in accelerated healing with faster renewal of cellular population and greater deterrence of the lesion.

Gorsky M, Raviv and (1992)[35] conducted a study on 6 patients to evaluate the maximum

permitted dose of etretinate (75 mg/day) as first mode of systemic treatment in patients with symptomatic OLP. But follow up of more than year showed that the patients had reverted to same frequency of recurrence as before the systemic use of etretinate.

5) Tacrolimus

Tacrolimus is a potent immuno-suppressive agent and can control symptoms and significantly improve refractory erosive LP.[18]

Olivier (2002)[36] and Kaliakatsou (2002)[37] conducted studies on OLP patients to investigate the efficacy and safety of 0.1% topical tacrolimus in erosive and ulcerative OLP and suggested that tacrolimus have rapid and significant effect in patients with erosive OLP that are refractory to other therapies.

6) Ultraviolet Irradiation

Ultraviolet irradiation, mainly in combination with psoralens, may suppress the cell mediated immune reactivity and thus forms the basis for use in lichen planus.[32]

Lundquist *et. al.* (1995)[38] in their controlled study investigated the treatment of severe oral lichen planus in 81 patients with 8 methoxypsoralen and long wave ultraviolet light. The result showed that 13 treated sites compared with 6 control sites responded significantly favorably to PUVA therapy.

7) MISCELLANEOUS TREATMENT

A) Antibiotics

Ronbeck *et. al.* (1990)[39] reported in their study that doxycycline monohydrate 100 mg/day for 6-8 weeks was used for the treatment of 14 patients suffering from desquamative gingivitis, out of which 6 were suffering from OLP. The group as a whole demonstrated a decreasing mucosal index and significant improvement after therapy.

Walchner (1999)[40] reported significant improvement in 1 patient treated with 250 mg capsule of tetracycline suspended in 100 ml of water and gargled for total of 15 minutes 2-3 times/day. After 6 weeks erosive lesion disappeared and reepithelization was seen.

B) Antimalarials

Eisen (1993)[41] treated 10 patients with OLP with hydroxychloroquine sulphate (Plaqunenil) an antimalarial agent. 7 of 10 patients improved by 50% or more. It was interpreted that hydroxychloroquine may be useful in treatment of OLP.

C) Azathioprine

Lozada (1981)[42] conducted a study to assess clinically the synergistic effect of azathioprine with prednisolone. Results of the study showed effective doses of prednisolone when combined with azathioprine were markedly lower and concluded azathioprine may be a successful steroid sparing adjunct.

D) Dapsone

Lodi *et. al.*(2005)[18] quoted in their study that dapsone has been used in treatment of erosive OLP with some benefits, but generally the use of dapsone is precluded.

E) Topical anesthetic

Dusek and Frick (1982)[6] in their article reported 40 ml of 0.01% synalar solution with 60

ml Benadryl elixir was found to be effective in relieving symptoms of the painful chronic lichen planus.

F) Interferon

They are being used as therapeutic agents because of their antiviral, antiproliferative and immunomodulatory effects.

Sato *et. al.* (1985)[43] conducted a pilot study on therapeutic effect of human fibroblast interferon on premalignant lesion (OLP and leukoplakia) arising in oral cavity and concluded that human interferon has a therapeutic effect on premalignant lesion.

Hildebrand *et. al.* (1995)[44] in their case report reported successful treatment of 3 patients suffering from generalized LP with recombinant interferon alfa 2b.

f) Levamisole

It has used as an immunomodulator in oral LP.[18]

Lu *et. al.* (1995)[45] found that levamisole 150 mg/day for 3 consecutive days in a week together with longer course of low dose systemic prednisolone 15 mg/day left patient symptom free for 6-9 months.

g) Mesalazine

Sardella *et. al.* (1998)[46] in his study found out that when topical mesalazine was compared with clobetasol propionate for treatment of symptomatic OLP, it was as effective as topical steroid.

h) Phenytoin

Bogaert and Sanchez (1990)[47] conducted a study on 30 OLP patients to evaluate systemic phenytoin as therapeutic modality on the basis of its ability to promote wound healing, moderate immune function and act as antipruritic agent. Phenytoin was given 100-200 mg daily. 40% had complete resolution of LP and 33% had substantial improvement. The result suggested a therapeutic role of phenytoin in OLP patients.

i) Surgery

Surgical excision has been recommended for isolated plaques or non healing erosions. Conventional surgical excisions, cryosurgery and CO_2 lasers all have been used.[18, 48, 49, 50]

CONCLUSION

Without adequate treatment the oral health declines along with person's quality of life. Diagnosis of OLP begins with careful medical history, examination of oral mucosa as well as skin. OLP can manifest without symptoms, therefore in routine examination oral mucosa should be thoroughly examined. Therapies are designed to prevent the recurrence and to relieve the symptoms of OLP. Current OLP treatment includes corticosteroids, antifungal, retinoid, tracrolimus, immunomodulators etc. However new research is required to find a complete cure of OLP. Efforts are going on but still in very early stage. Oral health professionals can play a vital role in identifying patient's with OLP and should provide appropriate preventive and therapeutic treatment that will help to preserve a person's health, function and quality of life.

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Dental Caries and Risk Assessment : A Review of Preventive Strategies and Management

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Abstract

Assessing risk for disease development is an important component of any disease prevention program. Risk susceptibility can be determined on a variety of levels, including community, individual, tooth and tooth surface. Therefore, an oral health risk assessment before 1 year of age affords the opportunity to identify high-risk patients and to provide timely referral and intervention for the child and allows an invaluable opportunity to decrease the level of cariogenic organisms in the mother with a significant caries risk before and during colonization of the infant. Caries risk is not stagnant in a patient and can vary from one point of time in his or her life to another. Such variation in susceptibility requires ongoing monitoring by the oral health care professional, since changes in health status, use of medications and other lifetime events can increase risk. This article reviews risk assessment for dental caries and the implication for developing preventive strategies.

Introduction

Dental problems such as tooth decay, periodontal disease and tooth loss, constitute a major public problem, in the world today.[1] In order to prevent oral health problems, the American Dental Association (ADA) and other organizations recommended that adults should thoroughly brush and floss their teeth at least once a day and get regular oral health check-ups.[2,3]

A key component of any preventive program is to assess a person's risk of developing a disease. In the case of dental caries, it is suggested that a risk profile be performed on a number of levels: community, individual, tooth and tooth surface.[4] This expanded approach considers risk implications from various factors that could influence carious activity and may help dental professionals better manage patients from a preventive perspective. More than 40% of children have tooth decay by the time they reach kindergarten.2 Infants who are of low socioeconomic status, whose mothers have a low education level, and who consume sugary foods are 32 times more likely to have caries at the age of 3 years than children in whom those risk factors are not present.[5] The risk for caries development varies significantly for different populations, age groups, individuals, teeth, and surfaces. Therefore caries-preventive measures must be integrated and based on predicted risk from age groups down to the individual tooth surfaces.

Goals

Based on this philosophy and on experiences from continuously ongoing researches evaluating and re-evaluating separate and integrated caries-preventive measures, as well as methods for prediction of caries risk, a needs related caries preventive program for 0- to 19-year-olds was introduced in the county of Varmland in 1979 .[6-8]

The goals for the subjects following the program from birth to the age of 19 years were:

1. To have no approximal restorations.
2. To have no occlusal amalgam restorations.
3. To have no approximal loss of periodontal attachment.
4. To motivate and encourage individuals to assume responsibility for their own oral health.

It was hoped that these goals would be attained for 19- year-old participants by 1999. The effect of the program is evaluated once every year on almost 100% of all 3 to 19-year-olds in a computer-aided epidemiologic program from 1979.[9,10]

Risk Groups for Dental Caries

Every child should begin to receive oral health risk assessments by 6 months of age by a qualified pediatrician or a qualified pediatric health care professional. In the case of the very young patient, a risk assessment to identify parents (usually mothers) and infants with a high predisposition to caries can easily be performed by taking a simple dental history from a new mother. An infant is assessed to be within 1 year of the following risk groups, the care requirements would be significant and surgically invasive; therefore, these infants should be referred to a dentist as early as 6 months of age and no later than 6 months after the first tooth erupts or 12 months of age (whichever comes first) for establishment of a dental home:

Establishing the Dental Home

The concept of the "dental home" is derived from the American Academy of Pediatrics concept of the "medical home." The American Academy of Pediatrics states, "the medical care of infants, children, and adolescents ideally should be accessible, continuous, comprehensive, family centered, coordinated, compassionate, and culturally effective. It should be delivered or directed by well-trained physicians who provide primary care and help to manage and facilitate essentially all aspects of pediatric care."[11] Pediatric primary dental care needs to be delivered in a similar manner. The dental home is a specialized primary dental care provider within the philosophical complex of the medical home. Referring a child for an oral health examination by a dentist who provides care for infants and young children.

Oral Health Risk Assessment and the Dental Home

Six months after the first tooth erupts or by 12 months of age establishes the child's dental home and provides an opportunity to implement preventive dental health habits that meet each child's unique needs and keep the child free from dental or oral disease.

The dental home should be expected to provide:

1- An accurate risk assessment for dental diseases and conditions
2- An individualized preventive dental health program based on the risk assessment
3- Anticipatory guidance about growth and development issues (ie, teething, digit or pacifier

habits, and feeding practices)

4- A plan for emergency dental trauma
5- Information about proper care of the child's teeth and gingival tissues
6- Information regarding proper nutrition and dietary practices
7- Comprehensive dental care in accordance with accepted guidelines and periodicity schedules for pediatric dental health
8- Referrals to other dental specialists, such as endodontists, oral surgeons, orthodontists, and periodontists, when care cannot be provided directly within the dental home.

Anticipatory Guidance and Parent and Patient Education

General anticipatory guidance for the mother (or other intimate caregiver) before and during the colonization process should include the following:

Oral hygiene—Parents should be instructed to brush thoroughly twice daily (morning and evening) and to floss at least once every day.

Diet—Parents should be instructed to consume fruit juices only at meals and to avoid all carbonated beverages during the first 30 months of the infant's life.

Fluoride— Parents should be instructed to use fluoride toothpaste approved by the American Dental Association and rinse every night with an alcohol-free over-the-counter mouth rinse with 0.05% sodium fluoride.

Caries removal—Parents should be referred to a dentist for an examination and restoration of all active decay as soon as feasible.

Delay of colonization—Mothers should be educated to prevent early colonization of dental flora in their infants by avoiding sharing of utensils (ie, shared spoons, cleaning a dropped pacifier with their saliva, etc).

Xylitol chewing gums—Recent evidence suggests that the use of xylitol chewing gum (4 pieces per day by mother) had a significant impact on decreasing the child's caries rates.[12]

General anticipatory guidance for the young patient

(0 to 3 years of age) should include the following:

Oral hygiene—the parent should begin to brush the child's teeth as soon as they erupt (twice daily, morning and evening) and floss between the child's teeth once every day as soon as teeth contact one another.

Diet—after the eruption of the first teeth, the parent should provide fruit juices (not to exceed 1 cup per day) during meals only. Carbonated beverages should be excluded from the child's diet. Infants should not be placed in bed with a bottle containing anything other than water. Ideally, infants should have their mouths cleansed with a damp cloth after feedings.

Fluoride—all children should have optimal exposure to topical and systemic fluoride. Caution should be exercised in the administration of all fluoride-containing products. The specific considerations of the judicious administration of fluoride should be reviewed and tailored to the unique needs of each patient. Review articles with applicable fluoride recommendations and supplementation algorithms are available.[13-16]

Recommendations

1. Early childhood caries is an infectious and preventable disease that is vertically transmitted from mothers or other intimate caregivers to infants.
2. All health care professionals who serve mothers and infants should integrate parent and care giver education into their practices that instruct effective methods of prevention of early childhood caries.
3. The infectious and transmissible nature of bacteria that cause early childhood caries and methods of oral health risk assessment, anticipatory guidance, and early intervention should be included in the curriculum of all pediatric medical residency programs and postgraduate continuing medical education curricula at an appropriate time.
4. Every child should begin to receive oral health risk assessments by 6 months of age from a pediatrician or a qualified pediatric health care professional.
5. Pediatricians, family practitioners, and pediatric nurse practitioners and physician assistants should be trained to perform an oral health risk assessment on all children beginning by 6 months of age to identify known risk factors for early childhood dental caries.
6. Infants identified as having significant risk of caries or assessed to be within 1 of the risk groups listed in this statement should be entered into an aggressive anticipatory guidance and intervention program provided by a dentist between 6 and 12 months of age.
7. Pediatricians should support the concept of the identification of a dental home as an ideal for all children in the early toddler years.

Summary

Dental caries is a bacteria-dependent, multifactorial disease, preventive measures such as sealants, can be implemented once those at risk are identified. Diagnostic tests and preventive therapies will be critical in the dental practice of the future, where health and wellness will be the primary goals. Dental caries is 5 times more common than asthma and 7 times more common than hay fever in children.[17] Early childhood dental caries emerges within all cultural and economic populations. Diagnostic tests and preventive therapies will be critical in the dental practice of the future, where health and wellness will be the primary goals.

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Bioconversion of Agricultural Wastes for Production of Milky Mushroom (*Calocybe indica*)

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Absract

In order to study the effect of different substrates on the growth and yield of *C. indica*, an experiment was carried out with four different substrates viz., wheat straw, paddy straw, chopped leaf of sorghum and saw dust. The substrate of paddy straw was found significantly superior than other substrates by producing (955.66 gm/bag). The mixture of paddy straw + wheat straw was second best substrate where it produced (893.66 gm) mushroom/bag followed by wheat straw (793.67 gm), mixture of wheat straw + chapped leaves of sorghum (584.33 gm) and mixture of mixture of saw dust + wheat straw (535.33 gm). Spawn run period between (23- 27days), initiated of pinhead also recorded in the range of (45 – 52 days). Then the crop was harvested at the stage of complete mature fruit bodies. The Ist flush was harvested in the range (34-37days). Maximum No. of fruit bodies per bag were obtained from paddy straw substrate. Paddy straw provide better yield among them. Paddy straw and wheat straw was highly significant. Thus, paddy straw was best substrate for the cultivation of the milky mushroom (*Calocybe indica*) because it was given better yield and good quality than the other substrate.

Key words: *Calocybe indica*, substrates, growth and yield

Introduction

Huge quantities of agricultural wastes and other organic wastes are generated annually through the activities of agricultural, forest and food processing industries. These agricultural wastes and other organic wastes are abundantly available in our country. In India, during 1993-94 production of wheat straw and paddy straw was approximately 110.6 million tones and 153.6 million tones respectively (Mathew 1996). The lignocellulosic wastes obtained from the production edible mushrooms will used as an animal nutritive feed stock. Among the various physical, chemical and biological methods used for upgrading the digestibility and nutritive value of agricultural wastes, biodegradation by using white rot fungi including mushrooms have been found promising. The species of Pleurotus has ability to secrete hydrolyzing and oxidizing enzymes (Toyama & Ogawa, 1974; Daugulis and Bone, 1977; and Rajarathnam *et al.*, 1979) which enables them to grow and flourish over wide range of natural lignocellulosic waste materials.

Besides it is estimated that about 355 million tonnes of agricultural wastes are generated

annually and about half (170 millons) of this residue is left out for burning and incorporating soil in manure form. Recently about 385 million tones of agricultural wastes are available in India and half of these agricultural wastes unused. Even if one per cent of this crop residue is used to produce mushrooms, that will help in residues management and India will become a major mushroom producing country in the world (Tewari and Pandey, 2002). The lignocellulosic wastes obtained from the production edible mushrooms will used as an animal nutritive feed stock. Among the various physical, chemical and biological methods used for upgrading the digestibility and nutritive value of agricultural wastes, biodegradation by using white rot fungi including mushrooms have been found promising. The species of Pleurotus has ability to secrete hydrolyzing and oxidizing enzymes (Toyama & Ogawa, 1974; Daugulis and Bone, 1977; and Rajarathnam *et al.*, 1979) which enables them to grow and flourish over wide range of natural lignocellulosic waste materials. The major parts of these agricultural wastes are bunt after harvesting, resulting multifaceted hazards including oxygen defiant environment, respiratory diseases such as allergic, bronchitis, asthama, tuberclusis and poor visibility at night. The useful insects, bio-agents, earthworm and soil microbe are also reduced due to burning of agricultural wastes and its heating on soil surface. According to survey, in some places these agricultural wastes are spreads on the cultivated land but it is not well decomposed and they created the problems during farming. The main concerned of our planners to remove these air pollution and soil pollution for environmental protection. This can be done by scientific utilization of resources and bioconversion for mushroom production.

Around 800 million people living in 46 countries are malnourished, 40,000 die everyday of hunger and hunger related diseases (Swaminathan 1995). In this context the mushroom cultivation represent one of the economically viable processes for the bioconversion of agricultural and agro-industrial wastes in to protein rich food making it a potent weapon against malnutrition in developing countries like India which has lowest per capita consumption of protein in the world (Sohi, 1982, Wood, 1989, Chang & Miles 1989, Buswell & Chang, 1993).

In this regard, Poppe (2000) have to considered the multilateral message of agricultural wastes conversion into mushrooms in the following categories:

1. Provision of food
2. Creation of jobs
3. Enhancement of family income
4. Control burning of waste and curb global warming
5. Lowering air pollution and CO2 level
6. Cleaning the field, road sides and forest margins
7. Protection of natural mushrooms flora
8. Forest fire prevention
9. Spent mushroom substrates for garden compost bioremediation purposes

Numerous reasons for growing more mushrooms by agricultural residues and by-products as approved by Chang & Miles 1989; Stamets 1993 & Poppe 1995. -

1. Decreasing the mountain range of wastes.
2. Most mushrooms are fast productive and can be grown allover the year in temperate zones with heating in winter and in tropical zones with protection against dry hot seasons.

3. No fight for field is necessary because mushroom cultivation needs only small spaces and can even grow in vertical layers or on the floor in forests where they don't need one centimeter extra place.
4. Converting the wastes in to mushroom proteins and vitamins, while also mushrooms represent one of the world's greatest untapped sources of tasteful food in the future. And let us not forget the medicinal value of mushrooms species.
5. Mushroom cultivation is labour intensive but labours are readily available in most tropical regions.

The milky mushroom (*Calocybe indica*) was first reported from India by Purkayastha and Chandra in 1974. This mushroom may be successfully cultivated during summer months and therefore, designated as summer mushroom also. It has stout fleshy, milky white; large sporophores with long shelf life. Consumers are liked this mushroom due to its attractive shape, colour and delicious flavour. This mushroom normally grows on the humus rich soil under road side trees and in agricultural fields. In some places they called as “Kudhu” but, popularly known as “Dudhi Chhata”. In rainy season, wild forms of *C. indica* were sold in Calcutta and their edibility was confirmed by Purkaystha and Chandra (1974), Purkyastha (1979), Natural occurrence of *C. indica* in plains of Tamil Nadu and Rajasthan has also been reported by Krishnamoorthy, 1995 and Doshi *et al.*, 1989 respectively.

The nutritive value of *Calocybe indica* is comparable with other mushrooms. Dried sporophores of C. indica contains 17.69 per cent protein, 4.1 per cent fat, 3.4 per cent crude fibre and 64.26 per cent carbohydrate. Matured sporocarps contain 4 per cent soluble sugars, 2.9 per cent starch and 7.43 per cent ash. In addition to this, it has most of the mineral salts required by human body such as potassium, sodium, phosphorous, iron and calcium. Due to its alkaline and higher fibre content; it highly suitable for people with hyperacidity and constipation (Doshi et al., 1988). Inspite of sincere efforts made by different workers only limited success was achieved on the cultivation of this mushroom until 1998. Krishnamoorthy (1995) and Krishnamoorthy et al. (1997) identified a potential strain of *C. indica* occurring in a sugarcane fields near Coimbatore. Later it was released as a new variety called APK-2 from Tamil Nadu Agricultural University and started cultivation under artificial condition (Krishnamoorthy *et al.*, 1998). Cultivation of milky mushroom is becoming popular in Tamil Nadu, Andhra Pradesh, Karnatake, Rajasthan and West Bengal.

Food and Nutritional value of Mushrooms

Mushrooms were believed by the Greeks to give strength to the warriors while Romans regarded them as the 'Food of the Gods'. The Chinese prized mushrooms as health food, the 'elixir of life' etc. In developing countries malnutrition is one of the major problems because most of the population remains under the economic line. Mushrooms are considered to be healthy food because of their relatively high and qualitatively good protein content and because of their good vitamins, minerals and low fat content (Table-1). Mushrooms have been recommended by FAO as food that contributes to the protein nutrition of developing countries which depend largely on cereals.

Mushrooms are recognized all over the world as a good source of protein, vitamins and

minerals for human consumption. It has been used for their medicinal and tonic properties, they have proved beneficial for the patients suffering from hypertension, diabetes and had ailments. Mushrooms contain all the essential amino acids well as the most commonly occurring non-essential amino acid and amides. Mushrooms are good source of vitamins such as vitamin 'B_1' (Thiamine), vitamin 'B_2' (Riboflavin), niacin, biotin and vitamin 'C' (Ascorbic acid). Mushrooms are also good source of minerals such as potassium, phosphorus and sodium and contain low but available form of Iron, Potassium and Sodium ratio is very high which is desirable for patients of hypertension.

Table 1: Proximate composition (Per cent fresh weight) of the cultivated mushrooms

Mushrooms /Vegetable	Moisture	Protein	Fat	Carbohydrate	Fibre	Ash	Calorie
Agaricus bisporus	90.1	2.9	0.3	5.0	0.9	0.8	36
Pleurotus sajor-caju	90.2	2.5	0.2	5.2	1.3	0.6	35
Volvariella volvacea	90.1	2.1	1.0	4.7	1.1	1.0	36
Cabbage	91.9	1.8	0.1	4.6	1.0	0.6	27
Cauliflower	90.8	2.6	0.4	4.0	1.2	1.0	30
Potato	74.7	1.6	0.1	22.6	0.4	0.6	97

Source: Rai and Sohi, 1988.

The success of any agricultural nation depends upon the ability of their people to sustainably convert the natural resources into economic wealth with judicious application of schemes and technologies without endangering the environment. The only solution to feed the coming generation without harming the environment is by organic farming cultivation. White button mushroom (*Agaricus bisporus*) and milky mushroom (*Calocybe indica*) is also growing seasonally by small and marginal farmers in rural area with good results. Today mushroom growing in Tarai region of Uttar Pradesh, Himanchal Pradesh and Haryana has taken the shape of a cottage industry and tones of mushroom reach Delhi from these areas at price reasonable enough for a common man to consumption. Milky mushroom (*Calocybe indica*) provides the good profit for this cultivation. Oyster mushroom and paddy straw mushroom can not grow in the month of April to July due to higher temperature in the hotter regions. Therefore milky mushroom (*Calocybe indica*) can be fitted for cultivation in higher temperature. Milky mushroom (*Calocybe indica*) provides much benefit to the small farmers by this cultivation.

Now the farming system has been changed during last 21st century and farmer taking an interest in latest cultivation techniques so as to get the maximum per capita income. The cost of cultivation is increasing day by day due to high cost of fertilizers, fungicides, insecticides, seeds, labour and irrigation. Fertile land under cultivation of crops is reducing due to urbanization, globalization and industrialization. Due to which many of the farmers becoming landless laborers'. Those farmers don't have land unable to purchase it for crop cultivation because of huge

increment in the cost of fertile land. Climate change and environmental degradation too are playing a substantial role. The world is losing between 5 and 10 million hectares of agricultural land annually due to severe degradation. About 592,000 sq.km of India's land has already deteriorated and this is likely to affect 177 million people.

Marginal land holding farmers unable to purchased all the agricultural and essential inputs because of their higher cost and try to find out an alternative over these problems by getting loans from either by government or private sectors. Due to unfavorable environmental conditions crop failure occurs this lads to suicides of farmers, malnutrition, poverty, hunger, unemployment. India can make rapid progress in mushroom industry by cultivating and commercializing of temperate and tropical mushrooms. But, they are still cultivated on small scale in some pockets on a specific substrates and yield potential is not satisfied due to specific substrates materials. There are need to evaluate various substrates and different casing materials for enhancing better growth behaviour and yield potential of mushrooms. Therefore, present investigation was on effect of substrates on growth behaviour and yield potential of milky mushroom (*Calocybe indica*).

Now these days many scientists gave a new dimension for mushroom production in India. Purkayastha and Chandra in 1974, Purkayastha and Nayak 1979, Krishnamoorthy *et al.* 1997, Kumar *et al.* 2001,Theradimani et al. 2001 Sherin *et al.* 2004, Amle *et al.* 2006, Usha 2007, Amle et al. 2007, Biswas and Singh 2009.

Materials And Methods

Mushroom culture: Culture of *Calocybe indica* (P.& C.) was obtained from G.B. Pant University of Agriculture and Technology. Udham Singh Nagar (Uttarakhand). This culture was sub-culture and maintained on PDA medium in a BOD incubator at 25 ± 2ºC temperature.

Spawn preparation: The spawns of *Calocybe indica* was prepared on wheat grains (*Triticum aestivum Linn.*). Well cleaned wheat grains were water soaked for over night and boiled until grains become soft. After boiling excess water was drained off and the grains were cooled in plastic tray. These cooled grains were mixed with 2% chalk (calcium carbonate) and 2% gypsum (calcium sulphate). Gypsum and chalk were added to avoid clumping of grains. This mixture (250 g/ bottle) was filled in the cleaned 500 ml saline bottles and sterilized in autoclave at 121ºC temperature (15 lb) for 1hr. These bottles were allowed to cool at room temperature and then inoculated with mycelial bits of 7 days old cultures of *Calocybe indica* respectively. These inoculated bottles were incubated at 25 ± 2°C temperature in a B.O.D. incubator for mycelial growth and development. The bottles were shaken at 4 days interval to spread the mycelium among the grains. The mycelium completely impregnated the grains within 2 weeks.

Substrates preparation: In order to study the effect of different substrates on the growth and yield of *C. indica*, an experiment was carried out with four different substrates viz., wheat straw, paddy straw, chopped leaf of sorghum, saw dust. These selected straw and saw dust were soaked in water for 15 hours and then excess water was drained off from substrates. These moist substrate were sterilized by autoclave at 10lb pressure for ½ hour.

Spawning: Well prepared paddy straw, wheat straw, chapped leaf + wheat straw (1:1), wheat straw + paddy straw (1:1) and saw dust + wheat straw (1:1) were spawned with spawn of *Calocybe indica* @ 3% moist weight basis. These spawned substrates were filled in polythene

bag, (50 x 55 cm). The upper surface of substrate was covered with paper sheet within the polythene bags. These mushroom bags were placed vertically in growing chamber where temperature ranges between 28-35^{0}C. Each bags contain 4 kg moist spawned substrate. Mushroom bags were completely colonized by mushroom mycelium within 15-20 days.

Casing: Milky mushroom needs casing for fruit body initiation. After completion of spawn run or mycelial growth in the beds, the 3 cm thick casing layer spread on the surface of mushroom bed. These casing materials were prepared by sterilized clay loam soil and two years old farm yard manure (1:1) ratio.

Cropping: Mushroom beds were sprayed regularly with water to maintain sufficient moisture level in the casing surface. Pinheads appear ranged between 44 to 52 days after spawning and they were ready for harvest within another one week. After the first harvest, the casing medium is gently ruffled, slightly compacted blank, sprayed daily with water.

Observation and measurement: Growth behaviour such as spawn run period, initiation of pinhead and harvesting of fruit bodies were observed in days. The morphological parameters of fruit bodies and yield of mushroom were also measured during investigation.

A. Time taken in days: Spawn run period, initiation of pinhead and harvesting of flushes
B. Morphological parameters of fruit bodies: Cap diameter (cm), stalk length (cm), weight of sporophore (gm), total no. of fruiting bodies per bag (gm), full pinhead (gm), premature fruit body (gm), half mature fruit body (gm), mature fruit body (gm), over mature fruit body (gm)
C. Yield in gm: Yield of different flushes and total yield

Statistical analysis of data: Each treatment was kept for three replications and data were analyzed statistically following standard procedure to draw the conclusion (Panse and Sukhatme, 1967).

Results and Discussion

Effect of substrates on growth behaivour of milky mushroom (*Calocybe indica*)

Spawn run period: The data presented in the (Table 4) indicated that spawn run period was very fast on Paddy straw substrate, where it took in (23 days) fallowed by mixture of Wheat straw and Paddy straw substrate (25 days). Mixture of Saw dust and Wheat straw was taken maximum time for spawn run period. Wheat straw and mixture of wheat straw + chapped leaf of sorghum were colonized by mushroom mycilium in (26 days). Paddy straw substrate was better among themselves because that requires minimum time spawn run period (i.e. 23 days). Paddy straw and Saw dust + Wheat straw have much significant differences.

Initiation of pin head: The mushroom pin head were first initiated (44 days) from Paddy straw substrate followed by mixture of paddy straw + wheat straw (47 days), wheat straw (49 days), mixture of wheat straw + chapped leaf of sorghum (51 days) and mixture of saw dust + wheat straw (52 day).Paddy straw, wheat straw + paddy straw and Saw dust +wheat straw have significant difference between each other.

Harvesting of flushes: The first flush from paddy straw substrate was harvested in (50 days) and it was followed by mixture of paddy straw +wheat straw where it harvested in (52 days). The wheat straw was taken much time (56 days) for flush harvesting. The result was significantly difference between paddy straw and mixture of saw dust + wheat straw. The second flush was first

harvested (66 days) from mixture of wheat straw + chapped leaf of sorghum followed by paddy straw (67 days). The maximum cropping period was recorded as (72 days) from mixture of wheat straw + saw dust. These finding confirmative with results of Tandan and Sharma (2006) and Sherin *et al.* (2004). They have sown spawn run period, flush harvesting and yield of milky mushroom on various substrates in their respective experiments.

Effect of substrates on weight of growth stages of milky mushroom (*Calocybe indica*)

The observations were recorded for weight of different growth stages such as initiated pinhead, full pinhead, pre mature, half-mature, mature and over-mature fruit body and the result are presented in the (Table 4) The paddy straw was best substrate for all above growth stages and it was produced (38.67 gm) mature fruit body for harvesting. The mixture of wheat straw + paddy straw was also superior than other substrates and these were weighted (240 mg, 7.33 gm, 15.33 gm, 18.67 gm, 36.67 gm and 123.67 gm) for initiated pinhead, full pinhead, pre-mature, half mature, mature and over mature fruit body respectively.

Effect of substrates on growth parameters and yield of milky mushroom (*Calocybe indica*)

Cap diameter: The growth parameters like cap diameter, stalk length, number of fruit bodies and yield of Calocybe indica on various substrate presented in the Table 2. The cap diameter of Calocybe indica ranged from (5.33 cm to 7.13 cm) according to utiliged various substrates. The maximum cap diameter (7.13 cm) was measured from paddy straw followed by mixture of wheat straw + paddy straw (6.83 cm), wheat straw + chapped leaf of sorghum (5.62 cm) and saw dust + wheat straw (5.33 cm). The paddy straw substrate and wheat straw + chapped leaf of sorghum have significant difference in both the substrates.

Stalk length: The maximum stalk length was measured from paddy straw substrate (8.67 cm) and wheat straw + paddy straw substrate (7.83 cm). Saw dust + wheat straw substrate was produced small stalk length (i.e. 7 cm). The result of mixture of saw dust + wheat straw and paddy straw have significantly difference.

Total number of fruit bodies: The maximum number of (14) fruit bodies were obtained from paddy straw substrates and minimum fruit bodies (7.67) obtained from saw dust + wheat straw and result was significant differences. The wheat straw + chapped leaves of sorghum and paddy straw was also highly significant difference among themselves. This finding is conformity with the result of Chakravorty and Sarkar (1978) reported that the performance of composed substrate was best in all the respects like spawn run period, number of buds and number of fructifications but the yield buds of mushroom on non composted substrates was quite satisfactory.

Yield of first and second flushes: Maximum yield of first flush was obtained from paddy straw (523.33 gm) than other substrates. The mixture of wheat straw + paddy straw was also better than wheat straw, whereas yield obtained (483.33 gm and 407.67 gm) respectively. The mixture of saw dust + wheat straw was yielded less (288.67 gm). The yield of second flush was reduced than yield was first flush among all substrates. The yield performances of all substrates were similar as like yield performance the fist flush.

Total yield: It is evident from the data (Table 2) on yield performance with various utilized substrates. The substrate of paddy straw was found significantly superior than other substrates by

producing (955.66 gm/bag). The mixture of paddy straw + wheat straw was second best substrate where it produced (893.66 gm) mushroom/bag followed by wheat straw (793.67 gm), mixture of wheat straw + chapped leaves of sorghum (584.33 gm) and mixture of mixture of saw dust + wheat straw (535.33 gm). Thus, paddy straw was best substrate for the cultivation of the milky mushroom (*Calocybe indica*) because its was given better yield and good quality than the other substrate.

This result is in conformity with the finding of Krishnomoorth et al. (2000) and Esmaran and Thomos (2003) reported paddy straw to be the best substrate for cultivation of *Calocybe indica*. Krishnamoorthy *et al.* (1997) also reported that Calocybe indica can be cultivated on a wide range of cellulosic substrate namely, paddy straw, maize stalk, sorghum stalk, vetiver grass, sugarcan bagasse, soybean hay and ground nut haulms. However, paddy straw and maize straw stalk found most suitable substrate for commercial production of milky mushroom. Amle et al. (2006) also studied the different agricultural wastes wheat straw, cotton stalk, soybean, sugarcane bagasse and its mixture for cultivation of *Calocybe indica*.

Table 2: Effect of substrates on growth parameters of milky mushroom (*Calocybe indica*)

Treatment	Cap diameter(cm)	Stalk length (cm)	No. of fruiting bodies per bag	Yield of 1st flush (gm)	Yield of 2nd flush (gm)	Total yield (gm)
Paddy Straw	7.13	8.67	14.00	523.33	432.33	955.66
Wheat Straw + Paddy Straw (1:1)	6.83	7.83	10.83	483.33	410.33	893.66
Wheat Straw	6.50	7.33	10.67	407.67	386.00	793.67
Wheat Straw + Chapped leaves of sorghum(1:1)	5.62	7.60	9.00	314.33	275.00	589.33
Saw dust + wheat straw (1:1)	5.33	7.00	7.67	288.67	246.67	535.33
$SEm_{\pm}$	0.41	0.52	1.19	20.67	26.65	-
CD(P=0.05)	1.31	1.62	3.75	65.13	83.96	-

Table 3: Effect of substrates on weight of growth stages of milky mushroom (*Calocybe indica*)

Treatment	Initiatied of pin head(mg)	Full pin head(gm)	Premature fruit body(gm)	Half mature fruit body(gm)	Mature fruit body(gm)	Over mature fruit body(gm)
Paddy straw	277.33	9.00	16.00	19.67	38.67	125.00
Wheat straw + Paddy straw (1:1)	240.00	7.33	15.33	18.67	36.67	123.67
Wheat straw	237.33	6.67	14.00	17.67	35.33	121.67
Wheat straw + Chapped leaves of sorghum (1:1)	235.33	7.00	13.67	15.67	33.33	120.60
Saw dust + wheat straw (1:1)	231.33	6.00	13.33	14.67	32.00	118.00
$SEm_{\pm}$	13.36	0.56	0.52	0.84	2.33	2.44
CD(P=0.05)	42.10	1.75	1.63	2.66	7.34	7.70

Table 4: Effect of substrates on growth behaviour of milky mushroom (*Calocybe indica*)

Time taken in (days)				
Treatments	Spawn run period	Initiation of pin head	Harvesting of flushes	
			1st flush	IInd flush
Paddy straw	23	45	50	67
Wheat straw + Paddy straw (1:1)	25	47	52	69
Wheat straw	26	49	56	68
Wheat straw + Chapped leaves of sorghum(1:1)	26	51	53	66
Saw dust + wheat straw(1:1)	27	52	55	72
$SEM_{\pm}$	0.49	0.80	1.66	3.44
CD (P=0.05)	1.55	2.53	5.23	10.82

Summary and Conclusion

Mushroom cultivation is the most suitable technology for creating wealth and health out of wastes from plants, animals and industries which are abundantly available on earth. Huge

quantities of agricultural wastes and other organic wastes are generated annually through the activities of agricultural, forest and food processing industries. These agricultural wastes and other organic wastes are abundantly available in our country. The major parts of these agricultural wastes are bunt after harvesting, resulting multifaceted hazards including oxygen defiant environment, respiratory diseases such as allergic, bronchitis, asthama, tuberclusis and poor visibility at night. The useful insects, bio-agents, earthworm and soil microbe are also reduced due to burning of agricultural wastes and its heating on soil surface. According to survey, in some places these agricultural wastes are spreads on the cultivated land but it is not well decomposed and they created the problems during farming. The main concerned of our planners to remove these air pollution and soil pollution for environmental protection. This can be done by scientific utilization of resources and bioconversion for mushroom production.

Mushroom production represented an attractive method of improving the nutritional quality of lignocellulosic wastes for use as an animal feed stock. Among the various physical, chemical and biological methods used for upgrading the digestibility and nutritive value of agricultural wastes, biodegradation by using white rot fungi including mushrooms have been found promising. There are need to evaluate various substrates for enhancing better growth behaviour and yield potential of mushrooms. Therefore, present investigation was on effect of substrates on growth behaviour and yield potential of milky mushroom. These studies will help to mushroom growers for selection of suitable substrates, for better growth behaviour and yield potential of milky mushroom.

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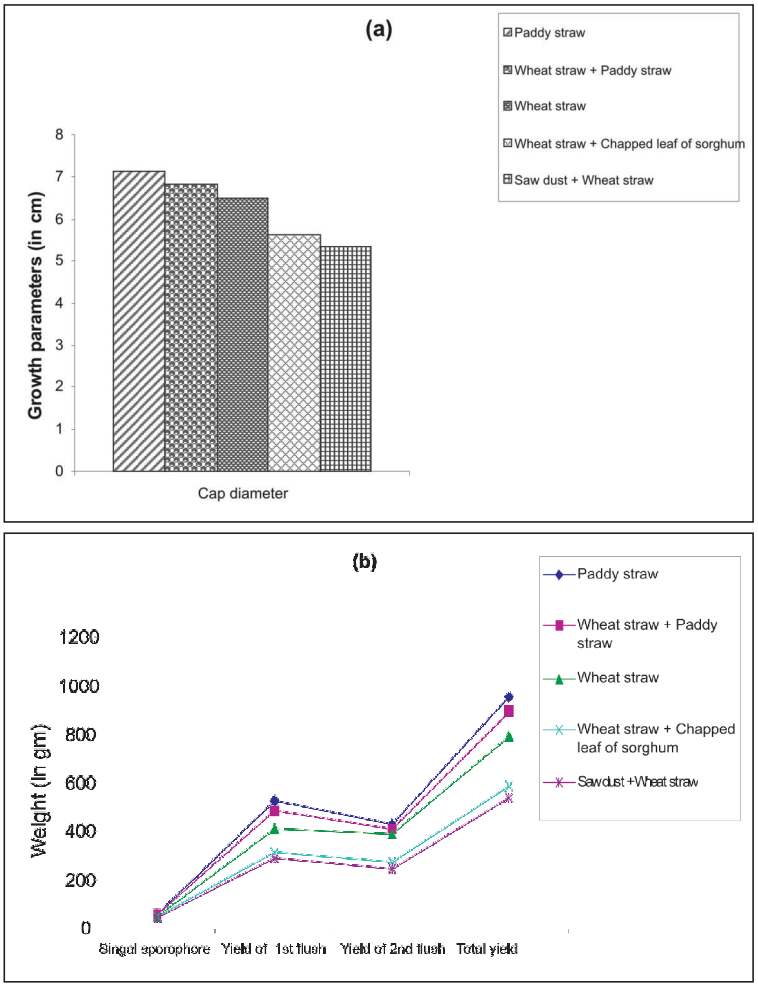
Fig. I: Effect of substrates on growth parameters of milky mushroom (*Calocybe indica*)



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Effect of Bisphenol A on Fertility of Male Mice

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Abstract

Bisphenol A (BPA) is a chemical widely used for the synthesis of polycarbonate plastics. The present study was undertaken to decipher the effects of BPA exposure, on fertility of male mice. A set of eight Swiss albino mice were treated with BPA intraperitonially at a dose of 100µg/kg body weight/day, for a period of two months. Control group was treated with vehicle.

Females mated with male mice that were exposed to BPA showed a 50% decrease in litter size compared to control. There was significant reduction in sperm count as well as testosterone level in the BPA exposed mice. The histological study of testes showed immense distortion of the seminiferous tubules in treated mice along with other deformities.

The present study gives strong evidence to support that BPA is capable of impairing male fertility by distressing the steroidogenesis and spermatogenesis process in male mice.

Keywords: Bisphenol A, xenoestrogen, male fertility, testosterone level, sperm count.

Introduction

Bisphenol A (BPA) is an important industrial compound employed as a monomer of polycarbonate plastics used in food packaging, manufacturing products such as baby bottles, water bottles, epoxy resins and white dental sealants (Colborn 1993, Brotons 1995, Kim 2010). BPA molecules are bound by "ester bonds" to form the polymer and this bond is disrupted by heat and acidic or basic conditions that release BPA into food or beverages in contact with the plastics (Krishnan 1993).

BPA is known to mimic the role of estrogen once inside the body, thus acting as a xenoestrogen. Hence, BPA has been placed in the group of chemicals known as endocrine disruptors (EDs). These EDs basically mimic the role of different hormones inside our body thereby causing severe disruption of the normal functioning of the endocrine system (Sekizawa

2008).

BPA is known to have weak estrogenic activities in vivo as well as in vitro, with approximately 10,000 times less affinity for estrogen receptors compared to 17β-estradiol (Kuiper 1998).

Indeed xenoestrogen like BPA are now being implicated in human infertility, genital tract malformations, and increased cancer rates in estrogen sensitive target tissues (Sharper 1993). Bisphenol A has been tested in male fertility studies in laboratory animals. Some studies have reported no effects on the reproductive function of male offspring following maternal exposure (Ashby 1999), whilst others have reported that exposure to low doses of BPA causes reproductive toxic effects (Nagel 1997). This raises some concern about the adverse effects of BPA on human fertility.

In view of the above literature, the present study was carried out to determine whether exposure to environmentally relevant BPA levels affects testicular steroidogenesis and if so, to identify the mechanisms associated with observed effects.

Material and Methods

Animal care and maintenance

Sixteen adult male *Swiss albino* mice used in the experiment were purchased from animal house facility of the Institute of Medical Sciences, BHU, Varanasi, India. They were kept in a controlled temperature of 22 ±3˚C on a 12-h light/dark cycle. Food pellets and water were made available to these animals *ad libitum*. Proper cleaning of cages and grills were done routinely.

BPA administration

Animals were divided into two groups and labeled as treated and control. BPA was administered intraperitonially at a dose of 100µg/kg body weight/day for a period of two months. Control group was injected with normal saline alone.

Fertility assessment

Fertility was estimated in adult male mice exposed to BPA. After two months of BPA injection, each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for 10 days, during which two estrus cycles should have elapsed, BPA treated and control males were then removed and sacrificed for further evaluations. The female mice were left isolated and allowed to give birth to their litters. The number of pups/litter were counted and compared among control and BPA exposed groups.

Weight of accessory sex organs

Bisphenol A-exposed and control mice were sacrificed after completion of the exposure, by cervical dislocation. Reproductive organs including the testes and seminal vesicles were dissected out and the paired weights were recorded.

Sperm Count

Sperm count was performed according to the method of Salian (2009a). Briefly, the cauda region of epidydymis was excised and collected. It was placed in 0.5 ml of phosphate buffer saline and homogenized using a manual glass homogenizer. The homogenate was mixed

using a vortex mixer, and the number of sperm was measured using a hemocytometer after a twenty fold dilution. Sperm counted were expressed as number of sperms in million per cauda epididymis.

Testosterone estimation

Radioimmunoassay (RIA) was used to quantify the levels of serum testosterone as described by D'Souza (2005) with some modifications. Briefly, blood was collected and allowed to stand at room temperature for 30 min to separate the sera. It was then centrifuged at 10,000 rpm for 10 min and, separated sera were then stored at -20°C for hormone assay.

Histological analysis

According to standard criteria given by Hess (1990), histological analysis was carried out. Briefly, the testes were dissected out and fixed in Bouin's fixative for 24 h. After primary fixation, 3 to 5 mm thick testicular slices were cut and refixed in fresh fixative for another 24 h. The paraffin embedded tissue blocks were sectioned at a thickness of 5μm, mounted on glass slide and stained with Hematoxylin and Eosin (Sigma, USA). The stained slides were reviewed under standard light microscope (Leitz orthoplan microscope- 054546).

Statistical analysis

The data were analyzed by using one way analysis of variance (ANOVA) through GraphPad prism software. All values were expressed as mean ± SEM. The significance of the data obtained was evaluated by using Student-Newman-Kules test. P-values of less than 0.05 ($p<0.05$) were considered significant.

Results

Females mated with male mice, that were exposed to BPA showed a significant reduction (***$p<0.001$) in the litter size. In control group number of pups varied from 10 to 12 whereas in treated group it varied from 5 to 6. The testis (***$p<0.001$) and seminal vesicle (** $p<0.01$) weight of BPA treated mice showed a significant reduction compared to control.

There was significant reduction (** $p<0.01$) in sperm count in the mice exposed to BPA. Sperm count was found to be in the range of 1.2 to 2.4 million in control mice whereas in BPA exposed mice it ranged from 0.54 million to 0.8 million. The testosterone level in control group was nearly 345 to 456 ng/ml and in treated group it was 156 to 262ng/ml. Thus, we saw a significant decrease (***$p<0.001$) in testosterone level after BPA exposure. The microscopic view of testes from BPA exposed mice showed immense distortion in seminiferous tubules with extensive vacuolization.

Discussion

The present study was designed to investigate any adverse effects of BPA on fertility and reproduction of adult male mice. This chemical was chosen because of its bulk production and huge consumption all over the world. Additionally, due to its estrogenic behavior there is a large possibility of its implication in male infertility.

The No Observed Adverse Effective Levels (NOAEL) of BPA has been found to be 5mg/kg body weight/day (NTP, 2001). Thus, we selected a dose much below this level in order to evaluate the effect of BPA exposure on male mice fertility.

Several reproductive parameters were adversely affected after exposure of BPA to adult male mice. First of all the reduction in number of pups/litter as a result of BPA exposure is a direct evidence of decrease in male fertility. Testis weight in adult mice is an important indicator of total germ cell number per testis. Simultaneously, the weight of seminal vesicle helps in indicating the testosterone level in adult male mice (Atanassova 1999). Thus, a decrease in both these end points due to BPA exposure gives a reliable indication of antiandrogenic effect of this chemical. Sperm count is an important parameter to analyze toxic effects of any substance on male reproduction. The result obtained here suggests that BPA causes a significant reduction in sperm production in male mice and it is in harmony with a previous study (Salian 2009b). Testosterone plays an important role in the process of steroidogenesis in male testis. An impaired histological architecture and a reduced testosterone level in BPA exposed mice gives a clear picture of impaired activity of Leydig cells.

Thus, the results obtained in the present study clearly demonstrate that long term BPA exposure to male mice at low doses is capable of adversely affecting the male fertility by causing severe damages to the spermatogenesis and steroidogenesis process.

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Figure legends

Fig 1: Decrease in number of pups/litter in the female mated with adult mice exposed to BPA compared to females mated with control mice. Data expressed as mean±SEM, n=8, and significant changes are shown as ***$p<0.001$.

Fig 2: Decrease in weight of testis in BPA treated mice compared to control. Data expressed as mean±SEM, n=8 and significant changes are shown as ***$p<0.001$.

Fig 3: Decrease in weight of seminal vesicle in BPA treated mice compared to control. Data expressed as mean±SEM, n=8, and significant changes are shown as **$p<0.01$.

Fig 4: Sperm count analysis of control vs BPA treated mice. The data is expressed as mean±SEM, n=8 and significant changes are shown as ** $p<0.01$.

Fig 5: Testosterone level in adult male mice exposed to BPA compared to control. The data is expressed as mean±SEM, n=8 and significant changes are shown as ***$p<0.001$

Fig 6: Photomicrographs of testis showing seminiferous tubules (ST) of (a) control and (b) BPA treated mice. The ST of BPA treated mice show extensive vacuolization with hypospermatogenesis whereas control mice show normal histological architecture.

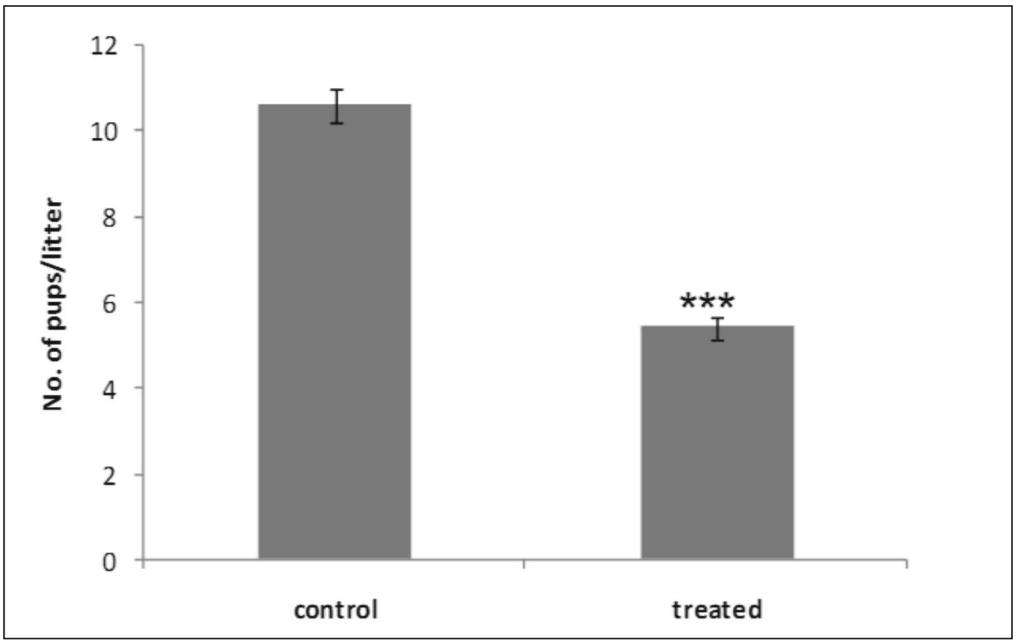

12
10
8
6
4
2
0
No. of pups/litter
***
control
treated

Fig. 1

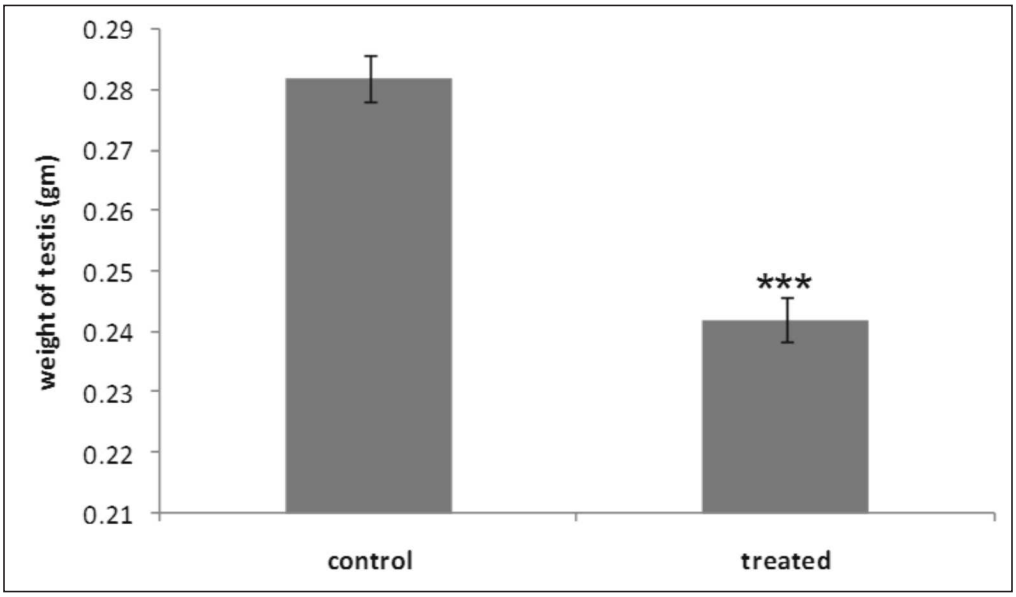

0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
weight of testis (gm)
***
control
treated

Fig. 2

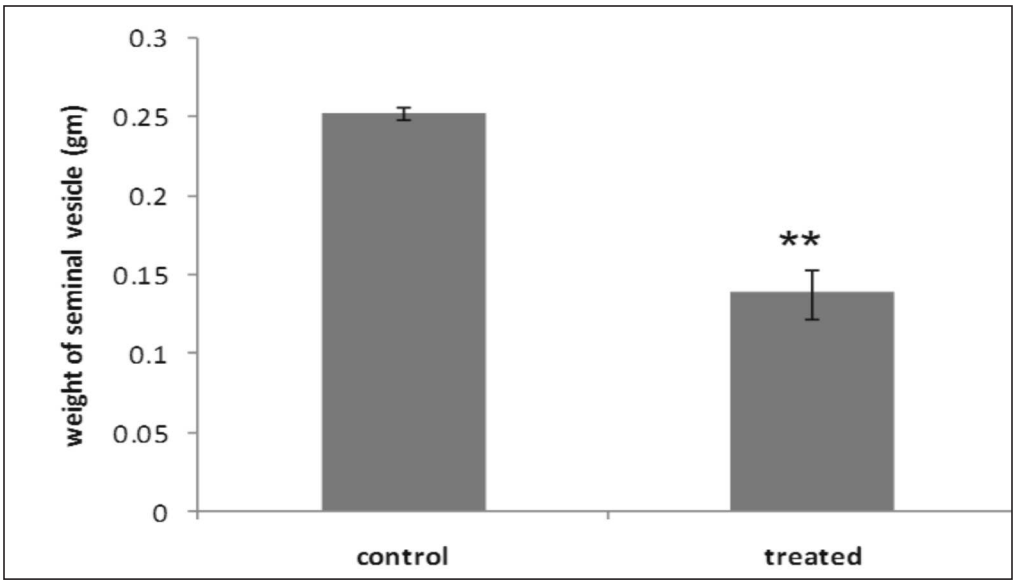

weight of seminal vesicle (gm)
0.3
0.25
0.2
0.15
0.1
0.05
0
**
control
treated

Fig. 3

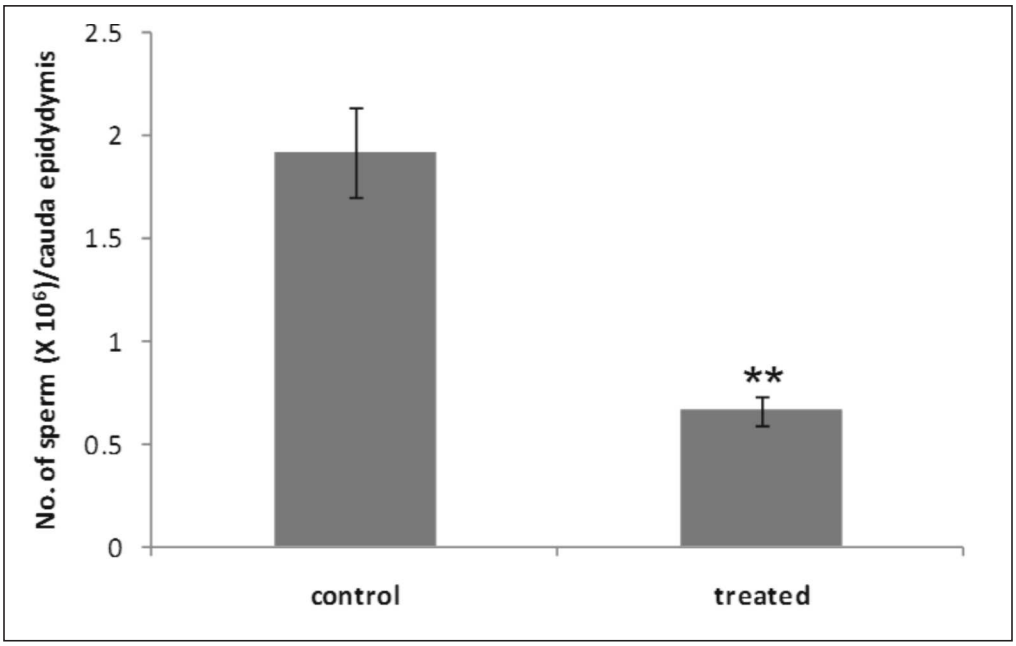

No. of sperm (X 10⁶)/cauda epidydymis
2.5
2
1.5
1
0.5
0
**
control
treated

Fig. 4

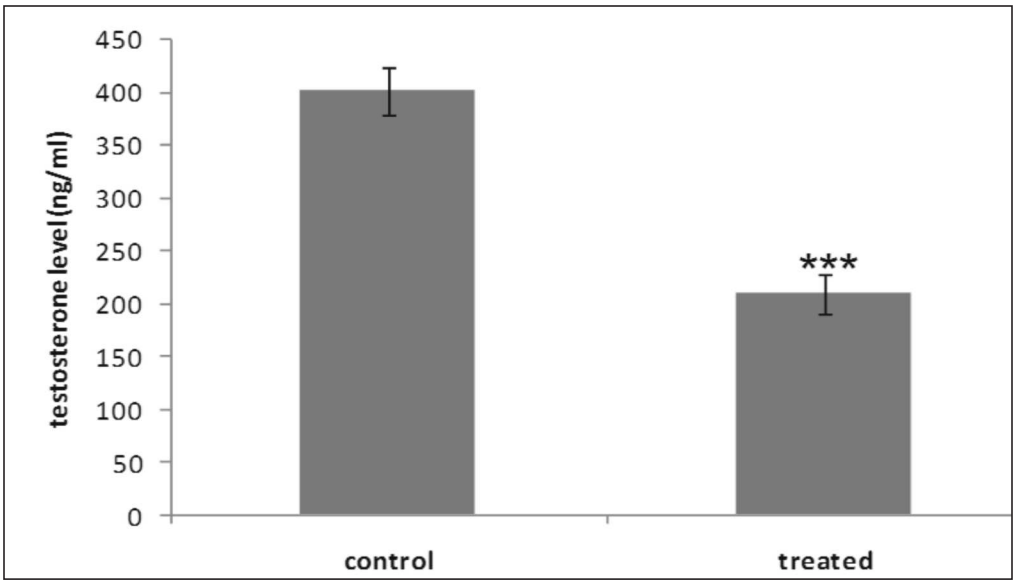
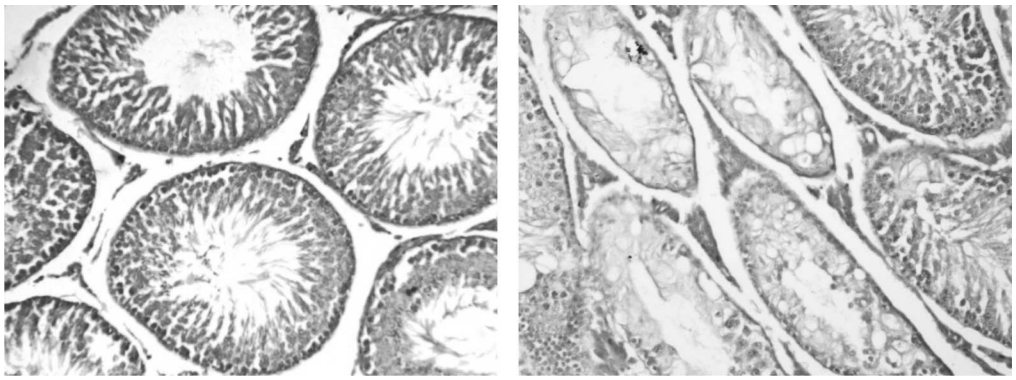

450
400
350
300
250
200
150
100
50
0
testosterone level (ng/ml)
***
control
treated

Fig. 5



(a) (b)

Fig. 6

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Interactive response of ultraviolet-B with other abiotic stress factors on plants

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Abstract

Depletion of stratospheric O_3 layer is leading to an increase in UV-B radiation on earth surface. Along with UV-B other abiotic stress factors are also changing simultaneously. Present review is summarizing the information available on the interactive effect of UV-B with other abiotic stress factors on various plant species. This article is an overview of literature of existing studies on the interactive effects of UV-B with water stress, nutrient stress, elevated carbon dioxide (CO_2), heavy metal and ozone (O_3). Experimental conditions along with doses of stress were also compared to make the clear view of difference in response of plants in natural and controlled conditions. Among all these studies only 25% studies were conducted in field conditions however rest of them were performed under controlled environment. Results of interactive effect at various levels as growth, anatomy, physiology, biochemical changes and yield were given in terms of increase and decrease. Mode of interaction was also discussed with other factors. Carbon dioxide and nutrient stress were found to alter the source and sink balance of carbon in plants which in turn provides protection against UV-B. Pathways for synthesis of UV-B and water stress induced secondary metabolites and signaling of defense gene expressions with heavy metals and UV-B were also compared in plants. Elevated carbon dioxide, nutrient stress were found to ameliorate the negative response of UV-B in most of the plant species however heavy metals, water stress and elevated level of O_3 were found to worsen the effect of UV-B in most of the studies. Interactive response of UV-B with other abiotic stresses is a broad area and results of few studies can't withdraw a definite conclusion. Field studies are also scanty and further needed to define the actual performance of plants in present and future environment.

Keywords: UV-B, CO_2, heavy metal, water stress, nutrient stress, O_3, growth, physiology, yield.

Introduction

Global climate is result of a complex system of various atmospheric processes and their products. Due to subsequent increase in industrialization, urbanization and agricultural practices

our atmosphere is undergoing a transition with the simultaneous increase in several abiotic factors such as UV-B, CO_2, O_3, temperature, heavy metals and excessive nutrients. With increasing trend of these abiotic factors the most important question to be answered is, whether these factors can counteract to nullify their negative effects or interaction may be antagonistic, synergistic or additive. During last few decades convincing evidences have been reported regarding the reduction in stratospheric O_3 layer due to emission of chlorine and bromine containing compounds. As these CFCs have a high half life ranging from 50 to 150 years and they can remain for the longer period in the upper atmosphere so it will take 2065 to return to the pre 1980 level if no further release will occur (UNEP, 2006). Since the discovery of ozone hole in 1979 by Farman and colleagues, consequential increase in solar UV-B is becoming a threat to all life forms on earth (Rozema et al., 2001). Goddard Institute of Space Studies (GISS) estimated that the maximum annual increase in Northern Hemispheric UV dose will be 14% in 2010-2020 (Taalas et al. 2000, 2002). Along with increase in UV-B other abiotic factors are also increasing simultaneously. Under natural field conditions it is common practice for a plant to encounter more than one environmental stress simultaneously. Depending on the mode of action of stress factors and plant species, net effect of two or more concomitant stresses can be antagonistic, additive or synergistic and it can also be possible that they can't influence each other's response. Present review is an attempt to summarize various studies pertaining with interaction of UV-B and other stress factors and emphasizing the possible mechanism behind their differential response.

Interaction of UV-B and CO_2

Increasing use of non-renewable natural resources especially fossil fuel is causing a steady increase of CO_2 concentration. Atmospheric CO_2 has increased from pre industrial value of 280 to the current level of 380 µmol mol^{-1} (IPCC, 2001) and according to predictions this may increase upto 700 µmol mol^{-1} by the end of this century (IPCC, 2007). Since CO_2 is a substrate of photosynthesis, its very important to assess how plants modify photosynthesis particularly Rubisco that catalyze CO_2 fixation. Various studies have been conducted to assess the impact of enhanced UV-B and CO_2 on plant. Lavola et al. (2000) have reported that 700 µmol mol^{-1} concentration of CO_2 is sufficient to ameliorate the harmful effect of UV-B (8.6 kJ m^{-2} day^{-1}) on birch seedlings however Tegelberg et al. (2008) have found similar level of CO_2 to be ineffective in ameliorating the harmful effect of UV-B (7.95 kJ m^{-2} day^{-1}) on birch plants. Under CO_2 enrichment the increased allocation of carbon is favored towards synthesis of condensed tannin than to other phenolic compounds. With the increases in carbon availiability under the enhanced UV-B more carbon allocation is reported for growth, lignifications, enhanced activity of enzymes and repairing processes (Lavola et al. 2000). Both UV-B and CO_2 are known to enhance flavonoid synthesis in plants but the quercetin glycosides were reported to be the most responsive flavonoid towards UV-B and CO_2 (Lavola et al. 1997, 2000). In a gymnospermic plant *Pinus taeda,* enhanced concentrations of CO_2 have modified the response of UV-B towards growth and biomass allocation of plant (Sullivan and Teramura, 1994). At CO_2 level of 350 µmol $mol^{-1,}$ biomass was preferentially allocated to shoot components while at elevated level of 650 µmol mol^{-1} it was preferred to root components at enhanced UV-B. Sullivan and Teramura, (1994) have

stated that increase in CO_2 favors carbon gain in plants by reducing diffusional limitation, lowering photorespiration and water use efficiency. Since UV-B also restricts growth of above ground part (leaf elongation, expansion etc.), both factors favor more allocation of biomass towards root and thus resulted a strong interactive effect of UV-B and CO_2 on biomass portioning.

Enhanced level of UV-B has reduced the stimulatory effect of CO_2 on biomass of *Vicia faba* plant but no interaction was noted with respect to photosynthetic parameters (Tosserams et al. 2001). The major responsive trait of plant towards elevated CO_2 is enhanced photosynthesis especially in C_3 plants. After a certain level of CO_2 plant shows acclimation response. Acclimation is nothing but down regulation of CO_2 fixation under elevated CO_2 due to the imbalance between supply and demand of assimilates. Increased accumulation of soluble carbohydrate and starch in leaves may down regulate the expression of nuclear photosynthetic genes including Rubisco (Pandurangam et al. 2006). Apart from direct end product feedback inhibition indirect decrease in photosynthesis also occurs through decrease in photosynthetic enzymes and reduced stomatal conductance (Sttit, 1991, Dijkstra et al. 1993). Acclimation can be recovered with demand of additional sinks for carbohydrate with the onset of flowering and fruiting. Tosserams et al. (2001) have also reported photosynthetic acclimation after 31 days of treatment and at that time total carbohydrate content was 11%. Similar response was noticed by Visser et al. (1997) in photosynthesis of *Vicia faba* but both of them changed the leaf optical properties of plant. Koti et al. (2005, 2007) have studied the growth, photosynthesis and floral attributes of another leguminous crop *Glycine max* and reported that elevated level of CO_2 may compensate the damaging effect of UV-B on growth and development of plants. However the damage caused by UV-B on flower, pollen morphology, production, germination and tube length can not be ameliorated by enhanced CO_2 (Koti et al. 2005). In C_3 plant *Dimorphotheca pluvialis* elevated CO_2 altered reproductive phenology (delayed) and reproductive success and this effect may be mitigated by enhanced UV-B conversely and no any interaction was observed under combined tretment (Wand et al. 1996). Different parameters of cotton plant responded differentially towards elevated UV-B and CO_2. Zhao et al (2003) have not found elevated CO_2 to be helpful in ameliorating the adverse effect of UV-B on growth and physiology of cotton plants especially in ball retention. However, on similar plant no interaction was reported for photosynthetic parameters by Zhao et al. (2004). Response on photosynthesis was very interesting; under the ambient UV-B condition acclimation was reported by elevated CO_2. Net photosynthesis was increased when elevated dose of both the factors were applied simultaneously and this response may be due to more utilization of photosynthate in protective measures. Similarly, Kakani et al. (2004) have also not found any interaction between UV-B and CO_2 in cotton plant. However, Qaderi et al. (2007) have reported that some of adverse effect of UV-B on reproductive parameters can be mitigated by elevated CO_2 in *Brassica napus*. In C_3 plant Helianthus annuus, Mark and Tevini (1997) have reported that doubling of CO_2 concentration may compensate or surpass the harmful effect of UV-B. Likewise Zhao et al. (2004), Staaij et al. (1993) have found elevated CO_2 acclimation under ambient UV-B and reverting the value of NAR back to the low CO_2 level while under elevated level of UV-B reduction in growth was reported and NAR value remained high which checks the negative feedback mechanism of an invasive plant *Elymus*

athericus. Teramura et al. (1990) have observed that in combination, enhanced UV-B has eliminated CO_2 induced increase in seed yield of wheat *seed yield* and total biomass of rice, however both were increased in soybean plants. Similar to the above result of rice Ziska and Teramura (1992) have also found elimination of CO_2 induced enhancement in biomass by elevated UV-B. In contrast to biomass yield was increased with elevated CO_2 and UV-B suggesting that yield can be the most conservative parameter with respect to CO_2 and UV-B interaction whereas the relative decrease in biomass would be more as compared to the present scenario of UV-B and CO_2. Unlike the other studies Deckmyn et al. (2001) also used two different levels of UV-B which are less than ambient (82 and 88%) under enhanced level of CO_2 and observed that elevated level of CO_2 stimulated growth at reduced level of UV-B (88%) in Trifolium repens.

Several mechanisms may be involved in modification of plant response to UV-B due to CO_2 enrichment. Elevated CO_2 induces the production of more leaves and thus enhances leaf area and in turn productivity of plants. UV-B induced damage to photosynthetic apparatus can also be compensated by enhancement of CO_2 by increasing carbon availability, water use efficiency, and Rubisco activity and also by reducing photosynthetic respiration (Sullivan and Teramura, 1994). Elevated CO_2 is also known to enhance secondary metabolism which may increase the amount of UV-B absorbing compounds (flavonoids, tannins, lignins etc.) which may reduce plant sensitivity towards UV-B (Rozema et al. 1997, Penuelas et al. 1997). Two hypotheses may function behind the CO_2 induced secondary metabolite synthesis. According to "carbon-nutrient balance hypothesis", increases in C/N ratio stimulate more production of carbon based secondary compounds (Bryant et al. 1983). Similarly the "growth differentiation balance hypothesis" says that any environmental condition (like elevated CO_2) which differentially affects photosynthesis (source) and growth (sink) will change the available carbon pool and synthesis of carbon based secondary compounds (Loomis, 1932). Increasing atmospheric CO_2 increases the strength of source and available carbon pool which in turn stimulate synthesis of secondary metabolites (Penuelas and Estiarte, 1998). Increase in the level of carbon based secondary compounds (tannin, lignin) provide protection to the plants against enhanced UV-B damage (Fig. 1).

UV-B and nutrient interaction

Various anthropogenic activities (industrial and agricultural) have significantly altered the global nutrient cycle. Excessive loading and deficiency both can strongly affect the sensitivity of plants towards other stresses. Mineral stress is defined as sub-optimal availability of essential nutrient or toxicity due to excess of nutrients to plants (Lynch and Clair, 2004). Majority of world agriculture is facing the problem of sub-optimal availability of nitrogen (N) and phosphorus (P). However N deposition is increasing in many European countries, north-eastern United States and China (Yao and Liu, 2006). Nitrogen is a major component for all the biochemical processes operating in plants and also important limiting factor in those zones where UV-B fluence rate are normally high (Riquelme et al. 2007). Some studies pertaining to interactive effect of UV-B and nutrients on tree plants reported that under low nutrient supply plants show tolerance against UV-B increment (Musil and Wand, 1994 on *Dimorphotheca pluvialis*), more than with optimal nutrients (De la Rosa et al. 2003 on *Betula pendula*) and with high nutrient supply (Tosserams et

al. 2001 *Plantago lanceolata*). Similar response was reported by Yao and Liu (2006) on tree species Acer mono maxim in which N supply made plant more sensitive towards UV-B. Nitrogen helped to increase the growth, antioxidants, lower the level of reactive oxygen species (ROS) and intensity of harm but was not able to totally alleviate the effect of UV-B. Gymnosperm plant *Picea asperata,* also responded similarly under same dose of UV-B (14.33 KJ/m^2/day) and N (20 g/m^2/area) (Yao and Liu, 2007). However, Yao et al (2008) doesn't found excess N to help in photosynthetic impairment in similar plant. These responses are also species dependent. Levizou and Manetas (2001) have noticed that slow growing *Ceratonia siliqua* doesn't respond against low/high nutrients in presence of UV-B and this may be due to the requirement of longer exposure time of both the stresses in order to get significant response. However, fast growing species *Phlomis fruticosa* showed improved growth under high nutrient and enhanced UV-B. Inherently slow growing species under nitrogen deficiency invest more carbon based secondary metabolites and their growth promotion by additional nutrients would result in less investment into phenolics and make plants more vulnerable to enhanced UV-B (Bryant et al. 1983, Levizou and Manetas, 2001). It is suggested that low nutrient availability induces synthesis of phenolics, condensed tannins and flavonoids (querecitin, myricitin) which may afford protection against UV-B radiation (De la Rosa et al. 2001., Lambers et al. 1993) According to carbon (C)/ nutrients balance hypothesis by Bryant et al. (1983), deficiency of nutrients affect growth of plant more than photosynthesis which result in diversion of assimilated carbon to production of secondary metabolites (phenolics/terpenoids). However the study of Lavola et al. (2003) made on a gymnosperm plant i.e. *Pinus sylvestris* reported that certain level of high nutrients (4 and 6%) may deliver protection against ambient and near ambient UV-B by increasing flavonoids and flavonols but carbon allocation to other branches of flavonoid pathway (catechin and tannin formation) remain unchanged. Mineral stress negatively affects the sink strength which favors synthesis of carbon based secondary compounds however according to Yeoman and Yeoman (1996) deficiency of N causes growth limitation which enhances the level of secondary metabolite (Fig 1). Enhanced level of secondary metabolite provides protection to plants against UV-B damage. Wheat is one of the most important cereal crops. And three different studies on wheat showed that increased level of nutrients provided protection against UV-B damage (Rathore et al. 2003), at both recommended and 1.5 times recommended NPK (Agrawal et al. 2004) however Agrawal and Rathore (2007) found only recommended dose of NPK helpful in ameliorating the negative effect of UV-B in wheat plants. Similar response was noticed by Singh et al. (2009) on *Amaranthus tricolor* in which 1.5 times recommended dose of NPK helped to minimize negative effect of UV-B while in *Solanum tuberosum* only recommended dose of NPK was found to be the best for reducing the effect of enhanced UV-B (Singh et al. 2010). They suggested that high nutrient supply enhanced the growth and thus invested more photosynthate for protection. Plants have strategies to trade off between productivity and tolerance to stress. Since high dose NPK increased plant tolerance to UV-B thus sustained higher yield (Singh et al. 2009). Correia et al. (2000, 2005) have observed that reduced N supply helped to minimize negative effect of UV-B on growth, photosynthesis and yield of maize plants. Nitrogen stressed plants generally have smaller leaves and low mersitematic activity. Since reduced level of cell division increases opportunity for repairement of DNA dimmers before cell enters its synthesis phase thus UV-B induced TT dimmers can be repaired to minimize its negative impact (Correia et

al., 2000). Similar response was noticed even in case of leguminous crops. Nitrogen stress rendered plant more tolerant towards UV-B by reducing leaf area and increasing amount of UV-B absorbing compounds in *Phaseolus vulgaris* (Requilme et al. 2007, Pinto et al.1999). Musil et al. (2003) have supplemented *Podolyria calyptrate* with nitrate which enhanced active metabolism (photosynthesis and respiration) and made plant more sensitive towards UV-B. However, Agrawal and Rathore (2007) have found recommended dose of NPK helping to alleviate the deleterious effect of UV-B in *Vigna radiata.* A conclusion could be drawn from the results of all the studies performed for low nutrient conditions especially N is a favoring condition to minimize the harmful effect of UV-B radiation. Pinto et al. (1999) has given a hypothesis that under low N, synthesis of protein was partially suppressed and turnover and catabolic protein degradation were favored which in turn stimulated the deamination of L-phenylalanine leading to overproduction of ammonia and cinnamic acid. Ammonia can be recycled into new proteins and cinnamic acid is used as substrate in phenyl propanoid pathway for synthesis of flavonoids, anthocyanin and various other secondary metabolites. Like NPK, iron (Fe) is an essential plant nutrient involved in synthesis of various antioxidants (SOD), non-specific peroxidases, ascorbate peroxidase and ascorbate-glutathione cycle. Zancan et al. (2008) have reported on *Hordeum vulgare* that Fe deficient conditions also make plant sensitive towards UV-B. Unlike the response of terrestrial plants to nutrients and UV-B, marine organisms showed a different trend. Under low level of N, *Myriophyllum spicatum* and *Dunaliella tertiolecta* both showed increased sensitivity towards enhanced UV-B (Li et al., 2005, Shelly et al., 2005).

UV-B and heavy metal interaction

Various studies have been conducted worldwide to evaluate the interactive effect of UV-B with different heavy metals and they faced that in general heavy metals have ameliorating effect to minimize the harmful effect of UV-B (Larsson et al., 2001, Liang et al., 2006, Chanjuan et al., 2006). On the other hand Rai et al. (1995, 1998) have studied effect of two metals Cu and Pb with UV-B on a cyanobacteria *Anabaena doliolum* and reported synergism between their responses. UV-B exposed cells lead to changes in membrane permeability by peroxidation of lipids and thus facilitated the uptake of Cu and Pb. Lipid peroxidation was identified to be the main phenomenon behind the synergistic interaction of UV-B with Cu and Pb (Rai et al. 1998). UV-B and Cu also altered the energy transfer system of phycobilisome, photosystem I and II, respiration rate and Na+ and K+ leakage (Rai et al. 1995). UV-B and Cd have reduced synergistically the level of photosynthetic pigments and in turn the photosynthetic electron transport activity and oxygen evolution of *Plectonema boryanum* (Prasad and Zeeshan, 2005). They suggested that involvement of similar and multiple sites of action by UV-B and Cd may be the possible reason for their synergistic interaction. Bryophytes also impart sensitivity to various changes in climate. Prasad et al. (2004) have reported additive effect of Cd and UV-B on *Riccia* sp. but the response was modified when the high concentration of Cd was applied in presence of similar dose of UV-B. Both the stress altered the photosynthetic activity of *Riccia* but the inhibition of PS II was only reported in case of UV-B while the water splitting complex was more susceptible towards Cd. Some other studies made with UV-B and Ni interaction on temperate leguminous plants showed some very interesting outcomes. Prasad et al. (2005) have noticed significant reduction in physiological characteristics and biomass production of soybean but

their mode of interaction was less than additive except catalase (CAT) which showed suppressed activity. However, Singh et al. (2009) have reported antagonistic response of UV-B and Ni on pigments, proteins and antioxidants of pea except CAT which showed synergistic response. Similar result was also reported by Mishra and Agrawal (2009) with UV-B and Cd on pea in which CAT showed its additive response against both the stress. Nandi et al. (1984) have suggested that degradation of tetrameric CAT molecule into monomeric subunits during stress may be a major reason for decreased activity of CAT. Utilization of CAT in hydrogen peroxide detoxification and its inactivation may be responsible for reduced CAT activity. In two different studies of Larsson et al (1998, 2001) on two members of Brassicaceae family *Brassica napus* and *Arabidopsis thaliana,* Cd was reported to be the more dominant stress as compared to UV-B and many of the stimulatory effects of UV-B were overridden by Cd. UV-B and Cd altered the balance of various nutrients such as Mg, Ca, P, Cu and K which was increased in shoots of both the test plants while the concentration of S decreased in *Brassica napus.* Larsson et al (1998) have also reported the reduction in concentration of UV screening pigments due to phytochelatin synthesis in presence of Cd. GSH acts as signal transducer of UV-B stimuli for induction of UV screening pigments, on the other hand GSH also act as precursor for phytochelatin synthesis therefore the simultaneous application of both Cd and UV-B may lower the level of UV screening pigments (Kalbin et al., 1997). The most pronounced effect of Cd+UV-B was reported on chl a/b ratio and non photochemical quenching in rapeseed which may be explained by the inhibition in activity of violaxanthin de-epoxidase in presence of Cd+UV-B (Larsson et al., 1998). Likewise the response of *Riccia,* Shukla et al. (2002) have also reported that low concentration of Cd (1 ppm) did not respond significantly in presence of UV-B however the higher dose (2.5, 5 ppm) caused retardation in growth and chlorosis of wheat plants. In another study made by Mishra and Agrawal (2006) on a leafy vegetable spinach, interactions of two heavy metals Ni and Cd individually and in combination with UV-B were evaluated and observed that their mode of interaction was always less than additive. Among both the metals Cd was found to be more deleterious as compare to Ni when provided with UV-B. To assess the effect of UV-B and heavy metal (Cd++), Nedunchezhian and Kulandaivelu (1995) have isolated chloroplast from *Vigna unguiculata* and observed that UV-B supported the inhibitory effect of all applied doses (3,6,9 mM). Both UV-B and Cd induced the severe loss of 17, 23, 33 and 43 kDa proteins which are responsible to inactivate oxygen evolving complex and thus affecting PS II activity. On the other hand PS I activity was only marginally affected. Rare earth metals are not serious environmental pollutants. Acidic condition can cause mobilization leading to their enrichment in ground water, river water etc. Neal et al (2005) have reported increased concentration of lanthanum (La), cerium (Ce), yttrium (Y) and praseodymium (Pr) in rain fall, cloud water and ground water in mid Wales, U.K. Some studies have also been conducted on interactive effect of rare earth metal and UV-B on plants (Chanjuan et al. 2006 a, b, Liang et al. 2006). In the study of Chanjuan et al. (2006 a, b) on soybean and rapeseed Ce helped to lower or alleviate the damage caused by low level of UV-B. In soybean Ce was capable of enhancing the capability of enzymes to scavenge free radicals and thus protected the membrane system. Similar response was also reported by Liang et al (2006) on soybean where La provided resistance to soybean towards UV-B with the help of increased levels of flavonoids, chlorophyll content and PAL (phenylalanine ammonia lyase) activity.

Both UV-B and heavy metal follow more or less similar pathways for signaling inside the plant cell. Being a nonionizing radiation UV-B infers both photomorphogenic and nonphotomorphogenic response which can be low and high fluence dependent. However the existence of UV-B receptors is potent question for decades. Previously it was thought that phytochromes and cryptochromes are putative UV-B receptor. But the study of mutants which are devoid of these photoreceptors showed influence of UV-B on hypocotyl elongation (Frohnmeyer and Staiger, 2003; Bocalandro et al. 2001). Inferences from some important studies suggest that UV-B receptor consist of a protein with a bound pterin or flavin as chromophores (Frohnmeyer and Staiger, 2003) or they can be a factor like ULI3 (found in Brassicaceae family) which encodes an unknown protein containing putative heme and diacylglycerol binding sites (Lariguet and Dunand, 2005). Evidence of some membrane bound cell surface receptors, SR 160 (a peptide systemin) was also given by Stratmann (2003) which is phosphorylated on the intracellular kinase domain in response to UV-B resulting in activation of several defense signaling steps. After the perception of signal photomorphogenesis can be induced by either UV Resistance Locus 8 (UVR8), Elongandated hypocotyl (HY5) or HY5 Homolog (HYH) dependent or independent pathways (Kaiserli and Jenkins, 2007; Brown and Jenkins, 2008).

Besides these undefined UV-B photoreceptors, existence of some cell surface bound receptors has also been noticed. NOS (Nitric oxide synthase) was also identified as factor responsible for upregulation of gene encoding chalcone synthase (CHS) (Jordan, 2002; Brosche and Strid, 2003). NADPH oxidase gene GP31 that encode a plasma membrane protein showed Ca^{++} dependent signaling of ROS in plants (Keller et al. 1998). NADPH induced ROS signaling has been noticed in case of both UV-B (Rao et al. 1996; Jordan, 2002) and heavy metal (Foreman et al. 2003; Maksymiec, 2007). Heavy metal induces H_2O_2 accumulation either by stimulating OXO (oxalate oxidase), NADPH oxidase or by displacing the transition metals from metallochaperones or metalloenzymes and these released transition metals induces oxidative stress (Polle and Schutzendubel, 2003). These transition metals can also activates genes responsible for chperones and metallothioneins. In case of UV-B these ROS perform signaling for the synthesis of jasmonic acid (JA), salicylic acid (SA) and ethylene (Mackerness et al. 1999). JA along with ethylene synergistically induces expression of pathogenesis related PDF 1.2 genes (Pannickx et al. 1998). However SA along with ethylene upregulate the expression of PR genes (Jordan, 2002). Heavy metal induced H_2O_2 accumulation can also trigger the mitogen activated protein kinase (MAPK) cascade involving histidine kinase which in turn activate transcription of defense genes (Polle and Schutzendubel, 2003). Some undefined cell receptors have also been recognized with UV-B which follows MAPK pathway (Fig 2).

UV-B and water stress interaction

Water stress is one of the most obvious global issues like temperature and salinity that affects the survival of agricultural crops. Drought is itself a metrological term that defines a particular period of an area without significant rain fall. Generally drought arises when available water in soil is reduced however the surrounding atmospheric condition causes continuous loss of water either through transpiration or evaporation. The International Water Management Institute estimates that by the year 2025, one third of the world population will inhabit regions of severe water stress scarcity (IWMI, 2005). Since UV-B and water stress are globally accepted

concurring problems of many parts of the world, their interaction should be discussed extensively. Numerous studies have been conducted worldwide on the interactive effect of UV-B and water stress on wheat (Feng et al. 2007, Alexiva et al. 2001), leguminous plants (Teramura et al., 1984, Allen et al., 1999) and aromatic plants (Nogues and Baker, 2000) and noticed various types of interactions. Net effects of these stresses are sometimes synergistic (wheat), additive (soybean), adaptive (sunflower) or without any interaction (lavender, rosemary). Table 4. represents overall type of interaction in different studies conducted so far. Teramura et al. (1984) have found that UV-B more effectively changed biomass allocation however water stress reduced leaf and node number of *Glycine max* and their combined effect was additive on dry matter production and photosynthesis. However, Sullivan and Teramura (1990) have reported that UV-B and water stress showed less than additive effect on photosynthetic parameters of same test plant. Water stress induced masking of effect of UV-B may be due to anatomical or biochemical adjustments (pigment accumulation) which ostensibly protect plants from UV-B through screening mechanism. Drought may also delay cell divison and reduces cell elongation (Boyer, 1970). Since UV-B directly affects cell division thus delay in cell divison may provide protection against UV-B. Another possibility is the development of reduced level of phosphorus in plant due to water stress. In soybean plant Sullivan and Teramura (1990) have reported that phosphorus deficiency in soybean plants directly reduces sensitivity against UV-B. On the other hand, Ren et al. (2009) have observed antagonistic response of UV-B and water stress induced response on yield of soybean. Another leguminous plant *Pisum sativum* showed differential response in photosynthesis and productivity under water stress and UV-B. Nogues et al. (1998) noticed synergistic mode of interaction of both the stresses in flavonoid production but UV-B induced severity of photosynthesis was delayed by UV-B through reducing water loss rates, stomatal conductance and leaf area. On the other hand, Allen et al. (1999) have observed that upto 30 % increase of UV-B doesn't affect the photosynthesis and productivity under well watered and droughted plants of pea. In the study of Yang et al. (2007) two different populations of a leguminous plant *Hippophae rhamnoides* were showed that water stress had moderate response of UV-B which is more pronounced in species growing at high altitude as compare to low altitude. In wheat, UV-B and water stress synergistically induced specific changes in leaf morphology and water relation leading to improved water economy which maintains photosynthetic performance, biomass and yield (Feng et al. 2009). This synergism doesn't show any detrimental effect as compared to their individual response. Increased root shoot ratio in response to UV-B may help to offset water deficit while reduction in leaf area, LAI and induction of flavonoids may help to counter balance effect of UV-B. However, Tian and Lei (2007) have reported that both the stresses produced excessive ROS production leading to increased oxidative stress. UV-B produced more severe response but their interactive response showed additive effect on wheat. Similar response was also reported by Zhao et al. (2009) at 15% field capacity in water relation of wheat plants. However, negative effect of UV-B was alleviated by mild water stress (0.5 MPa) in both pea and wheat plants (Alexieva et al. 2001). Cechin et al. (2008) have also noticed alleviation of negative effect of drought by UV-B on photosynthesis and transpiration. Cucumber is relatively susceptible to unfavorable environmental conditions and is often chosen for studies investigating the reaction to one or more stress factors. Kubis and Zajac (2008) have measured antioxidative system of cucumber and reported synergistic response of UV-B and water stress.

Enhanced activity of syringaldazine peroxidase (SPX) suggests intensification of cell wall component synthesis and consequent increase in cell wall rigidity which provides tolerance against drought stress. In Qurecus petraea two type of differential response were reported. UV-B and water stress showed positive correlation in reducing fluorescence of oak while Meszaros et al. (2005) have observed that UV-B radiation caused hardening of oak which ultimately provided tolerance against water stress. Ren et al. (2007) have also studied response of two species of Populas and reported that *P. kangdingensis* which is already adapted to drought condition exhibit more tolerance to UV-B as compared to *P. cathayana* found at lower altitude. However, another tree species *Salix myrsinifolia* showed additive effect on growth parameters (Turtola et al., 2006). They have taken hybrids of *Salix* (fast growing and slow growing) and found that fast growing species was more susceptible as compared to slow one. This response may be due to better adaptability towards UV-B because of slow growth. Exposure of drought stressed species to UV-B showed more allocation of biomass to root which improved water relation of plant and provided protection against UV-B. Study of Schmidt et al. (2000) also showed that exposure of UV-B moderates the response of water stress in *Arabidopsis* plants and mechanism behind this response underlies in the maintenance of leaf water relation due to induced biosynthesis of stress proteins and compatible osmolytes. On other hand Nogues and Baker (2000) have reported no any significant interaction of UV-B and water stress on three Mediterranean plants lavender, olea and rosemary.

Both UV-B and water stress alter the morphology, anatomy, photosynthesis and metabolism of plant however their mechanism and site of action may be different. Both the stress affects the light and dark reaction of photosynthesis at various steps; however their sites of action may be different. The major mode of UV-B induced damage to photosynthesis is photomodification of various components while for water stress the main deciding factor is stomatal limitation leading to carbondioxide deficiency and alteration of some structural components. Ability of any plant to tolerate stress condition also depends on multiple biochemical pathways and their important products (active metabolite and specific proteins) that may help to maintain plant homeostasis and to sustain their life. Plants have a common strategy for protection against water stress is by accumulating compatible solutes and electrolytes (osmolyte). Osmolyte are a group of biochemically inert compound which helps to maintain osmotic balance necessary for growth and cellular metabolism under dehydration. Water stress induces important metabolic changes including synthesis and accumulation of various polyamines, polyols, proteins, pigments, amino acids, sugar, phenolics and amines. Accumulation of these compounds in high concentrations raise cytoplasmic osmotic pressure without perturbing cellular function and they also stabilize enzymes and membranes of plants (Rathinasabapathi et al., 2000) which in turn provides protection against UV-B (Fig 3).

UV-B and O_3 interaction

Our present state of knowledge on combined effect of UV-B and O_3 on plants is very limited. Earlier studies reported more reduction after sequential treatment of UV-B and O_3 in pollen tube growth of *Nicotiana tobaccum* and *Petunia hybrida* as compared to their individual effect (Feder and Shrier). However, Rao and Ormorod (1995) have reported that pre-exposure of O_3 to Arabidopsis thaliana made plant more sensitive towards UV-B. Table 5. have summarized

effect of UV-B and O_3 interaction on some plant species along with their dose and experimental condition. Booker et al. (1994) have conducted three year field study (1989 to 1991) to assess the effect of UV-B and O_3 on soybean plant. UV-B was not reported to be harmful for growth and yield of soybean but O_3 showed significant reduction for all the studied parameters. UV-B reduced the intensity of O_3 induced visible injuries initially but in last year no such interaction was reported. Growth and yield parameters also showed no significant interaction between UV-B and O_3 of the same plant. Ozone treatment consistently induced visible injury suppressed net carbon exchange rate, growth, yield and accelerated reproductive development however enhanced UV-B didn't suppress any of the above parameters. To study the mechanism of differential response of both the factors, Rao et al. (1996) have used *Arabidopsis thaliana* and its flavonoid deficient mutant for separate exposure of UV-B and O_3 and reported that both the stresses induced oxidative stress and ROS production. UV-B preferentially enhanced NADPH-oxidase and peroxidase related enzymes while O_3 induced SOD and enzymes of ascorbate-glutathione cycle. Staaij et al. (1997) have studied effect of reciprocal exposure of UV-B and O_3 on *Elymus athericus* and found both the stresses negatively affecting the growth of plant. However, the mode of interaction was not clear but their combined response supported the hypothesis that when changes in climatic condition will subject the plants to elevated levels of UV-B and rising concentrations of tropospheric O_3, the total result of both stress factors on plant growth may be of an additive nature. Baumbusch et al. (1998) also explained that low UV-B induced the protection against elevated O_3 in two gymnospermic plant (pines and spruce) and found that pine was more sensitive however spruce is was protected by low level of UV-B. Similar response of amelioration of O_3 response even in presence of ambient UV-B was observed by Schnitzler et al. (1998) on similar coniferous plants. All these studies clearly indicate that amelioration of effect may be seen only when concentration of single factor is elevating and the other remain at ambient level. In another study of Tripathi et al. (2011) and Tripathi and Agrawal (2012) simultaneously exposed linseed plants with elevated dose of both the stresses and reported that these stresses lowered their negative effect in interaction as compared to individual exposures.

Among all the studies considered in the present review, most of them were performed under controlled and indoor conditions. Indoor experiments generally don't have sufficient photosynthetically active radiation and thus exhibit reduced photolyase activity and DNA repairing process (Caldwell et al. 1995). Since these studies are performed under laboratory conditions and controlled practices which are little different from what plants experience in natural field, further detailed research studies are needed to deepen the role of these abiotic stress factors in the adaptive or changed response of plants to an UV-B enriched environment.

From these studies it can be predicted that the overall response of UV-B may be modified in natural field conditions which is species specific. However from few studies, it is not possible to predict a clear conclusion whether the response will be additive, synergistic or antagonistic. Future interaction based studies are needed in natural filed conditions before we come to definite conclusion.

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Table 1. Interactive effects of UV-B radiation and carbondioxide on different plants

Plant material	Growth Conditions	UV-B dose	CO_2 dose	Result of different parameters				Conclusion	Reference
				Morphological	Growth and physiological	Biochemical	Yield		
Vicia faba	Pot experiment	Ambient	700 µmol mol^{-1}	Shoot length, leaf area and dry weight (+)	LWR, RSR (+) RWR, LAR, SLA (-) g_s and Pn (-)	carbohydrate, starch (+)	-	UV-B modified the response of elevated CO_2	Visser *et al* (1997)
Vicia faba	Green house	10.6 kJ m^{-2} day^{-1}	750 µmol mol^{-1}	Biomass, number of leaves (+), leaf area (-)	LWR, SLA LAR, Pn and g_s (-), (-) RWR (+)	Carbohydrate and starch content (-)	-	No stimulatory response of CO_2 under UV-B	Tosserams *et al* (2001)
Glycine max	SPAR chambers	10 kJ m^{-2} day^{-1}	720 µmol mol^{-1}	Size of floral organs (-)	-	-	-	amelioration	Koti *et al* (2005)
Glycine max	SPAR chambers	10 kJ m^{-2} day^{-1}	720 µmol mol^{-1}	Plant height and leaf area (-)	Pn (+)	chlorophyll, phenolics and wax contents (+)	-	Amelioration	Koti *et al* (2007)
Gossypium hirsutum	SPAR Chambers	15.1 kJ m^{-2} day^{-1}	720 µmol mol^{-1}	Stem elongation, branch length, leaf area, number of balls and dry weight (-), fruit abscission and number of fruiting branches (+)	Gs and net Pn (-)	Chlorophyll, carotenoid and non structural carbohydrate content (-)	-	Elevated CO_2 could not alleviate the detrimental effect of UV-B	Zhao *et al* (2003)
Gossypium hirsutum	SPAR chambers	16 kJ m^{-2} day^{-1}	720 µmol mol^{-1}	Canopy leaf area (-)	Pn, g_s, transpiration rate, water use efficiency, leaf dark respiration (-)	Starch and non structural carbohydrate (+)	-	No interaction of UV-B and CO_2	Zhao *et al* (2003)
Gossypium hirsutum	SPAR chambers	15.1 kJ m^{-2} day^{-1}	720 µmol mol^{-1}	-	Leaf Pn, g_s (-)	total chlorophyll, carotenoids (-), phenolics (+)	-	No interaction of UV-B and CO_2	Kakani *et al* (2004)
Betula pendula	Green house Pot experiment	8.16 kJ m^{-2} day^{-1}	700 µmol mol^{-1}	Biomass accumulation (+)	RSR (+)	Phenolics flavonoid condensed tannins, PAL and POD activity	-	Elevated CO_2 may ameliorate the effects of UV-B radiation	Lavola *et al* (2000)
Betula pendula	Closed top chamber	7.95 kJ m^{-2} day^{-1}	700 ppm	-	-	Peroxidase, polyphenol oxidase, total polyamines (+) while Chl a, Chl b and soluble protein (-)	-	Synergistic	Tegelberg *et al* (2008)
Pinus taeda	Green house	13.8 kJ m^{-2} day^{-1}	650 µmol mol^{-1}	Needle, root and stem biomass (-)	RSR (+) while SLW, Fv/Fm, O_2 evolution, Pn (-)	Total chlorophyll (+)	-	Elevated CO_2 may modified UV-B response	Sullivan and Teramura (1994)

Brassica napus	Green house	4.2 kJ m^{-2} day^{-1}	700 µmol mol^{-1}	-	CO_2 assimilation and water use efficiency (+) while transpiration (-)	Total chlorophyll, UV screening pigments (+)	Seed weight (-)	amelioration	Qaderi *et al* (2007)
Trifolium repens	Green house	Reduced level 82% and 88% than ambient	520 µmol mol^{-1}	UV-B favored shoot growth and CO_2 favored root growth, both enhanced number of flowers	-	-	-	Both stress enhanced their response	Deckmyn (2001)
Elymus athericus	Green house	16.8 kJ m^{-2} day^{-1}	720 µmol mol^{-1}	Plant dry weight, number of leaves, leaf area, shoot number and shoot length (-)	RSR, SLW, LAR (-) while SLA, NAR, RGR (+)	-	-	CO_2 modified response of UV-B	Staaij *et al* (1993)
Oryza sativa	Green house	13.8 kJ m^{-2} day^{-1}	660 µbars	biomass (+) number of tillers, leaf area and leaf weight (-)	RSR, SLW, Amax (+) while ACE and stomatal limitation (-)	Concentration of UV-B absorbing compound (+)	harvest index and yield (+)	UV-B modified the response of CO_2	Ziska and Teramura (1991)
Dimorphotheca pluvialis	Open top chamber	11.13 kJ m^{-2} day^{-1}	650 µmol mol^{-1}	Biomass, number of buds, open flowers and reproductive structure (+)	Pn, WUE (+) while g_s(-)	UV-B absorbing compound (+)	-	synergistic	Ward *et al* (1996)
Oryza sativa *Glycine max* *Triticum aestivum*	Green house	10% ozone depletion	650 µmol mol^{-1}	-	Pn (+) in wheat and soybean while (-) in rice	UV-B absorbing compounds (+)	Seed yield of rice, wheat (-) soybean(+)	UV-B modified the response of CO_2	Teramura *et al* (1990)

Negative impact (-), positive effect (+), non significant (ns)

Table 2. Interactive effect of UV-B and mineral nutrient stress along with experimental conditions on different plants

Plant material	Growth Conditions	UV-B dose	Nutrient dose	Results				Conclusion	Reference
				Morphological	Growth and physiological	Biochemical	Yield		
Acer mono maxim	Pot experiment	14.33 KJm^{-2}day^{-1}	20 g m^{-2} a^{-1} (Nitrogen)	total biomass, number of leaves, SLA (-)	Pn and gs and chlorophyll (-)	H_2O_2, O_2^-, Proline, POD, SOD, CAT, APX, GR (+)	-	N-supply made plant more sensitive to enhanced UV-B	Yao and Liu (2006)
Hordeum vulgare	Pot experiment	21 KJm^{-2}day^{-1}	100 µM (iron)	Fresh and dry weight (-)	-	Zeaxanthin (+), H_2O_2, ascorbate, protein CAT (-) APX (+)	-	UV-B enhances oxidative stress in iron deficient condition	Zaman et al. (2008)

Ceratonia siliqua ***Phlomis fruticosa***	field study	15% ozone depletion	10 mg N, 1 mg P, 1.3 mg K	No interaction on growth and morphology of *Ceratonia siliqua* However strong positive interaction on *Phlomis fruticosa*	-	Total phenolics and chlorophyll (+) in *Phlomis fruticosa* While decreases in *Ceratonia siliqua*	-	Low nutrient condition provide protection against UV-B stress	Levizou and Manetas (2001)
Betula pendula	Green house	7.3 KJ m^{-2} day^{-1}	Nutrient deficiency	% injury (+)	-	No interaction of UV-B and N on nutrient content of leaf and phenol except myricitin	-	additive	De la Rosa et al. (2005)
Plantago lanceolata	Green house and pot experiment	0, 4.6, 7.6 and 10.6 KJ m^{-2} day^{-1}	Low nutrient 0.5 g dm^{-2} High nutrient 4 g dm^{-2}	Root shoot and leaf biomass reduced at high N and UV-B	-	Chlorophyll and UV-B absorbing compounds (+)	-	amelioration	Tosserams (2001)
Pinus sylvestris	Green house	0-13.07 KJ m^{-2} day^{-1}	3.2-36.3 mg N L^{-1}	Shoot and needle weight were (+) at high UV-B	-	Low nutrient availability changes concentration of flavonoids and tannins	-	synergistic	Lavola et al. (2002)
Phaseolus vulgaris L.	Green house	3.2 KJ m^{-2} day^{-1}	12 or 1 mM nitrate	No change in leaf area in low N condition	Gs, Rubisco activity (-)	Starch, UV absorbing compound (+) chlorophyll (ns)	-	adaptation	Rique lme et al. (2007)
Picea asperata	Open semi field	+11.0 kJ m^{-2} day^{-1}	20 gm^{-2} a^{-1} nitrogen	Plant height, basal diameter (+) total biomass (-)	Pn, gs, transpiration rate, chlorophyll (-)	H_2O_2, O_2^- MDA, proline enzymatic activity (+)	-	synergistic	Yao and Liu (2007)
Picea asperata	Open semi field	14.33 kJ m^{-2} day^{-1}	20 gm^{-2} N	Plant height, basal diameter (+) biomass (-)	Pn, chlorophyll carotenoid (-)	-	-	amelioration	Yao et al. (2008)
Amaranthus tricolor	Field experiment	+7.2 kJ m^{-2} d^{-1}	Different NPK doses	Root, shoot length leaf area, biomass (-) number of leaves (+)	SLA (-) while SLW, NAR, RGR, (-)	-	Yield (+) only in 1.5 times recommended NPK while (-) in rest two nutrient doses	1.5 times recommended NPK showed amelioration	Singh et al. (2008)
Solanum tuberosum	Field experiment	+7.2 kJ m^{-2} d^{-1}	Different NPK doses	Root length, shoot length, leaf number, area and biomass (-)	Growth indices altered	-	Yield and quality deteriorated	No amelioration	Singh et al. (2010)
Zea mays	Field experiment	+6.84 kJ m^{-2} d^{-1}	N_0, N_{100}, N_{200}, N_{300} (nitrogen)	-	Pn, gs, transpiration rate (-)	chlorophyll, carotenoid, protein, soluble sugar, starch (-)	-	No amelioration	Cerezo et al. (2005)

Triticum aestivum ***Vigna radiata***	Field experiment	+7.1 kJ m^{-2} d^{-1}	Recommended dose of NPK and without NPK	Nutrient deficiency and UV-B results into maximum (-) biomass	-	Nutrient deficiency and UV-B result into more damage of chlorophyll and in thiol, SOD and POD (+)	-	Amelioration	Agrawal and Rathore (2007)
Triticum aestivum	Field experiment	Ambient +7.1) KJ m^{-2} day^{-1}	without NPK, Recommended NPK, 1.5 times NPK, 2 times NPK	Root, shoot length and biomass (-)	growth indices were minimum in additional NPK	-	Yield and harvest index (-) in UV-B and NPK amended plants	Mineral nutrient specially 1.5 times NPK is most suitable UV-B dose to overcome the effect of	Agrawal et al. (2004)
Zea mays	Field experiment	3.16(+6.84) KJ m^{-2} day^{-1}	N_0, N_{100}, N_{200}, N_{300} (nitrogen)	Maximum reduction in biomass in N0T	LAR, LWR, SLA (+) while NAR (-)	-	Ear length, ear perimeter, grain number, grain weight and grain yield (-)	UV-B lowered positive effect of N	Correia et.al. (2000)
***Cucumis sativus* L.**	UV-B transparent green house in Perlite pots	3.1(±2.5) KJ m^{-2} day^{-1}	four nitrogen treatments: 0.5, 2.0, 5.0, 10.0 mol m^{-3} of nutrient solution	Plant height, leaf area, total biomass reduced while epidermal thickness of leaf (+)	Fluorescence (-)	Chlorophyll and carotenoids (-) upto N level 5 mol m^{-3}	-	amelioration	Hunt and McNeil (1998)
***Dimorphotheca pluvialis* (L.)**	Green house and pot experiment	0,10,20,30% ozone depletion	Low and high nutrient dose N-5.8 mg, P-0.8 mg, K-1.7 mg	Biomass (-) in low N at 30% while number of leaves, leaf area (-) in high N at 30%	growth (ns), number of diaspore, inflorescence (+)	Foliar C:N have no effect while foliar P, thickness (-)	-	Low nutrient level enhances the effect of (+) UV-B	Musil and Wand (1994)
Myriophyllum spicatum (L)	Aquarium	0,0.3 W/m^2	0,2.3 mg/L of nitrogen	Growth (-)	-	phenolic content (ns)	-	Low N enhances the effect of UV-B	Li et al (2005)
Dunaliella tertiolecta	Auxenic culture	0.4W/ m^2	P starvation	Growth rate (-)	Pn and quantum yield reduced	-	-	Low P enhances the sensitivity towards UV-B	Shelly et al. (2005)

Negative impact (-), positive effect (+), non significant (ns)

Table 3. Interactive effects of UV-B radiation and heavy metals along with experimental conditions on different plants

Plant material	Growth Conditions	UV-B dose	Heavy metal dose	Result of different parameters				Conclusion	Reference
				Morphological	Growth and physiological	Biochemical	Yield		
Riccia sp.	Growth chamber	0.4 W m^{-2}	1-1000 mM (Cd)	Growth (-)	Oxygen evolution, PS2 activity and electron transport chain (-), respiration (+)	Chl a, b, carotenoids and phycocyanin (-), MDA content, SOD, CAT activity (+)	-	synergistic effect	Prasad *et al* (2004)
Triticum aestivum	Pot experiment	0.4 W m^{-2}	0.25-5.0 ppm (Cd)	Shoot length, fresh and dry weight (-)	-	Chl a, b, carotenoids soluble sugar (-) protein, free amino acid and starch content content (+)	-	amelioration	Shukla *et al. (2002)*
Spinacea oleracea	Pot experiment	+7.1 KJ m^{-2}	68 µ mol kg^{-1} Cd and Ni	Biomass (-)	-	Chl, carotenoid, ascorbic acid content, catalase activity (-) anthocyanin, flavonoid content, LPO, proline and peroxidase activity (+)	-	less than additive	Mishra and Agrawal (2006)
Glycine max	Growth chamber experiment	0.4 W m^{-2}	0.01, 0.10, 1.00 mM (Ni)	Height, leaf area, fresh weight and biomass (-)	PS I and II inhibited	Chl a,b, ascorbic acid and CAT activity (-) Carotenoid, H_2O_2, O_2^- ,proline, MDA content, electrolyte leakage, SOD and POD (+)	-	less than additive	Prasad *et al.* (2005)
Arabidopsis thaliana	Growth chamber	6 KJ m^{-2} day^{-1}	0,1 µM Cd	-	O_2 evolution, potential and maximum photochemical yield (-)	Total chlorophyll and carotenoid content (-), nutrient content affected Ca, Mg content (-)	-	amelioration	Larsson *et al* (2001)
Anabaena doliolum	Culture media	12.9 mW m^{-2}	0.3, 0.5 µg ml^{-1} Cu	Specific growth rate (-)	C-fixation, PS II and PS I, ATP pool, chlorophyll fluorescence and ETS (-) respiration rate (+) and complete loss of O_2^- evolution	LPO and Cu uptake (+)	-	Synergistic effect	Rai *et al.*(1995)
Anabaena doliolum	Culture media	12.9 mW m^{-2}	Cu 8.0 m mol l^{-1} Pb 70 m mol l^{-1}	-	-	Uptake of urea, NH_4, NO_3^{-2} and PO_4^{-3} (-)	-	synergistic	Rai *et al.* (1998)

Glycine max	Pot experiment	0.15, 0.45 W m^{-2}	La 20 mg/L	-	-	Pigments (-), flavonoid content and PAL activity (+)	-	amelioration	Bin *et al* (2006)
Pisum sativum	field experiment	+7.1 KJ m^{-2} day^{-1}	Cd 68 µmol kg^{-1}	Shoot length and biomass (-)	-	Chl, car, ascorbic acid and catalase (-) SOD,POD, thiol, proline and LPO (+)	Yield (-)	synergistic	Agrawal and Mishra (2007)
Plectonema boryanum	Culture media	0.4 W m^{-2}	2, 8 µM Cd	Growth and survival reduced	Pn, O_2 evolution, PS I and PS II activity (-) respiration (+)	Chl, car and phycocyanin (-) SOD, LPO, CAT (+)	-	synergistic	Prasad and Zeeshan (2005)
Pisum sativum	field experiment	+7.1 KJ m^{-2} day^{-1}	Ni 68 µmol kg^{-1}	-	-	Total chl, car, flavonoids (+), ascorbic acid, thiol, phenol, proline, LPO, SOD, POX (+) while protein and CAT activity (-)	-	antagonistic	Singh *et al* (2009)
Brassica napus	green house	15 KJ m^{-2} day^{-1}	0, 0.5, 2, 5 µM Cd	Leaf area root dry weight (-)	Fv/Fm, non photochemical quenching and photochemical yield (-)	Chl a, b, carotenoids (-) some nutrients (Cd, P,S,Cu, Zn) (+) some (-) (Mn)	-	synergistic	Larsson *et al* (1998)
Brassica juncea	Pot experiment	0.15, 0.35 W m^{-2}	Ce 12 mg/L	-	-	Chlorophyll (-), membrane permeability, SOD, CAT, POD activity (+)	-	amelioration	Chanjuan *et al* (2006)
Glycine max	Pot experiment	0.15, 0.45W m^{-2}	Ce 20 mg/L	-	All photosynthetic processes (-)	-	-	antagonistic	Chanjuan *et al* (2006)

Negative impact (-), positive effect (+), non significant (ns)

Table 4. Interactive effects of UV-B radiation and water stress along with experimental conditions on different plants

Plant material	Growth Conditions	UV-B dose	Water stress	Result of different parameters				Conclusion	Reference
				Morphological	Growth and physiological	Biochemical	Yield		
Pisum sativum	field study	2.4 kJ m^{-2} d^{-1}	-	Dry weight, height, leaf area, shoot number (+) Number of leaves (-)	Root shoot ratio (+)	Flavonoid and anthocynain (+)	-	No effect	Allen *et al* (1999)
Triticum aestivum	field study	4.25 kJ m^{-2} d^{-1}	15 % field capacity	Flowering, ripening delayed Growth, biomass (-)	Water potential (-) while relative water content (+)	Chlorophyll (-) while MDA and flavonoid (+)	Yield (-)	amelioration	Feng et al (2007)
Pisum sativum Triticum aestivum	Growth chamber	49 kJ m^{-2} d^{-1}	-0.5 MPa	Fresh, Dry weight, height (-)	relative water content (-)	Chlorophyll (-) anthocyanin, phenols, LPO, electrolyte leakage, proline (+) CAT, SOD, H_2O_2 (+)	-	synergistic effect	Alexieva *et al* (2001)
Triticum aestivum	Pot experiment	13.1 kJ m^{-2} day^{-1}	15 % field capacity	-	Water use efficiency, water consumption (-)	-	-	synergistic	Zhao *et al* (2009)
Glycine max	Green house	2.88 kJ m^{-2} day^{-1}	-2 MPa	Plant height, number of nodes, leaves, leaf area and dry weight(-)	SLW, net photosynthesis, stomatal conductance, dark respiration (-)	Chlorophyll a, b (-) UV-B absorbing pigments (+)	-	additive	Teramura *et al* (1984)
Glycine max	Field study	13.6 kJ m^{-2} day^{-1}	-2 MPa	Plant height, leaf area and dry weight (-)	SLW (+), AQE, g_s, photosynthesis and carboxylation efficiency (-)	-	Number of pods, seed number and seed yield (-)	additive	Sullivan and Teramura (1990)
Populus kangdingensis P. cathayana	Pot experiment	4.4 kJ m^{-2} day^{-1}	50% field capacity	Plant height, leaf number and leaf area (-)	Specific leaf mass (+)	UV-B absorbing pigments, proline, SOD, APX, CAT (+)	-	synergistic	Ren *et al* (2007)
Helianthus annuus	Green house	8.6 W m^{-2}		Stem, root, leaf dry weight (-)	Stomatal conductance, transpiration, internal CO_2 and Fv/Fm (-)	Chlorophyll a,b (-) and POD, MDA and proline (+)	-	amelioration	Cechin *et al* (2008)
Quercus petraea	Controlled chambers	150 µW m^{-2}		-	Water content, Sm, Fv/Fm (-), water potential (+)	Chlorophyll a,b (-) and carotenoids specially lutein (+)	-	amelioration	Meszaros *et al*

Lavandula stoechas Olea europea Rosmarinus officinalis	glass house	24 kJ m^{-2} day^{-1}		Plant height, dry weight, number of leaves, leaf area (-)	RWC, Ψw, Asat, Vc max, Fv/Fm and Φ_{II} (-), stomatal limitation and transpiration (+) SLA, LWR, LAR plant, soil water content (-)	Anthocyanin (+), flavonoid (+) in olive while (-) in other two	-	No interaction	Nogues and Baker (2000)
Salix myrsinifolia	glass house	7.2 kJ m^{-2} day^{-1}	20% field capacity	total biomass and stem height (-)	RSR (+)	-	-	additive effect	Turtola *et al* (2006)
Glycine max	Pot experiment	4.33, 12.8% UV-B	40%, 80 % water volume	-	-	-	yield (-)	antagonistic	Ren *et al.* (2009)
Pisum sativum	Green house	32 kJ m^{-2} day^{-1}	-	Biomass and growth (-)	LAR, RSR, plant water content all (-) photosynthetic (ns)	Anthocyanin and flavonoid contents (+)	-	UV-B radiation delayed effect of water stress	Nogues and Baker (1998)
Cucumis sativus	Growth chamber	16 kJ m^{-2} day^{-1}	40% water holding capacity	Dry weight (-)	Relative water content (-)	SPX, GR, GPX, SOD (+)	-	Synergistic	Kubis and Zajac (2008)
Arabidopsis thaliana	Controlled environment chamber	6 kJ m^{-2} day	-	No significant effect on biomass	Maintained leaf water content	Induction of some proteins	-	amelioration	Schmidt *et al.* (2000)
Quercus petraea	Phytotronic chamber	150 μW m^{-2}	-	-	Chlorophyll fluorescence (+)	xanthophyll cycle (+)	-	synergistic	Szellesi *et al.* (2008)
Hippophae rhamnoides	Green house	+8 kJ m^{-2} day	25 % field capacity	Total dry weight, leaf area (-)	SLA (-), RSR (+)	MDA, electrolyte leakage, proline, anthocyanin (+) , ABA (-)	-	Synergistic	Yang *et al.* (2005)
Triticum aestivum	Controlled environment	3.5 kJ m^{-2} day	- 5.0 MPa	Shoot growth (-)	-	H_2O_2, TBARS, CAT, APX, GPX, SOD,	-	additive effect	Tian and Lei (2007)

Negative impact (-), positive effect (+), non significant (ns)

Table 5. Interactive effects of UV-B radiation and Ozone along with experimental conditions on different plants

Plant material	Growth Conditions	UV-B dose	O_3 dose	Result of different parameters				Conclusion	Reference
				Morphological	Growth and physiological	Biochemical	Yield		
Glycine max	Open top chamber study	35 , 37 % depletion	83 nL L^{-1}	Visible injury reduced	Biomass (ns)	-	yield (ns)	No interaction	Miller et al. (1994)
Arabiodopsis thaliana	Growth chamber	18 kJ $m^{-2}d^{-1}$	200 ppb	Reduced growth	-	Enhanced SOD, GR, APX, POD	-	enhanced oxidative stress	Rao et al. (1996)
Elymus athericus	Growth chamber	16 kJ $m^{-2}d^{-1}$	190 µg m^{-3}	Number of shoots and leaves (-)	Pn (-)	-	-	additive effect	Staaij et al. (1997)
Pinus sylvestris Picea abies	Phytochambers	1.2 kJ $m^{-2}d^{-1}$	43 nL L^{-1}	-	-	POD, CAT, SOD, LPO, ascorbate, glutathione (+)	-	amelioration	Baumbusch et al. (1998)
Pinus sylvestris Picea abies	Growth chamber	1.2 kJ $m^{-2}d^{-1}$	Twice ambient	Visible injury (-)	Pn (-)	-	-	amelioration	Schnitzler et al. (1999)
Pinus sylvestris	Growth chamber	0.8 kJ $m^{-2}d^{-1}$	Twice ambient (52 to 192 nL L^{-1})	Visible injury (+)	-	Secondary metabolites (+)	-	additive	Zinsser et al. (2000)
Triticum aestivum	Field study	+7.6 kJ $m^{-2}d^{-1}$	0.07 µmol mol^{-1}	Biomass (-)	Photosynthesis, chlorophyll, carotenoids (-) anthocyanin , flavonoid (+)	CAT, phenol, POX (+) ascorbic acid (-)	Yield (-)	less than additive	Ambasht and Agrawal (2003)
Linum usitatissimum	Field study	+7. kJ $m^{-2}d^{-1}$	+ 10 ppb	Biomass (-)	-	antioxidants (+), protein profile and DNA showed alterations -	-	less than additive	Tripathi et al. (2011)
Linum usitatissimum	Field study	+7. kJ $m^{-2}d^{-1}$	+ 10 ppb	-	-		Seed yield, seed and oil quality (-)	less than additive	Tripathi and Agrawal (2011)

Negative impact (-), positive effect (+), non significant (ns)

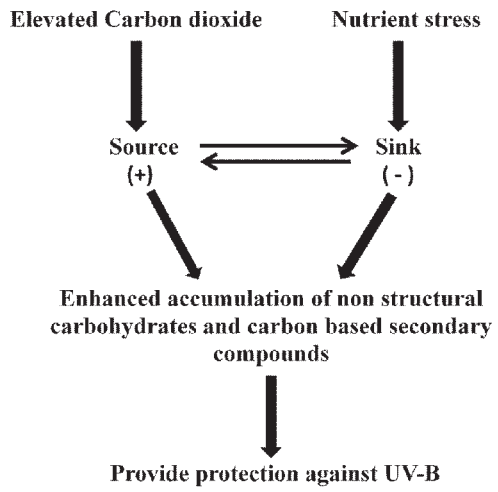

Fig 1. Carbon dioxide and nutrient induced alteration in source and sink balance resulting in protection against UV-B

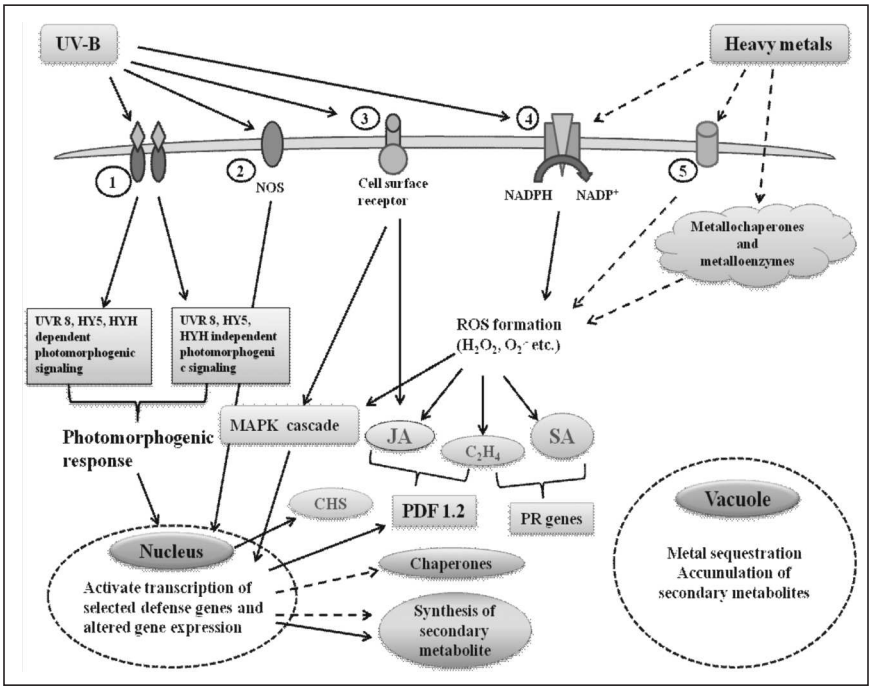

Fig 2. UV-B and heavy metal induced signaling in plant cell.

(Abbreviations; 1. Photoreceptors, 2.Nitric oxide synthase, 4. NADPH oxidase, 5. Oxalate oxidase, UVR 8; UV Resistance Locus 8, HY5; Elongandated hypocotyls, HYH; HY5 Homolog, MAPK; mitogen activated protein kinase, JA; jasmonic acid, SA; salicylic acid, C_2H_4; ethylene, ROS; reactive oxygen species, CHS; chalcone synthase)

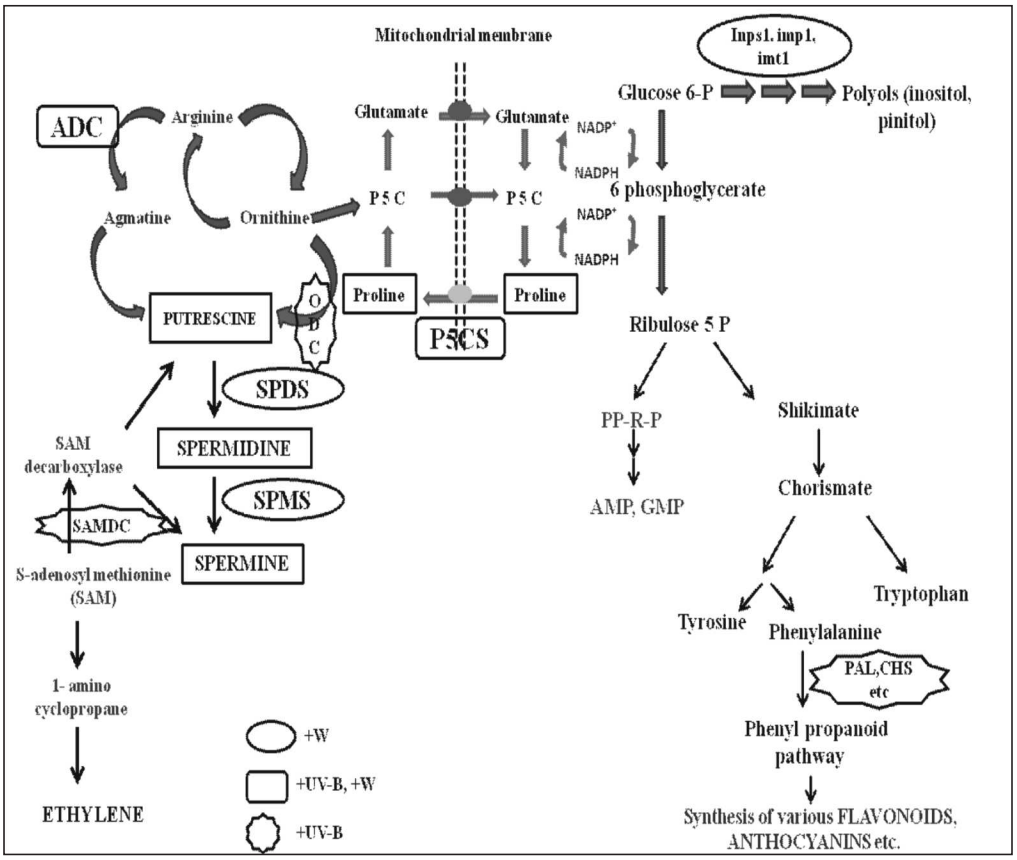

Fig 3. Induction of various enzymes after UV-B and water stress in synthesis of secondary metabolites.

(Abbreviations; SAMDC; S- adenosyl methionine decarboxylase, SPMS; spermine synthase, SPDS; spermidine synthase, P5C, [1]-pyrroline-5-carboxylate, P5CS; pyrroline-5-carboxylate synthetase, P5CR; pyrroline-5-carboxylate reductase, PP-R-P; phosphoribosyl pyrophosphatase, inps1; Inositol1-phosphate synthase, imp1, inositol monophosphatase, imt 1; inosititol O-methyltransferase, ADC; arginine decarboxylase, ODC; ornithine decarboxylase, PAL; phenyl alanine ammonia lyase, CHS, chalcone synthase)

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Random Genetic Drift Affecting Alcohol Dehydrogenase Polymorphism in Laboratory Populations of *Drosophila ananassae*

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Abstract

A mass culture stock of *Drosophila ananassae* established from Ranchi (Jharkhand) was analyzed for its Adh polymorphism and the stock was observed to be polymorphic at this locus as all the three genotypes (FF, FS and SS) could be recorded. From this stock, five separate lines were established by utilizing five pairs of flies. These five lines were maintained for at least five generations and in each generation twenty pairs were used for starting the next generation. It was observed that *Adh* polymorphism get affected very drastically in all the five lines as compared with the mass culture stock from which they were established. The present study thus clearly indicates the effect of random genetic drift on alcohol dehydrogenase polymorphism in laboratory populations of D. ananassae.

Keywords: Allozyme polymorphism, Alcohol dehydrogenase, genetic drift, *Drosophila ananassae.*

Introduction

Genetic polymorphism is maintained due to higher Darwinian fitness of heterozygotes (Dobzhoansky 1970). The factors which cause changes in the frequencies of alleles and genotypes in a population have been considered as important elemental forces of evolution. Among different elemental forces of evolution, natural selection and genetic drift are important in causing alterations in gene frequencies in populations (Hickey 1979, Hilbish and Koehn 1985). In a given environment, certain alleles or genotypes may be favored due to high adaptive values of their carriers by selection which will lead to gradual enhancement in the frequencies of those alleles in populations. However, in small populations gene frequencies may fluctuate significantly due to random genetic drift. The roles of evolutionary forces like selection and random genetic drift have been demonstrated in several animal species. It is very interesting to understand whether the phenomenon of polymorphism is affected more by random genetic drift or by natural selection (Kimura 1983, Koehn et al 1983).

Allozymes are allelic variants of an enzyme encoded by a particular locus. Numerous studies have been done on allozyme variation and its effect on the mechanism of evolution. It has been observed that allelic frequency changes at a particular allozyme locus in laboratory populations and are often considered as evidence for the occurrence of selection (Berger 1997, Ayala and Anderson 1973, Fontdevila et al 1975). Lewontin and Hubby (1966) undertook the first

extensive analysis of protein polymorphism in natural population of *Drosophila pseudoobscura* and studied 18 gene loci. Allozyme variation is widespread in most organisms including humans in which about 30 percent of all enzyme coding genes are polymorphic (Harris and Hopkinson 1976, Saastamoinen et al 2009, Flippov and Andronova 2011, Korshikov et al 2011).

Alcohol dehydrogenase is one of the most studied enzyme systems among proteins exhibiting allozyme polymorphism (Dickinson and Sullivan 1975). In natural populations of most of the *Drosophila* species, *Adh* gene is polymorphic for two allozymes designated as Slow (S) and Fast (F), on the basis of their electrophoretic mobility. Prakash and Shamina (1994) analyzed ten different geographical populations of *D. ananassae* from India to observe the allelic frequencies of Alcohol dehydrogenase (*Adh*), Octanol dehydrogenase (*Odh*) and Aldehyde oxidase (Ao). They reported genetic divergence pattern at Adh and Odh loci which they believed to be maintained by balancing natural selection varying spatially along the north- south axis at the Indian subcontinent.

Drosophila ananassae belongs to the *melanogaster* species group of the subgenus *Sophophora.* It is a cosmopolitan and domestic species. This species is known to possess many unique genetic properties and it is most prevalent in India. Alcohol dehydrogenase polymorphism has not been studied in the laboratory stocks of this species. In the present study we have analyzed one of the mass culture stocks and five drift lines for Adh polymorphism to study the role of evolutionary forces causing changes in the gene frequency of *Adh.*

Materials and Methods

Ranchi (RN) mass culture stock was established in 2010 from 17 isofemale lines. Polymorphism at Adh locus was tested in this stock and it was found to be polymorphic for all the three genotypes (FF, FS, and SS). From this mass culture stock, five drift lines (A, B, C, D and E) were established and maintained in culture bottles. The mass culture stock and all the five lines were maintained in yeast-agar culture medium in normal laboratory condition (12 hour light-dark cycle at 24 ºC). Drift lines were established by taking five pairs of flies from the mass culture stock and for further generations, only 20 pairs were used. All the five lines were maintained for five generations and then Adh polymorphism was tested. Flies were analyzed from each of the culture bottles for in- gel assay. For this purpose, single fly homogenate was run on 10% native Polyacrylamide gels at100 volts, for 4 hours at 4ºC. Staining procedure of Ayala et al. (1972) was followed, with a little modification in which isopropanol was replaced by 95% ethanol. To know whether the mass culture stock and the drift lines were in Hardy-Weinberg equilibrium for Alcohol dehydrogenase, chi square test was performed.

Results

Table 1 incorporates the observed and expected numbers of three genotypes (FF, FS and SS) and the allelic frequencies of S and F in the Ranchi mass culture stock. Out of 77 individuals analyzed from this stock, the heterozygotes were in maximum number followed by SS homozygotes. The frequency of S and F alleles were found to be 0.57 and 0.43 respectively. Chi-square analysis indicates that this population is not in Hardy-Weinberg equilibrium as there is significant difference between the observed and expected values ($p<0.01$). Table 2 incorporates the observed and expected numbers of genotypes, their allelic frequencies (F and S) and χ^2 values

in 5 different lines maintained in the laboratory for five generations. Populations reared in the bottles serialized as A, D and E were found to be polymorphic since SS and FS genotypes could be recorded in them. However, no individual having genotype FF could be detected in these stocks. Populations raised in bottles C and D became monomorphic at Adh locus since only SS genotypes could be detected. The deviation from Hardy–Weinberg equilibrium was insignificant in all the lines except in line E ($P<0.05$).

Table 1
Genotypic and allelic frequencies of flies analyzed from Ranchi mass culture stock.

Total No. of flies analysed		Genotypes			Allelic Frequencies	
		SS	FS	FF	S	F
77	obs.	19	50	8	0.57	0.43
	exp.	25.2	37.7	14.1		
$\sum\chi^2 = 8.176$, d.f=1, $P<0.01$						

Table 2
Genotypic and allelic frequencies of flies analyzed from 5 drift lines established from Ranchi mass culture stock

Lines	Total No. of flies analyzed		Genotypes			Allelic Frequency		χ^2	P
			SS	FS	FF	S	F		
A	35	obs.	32	3	0	0.96	0.04	0.09	>0.99
		exp.	32.26	2.68	0.06				
B	21	obs.	21	0	0	1	0	0	>0.99
		exp.	21	_	_				
C	21	obs.	21	0	0	1	0	0	>0.99
		exp.	21	_	_				
D	21	obs.	15	6	0	0.86	0.14	0.60	>0.20
		exp.	15.53	5.06	0.41				
E	21	obs.	7	14	0	0.67	0.33	5.30	<0.05*
		exp.	9.43	9.29	2.28				

* Significant

Discussion

Drosophila ananassae has been well studied for its chromosomal polymorphism but its protein polymorphism has not so substantially been studied (Singh 2000). We have started doing enzyme polymorphism in this species by selecting some of the enzymes which are known to be polymorphic in other species of *Drosophila* (Kumar & Singh 2012). In this study we have tried to see the founder effect on the persistence of enzyme polymorphism in this species. It is well documented that an allele may increase or decrease in its frequency through chance. in

populations, genetic drift favors either the loss or fixation of an allele. The rate at which an allele is lost or becomes fixed is completely dependent on the population size. The impact of random genetic drift is more significant in smaller populations. Random genetic drift may not have any effect in the fitness of a population or it may have a beneficial or detrimental effect on the population. In nature there are several interesting ways by which small population size and genetic drift affects the genetic composition of a population. By using inversions as chromosome markers, the phenomenon of genetic drift has been observed in *D. pseudoobscura* (Dobzhansky and Pavlovsky 1957) and *D. paluistoram* (Powell and Richmond 1974). They observed random changes in the frequencies of chromosome arrangements in the different experimental populations established with small numbers of individuals. Singh (1988) reported evidence for random genetic drift in laboratory populations of *D. ananassae.* He found variations in ST gene arrangement in contrast to its counterpart, AL arrangements in different laboratory lines started with less number of flies.

In this study, the mass culture stock showed polymorphism at Adh locus as all the three genotypes were recorded. The Adh locus in this mass culture stock is not in the Hardy-Weinberg equilibrium as there is significant deviation from expectation. The five founded populations raised from the mass culture stock showed very different genetic composition when analyzed after five generations. The five lines were established by just five founding females and were maintained by transferring only 20 pairs of flies in each generation. Therefore, the changes observed in the frequency of alleles are likely to be caused by random genetic drift. Fixation of the slow (S) allele occurred in bottles B and C in just five generations. In all the five lines, no fast homozygous (FF) individual was observed which indicates the elimination of this genotype. Although, heterozygotes (FS) were scored but their number was found to be less in frequency, indicating that the two alleles are still maintained in the population. It can be stated that the allele which was low in its frequency in the mass culture (i.e. the fast allele) decreased in its frequency in subsequent generations and even got eliminated completely in two of the lines (B and C). In fact the initial frequency of an allele, guides the path of random genetic drift more than it does to the other elemental forces of evolution. This is because, in a small population, where the role of random genetic drift is more pronounced, an allele having a low frequency is more likely to decrease in its frequency in the next generations and vice versa. Thus, in subsequent generations, it is likely to get eliminated and the other one to get fixed in the population. In this study each of these populations is assumed to undergo drift independently of the other populations.

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Bayesian Estimation of Three-Parameter Weibull Distribution under Asymmetric Loss Functions using Progressive Type-II Censored Data

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Abstract

We consider three-parameter Weibull distribution and derive Bayes estimators of the parameters under symmetric as well as asymmetric loss functions under progressive type-II censoring scheme. We use Lindley's approximation to obtain Bayes estimates and also perform simulation study for numerical illustrations.

Keywords: Bayes estimator; General Entropy loss function; LINEX loss function; Lindley's approximation; progressive type-II censoring; reliability function; squared-error loss function.

1. Introduction

The Weibull distribution is widely used as a lifetime model in reliability and survival analysis. Both, two and three parameter Weibull distributions have been extensively studied by several authors [see Cohen (1965, 1973, and 1975)]. Leone *et al.* (1960) obtained maximum likelihood estimators (MLEs), for the three parameter Weibull distribution using Newton Raphson Method. Harter and Moore (1965) provided iterative procedures for joint maximum likelihood estimation with complete and Type-II censored samples. Sinha and Solan(1988) considered this distribution and obtained Bayes estimates of parameters using Lindley's approximation. Kundu and Raqab (2009) considered the estimation of the stress strength model $P(X \le Y)$ when X and Y are independent and both follow three parameter Weibull distributions.

A random variable X is said to follow the three parameter Weibull distribution if its probability density function (pdf) is given by

$$f(x;\beta,\theta,\gamma) = \frac{\beta}{\theta}(x-\gamma)^{\beta-1}\exp\left(-\frac{(x-\gamma)^{\beta}}{\theta}\right), \quad 0 \le \gamma < x < \infty, \quad \beta,\theta > 0. \tag{1.1}$$

The reliability function and hazard rate functions for (1.1) at any time t, are given, respectively, by

$$R(t) = \exp\left(-\frac{(t-\gamma)^{\beta}}{\theta}\right); \qquad t > \gamma \tag{1.2}$$

and

$$h(t) = \frac{\beta}{\theta}(t-\gamma)^{\beta-1}; \qquad t > \gamma. \tag{1.3}$$

In lifetesting experiments, various kind of censoring schemes are frequently used to save time and cost. Among several existing censoring schemes, the progressive type-II censoring scheme has become very popular among workers in the field of reliability and survival analysis which is described as follows. Let n items are put to test and the numbers R_1, $R_2, \ldots, R_m$ are fixed such that, at the time of first failure, R_1 items are removed randomly from the experiment out of surviving n-1 items; at the time of second failure, R_2 items are removed out of n-2-R_1 surviving items; the process continue till the mth failure at which the test is terminated by removing the remaining $R_m (= n - m - \sum_{i=1}^{m} R_i)$ items. The observations x_1, $x_2, \ldots, x_m$ are called progressively type-II censored order statistics with progressive censoring scheme $(R_1, R_2, \ldots, R_m)$. For the detailed description and related methodology see Balakrishnan and Aggarwala (2000).

In the present paper, we consider the problem of ML and Bayesian estimation of parameters and reliability function of the three-parameter Weibull distribution under progressive type-II censoring scheme. The rest of paper is organized as follows. In the section 2, we obtain ML estimates of parameters using Newton Raphson Method. In section 3, we consider the Bayesian estimation of parameters and reliability function under squared error loss function (SELF), general entropy loss function (GELF) and LINEX loss function using Lindley's approximation. In Section 4, we perform simulation study.

2. Maximum Likelihood estimation

Suppose n identical items, the lifetime of each of which follow the pdf (1.1), are put to test. A sample of m observations $x_1, x_2, \ldots, x_m$ is obtained by following the progressive type-II censoring scheme $(R_1, R_2, \ldots, R_m)$, described in Section 1. The likelihood function of the observed sample $d = (x_1, x_2, \ldots, x_m)$ can be written as follows [see Balakrishnan and Aggarwala (2000).]

$$L(\beta, \theta, \gamma \mid d) = c\prod_{i=1}^{m} f(x_i; \beta, \theta, \gamma)\left(R(x_i; \beta, \theta, \gamma)\right)^{R_i}, \tag{2.1}$$

where, $c = n(n-R_1-1)(n-R_1-R_2-2)...(n-R_1-R_2-...-R_{m-1}-m+1)$.

Using (1.1) and (1.2), we obtain from (2.1), that

$$L(\beta,\theta,\gamma|d) = c\prod_{i=1}^{m}\frac{\beta}{\theta}(x_i-\gamma)^{\beta-1}\exp\left(-\left(\frac{(1+R_i)(x_i-\gamma)^{\beta}}{\theta}\right)\right).$$

$$= c\left(\frac{\beta}{\theta}\right)^{m}\left\{\prod_{i=1}^{m}(x_i-\gamma)^{\beta-1}\right\}\exp\left(-\frac{1}{\theta}\sum_{i=1}^{m}(1+R_i)(x_i-\gamma)^{\beta}\right). \quad (2.2)$$

Taking logarithm of both sides of (2.2), we obtain $\log L = l(say)$, given by

$$l \propto m\log(\beta) - m\log(\theta) + (\beta-1)\sum_{i=1}^{m}\log(x_i-\gamma) - \frac{1}{\theta}\sum_{i=1}^{m}(1+R_i)(x_i-\gamma)^{\beta} \quad (2.3)$$

In order to obtain MLEs of β,θ and γ we differentiate (2.3) partially w.r.t parameters and get

$$\frac{\partial l}{\partial\beta} = \frac{m}{\beta} - \sum_{i=1}^{m}\log(x_i-\gamma) - \frac{1}{\theta}\sum_{i=1}^{m}(R_i+1)(x_i-\gamma)^{\beta}\log(x_i-\gamma), \quad (2.4)$$

$$\frac{\partial l}{\partial\theta} = -\frac{m}{\theta} + \frac{1}{\theta^2}\sum_{i=1}^{m}(R_i+1)(x_i-\gamma)^{\beta} \quad (2.5)$$

and

$$\frac{\partial l}{\partial\gamma} = -(\beta-1)\sum_{i=1}^{m}(x_i-\gamma)^{-1} + \frac{\beta}{\theta}\sum_{i=1}^{m}(x_i-\gamma)^{\beta-1}. \quad (2.6)$$

We observe that the analytical solution of the likelihood equations (2.4), (2.5) and (2.6) is not possible. We, therefore, solve these equations using Newton-Raphson Method along with the condition that the MLE of γ is less than or equal to $x_{(1)}$, the minimum of $(x_1, x_2, ..., x_m)$ and present the obtained estimates in various tables at the end of the paper.

3. Bayesian Estimation using Lindley's Approximation

We consider the following non-informative prior for shape parameters

$$\pi_1(\beta) \propto \frac{1}{\beta},$$

The gamma prior for the scale parameter given by

$$\pi_2(\theta) = \frac{\mu^{\nu}\theta^{\nu-1}}{\Gamma(\nu)}\exp(-\mu\theta)$$

and uniform prior for the location parameter

$$\pi_3(\gamma) \propto \frac{1}{c}\text{; where c is a constant.}$$

Assuming all the parameters independent, the joint prior distribution of β, θ and γ can be written as follows

$$\pi(\beta,\theta,\gamma) = \pi_1(\beta)\pi_2(\theta)\pi_3(\gamma)$$

$$= \frac{\mu^{\nu}\,\theta^{\nu-1}}{\beta\, c\Gamma(\nu)}\exp(-\mu\theta);\ \beta,\theta,\upsilon,\mu > 0. \tag{3.1}$$

The posterior expectation of any parametric function of $\tau = (\beta,\theta,\gamma)$, say $\omega(\tau)$, can be obtained by solving the following ratio of integrals.

$$E(\omega(\tau) \mid d) = \frac{\int_{\tau} \omega(\tau)\pi(\tau)L(\tau \mid d)d\tau}{\int_{\tau} \pi(\tau)L(\tau \mid d)d\tau}, \tag{3.2}$$

which on using Lindley's approximation can be written in the following form.

$$\tilde{\omega}_S = \omega(\tau) + \psi + \omega_1\eta_1 + \omega_2\eta_2 + \omega_3\eta_3, \tag{3.3}$$

where,

$$\psi = (\delta_{12}\omega_{12} + \delta_{13}\omega_{13} + \delta_{23}\omega_{23}) + \frac{1}{2}(\delta_{11}\omega_{11} + \delta_{22}\omega_{22} + \delta_{33}\omega_{33}),$$

$$\eta_1 = (\delta_{11}g_1 + \delta_{12}g_2 + \delta_{13}g_3) + \frac{1}{2}(\delta_{11}\xi_1 + \delta_{12}\xi_2 + \delta_{13}\xi_3),$$

$$\eta_2 = (\delta_{21}g_1 + \delta_{22}g_2 + \delta_{23}g_3) + \frac{1}{2}(\delta_{21}\xi_1 + \delta_{22}\xi_2 + \delta_{23}\xi_3),$$

$$\eta_3 = (\delta_{31}g_1 + \delta_{32}g_2 + \delta_{33}g_3) + \frac{1}{2}(\delta_{31}\xi_1 + \delta_{32}\xi_2 + \delta_{33}\xi_3),$$

$$\xi_1 = \delta_{11}l_{111} + 2\delta_{12}l_{121} + 2\delta_{13}l_{131} + 2\delta_{23}l_{231} + \delta_{22}l_{221} + \delta_{33}l_{331},$$

$$\xi_2 = \delta_{11}l_{112} + 2\delta_{12}l_{122} + 2\delta_{13}l_{132} + 2\delta_{23}l_{232} + \delta_{22}l_{222} + \delta_{33}l_{332},$$

and

$$\xi_3 = \delta_{11}l_{113} + 2\delta_{12}l_{123} + 2\delta_{13}l_{133} + 2\delta_{23}l_{233} + \delta_{22}l_{223} + \delta_{33}l_{333},$$

All the subscripts 1, 2, 3 on the right hand sides refers to β, θ and γ, respectively,

$$g_i = \frac{\partial g}{\partial \tau_i}, \quad \omega_i = \frac{\partial \omega(\tau)}{\partial \tau_i}, \; l_{ij} = \frac{\partial^2 l(\tau)}{\partial \tau_i \partial \tau_j}, \qquad i = 1,2,3$$

$$l_{abc} = \frac{\partial^3 l(\tau)}{\partial \beta^a \partial \theta^b \partial \gamma^c}, \; a,b,c = 0,1,2,3 \;\; and \;\; a+b+c = 3,$$

where $g = \log(\pi(\tau))$ and δ_{ij}, is the $(i, j)^{th}$ element in the matrix $\{-l_{ij}\}^{-1}$, $i, j = 1,2,$; All the above expressions are to be evaluated at the MLE of the parameters.

3.1 Bayesian Estimation under SELF

Using the fact that, under SELF, the Bayes estimator of any parameter is its posterior mean, we obtain the Bayes estimators of β, θ, γ and R(t) by using (3.3) as follows.

1. When $\omega(\tau) = \beta$, we have $\omega_1 = 1, \omega_2 = 0, \omega_3 = 0$ and $\psi = 0$ substituting these values in (3.4), we get the following Bayes estimates of β .

$$\tilde{\beta}_s = \hat{\beta} + \eta_1. \tag{3.4}$$

2. When $\omega(\tau) = \theta$, we have $\omega_1 = 0, \omega_2 = 1, \omega_3 = 0$ and $\psi = 0$. Thus from (3.5), we get the Bayes estimate of θ given by

$$\tilde{\theta}_s = \hat{\theta} + \eta_2 . \tag{3.5}$$

3. When $\omega(\tau) = \gamma$, we have $\omega_1 = 0, \omega_2 = 0, \omega_3 = 1$ and $\psi = 0$. Then using (3.5), the Bayes estimates of γ is given by

$$\tilde{\gamma}_s = \hat{\gamma} + \eta_3 . \tag{3.6}$$

4. When $\omega(\tau) = R(t) = \exp\left(\frac{-(t-\gamma)^\beta}{\theta}\right)$, we have

$$\tilde{R}_s(t) = \hat{R}(t) + \psi_R + \omega_1\eta_1 + \omega_2\eta_2 + \omega_3\eta_3 , \tag{3.7}$$

where,

$$\omega_1 = \frac{-(t-\gamma)^\beta}{\theta}\exp\left(\frac{-(t-\gamma)^\beta}{\theta}\right)\log(t-\gamma),$$

$$\omega_2 = \frac{(t-\gamma)^\beta}{\theta^2}\exp\left(\frac{-(t-\gamma)^\beta}{\theta}\right),$$

$$\omega_3 = \frac{\beta(t-\gamma)^{\beta-1}}{\theta}\exp\left(\frac{-(t-\gamma)^\beta}{\theta}\right)$$

and

$$\psi_R = \left(\frac{\delta_{12}\partial^2\omega}{\partial\beta\partial\theta} + \frac{\delta_{13}\partial^2\omega}{\partial\beta\partial\theta} + \frac{\delta_{23}\partial^2\omega}{\partial\theta\partial\gamma}\right) + \frac{1}{2}\left(\frac{\delta_{11}\partial^2\omega}{\partial\beta^2} + \frac{\delta_{22}\partial^2\omega}{\partial\theta^2} + \frac{\delta_{33}\partial^2\omega}{\partial\gamma^2}\right).$$

3.2 Bayesian Estimation under GELF

The General entropy loss function for estimating a parameter (parametric function) $\omega = \omega(\tau)$ by $\hat{\omega}$ proposed by calebria and pulcini (2006) is given by

$$L(\hat{\omega}, \omega) = \left(\frac{\hat{\omega}}{\omega}\right)^q - q\log\left(\frac{\hat{\omega}}{\omega}\right) - 1, \qquad q \neq 0 \tag{3.8}$$

The Bayes estimator of ω under GELF in (3.8) is

$$\tilde{\omega}_G(\tau) = [E_\omega(\omega(\tau)^{-q})]^{-1/q}, \tag{3.9}$$

provided $E_\omega(\omega(\tau)^{-q})$ exist and finite. Thus, to obtain $\tilde{\omega}_G(\tau)$, the Bayes estimate of $\omega(\pi)$ under GELF, we first find the posterior expectation $E_\omega(\omega(\tau)^{-q})$ for given q, using (3.3). Then the Bayes estimates of β, θ, γ and and R(t) under GELF, can be obtained using (3.9).

1. When $\omega(\tau) = \beta^{-q}$, then using (3.5) we have

$$\tilde{\beta}_G = (\hat{\beta}^{-q} + \psi_\beta + \omega_1\eta_1)^{-1/q}, \tag{3.10}$$

where, $\omega_1 = -q\beta^{-q-1}$ and $\psi_\beta = \dfrac{q(q-1)\delta_{11}\beta^{-q-2}}{2}$.

2. When $\omega(\tau) = \theta^{-q}$, then we have

$$\tilde{\theta}_G = (\hat{\theta}^{-q} + \psi_\theta + \omega_2\eta_2)^{-1/q}, \tag{3.11}$$

where, $\omega_2 = -q\theta^{-q-1}$ and $\psi_\theta = \dfrac{q(q-1)\delta_{22}\theta^{-q-2}}{2}$.

3. When $\omega(\tau) = \gamma^{-q}$, then we have

$$\tilde{\gamma}_G = (\hat{\gamma}^{-q} + \psi_\gamma + \omega_3\eta_3)^{-1/q} \tag{3.12}$$

where, $\omega_3 = -q\gamma^{-q-1}$ and $\psi_\gamma = \dfrac{q(q-1)\delta_{33}\gamma^{-q-2}}{2}$.

4. When $\omega(\tau) = R(t)^{-q} = \exp\left(\dfrac{q(t-\gamma)^\beta}{\theta}\right)$, we have

$$\tilde{R}_G(t) = (\hat{R}(t)^{-q} + \psi'_R + \omega_1\eta_1 + \omega_2\eta_2 + \omega_3\eta_3)^{-1/q}, \tag{3.13}$$

where

$$\omega_1 = \frac{q(t-\gamma)^{\delta}}{\theta}\exp\left(\frac{q(t-\gamma)^{\delta}}{\theta}\right)\log(t-\gamma),$$

$$\omega_2 = \frac{-q(t-\gamma)^{\delta}}{\theta^2}\exp\left(\frac{q(t-\gamma)^{\delta}}{\theta}\right)\log(t-\gamma),$$

$$\omega_1 = \frac{q(t-\gamma)^{\delta}}{\theta}\exp\left(\frac{q(t-\gamma)^{\delta}}{\theta}\right)\log(t-\gamma),$$

and

$$\psi'_R = \left(\frac{\delta_{12}\partial^2\omega}{\partial\beta\partial\theta} + \frac{\delta_{13}\partial^2\omega}{\partial\beta\partial\theta} + \frac{\delta_{23}\partial^2\omega}{\partial\theta\partial\gamma}\right) + \frac{1}{2}\left(\frac{\delta_{11}\partial^2\omega}{\partial\beta^2} + \frac{\delta_{22}\partial^2\omega}{\partial\theta^2} + \frac{\delta_{33}\partial^2\omega}{\partial\gamma^2}\right).$$

3.3 Bayesian Estimation under LINEX Loss Function

The LINEX loss function, proposed by Zellner (1986), for estimating a parametric function $\omega(\tau)$ by its estimator $\tilde{\omega}(\tau)$ is given by

$$L(\hat{\omega}(\tau)-\omega(\tau)) = a\exp(c(\hat{\omega}(\tau)-\omega(\tau))) - c(\hat{\omega}(\tau)-\omega(\tau)) - 1, \qquad a>0,\ c\neq 0. \tag{3.14}$$

The Bayes Estimate of α under LINEX loss function is

$$\tilde{\omega}_l(\tau) = -\frac{1}{c}\log[E_{\omega(\tau)}(\exp(-c\omega(\tau)))], \tag{3.15}$$

Provided $E_{\omega(\tau)}(\exp(-c\omega(\tau)))$ exist and finite. Thus, to get $\tilde{\omega}(\tau)$, the Bayes estimate of $\omega(\tau)$ under LINEX loss function, we first obtain the posterior expectation $E_{\omega(\tau)}(\exp(-c\omega(\tau)))$ for given c, using (3.3), then the Bayes estimates of β, θ, γ and R(t) can be obtained using (3.15).

1. When $\omega(\tau) = \exp(-c\beta)$, then we have

$$\tilde{\beta}_l = -\frac{1}{c}\log E_{\beta}[\exp(-c\hat{\beta})],$$

$$= -\frac{1}{c}\log[\exp(-c\hat{\beta}) + \psi_{\beta} + \omega_l\eta_1], \tag{3.16}$$

where, $\omega_1 = -c\exp(-c\beta)$ and $\psi_\beta = \dfrac{c^2\delta_{11}\exp(-c\beta)}{2}$.

1. When $\omega(\tau) = \exp(-c\theta)$, then we have

$$\tilde{\theta}_l = -\frac{1}{c}\log E_\theta[\exp(-c\hat{\theta})],$$

$$= -\frac{1}{c}\log[\exp(-c\hat{\theta}) + \psi_\theta + \omega_2\eta_2], \tag{3.17}$$

where , $\omega_2 = -c\exp(-c\theta)$ and $\psi_\theta = \dfrac{c^2\delta_{22}\exp(-c\theta)}{2}$.

2. When $\omega(\tau) = \exp(-c\gamma)$, then we have

$$\gamma_l = -\frac{1}{c}\log E_\gamma[\exp(-c\hat{\gamma})],$$

$$= -\frac{1}{c}\log[\exp(-c\hat{\gamma}) + \psi_\gamma + \omega_3\eta_3], \tag{3.18}$$

where, $\omega_3 = -c\exp(-c\gamma)$ and $\psi_\gamma = \dfrac{c^2\delta_{33}\exp(-c\gamma)}{2}$.

3. When $\omega(\tau) = \exp(-cR(t)) = \exp\left(-c\exp\left(\dfrac{-(t-\gamma)^\beta}{\theta}\right)\right)$, then we have

$$\tilde{R}_l = -\frac{1}{c}\log E_R\left[\exp\left(-c\exp\left(\frac{-(t-\gamma)^\beta}{\theta}\right)\right)\right],$$

$$= -\frac{1}{c}\log\left(\exp\left(-c\exp\left(\frac{-(t-\gamma)^\beta}{\theta}\right)\right) + \psi_R'' + \omega_1\eta_1 + \omega_2\eta_2 + \omega_2\eta_3\right), \tag{3.19}$$

$$\omega_1 = \frac{c(t-\gamma)^\beta}{\theta}\log(t-\lambda)\exp\left(-(1+c)\exp\left(\frac{-(t-\gamma)^\beta}{\theta}\right)\right),$$

$$\omega_2 = \frac{-c(t-\gamma)^{\beta}}{\theta^2}\exp\left(-(1+c)\exp\left(\frac{-(t-\gamma)^{\beta}}{\theta}\right)\right),$$

$$\omega_3 = \frac{-c\beta(t-\gamma)^{\beta}}{\theta}\exp\left(-(1+c)\exp\left(\frac{-(t-\gamma)^{\beta}}{\theta}\right)\right),$$

and

$$\psi_R'' = \left(\frac{\delta_{12}\partial^2\omega}{\partial\beta\partial\theta} + \frac{\delta_{13}\partial^2\omega}{\partial\beta\partial\theta} + \frac{\delta_{23}\partial^2\omega}{\partial\theta\partial\gamma}\right) + \frac{1}{2}\left(\frac{\delta_{11}\partial^2\omega}{\partial\beta^2} + \frac{\delta_{22}\partial^2\omega}{\partial\theta^2} + \frac{\delta_{33}\partial^2\omega}{\partial\gamma^2}\right).$$

3.4 Simulation Study

In this section, we present simulation study for some numerical illustrations. For the values of the parameters β=2.5, θ=2, and γ =0.0001, we generate the type-II progressively censored samples using the algorithm of Balakrishnan and Sandu (2000) in software *R*. Since the evaluation of Bayes estimators through Lindley' approximation requires the MLEs of parameters, we first obtain the MLEs of β, θ, and γ using (2.4), (2.5) and (2.6), respectively. The obtained estimates are presented in the given tables. With the help of MLEs of parameters, we evaluate Bayes estimators taking the values of prior Hyper-parameters to be $\mu = 1$ and $\upsilon = 1$. In this study we generate 5000 samples and present the average estimates and corresponding RMSEs in various tables.

In this paper we have shown that the computations for the Bayes estimators for three-parameter family become easy with the present form of Lindley's approximation. Once the Bayesian estimation problem is set according to Section 3, we can compute the posterior expectation of any parametric function of parameters. The paper may be helpful for the workers in reliability and survival analysis who are not well aware with the advanced simulation techniques such as Markov Chain Monte Carlo, Gibbs Sampler etc.

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Table 1: Average Estimates and RMSEs (in the parenthesis) of β.

Sample Size		Progressive Censoring Scheme	$\hat{\beta}$	$\tilde{\beta}_S$	$\tilde{\beta}_G$		$\tilde{\beta}_l$	
n	m				q=-1	q=1	c=-1	c=1
00	100	All Items Failed	1.8681	1.8785	1.8785	1.8786	1.8908	1.8660
			(0.4327)	(0.4189)	(0.4189)	(0.4188)	(0.4046)	(0.4337)
	80	20*1,0*40,0*39	1.8131	1.8271	1.8271	1.8272	1.8419	1.8120
			(0.5059)	(0.4858)	(0.4858)	(0.4856)	(0.4673)	(0.5054)

		0*39,0*40,20*1	1.5437	1.5636	1.5636	1.5641	1.5757	1.5514
			(0.9518)	(0.9126)	(0.9126)	(0.9113)	(0.8910)	(0.9342)
		0*30,1*20, 0*30	1.8154	1.8282	1.8282	1.8283	1.8425	1.8135
			(0.5031)	(0.4848)	(0.4848)	(0.4847)	(0.4668)	(0.5037)
	60	40*1,0*30,0*29	1.7241	1.7452	1.7452	1.7455	1.7635	1.7260
			(0.6347)	(0.6012)	(0.6012)	(0.6007)	(0.5752)	(0.6290)
		0*29,0*30,40*1	1.3018	1.2750	1.2750	1.3372	1.3501	1.3131
			(1.5257)	(3.0299)	(3.0299)	(1.4532)	(1.4304)	(1.5391)
		0*10,1*40,0*10	1.7144	1.7342	1.7342	1.7345	1.7517	1.7161
			(0.6530)	(0.6212)	(0.6212)	(0.6207)	(0.5962)	(0.6478)
	40	60*1,0*20,0*19	1.5603	1.5883	1.5883	1.6007	1.6238	1.5733
			(0.9121)	(1.7748)	(1.7748)	(0.8357)	(0.7961)	(0.8877)
		0*19,0*20,60*1	1.0808	1.2268	1.2268	1.2496	1.2346	1.2150
			(2.0141)	(1.6211)	(1.6211)	(1.5636)	(1.6011)	(1.6511)
		0*10,3*20, 0*10	1.5799	1.6140	1.6140	1.6158	1.6374	1.5907
			(0.8804)	(0.8225)	(0.8225)	(0.8136)	(0.7782)	(0.8576)
60	60	All Items Failed	1.6828	1.7051	1.7051	1.7055	1.7226	1.6869
			(0.7047)	(0.6671)	(0.6671)	(0.6665)	(0.6412)	(0.6949)
	50	10*1,0*20,0*29	1.6187	1.6469	1.6469	1.6481	1.6673	1.6264
			(0.8103)	(0.7633)	(0.7633)	(0.7578)	(0.7282)	(0.7942)
		1*10,0*20,0*20	1.6343	1.6608	1.6608	1.6613	1.6796	1.6412
			(0.7849)	(0.7383)	(0.7383)	(0.7370)	(0.7083)	(0.7698)
		0*20,1*10, 0*20	1.6263	1.6502	1.6502	1.6538	1.6731	1.6323
			(0.7996)	(0.8605)	(0.8605)	(0.7504)	(0.7198)	(0.7870)

	40	20*1,0*20,0*19	1.5318	1.5522	1.5522	1.5739	1.5958	1.5469
			(0.9684)	(2.3860)	(2.3860)	(0.8863)	(0.8476)	(0.9420)
		1*20,0*10,0*10	1.5736	1.6036	1.6036	1.6066	1.6272	1.5830
			(0.8945)	(0.8644)	(0.8644)	(0.8324)	(0.7974)	(0.8749)
		2*10,0*20,0*10	1.5771	1.6085	1.6085	1.6100	1.6298	1.5874
			(0.8871)	(0.8321)	(0.8321)	(0.8254)	(0.7920)	(0.8650)

Note: $a*b$ indicates that a is repeated b times.

Table 2: Average Estimates and RMSEs (in the parenthesis) of θ ,

Sample Size		Progressive Censoring Scheme	$\hat{\theta}$	$\tilde{\theta}_S$	$\tilde{\theta}_G$		$\tilde{\theta}_l$	
n	m				q=-1	q=1	c=-1	c=1
100	100	All Items Failed	1.1519	1.1671	1.1671	1.1673	1.1751	1.1588
			(0.4599)	(0.4347)	(0.4347)	(0.4344)	(0.4229)	(.4471)
	80	20*1,0*40,0*39	1.1273	1.1462	1.1462	1.1465	1.1556	1.1364
			(0.8003)	(0.7682)	(0.7682)	(0.7676)	(0.7540)	(0.7832)
		0*39,0*40,20*1	1.2931	1.3108	1.3108	1.3110	1.3219	1.2992
			(0.5279)	(0.5034)	(0.5034)	(0.5031)	(0.4894)	(0.5182)
		0*30,1*20, 0*30	1.1706	1.1872	1.1872	1.1874	1.1965	1.1776
			(0.7294)	(0.7026)	(0.7026)	(0.7022)	(0.6892)	(0.7167)
	60	40*1,0*30,0*29	1.0878	1.1127	1.1127	1.1133	1.1240	1.1009
			(0.8681)	(0.8241)	(0.8241)	(0.8231)	(0.8063)	(0.8432)

		0*29,0*30,40*1	1.5645	1.5730	1.5730	1.5781	1.5987	1.5549
			(0.2594)	(0.2706)	(0.2706)	(0.2446)	(0.2349)	(0.2596)
		0*10,1*40,0*10	1.2135	1.2318	1.2318	1.2321	1.2444	1.2188
			(0.6622)	(0.6340)	(0.6340)	(0.6335)	(0.6166)	(0.6523)
	40	60*1,0*20,0*19	1.0204	1.0569	1.0569	1.0583	1.0708	1.0420
			(0.9908)	(0.9224)	(0.9224)	(0.9198)	(0.8987)	(0.9484)
		0*19,0*20,60*1	2.0968	2.0675	2.0675	2.0679	2.1243	2.0138
			(1.0094)	(1.0046)	(1.0046)	(1.0046)	(1.0154)	(1.0002)
		0*10,3*20, 0*10	1.3149	1.3331	1.3331	1.3331	1.3575	1.3070
			(0.5281)	(0.5040)	(0.5040)	(0.5200)	(0.4742)	(0.5345)
60	60	All Items Failed	1.0230	1.0468	1.0468	1.0474	1.0566	1.0365
			(0.8931)	(0.8482)	(0.8482)	(0.8472)	(0.8316)	(0.8660)
	50	10*1,0*20,0*29	0.9983	1.0267	1.0267	1.0276	1.0375	1.0152
			(1.0383)	(0.9838)	(0.9838)	(0.9821)	(0.9647)	(1.0043)
		1*10,0*20,0*20	1.0103	1.0377	1.0377	1.0385	1.0486	1.0262
			(1.0174)	(0.9652)	(0.9652)	(0.9638)	(0.9465)	(0.9854)
		0*20,1*10, 0*20	1.0463	1.0723	1.0723	1.0722	1.0833	1.0596
			(0.9502)	(0.9072)	(0.9072)	(0.9029)	(0.8846)	(0.9241)
	40	20*1,0*20,0*19	0.9683	1.0027	1.0027	1.0042	1.0156	0.9895
			(1.0972)	(1.0340)	(1.0340)	(1.0268)	(1.0061)	(1.0541)
		1*20,0*10,0*10	1.0373	1.0675	1.0675	1.0679	1.0811	1.0527
			(0.9709)	(0.9147)	(0.9147)	(0.9156)	(0.8922)	(0.9395)

		2*10,0*20,0*10	1.0036	1.0356	1.0356	1.0364	1.0484	1.0218
			(1.0341)	(0.9729)	(0.9729)	(0.9714)	(0.9509)	(0.9971)

Table 3: Average Estimates and RMSEs (in the parenthesis) of γ

Sample Size		Progressive Censoring Scheme	$\hat{\gamma}$	$\tilde{\gamma}_S$	$\tilde{\gamma}_G$		$\tilde{\gamma}_l$	
n	m				q=-1	q=1	c=-1	c=1
100	100	All Items Failed	0.1860	0.1860	0.1860	0.1860	0.1860	0.1860
			(0.0405)	(0.0405)	(0.0405)	(0.0405)	(0.0405)	(0.0405)
	80	20*1,0*40,0*39	0.1848	0.1848	0.1848	0.1848	0.1848	0.1848
			(0.0408)	(0.0408)	(0.0408)	(0.0408)	(0.0408)	(0.0408)
		0*39,0*40,20*1	0.1858	0.1857	0.1857	0.1857	0.1857	0.1857
			(0.0408)	(0.0408)	(0.0408)	(0.0408)	(0.0408)	(0.0408)
		0*30,1*20, 0*30	0.1860	0.1860	0.1860	0.1860	0.1860	0.1860
			(0.0410)	(0.0410)	(0.0410)	(0.0410)	(0.0410)	(0.0410)
	60	40*1,0*30,0*29	0.1852	0.1851	0.1851	0.1851	0.1851	0.1851
			(0.0405)	(0.0405)	(0.0405)	(0.0405)	(0.0405)	(0.0405)
		0*29,0*30,40*1	0.1855	0.1856	0.1856	0.1856	0.1856	0.1856
			(0.0409)	(0.0410)	(0.0410)	(0.0410)	(0.0410)	(0.0410)
		0*10,1*40,0*10	0.1859	0.1859	0.1859	0.1859	0.1859	0.1859
			(0.0407)	(0.0407)	(0.0407)	(0.0407)	(0.0407)	(0.0407)
	40	60*1,0*20,0*19	0.1851	0.1851	0.1851	0.1851	0.1851	0.1851

			(0.0404)	(0.0404)	(0.0404)	(0.0404)	(0.0404)	(0.0404)
		0*19,0*20,60*1	0.1684	0.1681	0.1681	0.1681	0.1681	0.1681
			(0.0583)	(0.0582)	(0.0582)	(0.0582)	(0.0582)	(0.0582)
		0*10,3*20, 0*10	0.1855	0.1855	0.1855	0.1855	0.1855	0.1855
			(0.0407)	(0.0407)	(0.0407)	(0.0407)	(0.0407)	(0.0407)
60	60	All Items Failed	0.2266	0.2266	0.2266	0.2266	0.2266	0.2266
			(0.0610)	(0.0610)	(0.0610)	(0.0610)	(0.0610)	(0.0610)
	50	10*1,0*20,0*29	0.2267	0.2266	0.2266	0.2266	0.2266	0.2266
			(0.0608)	(0.0608)	(0.0608)	(0.0608)	(0.0608)	(0.0608)
		1*10,0*20,0*20	0.2282	0.2282	0.2282	0.2282	0.2282	0.2282
			(0.0614)	(0.0614)	(0.0614)	(0.0614)	(0.0614)	(0.0614)
		0*20,1*10, 0*20	0.2277	0.2277	0.2277	0.2277	0.2277	0.2277
			(0.0615)	(0.0615)	(0.0615)	(0.0615)	(0.0615)	(0.0615)
	40	20*1,0*20,0*19	0.2267	0.2266	0.2266	0.2267	0.2266	0.2267
			(0.0607)	(0.0607)	(0.0607)	(0.0608)	(0.0607)	(0.0607)
		1*20,0*10,0*10	0.2273	0.2273	0.2273	0.2273	0.2273	0.2273
			(0.0611)	(0.0611)	(0.0611)	(0.0611)	(0.0611)	(0.0611)
		2*10,0*20,0*10	0.2297	0.2297	0.2297	0.2297	0.2297	0.2297
			(0.0622)	(0.0622)	(0.0622)	(0.0622)	(0.0622)	(0.0622)

Table 4: Average Estimates and RMSEs in the parenthesis of Reliability function at t=0.5.

Sample Size		Progressive Censoring Scheme	$\hat{R}(t)$	$\tilde{R}_S(t)$	$\tilde{R}_G(t)$		$\tilde{R}_I(t)$	
n	m				q=-1	q=1	c=-1	c=1
100	100	All Items Failed	0.8427	0.8434	0.8427	0.8417	0.8440	0.8436
			(0.0063)	(0.0062)	(0.0063)	(0.0065)	(0.0061)	(0.0062)
	80	20*1,0*40,0*39	0.8313	0.8326	0.8317	0.8303	0.8334	0.8329
			(0.0160)	(0.0157)	(0.0159)	(0.0162)	(0.0154)	(0.0156)
		0*39,0*40,20*1	0.8167	0.8189	0.8184	0.8172	0.8203	0.8198
			(0.0229)	(0.0223)	(0.0224)	(0.0228)	(0.0219)	(0.0220)
		0*30,1*20, 0*30	0.8384	0.8393	0.8388	0.8377	0.8405	0.8400
			(0.0076)	(0.0074)	(0.0075)	(0.0077)	(0.0072)	(0.0073)
	60	40*1,0*30,0*29	0.8132	0.8157	0.8146	0.8125	0.8171	0.8163
			(0.0201)	(0.0193)	(0.0196)	(0.0202)	(0.0189)	(0.0191)
		0*29,0*30,40*1	0.8084	0.7988	0.7986	0.8069	0.8097	0.8078
			(0.0244)	(0.0835)	(0.0835)	(0.0267)	(0.0309)	(0.0318)
		0*10,1*40,0*10	0.8305	0.8318	0.8316	0.8304	0.8344	0.8338
			(0.0126)	(0.0122)	(0.0123)	(0.0126)	(0.0117)	(0.0118)
	40	60*1,0*20,0*19	0.7755	0.7786	0.7773	0.7768	0.7853	0.7835
			(0.0413)	(0.1363)	(0.1368)	(0.0407)	(0.0374)	(0.0396)
		0*19,0*20,60*1	0.8250	0.8368	0.8370	0.8357	0.8413	0.8409
			(0.0434)	(0.0408)	(0.0407)	(0.0410)	(0.0499)	(0.0400)

		0*10, 3*20, 0*10	0.8244	0.8256	0.8263	0.8250	0.8321	0.8313
			(0.0411)	(0.0407)	(0.0405)	(0.0409)	(0.0490)	(0.0491)
60	60	All Items Failed	0.8264	0.8291	0.8281	0.8261	0.8305	0.8297
			(0.0184)	(0.0176)	(0.0179)	(0.0185)	(0.0173)	(0.0175)
	50	10*1,0*20,0*29	0.8125	0.8163	0.8151	0.8127	0.8182	0.8173
			(0.0189)	(0.0185)	(0.0186)	(0.0189)	(0.0184)	(0.0181)
		1*10,0*20,0*20	0.8179	0.8215	0.8206	0.8183	0.8237	0.8227
			(0.0290)	(0.0277)	(0.0280)	(0.0288)	(0.0270)	(0.0272)
		0*20,1*10, 0*20	0.8222	0.8244	0.8237	0.8225	0.8278	0.8267
			(0.0261)	(0.0334)	(0.0336)	(0.0260)	(0.0242)	(0.0251)
	40	20*1,0*20,0*19	0.7921	0.7926	0.7913	0.7938	0.8021	0.7997
			(0.0433)	(0.1825)	(0.1830)	(0.0427)	(0.0390)	(0.0430)
		1*20,0*10,0*10	0.8116	0.8151	0.8147	0.8127	0.8197	0.8184
			(0.0257)	(0.0262)	(0.0263)	(0.0254)	(0.0231)	(0.0238)
		2*10,0*20,0*10	0.8083	0.8128	0.8121	0.8096	0.8166	0.8154
			(0.0205)	(0.0194)	(0.0196)	(0.0201)	(0.0182)	(0.0185)

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Study of Some Improved Ratio Type Estimators Using Information on Auxiliary Attributes Under Second Order Approximation

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Abstract

Chakrabarty (1979), Khoshnevisan et al. (2007), Sahai and Ray (1980), Solanki et al. (2012) suggested some estimators to estimate unknown population mean of the study variable. These authors discussed the estimators along with their first order biases and mean square errors(MSE's). In this paper, we have tried to find out the second order biases and mean square errors of some estimators using information on auxiliary attribute. We have compared the performance of the estimators with the help of a numerical illustration.

Keywords: Simple Random Sampling, population mean, study variable, auxiliary attributes, ratio type estimator, product type estimator, Bias and MSE.

AMS classification : 62D05

1. Introduction

In the theory of sample surveys, the use of the auxiliary information can increase the precision or accuracy of an estimator of unknown population parameter of interest when study variable y is highly correlated with the auxiliary variable x. But there may be many practical situations when an auxiliary information is not available directly (it is qualitative in nature), that is, an auxiliary information is available in the form of an attribute. For example:

(a) The height of a person may depend on the fact that whether the person is male or female.
(b) The efficiency of a Dog may depend on the particular breed of that Dog.
(c) The yield of wheat crop produced may depend on a particular variety of wheat, etc.

In these situations by taking the advantage of point bi-serial correlation between the study variable y and the auxiliary attribute ϕ along with the prior knowledge of the population parameter of auxiliary attribute, the estimators of population parameter of interest can be constructed.

Consider a sample of size n drawn by simple random sampling without replacement (SRSWOR) from a population of size N. Let y_i and ϕ_i denote the observation on variable y and x respectively for the ith unit (i=1,2,3,……N) . We note that ϕ_i =1, if i^{th} unit possesses attributes ϕ and ϕ_i =0 otherwise . Let $A = \sum_{i=1}^{N} \phi_i$, and $a = \sum_{i=1}^{n} \phi_i$ denote the total number of units in the population and sample respectively possessing attribute x. Let $P = \frac{A}{N}$ and $p = \frac{a}{n}$ denote the proportion of units in the population and sample respectively possessing attribute ϕ .

Using the information of the point biserial correlation between the study variable and the auxiliary attribute, Naik and Gupta (1996), Shabbir and Gupta (2006), Ab-Alfatah *et al.* (2010) and Singh *et al.* (2007, 2008) have suggested improved estimators for estimating unknown population mean $\bar{Y}$. In this paper we have studied properties of some estimators under second order of approximation.

1. Some Estimators in Simple Random Sampling

For estimating the population mean $\bar{Y}$ of Y, adapting Chakrabarty (1979) ratio-type estimator in case of attribute auxiliary variable, we have

$$t_1 = (1-\alpha)\bar{y} + \alpha\bar{y}\frac{P}{p} \tag{2.1}$$

where $\bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i$.

Khoshnevisan et al. (2007) ratio- type estimator in case of attribute auxiliary attribute is given by

$$t_2 = \bar{y}\left[\frac{P}{\beta p + (1-\beta)P}\right]^g \tag{2.2}$$

where g is a constant. For $g=1$, t_2 is same as conventional ratio estimator whereas for $g=-1$, it becomes conventional product type estimator.

Adapting Sahai and Ray (1980) estimator for the situation when information is available in form of auxiliary attribute, we have

$$t_3 = \bar{y}\left[2 - \left\{\frac{p}{P}\right\}^w\right] \tag{2.3}$$

where w is a constant.

Adapting Solanki et al. (2012) estimator for case of attributes, we get an estimator t_4 as

$$t_4 = \bar{y}\left[2 - \left\{\left(\frac{p}{P}\right)^\lambda \exp\frac{\delta(p-P)}{(p+P)}\right\}\right] \tag{2.4}$$

where λ is a constant, suitably chosen by minimizing mean square error of the estimator t_4.

1. Notations used

Let us define,

$$e_0 = \frac{\bar{y}-\bar{Y}}{\bar{Y}} \quad \text{and} \quad e_1 = \frac{p-P}{P},$$

then $E(e_0) = E(e_1) = 0$.

For obtaining the bias and MSE expressions of the estimators, following lemmas will be used:

Lemma 3.1

(i) $V(e_0) = E\{(e_0)^2\} = \frac{N-n}{N-1}\frac{1}{n}C_{02} = L_1C_{02}$

(ii) $V(e_1) = E\{(e_1)^2\} = \frac{N-n}{N-1}\frac{1}{n}C_{20} = L_1C_{20}$

(iii) $COV(e_0, e_1) = E\{(e_0e_1)\} = \frac{N-n}{N-1}\frac{1}{n}C_{11} = L_1C_{11}$

Lemma 3.2

$$E\{(e_1^2e_0)\} = \frac{(N-n)}{(N-1)}\frac{(N-2n)}{(N-2)}\frac{1}{n^2}C_{21} = L_2C_{21}$$

(i) $E\{(e_1^3)\} = \frac{(N-n)}{(N-1)}\frac{(N-2n)}{(N-2)}\frac{1}{n^2}C_{30} = L_2C_{30}$

Lemma 3.3

(ii) $E(e_1^3e_0) = L_3C_{31} + 3L_4C_{20}C_{11}$

(iii) $E\{(e_1^4)\} = \frac{(N-n))(N^2+N-6nN+6n^2)}{(N-1)(N-2)(N-3)}\frac{1}{n^3}C_{30} = L_3C_{40} + 3L_4C_{20}^2$

(iv) $E(e_1^2e_0^2) = L_3C_{40} + 3L_4C_{20}$

Where $L_3 = \frac{(N-n))(N^2+N-6nN+6n^2)}{(N-1)(N-2)(N-3)}\frac{1}{n^3}$,

$$L_4 = \frac{N(N-n))(N-n-1)(n-1)}{(N-1)(N-2)(N-3)}\frac{1}{n^3}$$

and $C_{rs} = \frac{1}{N}\sum_{i=1}^{N}\frac{(P_i-\bar{P})^r(Y_i-\bar{Y})^s}{\bar{P}^r\bar{Y}^s}$

Proof of these lemma are straight forward by using SRSWOR (see Sukhatme and Sukhatme (1970)).

1. First Order Biases and Mean Squared Errors

The expressions for the biases of the estimators t_1, t_2, t_3 and t_4 are, respectively written as

$$Bias(t_1) = \bar{Y}\left[\frac{1}{2}\alpha L_1C_{20} - \alpha L_1C_{11}\right] \tag{4.1}$$

$$Bias(t_2) = \bar{Y}\left[\frac{g(g+1)}{2}L_1C_{20} - g\beta L_1C_{11}\right] \tag{4.2}$$

$$Bias(t_3) = \bar{Y}\left[-\frac{w(w-1)}{2}L_1C_{20} - wL_1C_{11}\right] \tag{4.3}$$

$$Bias(t_4) = \bar{Y}\left[-\frac{k(k-1)}{2}L_1C_{20} - kL_1C_{11}\right] \tag{4.4}$$

where, $k = \frac{(\delta+2\lambda)}{2}$.

The expressions for the MSE's of the estimators t_1, t_2 t_3 and t_4 are , respectively, given by

$$MSE(t_1) = \bar{Y}^2\left[L_1C_{02} + \alpha^2 L_1C_{20} - 2\alpha L_1C_{11}\right] \tag{4.5}$$

$$MSE(t_2) = \bar{Y}^2\left[L_1C_{02} + g^2\beta^2 L_1C_{20} - 2g\beta L_1C_{11}\right] \tag{4.6}$$

$$MSE(t_3) = \bar{Y}^2\left[L_1C_{02} + w^2 L_1C_{20} - 2wL_1C_{11}\right] \tag{4.7}$$

$$MSE(t_4) = \bar{Y}^2\left[L_1C_{02} + k^2 L_1C_{20} - 2kL_1C_{11}\right] \tag{4.8}$$

The MSE's of the estimators t_1, t_2 t_3 and t_4 under optimum conditions are equal to the MSE of the regression estimator when we consider the terms up to first order of approximations. In search of the optimum estimator, we have extended this study to second order of approximation.

1. Second Order Biases and Mean Squared Errors

Expressing estimator t_1 in terms of e's (i=0,1), we get

$$t_1 = \bar{Y}(1+e_0)\left\{(1-\alpha) + \alpha(1+e_1)^{-1}\right\}$$

or

$$t_1 - \bar{Y} = \bar{Y}\left\{e_0 + \frac{e_1}{2} + \frac{\alpha}{2}e_1^2 - \alpha e_0e_1 + \alpha e_0e_1^2 - \frac{\alpha}{6}e^3 - \frac{\alpha}{6}e_0e_1^3 + \frac{\alpha}{24}e^4\right\} \tag{5.1}$$

Taking expectations, we get the bias of the estimator t_1 up to the second order of approximation

as

$$\text{Bias}_2(t_1) = \bar{Y}\left[\frac{\alpha}{2}L_1C_{20} - \alpha L_1C_{11} - \frac{\alpha}{6}L_2C_{30} + \alpha L_2C_{21} - \frac{\alpha}{6}(L_3C_{31} + 3L_4C_{20}C_{11}) + \frac{\alpha}{24}(L_3C_{40} + 3L_4C_{20}^2)\right] \tag{5.2}$$

Similarly, we get the expressions for the biases of the estimator's t_2, t_3, and t_4 up to second order of approximation as follows

$$\text{Bias}_2(t_2) = \bar{Y}\left[\frac{g(g+1)}{2}\beta^2L_1C_{20} - g\beta L_1C_{11} - \frac{g(g+1)}{2}\beta^2L_2C_{21} - \frac{g(g+1)(g+2)}{6}\beta^3L_2C_{30}\right.$$

$$-\frac{g(g+1)(g+2)}{6}\beta^3\left(L_3C_{31}+3L_4C_{20}C_{11}\right)$$

$$+\frac{g(g+1)(g+2)(g+3)}{24}\beta^4(L_3C_{40}+3L_4C_{20}{}^2)\Bigg] \tag{5.3}$$

$$\text{Bias}_2(t_3)=\bar{Y}\Bigg[\frac{w(w-1)}{2}L_1C_{20}-wL_1C_{11}-\frac{w(w-1)}{2}L_2C_{21}-\frac{w(w-1)(w-2)}{6}L_2C_{30}$$

$$-\frac{w(w-1)(w-2)}{6}\left(L_3C_{31}+3L_4C_{20}C_{11}\right)$$

$$-\frac{w(w-1)(w-2)(w-3)}{24}(L_3C_{40}+3L_4C_{20}{}^2)\Bigg] \tag{5.4}$$

$$\text{Bias}_2(t_4)=\bar{Y}\Bigg[-\frac{k(k-1)}{2}L_1C_{20}-kL_1C_{11}-\frac{k(k-1)}{2}L_2C_{21}-ML_2C_{30}-M\left(L_3C_{31}+3L_4C_{20}C_{11}\right)$$

$$-N(L_3C_{40}+3L_4C_{20}{}^2)\Bigg] \tag{5.5}$$

where, $M=\frac{1}{2}\left\{\frac{\left(\delta^3-6\delta^2\right)}{24}+\frac{\left(\alpha(\delta^2-2\delta\right)}{4}+\frac{\lambda(\lambda-1)}{2}\delta+\frac{\lambda(\lambda-1)(\lambda-2)}{3}\right\}$ and

$k=\frac{(\delta+2\lambda)}{2}$

N=

$$\frac{1}{8}\left\{\frac{\left(\delta^4-12\delta^3+12\delta^2\right)}{48}+\frac{\left(\alpha(\delta^3-6\delta\right)}{6}+\frac{\lambda(\lambda-1)}{2}(\delta^2-2\delta)+\frac{\lambda(\lambda-1)(\lambda-2)(\lambda-3)}{3}\right\}$$

Following are the expressions of the MSE's of the estimator's t_1, t_2, t_3 and t_4 respectively, up to second order of approximation

$$\text{MSE}_2(t_1)=\bar{Y}^2\Big[L_1C_{02}+\alpha^2L_1C_{20}-2\alpha L_1C_{11}-\alpha^2L_2C_{30}+(2\alpha^2+\alpha)L_2C_{21}$$

$$-2\alpha^2\left(L_3C_{31}+3L_4C_{20}C_{11}\right)$$

$$+\alpha(\alpha+1)\left(L_3C_{22}+3L_4(C_{20}C_{02}+C_{11}^2)\right)+\frac{5}{24}\alpha^2(L_3C_{40}+3L_4C_{20}{}^2)\Bigg] \tag{5.6}$$

$$MSE_2(t_2)=\bar{Y}^2\Big[L_1C_{02}+g^2\beta^2L_1C_{20}-2\beta gL_1C_{11}-\beta^3g^2(g+1)L_2C_{30}+g(3g+1)\beta^2L_2C_{21}$$
$$-2\beta gL_2C_{12}-\left\{\frac{7g^3+9g^2+2g}{3}\right\}\beta^3\left(L_3C_{31}+3L_4C_{20}C_{11}\right)$$
$$+g(2g+1)\beta^2\left(L_3C_{22}+3L_4(C_{20}C_{02}+C_{11}^2)\right)$$
$$+\left\{\frac{2g^3+9g^2+10g+3}{6}\right\}\beta^4(L_3C_{40}+3L_4C_{20}{}^2\Big] \tag{5.7}$$

$$MSE_2(t_3)=\bar{Y}^2\Big[L_1C_{02}+w^2L_1C_{20}-2wL_1C_{11}-w^2(w-1)L_2C_{30}+w(w+1)L_2C_{21}-2wL_2C_{12}$$
$$+\left\{\frac{5w^3-3w^2-2w}{3}\right\}\left(L_3C_{31}+3L_4C_{20}C_{11}\right)$$
$$+w\left(L_3C_{22}+3L_4(C_{20}C_{02}+C_{11}^2)\right)+\left\{\frac{7w^4-18w^3+11w^2}{24}\right\}(L_3C_{40}+3L_4C_{20}{}^2)\Big] \tag{5.8}$$

$$MSE_2(t_4)=\bar{Y}^2\Big[L_1C_{02}+k^2L_1C_{20}-2kL_1C_{11}+kL_2C_{21}-2kL_2C_{12}+k^2(k-1)L_2C_{30}$$
$$+2k^2(k-1)\left(L_3C_{31}+3L_4C_{20}C_{11}\right)+k\left(L_3C_{22}+3L_4(C_{20}C_{02}+C_{11}^2)\right)$$
$$+\frac{(k^2-k)^2}{4}(L_3C_{40}+3L_4C_{20}{}^2)\Big] \tag{5.9}$$

where, $k=\dfrac{(\delta+2\lambda)}{2}$.

The optimum value of w for the constant involved in the estimator t_3, we get by minimizing $MSE_2(t_3)$. But theoretically the determination of the optimum value of w is very difficult, so we have calculated the optimum value by using numerical techniques. Similarly, the optimum value of k which minimizes the MSE of the estimator t_4 is obtained by using numerical techniques.

1. Numerical Illustration

The various result obtained in the pervious sections are now examined with the help of the following data set:

Source of the Data

The data for the empirical analysis is taken from Sukhatme and Sukhatme (1970), p.256
Y= number of villages in the circles and

ϕ = A circle consisting more than five villages

N=89, $\overline{Y} = 3.36$, $P = 0.1236$, $\rho_{pb} = 0.766$, $C_y = 0.604$, $C_x = 2.19$.

Table 6.1: Biases and MSE's of estimators

Estimators	Bias		MSE	
	First order	Second order	First order	Second order
t_1	23.385388	24.42412947	17597.0515	23161.50715
t_2	-9.11939E-09	-0.84723967	17597.0515	17653.75783
t_3	18.30588379	26.44381147	17597.0515	19089.85861
t_4	18.30588379	55.4734888	17597.0515	18104.07826

In the Table 6.1 the biases and MSE's of the estimator's t_1, t_2, t_3, and t_4 are written under first order and second order of approximations. For all the estimators t_1, t_2, t_3, and t_4 it is observed that the value of the biases are increasing. For all estimators MSE's up to the first order of approximation under optimum conditions are equal, which encourage us to study the properties of the estimators up to the second order of approximation. On the basis of study up to the second order of approximation we conclude that estimator t_2 is best followed by t_4, and t_3 among the estimators considered here for the given data set.

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A Generalized Chain Ratio in Regression Estimator for Population Mean Using two Auxiliary Characters in Sample Survey

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Abstract

A generalized chain ratio in regression estimator for population mean using two auxiliary characters has been proposed and its properties have been studied. A comparative study of the proposed estimator has been made with the relevant estimators. An effective range for α has been obtained for which the proposed estimator is more efficient than relevant estimators. For optimum value of α , the proposed estimator is found to be more efficient than the relevant estimators which is supported by the empirical study.

Key words: Bias, mean square error, two phase sampling, auxiliary characters, chained estimators.

1. Introduction

The information on auxiliary character is used to increase the efficiency of the estimators. Such information is generally used in ratio, product and regression type estimators for the estimation of population mean of study character y . Several research works have been done in developing ratio, product and regression type estimators by using an auxiliary character. Using the information on two auxiliary characters x and z , in which the population mean of first is unknown and second is known, chain ratio type and regression type estimators for population mean of study character y have been proposed by Chand (1975) and Kiregyera (1980,84). Further, Srivastava et al. (1990) proposed generalized chain ratio estimator for population mean of study character.

In the present paper, we have proposed a generalized chain ratio in regression estimator for population mean using auxiliary characters. We obtained the expressions for bias and mean square error of the proposed estimator. A comparative study of the proposed estimator is carried out with the relevant estimators. An empirical study is given to show the performance of the proposed estimator.

2. The estimators

Let $\bar{Y}$, $\bar{X}$ and $\bar{Z}$ denote the population mean of study character y, auxiliary character x and additional auxiliary character z having jth values Y_j, X_j and Z_j: $j = 1,2,3...N$.

In the case when population mean of auxiliary character is not known, we draw a large preliminary sample of size $n'(< N)$ from population of size N by using SRSWOR scheme of sampling and estimate the population mean $\bar{X}$ by first phase sample mean $\bar{x}'$ based on n' units. Further, we draw a sub-sample of size $n(< n')$ from large preliminary sample of size n' and compute $\bar{y}$ and $\bar{x}$ which are the sub-sample means based on n units. In such case, the double sampling ratio and regression estimators are defined by

$$T_1 = \bar{y}\frac{\bar{x}'}{\bar{x}} \tag{2.1}$$

and

$$T_{l1} = \bar{y} + b_{yx}(\bar{x}' - \bar{x}), \tag{2.2}$$

where $b_{yx} = \frac{\hat{S}_{yx}}{\hat{S}_x^2}$, $\bar{x}' = \frac{1}{n'}\sum_{j=1}^{n'} x_j$, $\hat{S}_{yx}$ and $\hat{S}_x^2$ denote the estimates of S_{yx} and S_x^2 based on n units.

Further, Srivastava (1970) has proposed a generalized two phase sampling estimator for estimating population mean $\bar{Y}$ using information on auxiliary character x, which is given as follows:

$$T_2 = \bar{y}\left(\frac{\bar{x}'}{\bar{x}}\right)^{\alpha_1}, \tag{2.3}$$

where α_1 is unknown constant.

The mean square errors of the estimators T_1, T_2 and T_{l1} are given as follows:

$$MSE\ (T_1) = V(\bar{y}) + \bar{Y}^2\left(\frac{1}{n} - \frac{1}{n'}\right)\left(C_x^2 - 2\rho_{yx}C_yC_x\right), \tag{2.4}$$

$$MSE\ (T_2)_{\min} = V(\bar{y}) - \bar{Y}^2\left(\frac{1}{n} - \frac{1}{n'}\right)\rho_{yx}^2 C_y^2 \tag{2.5}$$

and

$$MSE(T_{l1}) = V(\bar{y}) - \bar{Y}^2\left(\frac{1}{n} - \frac{1}{n'}\right)\rho_{yx}^2 C_y^2, \tag{2.6}$$

where $V(\bar{y}) = \left(\frac{1}{n} - \frac{1}{N}\right)S_y^2$

In the case when the population mean $(\bar{X})$ of an auxiliary character x is not known but the population mean $(\bar{Z})$ of an additional auxiliary character z is known. It is suggested to take a large preliminary sample of size $n'(< N)$ from population of size N by using simple random sampling without replacement scheme of sampling and estimate the population mean $\bar{X}$ by $\hat{\bar{X}} = \bar{x}'\frac{\bar{Z}}{\bar{z}'}$ which is more efficient in comparison to $\bar{x}'$ if $\rho_{xz} > \frac{1}{2}\frac{C_z}{C_x}$, where $\bar{x}'$ and $\bar{z}'$ are the preliminary sample means based on n' units. Further, a sub-sample of size $n(< n')$ is selected from large preliminary sample of size n' and compute $\bar{y}$ and $\bar{x}$ based on n units. In this case, Chand (1975) and Kiregyera (1984) proposed chain ratio type and ratio in regression estimators, which are given as follows:

$$T_3 = \bar{y}\frac{\bar{x}'}{\bar{x}}\frac{\bar{Z}}{\bar{z}'} \qquad \text{and} \qquad T_{l2} = \bar{y} + b_{yx}\left[\bar{x}'\frac{\bar{Z}}{\bar{z}'} - \bar{x}\right]. \tag{2.7}$$

Now, we propose generalized chain ratio in regression estimator for population mean by using auxiliary characters which is given as follows:

$$T_{l3} = \bar{y} + b_{yx}\left[\bar{x}'\left(\frac{\bar{Z}}{\bar{z}'}\right)^{\alpha} - \bar{x}\right], \tag{2.8}$$

where α is unknown constant which is determined later.

3. Bias and mean square error of the estimator T_{l3}

Using the large sample approximation, the expressions for bias and mean square error of the estimator T_{l3} up to the terms of order (n^{-1}) are given as follows:

$$Bias(T_{l3}) = \theta\left[-\mu_{14}+\mu_{15}+\mu_{24}-\mu_{25}-\alpha\mu_{34}+\alpha\mu_{35}+\frac{f'}{n'}\left\{\frac{\alpha(\alpha+1)}{2}C_z^2-\alpha\rho_{xz}C_xC_z\right\}\right], \quad (3.1)$$

and

$$MSE(T_{l3}) = MSE(T_{l1}) + \bar{Y}^2\frac{f'}{n'}\left[\alpha^2\frac{\rho_{yx}^2C_y^2C_z^2}{C_x^2} - 2\alpha\frac{\rho_{yx}\rho_{yz}C_y^2C_z}{C_x}\right], \quad (3.2)$$

where

$$\theta = \bar{X}\frac{S_{yx}}{S_x^2}, \mu_{14} = Cov(\bar{x}, \hat{S}_{yx}), \mu_{15} = Cov(\bar{x}, \hat{S}_x^2), \mu_{24} = Cov(\bar{x}', \hat{S}_{yx}),$$

$$\mu_{25} = Cov(\bar{x}', \hat{S}_x^2),\ \mu_{34} = Cov(\bar{z}', \hat{S}_{yx}),\ \mu_{35} = Cov(\bar{z}', \hat{S}_x^2),$$

$$f' = 1-\frac{n'}{N}, C_y = \frac{S_y}{\bar{Y}}, C_x = \frac{S_x}{\bar{X}}, C_z = \frac{S_z}{\bar{Z}}\ ;\ S_y^2 = \frac{1}{N-1}\sum_{j=1}^{N}(Y_j-\bar{Y})^2,$$

$$S_x^2 = \frac{1}{N-1}\sum_{j=1}^{N}(X_j-\bar{X})^2, \quad S_z^2 = \frac{1}{N-1}\sum_{j=1}^{N}(Z_j-\bar{Z})^2$$

ρ_{yx} and ρ_{yz} are the correlation coefficients between (y, x) and (y, z).

The optimum value of α for which $MSE(T_{l3})$ is minimum, given by

$$\alpha_{opt} = \frac{\rho_{yz}}{\rho_{yx}}\frac{C_x}{C_z} = Q_1 \quad (3.3)$$

and the minimum mean square error of the estimator T_{l3} is given by

$$MSE\ (T_{l3})_{\min} = MSE\ (T_{l1}) - \bar{Y}^2\frac{f'}{n'}\rho_{yz}^2C_y^2. \quad (3.4)$$

The optimum value of α may be obtained from past data. If past data is not available then one may estimate it on the basis of sample observations without having any loss in the efficiency of the estimator [Reddy (1978)]. If we estimate the optimum value of the constant by using the sample value, the minimum value of the mean square error of the estimator up to the terms of order (n^{-1}) are unchanged [Srivastava and Jhajj (1983)].

The mean square errors of the estimators (T_3) and (T_{l2}) are given by

$$MSE(T_3) = MSE(T_1) + \bar{Y}^2 \frac{f'}{n'}(C_z^2 - 2\rho_{yz}C_yC_z) \tag{3.5}$$

and

$$MSE(T_{l2}) = MSE(T_{l1}) + \bar{Y}^2 \frac{f'}{n'}\left[\frac{\rho_{yx}^2 C_y^2 C_z^2}{C_x^2} - 2\frac{\rho_{yx}\rho_{yz}C_y^2 C_z}{C_x}\right], \tag{3.6}$$

4. Some special cases of the proposed estimator

(i) If $\alpha = 0$ then T_{l3} reduces to $T_{l1} = \bar{y} + b_{yx}(\bar{x}' - \bar{x})$. (4.1)

(ii) If $\alpha = 1$ then T_{l3} reduces to $T_{l2} = \bar{y} + b_{yx}\left[\bar{x}'\frac{\bar{Z}}{\bar{z}'} - \bar{x}\right]$. (4.2)

(iii) If $\alpha = -1$ then T_{l3} reduces to $T'_{l2} = \bar{y} + b_{yx}\left[\bar{x}'\frac{\bar{z}'}{\bar{Z}} - \bar{x}\right]$. (4.3)

5. Comparison of the proposed estimator T_{l3} with T_1, T_2, T_3, T_{l1}, T_{l2}

$$MSE(T_{l3}) < MSE(T_1) \text{ if } \frac{B - \sqrt{B^2 + AC}}{A} < \alpha < \frac{B + \sqrt{B^2 + AC}}{A} \tag{5.1}$$

$$MSE(T_{l3}) < MSE(T_2)_{\min} \text{ if } 0 < \alpha < 2\frac{B}{A} \tag{5.2}$$

$$MSE(T_{l3}) < MSE(T_3) \text{ if } \frac{B - \sqrt{B^2 + AC'}}{A} < \alpha < \frac{B + \sqrt{B^2 + AC'}}{A} \tag{5.3}$$

$$MSE(T_{l3}) < MSE(T_{l1}) \text{ if } 0 < \alpha < 2\frac{B}{A} \tag{5.4}$$

$$MSE(T_{l3}) < MSE(T_{l2}) \text{ if } 1 < \alpha < 2\frac{B}{A} - 1 \tag{5.5}$$

where $A = \frac{f'}{n'}\frac{\rho_{yx}^2 C_y^2 C_z^2}{C_x^2}$, $B = \frac{f'}{n'}\frac{\rho_{yx}\rho_{yz}C_y^2 C_z}{C_x}$,

$$C = \left(\frac{1}{n} - \frac{1}{n'}\right)\left\{C_x^2 + \rho_{yx}^2 C_y^2 - 2\rho_{yx}C_yC_x\right\},$$

and $C' = \left[\left(\frac{1}{n} - \frac{1}{n'}\right)\left\{C_x^2 + \rho_{yx}^2 C_y^2 - 2\rho_{yx}C_yC_x\right\} + \frac{f'}{n'}\left(C_z^2 - 2\rho_{yz}C_yC_z\right)\right]$

6. Empirical study

Data Set I ($N = 82$, $n' = 43$, $n = 25$)

The data used by Srivastava et al. (1989), 82 children of age 3 months of Varanasi, India have consider for the present study,

y : mid arm circumference of the children,

x : chest circumference of the children ,

z : skull circumference of the children.

The values of the parameters of the y, x and z characters for the given data are given as follows:

$\bar{Y} = 11.90$, $\bar{Z} = 39.80$, $C_y^2 = 0.0052$, $C_x^2 = 0.0011$, $C_z^2 = 0.008$, $\rho_{yx} = 0.87$,

$\rho_{yz} = 0.86$

Data Set II ($N = 55$, $n' = 30$, $n = 18$)

The data used by Srivastava et al. (1989), 55 children of age 5 years of Varanasi, India have consider for the present study

y : weight of the children,

x : chest circumference of the children,

z : skull circumference of the children.

The values of the parameters of the y, x and z characters for the given data are given as follows:

$\bar{Y} = 17.08$, $\bar{Z} = 50.44$, $C_y^2 = 0.0161$, $C_x^2 = 0.0027$, $C_z^2 = 0.007$, $\rho_{yx} = 0.84$,

$\rho_{yz} = 0.51$

Table1

Relative efficiency of the estimators $\bar{y}$, T_1 , T_2 , T_3 , T_{l1} , T_{l2} and T_{l3} (in %) with respect to $\bar{y}$.

Estimators	Data Set 1	Data Set 2
$\bar{y}$	100.00 (0.02047)*	100.00 (0.17553)*
T_1	154.96 (0.01321)	144.79 (0.12123)
T_2	183.75 (0.01114)	172.27 (0.10189)

T_3	228.21 (0.00897)	160.76 (0.10919)
T_{l1}	183.75 (0.01114)	172.27 (0.10189)
T_{l2}	391.39 (0.00523)	209.31 (0.08386)
T_{l3}	400.59 (0.00511)	210.52 (0.08338)

*Figures in parenthesis give the $MSE(.)$.

From table 1, we observe that the proposed estimator T_{l3} is more efficient than the relevant estimators $\bar{y}, T_1, T_2, T_{l1}$ and the chained estimators T_3 and T_{l2} proposed by Chand (1975) and Kiregyera (1984).

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Some Improved Estimators For Estimating Population Mean In Stratified Random Sampling

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Abstract

Some improved estimators are proposed for estimating the population mean in stratified sampling in the presence of auxiliary information. Mean squared error (MSE) of the proposed estimators have been derived under large sample approximation. It has been shown that under optimum conditions proposed estimators are better than usual unbiased estimator and Hansen et al. (1946) estimator. Both theoretical and empirical findings are encouraging and support the soundness of the proposed procedure for mean estimation.

Keywords: Finite population mean, mean squared error, Optimum estimator, Auxiliary variable, study variable.

1. Introduction

Stratified sampling has often proved useful in planning surveys for improving the precision of other unstratified sampling strategies to estimate the finite population mean $\bar{Y} = \frac{1}{N}\sum_{h=1}^{L}\sum_{i=1}^{N_h} y_{hi}$.

A ratio-product estimation of finite population mean $\bar{Y}$ can be made in two ways. One is to make a separate ratio-product estimate of the total of each stratum and add these totals. An alternative estimate is derived from a single combined ratio-product.

Consider a finite population of size N. Let y and x respectively, be the study and auxiliary variates on each unit U j (j = 1,2,3,..., N) of the population U. Let the population be divided into L strata with the hth-stratum containing N_h units, h=1,2,3,…,L so that $\sum_{h=1}^{L} N_h = N$.

Suppose that a simple random sample of size n_h is drown without replacement from h^{th} stratum such that $\sum_{h=1}^{L} n_h = n$.

We compute the sample mean of the variates in stratified sampling method as,

$$\bar{y}_{st} = \sum_{h=1}^{n_h} W_h \bar{y}_h \quad \text{and} \quad \bar{x}_{st} = \sum_{h=1}^{n_h} W_h \bar{x}_h$$

where,

$\bar{x}_h$ is the sample mean of auxiliary variates of hth stratum

$\bar{y}_h$ is the sample mean of study variates of hth stratum

$W_h = \frac{N_h}{N}$ is stratum weight.

The variance of usual unbiased estimator Yst, us given as-

$$V(\bar{Y}st) = \frac{L}{Z} \quad S\,Y^2h\,wh^2\,\gamma h$$
$$h=1$$

When the population mean $\bar{X}$ of the auxiliary variate x is known, Hansen, et al. (1946) suggested a "combined ratio estimator" as:

$$y_{Rc} = \bar{y}_{st}\left(\frac{\bar{X}}{\bar{x}_{st}}\right) \tag{1.1}$$

The combined product estimator for $\bar{Y}$ is defined by,

$$\bar{y}_{Pc} = \bar{y}_{st}\left(\frac{\bar{x}_{st}}{\bar{X}}\right) \tag{1.2}$$

To the first degree of approximation, the variances of $\bar{y}_{Rc}$ and are $\bar{y}_{Pc}$ are respectively given by

$$V(\bar{y}_{Rc}) = \sum_{h=1}^{L} W_h^{\,2}\gamma_h \;(S_{hy}^{\;2} + R^2 S_{hx}^{\;2} - 2RS_{hxy}) \tag{1.3}$$

$$V(\bar{y}_{Pc}) = \sum_{h=1}^{L} W_h^{\,2}\gamma_h \;(S_{hy}^{\;2} + R^2 S_{hx}^{\;2} + 2RS_{hxy}) \tag{1.4}$$

where,

$$C^2_{hx} = \frac{S^2_{hx}}{\bar{X}^2}, \quad C^2_{hy} = \frac{S^2_{hy}}{\bar{Y}^2}, \quad R = \frac{\bar{Y}}{\bar{X}}, \rho_{hxy} = S_{hxy}\frac{S_{hxy}}{S_{hx}S_{hy}}, \quad \gamma_h = \left(\frac{1}{n_h} - \frac{1}{N_h}\right)$$

$$S^2_{hy} = \frac{1}{N_h - 1}\sum_{i=1}^{N_h}(y_{hi} - \overline{Y}_h)^2 \ , \ S^2_{hx} = \frac{1}{N_h - 1}\sum_{i=1}^{N_h}(x_{hi} - \overline{X}_h)^2 \ ,$$

$$S_{hxy} = \frac{1}{N_h - 1}\sum_{i=1}^{N_h}(x_{hi} - \overline{X}_h)^2 \ (y_{hi} - \overline{Y}_h)$$

In this study, under stratified random sampling without replacement scheme, we suggest some improved estimators which are more efficient than estimator proposed by Hansen, et al. (1946) estimator.

1. Proposed estimators

Adapting Sahai and Ray (1980) estimator in stratified random sampling we propose an estimator t_1 as:

$$t_1 = \overline{y}_{st}\left[2 - \left\{\frac{\overline{x}_{st}}{\overline{X}}\right\}^w\right] \tag{2.1}$$

We propose another estimator t_2 as:

$$t_2 = \overline{y}_{st}\left[\frac{\overline{x}_{st} + a(\overline{X} - \overline{x}_{st})}{\overline{x}_{st} + b(\overline{X} - \overline{x}_{st})}\right]^p \tag{2.2}$$

To improve the efficiency of the estimators several authors have suggested combining ratio estimator with difference estimator in different ways. Some important references are Ray and Singh (1981), Singh et al. (2008), Gupta and Shabbir (2008), Grover and Kaur (2011) and Singh and Solanki (2012). Motivated by these authors we suggest some improved estimators combining ratio estimator with difference estimator as:

$$t_3 = \left(k_{31}\overline{y}_{st} + k_{32}(\overline{X} - \overline{x}_{st})\right)\left[2 - \left\{\frac{\overline{x}}{\overline{X}}\right\}^W\right] \tag{2.3}$$

$$t_4 = \left[k_{41}\overline{y}_{st} + k_{42}(\overline{X} - \overline{x}_{st})\right]\left(\frac{\overline{x}_{st} + a(\overline{X} - \overline{x}_{st})}{\overline{x}_{st} + b(\overline{X} - \overline{x}_{st})}\right)^P \tag{2.4}$$

$$t_5 = k_{51}\overline{y}_{st}\left(2 - \left(\frac{\overline{x}_{st}}{\overline{X}}\right)^W\right) + k_{52}(\overline{X} - \overline{x}_{st}) \tag{2.5}$$

$$t_6 = k_{61}\bar{y}_{st}\left[\frac{\bar{x}_{st} + a(\bar{X} - \bar{x}_{st})}{\bar{x}_{st} + b(\bar{X} - \bar{x}_{st})}\right]^p + k_{62}(\bar{X} - \bar{x}_{st}) \tag{2.6}$$

To obtain the biases and MSE's of the proposed estimators, we use the following notations in the rest of the article:

$$\bar{y}_{st} = \sum_{h=1}^{L} w_h \bar{y}_h = \bar{Y}(1 + e_0),$$

$$\bar{x}_{st} = \sum_{h=1}^{L} w_h \bar{x}_h = \bar{X}(1 + e_1),$$

Now expressing estimators in the terms of e_i's (i=0,1), we have

$$t_1 = \bar{Y}\left[1 - we_1 - w(w-1)\frac{e_1^2}{2} + e_0 - we_0e_1\right] \tag{2.7}$$

$$t_2 = \bar{Y}\left[1 + \{e_0 + e_1 D\} + e_1^2 C + e_0 e_1\right] \tag{2.8}$$

$$t_3 = k_{31}\bar{Y}e_0 + k_{31} - wk_{31}e_0e_1\bar{Y} - wk_{31}e_1\bar{Y} - \frac{w(w-1)}{2}k_{31}e_1^2\bar{Y} - k_{32}e_1\bar{X} + wk_{32}e_1^2\bar{X} \tag{2.9}$$

$$t_4 = \bar{Y}k_{41}[1 + e_0 - pe_1(1-b) + pe_1(1-a)] + k_{41}\bar{Y}e_1^2\frac{[p^2(a-b)^2 + p(b^2 - a^2 + 2ab)]}{2}$$
$$+ k_{41}\bar{Y}e_0e_1p(b-a) - k_{42}e_1\bar{X} + k_{42}pXe_1^2(1-b) - k_{42}p\bar{X}(1-a)e_1^2 \tag{2.10}$$

$$t_5 = k_{51}\bar{Y}[1 + e_0 - we_0e_1 - we_1 - w(w-1)\frac{e_1^2}{2}] - k_{52}\bar{X}e_1 \tag{2.11}$$

$$t_6 = k_{61}Y[1 + (E_0 + p(b-a)e_1] + e_{61}^2 C - Ae_0e_1 - k_{62}\bar{X}e_1 \tag{2.12}$$

Taking expectations and than substracting $\bar{Y}$, we get the biases of the above estimators, respectively as:

$$B(t_1) = \bar{Y}\left[\frac{w(1-w)}{2}\frac{v(\bar{x}_{st})}{\bar{X}^2} - \frac{w\,cov(\bar{y}_{st}, \bar{x}_{st})}{\bar{Y}\bar{X}}\right] \tag{2.13}$$

$$B(t_2) = \bar{Y}\left[C\frac{v(\bar{x})}{\bar{X}} - A\frac{cov(\bar{x}_{st}, \bar{y}_{st})}{\bar{X}\bar{Y}}\right] \tag{2.14}$$

$$B(t_3) = \bar{Y}(k_{31}-1) - k_{31}\bar{Y}\left\{\frac{w(w-1)v(\bar{x}_{st})}{2\bar{X}^2} + \frac{w\,cov(\bar{x}_{st}\bar{y}_{st})}{\bar{X}\bar{Y}}\right\} + k_{32}\bar{X}w\frac{v(\bar{x}_{st})}{\bar{X}^2} \tag{2.15}$$

$$B(t_4) = \bar{Y}(k_{41}-1) + k_{41}\bar{Y}\left\{\frac{v(\bar{x})}{\bar{X}^2}C + D'\frac{cov(\bar{x}_{st}, \bar{y}_{st})}{\bar{X}\bar{Y}}\right\} - k_{42}D'\frac{v(\bar{x}_{st})}{\bar{X}} \tag{2.16}$$

$$B(t_5) = \bar{Y}(k_{51}-1) + k_{51}\bar{Y}\left\{\frac{w\,cov(\bar{x}_{st}\bar{y}_{st})}{\bar{X}\bar{Y}} - w(w-1)\frac{v(\bar{x}_{st})}{2\bar{X}^2}\right\} \tag{2.17}$$

$$B(t_6) = \bar{Y}(k_{61}-1) + k_{61}\bar{Y}\left\{\frac{v(\bar{x}_{st})}{\bar{X}^2}C - A\frac{cov(\bar{x}_{st}\bar{y}_{st})}{\bar{X}\bar{Y}}\right\} \tag{2.18}$$

where,

$A = p(1-a)$

$B = p(1-b)$

$C = \dfrac{p^2(a-b)^2 + p[b^2 - a^2 + 2(a-b)]}{2}$

$D' = p(a-b).$

The MSE expressions of the above estimator's are respectively given by

$$MSE(t_1) = v(\bar{y}_{st}) + w^2R^2v(\bar{x}_{st}) - 2wR\,cov(\bar{y}_{st}, \bar{x}_{st}) \tag{2.19}$$

$$MSE(t_2) = v(\bar{y}_{st}) + D'^2R^2v(\bar{x}_{st}) + 2D'R\,cov(\bar{y}_{st}, \bar{x}_{st}) \tag{2.20}$$

$$MSE(t_3) = \bar{Y}^2(K_{31}-1)^2 + K_{31}^2\{v(\bar{y}_{st}) + w\ R^2 v(\bar{x}_{st}) - 4wR\,cov(\bar{y}_{st},\bar{x}_{st})\}$$
$$+ K_{32}^2 v(\bar{x}_{st}) + 2K_{31}\left\{wR\,cov(\bar{y}_{st},\bar{x}_{st}) + \frac{w(w-1)R^2 v(\bar{x}_{st})}{2}\right\} - 2K_{32}wRv(\bar{x}_{st})$$
$$+ 2K_{31}K_{32}\{2wRv(\bar{x}_{st}) - cov(\bar{y}_{st},\bar{x}_{st})\} \tag{2.21}$$

$$MSE(t_4) = \bar{Y}^2(K_{41}-1)^2 + K_{41}^2 A_4 + K_{42}^2 B_4 - 2K_{41}C_4 + 2K_{42}D_4 - 2K_{41}K_{42}E_4 \tag{2.22}$$

$$MSE(t_5) = \bar{Y}^2(K_{51}-1)^2 + K_{51}^2\{v(\bar{y}_{st}) + w^2R^2v(\bar{x}_{st}) - 4wRcov(\bar{y}_{st},\bar{x}_{st}) - w(w-1)R^2v(\bar{x}_{st})\}$$
$$+ K_{52}^2 v(\bar{x}_{st}) + 2K_{51}\left\{wRcov(\bar{y}_{st},\bar{x}_{st}) + \frac{w(w-1)R^2v(\bar{x}_{st})}{2}\right\}$$
$$-2K_{51}K_{52}\{cov(\bar{y}_{st},\bar{x}_{st}) - wRv(\bar{x}_{st})\} \tag{2.23}$$

$$MSE(t_6) = \bar{Y}^2(K_{61}-1)^2 + K_{61}^2\{v(\bar{y}_{st}) + D'^2R^2v(\bar{x}_{st}) + 4D'Rcov(\bar{y}_{st},\bar{x}_{st}) + 2R^2v(\bar{x}_{st})C\}$$
$$+ K_{62}^2 v(\bar{x}_{st}) - 2K_{61}\{R^2v(\bar{x}_{st})C + RD'cov(\bar{y}_{st},\bar{x}_{st})\}$$
$$-2K_{61}K_{62}\{cov(\bar{y}_{st},\bar{x}_{st}) + D'Rv(\bar{x}_{st})\} \tag{2.24}$$

Where,

$$A_4 = \bar{Y}^2\left(\frac{v(\bar{y}_{st})}{\bar{Y}^2} + D'^2\frac{v(\bar{x}_{st})}{\bar{X}^2} + 4D'\frac{cov(\bar{y}_{st},\bar{x}_{st})}{\bar{X}\bar{Y}} + 2C\frac{v(\bar{y}_{st})}{\bar{X}^2}\right)$$

$$B_4 = \bar{X}^2\frac{v(\bar{x}_{st})}{\bar{X}^2}$$

$$C_4 = \bar{Y}^2\left(C\frac{v(\bar{x}_{st})}{\bar{X}^2} + D'\frac{cov(\bar{y}_{st},\bar{x}_{st})}{\bar{X}\bar{Y}}\right)$$

$$D_4 = \bar{X}\frac{v(\bar{x}_{st})}{\bar{X}^2}D'$$

$$E_4 = \bar{X}\bar{Y}\left(D'\frac{v(\bar{x}_{st})}{\bar{X}^2} + \frac{cov(\bar{y}_{st},\bar{x}_{st})}{\bar{X}\bar{Y}}\right)$$

Partially differentiating equation (2.21) with respect to K_{31} and K_{32}, we get the optimum values as:

$$K_{31}(opt) = \frac{D_3E_3 - B_3(\bar{Y}^2 - C_3)}{E_3^{\,2} - B_3(\bar{Y}^2 + A_3)} \quad , \quad K_{32}(opt) = \frac{E_3(\bar{Y}^2 - C_3) - D_3(\bar{Y}^2 + A_3)}{E_3^{\,2} - B_3(\bar{Y}^2 + A_3)} \qquad (2.25$$

Where,

$$A_3 = \left\{v(\bar{y}_{st}) + w\ R^2 v(\bar{x}_{st}) - 4wR\ \mathrm{cov}(\bar{y}_{st}, \bar{x}_{st})\right\}$$

$$B_3 = v(\bar{x}_{st})$$

$$C_3 = \left\{wR\ \mathrm{cov}(\bar{y}_{st}, \bar{x}_{st}) + \frac{w(w-1)R^2 v(\bar{x}_{st})}{2}\right\}$$

$$D_3 = wRv(\bar{x}_{st})$$

$$E_3 = \left\{2wRv(\bar{x}_{st}) - \mathrm{cov}(\bar{y}_{st}, \bar{x}_{st})\right\}$$

Similarly, partially differentiating equation (2.22) with respect to K_{41} and K_{42}, we get the optimum values as:

$$k_{41}(opt) = \frac{B_4(\bar{Y}^2 + C_4) - D_4E_4}{B_4(\bar{Y}^2 + A_4) - E_4^{\,2}} \quad , \quad k_{42}(opt) = \frac{E_4(\bar{Y}^2 + C_4) - D_4(\bar{Y}^2 + A_4)}{B_4(\bar{Y}^2 + A_4) - E_4^{\,2}} \qquad (2.26)$$

where,

$$A_4 = \bar{Y}^2\left(\frac{v(\bar{y}_{st})}{\bar{Y}^2} + D'^2\frac{v(\bar{x}_{st})}{\bar{X}^2} + 4D'\frac{\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st})}{\bar{X}\bar{Y}} + 2C\frac{v(\bar{y}_{st})}{\bar{X}^2}\right)$$

$$B_4 = \bar{X}^2\frac{v(\bar{x}_{st})}{\bar{X}^2}$$

$$C_4 = \bar{Y}^2\left(C\frac{v(\bar{x}_{st})}{\bar{X}^2} + D'\frac{\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st})}{\bar{X}\bar{Y}}\right)$$

$$D_4 = \bar{X}\frac{v(\bar{x}_{st})}{\bar{X}^2}D'$$

$$E_4 = \bar{X}\bar{Y}\left(D'\frac{v(\bar{x}_{st})}{\bar{X}^2} + \frac{\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st})}{\bar{X}\bar{Y}}\right)$$

Now, partially differentiating equation (2.23) with respect to K_{51} and K_{52}, we get the optimum values as:

$$k_{51}(opt) = \frac{B_5(\overline{Y}^2 - C_5)}{B_5(\overline{Y}^2 + A_5)D_5^{\,2}} \quad K_{52}(opt) = \frac{D_5(\overline{Y}^2 - C_5)}{B_5(Y^2 + A_5)D_5^{\,2}} \tag{2.27}$$

Where,

$$A_5 = \left\{v(\bar{y}_{st}) + w^2R^2v(\bar{x}_{st}) - 4wR\,\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st}) - w(w-1)R^2v(\bar{x}_{st})\right\}$$

$$B_5 = v(\bar{x}_{st})$$

$$C_5 = \left\{wR\,\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st}) + \frac{w(w-1)R^2v(\bar{x}_{st})}{2}\right\}$$

$$D_5 = \left\{\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st}) - wRv(\bar{x}_{st})\right\}$$

Finally, partially differentiating equation (2.24) with respect to K_{51} and K_{52}, we get the optimum values as:

$$k_{61}(opt) = \frac{(\overline{Y}^2 + C_6)B_6}{B_6(\overline{Y}^2 + A_6) - D_6^{\,2}} \quad , \; k = \frac{(\overline{Y}^2 + C_6)D_6}{B_6(\overline{Y}^2 + A_6) - D_6^{\,2}} \tag{2.28}$$

Where,

$$A_6 = \left\{v(\bar{y}_{st}) + D'^2R^2v(\bar{x}_{st}) + 4D'R\,\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st}) + 2R^2v(\bar{x}_{st})C\right\}$$

$$B_6 = v(\bar{x}_{st})$$

$$C_6 = \left\{R^2v(\bar{x}_{st})C + RD'\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st})\right\}$$

$$D_6 = \left\{\mathrm{cov}(\bar{y}_{st}, \bar{x}_{st}) + DRv(\bar{x}_{st})\right\}$$

3. Empirical Study

To see the performance of various estimators of population mean $\overline{Y}$, with respect to

Usual unbiased estimator $\bar{y}_{st}$, we have considered two data sets. Summaries of the Data are given below:

Data set 1: Source Singh and Mangat:

y_h: Juice quantity , x_h: Weight of cane.

Total	***Stratum***	***1***	***2***	***3***
N=25	N_h	6	12	7
n=10	n_h	3	4	3
$\bar{X}$=326	$\bar{X}_h$	366.666	310.883	317.143
$\bar{Y}$=102.6	$\bar{Y}_h$	135	99.166	80.714
S_x^2=2700	S_{xh}^2	2706.666	1881.06	2890.476
S_y^2=558.583	S_{yh}^2	80	226.515	120.238
ρ=.7314955	ρ_h	0.9455626	0.948196	0.7523324
R=0.314723	γ_h	0.1666667	0.1666667	0.1904762
ρ_c=0.8676778	W_h^2	0.0576	0.2304	0.0784

DATA SET 2: Source Singh and Chaudhary (1986, pg.162)

The data were collected in a pilot survey for estimating the extent of cultivation and production of fresh fruits in three districts of U.P .

x_h : area under orchards in heet.

y_h : total no of trees

Stratum	**1**	**2**	**3**
N_h	985	2196	1020
n_h	6	8	11
$\bar{X}_h$	11253	25115	18870
S_{xh}^2	15.97	132.66	38.44
S_{yh}^2	74775.47	259113.7	65885.6

S_{xyh}	1007.75	5709.16	1404.71
γ_{h}	0.16598	0.12454	0.08902

and

$R=49.03, \alpha_{opt}=0.9422$

Table- 3.1: MSE's and PRE's of estimators

		Data-1		Data-2	
	ESTIMATORS	**MSE**	**PRE**	**MSE**	**PRE**
1	t_1	701.546	1403.318	2.782946	404.6695
2	t_2	701.54	1403.318	2.782946	404.6695
3	t_3	629.0631	1565.013	2.77094	404.9483
4	t_4	874.5025	1125.774	3.051538	369.0511
5	t_5	601.846	1635.864	2.77668	405.5826
6	t_6	524.6948	1876.314	2.77092	406.4257
7	$\bar{y}_{RC}$	857.37974	1148.2567	3.47243	324.3185
8	$\bar{y}_{PC}$	21953.129	44.84	47.0589	23.93111
9	$\bar{y}_{st}$	9844.9203	100.00	11.26173	100.00

Conclusion

From Table 3.1, we see that all the proposed estimators perform better than usual mean estimator and combined ratio estimator. For data set 1 estimator t_6 is best followed by the estimators t_5 and t_3. For data set 2 t_6 is the best estimator followed by the estimator t_5.

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Lanczos derivative applied to Fourier series

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Abstract.

It is very known that if the operator $\frac{d}{dx}$ acts on each term into a convergent Fourier series (FS), then it may result a divergent series. This situation is remedied applying the symmetric derivative to FS, which implies the existence of the important Fejér-Lanczos Factors. In this note, we show that the Lanczos derivative also leads to these Factors.

1.- Introduction.

If on the Fourier series [1]:

$$f(x) = \frac{1}{2}\, a_0 + \sum_{k=1}^{\infty} \left[a_k \cos(kx) + b_k \sin(kx)\right] , \tag{1}$$

convergent in $[-\pi, \pi]$, we apply the operator $\frac{d}{dx}$ results: $\frac{d}{dx} f(x) = \sum_{k=1}^{\infty} k \left[-a_k \sin(kx) + b_k \cos(kx)\right]$, (2) which it may be divergent [2,3]. This problem was remedied by Lanczos [3] with $f'(x)$ defined as a Symmetric Derivative:

$$f'(x) \equiv \lim_{n \to \infty} \frac{1}{\frac{2\pi}{n}} \left[f_n\left(x + \frac{\pi}{n}\right) - f_n\left(x - \frac{\pi}{n}\right)\right] , \tag{3}$$

with the partial sums:

$$f_n(x) = g_n(x) + h_n(x) ,$$

$$g_n(x) = \frac{1}{2}\, a_0 + \sum_{k=1}^{n} a_k \cos(kx) \ , \ h_n(x) = \sum_{k=1}^{n} b_k \sin(kx) \ , \tag{4}$$

resulting the convergent expression:

$$f'(x) = \lim_{n \to \infty} \sum_{k=1}^{n} \sigma_k \frac{d}{dx} \left[a_k \cos(kx) + b_k \sin(kx)\right] , \tag{5}$$

with the Fejér-Lanczos Factors [3,4]:

$$\sigma_0 = 1 \ , \ \sigma_k = \frac{\sin\left(\frac{k\pi}{n}\right)}{\frac{k\pi}{n}} \ , \ k = 1, \ldots, n \ , \ \sigma_n = 0 \ . \tag{6}$$

The set of factors σ_k, for a given n, it is equivalent to a discrete sampling function.

In (2) and (3), we employ two derivatives, however, also there is the Lanczos derivative [3, 5-11], then it is natural to ask if this ultimate derivative leads to relation (5). The answer is yes, to see the next section.

2.- Lanczos generalized derivative.

Lanczos [3] used the least square method of Gauss-Legendre to obtain an integral expression for the derivative of a function, that is, differentiation by integration:

$$F'(x) = \lim_{\epsilon \to 0} \frac{3}{2\epsilon^3} \int_{-\epsilon}^{\epsilon} t\, F(x+t)\, dt\,, \tag{7}$$

which it may be applied to Fourier case:

$$\frac{3}{2\epsilon^3} \int_{-\epsilon}^{\epsilon} t\, g_n(x+t)\, dt \overset{(4)}{=} \frac{3}{2\epsilon^3} \sum_{k=1}^{n} a_k \int_{-\epsilon}^{\epsilon} t \cos(kx+kt)\, dt\,,$$

$$= -3 \sum_{k=1}^{n} a_k \frac{\sin(kx)}{k^2} A_k \quad \text{with} \quad A_k(\epsilon) = \frac{1}{\epsilon^3}\left[\sin(k\epsilon) - k\,\epsilon \cos(k\epsilon)\right]\,; \tag{8}$$

similarly:

$$\frac{3}{2\,\epsilon^3} \int_{\epsilon}^{\epsilon} t\, h_n(x+t)\, dt \overset{(4)}{=} \frac{3}{2\,\epsilon^3} \sum_{k=1}^{n} b_k \int_{\epsilon}^{\epsilon} t\, \sin(kx+kt)\, dt\,,$$

$$= 3 \sum_{k=1}^{n} b_k \frac{\cos(kx)}{k^2} A_k\,. \tag{9}$$

Therefore, the Lanczos derivative applied to partial sum (4) gives, taking $\epsilon = \frac{\pi}{n}$;

$$f'(x) = \lim_{n \to \infty} \frac{3}{2\epsilon^2} \int_{-\epsilon}^{\epsilon} t\, f_n(x+t)\, dt\,,$$

$$\overset{(8)\,and\,(9)}{=} \lim_{n \to \infty} 3 \sum_{k=1}^{n} \frac{1}{k^2} A_k \left[-a_k \sin(kx) + b_k \cos(kx)\right],$$

$$= \lim_{n \to \infty} \sum_{k=1}^{n} \frac{3A_k}{k^3} \frac{d}{dx}\left[a_k \cos(kx) + b_k \sin(kx)\right]\,, \tag{10}$$

but the Bernoulli–Hôpital rule permits to observe the behavior:

$$A_k\left(\epsilon = \frac{\pi}{n}\right) \xrightarrow[n >> 1]{} \frac{k^3}{3} \frac{\sin(k\,\epsilon)}{k\,\epsilon} = \frac{k^3}{3} \frac{\sin\left(\frac{k\pi}{n}\right)}{\frac{k\pi}{n}} \overset{(6)}{=} \frac{k^3}{3}\sigma_k\,, \tag{11}$$

and this value employed in (10) implies (5), q.e.d.

Thus, it is proved that the Symmetric and Lanczos derivatives give us the same expression for the derivative of a infinite Fourier series, with the important participation of the Fejér–Lanczos factors.

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