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22 & 23 September, 2015

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RESEARCH ARTICLES

RESEARCH ARTICLE

Contribution of water management in sustainable Agriculture development in Ahmednagar district, MS, India

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ABSTRACT

India is developing country and in India 64.72% people are depend on agriculture and Indian agriculture is depend on rainfall. Agriculture remains the backbone of the Indian economy through there is fast growth in various sectors. It is not an equal in every year and place. Even this is true though there are some reasons due to which agriculture is still not sustainable. Farmers are facing many difficulties in agriculture. Water management has very much importance in sustainable agricultural development. The effect, need and method are water management on sustainable development is studied in this research paper.

Keywords: Sustainable Development, Agriculture, Water Management.

INTRODUCTION

Sustainable development is one which exists for long time. Development in agriculture field which exist for long time is called as sustainable agriculture development. According to Braithland commission without compromising with the needs of generation fulfilling the needs of present generation is called integral development. This definition is stated by this commission. Integral development is flexible process and it consists of complete use of equipment, direction of investment, orientation of technical development, organic or statistical change and compatibility of all these things and fulfilling needs and demands.

Water management is an important medium for sustainable agricultural development, on the Earth usable water will be very few so need to developed watershed or drainage basin. A drainage basin or watershed is an extent or an area of land where surface water from rain, melting snow, or ice converges to a single point at a lower elevation, usually the exit of the basin, where the waters join another water body, such as river, lake, reservoir, estuary, wetland, sea or ocean.

Agriculture in the district depends mainly on the rainfall from south-west monsoon. The distribution of rainfall is most uneven. The major part of precipitation is experienced in western portions of Akola taluka, whereas rains in southern part of the district are most erratic. A major portion of the district lies in the zone of low rainfall ranging from 508 mm. to 635 mm. annually. The district can be divided into three zones according to rainfall at

taluka headquarters, *viz.,* the northern part comprising Kopargaon, Sangamner and Shrirampur talukas with a rainfall of about 500 mm. or less, the south-eastern part consisting of Shevgaon, Ahmadnagar, Pathardi and Jamkhed talukas with normal rainfall of 600 mm. and the third zonecomprising the remaining talukas with rainfall between 500 mm. and 600 mm. The rains usually start in the second week of June and last till the end of September. The intensity of rainfall is the highest in July. Sometimes thunder-showers in March and April are recorded. In the plain areas of the district the rains are erratic and mostly from the north-east monsoon.

Irrigational facilitiesin the district mainly included wellwatering or *motasthal* and small channel-watering or *patasthal.* The area of neither class was large. At the same time they want of a large enough supply of water and of land at a suitable level made the area of channel watered land much less than the area of well-watered land. Most of the dams or *bandharas* were built of mud and had to be repaired every year after the rains. Such *bandharas* were found throughout the district especially in Parner, Shrigonda, Karjat, Ahmadnagar, Kopargaon and Sangamner talukas, built across the many small early-dry streams which seam the country. Even now this practice is in vogue. Besides, wells were also used for irrigating all over the district. Day today ground water level decries and many irrigation problems incises, so water management is very important to agriculture development.

RESEARCH METHODOLOGY

In the relevant research paper researcher has used secondary data collection method. Topographical maps and survey of India sheets are used for physiographical study. For that purpose researcher has used agricultural related web sites, schemes magazines and government reports for data collection. The first step was successful construction of nullah bunds to increase the water levels. The villagers renovated tanks and recharged the groundwater by the tank water. Due to the steady percolation of water, the groundwater table began to rise. Ahmednagar was divided into four watershed zones. In order to conserve soil and water by checking the runoff, contour trenches and gully plugs were constructed along the hill slopes. This process was supplemented by a forestation, nullah bunds, underground check dams and cemented bandhras at strategic locations. Government social forestry schemes were utilised around the district. The environmental model is capable of dealing with recurrent drought. Soon, water was available even in summer.

SUSTAINABLE AGRICULTURAL DEVELOPMENT

The issues of sustainable development can be classified under three types of farming systems that are traditional production system, modern agriculture system and sustainable agriculture system. Further they can compare as three dimensions, ecological, economic and social sustainability. To increase the organic matter content of the soil, thus raising its ability to preserve and store water that falls as rain. Sustainable agriculture increases the diversity of crops produced and raising the diversity of insects and other animals and plants in and around the fields. Indiscriminate use of pesticides, improper storage etc. may lead to health problems.

Besides the small *bandharas* and irrigation wells, there were a few Government water works which mainly included the Bhatodi lake and the Ojhar and Lakh canals of the Pravara river water scheme. Table 1 gives Net area irrigated by various sources of water-supply in the district

In backbones of agriculture irrigation in ahmednagar are well and canals. Wells are used in all over the district. Canal are used in sangamner, Kopargoan, Shrirampur, Rahuri, Newasa and Shrigonda Tahsil. As well As private canal used in irrigation Akole, Sangamner, Newasa and Parner Tahsil. Tank irrigation will be used in Ahmednagar and Karjat Tahsil. The source of water Supply change in day by day.

CAUSES OF DEGRADATION OF WATER RESOURCES:

The major causes are:

1. Depleting forest and grass cover, particularly in catchments areas.
2. Neglect of traditional water harvesting and conservation techniques.
3. Increased pollution of both surface and ground water.
4. Improper water resources management.
5. Absence or improper functioning of industrial and municipal treatment

Plants are non-implementation of environment laws. Therefore substantial water resources development is the prime need of our Country.

Table 1: Net area irrigated by various sources of water-supply

Taluka	Year	Net area irrigated by					
		Government canals	Private canals	Tanks	Wells	Other Source	Total
Ahmadnagar	1961-62	--	463	1,814	33,880	--	36,157
	1965-66	--	--	699	24,339	--	25,038
Sangamner	1961-62	3,135	183	--	13,658	--	16,976
	1965-66	1,417	--	--	18,623	--	20,040
Akola	1961-62	--	1,297	--	1,504	--	2,801
	1965-66	189	1,093	--	1,469	189	2,940
Kopargaon	1961-62	47,765	--	--	28,794	--	75,559
	1965-66	63,612	--	--	23,341	--	86,953
Shrirampur	1961-62	36,542	--	--	25,922	--	62,464
	1965-66	45,186	--	--	26,975	--	72,161
Rahuri	1961-62	16,532	--	--	15,650	--	32,182
	1965-66	19,143	--	--	25,743	200	45,086
Nevasa	1961-62	1,817	315	--	12,145	--	14,277
	1965-66	1,810	--	--	13,544	--	15,354
Shevgaon	1961-62	--	--	--	11,474	--	11,474
	1965-66	--	--	--	13,573	--	13,573
Pathardi	1961-62	--	--	--	14,333	--	14,333
	1965-66	--	--	--	15,643	--	15,643
Parner	1961-62	--	1,540	--	19,536	--	21,076
	1965-66	--	230	--	20,846	--	21,076
Shrigonda	1961-62	13,164	--	--	25,032	--	38,196
	1965-66	14,362	--	--	20,311	--	34,673
Karjat	1961-62	--	--	899	20,523	1,424	21,422
	1965-66	--	--	4,713	20,878	1,100	26,691
Jamkhed	1961-62	--	--	--	13,096	--	13,096
	1965-66	--	--	--	17,664	--	17,664
District Total.	1961-62	1,18,955	3,798	2,713	2,35,547	--	3,61,013
	1965-66	1,45,719	1,323	5,412	2,42,949	1,489	3,96,892
	1967-68	1,31,900	800	900	2,20,200	--	3,53,800

Fig. 1: graph show the increases irrigation source of water supply.

'Sustainability' is the Central concept, which must address economic, social and environmental issues. Sustainable water resources Management means 'meeting the water requirements of the people, at the same time, conserving water resources for the benefits of future generations.' Technological cooperation and the growth of indigenous capacities at the grass root level and in the hierarchy of super structures are integral to that design and its implementation, particularly in the context of our country.

TIPS FOR WATER CONSERVATION

Each and every drop of water needs to be saved following the principle "No crop without a drop". A little thought and attention followed by an action from us can go a long way in conserving this precious natural resource.

- Encourage your family, community and local govt. to conserve water by providing water literacy.
- Water should not be wasted at any cost. Taps should be closed when not in use and leaky taps must be repaired without any delay.
- Rainwater should be harvested by using various rainwater harvesting techniques and can be used for various domestic, agricultural purposes and also for groundwater recharging.
- Water supply lines must be maintained properly and faulty and leaking pipes replaced. Plumbing should be done correctly, seeking advice from experts.
- Always install low volume flush toilets.
- Hand pumps should be well maintained to perform effectively and efficiently.
- HP matching in accordance with water demand is essential. High HP motors should not be used to draw water from the well (open or bore) when low HP pumps can perform the same function.
- Avoid installing ornamental water features unless the water is being recycled.
- Overhead storage tanks should be maintained in good condition, PVC tanks which are cheaper and lighter in weight and do not corrode or rust are preferable to metal tanks.
- Ensure that your swimming pools, fountains and ponds are equipped with reticulating pumps.
- Don't forget to weed your crop fields, lawns and gardens.
- Always irrigate plants during morning or evening to minimize evaporation losses.
- Desalting of canals, tanks, ponds, etc. must be done regularly during the summer months. People should be encouraged to revive the traditional practices of protecting trees around tanks, ponds or other water reservoirs.
- Deforestation of vegetation without compensatory a forestation is a short-sighted approach for solving immediate needs. A forestation of barren & hilly slopes should be carried out. Trees withstand drought better than crops. They check dust, replenish streams, provide fodder for cattle, shade to cattle &
- Watershed management i.e creation of small reservoirs and Percolation tanks to hold run-off water must be implemented and maintained.
- In hilly areas terrace cultivation should be practiced to prevent surface run off.
- Contour plugging and planting of grasses and trees in catchment area check run-off water and increase the soil's capacity to retain moisture.
- Sprinkler irrigation should be used for closely-spaced crops like wheat, millet, pulses, groundnuts, etc., as it conserves 30 to 40 per cent of the water as compared to surface method of irrigation.
- Drip irrigation is most suited for closely spaced row crops like vegetables, cotton, sugarcane. The efficiency of this system is around 25 to 30 per cent in conserving soil moisture.
- Pitcher irrigation can also be used for irrigating the plants. In this method holes are drilled in a mud pot and it is partially buried in the soil in proximity to the plant. The water in the pot oozes out slowly, ensuring that the soil is continuously moist and the plant gets a constant supply of water.
- Deep trenches can be dug adjacent to bunds to collect runoff water and soil especially in water deficient regions.
- Wastewater generated in industries should be treated carefully and should be recycled.

FACTS ABOUT WATER RESOURCES

1. Water is the most abundant single substance in the biosphere, 150,000,000 cubic kilometers in volume.
2. Our earth is covered with 71% water of which the oceans and seas hold 97% which is salty. Another 2% is locked up in the ice caps and snow and is thereby unusable. Only 1% is found in the rivers,

lakes and underground reservoirs and can be used by man.

3. India is one of the wettest countries in the world. Its average annual rainfall is 1,170 mm. India gets about 400 million hectare-metres (mham) of precipitation annually, in the form of rain and snow.
4. 1,683 million cubic meters of water flow through Indian rivers every year.
5. 85-90% of the rain water flows into the sea.
6. If we don't stop constructing dams, there will be hardly any free-flowing rivers left in the country.
7. 71% of water is lost from unlined canals, due to seepage.
8. Deforestation and destruction of wetland areas are the causes of increased sediments in water.
9. Irrigation accounts for 92% of the water consumed and the remaining 8% is used for domestic and industrial needs.
10. 450 km3 of waste water enters the world's rivers. 600 km3 of water is needed to transport this waste away and dilute it.
11. About 70% of India's surface waters are polluted: Out of some 3,119 towns and cities, only 217 have partial or complete sewage treatment facilities.
12. Many lakes and reservoirs are becoming atrophied (enrichment of organic nutrients) and their ability to support aquatic life is being cost.
13. Excessive deforestation results in silting of the rivers, thereby reducing their water holding capacity, which in turn, results in the spilling over the flooding of adjacent areas.
14. Water-borne diseases such as typhoid, jaundice, cholera, diarrhea and dysentery account for 66% of all illnesses in India.
15. With 70% of available drinking water being polluted, two-thirds of all diseases in India are water borne. As a result, we lose 73 million work days annually together with production worth Rs. 600 corers.
16. Industrial wastes, drained into waterways, have created the nightmare of paralysis and other crippling diseases caused by slow pollution due to mercury and other metals which creep up the food chain into fish as well as cow's milk.
17. India's groundwater resources are about 10 times its annual rainfall. But this water is declining in many areas due to the increasing number of tube wells.
18. Wetlands which act as a buffer for floods, purifiers of wastewater and nurseries for fish and wildlife, are being drained with no regards to their economic values.
19. The biggest problem with India's water resources is that it varies greatly over both time and space. Nearly three-quarters of India's rain comes pouring down during the four monsoon months from June to September. For the rest of the year, the country remains relatively dry.

WHAT SHOULD A COMMON MAN DO TO SAVE OUR WETLANDS?

Public can help for protection and preservation of wetlands and other vital ecosystems only if they know about their significance. We should:

1. Be aware and make others aware about the values, functions, uses and attributes of such ecosystems.
2. Create local database for information on wetlands, wildlife, natural resources and endangered, rare and vulnerable species. Particularly learn about local species.
3. Avoid unnecessary environmental damage.
4. Create and support local conservation groups and discourage poaching activities. Joining conservation groups would help you learn more about local, regional and global wetlands issues.
5. Enhance public consciousness by carrying environmental conservation messages amongst colleagues, friends, neighbors and relatives.
6. Take care of the plants and plant more trees & shrubs. Protect wildlife habitats.
7. Learn about man and biosphere relations, role of man for environmental management. Also learn about ecosystems and sustainability.
8. Reduce pressure on natural sources of water by suitably recycling and reusing waste waters.
9. Monitor local water systems to ensure they are not misused, polluted and used as sinks for waste.
10. Keep vigilant for new projects in your area and ask about possible pollution of the environment. Also enquire about protective measures. This will help in preserving the sensitive habitats.
11. Understand and make others to understand the threats, visible or invisible, direct or indirect, manmade or natural that may lead to the degradation and loss of such habitats.
12. Teach others what you know about natural ecosystems.
13. Take time to visit and explore the functions and the contribution of wetlands towards human well being.

14. Ask yourself and others about the significance of wetlands for humans and other living beings and what benefits you/they have been and are deriving from the resource.
15. Try to learn about the birds including migratory waterfowl that have been inhabiting the areas a couple of decades ago and are seen now. Explore the reasons for change.
16. Learn about the source of water, status of biological resources, etc. in the area.
17. Resolve to find out ways and means and to involve one and all for conservation, protection and improvement of the habitat for you and other living beings including migratory waterfowl.
18. Do not throw hazardous chemicals and solid wastes into water bodies. This may endanger sensitive flora, fauna and even the human populations dependant on the water body.
19. Do not litter and defecate along water courses. Major population of Punjab and adjoining states is dependent on surface water sources for drinking water.
20. Do not burn agro wastes- this could cause acid rains that may then cause irreparable and unaccountable loss and damage to the terrestrial as well as aquatic flora and fauna.
21. Learn and make others to learn about our environmental obligations for securing safe and enjoyable surroundings for our descendants.

CONCLUSION

The physical landscape of the region is marked by the mountain and hill ranges, reiver plains and undulating topography of plateaus in the study area. Sahyadri ranges are well defined by the watershed and available water in various dams. About 70% Population depend directly upon land middle and eastern part of the Ahmednagar district. In India is mainly use of the agricultural land and converted into residential and other uses for the growth and development of the facilities. Farmers have adopt modern technology i.e. fruits and vegetables drip irrigation facilities, variety seeds material, increasing use by composting biomass, improved planting technology and micro irrigation systems, crop loans, good network of transports and markets, agricultural advisory centers and also available facilities in the study area.

Therefore, recently cropping pattern is change and day to day positive increased but eastern part of the study area is concentrate in the rice crop because of these areas situated in the hilly and heavy rainfall. Normally, groundwater and surface water are used for irrigation and when water available in these sources is taken away artificially by flowing it for supplying water in required quantity to crops, it is called irrigation. In general, the goal is to supply the entire field uniformly with water, so that each plant has the amount of water it needs, neither too much nor too little.

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RESEARCH ARTICLE

Studies on indoor Aeromycoflora of Arva rice mill industry with its effects on human beings in and around Desaiganj (Wadsa) district Gadchiroli, MS, India

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ABSTRACT

Monitoring of indoor air of Arva rice mill for airborne fungi at Desiaganj (Wadsa) district - Gadchiroli in respect to effects on human being was undertaken during the period from Jan- 2012 to Dec-2013 using petriplate method and Hi Air sampler. Altogether 64 fungal organisms were isolated and identified from four different sections of Arva rice mill, belonging to Oomycota, Zygomycota, Ascomycota and Deuteromycota.The important fungal types identified were *Aspergillus niger, A. candidus, A. fumigatus, A. sydowii, A. flavus, penicillium notatum, P. glabrum, P. funiculosum, P. citrinum, Alternaria solani, A. alternata, Fusarium monoliforme, F. solani, Curvularia geniculata, C. lignicola, C. lunata, Bipolaris oryzae Cercospora sp.., Mucor sp., Rhizopus sp., Cunninghamella sp., Pithomyces sp., Epicoccum sp. Trichoderma sp., Torula sp., etc. during period of investigation.*

Keywords: Arva rice mill, aeromycoflora,effects on human, indoor air, Desaiganj (Wadsa)

INTRODUCTION

Aeromycology concerns to scientific study of sources, dispersion and effects of airborne micro - propagules of fungal origin. It involves aerial transport of potent fungal micro propagules including variety of magnitude of fungal spores, acervuli, cleistothecia, fragments of sterile mycelia, etc. in many different parts of the world (Wikipedia, 2014). The fungal spores are among the most abundant airborne bioparticles in the atmosphere of the earth and prominent allergens than air borne pollengrains, viruses, bacteria, protozoa, different propagules and vegetative cells of algae, lichens, bryophytes and pteridophytes, insect debris, house dust, mites, animals danger chemicals and food. Airborne fungal spores are ubiquitous in nature and can survive in both wet and dry environment through scavenging nutrients from the atmosphere (Verma et al, 2013). About 80,000 fungal species airborne reported and most of which are cosmopolitan in origin (Kendrick, 2000). The spores of fungal origin surviving in atmosphere are important components of bioaerosol as well as considered to act as indicator of the level of atmospheric biopollution (Ananna et al., 2013). These airborne spores can be cell cultures and have

property to undergo mutation producing genetically modified strains (EFSA, 2011). Human endoparasites may able to provoke any infection, childhood asthma, allergies, mycotoxicity while saprobes play a significant role in biodegradation or organic wastes (Aimanianda *et at*., 2010). This increased awareness has made the study of fungal propagules prevalent in air, important and hence the study of aeromycology has acquired a prominent place in various fields of environmental science.

Fungi produce a number of toxic chemicals such as poisonous compound found in some species of microfungi. Some fungi are known to produce secondary metabolites that are harmful to animals and human when ingested inhaled (Croft et al., 1986; Miller, 1992) or brought in contant with the skin (Schiefer, 1990). These toxic metabolites including alkaloids, cyclopeptides are called as Mycotoxins. The international agency for reseach on cancer, IARC (1993) classified aflatoxin, a toxin discovered in 1961 in *Aspergillus niger and A. parasiticus* as yielding, 'Sufficient evidence for human and animal carcinogenicity'. Peoples in India, working in Rice industries come in contact with grains. All durable and dried agricultural commodities, if not properly dried after harvest, are subject to attack by fungi and nearly 20% of the harvested crops in the developing countries including India have been lost due to post harvest diseases. Paddy Grain have generally high percentage of moisture at harvest becomes mouldy during storage and because high fragrance in milling and other post harvest technological processes. Many moulds colonizing grains besides degrading the grain and making in less palatable, may give rise to health hazards to workers handling the grain with mouldy or dirty grain. The dust when inhaled causes respiratory disorders to workers.

MATERIALS AND METHODS

Study area: Desaiganj (Wadsa) is a town and a municipal council in the Gadchiroli district in the state of Maharashtra, India. There are many small scale industries cropping up, beside some existing one's like a 10 MW power plant, a sugar factory, a medicine factory and two fertilizer plants. It's a center market for rice trading. Shree Sai Arva Rice Mill and Rajmata steam Rice Mill both are located at Lakhandur Road in Wadsa town of Gadchiroli district of Maharashtra. But the present study was orient on selected Shree Sai Arva Rice Mill from its 4 sections (Paddy godown, machine section, rice godown and husk storage section).

Air sampling was conducted inside the four different sections of Arva rice mill Industry at Desaiganj, (Wadsa) district, Gadchiroli for two consecutive years (Jan., 2012 - Dec., 2013) using Hi Air sampler (Mark II), Hi media Laboratories, India,. for five minutes on Agar strips, fortnightly. Simultaneously exposure petriplate method containing CDA (Czapek's Dox Agar) with streptomycin, two times in a month, by keeping them at the height of five feet from the ground level. Petriplates were incubated at room temperature. After 3 - 4 days colonies were observed, counted and sub cultured for identification.

RESULTS AND DISCUSSION:

Near about total 64 species belonging to 24 fungal genera were identified from the four different sections of Arva Rice Mill Industry (Table 1). Out of 64 fungal types trqpped, one fungal type belonged to Oomycota, 8 belonged to Zygomycota, 27 belonged to Ascomycota and 28 belonged to Deuteromycota. The maximum numbers of fungal types were contributed by Deuteromycota which was followed by Asomycota, Zygomycota and Oomycota. The fungal organisms were found present throughout the year in the indoor environment of Arva rice mill Industry.

The concentration of fungal airspora was increased during warmer and humid condition followed by seasonal trend in relative humidity, rainfall & temperature. During the period of investigation (Jan. 2012 - Dec. 2013), 14 species of- *Aspergillus,* 6 species of *penicillium*, 5 species of - *Curvularia,* 4 species of *Fusarium, Alternaria, Mucor* each, 3 species of *Trichoderma, Cladosporium, Rhizopus* each, 2 species of *Chaetomium, Bipolaris, Torula* each, and single species of *Cuninghamella, phytophthora, Drechslera, Epicoccum, Pithomyces, Phoma, Scicaria, Botrytis, Cercospora, Nigrospora, Pyricularia, Tricothecium,* etc were identified. The dominant fungal types identified were *Aspergilhus niger, A. condidus, A. fumigatus, A. sydowii, A flavus, A. versicolar, A. terreus*, *Penicillium notatum, P. glabrum, P. funiculosum, P. citrinum, Alternaria solani, A. alternata, Fusarium monoliforme, F. solani, Curvularia geniculata, C. lignicola, C. lunata, Bipolaris oryzae, Rhizopus stolonifer, R. oryzae, Mucorhiemalis, M. pusillus, Cladosporium sp.*, etc. The present finding clearly showed that *Aspergillus sps* were found to be the dominant among aeromycoflora throughout the year.

Table No. 1. Exposure Petriplate Method: Fungal genera/species identified in four sections of Arva Rice Mill, during Jan. 2012 – Dec. 2013

Sr. No.	Genera/Species	Paddy Godown	Machine Section	Rice Godown	Husk Storage Section
A)	**Oomycota**				
1	*Phytophthora infestans* de Bary	+	-	-	+
	Total	**1**	**-**	**-**	**1**
B)	**Zygomycota**				
2	*Cunninghamella sp.*	+	+	+	+
3	*Mucor hiemalis* Wehmer	+	+	+	+
4	*M. pusillus* Lindt.	+	+	+	+
5	*M. racemosus* Fresen.	+	+	+	+
6	*M. plumbeus Bonord.*	+	+	+	+
7	*Rhizopus stolonifer* (Ehrenb.)Vuill.	+	+	+	+
8	*R. oryzae* Went & Prins.	+	+	+	+
9	*R. oligospora* R. microsporus Tiegh	+	+	-	+
	Total	**08**	**08**	**07**	**08**
C)	**Ascomycota**				
10	*Aspergillus flavus* Link.	+	+	+	+
11	*A. fumigatus* Fresen.,	+	+	+	+
12	*A.niger* Tiegh.	+	+	+	+
13	*A.flavus var. oryzae*(Ahlb.) Kurtzman, Smiley, Robnett & Wicklow.	+	+	+	+
14	*A.versicolor* (Vuill.) Tiraboschi.	+	+	+	+
15	*A. terreus* Thom.	+	+	+	+
16	*A.flavipes* (Bainier sartory) Thom and church	+	+	+	+
17	*A.ochraceus With.*	+	+	-	-
18	*A.glaucus* Link.	+	+	-	+
19	*A.candidus* Link.	+	+	-	+
20	*A.nidulans* Fennell & Raper	+	+	+	+
21	*A.sydowii* (Bainier & sartory) Thom church.	+	+	-	+
22	*A.humicola* Choudhuri & Sachar	+	+	-	-
23	*A.carbonarius* Thom.	+	-	-	+
24	Chaetomium *glabosum* Kunze	+	+	+	+
25	*C.globosum*	+	+	+	+
26	*Drechslera sp.*	+	-	-	-
27	*Epicoccum sp.*	+	-	+	+
28	*Penicillium notatum* Westling.	+	+	+	+
29	*P.chrysogenum* Thom.	+	+	+	+
30	*P.citrinum* Thom.	+	-	+	+
31	*P.glabrum* Westling	+	+	+	+
32	*P. corylophilum* Dierckx	+	+	+	+
33	*P. funiculosum* Thom.	-	+	+	+
34	*Pithomyces sp.*	+	+	+	+
35	*Phoma glomerata* (Carda) wollenw. & Hochapfel	-	+	+	+
36	*Scicaria sp.*	-	-	+	+
	Total	**24**	**22**	**20**	**24**
D)	**Basidiomycota**				
	Total	**-**	**-**	**-**	**-**
E)	**Deuteromycota**				
37	*Alternaria solani* Sorauer	+	+	+	+
38	*A.alternata* (Fr.) Keissl.	+	+	+	+
39	*A. longipes* (Ellis & Everh.) E.W.Masan	-	-	+	+
40	*A.brassicicola(schwein.)* wiltshire	+	+	-	+
41	*Botrytis sp.*	+	+	-	-
42	*Cladosporium cladosporioides* (Fresen.) devries	+	+	+	+

Table 1: Continued…

Sr. No.	Genera/Species	Paddy Godown	Machine Section	Rice Godown	Husk Storage Section
43	B.herbarum (Pers.) Link.	+	+	+	+
44	C.lignicola Link.	+	+	+	+
45	Curvularia geniculata Boedijn	-	-	-	+
46	Bipolaris specifera subram. (Curvularia tetramera)	+	+	+	+
47	C.lunata Boedijn	+	+	+	+
48	C.branchyspora Boedijn	-	-	-	+
49	C.subulata Boedijn ex.J.C. Gilman	+	+	-	-
50	Cercospora sp.	+	+	+	+
51	Fusarium oxysporum Schlecht	+	+	+	+
52	F. monoliforme J. Sheldon.	-	-	+	-
53	F.solani Appel & Wollonweber.	+	+	+	+
54	F.equiseti Saecardo	-	-	+	+
55	Bipolaris oryzae shoemaker	+	+	+	+
56	Bipolaris tetramera Shoemaher	+	+	+	+
57	Nigrospora Sp.	+	+	+	+
58	Pyricularia sp.	+	-	-	+
59	Trichothecium roseeum Link.	+	+	+	+
60	Torula graminis Desm. ex Fr.	+	+	-	+
61	T. herbarum Link.	+	+	-	+
62	Trichoderma Viride Pers.	+	+	+	+
63	T. Koningii Oudem.	-	-	-	+
64	T. Iignorum Tode	-	+	-	-
	Total	**21**	**21**	**18**	**24**
F)	***Other types***				
65	*Sterile mycelia*	+	+	+	+
66	*Unidentified*	+	+	+	+

CONCLUSION

The present study concluded that air borne fungi can play important role in producing respiratory allergies in humans. The most common health hazard due to continuous exposure to such aero-biota which is heavily infested with pollen, fungal spores is allergy. Many workers reported that species of *cladosporium, penicillium, Aspergillus, Mucor, Rhizopus, Fusarium* contain a variety of antigens which induce the synthesis of antibodies in the human beings the allergens, these species are frequently abundant in indoor air of Arva rice mill which causes asthma, allergic rhinitis, respiratory allergies and other allergic diseases.

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RESEARCH ARTICLE

Biodiversity of Mite Fauna in the Intramural Environment of Rat House at Pune

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ABSTRACT

A fortnightly investigation had been carried out in the intramural environment of Rat House of Educational Complex, Erandwane, Pune from January 2015 to August 2015 to analyze the environmental impact on the incidence, population density, monthly variation, seasonal variation and dynamics of mite fauna to explore biodiversity with constant environmental parameters using simple 'Pickup Technique' from husk samples under stereo binocular dissecting microscope. In all 356 mite specimens have been screened, identified and classified into male and females under four genera named as *Haemolaelaps glasgowi* abbreviated as Hg, *Echinolaelaps echidninus* abbreviated as Ee belonging to family Laelaptidae and Unidentified (UI_1) and (UI_2). Interesting findings and catchy specimens have been reported exhibiting fortnightly, monthly and seasonal variation during the study period. Quantitative analysis revealed that *Echinolaelaps echidninus* (78.36%) has been recorded as dominant species followed by *Haemolaelaps glasgowi* (20.78%) and UI_1, UI_2 have been reported very rare. Percentage of *Echinolaelaps echidninus* female had been found to be 44.93% which was more than males (33.39%). The percentage of *Haemolaelaps glasgowi* male was found to be 11% and 9% of *Haemolaelaps glasgowi* female has been observed. Also the overall population of *Echinolaelaps echidninus* has been reported more as compared to *Haemolaelaps glasgowi.* These mites have been reported during all the months but exhibited impacts of environmental parameters like temperature, relative humidity and rainfall on their population load. Maximum load has been recorded during August 2015 (83/gm) with breeding, egg laying, hatching into larvae, moulting into nymphs and adults while minimum load has been recorded during February 2015 (19/gm).

Keywords: Rat Mites, Diversity, Allergenic, *Haemolaelaps glasgowi, Echinolaelaps echidninus,* Unidentified.

INTRODUCTION

Ticks and mites belong to *Arachnida* and have been focused for the studies very recently as a new branch of zoology or veterinary science under Acarology. To a little more extent House Dust Mites have been explored

during last few decades as significant offending intramural allergens causing prominent allergy among sensitive victims. About 20% Indian and world population has been recorded today to suffer from allergic ailments like respiratory or skin allergy.

Hence scientists focused their attention on role of environment on the incidence, population dynamics, fluctuation or rhythmic seasonal dynamics of the house dust mite for their management and treatment of allergy patients to give them good health.

Kern (1921) was the first American Scientist to discover *Dermatophagoides pteronyssinus,* a house dust mite as prominent cause of house dust mite allergy and not the proper dust in the homes as presumed earlier. Subsequently different groups of HDM like Pigeon mites, poultry mites, bed mites, floor mites, animal mites like rat, cat, pig, cattle mites and other bird mites like fowl mites etc. had been studied on top focus to analyze the allergic ailments among the patients by several workers from abroad and also in India.

Accordingly, through further studies in all 36 species have been recorded world over as offending allergens (Spieksma, 1997), 29 species from West Bengal i.e. India, 23 from Karnataka (Rao and Ranganath, 1981), 20 from Maharashtra (Jogdand, 2012) and 17 from Kerala (Haq and Ramani, 2010). Now the Indian record has been raised to 36 and record of Maharashtra to 27, updating the world record to 43 instead of 36 (Jogdand, 2013).

Fain A (1957), Spieksma (1997), Maunsell (1968), Kern (1921) etc.. were prominent researchers from abroad and Shivpuri (1977), Modak *et al.* (1991), Rao and Ranganath (1981), Channabassavana (1981), Lal *et al.* (1973), Maurya *et al.* (1982), Tilak and Jogdand (1989a; 1989b; 1989c;1989d), Jogdand (1986), Jogdand (1994,1995,1996,2000,2007,2010), Rao Krishna SN *et al* (1980), Tilak and Jogdand *et al* (1994), Tilak and Jogdand (2009) etc from India who gave valuable contributions in Acarology. Reviewing this work the present study has been undertaken to explore 'Biodiversity of mite fauna in the intramural environment of rat house at Pune.

MATERIALS AND METHODS

House dust has been considered as intramural detritus ecosystem comprising biotic components like microbes of bacterial and fungal or some times of viral origin from plant kingdom and mites, eggs of mosquitoes, cockroaches, lice, bed bugs, protozoan cysts etc from animal kingdom. Dust in the houses constitute abiotic components including dandruff, cosmetics, debris, paints, colors etc. These biotic and abiotic components of detritus ecosystem constitute the material for the subject. Here we have selected study of mites from the husks of 'Rat House' from Pune.

Simple pickup method (Jogdand 1988) from husk samples under the stereobinocular dissecting microscope has been used for the separation of mite specimens from rat house husk. The fresh samples of rat house husk have been collected fortnightly, regularly on the fixed dates for i.e. 1st and 16th of every month at a fixed time i.e.at 8 a.m. sharply, in sufficient quantity using paper envelope and immediately brought to the research laboratory in the Department of Zoology, BVDU, Yashwantrao Mohite College, in the premises of More Vidyalaya, Pune, Maharashtra.

Examination of Rat husk samples :

Two grams of these fresh samples have been spread on a clean, dry and sterile petridish with a uniform thin layer and observed under a stereobinocular dissecting microscope with illumination device below, so that mites get exposed and illuminated when they get agitated and start their movements and thus easily detected in the husk due to their prominent shape, size and color.

Isolation of mites:

These mites have been manually picked up and collected using a moist needle dipped in 4% lactic acid and stored in lactic acid in a cavity block. Because on touching the mite in the husk by tip of moist needle, it sticks to needle and thus collected.

Clearing and mounting:

Depending upon the extent of sclerotization of body cover, they have been kept safe inside the lactic acid for one to three days and mounted when they become transparent in the melted glycerin jelly, keeping ventral posture up. The cover slip is then gently pressed with blotting paper to remove excess jelly and for proper spreading of body parts and leg pairs. They get solidified on cooling to normal temperature and the slides are ready for microscopy and photography.

Identification:

The mites were identified according to the keys given by Baker and Wharton (1958), Hiware *et al*.

(2003), Hughes (1976), Spieksma (1997) and other available literatures. These specimen slides have been screened under Leica binocular research microscope with camera, monitor and measurement facility in a special Leica room.

RESULT AND DISCUSSION

During the study period in all, 356 specimens have been screened under 4x or 10x X 10x combination magnification and date wise measurements of specimens have been recorded. And these specimens have been identified and classified into male and females under four genera named as *Haemolaelaps glasgowi* (Ewing, 1925), *Echinolaelaps echidninus* (belonging to family *Laelaptidae* and Unidentified (UI_1) & (UI_2) as very rare specimens (Plate I).

Interesting findings have been recorded as briefly described and discussed here. The colour of adult female *Echinolaelaps echidninus* is reddish brown and attains a size of about (1137.54 X 580.51µm) Male and Female (1122.80X594.12µm). The genitoventral plate is widely expanded posterior to coxa IV and extends nearly to the anal plate from which it is separated by a very thin strip of integument. The colour of *Haemolaelaps glasgowi* is dark brown and attains a size of about (508.78X314.41µm) Hg male and female (1114.5X554.08 µm). The genitoventral plate is separated from the anal plate by a distance distinctly greater than the length of the anus. The shape of (UI1) has been found to be oval with stunted leg pairs with terminal disc. It is brownish in colour having 636X393µm. The another species (UI2) is brown in colour having size 502X 381µm.bearing slender and very long leg. (Plate I)

The fortnightly and monthly percentage contribution of all species has been described here. Highest total fortnightly load of rat mites has been recorded during first fortnight of August (83) followed by second fortnight of July (44) and second fortnight of June (41). And lowest total load (4) has been recorded during second fortnight of January 2015.During both March and April first fortnight the load has been found to be same i.e.20. (Table 1 and 2)

The ratio of female: male in both the genera shows interesting findings. The ratio of fortnightly Ee females: Ee males has been found to be 44.93% : 33.39% where as on contrary the ratio of fortnightly Hg female : Hg males has been found to be 8.69% : 12.05%. (Fig.1 and 2)

The quantitative analysis revealed that Ee (78.36%) is dominant species as compared to Hg (20.78%) and UI_1 (0.56%),UI_2 (0.28%). Highest load has been recorded during August i.e. 63 of Ee and 17 of Hg followed by UI_1 (2) and UI_2 (1). Hg contributed 1.12% during January and 1.96% during February while Ee has been found absent during January and contributed 1.69% during February. The highest load of Ee has been found in the month of July (20.78%) comprising 13.48% females and 7.3% males. Whereas highest load of Hg has been observed in the month of August (4.77%) comprising 3.37% males and 1.4% females (Table 3 and 4)

Table1: Fortnightly contribution of *Echinolaelaps echidninus* in the Intramural Environment of Rat House at Pune.

Particulars	% Ee Total Load	%Ee Male	%Ee Female
2nd fortnight Jan.	0	0	0
1st fortnight Feb.	0	0	0
2nd fortnight Feb.	1.69	1.40	0.28
1st fortnight March	5.06	3.93	1.12
2nd fortnight March	1.40	1.40	0
1st fortnight April	3.93	3.93	0
2nd fortnight April	3.65	1.40	2.25
1st fortnight May	5.34	0.84	4.49
2nd fortnight May	4.78	2.81	1.97
1st fortnight June	3.37	1.40	1.97
2nd fortnight June	10.67	3.09	7.58
1st fortnight July	10.11	3.09	7.02
2nd fortnight July	10.67	4.21	6.46
1st fortnight August	17.69	5.89	11.79
Total	**78.36**	**33.39**	**44.93**

Table 2: Fortnightly contribution of *Haemolaelaps glasgowi* in the Intramural Environment of Rat Houseat Pune.

Particulars	% Hg Total Load	%Hg Male	%Hg Female
2nd fortnight Jan.	1.12	1.12	0
1st fortnight Feb.	1.97	0.56	1.40
2nd fortnight Feb.	1.69	1.12	0.56
1st fortnight March	0.56	0.56	0
2nd fortnight March	0.28	0.28	0
1st fortnight April	1.69	1.40	0.28
2nd fortnight April	2.81	0.84	1.97
1st fortnight May	1.69	0.28	1.40
2nd fortnight May	0.56	0.56	0
1st fortnight June	0.28	0.28	0
2nd fortnight June	0.84	0.56	0.28
1st fortnight July	0.84	0.28	0.56
2nd fortnight July	1.68	0.84	0.84
1st fortnight August	4.77	3.37	1.40
Total	**20.78**	**12.05**	**8.69**

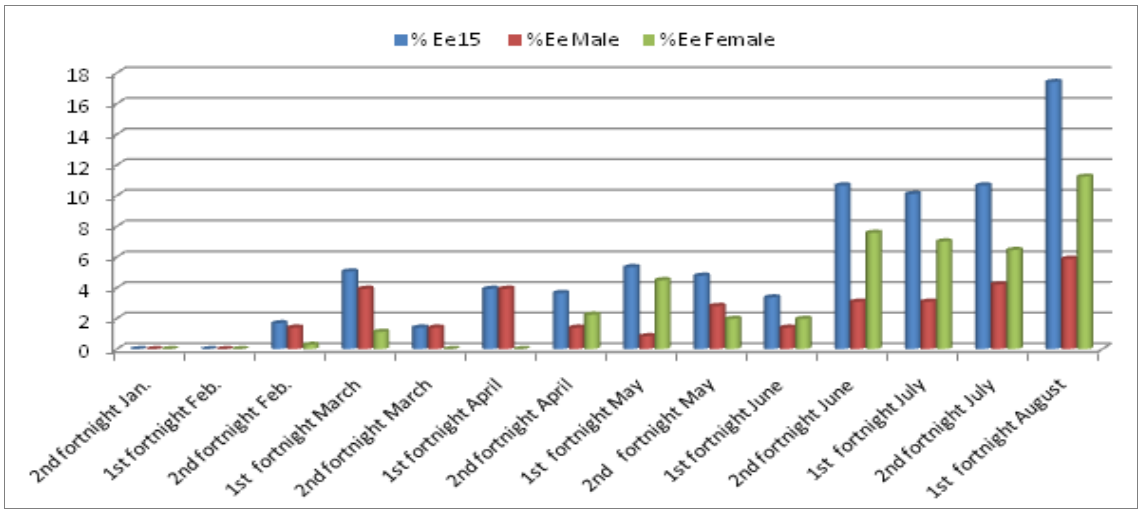


Fig. 1: Fortnightly contribution of *Echinolaelaps echidninus* in the Intramural Environment of Rat House at Pune.

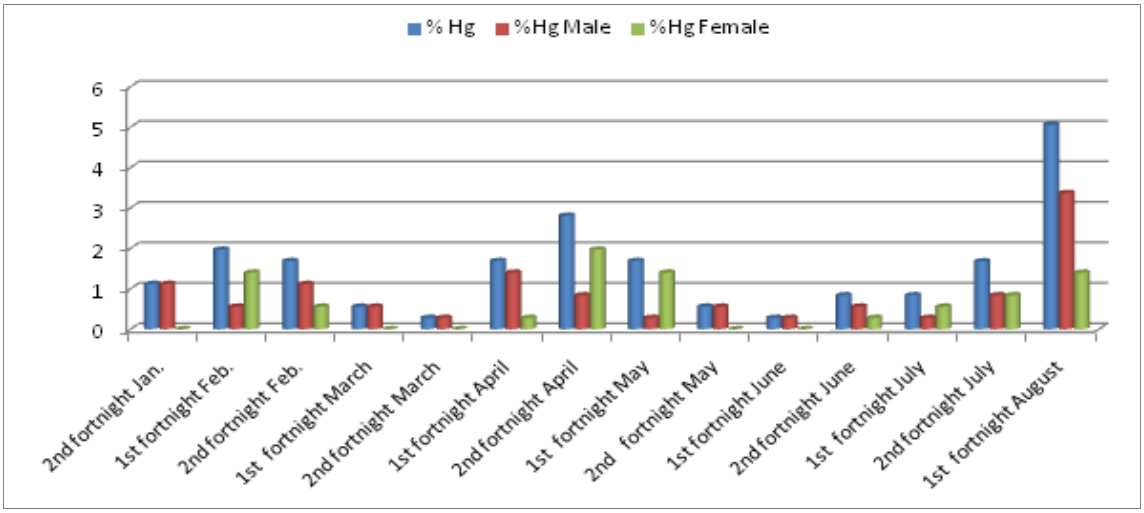


Fig. 2: Fortnightly contribution of *Haemolaelaps glasgowi* in the Intramural Environment of Rat House at Pune.

Table. 3: Monthly contribution of *Echinolaelaps echidninus* in the Intramural Environment of Rat House at Pune.

Echinolaelaps echidninus			
Month	**% Ee Total Load**	**% Ee male**	**% Ee Female**
Jan	0	0	0
Feb	1.69	1.4	0.28
March	6.46	5.33	1.12
April	7.58	5.33	2.25
May	10.12	3.65	6.46
June	14.04	4.49	9.55
July	20.78	7.3	13.48
Aug	17.69	5.89	11.79
Total	**78.36**	**33.39**	**44.93**

Fig.3: Monthly contribution of *Echinolaelaps echidninus* in the Intramural Environment of Rat House at Pune

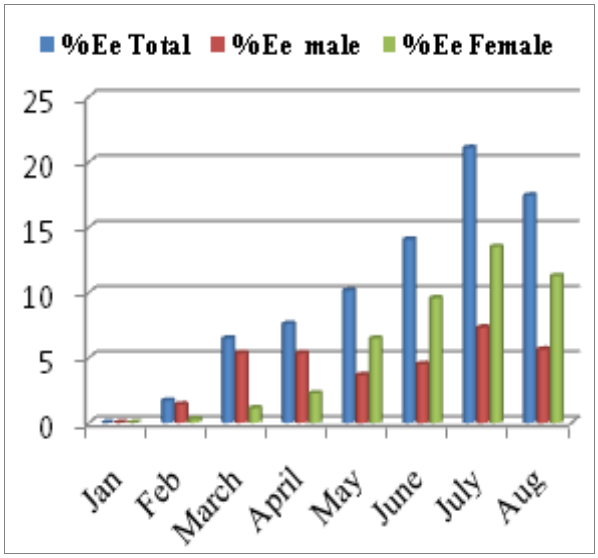
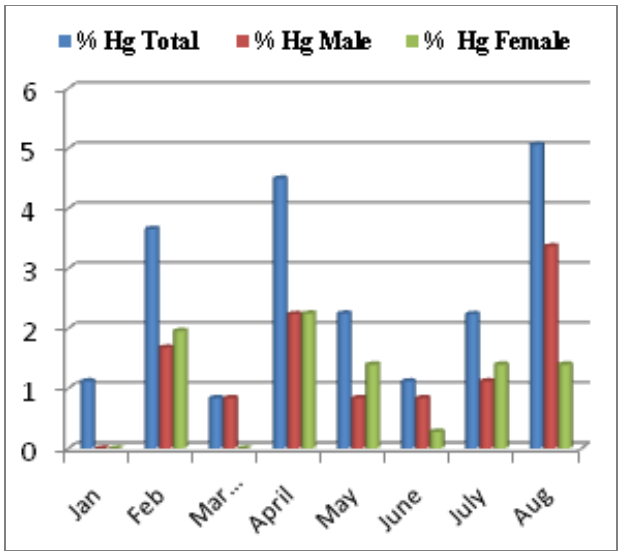


Table 4: Monthly contribution of *Haemolaelaps glasgowi* in the Intramural Environment of Rat House at Pune.

Haemolaelaps glasgowi			
Month	**% Hg Total Load**	**% Hg Male**	**% Hg Female**
Jan	1.12	0	0
Feb	3.66	1.68	1.96
March	0.84	0.84	0
April	4.5	2.24	2.25
May	2.25	0.84	1.4
June	1.12	0.84	0.28
July	2.52	1.12	1.4
Aug	4.77	3.37	1.4
Total	**20.78**	**10.93**	**8.69**

Fig.4: Monthly contribution of *Haemolaelaps glasgowi* in the Intramural Environment of Rat House at Pune.



The monthly ratio during August of Ee females : Hg females has been found to be 11.79%:1.4% and Ee males : Hg males has been found to be 5.89%:1.4%. During both March and April the percentage contribution of Ee males has been found to be same i.e. 5.33%. (Fig. 3 and 4).

The quantitative analysis of egg bearing and non egg bearing females has been carried out. The total number of egg bearing females of Ee and Hg, observed was (163-45.78%),while the highest number of egg bearing females has been observed in the month of July i. e. 47 and in March only 3 females have been found carrying eggs and no egg bearing female has been recorded during January. The Ee females carrying egg showed more contribution (141-39.60%) as compared to Hg (22-6.17%)).Hence The total ratio of Ee egg bearing female: Hg egg bearing female has been found to be 39.60%: 6.17%. and (28-7.86%) females without egg have been observed during study period. Two unidentified species have been recorded during August 2015 as UI_1 and UI_2 consisting a larva and an adult. (Plate I).

***Echinolaelaps echidninus* (Male)**

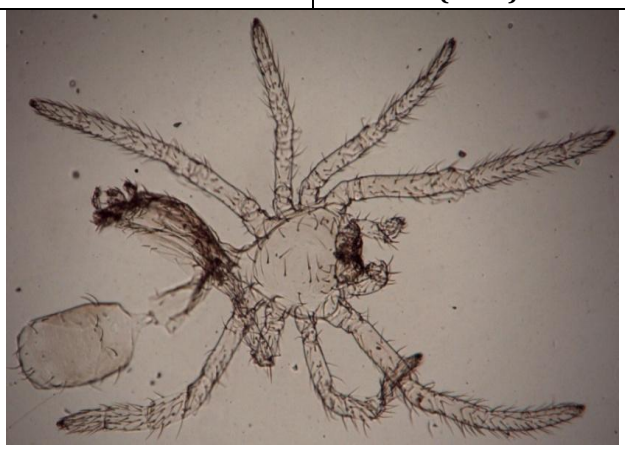
***Echinolaelaps echidninus* (Female)**

Unidentified 1

***Haemolaelaps glasgowi* (Male)**

***Haemolaelaps glasgowi* Female**

Larva of Unidentified 2



Unidentified 2
Identification of UI1, UI2 and larva is in process

Plate1:Identified and unidentified species of Rat mite.

The study on house dust mites has been mostly restricted to human houses/ patients houses further extended to poultry house dusts or different types of dwellings with human association. But this type study is totally new as so far the common word "Rat mites" has been recorded in the published literature. But In this study a continuous study of rat house husks has been carried out for eight months fortnightly. No such study has been carried earlier on Rat House husk mites. But a similar type of study in House Dust Mites in the human dwellings have been carried out (Jogdand, 1988) which revealed variation in mite load as per the types of dwellings (Huts, slums, well built and well ventilated buildings etc.), seasons, months, localities etc. and recorded highest mites load during rainy season particularly in dark damp and illventilated poorly constructed houses as compared to well ventilated posh buildings. Moderate mite load during winter and minimum load during summer seasons (Jogdand 1997a; 1997b) has been reported. 20 species of HDM in Maharashtra (Jogdand, 2012) have been recorded and established role of environment on dynamics of house dust mites at Pune (Jogdand *et al*., 2013). But in this study of rat house husk mites, the usually recorded house dust mites like *D. pteronyssinus, D.farinae, Caloglyphus, Blomia* etc. have been surprisingly found absent totally. Instead of these Ee and Hg have been recorded mostly during rainy and summer season in good numbers, showing seasonal fluctuations and less monthly variation in contrast to HDM which showed prominent variation (Jogdand, 2009).

Biodiversity is specific limited to Ee, Hg and UI1 and UI2 showing more abundance of Ee and Hg while incidence of UI1(2specimens) and UI2(only one specimen) is very rare. The identification of UI1and UI2 is in process and study is continued to determine lower stratum mite fauna below the husk in the rat house dust and elaborate study will be presented in future. However this study has opened a new thrust

area in acarology with good scope to study rat house dust mites from allergy point of view.

CONCLUSION

From the present study it can be concluded that, 356 mites have been recorded from the rat house. These have been classified and identified into 4 genera out of which Ee has been recorded dominant followed by Hg, UI1and UI2 are unidentified and rare and may be new records thus showing biodiversity.

Mites in the house dust are proved allergens causing severe allergy, comparatively in more patients sensitive to them. They show seasonal variations and great species biodiversity. Control of mite populations by various methods including chemical control helps to solve the problem in the management of house dust mite allergy. Thus these studies have been found useful to give relief to ailing allergy patients to afford them good health in twenty first century.

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RESEARCH ARTICLE

Qualitative Assessment of airborne deuterospores over pomegranate (*Punica granatum* L.) field

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ABSTRACT

Pomegranate (*Punica grantum* L.) is a high value commercial horticultural crop grown extensively throughout Western Maharashtra. The aerobiological survey was undertaken to understand the qualitative and quantitative incidence of fungal spores over pomegranate field for the first time from this unexplored locality by operating Continuous Volumetric Tilak Air Sampler. Out of total air spora, 42 spore types of deuterospores were recorded. Percentage contribution of each spore type of deuteromycetes to the total air spora revealed that *Cladosporium* contributed the highest toll (29.49%) followed by *Epicoccum* (9.27%), *Nigrospora* (2.86%), *Alternaria* (2.30%), *Botrytis* (2.18%), *Botrydipodia* (2.00%). Influence of meteorological factors on incidence of deuterospores was observed. The present paper provides a critical account of airborne deuterospores over pomegranate field.

Keywords: Airspora, Tilak air sampler, deuterospores, pomegranate field.

INTRODUCTION

Pomegranate (*Punica granatum* L.) Var. Mrudula is an economically important fruit crop. The fruits are perishable in nature and have high water contents. In Maharashtra, 25000 hectors of land is under cultivation of pomegranate. However, it is subjected to various air borne fungal diseases like leaf spot disease, caused by *Curvularia*, fruit rot caused by *Colletotrichum*, spot on fruits caused by *Cercospora*, burning of fruits and leaves by *Alternaria alternate*. The disease is more prevalent during mrug and hasta bahar. These disease cause enormous loss to the farmers. Fungal diseases are responsible for rotting of fruits, irregular spots on fruits and leaves, discolouration of fruits, etc. Ultimately there is reduction in the prizes to the fruits in the market. Pomegranate production has increased for both fresh market and juice in the last several years and fungal diseases has become a major concern to the growers.

Aerobiology is the science which studies the atmospheric dispersion of biological materials and their impact on the environment and organism. Aerobiology could help agriculture in different ways to optimize the production of many crops. Aerobiological studies are of great importance as they provide with qualitative and quantitative information about

airborne fungi. Composition and concentration of airborne mycoflora depend on several factors including topography, time of day, meteorological parameters, type of vegetation, air pollution, agricultural, industrial and other human activities (Pepeljnjak and Segvic Klaric, 2003; Mali *et al.*, 2006). Pande (1976) studied the airspora over orange field. Tilak and Babu (1981) made intensive and critical study of pathogen and reported the concentration of urediniospores of *Puccinia penniseti* in the air relation to disease incidence and growth stages of the crop. Aher and Pande (2004) made an intensive study on pathogenic fungal spores over groundnut field. Reddy (2014) carried out qualitative assessment of ascospores over sugarcane field. The aeromycoflora of the pomegranate crop is not studied so far. Present study was undertaken with a view to study the qualitative and quantitative assessment of deuterospors over pomegranate field.

MATERIALS AND METHODS

The aerobiological investigation has been carried out with the help of Continuous Volumetric Tilak Air Sampler (Tilak and Kulkarni, 1970). The air sampler runs on 230 v current and drum present inside the sampler complete one rotation in 8 days. The air sampler provides continuous sampling for 8 days and constant volume of air measuring 5 litre per minute enters through the orifice. The spore number trapped in the sampler was expressed as number of spores per cubic meter of air. For estimating the spore types, their concentration and percentage contribution, slides were scanned and were calculated for specific count by multiplying the actual number of spores encountered with the conversion factor of the sampler which is 14. Scanning of slides was done under binocular microscope, the identification was based mainly on microscopic characters, comparative spore morphology and spore description. Spore types were identified upto generic level with the help of relevant literature (Barnett and Hunter, 1972 and Tilak, 1989).

RESULTS AND DISCUSSION

During the period of present investigation the group deuteromycotina contributed a total of 42 spore types. *Cladosporium* contributed 29.49% to the total air spora from the group followed by *Epicoccum* (9.27%), *Nigrospora* (8.33%), *Alternaria* (2.30%), *Curvularia* (1.53%) (Table 1). The high incidence of

Table 1. Percentage contribution of each spore type of deuteromycetes to the total airspora.

Sr. No.	Spore type	Percentage contribution
1	*Cladosporium*	29.49
2	*Epicoccum*	9.27
3	*Nigrospora*	8.33
4	*Alternaria*	2.30
5	*Botrytis*	2.18
6	*Botrydiplodia*	2.00
7	*Papularia*	2.00
8	*Zygosporia*	1.95
9	*Ceratophorum*	1.77
10	*Claviceps*	1.71
11	*Tetraploa*	1.59
12	*Curvularia*	1.53
13	*Nodulosphaeria*	1.53
14	*Phacorchoconis*	1.53
15	*Geotrichum*	1.47
16	*Gleosporium*	1.47
17	*Arthrnium*	1.41
18	*Harknessia*	1.41
19	*Monodictys*	1.41
20	*Phaeothrichoconis*	1.41
21	*Fusarium*	1.36
22	*Myrothicium*	1.36
23	*Periconia*	1.35
24	*Beltraniella*	1.30
25	*Colletotrichum*	1.30
26	*Excipularia*	1.30
27	*Dreschslera*	1.24
28	*Cheatomella*	1.18
29	*Helminthosporium*	1.18
30	*Oidium*	1.18
31	*Cercospora*	1.12
32	*Diplodia*	1.12
33	*Haplosporella*	1.12
34	*Dictyoarthrium*	1.06
35	*Cordana*	1.00
36	*Graphium*	1.00
37	*Spegazzinium*	1.00
38	*Sirodesmium*	1.00
39	*Corynespora*	0.88
40	*Pestalotiopsis*	0.88
41	*Deightoniella*	0.82
42	*Pestalotia*	0.59

Cladosporium, Epicoccum, Nigrospora, Botrytis, Alternaria, etc. in the air was simply because of their saprobic as well as parasitic habit, their high degree of vegetative reproduction by fragmentation, budding, etc., asexual reproduction by developing conidia and also having capacity of high fruiting with passive spore liberation. The potential harm of fungal aerosol is mainly decided by the concentration and distribution of the pathogenic fungi.

The spore types of *Alternaria, Cercospora, Helminthosporium, Curvularia, Colletotrichum Dreschleria* are pathogenic to pomegranate crop. The highest concentration of *Curvularia* spores was recorded on 8th October 2005 when there was a record of 26^{OC} mean temperature, 90% relative humidity and 27.7 km/hr wind velocity. These reports are in accordance with earlier reports of Pady (1957), Tilak and Bhalke (1979), Jogdand (1987) and Karne (2008). The spores of *Alternaria* with their maximum number was recorded on 9th November 2005 when there was record of 20^{OC} mean temperature, 76% relative humidity and 34.3 km/hr wind velocity. Calvo *et al.* (1981) reported the occurrence of *Alternaria* spores in atmosphere greatly affected by climatic conditions.

The spores of *Colletotrichum* and *Cercospora* with its maximum concentration were recorded on 5th October 2005 on which temperature was 26^{OC}, relative humidity was 76% and wind velocity was 26.6 km/hr. Aher (1993) reported similar observations over groundnut field at Ahmednagar.

Highest spore concentration of *Claviceps* was recorded on 7th October 2005 when there was record of 25^{OC} temperature, 66% relative humidity and 56.4 km/hr wind velocity. Spores of *Nigrospora* showed their highest concentration on 20th October 2005 when there was record of 23.5^{OC} temperature, 82% relative humidity and 19.7 km/hr wind velocity. Similar reports have been made by Karne (2013), Murdhankar and Pande (1991) and Aher *et al.* (2004) over various crop fields.

Although weather parameters were favourable for the initiation of the leaf spot disease, the crop variety grown on the field "Mrudula" was found to be highly resistant for disease incidence and therefore the crop was found quite healthy. This collected information may provide basic data which is very useful for the disease forecasting and also will bring forth many useful and meaningful results for implementing cheaper and better preventive measures of crop plant disease management.

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RESEARCH ARTICLE

Antimicrobial activity of Rhizospheric Bacteria of *Azadirachta indica* Producing Metabolites against Human Bacterial Pathogens

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ABSTRACT

Medicinal plants are widely used all over the world for natural medicines. *Azadirachta indica* are known as "mother medicine of nature"; they have chemical compounds for curing and preventing diseases. These plants have valuable antimicrobial resources and can produce a large number of metabolites which having antibacterial properties, regulating their own growth and development to encourage other organism beneficial to them and suppress organisms that are harmful. Soil microorganism provides an excellent resource for isolation and identification of therapeutically important products; Antimicrobial metabolites were produced by different bacteria present in soil. In present study 21 rhizospheric soil samples of *Azadirachta indica* were collected from western Vidharbh region of Maharashtra state and were analyzed for presence of bacteria which can produce metabolites, isolation of desired bacteria were carried out by serial dilution method, Total 27 bacteria have been isolated from rhizospheric soil samples and out of 27 only 3 were potent isolates whose have been characterized on the basis of antibiogram test that revealed the activity of isolates, further characterization was done by following the Bergey's Manual of Systematic Bacteriology. Accordingly *Azadirachta indica* rhizospheric characterized isolates were *Sporosarcina*, *Micrococcus luteus* and *Staphylococcus epidermis*. These potent isolates could be further exploited for the production of metabolites in production media.

Keywords: *Azadirachta indica*, Medicinal plants, Metabolites, Rhizospheric.

INTRODUCTION

Medicinal plants are part of human society to combat disease, from the down of civilization. *Azadirachta indica* is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. *A. indica* and *M. azedarach* are two closely related species of *Meliaceae*. The former is popularly known as Indian neem, and the latter as the Persian lilac. Neem is an evergreen tree cultivated in various part of the Indian

subcontinent. A very part of the tree has been used as traditional medicinal for house hold remedy against various human ailments (Biswas *et al.,* 2002).

India has one of the richest medical cultures in the world. Indian literature incorporated in remarkably broad definition of medicinal plant and considers all plants are potential sources of medicinal substance. The plants containing medicinal substance which substances which can be use as antifungal, antibacterial, anticancerous etc. are term as medicinal plant. The world health organization (WHO) has listed 21000 plants which are used for medicinal purposes around the world. Among this 2500 species are in India, out of which 150 species are use commercially on a fairly, large scale, India is the largest producer of medicinal herbs and is called as botanical garden of the world. Plants are primary source of medicine, among the plants known for their medicinal values (Yadav *et al*., 2013)

Medicinal plants are considered to be very rich sources of metabolites. Microorganisms live in a world of chemical signals; they use small molecular weights compounds known metabolites, to regulate their own growth and development, to encourage other organisms that are helpful. Microbial metabolites are exquisitely selective; others are broadly active against many species. Organisms resistant to the effects of metabolites thrives microbes use metabolites to regulate the environment in which they live and form this platform they control the function.

The plant chemicals are classified as primary and secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are offer concentration in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolites. Primary metabolites obtain from higher plants for commercial use is high volume-low value bulk chemical (e.g. vegetable oil, fatty acid, carbohydrates etc) (Wink *et al.,* 2005).

Secondary metabolites are classically organic compounds produce from micro-organism during the alteration of primary metabolites synthesis. Secondary metabolites have a role in the growth and development of microbes and are usually form in the stationary phase. Many among secondary metabolites have ecological function; which include defense mechanism also function as antimicrobial agents or antibiotics and by producing various pigments. Antibiotics are one of the most important and wide employed secondary metabolites produce by bacteria. The soil microbes are a major source of antibiotics various bacterial strains are selected for antibiotics production as its isolation, maintenance and strain improvements is easy (Pande and Malviya 2014).

Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been broadly used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally multifaceted. More than 140 compounds have been isolated from diverse parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used conventionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been verified to exhibit immunomodulatory, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. (Biswas *et al*., 2002). The presence study is carried out by the antibacterial activity of rhizospheric bacteria of *Azadirachta indica* producing metabolites against human bacterial pathogens.

MATERIALS AND METHODS

Sample Collection*:* Soil samples were collected from rhizospheric region of *Azadirachta indica* plant located in different region in Akola city western Vidharbh region of Maharashtra. Total 21 rhizospheric soil samples were collected in the sterilized polythene bag containing soil sample were transfer immediately to laboratory.

Isolation of Bacteria: The collected Rhizospheric soil samples of *Azadirachta indica* were weight 1 gm aseptically and immediately transfer to 9 ml saline suspension that is called as Stock culture. After the rhizospheric soil was added to prepare stock solution further Serial dilution method was performed to get reduce number of bacteria. Dilution was made up to 10^{-8} to reduce the load of bacteria for better isolation of colonies. After inoculating and incubation period different colonies were observed on Nutrient agar plates and Selective medium plates. Colony characteristics were observed and noted. Single colony

was streak on nutrient agar slant for the isolation of pure culture.

Isolation of crude extracts producing antimicrobial substances: For the isolation of antimicrobial crude extract the test bacterial sample was inoculated in nutrient broth fermentation medium & incubated at 37^0c for 48 hrs. Generally the antimicrobial substances produced by bacteria in their maximum stationary phage so after incubation period. The fermented broth was then treated to separate the biomass from broth. The broth was then centrifuged at 5000 rpm on for 15 minutes and then subjected to extraction with ethyl acetate by solvent extraction procedure equal volume of ethyl acetate was added to the filtrate and mixed well by vigorous shaking for 10 minutes. Tubes were allowed to settle for 5 minutes till two clear immiscible layers are formed. The upper layer containing the extracted compounds was separated and collected in another tube. This filtrate extract was evaporated to dryness in hot air oven. The extract residue was dissolved in dimethyl sulfoxide (DMSO) and stored at 4^oc to be used as stock solution for antimicrobial assay.

Antibiogram test: Microorganisms are found in their natural habitat and are in constant exposure of undesirable chemicals, which may have antimicrobial activity against various microbes other than itself. To check the resistivity or sensitivity of a microbe against the various pathogens antibiotic sensitivity test is used to perform. This test is also termed as Antibiogram test. Nutrient agar plates were prepared. 20 µl of selected test pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*) were spread on to the solidified nutrient agar plates. Three wells were made at appropriate distance onto the agar plate with the help of gel puncture and filled using different concentration like 25 µl, 50µl, and 75 µl of the bacterial isolates' broth extracts obtained from different strains. Petri plates were incubated at 37ºC for overnight (Malviya and Pandey 2014). Then the diameter of the zone of inhibition was measured in mm and noted. The antimicrobial activity was determined by measuring the clear zone around the wells.

Identification: Different techniques and tests were performed such as Simple staining, Gram staining, Endospore staining, Motility, Acid fast staining, Biochemical Test, Sugar Fermentation Glucose, Lactose, Mannitol, IMViC test, Indole test, Methyl Red test, Vogas proskauer test, Citrate utilization test, Enzymes test Catalase, Oxidase, Urease, NO3 Reduction, H_2S Production, Starch hydrolysis, etc. for the identification of potent isolates (Pandey and Singh, 2013).

RESULTS AND DISCUSSION

Isolation, Purification and characterization of rhizospheric soil sample of *Curcuma longa.*

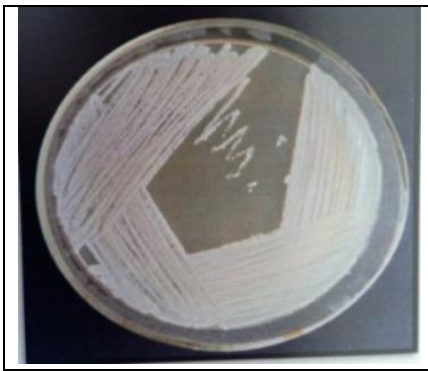
Soil samples of the *Azadirachta indica* rhizosphere regions were collected from the different region in Akola City, Western Vidharbh region of Maharashtra. The bacterial culture from the soil samples were collected by the serial dilution and spread plate technique. The total 27 culture have been isolated from the soil samples and out of total 27 only 3 have been characterized which are potent isolates.

Rhizosphere microorganisms increase root exudation through production of plant hormones or more directly by physically damaging the roots (Grayston *et al*. 1996). In general, the nutrient-rich rhizosphere is naturally colonized by many beneficial or pathogenic bacteria which may have a considerable impact on plant growth, development and productivity. The numerous interactions between bacteria and roots may have beneficial, harmful or neutral effects on the plant, the outcome being dependent on the type of symbiotic interaction and the soil conditions.

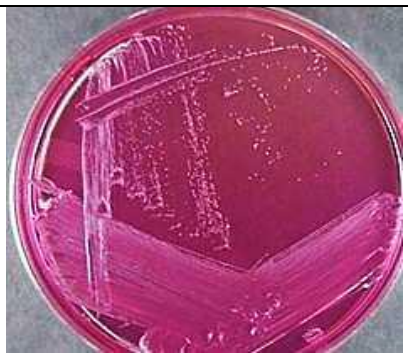
In the present study, medicinal plant *Azadirachta indica* has been selected, the rhizospheric region have been targeted to take the soil sample. The rhizosphere is the region adjacent to the plant root. The root exudates and the secondary metabolites secreted by the micro flora of the soil may affect each other and also to the plant health. There are total 27 cultures were isolated from these soil samples in which only 3 cultures have been screened. These 3 isolates are active against the selected pathogens, *E. coli*, *P. aeruginosa* and *S.aureus.* The characterized 3 cultures were *Sporosarcina, Micrococcus luteus and Staphylococcus epidermis*. To characterize these cultures, Bergey's manual has been followed. According to this, gram's staining, Catalase test, Endospore test, acid fast staining, glucose fermentation test, Mannitol fermentation test, lactose fermentation test, citrate utilization test, Oxidase test, glucose oxidation test, and nitrate reduction test have been performed. The analysis of antibiogram of the

entire characterized isolates has been observed before identification against selected pathogens. This has shown the drastic change in the activity of the isolates. The potent isolates were found able to produce metabolites on the basis of their specificity and hence the metabolites have shown the many fold increment in the activity of the isolates. Further, the isolates have been tested for the activity to inhibit the growth of the selected human pathogens by antibiogram test (Malviya and Pandey, 2014).

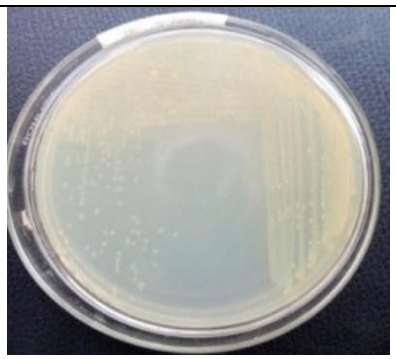
There are two potent isolates have been found, which has shown the best activity against selected pathogens. Those isolates are N2 (*Micrococcus luteus*) has shown zone of the inhibition 16 mm against *E. coli* and N3 (*Staphylococcus epidermis*) has shown zone of the inhibition 16 mm against *S.aureus*. The N1 (*Sporosarcina*) is the much more potent culture in comparison to other one. This culture has shown the best result against *S. aureus* (16.5 mm) in contrast to other pathogens. Between both the potent isolates, the N1 culture has the maximum activity against all the selected pathogens. This has been observed by comparing all the isolates activity of *Azadirachta indica* rhizospheric soil sample.



(Fig 1.1). Tryptic Soya Agar



(Fig 1.2). Mannitol Salt Agar



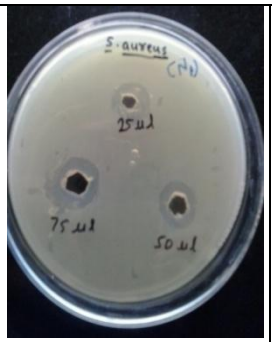
(Fig 1.3). Nutrient Agar

Fig 1: Showing results of isolated colonies on different selective media

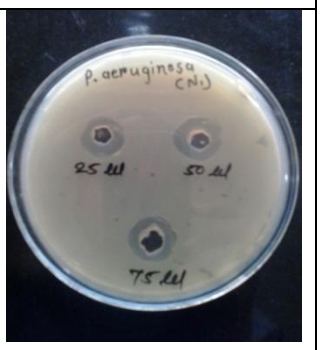
(Fig 4). Isolated pure culture in form of slants

Results showing zone of inhibition against pathogenic bacteria

(Fig 5). Against *E.coli*



(Fig 6). Against *S.aureus*



(Fig 7). Against *P.aeruginosa*

Table: 1: Antimicrobial activity of N1, N2, and N3 at different concentration

Test bacterial strains	Concentration /Diameter (µl/mm)								
	N1			N2			N3		
	25µl	50µl	75µl	25µl	50µl	75µl	25µl	50µl	75µl
E.coli	12	15	16	8	11	14	9	13	14
S.aureus	13	14	16.5	10	13	13	10	12	15
P.aeruginosa	12	13	16	11	12	13	11	12	14

❖ Abbreviations : N1- Sample No.1 | N2- Sample No.2 | N3- Sample No.3

Table 2: Morphological & Biochemical Characteristics

Sample	Gram Character	Motility	Endospore	Acid Fast	Sugar fermentation			IMVIC				Enzymes			No3 Reduction	H_2S prouction	Starch hydrolisis
					Glucose	Lactose	Mannitol	Indol	MR	VP	Citrate	Catalase	Oxidase	Urease			
N1	+	+	+	+	+	+	–	–	–	–	+	+	–	+	–	–	–
N2	+	–	–	NA	–	–	–	–	+	+	+	+	+	+	–	–	–
N3	+	–	–	NA	+	+	–	–	+	+	–	+	–	+	+	–	–

Where, +: Positive, – : Negative, MR: Methyl red, N1;N2 and N3: Sample Numbers
VP: Voges Proskaur, NA: Not Applicable

On the basis of cultural, Morphological and Biochemical characteristics.

The potent isolates were identified by using Bergey's manual of systematic Bacteriology.

N1: *Sporosarcina*, N2*: Micrococcus luteus* and **N3*: Staphylococcus epidermis***

CONCLUSION

The present study was an attempt to identify and pick out the versatile bacterial strains that display antimicrobial activity against variety of microbial pathogens intrinsically. Total 27cultures were isolated from rhizospheric region of *Azadirachta indica* out of 3 were potent isolates characterized as *Sporosarcina, Micrococcus luteus and Staphylococcus epidermis*.

The Rhizospheric bacterial crude extract of *Sporosarcina, Micrococcus luteus and Staphylococcus epidermis* were found to be more or less active against almost all tested pathogenic strains. Hence *Azadirachta indica* can be employed as source of natural antimicrobials that can serve as an alternative to conventional medicines. It was concluded that the best activity have been shown by the *Azadirachta indica* rhizospheric isolates (N1) which is of *Sporosarcina* against all three human pathogenic organisms *(E.coli , S.aureus , P.aeruginosa*). The activity of rhizospheric isolates was showing best results against *S. aureus*

The result of this study strongly supports that the bacterial isolates produces metabolites and may be used in the management of microbial infection and the present findings highlights the important for further investigation towards the goal of obtaining novel antimicrobial agent.

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RESEARCH ARTICLE

The antimicrobial activity in the crude honey samples from the Chalisgaon region

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ABSTRACT

The present research work was carried out to study the antimicrobial activity of honey sample collected from chalisgaon region during 2012 to 2015. During study, four crude honey samples were applied to evaluate the antifungal and antibacterial study on the basis of zone of inhibition. It was concluded that *Escherechia coli* and *Bacillus subtillis* are more susceptible than other experimental bacteria. In the antifungal activity, the *Aspergillus terrens* had shown more susceptibility than other experimented fungi.

Keywords: Crude honey, Antibacterial activity, antifungal activity, Zone of inhibition.

INTRODUCTION

Honey is the natural sweet substance produced by honey bees from nectar or blossoms from the secretion of living parts of plants. It is most primitive and nourishing agent. It is unique mixture of invert sugar (62-83%) sucrose (0-8 %), dextrin (0.8-7%), vitamin together with water and trace of other nutrients (Somai *et al.*, 1994; Mandal and Mandal, 2011).

In the medical field, antimicrobial agents are responsible to minimize the various microbial diseases. However, day by day resistance power reduced the effect of antibiotics and shows very serious problem to the public health (Mandal *et al*., 2010). For this reason, it is a need to developed new antimicrobial tactics and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey (Basualdo *et al.*, 2007; Mandal *et al*., 2009,2010; Mandal and Mandal, 2011). Recently, many researchers have reported the antimicrobial activity of honey and found that natural crude honey has some broad-spectrum antimicrobial activity when tested against pathogenic bacteria, fungi and food spoilage microorganisms (Basualdo *et al.,* 2007; Levy and Marshall, 2004; Lusby *et al*., 2005; Mundo *et al*., 2004).

The antimicrobial activity is variable among the different honey depending on its geographical, seasonal and botanical source as well as through harvesting, processing and storage conditions. According to Mavric *et al.* (2008), the antimicrobial activity of honey is recognized largely by osmolality, pH, Hydrogen peroxide production and the presence of other photochemical components eg. methylglyoxal.

As per the importance of honey in medical and pharmaceutical field it is necessary to evaluate the various components which show the antimicrobial activity in the honey samples. Here we report initially the present status of the antimicrobial activity against the crude honey samples of *Apisdorsata* originating from nearby area of Chalisgaon region.

MATERIALS AND METHODS

Honey sample: Natural crude honey samples were collected from the tribes from five different sites (S1, S2, S3, S4 and S5) of Chalisgaon region. They were stored in tightly closed glass bottles wrapped in aluminum foil and kept at room temperature. Each honey solution was prepared just before use to insure that there was no loss of hydrogen peroxide. Sample of 10 gm. of honey was added to 10 ml distilled water and mixed to achieve 50 % (w/V) solution.

Determination of antimicrobial activity*:* For this study four bacterial strains such as *Escherichia coli, Pseudomonas auriginosa, Lactobacillus sporogense* and *Staphylococcus aurius* and three fungal strains *Aspergillus fumigense, Aspergillus terrens, Penicilium chrysogenum* were used. The antimicrobial activity of honey sample was evaluated by agar diffusion method. 100µ of diluted bacterial suspension were spread onto the surface of plate L.B. agar medium. Wells (0.6mm in diameter) were cut from agar with a sterile cork borer. Then 100µ of honey solution were added to each well. Water was used as negative control in all experiments. All the Plates were incubated at 37^0C for 24 hrs. Then antimicrobial activity was evaluated by measuring the diameter of the clear inhibition zone expressed in millimeters (mm) around each tested substance.

RESULTS

The antimicrobial effect of honey has been reported earlier by a number of workers (Sheikh *et al*, 1995; Basualdo *et al*, 2007; Temaru *et al.*, 2007; Mandal *et al*., 2009, 2010; Chauhan *et al*., 2010). The present study was carried out to determine the antimicrobial effects of honey around the Chalisgaon region. Around five selected sites, four bacterial strains were tested with 50 % (w/V) honey solution; this killed the inocula of all bacteria at specific level which shows the antibacterial effect against the selected honey sample (Table1). The zone of inhibition of selected bacteria showed variation in five different experiments when treated with the honey sample (fig. 1). *E. coli* and *B. subtillis* showed effect in the form of zone of inhibition is 14.9 and 14.8 mm respectively while in *L. sporogens* and *P. auriginosa* it vary as 10.4 and 9.4 respectively. These observations also supported by earlier workers (Cavanagh *et al.,* 1968; Sheikh *et al*, 1995; Levy and Marshall, 2004; Basualdo *et al.,* 2007; Chauhan *et al.*, 2010; Ghanem, 2011).

Fungi when tested with honey diluted to 50%, which showed since this killed the entire inocula of all fungi at specific level which shows the antibacterial effect against the selected honey sample. (Table: 2). The zone of inhibition of selected fungi showed variation in every experiment when treated with the honey sample. *A. terrens* form 7.2 mm zone of inhibition while in *A. fumigense* forms 4.8 mm, it shows that *A. terrens* is more susceptible than other two.

Table 1: Antibacterial activity of honey

Sr. No.	Microorganism	Zone of inhibition(mm)					Mean
		S1	S2	S3	S4	S5	
1	*Bacillus subtillis*	16	14	19	12	12	14.6
2	*Escherechia coli*	18	13	15	15	13	14.8
3	*Lactobacillus sporogens*	12	12	08	10	10	10.4
4	*Pseudomonas auriginosa*	17	08	06	06	10	9.4

Table 2: Antifungal activity of honey

Sr. No.	Microorganism	Zone of inhibition(mm)					Mean
		S1	S2	S3	S4	S5	
1	*Aspergillusfumigense*	07	04	05	03	05	4.8
2	*Aspergillusterrens*	08	08	07	07	06	7.2
3	*Peniciliumchrysogenum*	-	-	-	-	-	--

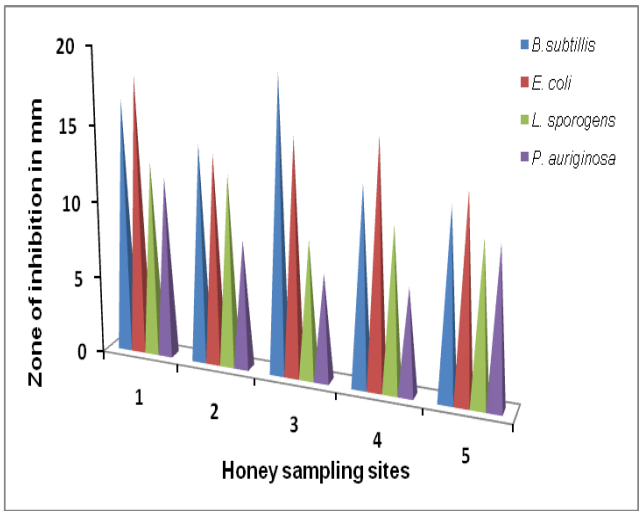

Fig 1: showing antibacterial activity of honey

These results also supported by many workers (Haffeejee and Moosa, 1985; Jeddar *et al.,*1985; Kim *et al.,*1995;Sheikh *et al*, 1995; Hasanain, 1997; Levy and Marshall, 2004; Chauhan*et al.,*2010;Ghanem, 2011).

CONCLUSION

In antibacterial activity, it is concluded that *E.coli* and *B.subtillis* are more susceptible than *L. sporogens*and *P. auriginosa* while in antifungal activity, *A.terrens* is more susceptible than *A.fumigense*. The fungus, *P.chrysogenum* shows no any inhibitory activity against the honey samples. This preliminary research may be informative for further research especially in nutritional supplements and cosmetics as well as for pharmaceutical and medical use.

At present a number of honeys are available in the market with standardized levels of antibacterial activity. It may be noted that crude honey possesses excellent antibacterial activity comparable to the commercial honeys. Therefore it is necessary to study other locally produced crude honeys for their antimicrobial activities.

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RESEARCH ARTICLE

Cytological changes in the brain of fourth and fifth instar of worker honey bee *Apis cerana indica* during post-embryonic development

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ABSTRACT

During the post-embryonic development of Indian honey bee, *Apis cerana indica*, brain undergoes enormous change in its anatomical organization. Brain of fourth and fifth instar larva consist of MNC, LNC, PNC and VNC group of neurosecretory cells. Cell types B, C1, C2 are found in LNC, PNC, and VNC while in MNC all cell types A, B, C1, C2 are found. Variation was observed in distribution and size of cell cytoplasm in each hemisphere of brain of fourth and fifth instar larva.

Keywords: brain, Honeybee, Neurosecretory cell, Post-embryonic development,

INTRODUCTION

Indian Honeybee, *Apis cerana indica* can be easily kept in hive and are domesticated for commercial production of honey, other products and as a good pollinator due to its non-aggressive behavior and rarely exhibiting swarming behavior. Under well condition, it built 5-7 vertical and parallel comb in hallow of tree trunk rock crevices under shadow. Single colony per annum on an average may yield 5-6 Kg honey. Most of the research work on Indian honeybee is confined to its distribution, behavior, morphology and social life, comb formation while no significant contribution has been made towards histomorphology of brain, physiology of neurosecretory cell and hormonal activity during post-embryonic development. Snodgross (1956) described the anatomical organization of central and sympathetic nervous system in hymenoptera. Wayer (1987) observed some neurosecretory cell inthe adult brain of worker, drone and queen honeybee, *Apis mellifera* first time. Itis now well established from research of certain worker that the central nervous system play an most important role during post-embryonic development, caste differentiation and reproduc- tion in insect (Raabe, 1982; Mishra and Dogra, 1983; Farris *et al*., 1999; Tembhare and Barsagde, 2000; Norbert and Karl, 2005).

MATERIAL AND METHOD

The fourth and fifth instar larva of *Apis cerana indica* were collected in saline solution during the month of October to April 2006-07 from the well maintained honey bee culture at the department of zoology, RTM Nagpur university campus, Nagpur. The cephalic neuroendocrine organ was dissected out from the larva of*Apis cerana indica* under stereoscopic binocular microscope in insect saline solution. Tissue was fixed in aqouse Bouins fixative about 16-24hrs duration for histological studies. Thereafter tissue were dehydrated in alcohol grade, cleared in xylene and embedded in paraffin wax (58-60 0C). Serial section were cut at 4-5-micron thickness and stained with Chrome Alum Haematoxylin-Phloxine (CAHP) and Cameron and Steel's Adehyde Fuschn-Halmi's mixtue (AF).

RESULT AND DISCUSSION

Present study has been undertaken to provide cytology, distribution and classification of cerebral neurosecretory cell and transport of neurosecretory material with their neurosecre- tory pathway in worker honeybee *Apis cerana indica* during post-embryonic development. Most of the earlier worker describe structure and function of cephalic neuroendocrine system in the honeybee *Apis mellifera* and hormonal regulation of polymorphism (Hannan, 1955; Snodgrass,1956; Canetti, *et al.* 1964; Thomsen,1965; Dogra *et al*.,1977; Laere,1970; Breed,1983; Ritcey and Dixon, 1996a; Farris *et al*.,1999; Wheeler *et al*.,2006).

Brain of *Apis cerana indica*, undergoes enormous change in its anatomical organization during the post-embryonic development. Variation was observed in distribution and size of cell cytoplasm in each hemisphere of brain of fourth and fifth instar larvae. All the four type of neurosecretory cells are observed in brain of fourth and fifth instar larva consist of MNC, LNC, PNC and VNC group. In honeybee, some worker described the presence of single pair of MNC groups in the pars cerebralis region of brain (Weyer, 1935; Scaller, 1937; Laere, 1970; Mishra and Dongra, 1983).

In MNC of fourth instar larva A, B, C1, C2, are measuring about 9.06±0.16, 6.78±0.05, 16.58±0.30, and 11.52 ±0.04 µm in diameter respectively. Ritcey and Dixon (1996a) reported three group of neurosecretory cell, MNC, LNC in protocerebrum and VNC group in tritocerebrum in the brain of *Apis mellifea.* Tembhare and Paliwal (1993) described the six paired groups viz medial, lateral, posterior, deutocerebral, ventral and optic groups of NSC in the brain of done and queen of *Apis dorsata*. The present study demonstrate the presence of paired group of MNC, LNC, PNC, in protocerebrum and VNC in tritocerebrum of the 4th and 5th instar larva of *Apis cerana indica.*

The B, C1, and C2 cell type LNC measure about 6.10±0.24, 16.40±0.48, 10.08±0.32 µm in diameter. In PNC B, C1, C2 cell type measuring about 6.78±0.50, 16.40±0.45, and 10.58±0.52 µm in diameter respectively. VNC group cell type B, C1, C2 are measuring about 6.78 ±0.46, 16.58±0.37, 10.58±0.98 µm in diameter respectively. The brain of fifth instar larva is slightly larger than fourth instar larva. A,B,C1,C2 cell types of MNC group measuring about 12.30±0.44, 8.25±0.38, 20.31±0.06, 12.98±0.32 µm in

Table 1: Distribution and size of cell cytoplasm of cerebral neurosecretory cells of brain in 4th *and* 5th instar of worker honeybee *Apis cerana indica*

NS Cells	4th instar				5th instar			
	Cell type				Cell type			
	A	B	C1	C2	A	B	C1	C2
MNC	9.06 ± 0.16	6.78 ± 0.05	16.58 ±0.30	11.52 ±0.04	12.30 ±0.44	8.25 ±0.38	20.31 ±0.06	12.98 ±0.32
LNC		6.10 ±0.24	16.40 ± 0.48	10.08 ± 0.32		6.06 ±0.07	42 ±0.50	12.08 ±0.32
PNC		6.78 ± 0.50	16.40 ±0.45	10.58 ±0.52		9.08 ±0.56	20.05 ±0.62	12.05 ±0.64
VNC		6.78 ±0.46	16.58 ±0.37	10.58 ±0.98		9.08 ±0.58	16.40 ±0.28	12.04 ±0.28

Abbr : **A,B,C1,C2**- Neurosecretory cell types. **LNC-** Lateral neurosecretory cell, **MNC**- Mediam neurosecretory cell, **PNC**-Posterior neurosecretory cell, **VNC-**Ventral neurosecretory cell.

diameter respectively. In LNC B, C1, C2 cell measuring about 6.06±0.07, 16.42±0.50, 12.08±0.32 µm in diameter respectively. In PNC B, C1, C2 cell type measuring about respectively 9.08±0.56, 20.05±0.62, and 12.05±0.64 µm in diameter. VNC group of neurosecretory cell consist of B, C1 C2 cell type measuring about 9.08±0.58, 16.40±0.28, 12.04±0.28 µm in diameter respectively (Table 1).

In hymenoptera Thomsen (1954a) and Nayar (1955) have classified the cerebral neurosecre- tory cell into A and B type and suggested that A cell represent the active while B cell represent inactive during secretory cycle. The cerebral NSC in the brain of *Apis* where however classified as the large, small and intermediate cells mostly on the basis of their staining affinities and other characteristic (Ritcey and Dixon, 1996a). Breed (1983) categorized NSC simply on the basis of position in the brain: medial, lateral I and lateral II. In the present study, vertebral neurosecretory cells has been classified into A,B,C1,C2 on the basis of their staining affinity to the various selective stains and some variation in their cytomorphological feature. Median neurosecre- tory pathway joined with lateral neurosecretory pathway, posterior neurosecretory pathway and ventral neurosecretory pathway in the tritocerebral part of brain and emerge out as nervi corpori cardiaci.

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RESEARCH ARTICLE

Contaf (a systemic fungicide) induced histopathological changes in the target organs of Freshwater Teleost Fish, *Barbus carnaticus*

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ABSTRACT

The study deals with the effect of Contaf, (a systemic fungicide) on histological studies in *Barbus carnaticus*. LC 50 concentration of Contaf for *Barbus carnaticus* was found to be 5.9439 ppm for 24 h, 5.2142ppm for 48 h, 5.2469ppm for 72h and 5.2516 ppm for 96 h. and 1/10th of the corresponding LC50 values were treated as sublethal concentrations. The fish showed severe histological changes like severe necrosis i.e. local death of cells. Swelling, hypertrophy of cells, as the period of exposure go on increasing.

Keywords: *Barbus carnaticus*, Contaf, Histology.

INTRODUCTION

Pesticides are being used to control the population of the pests and to increase the crop yield. Indiscriminate use of pesticides in farm industry producing harmful effect on fish and other non target organisms of environment. Toxicants are known to changing the tissue organization, impair the normal structure and function of the body by inducing histopathological lesions. Many workers have reported toxicity induced by insecticides on various organs of fish (Ishihara and Tamura 1967; Mukherje and Bhattacharya, 1975; Dubale and Shah 1979; Dubale and Awasthi, 1982) Liver, kidney, ovary and testis are the worst organs affected by pesticides.

The kidney as an organ for the excretion of waste materials from the body itself is exposed to harmful effects of the foreign materials including insecticides Liver is the main metabolic centre where detoxification and drug metabolism take place which makes it greatly vulnerable to changes by toxic substances. Since liver is the centre of metabolic activities and all toxic compounds are likely to be metabolized in this organ.

The various hepatic enzymes are prone to toxic effects and can be used as indicators of sublethal exposure of fish to toxic metabolites. Liver is the largest gland of the body having several functions, it has no direct contact with the environmental pollutants dissolved in water but due to contact with

blood it is indirectly affected. Fish exposed to sublethal concentrations of Contaf for different exposure periods showed considerable degree of alternations in the histology of gonads. The seminiferous tubules are normally of varying shapes and sizes. Each tubule has a definite thin fibrous wall which is not distinguishable after spawning phase. The testis of *Barbus carnaticus* showed significant changes on exposure of spermatogonic cells as well as inflammation of cells, contraction and vacuolization of tubules. It also affected ovary by disrupting the ovary and developing oocytes at various stages and cause breeding strength of fish.

Since there is dearth of information on histopathological studies of fish induced by insecticides an attempt has been made here to assess the histopathological lesions in the kidney, liver, ovary and testis of fish *Barbus carnaticus* induced by Contaf.

MATERIALS AND METHODS

Barbus carnaticus (approx.. wt. 100 g.) were collected from the Girna Dam, constructed on Girna river in Dist: Nasik, Maharashtra and acclimated to the laboratory conditions for a period of 15 days in a large tank of 1000 liter, previously washed with potassium permanganate and water temperature was 26 ± 35 ^{0c} and pH 7.0 – 7.2maintained in aquarium.

Toxicity Assay :

Ten *Barbus carnaticus* were kept in a glass tank of dechlorinated tap water. The fish were treated with varying concentrations of Contaf. The $1/10^{th}$ of the LC50 values were taken as sublethal concentrations for the 24, 48, 72 and 96 hours, respectively. To observe the histopathological changes in target organs of *Barbus carnaticus* a group of ten individuals exposed to different sublethal concentrations of Contaf for 24, 48, 72 and 96 hours, respectively. All individuals in control were maintained in pesticide free dechlorinated water in the separate tank. After exposure and completion of treatment, *Barbus carnaticus* were dissected liver, kidney, ovary and testis were removed and fixed for routine microtechnique procedure. Sections were cut (8 µ) and stained with a haematoxyline and eosin.

RESULTS AND DISCUSSION:

Histology of fish liver under control is given in (Fig. 1). The normal liver showed external structure of hepatic cells and connective tissue. Histopathology of experimental fish showed appearance of small vacuoles, degenerations of hepatocytes and necrosis of cells, proliferations of ducted cells (Fig.2). Hepatic cells are scattered and showed large vacuoles. In many places, necrosis is observed in liver at 96 hours stage. All these results of liver are in agreement with those of Saxena *et al*; (1989). They said that Malathion is more toxic than Carbarylin inhibition and the *de novo* synthesis of lipid and protein in the liver of *Ophiocepphalus punctatus*.

Histology of fish kidney under control (Fig. 3) showed many nephrons and each nephron consists of two parts the glomerulus and urinary tubule, normal distinct glomerulus with proximal tubule, conducting tubule with sinus appeared in connective tissue, necrosis swelling in renal tubules etc. (Fig.4) As toxic products are eliminated through the kidney, kidney is suspetible to sublethal doses of Contaf. It might have caused degenerative changes in renal tubules and glomerulus i.e. necrosis in the proximal tubules and glomerulus of kidney. Degenerative changes in epithelial cells of collecting tubules of *Tilapia mossambica* exposed to Fenvalerate, has been reported by Radhaiah (1985). Shrinkage of glomerulus was reported in *Nemachelius denisoni* (Day) exposed to phosphomidan Rashatwar and Ilyas (1984). Similar results on fresh water teleosts were reported by Rao (2003) and Tilak *et al*. (2004). During the present investigation, changes were observed in the kidney of *Barbus carnaticus* exposed to different concentrations of Contaf. Histopathological changes were found in the glomerulus, renal tubules and haemopoitic tissue. A gradual increase in the damage was noticed and the severe histological lesions caused by physio- logical and biochemical disturbances in fish.

The ovary of normal *Barbus carnaticus* reveals that it is surrounded by an ovarian wall which is differentiated into an outer thin peritoneum a thicker tunica albuginea made up of connective tissue, muscle fibers and blood capillaris. The innermost layer is the germinal epithelium which joins with the tunica albuginea at several places and projects into the central lumen of the ovocoelin the form of finger like projections called *ovigerous lamellae* (Fig. 5). The histology of experimental fish ovary showed disrupted follicular epithelial cells. Nucleolus showed condensation of crescent shaped dark granules at one side. Degeneration of epithelial cells causes vacuolization, breakdown of germinal vesicles, many disrupted oogonia (Fig 6). Most of the workers have

shown that the fishes exposed to pesticides led to lowered steroid genesis Kapur *et al.,*1978. Stopage of development of advanced oocyte stages and thus reducing the number of viable oocytes (Saxena and Garg, 1978; Yasuno *et al*., 1980; Mani and Saxena, 1985). The increase in follicular atresia was obvious due to effect of pesticides on fish ovary. Both inhibited the growth of oocytes and raised incidences of follicular atresia were evident in ovary of *Channa orientalis* exposed to Nuvan Dimecron as have been observed in the case of certain fishes (Mani and Saxena 1985; Ghosh 1986; Singh and Sahai 1986, Khillare and Wagh 1987; Patwardhan and Gaikwad 1990; Dutta *et al.,* 1994). The histological abnormalities in ovaries may be due to factors like ionizing radiations, electric current, parasitic infections. Xenobiotic toxicants, Sarojini and Victor (1985) and by variety of effluents and aquatic pollutants (Shukla *et al*., 1984; Saxena and Garg,1978; Johnson *et al*., 1998; Mc Comic *et al.,* 1989 Kumar *et al.,* 2000.)

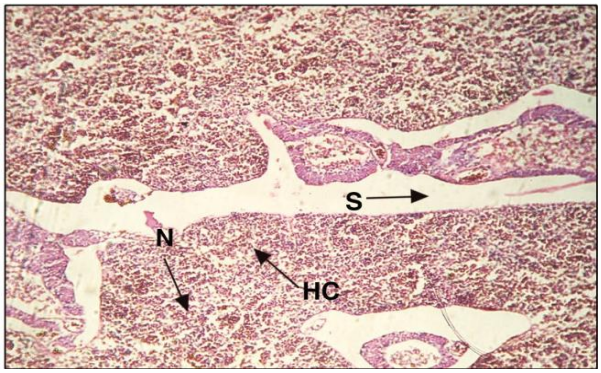

Fig 1: T.S. of liver from *B.carnaticus* (Control).Hc-Hepatocyte, N-Nucleus, NC-Nucleolus, S-Sinuside

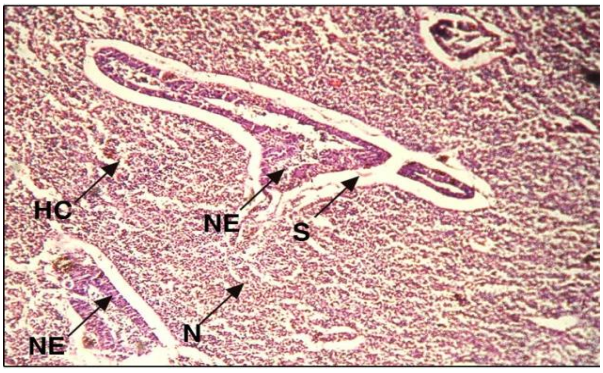

Fig 2: T.S. of liver from *B.carnaticus* (Exposed to Contaf for 96h). HC-hepatocyte,S-sinuside, N- nucleus

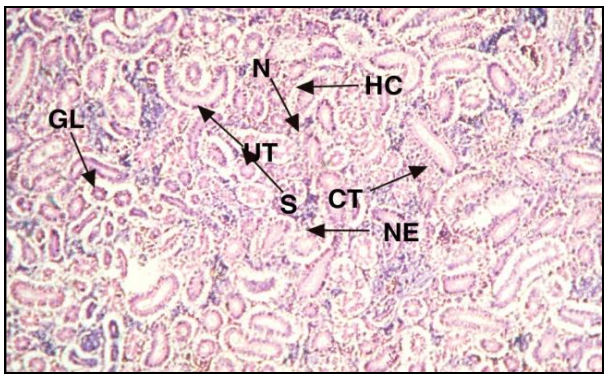

Fig 3: T.S. of kidney from *B.carnaticus* (Control). GL-Glomerulus, UT-Uriniferous Tubule, S-Sinus

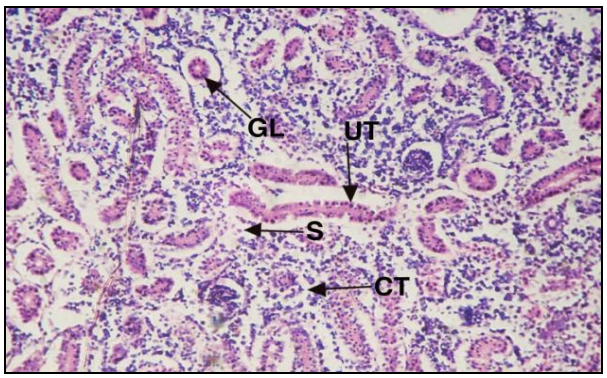

Fig 4: T.S. of kidney from *B.carnaticus* (Exposed to Contaf for 96h). GL-Glomerulus, UT-Uriniferous Tubule, S-Sinus.

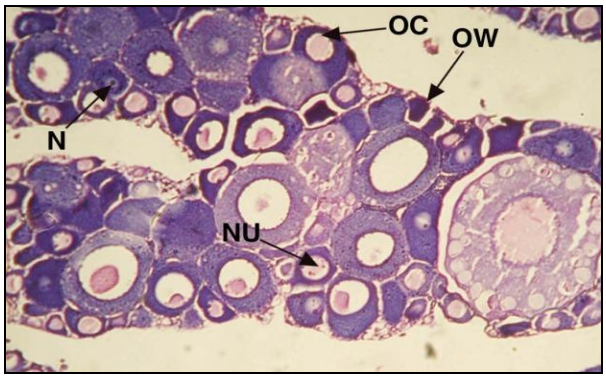

Fig 5 : T.S. of ovary from *B.carnaticus* (Control). OC-Oocyte, NU-Nucleolus,N-Nucleus, OW-Ovarian Wall.

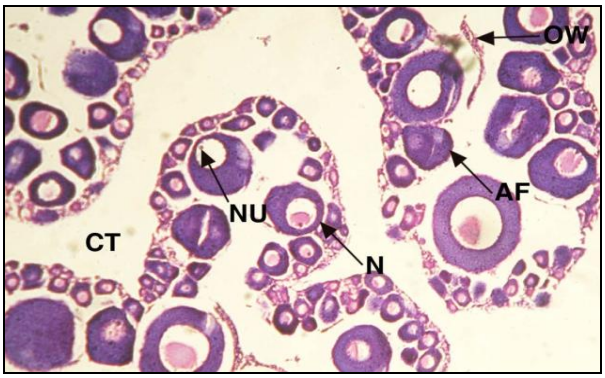

Fig 6: T.S. of ovary from *B.carnaticus* (Exposed to Contaf for 96 h). OC-Oocyte, NU-Nucleolus, N-Nucleus, OW-Ovarian Wall, AF-Atretic Follicle.

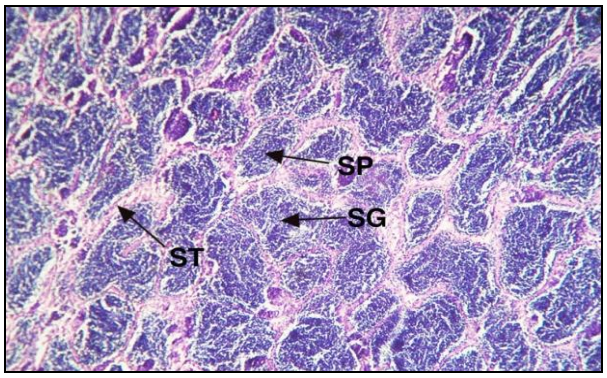

Fig 7: T.S.oftestis from *B.carnaticus* (control). SL-Seminiferous Lobule, SG- Spernatogonia, SP-Spermatids.

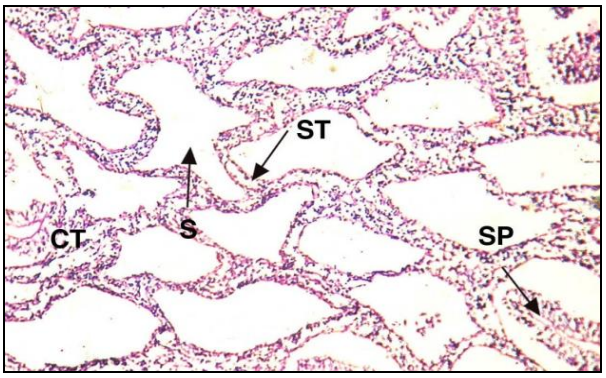

Fig 8-T.S.of testis from *B.carnaticus* (Exposed to Contaf for 96 h).SL-Seminiferous Lobule, SG-Spernatogonia, SP- Spermatids.

Almost all similar histopathological findings were reported by Hossain *et al.,* 2002 in the ovaries *Anabas testudineus.* The histological abnormalities in ovaries may be caused by several factors viz. ionizing radiations, electric current, parasitic infections, xenobiotic toxicants (Sarojini and Victor, 1985) and by a variety of effluents and aquatic pollutants (Shukla *et al.,* 1984; Saxena and Garg, 1978; Johnson *et al.,*1988; Mc Comic *et al*., 1989; Kumar *et al.,* 2000).

The histology of fish testis under control is given in (Fig.7). The normal testis showed healthy seminiferous tubules which is internally lined by tubular epithelium which gives rise to spermatocytes. Histopathology of experimented fish testis, showed disrupted seminiferous tubules and immature spermatogonia and general inflammatory response (Fig.8). Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants (Sokal*et al.,* 1985 Ruby *et al.,* 1986 1987). Exposure of Contaf is responsible for histopathological damage of fish testis and vacuolization of tubular cells and distortion of seminiferous tubules, enlarged interstitium and haemorhage in inter tubular area in albino rats exposed to pesticides have been reported. Dutta and Dikshith, 1973; Ghosh *et al.,*; 1979; Baronia and Sahai 1993; Katti and Sathyanesan (1985) observed exposure dependent on concentration mediated changes in testis of *C. batrachus* treated with lead.

Kinnberg *et al.* (2000) have also documented concentration dependant effects on nonylphenol on testicular structure of the fish *Xiphophorus maculates* and Zusthi (2005) observed that the effect of fenthion on testes of *Glassogobiousgiuris*. They observed reduction in size with spermatids and sperms in degenerating condition.

It is evident from results that Contaf is moderately toxic to *Barbus carnaticus*. The fish behaved normal in natural manner with coordinated movements. They were alert at the slightest disturbance but in the toxic environment and they showed irregular erratic and darting swimming movements and loss of equilibrium. The present investi- gation evidenced that Contaf is toxic and had profound effect on the behavior and histology of liver, kidney, ovary and testis of *Barbus carnaticus* in sublethal concentrations.

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RESEARCH ARTICLE

Protective effect of dietary supplementation of *Spirulina platensis* on improvement of growth parameters in mercuric chloride exposed fish, *Labeo rohita*

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ABSTRACT

Metal contamination in freshwater bodies is a matter of serious concern from the human health point of view since many aquatic organisms, particularly fish, form an integral part of the human diet. *Labeo rohita* is one of the important cultured carp species in Asia. Among the freshwater fishes, carps are most affected to environmental contamination. The reduction of toxic elements in aquatic systems and organisms by acceptable methods is a need of the hour. The effect of spirulina supplementation on reduction of mercuric chloride toxicity based on food utilization was studied in a freshwater fish, *Labeo rohita*. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. The results showed that sublethal exposure of *Labeo rohita* fed with *spirulina* free diet (T1 groups) significantly reduced the food utilization parameters than those exposed to sublethal level of mercuric chloride and fed *Spirulina* supplementation diet (T2 - T5 groups).

Keywords: Protective effect, *Spirulina platensis*, Growth parameters, Mercuric chloride, *Labeo rohita.*

INTRODUCTION

Public health concern over mercury exposure to human beings due to consumption of contaminated fish has been a topic of political and medical debate. Pathological effects due to inorganic mercury in certain fishes have been reported. (Sastry and Gupta 1978, Naidu *et al*., 1983). In the aquatic environment inorganic mercury is converted to methyl mercury and is the predominant form of mercury reported in fishes caught from contaminated waters.

Spirulina is one of the most concentrated natural sources of nutrients for all animals. *Spirulina* contains protein (60-70%), essential amino acids and fatty acids, phycocyanin (14%), chlorophyll (1%) and carotenoid pigments (0.37%), vitamin B-12, and minerals that play important roles in animals in various ways (Venkataraman 1993).

Spirulina improves the intestinal flora in fish by breaking down indigestible feed components (Ramakrishnan et al., 2008). It stimulates the production of enzymes that transport fats in fish for growth instead of storage (Henrikson, 1994). β-carotene in spirulina firmly maintains the mucous membrane and thereby prevents the entry of toxic elements into the body (Henrikson, 1994). Chlorophyll in *Spirulina* acts as a cleansing and detoxifying factor against toxic substances (Henrikson, 1994). Researchers have reported the therapeutic effects of *Spirulina* as a growth promoter, probiotic, and booster of the immune system in animals including fishes (Venkataraman, 1993). So far, *Spirulina* is known for its nutritive value only; its role in alleviating metal toxicity in fishes and other cultivable organisms remains unexplored. In the present study, experiments were designed to investigate the impact of dietary *Spirulina* supplementation on the growth, and alleviation of mercuric chloride in carp, *Labeo rohita*. In the present investigations concentrated on damage induced by inorganic mercury in the Indian major carp *Labeo rohita* and protective role done by *Spirulina platensis.*

MATERIALS AND METHODS

The live major carp, *Labeo rohita* were obtained from a Girna river dams near Chalisgaon city. They were acclimatised in laboratory condition for more than two weeks. The temperature, PH, Salinity and dissolved oxygen of the water were found to be 27 ± 1Ċ, 7.55 ± 0.1, 0.76 ± 0.09% and 7.20 ± 0.12 ml/l respectively. During the acclimatisation, water was changed daily and fish were fed ad libitim with pelletised diet containing 35% protein. Acclimatized fish (1.30 ± 0.10g) were exposed to different concentrations (0, 0.03, 0.06, 0.09., 0.12, 0.15, 0.18 ppm) of mercuric chloride $HgCl_2$ obtained from Merk India Ltd. (Mumbai, India) and mortality was observed for 96 h. A static bioassay method was adopted for the determination of 96 h median lethal concentration. Probit analysis was followed for the calculation of 96 hours LC_{50} Control group of fish was maintained in mercury free freshwater.

Feed: In the present experiment, 35% protein diet was used as basal diet for *Spirulina patensis* supplementation. The intergradient of dried fish meal, ground oil cake, cod liver oil, egg yolk, tapioca flour, vitamins and mineral mixtures were used to prepare the 35% protein diet, with appropriate proportion by square method. In addition to the control diet, five diets (0, 2, 4, 6, 10 %) were prepared with different *Spirulina patensis* levels. The experimental diets were by adding the appropriate level of *Spirulina patensis* with chosen intergradient to boiled water, mixed well and steam cooked for 15-20 min. After moderate cooling, pellets (2mm) were prepared with operated pelletizer and dried in sunlight. After drying diets were separated stored in refrigerator. Active and healthy fish (1.30 ± 0.10 g) were chosen from the acclimatisation tank and starved for 24 h. prior to the commencement of experiment. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. Triplicates were maintained for each group.

Group-I: served as control and reared in mercuric chloride free freshwater and fed with *Spirulina patensis* free diet. Test animals belonging to 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} groups were exposed to 0.12 ppm of mercuric chloride.

Group-II: Individuals was fed with *Spirulina patensis* free diet, however 3^{rd} , 4^{th} , 5^{th} and 6^{th} groups were fed with 2, 4, 6, and 10% *Spirulina patensis* diets respectively. The experimental groups 1, 2, 3, 4, 5, and 6 are designated as C, E1, E2, E3, E4 and E5 respectively. The experiment was conducted in glass aquaria containing 100L water. The water was not changed during the experiment but was aerated for 14 h. to avoid depletion of oxygen. The hydrobiological parameters like dissolved oxygen, temperature, PH, salinity and hardness of water were estimated during non- aeration period. Two series of experiment were conducted in the present study.

Exp.I: Feeding and growth- During the experiment period, the chosen groups were fed with weighed quantities of experimental diets twice a day at 07:00 and 18:00 hrs. Unconsumed feed was removed after 1 h feeding and dried in hot air oven at 80Ċ for two days. Feed intake was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of the offered feed. The feeding rate (mg/g live fish/day) was computed as the amount of feed consumed / (initial wet wt of the fish × no. days). Feed samples and unconsumed feed were weighted in an electric monopan balance to 1 mg accuracy. The duration of

the experiment was 7, 14, 21 days. The sacrifice method was adopted to estimate the growth of the experimental fish. Calculation of selected food utilisation parameters has been described in detail.

At the beginning the experiment, the total weight of the fish in each groups was weighed in an electric monopan balance. Five fish from the stock were sacrificed to estimate water content and determine the initial dry weight of the fish. All fish in each group were weighed at the end of the experiment and dry weight was calculated using the percent water content of fish sacrificed at the beginning of the experiment. Weight gain (growth) was calculated as the difference between initial and final dry fish weight. Growth rate (mg/g live fish/day) was calculated as growth / (initial weight of fish × no. of days). Gross conversion efficiency (%) was calculated as growth /feed intake × 100. Feed conversion ratio (FCR) was computed as the relation between feed intake and growth.

RESULTS AND DISCUSSION

Inorganic mercury salts are unable to cross tissue blood barriers and is eliminated at a faster rate than methyl mercury (Ulfvarson, 1966). Animals start accumulating mercury when the rate of uptake exceeds the rate of elimination. When inorganic mercury treatment was stopped, the elimination of accumulated mercury resulted in the decline of its residue level in liver and this brought about a corresponding histological recovery studied by (Paulose , 1988).

The accumulation of heavy metals in the tissues of fishes may cause various physiological defects and mortality (Torres *et al.,* 1987). Heavy metals accumulated in the tissues of aquatic animals may become toxic when accumulation reaches a substantially high level (Kalay and Canli, 2000). The pattern of bioaccumulation of metals in animals differs from metal to metal and organ to organ during their functional status. Most of the investigations pertaining to heavy metals contaminants in aquatic systems are dealt either with toxicity or with accumulation (Rushforth *et al.,* 1981; Khadiga *et al.,* 2002). Heavy metals have been shown to be concentrated in the liver of various fishes (Sorensen, 1991 and Rao *et al.,* 1998).

The Dietary ascorbic acid supplementation at a level of 2000 mg kg-1 diet resulted in decreased copper accumulation in the gills and liver of rainbow trout and also decreased copper levels in the gills, haepatopancreas, Kidney and intestine. These results demonstrated that dietary ascorbic acid decreased the toxicity of water borne copper accumulation in the tissues. It is likely that, dietary *spirulina* may also reduce the metal level in tissues (Lanno *et al.,* 1985) and protect *Labeo rohita* from mercuric chloride toxicity.

In the present work the feed intake was decreased in the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet but it was significantly increased in the mercuric chloride with *Spirulina platensis* diet from 20.49 to 28.32 (g dry matter) and the consumption rate was increased from 32.38 to 41.90 (mg/g live fish/day) as the percent dose of *Spirulina platensis* increased.

Table 1 : Number and Percentage of Floral elements of Patnadevi Forest.

Parameters	**Diet (*Spirulina* content)**					
	Control	**T_1 (0%)**	**T_2 (2%)**	**T_3 (4%)**	**T_4 (6%)**	**T_5 (10%)**
Food intake (g dry matter)	31.21 3.43	15.82 1.36	20.49 2.03	23.11 2.32	27.87 2.69	28.32 2.45
Consumption rate (mg/g live fish/day)	56.38 5.43	25.26 2.16	32.38 3.47	39.48 4.02	42.11 4.53	41.90 4.21
Weight gain (g wet wt.)	6.76 0.71	1.71 0.19	3.61 0.36	5.20 0.57	5.58 0.51	5.90 0.43
Weight gain (%)	26.06 2.17	3.61 0.36	6.89 0.65	18.96 1.75	20.60 0.55	19.53 1.53
Growth rate (mg/g live fish/day)	12.95 1.15	2.88 0.32	3.09 0.28	6.89 0.65	8.36 0.78	7.05 0.38
Gross conversion Efficiency (%)	23.72 2.38	6.00 0.60	11.00 1.29	20.49 2.03	19.95 1.83	18.56 1.39
Feed conversion ratio	5.28 0.51	5.92 1.73	9.81 0.61	7.05 0.38	5.10 0.43	5.09 0.48

The sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet resulted in significant decrease in weight gain (%) but the weight gain were increased in the mercuric chloride with *Spirulina platensis* diet from 6.89% to 19.53 % and the growth rate was increased from 3.09 to 7.05 (mg/g live fish/day) as the percent dose of *Spirulina platensis* increased. Mercuric chloride exposed *Labeo rohita* fed *spirulina* supplemented diets might have eliminated the copper from the body tissues through feces, *Spirulina* reduced genotoxicity and oxidative stress of several antibiotics in mice (Premkumar, 2004) and lead (Pb) toxicity in rats (Upasani, 2003).The present study showed that feeding and growth parameters improved in mercuric chloride exposed fish fed *Spirulina*-supplemented diets. *Spirulina* reduced mercuric chloride accumulation in tissues and increased mercuric chloride elimination through feces, lessening the metal burden and its toxicity to fish. The reduced growth rate in fish given a sublethal level of mercuric chloride was probably due to the tissue burden of mercuric chloride which, in turn, could have caused a reduction in feed intake, an increase in metabolic cost, or poor food conversion efficiency. Growth reduction in copper-exposed *Salmo gairdneri* was partly due to increased metabolic costs and reduced food consumption (Lett *et al.,* 1976).

Supplementation of *Spirulina* in the diet, improved the food utilisation parameters in mercuric chloride exposed fish. The feed conversion ratio (FCR) value of fish belonging to T4 groups was low as (5.10) as compared to other groups other groups and close to the FCR value of control fish. (Table-1). It was due to the *Spirulina palatensis* which reduced the accumulation of mercuric chloride in tissues in elimination of accumulated metal through faces, lessening the metal burden and its toxicity on fish. Spirulina contains phycocynin (14%) cholorophyll (1%) and carotenoid (0.37%) pigments. (Henrikson, 1994) B-carotene of Spirulina maintains the mucous membrane firmely (Henrikson, 1994) and thereby entry of toxic elements in to the body is prevented. Chlorophyll of *Spirulina* acts as a cleasing and detoxifying phytonutrient against the toxic substances (Henrikson, 1994).

It indicates that *Spirulina* has the ability to eliminate and detoxify and accumulated mercuric chloride and it was proved by improvement of feeding and growth parametrers in sublethal exposure of *Labeo rohita* fed *Spirulina patensis* supplementation diets. Working on rainbow trout *Salmo gaiedheri*, (Lanno *et al.,*1985) found that high level of dietary ascorbic acid (10g Kg-1 diet) improved the body weight gain in copper exposed fish as compared to fish fed on low levels on ascorbic acid (0.9g kg-1 diet).

CONCLUSION

The present study shows that, the dietary supplementation of *Spirulina* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita* and improved the food utilization parameters like feed intake, consumption rate, weight gain, growth rate and feed conversion ratio (FCR) value significantly as the percent dose of *Spirulina platensis* was increased in a short period of time.

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RESEARCH ARTICLE

Biodiversity and prevalence of Helminth parasites of Girna Dam Fishes

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ABSTRACT

The objective of the present study was to study the biodiversity and prevalence of helminth parasites of Girna dam fishes. A total of 500 fishes were examined, in which 249 fishes (49.8 %) were infected with 228 cestode parasites and 172 nematode parasites, which belongs to four and seven genera respectively. The result of the present study suggest that cestodes mainly *Circumoncobothrium spp., Senga spp., Lytocestus spp., Polyoncobothriumspp*., and nematodes mainly *Eustrongylides spp., Rhabdochona spp., Syphaciella spp*., *Contracaecum spp*., *Ascardiaspp*., *Camallanusspp*.,and *Trichurisspp*., are the main intestinal parasites of girna dam fishes(viz. *Mastacem bellusarmatus, Wallago attu, Ophiocephalus punctatu, and Clarius batrachus*). This report summarizes the data of incidence, intensity, density and index of infection in fresh water fishes in relation to environmental factors. Incidence of infection was higher in winter season. While intensity of infection was higher in Monsoon and density of infection was higher in winter season. The present study will be helpful to the status of diversity of cestode and nematode parasites from Girna dam.

Keywords: Cestodes, Nematodes, Girna dam, biodiversity, prevalence.

INTRODUCTION

The Girna river is originate at Kem Peak in the Western Ghats range of Nashik District. The name Girna derives from the name of the goddess Giraja (Parvati).Girna Dam is an earth fill dam on Girna river located in Nandgaonnear Malegaon, district Nasik of Maharashtra state. It was built in 1969. Irrigation and hydroelectricity are two major needs for which Girna Dam had been created. It has catchment area 4729.34 sq.km.

India is the third largest producer of fish in the world and second in inland fish production. Fisheries are important for the Indian economy as it provides employment opportunities; it is a source of nutritional food and foreign exchange earnings. The fishes are said to be "gold" from water. *Mastacem bellusarmatus* Lacepede (1800), *Wallago attu* Bleeker (1851), *Ophiocephaluspunctatu,* Hamilton (1822) *and Clarius batrachus*

Linnaeus (1758) are highly demanded market fish in India as a table fish for high quality of nutritional value. It contains protein, lipid, minerals and vitamins. But fish farming remains a high risk investment, mainly due to the disease problems caused by parasitic infection. A fish disease due to the helminth parasite is one of the most important problems in fish culture and fish farming. The common cestodes and nematodes of these fishes causing the economic loss includes the parasites like *Lytocestus, Polyonco bothrium, Senga, Gangesia, Circumonco bothrium* and nematodes mainly *Trichuris*. *Eustrongylides, Ascardia*, *Contracaecum*, *Camallanus, Rhabdochona* and *Syphaciella*, However, very little is known about the parasitic fauna of fishes of India in comparison with the information available from other regions of the continent. Several investigations have studied helminth parasites of fresh water fishes. The work of these investigations concerns the survey, population dynamics, host specificity and organ specificity. The environmental factors including climate, season and rainfall play an important role in the development of helminth parasites. Due to the environmental factors the natures of helminth infection of different group of livestock have been studied by workers from particular region of the country.

Work of Yamaguti (1959; 1961) related to the occurrence of helminth parasite in vertebrate host is of immense importance with regard to different zones of the world. Chubb (1982) illustrated the studies of seasonal occurrence of helminth in freshwaterfishes in different climatic zones of the world. Agrawal (1990) described some nematode parasites of freshwater fishes from Lucknow. Shomorendro and Jha (2003) also studied some of the nematode parasites. Karand Barbhuiya (2009) studied the effect of length of fish on the occurrence of nematode and acantocephalan parasites. Geetaraniet al. (2010) has studied the intensity of helminth infections in fishes of Manipur. While Dhole *et al*., (2010) has done survey of helminth parasites in freshwater fishes from Marathwada region. Recently, Jadhav*et. al.,* (2011) studied incidence of helminth parasites in freshwater fishes from SinaKolegoan Dam, Dist. Osmanabad.

The infection of helminth parasites are found in numbers of fresh water fishes. Due to immense infection it enhances the rate of mortality therefore in order to avoid loss of economical, nutritional and medicinal value also to preserve endangered species of fishes. The study is an important specially Helminth parasites which also liable to spread their effect upon human beings. This warrants serious attention of biologists for having knowledge of helminth parasites.

Keeping this view in mind, the author studied the biodiversity and prevalence of helminth parasites in *Mastacem bellusarmatus, Wallago attu, Ophiocephalus punctatus, and Clarius batrachus* from Girna dam for three seasons i.e. monsoon, winter and summer during February 2010 to January 2012.

MATERIAL AND METHODS

The freshwater fishes were collected from Girna dam for three seasons i.e. monsoon, winter and summer during February 2010 to January 2012. Fishes were opened up ventrally and the internal organs examined. The entire digestive system was removed and placed in a petri dish with physiological saline. Infection of each group of parasites was treated as follows: Collected cestodes were first relaxed and then fixed in 4% formalin and stained by using Harris haematoxyline. Stained parasites were washed in water, dehydrated in ascending grades of alcohol, cleared in xylene, mounted in D.P.X. Collected nematodes were first relaxed and then fixed in hot 10% glycerol or 70% alcohol, cleared in lactophenol and mounted in glycerinegelly. Drawings are made by using a camera lucida. The identification of helminth is made with the help of "SystemaHelminthum" Vol. II and III by Yamaguti(1959, 1961); Advances in the Zoology of Tapeworms, 1950-1970, by Wardle *et al.,* (1974) and Keys to the cestode parasites of vertebrates by Khali *et al.,* (1994). Collected the data month wise and calculate the percentage of incidence, intensity, density and index of infection seasonally i.e. monsoon, winter and summer.

Population dynamics of helminth parasites were determined by following formulae,

$$1.\ \text{Incidence of Infection} = \frac{\text{Infected host}}{\text{Total hosts examined}} \times 100$$

$$2.\ \text{Intensity of Infection} = \frac{\text{Number of parasite collected}}{\text{Number of infected hosts}}$$

$$3.\ \text{Density of Infection} = \frac{\text{No. of parasite collected}}{\text{Total hosts examined}}$$

$$4.\ \text{Index of Infection} = \frac{\text{No. of infected hosts} \times \text{No.of parasite collected}}{(\text{Total hosts examined})^2}$$

RESULTS AND DISCUSSION

Helminth parasite infection is the common problem of fresh water fishes all over the world. The study is related to taxonomy, statistical application and population of helminth parasites. The collection of the helminth parasites was carried out from the fresh water fish *Mastacem bellusarmatus, Wallagoattu, Ophiocephalus punctatus and Clarius batrachus* from Girna dam during study period i.e. February 2010 to January 2012.

After closer observation the collected helminth were found belongs to the genus of cestodes *Circumonco bothrium* Shinde (1968), *Senga* Dollfus (1934), *Lytocestus* (Cohn, 1908) Hunter (1927), *Polyoncobothrium* Diesing (1854) and the genus of nematodes *Eustrongylides* Jagerskiold (1909), *Syphaciella* Monnig (1924), *Ascardia* Dujardin (1845), *Camallanus* Raillet et Henry (1915), *Rhabdochona* Railliet (1916), *Contracaecum* Railliet and Henry (1912) and *Trichuris* Roederer (1761).

Out of 500 samples examined 249 specimens (49.8%) were positive for various helminth parasites Table 1 and Table 2. The present investigation indicates that a total 228 cestodes and 172, nematodes were collected. The values for the incidence, intensity, density and index of infection are given in Table 3 whereas the Table 4 and Table 5 shows influence of season on parasitic infection of helminth parasites from freshwater fishes.

The incidence of infection of cestode parasite during 2010-11 was maximum (71.62%) in summer season, followed by (69.33%) in monsoon season and slightly lower (63.51%) in winter season. The intensity of infection was maximum (0.96) in winter season, followed by (0.89) in summer season and lower (0.75) in monsoon season. The density of infection was maximum (0.64) in summer season, followed by (0.61) in winter season and lower (0.52) in monsoon season. The index of infection was maximum (0.45) in summer season, followed by (0.39) in winter season and lower (0.36) in monsoon season.

Table 1: Incidence of Helminth parasites in fresh water fishes from Girna Dam during 2010-2012

Sr.No.	Parasitic species	No. of sample +ve	Locality
01	*Lytocestus sp.*	10	Intestine
02	*Polyoncobothrium sp.*	22	Intestine
03	*Senga sp.*	84	Intestine
04	*Circumoncobothrium sp.*	112	Intestine
05	*Trichuris sp.*	10	Large Intestine
06	*Eustrongylidessp.*	48	Subcutaneous tissues & Intestine
07	*Ascardiasp.*	54	Intestine
08	*Contracaecum sp.*	15	Stomach wall, mesentery & Intestine
09	*Camallanussp.*	09	Intestine
10	*Rhabdochonasp.*	18	Intestine
11	*Syphaciellasp.*	18	Body cavity, mesentery & Intestine
	Total	**400**	

Table 2: Incidence, Intensity, Density and Index of Helminth infection during 2010-2012

Sr. No.	Genus	No. of host dissected	No. of host infected	No. of parasite collected	Incidence of Infection	Intensity of Infection	Density of Infection	Index of Infection
01	*Lytocestus*	500	249	10	49.8	0.04	0.02	0.01
02	*Polyoncobothrium*	500	249	22	49.8	0.09	0.04	0.02
03	*Senga sp.*	500	249	84	49.8	0.34	0.17	0.08
04	*Circumoncobothrium*	500	249	112	49.8	0.45	0.22	0.11
05	*Trichuris*	500	249	10	49.8	0.04	0.02	0.01
06	*Eustrongylides*	500	249	48	49.8	0.19	0.10	0.05
07	*Ascardia*	500	249	54	49.8	0.22	0.11	0.05
08	*Contracaecum*	500	249	15	49.8	0.06	0.03	0.01
09	*Camallanus*	500	249	09	49.8	0.04	0.02	0.01
10	*Rhabdochona*	500	249	18	49.8	0.07	0.04	0.02
11	*Syphaciella*	500	249	18	49.8	0.07	0.04	0.02

Table 3: Showing helminth parasites of fishes collected from Girna Dam during 2010-2011

Month	Helminth group	No. of host dissected	No. of host infected	Total No. of parasite collected	Incidence %	Intensity %	Density %	Index of infection
Feb. 2010	Cestode	18	8	10	44.64	1.25	0.55	0.25
	Nematode		4	6	22.22	1.50	0.33	0.07
March 2010	Cestode	19	9	11	47.37	1.22	0.58	0.27
	Nematode		7	8	36.84	1.14	0.42	0.16
April 2010	Cestode	18	9	17	50.00	1.88	0.94	0.47
	Nematode		5	10	27.77	2.00	0.55	0.15
May 2010	Cestode	19	6	9	31.58	1.50	0.47	0.15
	Nematode		5	6	26.32	1.20	0.32	0.08
June 2010	Cestode	17	9	12	52.94	1.33	0.71	0.37
	Nematode		5	8	29.41	1.60	0.47	0.14
July 2010	Cestode	19	4	9	21.05	2.25	0.47	0.09
	Nematode		5	9	26.32	1.80	0.47	0.12
Aug. 2010	Cestode	18	8	14	44.44	1.75	0.77	0.35
	Nematode		4	5	22.22	1.25	0.27	0.06
Sept. 2010	Cestode	20	5	10	25.00	2.00	0.50	0.12
	Nematode		7	6	35.00	0.86	0.30	0.10
Oct. 2010	Cestode	19	7	8	36.84	1.14	0.42	0.15
	Nematode		5	4	26.32	0.80	0.21	0.05
Nov. 2010	Cestode	18	7	8	38.88	1.14	0.44	0.17
	Nematode		4	6	22.22	1.50	0.33	0.07
Dec. 2010	Cestode	20	9	11	45.00	1.22	0.55	0.24
	Nematode		7	7	35.00	1.00	0.35	0.12
Jan. 2011	Cestode	18	7	12	38.88	1.71	0.66	0.26
	Nematode		6	8	33.33	1.33	0.44	0.15
Total		**223**	**152**	**214**	**68.16**	**1.40**	**0.96**	**0.65**
During 2011-2012								
Feb. 2011	Cestode	25	4	8	16.00	2.00	0.32	0.05
	Nematode		4	7	16.00	1.75	0.28	0.04
March 2011	Cestode	20	4	9	20.00	2.25	0.45	0.09
	Nematode		5	8	25.00	1.60	0.40	0.10
April 2011	Cestode	22	5	8	22.73	1.60	0.36	0.08
	Nematode		3	7	13.64	2.33	0.32	0.04
May 2011	Cestode	21	3	8	14.29	2.66	0.38	0.05
	Nematode		5	7	23.81	1.40	0.33	0.08
June 2011	Cestode	26	4	9	15.38	2.25	0.35	0.05
	Nematode		5	8	19.23	1.60	0.31	0.06
July 2011	Cestode	20	3	6	15.00	2.00	0.30	0.04
	Nematode		3	7	15.00	2.33	0.35	0.05
Aug. 2011	Cestode	24	4	7	16.66	1.75	0.29	0.05
	Nematode		3	7	12.50	2.33	0.29	0.04
Sept. 2011	Cestode	22	5	8	22.73	1.60	0.36	0.08
	Nematode		3	7	13.64	2.33	0.32	0.44
Oct. 2011	Cestode	26	5	**9**	19.23	1.80	0.35	0.07
	Nematode		4	**8**	15.38	2.00	0.31	0.05
Nov. 2011	Cestode	25	4	**8**	16.00	2.00	0.32	0.05
	Nematode		4	**7**	16.00	1.75	0.28	0.04
Dec. 2011	Cestode	24	5	**9**	20.83	1.80	0.38	0.08
	Nematode		4	**8**	16.66	2.00	0.33	0.05
Jan. 2012	Cestode	22	4	8	18.18	2.00	0.36	0.07
	Nematode		4	8	18.18	2.00	0.36	0.67
Total		**277**	**97**	**186**	**35.01**	**1.92**	**0.67**	**0.24**

Table 4: Showing influence of seasons on helminth infection during 2010-2011

Helminth group	Seasons	No. of host dissected	No. of host infected	Total No. of Parasite collected	Incidence %	Intensity %	Density %	Index of infection
Cestode	**Monsoon**	75	52	39	69.33	0.75	0.52	0.36
	Winter	74	47	45	63.51	0.96	0.61	0.39
	Summer	74	53	47	71.62	0.89	0.64	0.45
Total		**223**	**152**	**131**	**68.16**	**0.86**	**0.59**	**0.40**
Nematode	**Monsoon**	75	52	25	69.33	0.48	0.33	0.23
	Winter	74	47	28	63.51	0.60	0.38	0.24
	Summer	74	53	30	71.62	0.57	0.41	0.29
Total		**223**	**152**	**83**	**68.16**	**0.55**	**0.37**	**0.25**

Table 5: Showing influence of seasons on helminth infection during 2011-2012

Helminth group	Seasons	No. of host dissected	No. of host infected	Total No. of Parasite collected	Incidence %	Intensity %	Density %	Index of infection
Cestode	**Monsoon**	92	30	30	32.60	1.00	0.33	0.11
	Winter	97	34	34	35.05	1.00	0.35	0.12
	Summer	88	33	33	37.50	1.00	0.37	0.14
Total		**277**	**97**	**97**	**35.00**	**1.00**	**0.35**	**0.12**
Nematode	**Monsoon**	92	30	29	32.60	0.96	0.31	0.10
	Winter	97	34	31	35.05	0.91	0.32	0.11
	Summer	88	33	29	37.5	0.88	0.33	0.12
Total		**277**	**97**	**89**	**35.00**	**0.92**	**0.32**	**0.11**

The incidence of infection of nematode parasite during 2010-11 was maximum (71.62%) in summer season, followed by (69.33%) in monsoon season and lower (63.51%) in winter season. The intensity of infection was maximum (0.60) in winter season, followed by (0.57) in summer season and lower (0.48) in monsoon season. The density of infection was maximum (0.41) in summer season, followed by (0.38) in winter season and lower (0.33) in monsoon season.

The index of infection was maximum (0.29) in summer season, followed by (0.24) in winter season and slightly lower (0.23) inmonsoon season.

The incidence of infection of cestode parasite during 2011-12 was maximum (37.50%) in summer season, followed by (35.05%) in winter season and lower (32.60%) in monsoon season. The intensity of infection was same (1.00) in all seasons. The density of infection was maximum (0.37) in summer season, followed by (0.35) in winter season and lower (0.33) in monsoon season. The index of infection was maximum (0.14) in summer season, followed by (0.12) in winter season and lower (0.11) in monsoon season.

The incidence of infection of nematode parasite during 2011-12 was maximum (37.50%) in summer season, followed by (35.05%) in winter season and lower (32.60%) in monsoon season. The intensity of infection was maximum (0.96) in monsoon season, followed by (0.91) in winter season and lower (0.88) in summer season. The density of infection was maximum (0.33) in summer season, followed by (0.32) in winter season and lower (0.31) in monsoon season. The index of infection was maximum (0.12) in summer season, followed by (0.11) in winter season and lower (0.10) in monsoon season.

It was observed that the cestode and nematode species were present throughout the period of investigations but the intensity varied. Specially, large numbers of cestode and nematodes were recovered mainly from the intestine throughout the period. The development of parasites needs temperature and sufficient moisture. Environmental variations are reflected in seasonal difference in the incidence of diseases. The infections caused by the nematode parasites may be a major problem in the mortality of fishes. Such infections not only deteriorate the muscle quality, stunt growth but even sometimes prove fatal due to internal injury. In addition to this we may also suffer from many diseases if we ingest improperly cooked fishes.

The present investigation provides a good deal of information on the occurrence of cestode and nematodes from fresh water fishes of Girna Dam. The fish host is infected by ingesting invertebrates or fish intermediate host carrying the last larval or infective stage of the parasite. The level and periodicity of infection of these intermediate hosts, their availability to the definitive fish hosts, the feeding behavior and migrations of these fishes and the success of the parasites larva in establishing itself in the appropriate niche in the fish host all play a part in determining the ultimate biology of the parasites.

Feeding activity of the host also be one of the reasons for the seasonal fluctuation of infection according to the fishes were infected with large number of parasites in late winter to end of summer months, because the environmental conditions are favorable in such months. Thus the temperature and seasons play an important role in the recruitment of parasitic fauna.

The above results were compared with many earlier workers as Anderson (1976) who worked on seasonal variation in the population dynamics of *Caryophyllaeus luticeps.* Availability of food and feeding activity, distribution and environment of host, are influence the parasitic development Kennedy (1978) and Lawrence (1970). The parasites causes depletion of the nutritional contents in host's body and results in the low productivity, loss in fish industry Hiware (1999). Moller and Anders (1986) concluded that fish from more polluted water tend to harbor more helminth parasites than those from less polluted waters. Fresh water fishes was the most heavily infected, it was observed they feed mainly on a particular type of zooplankton and other small fishes. Some of these parasitize cause diseases to fish, affecting their health and reproduction, making them fall easy prey to predators and some infect man. In fish farming, parasites may lead to epidemics and mortalities, resulting in economic losses Khalil and Polling (1997).

Thus the present study gives the idea of damage caused by these helminth parasites to the fish economy. This study also adds some data regarding the taxonomy and diversity of parasites so that it will provide them preliminary literature to the researchers in the field of fish parasites.

CONCLUSION

The two year survey has shown that fresh water fishes from Girna Dam harbor a wide range of cestode and nematode parasites. After the analysis of data, it can be concluded that the high infection of Cestode and Nematodes (incidence, intensity, density and index of infection) occurred in summer and monsoon seasons followed by winter during 2010-11 and high infection of Cestode and Nematodes occurred in summer and winter seasons followed by monsoon during 2011-12 This type of results indicates that environmental factors influencing the seasonality of parasitic infection either directly or indirectly. However, the above study can only be complete if it covers a whole season to investigate the variation in parasite fauna with the diet of the host and variation in infection with the habitat type.

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RESEARCH ARTICLE

Lancergold induced toxic impacts on glycogen content of liver and gonads of a freshwater fish, *Labeo rohita*

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ABSTRACT

In the present study, effects of an insecticide Lancergold on glycogen content in the tissues like liver and gonads of a freshwater fish, *Labeo rohita* were studied during acute period (24 to 96 hrs). The LC_{50} value of Lancergold was found to be 3.1ppm for 96 hrs. Fishes were exposed to lethal concentration of Lancergold under laboratory conditions. Then tissues like liver and gonads were removed carefully from the control and experimental groups. These tissues were used for glycogen estimation. Results of the present investigation showed decreased level of glycogen in both the experimental tissues as compared to the control.

Keywords: Lancergold, *Labeo rohita*, Liver, Gonads, Glycogen.

INTRODUCTION

Freshwater is one of the most important resource required essentially for the life but the different types of pollutants like agricultural, industrial and municipal wastes change the quality of water. Pesticides used in agroecosystem for killing insect pests and better crop production leave residues in water and soil even after several days of the spray in the crop fields (Remia *et al*., 2008). These pesticides reach to the aquatic ecosystems by many ways like rainfall, floods etc. and pollute them. Many non-target organisms like fish, bivalves, crabs etc. get adversely affected. These pollutants enter in the body of aquatic animals through mouth, gills, skin and produce many hazardous effects on their vital processes. They damage their different organs as well as systems and produce disturbances in physiological and biochemical processes.

Water pollution is recognized globally as a potential threat to both human and other animal populations which interact with the aquatic environment (Bose *et al*., 2011). Today, Lancergold used widely in fields. It is an insecticide. It contains acephate, imidacloprid, alkyl naphthalene sulphonate (surfactant) and precipited silica (inert).

Imidacloprid is a systemic insecticide which is widely used in the whole world at current period. Fishes are mainly utilized as food materials amongst all the aquatic animals. *L. rohita* is common edible and one of the prime cultured fish. *L. rohita* also has more tremendous economic importance. Most of the pesticides are metabolic depressors so they disturb

the biochemical procedure and affect the activity of biomolecules like proteins, carbohydrates and lipids. Hence, the present investigation has been undertaken to study the biochemical alteration in tissues like liver and gonads of a freshwater fish, *Labeo rohita* specifically in glycogen content induced by an insecticide, Lancergold.

MATERIALS AND METHODS

The fishes *Labeo rohita* were collected from the Ganeshpur, Gadad and Girna river dam near Chalisgoan city, Dist. Jalgoan, Maharashtra, India. They were collected from their natural habitat and brought to the laboratory. The LC_{50} values are determined by Finney, (1971) method. The fishes were acclimatized to the laboratory conditions for 10 to 15 days prior to subjecting them to experiments. Well aeration is maintained for oxygen. Healthy and active fishes were chosen for experiments. Two groups of these fishes were formed. One group was considered as experimental group exposed to reagent grade of Lancergold for acute exposure (24 to 96 hrs). Another group was treated without pollutants and was considered as control. Biochemical parameter was assessed in five individual animals, pesticide treated and control groups were prepared. The fishes were starved for one day prior to experimentation in order to avoid the metabolic differences, if any due to differential feeding and food reserves.Glycogen was estimated from liver and gonads of *L. rohita* by Anthrone reagent method (Dizwann and Zandee, 1972).

RESULTS AND DISCUSSION

The results of present study revealed that, after acute (24 to 96 hrs) exposure to Lancergold, glycogen content of liver and gonads of a freshwater fish, *L. rohita* was decreased as compared to the control. Results are summarized in the table 1 and Fig. 1.

Glycogen is stored in the tissues in the form of carbohydrates which may provide a reserve food energy during stress. Glucose and glycogen are the main sources of energy for all vital activities of the body which are present in the carbohydrates. Carbohydrates are considered to be the first among the organic nutrients to be depleted and degraded in response to stress conditions imposed on animal (Nagaraju and Venkata, 2013).

Reduction in glycogen level may be due to inhibition of hormones which are involved in the synthesis of glycogen. Depletion of glycogen may also be due to direct utilization for energy demand caused by pesticidal stress. Similar reduction in glycogen concentration in tissues like liver and gonads of a fish, *Channa gachua* (Ham) after chromium toxicity was reported by Kawade and Khillare, (2012). Reduction in glycogen level were also reported earlier by Dubale and Shah, (1981); Sastry and Subhadra, (1982); Bedi and Kanan, (2005); Ganeshwade *et al*., (2011); Chezhian *et al*., (2010) and Sreenivasa, (2002).

Glycogen mobilization is maximum in the liver because liver is the seat of glycogen metabolism supplies glycogen for producing more energy to combat pesticidal stress. According to Dezwan and Zandee, (1972), a drop in tissue glycogen content may

Table 1: Toxic impact of insecticide, Lancergold on Glycogen contents in Liver and Gonads (Testis and Ovary) of *Labeo rohita* after acute (24 to 96 hrs) exposure.

Tissue	Treatment	Acute			
		24 hrs	**48hrs**	**72hrs**	**96hrs**
Liver	Control	8.4505 ±0.008246***	8.3939 ±0.010392***	8.3548 ±0.145602***	8.2829 ±0.000078***
Testis	Control	7.6851 ±0.006164***	7.6249 ±0.009165***	7.5536 ±0.008831***	7.5431 ±0.02607***
Ovary	Control	7.3768 ±0.034058***	7.3764 ±0.03286***	7.3762 ±0.02898***	7.2961 ±0.05639***
Liver	Lancergold	7.4768 ±0.03405***	7.3264 ±0.03286***	7.0124 ±0.02898***	6.5489 ±0.05639***
Testis	Lancergold	6.8671 ±0.006928***	6.4623 ±0.006164***	5.9402 ±0.01077***	5.0124 ±0.008246***
Ovary	Lancergold	7.0001 ±0.04049***	6.7631 ±0.04795***`	6.5892 ±0.03714***	6.0271 ±0.02683***

Values expressedas mg/100mg of wet wt. of tissues, ± indicate S.D. of five observations, values are significant at P<0.001***.

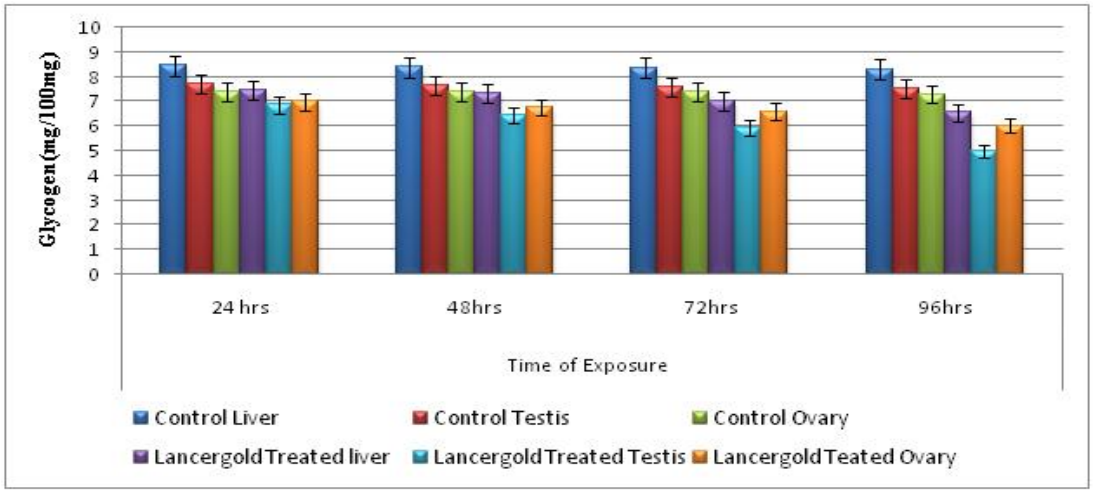

Fig.1 Variation in Glycogen content of liver and gonads of freshwater fish, *Labeo rohita* after acute exposure to an insecticide, Lancergold.

also be either due to decreased synthesis as a consequence of toxic stress and breakdown. Decreased glycogen level in various tissues of fish due to pollutants were observed by Asifa and Vasantha, (1994) in *Clarias batrachus* after endosulfan exposure; James and Sampath, (1995) in *Heteropneustes fossilis* (Bloch) after copper and ammonium mixture toxicity; Shobha *et al*., (2000) in *Tilapia mossambica* due to sodium arsenate intoxication; Rawat *et al*., (2002) in *Heteropneustes fossilis* after endosulfan exposure; Binukumari and Vasanthi, (2013) in *Labeo rohita* after dimethoate 30% EC intoxication.

According to Nagaraju and Venkata Rathnamma, (2013), depletion in the total glycogen in all the vital tissues may be attributed toxic stress resulting in the disruption of enzymes associated with the carbohydrate metabolism. Stepped up glycogenolysis leads to a decrease in glycogen content (Koundinya and Ramamurthy, 1980). Whereas Martin and Arivoli, (2008) suggested that, the depletion of glycogen explains the increased demand of these molecules to provide energy for the cellular biochemical process under toxic manifestation.

CONCLUSION

In the present investigation, an insecticide Lancergold caused decrease in glycogen level of the liver and gonads of freshwater fish, *Labeo rohita* during acute exposure period. It can be concluded that the toxicity of this insecticide may lead to severe effect on aquatic animals.

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RESEARCH ARTICLE

Protective effect of dietary *Spirulina platensis* on haematological parameters of *Labeo rohita* exposed to sublethal concentration of mercuric chloride

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Shelke AD and Wani GP (2015) Protective effect of dietary *Spirulinaplatensis* on haematological parameters of *Labeo rohita* exposed to sublethal concentration of mercuric chloride., *International J. of Life Sciences,* Special issue, A3: 52-58.

ABSTRACT

A considerable number of contaminants of environment contain heavy metals as their constituents. Mercury is considered to be one of the most toxic metals. Industries discharge heavy metals recklessly in to the environment. The consumption of heavy metalsparticularly mercury through food chain via fish by human being cause severe disorder in the physiology. Heavymetals causing serious damages to entire ecosystem including fishes. To reduce the toxicity of heavy metals of fishes, it is one of the serious and major problems in fish culture. There is a lack of study about the effects of phytoplankton especially micro algae on fishes. The protective effects of *Spirulina* applied on other animal's however it has yet to be applied for fishes. The effect of spirulina supplementation on reduction of mercuric chloride toxicity based on haematological parameters was studied in a freshwater fish, *Labeo rohita*. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. The results showed that sublethal exposure of *Labeo rohita* fed with *spirulina* free diet (T1 groups) significantly reduced the hamotological parameters than those exposed to sublethal level of mercuric chloride and fed *Spirulina* supplementation diet (T2- T5 groups).

Keywords: Protective effect, *Spirulina platensis*, Haematologial parameters, Mercuric chloride, *Labeo rohita.*

INTRODUCTION

The natural aquatic systems is getting extensively contaminated with heavy metals released from domestic, industrial and other anthropogenic activities (Velez and Montoro, 1998, Conacher *et al.,* 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.,* 2007; Vosyliene and Jankaite, 2006). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.,* 2004). Recent investigations have shown that a small amount of metal is sufficient to bring about severe biochemical, physiological and haematological consequences.

Effect of *Spirulina* in channel catfish *Ictaluruspunctatus,* (Duncan and Klesius, 1996) found that fish fed *Spirulina* had a lower percentage of erythrocytes and a higher percentage of lymphocytes than fish fed a control diet. There was no difference in thrombocytes and macrophages in the *Spirulina* and control diet. However, peritoneally elicited phagocytes from fish fed *Spirulina* showed enhanced phagocytosis to zymosan and increased chemotaxis to *Edwardsiellaictaluri* exoantigen (5.9 times). *Spirulina* also enhanced production of antibodies to key limpet hemocyanin (KLH) but not to *E ictaluri*. (Hayashi *et al*., 1993*),* who showed in mice that *Spirulina* increased the antibody response to a thymus-dependent antigen but not to a thymus independent antigen. Inastudy by (Lee, 1999) the activity of granulocytes and hyaline cells was enhanced significantly in tiger prawns *Penaeusmonodon* supplied with a feed containing as low as 0.1% (w/w) dry *Spirulina*. The increase in phagocytic activity of haemocytes was a function of *Spirulina* content and time. Prawns fed with *Spirulina* couldclear *Vibrio parahaemolyticus*, a pathogen of prawns, from the hemolymphat half the time taken by control prawns fed with basal diet.

In the present study, experiments were designed to investigate the impact of dietary *Spirulina* supplementation on the haematological, and alleviation of mercuric chloride in carp, *Labeorohita*. In the present investigation, focus on damage induced by inorganic mercuryin the Indian majorcarp *Labeo rohita,* and protective role done *Spirulina Plantensis*.

MATERIALS AND METHODS

The live major carp, *Labeorohita*were obtained from a Girna river dams near Chalisgaon city. They were acclimatized in laboratory condition for more than two weeks. The temperature, PH, Salinity and dissolved oxygen of the water were found to be 27 ± 1Ċ, 7.55 ± 0.1, 0.76 ± 0.09% and 7.20 ± 0.12 ml/l respectively. During the acclimatization, water was changed daily and fish were fed ad libitim with pelletised diet containing 35% protein. Acclimatized fish (1.30 ± 0.10g) were exposed to different concentrations (0, 0.03, 0.06, 0.09., 0.12, 0.15, 0.18 ppm) of mercuric chloride $HgCl_2$ obtained from Merk India Ltd. (Mumbai, India) and mortality was observed for 96 h. A static bioassay method was adopted for the determination of 96 h median lethal concentration Probit analysis was followed for the calculation of 96 hours $LC_{50.}$ Control group of fish was maintained in mercury free freshwater.

Feed: In the present experiment, 35% protein diet was used as basal diet for *Spirulina patensis* supplementation. The intergradient of dried fish meal, ground oil cake, cod liver oil, egg yolk, tapioca flour, vitamins and mineral mixtures were used to prepare the 35% protein diet, with appropriate proportion by square method. (Hardy, 1980). In addition to the control diet, five diets (0, 2, 4, 6, and 10 %) were prepared with different *Spirulina patensis* levels. The experimental diets were by adding the appropriate level of *Spirulina patensis* with chosen intergradient to boiled water, mixed well and steam cooked for 15-20 min. After moderate cooling, pellets (2mm) were prepared with operated pelletizer and dried in sunlight. After drying diets were separated stored in refrigerator.

Active and healthy fish (1.30 ± 0.10 g) were chosen from the acclimatization tank and starved for 24 h prior to the commencement of experiment. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. Triplicates were maintained for each group.

Group-I: served as control and reared in mercuric chloride free freshwater and fed with *Spirulina patensis* free diet. Test animals belonging to 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} groups were exposed to 0.12 ppm of mercuric chloride.

Group-II: individuals was fed with *Spirulina patensis* free diet, however 3^{rd} , 4^{th} , 5^{th} and 6^{th} groups were fed with 2, 4, 6, and 10% *Spirulina patensis* diets respectively.

The experimental groups 1, 2, 3, 4, 5, and 6 are designated as C, E1, E2, E3, E4 and E5 respectively. The experiment was conducted in glass aquaria containing 100L water. The water was not changed during the experiment but was aerated for 14 h to avoid depletion of oxygen. The hydrobiological parameters like dissolved oxygen, temperature, PH, salinity and hardness of water were estimated during non-aeration period. Two series of experiment were conducted in the present study.

Exp-II: Like the first series of experiment, a parallel experiment was conducted simultaneous- sly for 7, 14, 21 days to study the impact of dietary *Spirulina*

patensis on selected parameters in *Labeo rohita.* Test animals fed ad libitum with chosen experimental diets to respective exposures twice a day at 07:00 and 18:00 hrs. for 1h each. Test animals were starved for 24h prior to the conclusion of the experiment for the estimation of haematological parameters.

Three fish were removed from each experimental group at the end of the experiment; blood was collected and analyzed for selected haematological parameters. Blood was collected in watch glass containing required amount of 6% EDTA as an anticoagulant from three experimental fish at a time by cutting the caudal peduncle using a sharp knife. Haematological parameters were estimated according to routine clinical method RBC was counted by using an improved Neubauer counting chamber. Haemoglobinometer was used to determine the haemoglobin content of blood.

RESULTS AND DISCUSSION

The haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. Short-term exposures to low concentrations of heavy metals mostly induce an increase in these hematological indices. Increase in RBC number and hematocrit level was reported in *Mystusvittatus* exposed to sub-lethal and lethal concentrations of copper (Singh and Singh, 1982). Some workers also reported a fall in RBC count, haemoglobin percent and packed cell volume and decrease in MCH, MCHC and MCV in freshwater fishes exposed to cadmium, zinc and nickel indicating anemia, erythropenia and leucopoiesis (Vincent *et al.,*1996). The TEC, hemoglobin percent and mean cell hemoglobin (MCH) were appreciably declined in *Labeo rohita* exposed to chromium reflecting the anemic state of the fish which could be possibly due to iron deficiency and its consequent decreased utilization for hemoglobin synthesis. This is in accordance with a similar study on *Labeo rohita*, which also reported hypo chromic microlytic anemia under lead chloride stress, Reddy *et al.* (1998). Anemia in fish is an early manifestation of acute and chronic intoxication of chromium. Further, a significant decrease in TEC, hemoglobin per cent, MCH and hematocrit were also reported in *Channa punctatus* exposed to both copper and chromium and this decrease is more pronounced in fishes exposed to chromium suggesting that the metal induces acute anemia under toxic conditions (Singh., 1995). The anemia could be probably due to structural alterations of heme leading to disturbed haemoglobin synthesis and also the inhibitory effect of mercuric chloride on the enzyme system in the synthesis of haemoglobin cannot be ruled out. (Johansson-Sjobeck and Larrsson, 1979).

Table-1: Effect of supplementation of dietary *Spirulina platensis* on Red blood corpuscles (RBC) count ($\times 10^6$ mm^{-3}) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

Sr. No.	Treat.	g % *S.p.*	Red blood corpuscles count ($\times 10^6$ mm^{-3})		
			7 days	14 days	21 days
1	Control	0%	2.58±0.13	2.61±0.18	2.56±0.02
2	T_1	0%	1.45±0.10** -43.57	1.41±0.1* -46.15	1.34±0.11*** -47.85
3	T_2	2%	1.48±0.05** -42.41	1.63±0.13* -37.54	1.64±0.06*** -36.18
4	T_3	4%	1.51±0.06** -41.24	$1.73±0.14^{NS}$ -33.71	1.80±0.12** -29.96
5	T_4	6%	1.56±0.04** -39.29	$2.00±0.13^{NS}$ -23.37	2.1±0.07** -18.28
6	T_5	10%	$2.00±0.11^{NS}$ -22.17	$2.06±0.23^{NS}$ -21.07	2.08±0.11* -19.06

i) Each value are mean ± S.D. of three estimations.
ii) (+) or (-) signs indicate % variation over control.
iii) Values are significant at * = P<0.05, *** = P<0.001, NS = Non significant.

Table 2: Effect of supplementation of dietary *Spirulina platenis* on Haemoglobin content (%) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride.

Sr. No.	Treat.	g% S.p.	Haemoglobin content (g %)		
			7 days	14 days	21 days
1	Control	0%	8.58±0.42	8.51±0.32	8.41±0.37
2	T_1	0%	4.92±0.09** -42.65	4.40±0.55** -48.29	4.03±0.46** -52.08
3	T_2	2%	5.27±0.35** -38.57	5.51±0.32** -35.25	5.80±0.62* -31.03
4	T_3	4%	5.72±0.33** -33.33	6.27±0.39* -26.32	6.33±0.27* -24.73
5	T_4	6%	6.38±0.44* 25.64	6.86±0.19* -19.38	$6.80±0.76^{NS}$ -18.29
6	T_5	10%	$6.82±0.60^{NS}$ -20.51	$7.05±0.57^{NS}$ -17.15	$7.00±0.67^{NS}$ -13.79

i) Each value are mean ± S.D. of three estimations.
ii) (+) or (-) signs indicate % variation over control.
iii) Values are significant at * = P<0.05, *** = P<0.001, NS = Non significant.

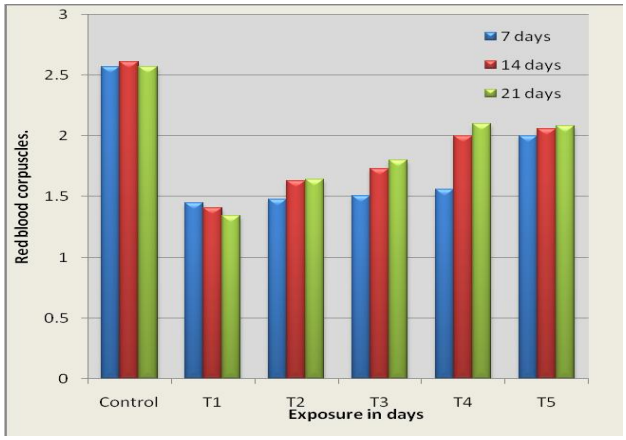

Fig1: Variations in effect of supplementation of dietary *Spirulina platensis* on Red blood corpuscles (RBC) count (×10^6 mm^{-3}) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

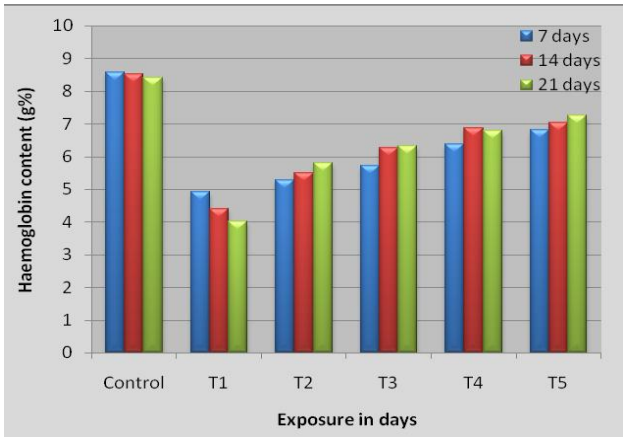

Fig 2: Variations in effect of supplementation of dietary *Spirulina platenis* on Haemoglobin content (%) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride.

In the present wok the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet resulted in significant decrease in RBC count but RBC count were increased in the mercuric chloride with *Spirulina platensis* diet from 2% to 10% and the RBC count was increased from 1.64 to 2.08 (×10^6 mm^{-3})as the percent dose of *Spirulina platensis* increased. Haemoglobin content was decreased in the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet but it was significantly increased in the mercuric chloride with *Spirulina platensis* diet from 2% to 10% and the haemoglobin content was increased from 5.80 to 7.00 g % as the percent dose of *Spirulina platensis* increased. In mercuric chloride exposed *Labeo rohita* and it may be due to the reduction of RBC count and HB content which reflected on tissue respiration. This may be evidently reflected on overall oxygen consumption of animals exposed to mercuric chloride.

The present study revealed that, the haematological parameters were improved in mercuric chloride exposed *Labeo rohita* fed *Spirulina* supplementation diets as suggests the protective role of *Spirulina* against mercuric chloride toxicity in

Labeorohita has 14% phycocynin pigment and it stimulates the erythropoesis (EPO) hormone production for hematopoesis. Phycocynin pigment also regulates the production of white blood cells even when bone marrow stem cells are damaged by toxic chemical or radiation reported by (Henrikson, 1994). (Sharma and Sharma, 2005), reported that *Spirulina* added feeds improved the tolerance of Poecilla reticulate when exposed to an azo-dye methyl red by considerable reduction in the cytotoxic effects on RBC'S count at higher concentration of the dye. Hayashi et al 1996 made similar findings and reported enhancement and proliferation of haematopoietic cells in the bone marrow.Inorganic mercury salts are unable to cross tissue blood barriers and is eliminated at a faster rate than methyl mercury (Ulfvarson, 1966). Animals start accumulating mercury when the rate of uptake exceeds the rate of elimination. When inorganic mercury treatment was stopped, the elimination of accumulated mercury resulted in the decline of its residue level in liver and this brought about a corresponding histological recovery studied by (Paulose , 1988).

The accumulation of heavy metals in the tissues of fishes may cause various physiological defects and mortality (Torres *et al.,* 1987). Heavy metals accumulated in the tissues of aquatic animals may become toxic when accumulation reaches a substantially high level (Kalay and Canli, 2000). The pattern of bioaccumulation of metals in animals differs from metal to metal and organ to organ during their functional status. Most of the investigations pertaining to heavy metals contaminants in aquatic systems are dealt either with toxicity or with accumulation (Rushforth *et al.,* 1981; Khadiga *et al.,* 2002). Heavy metals have been shown to be concentrated in the liver of various fishes (Sorensen, 1991 and Rao *et al.,* 1998).

The Dietary ascorbic acid supplementation at a level of 2000 mg kg-1 diet resulted in decreased copper accumulation in the gills and liver of rainbow trout and also decreased copper levels in the gills, haepatopancreas, Kidney and intestine. These results demonstrated that dietary ascorbic acid decreased the toxicity of water borne copper accumulation in the tissues. It is likely that, dietary *spirulina* may also reduce the metal level in tissues (Lanno *et al.,* 1985) and protect *Labeo rohita* from mercuric chloride toxicity.

In the present work the feed intake was decreased in the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet but it was significantly increased in the mercuric chloride with *Spirulina platensis* diet from 20.49 to 28.32 (g dry matter) and the consumption rate was increased from 32.38 to 41.90 (mg/g live fish/day) as the percent dose of *Spirulina platensis* increased. The sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet resulted in significant decrease in weight gain (%) but the weight gain were increased in the mercuric chloride with *Spirulina platensis* diet from 6.89% to 19.53 % and the growth rate was increased from 3.09 to 7.05 (mg/g live fish/day) as the percent dose of *Spirulina platensis* increased. Mercuric chloride exposed *Labeo rohita* fed *spirulina* supplemented diets might have eliminated the copper from the body tissues through feces, *Spirulina* reduced genotoxicity and oxidative stress of several antibiotics in mice (Premkumar, 2004) and lead (Pb) toxicity in rats (Upasani, 2003).

The present study showed that feeding and growth parameters improved in mercuric chloride exposed fish fed *Spirulina*-supplemented diets. *Spirulina* reduced mercuric chloride accumulation in tissues and increased mercuric chloride elimination through feces, lessening the metal burden and its toxicity to fish. The reduced growth rate in fish given a sublethal level of mercuric chloride was probably due to the tissue burden of mercuric chloride which, in turn, could have caused a reduction in feed intake, an increase in metabolic cost, or poor food conversion efficiency. Growth reduction in copper-exposed *Salmo gairdneri* was partly due to increased metabolic costs and reduced food consumption (Lett *et al.,* 1976).

Supplementation of *Spirulina* in the diet, improved the food utilisation parameters in mercuric chloride exposed fish. The feed conversion ratio (FCR) value of fish belonging to T4 groups was low as (5.10) as compared to other groups other groups and close to the FCR value of control fish. (Table-1). It was due to the *Spirulina palatensis* which reduced the accumulation of mercuric chloride in tissues in elimination of accumulated metal through faces, lessening the metal burden and its toxicity on fish. Spirulina contains phycocynin (14%) cholorophyll (1%) and carotenoid (0.37%) pigments. (Henrikson, 1994) B-carotene of Spirulina maintains the mucous membrane firmely (Henrikson, 1994) and thereby entry of toxic elements in to the body is prevented. Chlorophyll of *Spirulina* acts as a cleasing and

detoxifying phytonutrient against the toxic substances (Henrikson, 1994).

It indicates that *Spirulina* has the ability to eliminate and detoxify and accumulated mercuric chloride and it was proved by improvement of feeding and growth parametrers in sublethal exposure of *Labeo rohita* fed *Spirulina patensis* supplementation diets. Working on rainbow trout *Salmo gaiedheri*, (Lanno *et al.,*1985) found that high level of dietary ascorbic acid (10g Kg-1 diet) improved the body weight gain in copper exposed fish as compared to fish fed on low levels on ascorbic acid (0.9g kg-1 diet).

CONCLUSION

The present study shows that, the dietary supplementation of *Spirulina* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita* and improved the haematological parameters like RBC count and haemoglobin content significantly as the percent dose of *Spirulina platensis* was increased in a short period of time.

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RESEARCH ARTICLE

Seasonal activity rhythms in a land Slug, *Semperula maculata*

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ABSTRACT

Mollusca is the largest group of organisms after Arthropoda. Slugs, *Semperula maculata*are molluscs without shell. Field survey was made from Satpuda Mountain area and Residential / plane area near and around Ranipur dist-Nandurbar during 2014. These slugs are hermaphrodite and lay eggs in clutches. They lay about 25-30 eggs in a chain found in decaying humus or under stones and decaying leaves of surrounding plants in their habitat. Slugs in various habitats were observed with various length and sizes groups. Studies on maturation and correlation of relationship between diameter and standard length of a slug is r^2=0.0021 whereas between PH of soil and number in plane area is r^2=0.85 and in mountain area is 0.53.

Keywords: Satpuda, seasonal changes, correlation, length-size groups.

INTRODUCTION

Slugs are economically important to man as they cause damage to crop plants, garden plants and forestry. Slugs prefer moist shady and decaying zone of land. Slugs predominantly preferscold placesof environment (Moens and den bruel, 1960, Godan 1983). Slugs are moist and slimy and are more active in monsoon. Slugs,*Semperula maculata*feeds on plant juices from a variety of different species, including some commercial crops. This slug releases much mucus as on offence when disturbed. Many slugs in India and Europe lay eggs in late monsoon and in winter. (Getz, 1959, Kulkarni, 1973).

The biological rhythms of course aredependent on the varied photoperiods and seasonal cycles. Photoperiods play a key role in metabolism (Newell, 1966, Morton, 1979; Magare and Kulkarni, 1993a; 1993b and Panigrahi, 2000). *Semperula maculata* is a pest land slug feeds mostly on cabbage and potatoes. This slug also serves as a pest of horticulture and forestry as it feeds on varieties of vegetables like, tomato, brinjal, cucumber, etc. and some germinating seeds in forest. These are mostly found in association with the slug, *Laevicaulis alte* which is a pest slug of agriculture and horticulture. Raut and Panigrahi, 1988).

In most of the invertebrates a large number of environmental factors affects in various ways. (Magare, 1993.) The relationship between temperature changes and metabolism in a land slug, *Laevicaulis alte*was studied by Kulkarni (1973). As the climate is different in plane and mountain area, the shape and size of slugs found to be different. In present work an

attempt was made to study the seasonal activity pattern in a slug, *Semperula maculata*. By studying maturation, length size relation in plane and mountain area in and around Satpuda Mountains of Nandurbar district.

MATERIAL AND METHOD

Study area: Randomly scattered in and around the area of Ranipur, Mhasawad and Toranmal in Maharashtra, India. The area covers Satpuda Mountains, dense forest, plane are with some residential area distributed frequently at the foot of Satpuda Mountain area. Toranmal is a dense forested mountain area towards North to Ranipur and Mhasawad area is on plane ground and towards south to Ranipur. Both sites have good habitat for slugs feeding and breeding. Observations and data collection were made in field area during July to December, 2014.

Sample Collection and Observation: The slugs, *Semperulamaculata* were collected by hand picking using gloves to prevent infection from slugs. Sample collection was made in field area from two sites i.e. Mountain area (Toranmal and Lenghapani) and Plane area (Mhasawad and Ranipur). The catch from each sampling stations was recorded from 1x1 sq. meter quadrant. The average of three quadrants was taken as a unit of study. After counting the numbers the data of length and diameter of the creeping slug is noted. The identification of slug was carried out as per the records of samples previously identified by Zoological Survey of India, Kolkata. During study period the number of eggs laid by slug is noted. Simultaneously the data on environmental parameters like temperature, PH of soil, humidity, etc. recorded. Observations on copulation and egg laying were made at night also by using torch.

RESULT AND DISCUSSION

Taxonomy:

Land slug *Semperula maculata* is a gastropod mollusk belongs to family veronicellidae of the order systellomopha. The species found randomly scattered in Ranipur area and are abundant in plane area than in mountain area. *Semperula maculata* is an endemic to India and found widely distributed throughout India. (Fig.1)

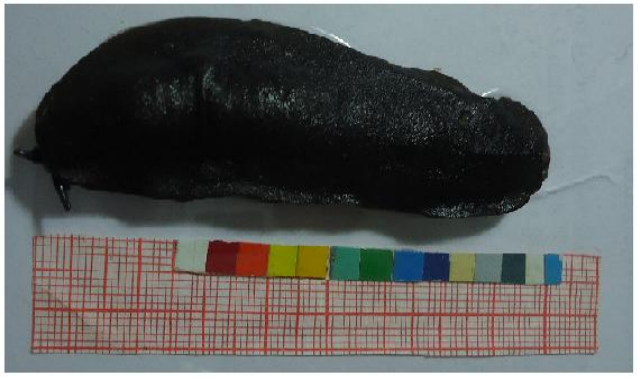

Fig. 1: Land slug, *Semperula maculata* found in satpuda mountain area.

Soil Parameters:

To study the ecology of slug, *Semperula maculata*the soil parameters from the study site was made. Mostly the slugs prefer low temperature ranges between 22-26 °C. and alkaline soil rich in organic carbon.

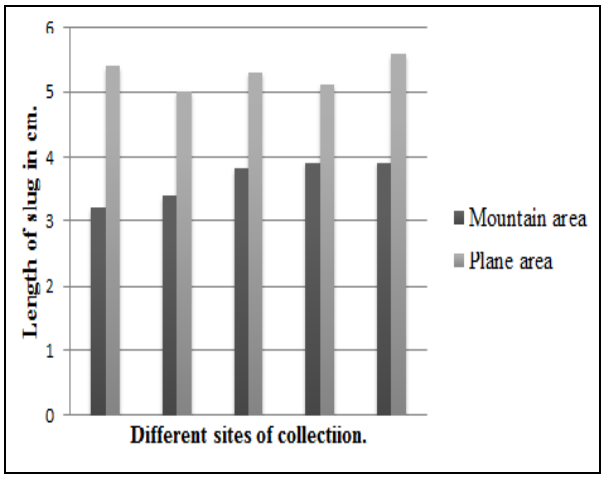

Fig. 2: length size relationship of a slug, *Semperula maculata* form Satpuda mountains

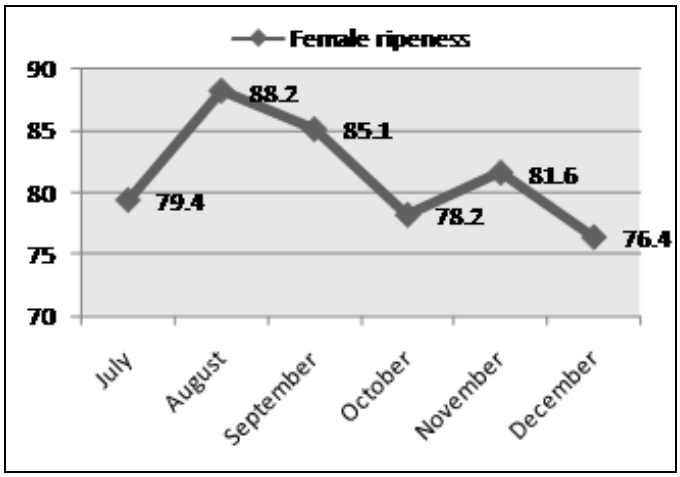

Fig.3: Monthly changes in Female ripeness of a slug, *Semperula maculata*.

The PH of soil ranges between 7.1 to 7.9 in various sites of collection in mountain are whereas it was less alkaline in plane are and ranges between 6.6 to 7.4 The slugs are abundant in moist and humus rich soil. Slugs are few in mountain area whereas are abundant in plane area.

Shape and size relationship:

The slugs were collected from plane area ranges in length size between 5.0-5.6 cm. whereas those from Mountain area are ranges between 3.2-3.9cm. The significant differences between the standard length sizes of the slugs might be due to feeding competition as the population is more, available food quality, more enemies and changed climate in their habitat. The population correlation of the slugs in both the area is r^2 = 0.0021 in plane area and r^2 = 0.5329 in mountain area (Fig.2).

Ripening and egg laying:

The slugs are nocturnal. During day time they hide inside stones, wood or decaying leaves and come out their dwellingsan hour after the sun set and remains active up to early morning. They prefer late monsoon and early winter for breeding and egg laying. Slugs lays about 25-30 eggs in the form of a beaded string in a mucous jelly. The number of adult slugs, *Semperula maculate* in a set of samples showing growth and development of body and reproductive organs as per the arbitrary criteria and development of reproductive organs.

Slugs were collected and maintained in an animal house for observation in nylon net of 1x2 meter on natural grassland and vegetation ground. They were fed with pieces of vegetables like potatoes, brinjal, tomato, cabbage, etc. The reproductive behavior of slugs shows secretion of dart with swellings of erected penial apparatus in body. The excited partners are approaches towards one another and communicate the confirmation by tentacles. Secondly they release excess mucus and moving both mating partners one behind another in a small circle on a ground. Finally male matured releases sperms in the vagina of female matured slug. Copulation lasts for about 40-50 minutes (Fig.3).

Rainfall is more apparent among all environmental factors which cause meaningful changes in reproductive activities of slugs. The mating process among terrestrial slugs was observed in a slug, *Arion empiricorum* (Kunkel, 1900), and*Limax* and other limacids (Gerhardt, 1934). In *Semperula maculata* usually courtship occurs after circular movement on ground and then winds both partners close together for copulation. The present results partly correlated with the finding of Kunkel (1900) on Limax slug.

Seasonal changes in Temperature and PH influences reproduction in a slug, *Semperula maculata*. They also prefer alkaline soil and show more population density whereas in area where humidity is not in favorable range the density of slugs is less. They prefer moist and cold environment which is in favorable range during monsoon, so rainfall is the key factor regulating maximum activity of a slug, *Semperula maculata*. Present findings correlates with the work of Panigrahi (2000).

Slugs are more careful and sensitive regarding the maintenance of body water percentage. Huddling of slugs during aestivation might be an act of conserving body water (Richter, 1976)*Semperula maculata* prefers 17-20 $^{\circ}$ C. (Moens and dVan elen Bruel, 1960) Whereas slugs *Limax flavus*prefers, 21-27 $^{\circ}$ C. range of temperature. The slugs cannot tolerate continuous high temperature and they undergoes deep in soil or inside stone, wood or any suitable substratum.

The activity of many slugs shows rhythmicity which is endogenous (Lewisa, 1969). Slugs *Semperula maculata*are very active at night and in shady and cloudy climate. The results correlate with the findings of white (1959). The activity rhythms of *Semperula maculata*are more in evening and night. The greatest activity rhythms are fond in a slug, *Deroceros reticulatus* during night and mid night. The rhythmic activities of slugs are also controlled by humidity and rainfall.

CONCLUSION:

Field survey of Land Slugs, *Semperulamaculata* was made from Satpuda Mountain area from Residential / plane area near and around Ranipur dist. Nandurbar during 2014. These slugs are hermaphrodite and lay about 25-30 eggs eggs in decaying humus. Slugs in various habitat were studied on maturation and correlation of relationship between diameter and standard length of a slug. The statistical correlation observed between length size is r^2=0.0021 whereas between PH of soil and number of samples in plane area is r^2=0.85 and in mountain area isr^2= 0.53.

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RESEARCH ARTICLE

Histomorphological study of the male reproductive system in the Indian drone Honeybee, *Apis cerana indica* (Hymenoptera)

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ABSTRACT

The internal male reproductive system in *Apis cerana indica* was consists of a paired testis, vasa deferentia, mucus gland and a median ejaculatory duct. It was observed that each testis internally packed with seven tube-like follicles compactly filled with the cysts. The spermatogenic stages mainly occurred in the pupal stages. The seminal vesicle represented by a distal large sac-like region of the vas deferens. It consists of an inner epithelial and outer thick muscle layers. The epithelial layer was consists of tall, columnar cells with brush border towards the lumen filled with a large mass of sperm bundles. The paired mucus glands were large, sac-like structures representing a peculiar type of male accessory glands. The epithelial layer of mucus gland was composed of tall, columnar glandular cells which showed their secretory nature. It was noticed that the mucus gland and the seminal vesicle has mesodermal in origin. The mucus glands open into a common ejaculatory duct via lateral ejaculatory ducts. The ejaculatory duct was a long slender tube opens distally into an aedeagus. The wall of the ejaculatory duct was differentiated into outer broad epithelial layer and inner cuticular layer. The inner thin cuticular layer bears elongated spines in the lumen. The presence of cuticular layer showed that the ejaculatory duct is ectodermal in origin.

Keywords: drone honeybee, *Apis cerana indica*, male reproductive organs

INTRODUCTION

In social hymenopterans (ants, bees and wasps), the reproductive systemin males has received little attention when compared to that of queens and workers on the basis of colony performance. They play the major role in colony formation, but still neglected (Bishop, 1920; Snodgrass, 1956). The male reproductive system in these social insects constitutes paired testes opens into the male gonopore through variable modified reproductive tract. Certain glands also associated to the reproductive tract, which produce secretions that are helpful to the sperm (Chen, 1984; Davey, 1985; Gillot, 1988; Chapman, 1998). Also in bees, the seminal vesicle and the sex accessory gland represents the primary sperm storage bag where the sperms are stored until mating. Their secretion may affect physiological and

behavioral changes in mated female (Bishop, 1920;Snodgrass, 1956; Ferreira *et al*., 2004). Physiological and biochemical studies of reproductive glands in hymenopterans have shown variety of basic features in spite of the morphological diversity of these glands (Davey, 1985; Happ, 1992; Baer *et al*., 2003; Sawarkar and Tembhare, 2010; 2014).

Ferreira *et al* (2004) noticed that the internal reproductive organs of 51 bees are variable histo-morphologically. On the basis of origin, morphological pattern and physiological role, the reproductive apparatus differentiated into Type I, Type II and Type III.

As per the focus on recently demonstrated importance of the male secretory substance and their role associated to the sperm and female tract, the present study undertake the histomorphological differentiation of the internal reproductive organs of drone honeybee in *Apis cerana indica*.

MATERIAL AND METHOD

During the present study, the adult drones of *Apis cerana indica* were collected from the hive established at the premises of the CES College, Chalisgaon, Dist-Jalgaon (India) during the year 2010 to 2013. The internal reproductive organs of the drone honeybees were dissected in the insect Ringer solution. The tissues then fixed immediately in Bouin's fixative for 18-24 h, dehydrated in ethanol, cleared in xylene and then embedded in paraffin wax at 58-60º C. The sections were cut at 4-6 µm thickness. The sections were stained with Ehrlich's Haematoxylin Eosin (HE) and Heidenhain's Iron haematoxylin-orange G (Fe-H) histological techniques (Tembhare, 2006).

RESULT AND DISCUSSION

The internal male reproductive system in *Apis cerana indica* consists of a pair of testes, a pair of vasa deferentia, a pair of accessory sex glands and a median ejaculatory duct (Fig. 1). It is also observed in other honeybees as *Apismellifera* (Bishop, 1920; Snodgrass, 1956; Woyke, 1958; Simpson, 1960; Koeniger, 1986), *Apisdorsata* (Paliwal, 1993) and *Apisflorea* (Koeniger *et al*., 1989).

The testes are creamy, oval-shaped bodies lying at anterior side of the mucus glands and situated in between the 2nd and 3rd abdominal segments. The numbers of testicular follicle are seven in *A.c. indica*and arevariable in number in other hymenopterans (Snodgrass, 1956; Wheeler and Krutzsch, 1992; Duchateau and Mariën, 1995; Ferreira *et al*., 2004). Each follicle is composed of an inner layer of epithelial cells and an outer layer of muscle fibres. At the inner side consist of number of cysts with full of spermatogenic stages (Fig. 2,2a). Each follicle had opened posteriorly into the vas eferens and then into a long, coiled vas deferens. It is differentiated into three regions, the apical short coiled tube, middle cylindrical seminal vesicle (SV) and distal straight duct. The apical part of vas deference consists of outer circular muscle layer and inner epithelial layer with large lumen (Fig. 3). The distal part opens into the basal region of the mucus gland (MG) with the muscular valve (Fig. 4). The SV represents distal large sac-like region of the vas deferens which bears an inner epithelial and outer thick muscle layers and externally covered with a thin peritoneal sheath. The muscle layer is composed of outer longitudinal and inner circular muscle layers. The epithelial cells are tall and columnar with brush border towards the lumen. The lumen is filled with a large mass of sperm bundles, keeping their heads towards the wall and tails at the center of the lumen (Fig. 5, 5a).

The paired mucus glands (MG) are large, kidney-shaped, sac-like structures representing a peculiar type of male accessory glands in the bee. Each gland consists of larger distal and a narrow proximal part with a constriction in between. Each gland is tapering basally and opens into the ejaculatory duct (ED) through a well-defined valve. The wall of the MG is composed of an inner epithelial layer and outer thick muscle coat and externally covered with thin peritoneal sheath. The epithelial layer is formed of tall, columnar glandular cells. The muscle coat is formed of three sublayers, the outer longitudinal, middle circular and inner longitudinal muscle layer. The lumen of the MG is filled with a variable amount of secretory material (Fig. 6, 6a).

The MG opens into the paired, lateral ED and then into a long slender common ED. The wall of the ED is composed outer broad epithelial layer and inner cuticular layer. It does not represent outer muscular layer. The epithelial layer is composed of squamous epithelial cells bearing nuclei at the base and scanty cytoplasm towards the perikarya. The inner thin cuticular layer bears elongated spines in the lumen. The lumen is large measuring about 289.7±2.763 µm in diameter and filled with a mass of sperms in the mature drones (Fig. 7, 7a).

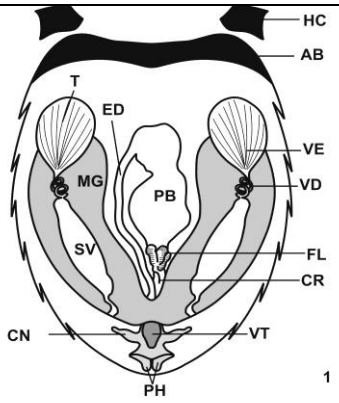

Fig. 1: Anatomical organisation of the male reproductive system (MRS) in *A. cerana indica*. (diagramatic)
Abbr.: AB- - Abdomen, CR-Cervix, CN-Cornua, ED-Ejaculatory duct, FL-Fimbriated lobe, HC- Hind coxa, MG-Mucus gland, PB-Penis bulb, PH-Phallotrem, SV-Seminal vesical, T-Testis. VD-Vas deferens, VE-Vas eferens, VT-Vestibule.

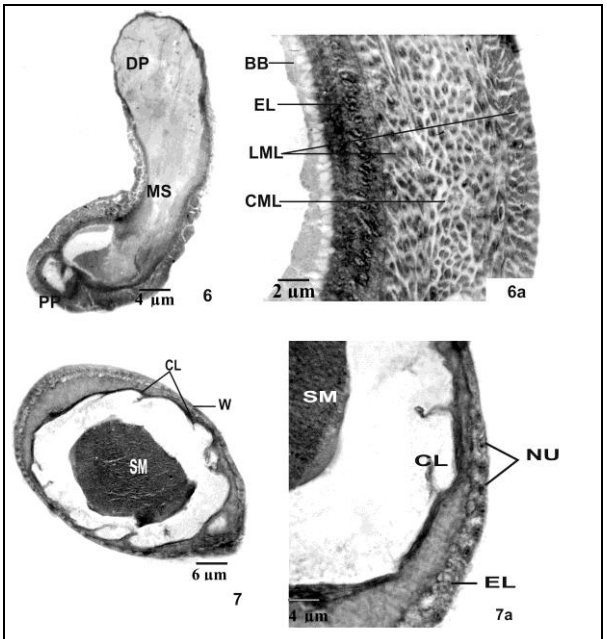

Fig. 6 : Longitudinal section of Mucus gland, Fig. 6a: Magnified view MG
Fig. 7 : Cross section of Ejaculatory duct, Fig. 7a : Magnified view of Fig. 7
Abbr. : BB- Brush border, CL- Cuticular layer, CLM- Circular muscle layer, DP-Distal part, EL- Epithelial layer, F-Follicle, L-Lumen, LML- Longitudinal muscle layer, MS- mucus secretion, NU- Nucleus, PP-Proximal part, SM-Secretory material, W-Wall.

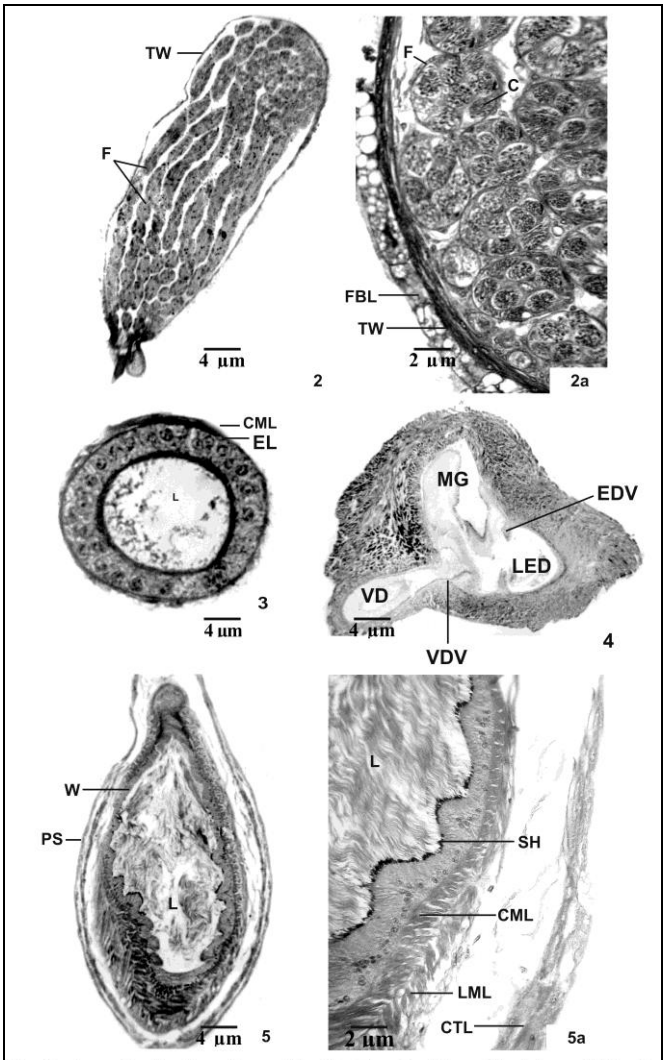

Fig. 2 : Longitudinal section of Testis, Fig. 2a : Magnified view of Fig. 2
Fig. 3 : Cross section of vas deferens, Fig. 4 : Basal region of MG
Fig. 5 : Longitudinal section of SV, Fig. 5a : Magnified view of Fig. 5
Abbr. : CML- Circular muscle layer, CTL-Connective tissue layer, EL-Epithelial layer, F-Follicle, FBL- Fat body layer, L-Lumen, LML-Longitudinal muscle layer, PS-Peritoneal sheath, SH-Sperm head, SM- Secretory material, TW- Testis wall, W-Wall.

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The histological structure of the vas deferens (VD), seminal vesicle (SV) and mucus gland (MG) suggests their mesodermal origin while that of the ejaculatory duct (ED) as the ectodermal origin in *A. c.indica*. The epithelium of the SV and MG of *A. c. indica* is secretory and the inner surface bears the brush border, similar to that in *A. mellifera* (Bishop, 1920; Snodgrass, 1956) and *A. dorsata* (Paliwal, 1993). It is also noted that the wall of SV as well as MG in *A. c.indica* becomes thick and muscular due to the presence of longitudinal and circular muscle fibres which may initiate rhythmic contraction to facilitate transport of spermatozoa towards the ejaculatory duct. The opening of MG and median ED are moreover, provided with well-defined valves in *A. c. indica*, which seems to be a common feature in the honeybees (Snodgrass, 1956; Paliwal, 1993) in order to control consecutive release of semen as well as mucus gland secretion.

CONCLUSION:

It is concluded that in *A. c. indica* the internal male reproductive system consists of a paired testis, seminal vesicle, mucus gland and an ejaculatory duct.

The testes are elongate, oval-shape, creamy white bodies observed during the pupal stages and consists of seven follicles.

The testis becomes shrink due to complete release of spermatozoa shown in adult drones. It is also noticed that the size of the SV and the MGincreases in adults which may associated to the storage of sperms until the mating period. While the secretion of the mucus glands which may involve in the longevity of sperm and sperm storage. The SV and the MG consist of outer muscle coat and inner smooth columnar epithelial layer. While the ejaculatory duct composted of outer epithelial and inner cuticulin layer which shows that the SV and MG are mesodermal and ED is ectodermal in origin.

It may helpful to understand the reproductive physiology of drone in this species and other related Hymenoptera species.

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RESEARCH ARTICLE

Effect of Vincristine on some biochemical parameters in male Albino Rat

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ABSTRACT

Vincristine ((C_{46} H_{56} N_4 O_{10})) is one of the most widely used effective oncostatic drug. It is an indole-indolin alkaloid from periwinkle plant vincarosea. The drug was administered intravenously to six adult male rats at dose levels of 0.06 and 0.12 mg/KgBW/day respectively. The object of the present work is to study the Vincristine effect on the reproductive accessory gland and testes. Vincristine prevents metastatic growth by preventing the formation of spindle fibers, thereby arresting mitosis without affecting replication of DNA. Vincristine affect on biochemical parameters. The total protein, citric acid and fructose decreased significantly in low dose and high dose treatment as compare to control ($P < 0.001$)). The body weight and organ weight also significantly decreased in low and high dose regimen as compare to vehicle treated control. From the foregoing mono-therapeutic study, it is concluded that this chemotherapy schedule belongs to the category of anti-gonadotropic and anti-androgenic.

Key words: Vincristine, Biochemical, Albino rat

INTRODUCTION

The vinca alkaloids are cell-cycle specific agents and, in common with other drugs such as colchicine, podophyllotoxin and taxanes, block cells mitosis (George *et al*., 1965; Bensch and Malawista, 1968; Dustin, 1984). The biological activities of these drugs can be explained by their ability to bind specifically to tubulin and to block the ability of the protein to polymerize into microtubules. Through disruption of the microtubules of the mitotic apparatus, cell division is arrested in metaphase. In the absence of an intact mitotic spindle, the chromosomes may disperse throughout the cytoplasm (exploded mitosis) or may clump in unusual groupings, such as balls or stars. The inability to segregate chromosomes correctly during mitosis presumably leads to cell death.

MATERAILS AND METHOD

The six adult male Rats weighing between 284 to 360 gms were selected for the present study collected from R.C. Patel Pharmacy College Shirpur.

Table 1: Experimental Design for Vincristine Treatment

Number of animals and sex	Treatment	Dose (mg/Kg BW)	Route	Duration
6 males (Experimental)	Vincristine	0.06 mg daily	I.V.	10 days
6 males (Experimental)	Vincristine	0.12 mg daily	I.V..	10 days
6 males (Control)	Saline	E.V.	I.V.	10 days

(Abbreviations: - E.V. = Equal Volume, I.V. = Intra Venous, BW=Body weight)

Even though the order Rodentia is highly exploited for the experimental purposes are easily available and can undergo all the laboratory tests without any difficulty, hence is suitable for experimental work. For the present study adult male rat weighing between 284 to 360gms were collected in wire mesh cages. The animals were kept in captivity in the wire mesh cages experiencing natural daylight and temperature. After a week of acclimatization to laboratory conditions, the animals were used for different sets of experiments.

Biochemical Assay

The concentration of proteins, fructose and citric acid in the testis and accessory reproductive organs were carried out.

Preparation of homogenate

Dry weights of the reproductive organs were taken before proceeding for the bio-chemical analysis so that the estimated value of enzymes and substrates can be calculated / gram dry weights of the reproductive tissue. The homogenate was prepared in 10 ml of distilled water as well as 10ml of saline. The tissue was ground with mortar and pestle. Clear supernatant obtained after centrifugation at 3000 r.p.m. was used for various biochemical assays.

a) Estimation of Protein

Biochemically protein was estimated by the method of Lowry *et al*., (1951).

b) Estimation of Citric Acid

Biochemically citric acid was estimated by the method of Ettinger *et al*., (1952).

c) Estimation of Fructose

Biochemically fructose was estimated by the method of Foreman *et al*., (1973).

RESULT AND DISCUSSION

For the present work anti cancer drugs, Vincristine (VCR) was studied.

Vehicle treated control

Body Weight :

The body weight varies from 284 gms to 360 gms in a mature adult.

Organ Weight:

During active breeding period, the weight of testis was 1.385 to 1.390 gms., seminal vesicle was varied from 0.210 to 0.240 gms . and prostate was 0.320 to 340 gms.

Biochemical study

Protein

The value of protein in control animal was found to be 2.370 (range 2.360 – 2.380 mg/gm, Table-4 & bar diagram) was recorded (P<0.001).

Citric acid

In vehicle treated control the value of citric acid was 0.545 mg/gm (range 0.535-0.555) Table-4 & bar diagram) (P<0.001).

Fructose

Significant decrease (range 1.118-1.138.920 mg/gm, Table-4 & bar diagram) in the fructose content was noticed (P<0.001).

Low dose treatment

Body weight

The total body weights of all the animals treated with 0.06 mg/kg BW/day showed significant decrease (P<0.05) as compared to control animals (Table-2 & bar diagram).

Organ weight

0.06 mg/kg BW/day for 10 days registered a decrease in the testis, prostate and seminal vesicle as compared to control animals (Table-3 & bar diagram).

Biochemical study

Protein

A significant decrease in the protein concentration (range 1.910 – 0.930 mg/gm, Table-4 & bar diagram) was recorded (P<0.001).

Citric acid

This treatment resulted into significant decrease (range 0.420-0.440 mg/gm, Table-4 & bar diagram) in its citric acid content (P<0.001).

Fructose

Significant decrease (range 1.097-1.117 mg/gm, Table-4 & bar diagram) in the fructose content was noticed (P<0.001).

High dose treatment

Body weight

The total body weight of all the animals treated with 0.12mg/kgBW/day for 10 days showed significant decrease (P < 0.01) in their body weight as compared to low regimen treated group (Table-2 & bar diagram).

Organ weight

In high dose treatment of Vincristine with 0.12mg/kgBW/day for 10 days registered a decrease in testes, seminal vesicle and prostate weights (P<0.05)) as compared to low dose treated animals. (Table-3 & bar diagram).

Biochemical study

Protein

The total protein content after treatment dropped significantly (range 0.620-0.640 mg/gm, Table-4 & bar diagram) (P<0.001).

Citric acid

The concentration of citric acid showed significant decline (range 0.230-0.250, Table-4 & bar diagram) (P<0.001).

Fructose

The concentration of fructose after administration of VCR registered an increase (range 0.965-0.985 mg/gm, Table-4 & bar diagram) (P<0.001).

Table 2 & Fig. 1: Effect of 0.06 mg and 0.12 mg Vincristine daily for 10 days on initial and final body weights of male albino rats (values are mean ± SE, figures in parenthesis are number of animals used)

Treatment	Total body weight	
	Initial	Final
Control (6)	324±6.4	330 ± 14
0.06mg/kg BW daily 10 days (6)	310 ± 6.4	303 ±14
0.12mg / Kg BW daily 10 days (6)	340 ± 7.6	320± 12*

P value < 0.05

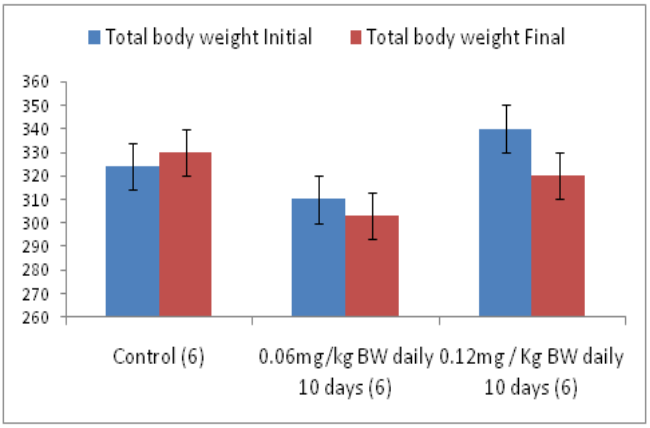

Table 3: Effect of 0.06 mg and 0.12 mg/kg BW Vincristine daily treatment for 10 days on organs weight of male albino rats (values are mean ± SE, figures in parenthesis are number of animals used).P value < 0.05

Treatment	Total Organ weight		
	Testes	Seminal vesicle	Prostate
Control (6) Control	1.385±0.33	0.210±0.028	0.320±0.0081
0.06mg/kg BW daily 10 days (6)	1.373±0.034	0.206±0.027	0.315±0.0081*
0.12mg / Kg BW daily 10 days (6)	1.360±0.32	0.202±0.020	0.308±0.0081

Table 4: Effect of 0.06 mg and 0.12 mg/kg BW Vincristine daily treatment for 10 days on biochemical parameters of male Albino rats (values are mean ± SE, figures in parenthesis are number of animals used).

Treatment	Protein	Citric acid	Fructose
Control (6)	2.370±0.02	0.545±0.04	1.128±0.02
0.06mg/kg BW daily 10 days (6)	0.920 ±0.03	0.430±0.03	1.107±0.02
0.12mg / Kg BW daily 10 days (6)	0.630 ±0.02	0.240±0.03	0.975±0.02

P value – P < 0.001

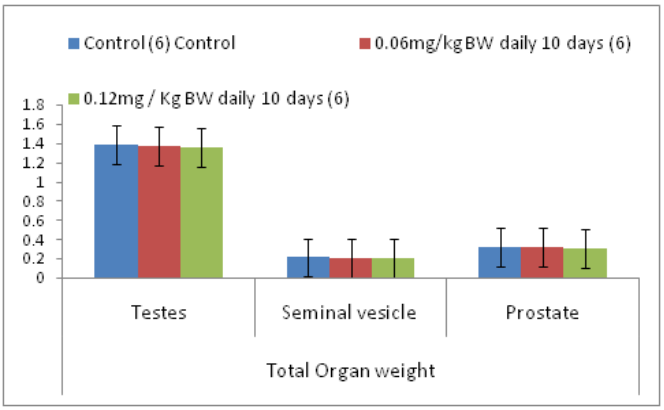

Fig. 2: Effect of 0.06 mg and 0.12 mg/kg BW Vincristine daily treatment for 10 days on organs weight of male albino rats (values are mean ± SE, figures in parenthesis are number of animals used).P value < 0.05

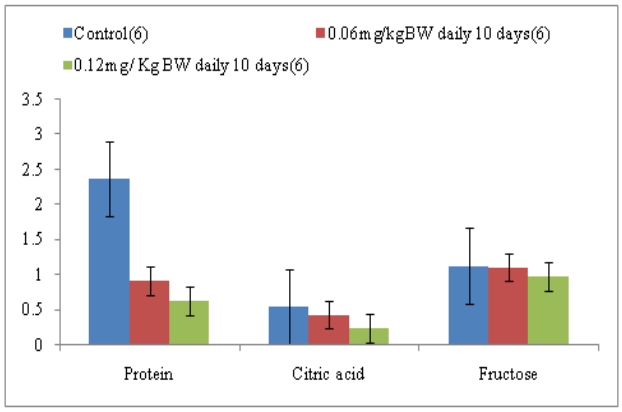

Fig. 3: Effect of 0.06 mg and 0.12 mg/kg BW Vincristine daily treatment for 10 days on biochemical parameters of male Albino rats (values are mean ± SE, figures in parenthesis are number of animals used).

DISCUSSION

Body weight

It may be suggested that a reduction in the total body weight with these chemotherapeutic drugs (VCR) treatment may be due to decline in the circulating blood serum androgen since androgen are a potent stimulant of nitrogen retention and their administration readily leads to an increase in body weight in both men and women (Kochakian, 1950; Forbes, 1985; Bhasin *et al*., 1997).

Organ weight

A reduction in the weights of testis and the accessory organs or glands of the Vincristine treated rats (both the low and high dose treated groups) points to reduced androgen levels of androgen binding protein (ABP) in the testis and a reduction in the circulating androgen. This is because the biosynthesis and secretion of ABP appears to be regulated by both FSH and androgens (Tindall and Means, 1976; Buchanan and Riches, 1986).

Proteins

Proteins are major constituent of animal tissues and show considerable variation during different metabolic states and play important role in reproductive physiology. It also acts as a source of plastic material and carriers of enzymes, antigens, hormones and other essential substances also play important physiological role for the tissue development. Proteins are involved in a number of important cellular activities (Brachet, 1940; Caspersson, 1941). Vincristine resulted in to decreased in protein content in male squirrel (Chaudhari and Sastry, 2014).

In the present study treatment with vincristine, the significant decrease ($P < 0.001$) in the total protein content with low dose treatment (0.06 mg / Kg BW/day) and with high dose Vincristine (0.12 mg/Kg BW/day) was observed. VCR treatment studies manifested antiandrogenic and antifertility effects in intact male albino rats.

Citric Acid

Citric acid is a tri-carboxylic acid and like dicarboxylic acids, it is not metabolized by sperm since it is unable to cross the plasma membrane. Even though it is frequently included as a buffer in diluents used for sperm storage, its normal role in seminal biochemistry is not at all clear. Citric acid is a well known chelating agent of divalent cations and may protect sperm from heavy metal poisoning, to which sperms are particularly susceptible (White and Holland, 1977). In view of the importance of calcium in uptake for the acrosome reaction seminal citrate may, due to its chelating role, prevent the acrosome reaction from occurring prematurely as studied by Yanagimachi and Usai (1974) in bull.

A significant decline in citric acid ($P < 0.001$) content with low dose and high dose VCR resulted. Decrease in the citric acid concentration is associated with the degenerative changes of spermatogenic elements (Dixit *et al*., 1979).

Fructose

Estimation of fructose values in very important during the study in male reproduction because fructose concentrations have been assumed to be good and easily accessable indices of androgenic activity and Leydig cell function (Phadke *et al*., 1973). Beside this seminal fructose provides an indication of size, storage and secretory activity of seminal vesicle (Mann and Lutwak – Mann, 1951).

In the present study treatment with vincristine, the significant decrease ($P < 0.001$) in the fructose content with low dose treatment (0.06 mg / Kg BW/day) but significant increase ($P < 0.001$) with high dose Vincristine (0.12 mg/Kg BW/day) was observed.

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RESEARCH ARTICLE

Effect of anticancer drug Vincristine on sperm morphology in Albino rat

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ABSTRACT

Vincristine (C_{46} H_{56} N_4 O_{10}) is one of the most widely used effective curative for cancer. It is an indole-indolin alkaloid from periwinkle plant. The drug was administered intravenously to six adult male rats at dose levels of 0.06 and 0.12 mg/KgBW/day respectively. The object of the present work is to study the Vincristine effect on the reproductive accessory gland and testes. This drug prevents metastatic growth by preventing the formation of spindle fibers, thereby arresting mitosis without affecting replication of DNA. Vincristine affect on sperm morphology and resulting into primary and secondary sperm abnormality. The different sperms abnormalities were found to be in head, mid-piece and tail. The higher percentages of abnormalities are amorphous head, coiled tail and hook less head. The percentages of sperm abnormalities are more significant in low dose and high dose treatment as compare to vehicle treated control. From the foregoing mono-therapeutic study, it is concluded that this chemotherapy schedule belongs to the category of anti--androgenic.

Keywords: Vincristine, Sperm study, Albino rat

INTRODUCTION

Vincristine (C_{46} H_{56} N_4 O_{10}) is one of the most widely used effective curatives for cancer. It is an indole-indolin alkaloid from periwinkle plant. Vincristine isan important clinical agent for treatment of leukemia's, lymphomas, and testicular cancer (Jorden *et al*., 1985). The biological activities of these drugs can be explained by their ability to bind specifically to tubulin and to block the ability of the protein to polymerize into microtubules. Through disruption of the microtubules of the mitotic apparatus, cell division is arrested in metaphase. In the absence of an intact mitotic spindle, the chromosomes may disperse throughout the cytoplasm (exploded mitosis)or may clump in unusual groupings, such as balls or stars. The inability to segregate chromosomes correctly during mitosis presumably leads to cell death due to chromosomal mutation.

MATERIALS AND METHODS

Animals and Treatment: Adult male albino rats of 200 to 250gms were obtained from the animal house facility of R.C.Patel Pharmacy College Shirpur

Shirpur. After a week of acclimatization to laboratory conditions Vincristine (C_{46} H_{56} N_4 O_{10}) dissolved in saline was administered intravenously. The control animals received same amount of saline (Table-1).The animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the organs were excised. Both the cauda epididymis wasutilized for sperm analysis.

The spermatozoa present in the cauda epididymis were collected after mincing/slicing the tissue in a cavity block containing 1ml of physiological saline and centrifuged at 600 rpm for 1 minute with a drop of 5% aqueous eosin (WHO, 1999). Coslab digital microscope with Phase contrast adjustment was used to observe the sperms. All evaluations were done at 25X, 45X and 100X.

Assessment of sperm morphology

The saline solution of cauda epididymis prepared for studying the sperm concentration was directly observed several times for assessing the sperm morphology.

OBSERVATIONS AND RESULTS

In the present study the Vincristine (VCR, Cytocristin) drug was used to find out the changes in the sperm of albino rat *Rattus rattus*. They include:

Sperm morphology

1 Head defects (Table-2)

Hook less head: The population of spermatozoa with hook less head sperm abnormality fig.3)was significantly increases in low dose ($p<0.01$) and high dose ($p<0.001$).

Banana shape head: The population of sperms with banana shape head abnormality (fig.4) was significantly decreased in low dose ($p<0.05$) and increased in high dose ($p<0.01$).

Amorphous head: The population of spermatozoa with amorphous head abnormality (fig.5) was significantly increased in low dose and high dose as compare to vehicle treated control ($p<0.001$).

Table 1: Experimental Design for Vincristine Treatment

Number of animals and sex	Treatment	Dose (mg/Kg BW)	Route	Duration
6 males (Experimental)	Vincristine	0.06 mg daily	I.V.	15 days
6 males (Experimental)	Vincristine	0.12 mg daily	I.V.	15 days
6 males (Control)	Saline	E.V.	I.V.	15 days

Abbreviations:- E.V. = Equal Volume, I.V. = Intra muscular, BW=Body weight.

Table 2: Effect of 0.06 mg and 0.12 mg VCR / day for 15 days on sperm morphology and percentage occurred of different sperm abnormalities (values are mean ± SE).

Sr. No.	Mean Sperms Abnormality(%)	Control	0.06mg / kg BW/ day for 10 days	0.012 mg / kg BW / day for 10 days
1	Hook less head	2.20±0.27	2.52±0.16 #	5.91±0.15###
2	Banana shape head	2.61±0.29	2.02±0.18 #	3.27±0.16##
3	Amorphous head	1.90±0.28	1.94±0.21#	13.30±1.44###
4	Pin head	0.99±0.11	1.51 ±0.11###	0.75 ±0.13#
5	Tailless head	0.43±0.19	2.53±0.17##	0.65±0.07#
6	Bent mid piece	3.80±0.17	3.73±0.23*	3.76±0.22*
7	Curved mid piece	2.71±0.23	1.53±0.26##	3.56±0.13##
8	Headless tail	1.26±0.21	1.35±0.25*	1.35±0.08*
9	Bent tail	3.10±0.12	4.06±0.72#	2.46±0.34#
10	Curved tail	0.95±0.10	4.27±0.18###	5.70±0.31###
11	Coiled tail	2.48±0.35	6.46±0.35##	4.44±0.16##
12	Looped tail	3.32±0.19	2.50±0.30#	2.43±0.14##
	Total mean sperm abnormality (%)	**25.75±0.35**	**34.42±0.36**###	**47.58±0.62**###

#p<0.05, ##p<0.01, ###p<0.001 and *Insignificant

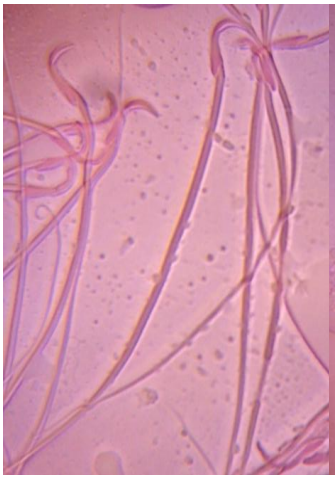

Fig.1a: Microphotograph showing swarms of normal sperms (arrow) X 1000.

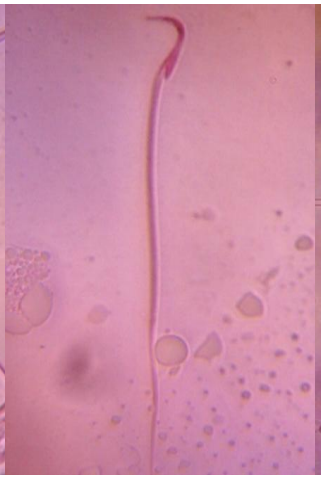

Fig. 1b: showing single sperm with normal head(arrow), mid piece (arrow head) and tail(long arrow)X 1000.

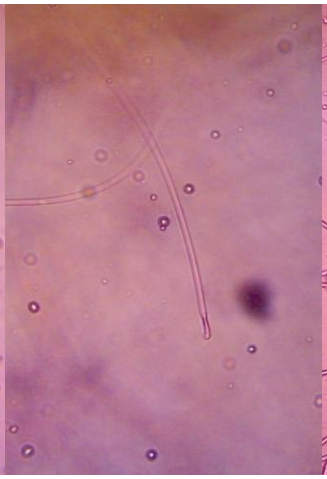

Fig. 3: Microphotograph of sperm with hook less head (arrow) X 400.

Fig. 4: Photograph of few sperms showing banana shape head (arrow) X400

Fig. 5: Photograph of sperm showing amorphous head (arrow) X 400.

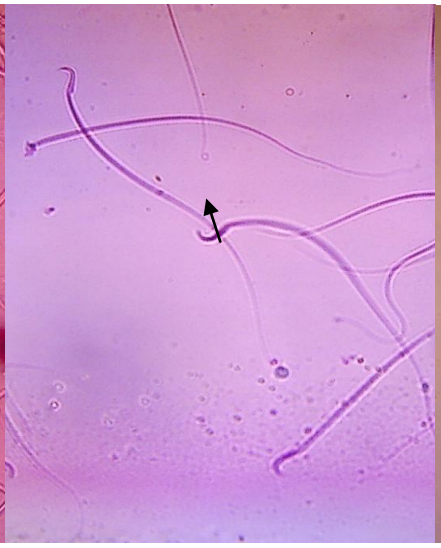

Fig. 6: Photograph with pin headed sperm (arrow) X 400.

Fig.7: Photograph of sperm showing tailless head (arrow) X 400.

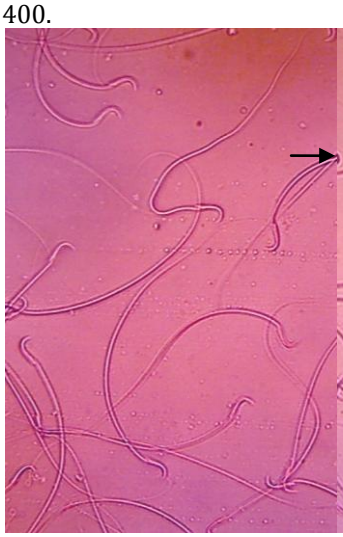

Fig. 8: Microphotograph showing sperm with bent mid piece (arrow) X 400.

Fig.9 : Photograph of sperm with curved midpiece (arrow) X 400.

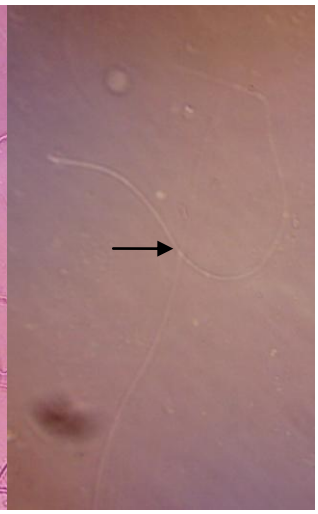

Fig.10: Photograph of sperm with headless tail (arrow head) X 400.

Fig.11: Photograph of sperm with bent tail (arrow) X 400.

Fig.12: Photograph of sperm with curved tail (arrow) X 1000.

Fig. 13: Microphotograph showing sperm with coiled tail (arrow) X 400.

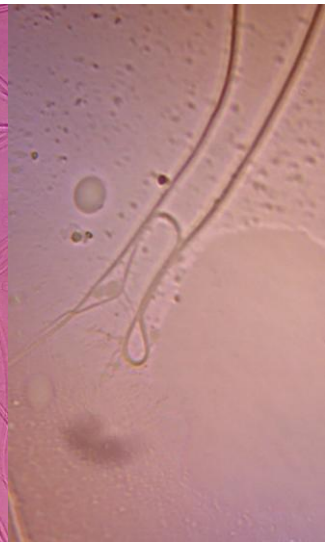

Fig.14: Photograph of sperm with looped tail (arrow) X 1000.

Pin head*:* Occurrence of pin head sperm abnormality (fig.6) was significantly ($p<0.001$) higher in low and high dose treatment as compare to control.

Tailless head*:* Sperm abnormality with tailless head (fig.7) had lesser in control group and it was significantly increased ($p<0.01$) in low dose and high dose treatment.

2 Mid-piece defect (Table 2)

Bent mid- piece: The spermatozoa of rats in control group show more sperms withbent mid piece(fig.8). The differences of means were not significant in low dose and high dose treatment.

Curved mid-piece: Occurrence of Curved mid-piece abnormality of sperms (fig.9) were significantly decreased ($p<0.01$) in low dose and further increased in high dose.

3. Tail defects:

Headless tail*:* Occurrence of headless tail sperm abnormality(fig.10) was not significant in low dose and high dose treatment.

Bent tail*:* The spermatozoa with bent tail abnormality (fig.11) was significantly increased (**$p<0.01$**) in low dose and decreased in high dose.

Curved tail *:* The sperm abnormality with curved tail (fig.12) was significantly increased (**$p<0.01$**) in low dose and high dose treatment.

Coiled tail*:* The population of spermatozoa with coiled tail (fig.13) was significant (**$p<0.01$**) in low and high dose regimen.

Looped tail*:* Occurrence of looped tail (fig.14) abnormality of sperms in control was significant ($p<0.01$) in low and high dose treatment.

DISCUSSION

The sperm morphology was used in this study to evaluate the effects of Vincristine by using albino rat model. The drug can affect on reproductive system resulting in the sperm production. Abnormal forms of spermatozoa occurred in all mammals (Mann & Mann, 1981). Vinca alkaloid is an anti-neoplastic and anti-carcinogenic drug. The drug arrest cell growth through its effects on cytoskeletal elements and inhibits spindle formation essential for normal cell division. Vincristine acts as a cytotoxic agent to differencing spermatogonia (Lu & Meistrich, 1979). Vincristine works as ancolytic agent, preferentially kill cells of specific stages of the spermatogonic pathway at doses with clinical range for human.

Abnormalities induced due to Vincristine in present study included primary and secondary abnormalities. In present study observed both the types, primary types reported in our results were hook less head, headless tail and tailless head. Secondary types of abnormality obtained in present study were, banana shape head, amorphous head, bent tail, curve tail, bent mid piece, coiled tail, looped tail and curved mid piece. Our results were in accordance to Saba *et al.,* 2009. In recent study I got one more additional types of abnormality includes pin head. Thus in the

present study Vincristine at dose level 0.06 mg and 0.12 mg resulted in abnormal morphology. The results were more predominant in high dose as compared to control and low dose treatments.

Vincristine is an anti-proliferative, radiomimetic, anti-carcinogenic drug. This drug arrests cell growth through their effect on cyto-skeletal elements and tubulin formation. As a result there is no formation of spindle which is essential for normal cell division. Acrosomal head shape sperms is disrupted from the normal by affecting the tubulin polymerization in the microtubule and by inhibiting axoplasmic flow (Avadhani and Kumar, 1994). Vincristine induced all the wide range of abnormalities depending upon dose level, amorphous head followed by bent mid piece and bent tail.

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RESEARCH ARTICLE

Species inventory of land Molluscs from Satpuda Mountains, India

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ABSTRACT

Diversity, Ecology and systematic of land molluscan species from Satpuda mountain area was studied during 2014-2015. Study area comprises 20 different sampling stations in about 200 sq. km zone of Satpuda Mountains in North Maharashtra, India. A total of 88 individuals belonging to 11 different species were collected from 20 collection spots in 10 localities. Eight families represent 11 species. A maximum of 03 species represented from Ariophantidae family, 02 from Cerastuidae and only 01 from Endodontidae, Vertiginidae, Pyramidulidae, Subulinidae, Camaenidae and Veronicellidae. Changes in species composition and abundance are related to natural forest site variations area (FA) and inresidential area in satpuda mountains (RA). The Shannon's diversity index shows maximum diversity index with 11 numbers of species is 2.13 in Forest area (FA) and 2.15 in Residential area (RA). The evenness values calculated statistically are 0.89in FA and 0.90 in RA. Need for biodiversity conservation is emphasized. These land mollusks are quite common in humified areas among Satpuda, mountain belts.

Keywords: Land molluscs, Satpuda, forest, Shannon's index.

INTRODUCTION

Study of fauna of mollusks provides crucial information on ecology of the region. In recent times the biotic fauna is greatly threatened by various human activities. Biodiversity conservation necessitates knowledge on the diversity of animals and plants with their distribution and ecological status. Satpuda mountain ranges of Maharashtra comprises forest zone close to Western Ghats. The estimated number in Indian subcontinent is around 5041 species out of the estimated species of 66, 535 species of the world. (Ramakrishna and Mitra, 2002).

Till date about 1487 species of land snails belonging to 32 families and 140 genera have been reported in India (Ramakrishna and Mitra, 2002). Indian gastropods have been studied by number of workers. Anandale (1919) and Prashad (1925) and Hora (1925 & 1926), Kulkarni (1971) Godan (1983) and Magare (2007) made some interesting observations on the hill stream molluscan fauna of various regions of Maharashtra. Mulkhedkar and Tonapi (1963) and Kulkarni (1973) published an account of landand freshwater Molluscs from Marathwada region. Raut and Ghose (1984) described various terrestrial mollusks of Nepal. Cooke et. Al, (1986) studied on mollusks. Recently Rao and Ghose (2001) have made survey of terrestrial mollusks from Nepal. Magare (2002 & 2006) studied on biodiversity of mollusksfrom tribal Zone of Nandurbar district.

Considering the paucity of information on land snails and severity of threat to them in India especially in satpuda mountain ranges, the need of the hour is to make an inventory and to study the distribution pattern of them. These results prompted me to make detailed survey and population dynamic study of land snails to fill up the lacuna of survey of landmolluscs fromForest and Residential area of Satpuda Mountains.

MATERIAL AND METHOD

For present study the survey was made from different vegetation types in and around satpuda mountain area. Grasslands, Forests, Rocky Mountains, marshy places, moist soil, agricultural lands, horticultural Zones, gardens, roadside dense vegetation, river-sides, grasslands and paddy fields were the exact locations of occurrence of shells and snails. The gardens, parks, cultivation area, vermin-culture center area and nurseries were always surveyed during different seasons of the year 2014-2015 from the foot of satpuda mountins and allied areas. The preliminary studies carried out at forest area of satpuda mountain area includes, Toranmal, Dhadgaon, Molgi, Walamba and Mandava whereas for residential area (Plane area) studies were carried out from Ranipur, Akkalkuwa, Taloda, Shahada and Nandurbar. Collection and observation from each site was made and from each site a quadrant of 1x1 meter was taken as the unit for population estimation of the snails and slugs. The snails and slugs found in these quadrants were collected manually by hand picking, using gloves to prevent infection. The snails were counted quadrant wise and species wise. The mean was calculated for each species and the number calculated for actual snail population per quadrant area. The shells were also collected and the study of the shells was made. The shape, colour and habitat of the gastropods samples from these collection sites were recorded for further morphological studies. Specimens were washed, dried and kept in plastic containers in small vials with cotton, for identification. The identification was made with the help of previously identified reports of Zoological Survey of India, Kolkata. From the survey sites the soil parameters and atmospheric temperature are recorded. The time spent in minutes per area in square Hectometers was recorded in searching the snails and slugs from 20 collection sites of 10 localities at the base of Satpuda Mountains.

RESULT AND DISCUSSION

Species diversity:

A total of 88 individuals belonging to 11 different species were collected from 20 collection spots in 10 localities distributed along Forest area (FA) and Residential area (RA) of Satpuda Mountains (Table-2). The occurrence of these species in Satpuda mountain is the first record but are recorded in other parts of India. Eight families represent 11 species. A maximum of 03 species represented from Ariophantidae family, 02 from Cerastuidae and only 01 from Endodontidae, Vertiginidae, Pyramidulidae, Subulinidae, Camaenidae and Veronicellidae. Changes in species composition and abundance are related to natural forest site variations area (FA) and in residential area in satpuda mountains (RA). The time spent was 2100 minutes in searching the snails and slugs from an area of 6400 square Hectometers distributed in 20 collection sites of Satpuda Mountain and allied plane area. (Fig 2).

Genera with maximum number of species include *Subulina* and *Cerastus.* The occurrence of these mollusks was reported from various sites, represent-ting moist land, fallen leaves of plants, humid, shady and rocky places of forests, agricultural fields and gardens. The majority of land mollusks were found in Taloda in plane area and in Toranmal in mountain area. Minimum numbers of species found in the study are *Euplecta* which are very rare. The greater density and species richness accounted in plane area. Calculations for diversity assay was done using Shannon-Weiner index (H,) formula, $H' = -\sum Pi \times In (Pi)$, Where Pi=proportion of individual species. The Shannon's index follows the same pattern as that of species richness. The Shannon's diversity index shows maximum diversity index with 11 numbers of species is 2.13 in Forest area (FA) and 2.15 in Residential area (RA). The eveness values calculated statistically by calculator software are 0.89 in FA and 0.90 in RA.

Variations in species composition and abundance:

Malacofauna and population density of species particularly of the most abundant mollusks varied in Mountain as well as in plane area. *Cerastua fairbanki* were tremendous in mountain area whereas *Cerastus jerdoni* were more in plane area, even though they are rare. *Subulina octona* are dominant in both area of satpuda studied in present work. Variation in topography and small scale elevation difference and the extent of human impacts (plane area) could contribute to this difference. (Fig.1). Area with high

organic content had more species like Taloda i.e. Riverside locations with agricultural land having dense vegetation and also in some other locations. Generally snails and slugs preferred moist alkaline soil. They were very few in acidic soil conditions and rare in an area without organic content.

Systematic account:

The second largest phylum in animal kingdom is Mollusca. Gastropos are more in Mollusca than any other classes. In Gastropoda, Stylommatophora is an order of sub-class Pulmonata. The stlommatophorans are with or without shells, with two pairs of tentacles of which the posterior pair is having an eye at its tip and usually with a common gonoore near base.

The order systellomorpha comprises slugs and shelled molluscs.

Phyllum- Mollusca
Class- Gastropoda.
Sub-class- Pulmonata.
Order-Stylommatophora.
Family- Cerastuidae.
Genus- *Cerastus*.
Species-*fairbanki*
Species*-jerdoni.*
Family- Ariophantidae.
Genus-*Cryptaustenia*.
Species- *bensoni.*
Genus- *Euplecta.*
Species- *subdecussata.*
Genus-*Macrochalmys*
Species-*Petrosa*.
Family- Endodontidae.
Genus- *Philalanka.*
Species- *quinquelirata.*
Family- Vertiginidae.
Genus- *Pupisome.*
Species- *evezardi.*
Family- Pyramidulidae.
Genus- *Pyramidula.*
Species- *humilis.*
Family- Subulinidae.
Genus- *Subulina.*
Species- *octona.*
Family- Camenidae.
Genus- *Trachia.*
Species- *fallociosa.*
Order- Systellomorpha.
Family- Veronicellidae.
Genus- *Laevicaluls.*
Species- *haroldi.*

Soil parameters:

To study the ecology of land mollusks simply an account of soil parameters from study site was made. Most of the snails prefer low temperature i.e. 25 C to 30 C and soil rich in organic carbon. In rainy season they occurred from soil surface to a depth of around 4-6 cm. They are more in numbers in moist and humus rich soil. Molluscs were few in mountain area up to an elevation of about 1000 meters from sea level and are more in orchards and in plane area. There are dissimilarities in soil parameters of study site preferred by different terrestrial gastropod mollusks. (Table 3).

In North Maharashtra Satpuda mountain is an allied zone of western Ghats, which is an important global hot spot of biodiversity in India. Nowadays overcrowding, overgrazing, overexploitation of natural resources and deforestation causes destruction of various natural habitats, particularly of invertebrates. From the present study it appeared that the abundance of molluscan species is more from the plane area which was quantified from terrestrial habitats of 10 different localities. There is a much variation observed in forest and plane area sites. The molluscan fauna of Satpuda Mountain is much but still unexplored therefore a systematic work to describe the members of mollusks from 20 sites of 10 localities was adjourned with 10 genera and 11 species, belonging to eight families and 03 orders. Hora (1925) studied on the habits of succineid mollusks from the Western Ghats and collected interesting data on hill stream mollusks of Pune, Maharashtra. Survey and ecological aspects of pulmonates from Marathwada region were studied by Kulkarni and Nagabhushanam (1973). Habitat studies, locations with their soil parameters, diversity index and mean density have been carried from land mollusks in Satpuda mountain areas of North Maharashtra is carried out in present investigation.

Kulkarni (1973) studied a detailed systematic on freshwater and land pulmonates of Marathwada region of Maharashtra and represented by six families with ten genera and fifteen species. Rao and Ghose (2001) studied on terrestrial mollusks of Nepal. Raheem et al. (2009) have made an illustrated guide to provide a brief introduction to the rich and fascinating land snail fauna of Western Ghats, India. Many of the species featured in this guide also occur in North Maharashtra. Magare (2002 & 2006) studied on terrestrial pulmonates from Nandurbar districts and provided a detailed systematic account of 24 different

Table 1: Species inventory of terrestrial molluscs in Forest Area (FA) and Residential Area (RA) sites of Satpuda Mountains.

Sr. No.	Species	Family	Number of individuals	
			FA	RA
1	*Cerastuafairbanki*	Cerastuidae	12	04
2	*Cerstusjerdoni*	Cerastuidae	04	01
3	*Cryptausteniabensoni.*	Aripophantidae	02	01
4	*Euplectasubdecussata.*	Ariophantidae	01	01
5	*Macrochlamyspetrosa*	Ariophantidae	08	04
6	*Philalankaquinquelirata.*	Endodontidae	09	06
7	*Pupisomeevezardi*	Vertiginidae	02	01
8	*Pyramidulahumilis*	Pyramidulidae	02	01
9	*Subulinaoctona*	Subulinidae	12	07
10	*Trachiafallasciosa*	Camaenidae	06	01
11	*Laevicaulisharoldi*	Veronicellidae	02	01
		Total	60	28
		Mean	5.54	2.54
		SD	3.16	1.60
		Shannon's Index	2.13	2.15
		Eveness	0.89	0.90

Table 2: Area wise different sampling stations (20) from 10 various localities from Satpuda mountain area.

Sr No	Mountain Area (Sector-I)		Plain Area (Sector-Ii)	
	Name of locality	Sampling station	Name of locality	Sampling station
1.	Toranmal	Lenghapani	Ranipur	Sultanpur
2.	Dhadgaon	Mungabari	Akkalkuwa	Khapar
3.	Molgi	Kathi	Taloda	Kothar
4.	Walamba	Dab	Shahada	Mhasawad
5.	Mandawa	Gadhwani	Nandurbar	Prakasha.

Table 3: Soil parameters from study sites.

Sr. No.	Locality	Soil Temp. (ºC)	PH	Electrical Conductivity (Ds/m)	Relative humidity	Organic C %	Total N %	Available S (PPM)
1.	Toranmal	25.0	7.06	0.71	85.0	2.05	0.32	45.1
2.	Ranipur	25.8	7.09	0.84	84.0	2.48	0.38	49.2
3.	Dhadgaon	25.5	7.04	0.65	77.0	4.46	0.07	55.1
4.	Akkalkuwa	25.2	7.05	0.68	78.0	4.18	0.47	54.4
5.	Molgi	26.2	6.64	1.23	57.3	5.50	0.49	53.1
6.	Taloda	26.0	6.48	0.89	61.3	5.92	0.23	37.3
7.	Walamba	24.5	6.50	0.60	89.0	3.63	0.07	23.5
8.	Shahada	24.4	6.45	0.25	87.0	3.69	0.25	44.5
9.	Mandawa	24.3	6.36	0.61	84.4	4.25	0.45	19.9
10.	Nandurbar	24.0	6.56	0.59	90.9	4.07	0.15	31.2
	Total	250.9	67.23	7.05	793.9	40.23	2.88	413.3
	Mean	25.09	6.72	0.70	79.39	4.02	0.28	41.33
	SD	0.7738	0.2989	0.2521	11.467	1.1826	0.1593	12.8287

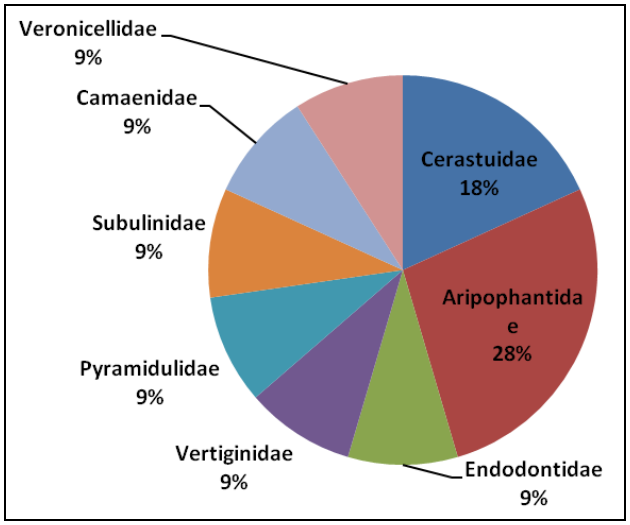

Fig. 1: Percentage of species composition of mulluscs from Satpuda mountain area

species of mollusks including 13 terrestrial species. In present work apart from published many terrestrial species of molluscs, 11 other terrestrial mollusks were studied and recorded first time from SatpoudaMountain area in India fromforested (Mountain) and residential (Plane) area.

CONCLUSION

The present work is undertaken to explore the Diversity, Ecology and systematics of land molluscan species from Satpuda mountain area. The work was undertaken during 2014-2015. The Study area consists of 20 different sampling stations in about 200 sq. km zone of Satpuda Mountains in North Maharashtra, India. A total of 88 individuals belonging to 11 different species were explored from 10 localities. The statistical data of Changes in species composition and abundance are related to natural forest site variations area (FA) and in residential area in Satpuda mountains (RA). The Shannon's diversity index 2.13 in Forest area (FA) and 2.15 in Residential area (RA). The eveness values calculated statistically are 0.89in FA and 0.90 in RA. These land mollusks are quite common in humified areas.

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RESEARCH ARTICLE

Changes in the RNA content of gill and gonad tissues of *Corbicula striatella* due to 5-fluorouracil toxicity

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ABSTRACT

During the study of RNA contents of gill and gonad tissues in *corbicula striatella* shows significant decrease in gill from 2.23±0.059to 1.96±0.109 *(-12.107) for 15th day and on 30th day. There is significant decrease from 1.75±0.113 to 1.31±0.102** (-19.714) on gonad shows significant and decrease from 8.16±0.951 7.16±0.312 (-12.254 on 15th days and on 30th days there is a significant decrease from 7.33±0.654 to 6.00±0.328** (-18.144) on 30th day This is due to toxicity of 5- Fluorouracil and changes of RNA in mobilization of tissue in the cellular metabolism.

Keywords: RNA content, *Corbicula striatella*, 5- Fluorouracil

INTRODUCTION

RNA is capable of carrying out a multitude of diverse biological functions. Many biologically active RNA have to adopt intricate 3D structures that rival protein structures in their complexityto be functional in a cellular environment. 5- fluorouracil and Heavy metals may interact with RNA polymerases which causes adverse effect. RNA polymerase must bind site specifically to its DNA template, binds its nucleotide and primer substrate, and form new phospho diester bond in elongating the growing RNA. Zinc ion appears to be essential for the function of both RNA polymerase and DNA topoisomerase, (Giedroc and Coleman, 1989). Biochemical changes such as inhibition of enzymes, metabolic disorder, genetic damage, hypertension and cancer. (Underwood, 1971; Zemasky, 1974; Lucky and Venugopal, 1977). RNA is the polymer of the ribonucleotides held together by 3',5' phosphodiester bridge. RNA has certain similarities with DNA structure.

MATERIALS AND METHODS

Attempts have been made in this study to select Fresh water bivalves, *Corbicula striatella* were collected from of Girna dam which is about at the distance of 50 K.M. away from Chalisgaon City of Maharashtra state. First they are made acclimatized to laboratory condition and they are washed. The water in the aquarium was changed regularly after every 24 hours.

After the acclimatization, bivalves , *Corbicula striatella* were divided into two groups with equal numbers of animals They were kept in seprate aquarium for 15 and 30 days out of remaining one groups treated by chronic Concentration $LC_{50/10}$ value of 96 hrs.). Of 5-fluorouracil (3.716ppm). On 15th and 30th day of exposure, bivalves from each experimental group were sacrificed and gill and gonad, were removed. These tissues were dried in oven at 75 ^{0}C to 80 ^{0}C till constant weight was obtained and blended into dry powder. These powders were used for the estimation of biochemical components of RNA to observe efficacy of 5-fluorouracil.

Procedure- Estimation of RNA:-

RNA content of the tissue was estimated by following Orcinol method of Volkin and Cohn (1954). 10 mg of dry tissue powder was homogenized by adding 10ml distilled water. Then it was centrifuged at 3000 rpm for 10 minutes. The supernatant contains RNA. 1ml supernatant was taken in test tube and 3ml Orcinol reagent was added. Then the mixture in the test tube was boiled in boiling water bath for 15 minutes. After boiling the solution in the test tube was allowed to cool. The optical density of the colour developed was read at 665 nm filter. Array of increasing concentration of standard RNA solution was processed in the same way and the optical densities were read to calculate the concentration of RNA from the sample powder.

RESULTS

The above Experiment has concluded that the result obtained on 15 & 30 days of gill, and gonad with5-fluorouracil are as Follows.

15 Days treatment teriod (Subchronic)

The gill and gonad of *Corbicula striatella* shows a significant ($P < 0.01$) decrease. The gill shows control 2.23±0.059 to treated with 5-fluorouracil 1.96±0.109* mg/g wet tissues in treated .The total RNA content in gill and gonad corresponds to an decrease by 12.10% to 12.54% The profile of total RNA content in gonad shows significant decrease from 8.16±0.951 to 7.16±0.312* mg/g wet tissues in gonad respectively. The gonad shows a decrease 12.54%. In both cases significantly decreases is recorded.

30 Days treatment period (Chronic)

The total RNA content of gill and gonad in control *Corbicula striatella* has been assessed. The result obtained 30th day after treating gill with the dose has shown significant in from 1.75±0.113 to 1.31± 0.102**mg/g wet tissues. Secondly the result of gonad treated with 5-fluorouracil is significant decreases 7.33±0.654 to 5.00 ± 0.358** mg/g wet tissues respectively.

Similarly 15 days subchronic exposure of treatment as compare to 30 days exposure shown that they are both tissues reveals significant depletion in RNA content. In the present comparative study gill, and gonad shows significant elevation in to the RNA level depletion in the exposure periods. This reveals a large variety of chemotherapeutic drugs used to treat cancer, but unfortunately many organic compounds shows limited efficacy problems of delivery and development of 5-fluorouracil is a conventional chemotherapeutic agent that binds co-valently to purine RNA bases and cellular apoptosis (Kerbel 1997).

The distinct types of the RNA with their cellular compositions are - messenger RNA 5-10%, Transfer RNA 10-20%, and ribosomal RNA 50-80 %. (Satyanarayana, 1999).

Table 1: Alteations in the RNA Content mg/100mg dry weight+ S.E. in digestive gland, and foot tissues of *Corbicula striatella* Treatment with 5-fluorouracil .

Sr no.	Tissues	Days	Control	Experimental	Student 't' test 'p' value	% increases (+) or decreases (-)
1	Gill	15 30	2.23±0.059 1.75±0.113 4.61± 0.451	1.96±0.109* 1.31± 0.102**	P < 0.01 P < 0.01	12.10% 19.14%
2	Gonad	15 30	8.16±0.951 7.33±0.654	7.16 ± 0.312* 5.00± 0.328**	P < 0.01 P < 0.001	12.54% 18.14%

Divalent metal ions in general and Mg^{++} with its favorable charge/size ratio in particular (Woodson, 2005) play an important role in RNA folding. Mg^{++} ions not only stabilizethe final structure through either direct coordination with negatively charged groups of the RNA orin a water-mediated interaction with the hexahydrated ion ($Mg(H_2O)_6^{++}$). They also influence the rate of folding, stabilize folding intermediates or destabilize alternative conformations (Wu and Tinoco, 1998). The effect of cisplatin damage on RNA pol II elongation was investigated using site-specifically-placed cisplatin adducts. In effective block to RNA pol. II elongation, inhibiting the polymerase by 80%. In contrast, RNA pol. II completely bypassed the cisplatin. These studies suggest the inhibition of RNA pol. II transcription following the treatment of cells with cisplatin (Carleen Cullinane *et al.,* 1999). In the light of above fact that *Corbicula striatella* was selected for biochemical study under sublethal concentration of Cisplatin for the subchronic (15 days) and chronic (30 days) period.

CONCLUSION

The present study indicates that effect of toxicity on *corbicula stritela* in the tissue of gills and gonads during the period 15 day and 30 days exposures of 5-fluorouracil anticancer drug toxicity to determine RNA reduced growth rate in enzymes of polymerase in RNA synthesis, which is the action of inhibit uracil base pair in subgroup of rRNA, tRNA, mRNA it has adverse effect of inhibitant of RNA transcription due to the period exposures. The toxicity of 5-fluorouracil in RNA reduced growth rate in polymerase enzymes. Then impact of toxicity determined in genetical disorders inhibit the uracil base pair in RNA. Which is the adverse effect stop RNA transcription due to the period of 15 days and 30 days exposure.

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RESEARCH ARTICLE

Morphological studies on soil protozoa *Euplotese urystomus* from Godavari basin area at Paithan District

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ABSTRACT

The majority of protozoan species are free living, and they are indicators of pollution. Free living protozoan can be found throughout the environment and are particularly abundant in soil and water. A study had been undertaken on the soil protozoan fauna of Paithan. During the present study *Euploteseurystomus* species was recorded in Paithan district. The morphology of *Euploteseurystomus* species was investigated using living observations at light microscopic level and by staining method. Additional data and details on the morphology of *Euploteseurystomus* described and illustrated. This paper is helpful for their identification of *Euploteseurystomus.*

Keywords: Protozoa, soil, *Euploteseurystomus*

INTRODUCTION

Protozoa play an important role in mineralizing nutrient, making them available for use by plant and other soil organisms. Protozoa have a lower concentration of nitrogen in their cells than the bacteria they eat. (The ratio of carbon to nitrogen for protozoa is 10:1 or much more and 3: to10:1for bacteria.) Bacteria eaten by protozoa contain too much nitrogen for the amount of nitrogen protozoa need .They release the excess nitrogen in the form of ammonium (NH_4). This usually occurs near the root system of a plant .Bacteria and other organisms rapidly take up most of the ammonium. Protozoa are also an important food source for other soil organisms and help to suppress disease by competing with or feeding on pathogen .Protozoa also play an important role in regulating the bacterial population. For proper quality and texture of a soil, protozoa have their definite role. For the present study the selected area of PaithanTaluka is fully supported by the Godavari basin. Now a day Godavari is polluted by various ways, hence the aim of this work is to find out the various species of soil protozoa.

MATERIALS AND METHODS

Soil sample was collected in plastic bags. Most of the samples will be collected in morning time as the high temperature affects the abundance of protozoa and they found more abundant at low temperature. These

samples were brought to laboratory and examined under the microscope for the further study and observation.. As the soil protozoa need water to move and that plays a big role in determining them, soil was diluted with chlorinated water and observed under the microscope by taking a drop on a slide. Protozoa are usually swim rapidly in water and hence unable to identify. To immobilize those, 10% methyl cellulose will be added to the water drop on slide. This slows the movement of organism without immediate death or bursting.

Culture method

When protozoa are less abundant in the water samples their population can be increased by culturing them. For cultivation of these organisms following methods are used.

1. Hay infusion
2. Wheat infusion
3. Rice infusion

RESULTS AND DISCUSSION

Description of Genus

Peristome large with well-developed adrenal zone; ventral in group and reduced; anal of five cirri conspicuous. Genus *Euplotes* Ehrenberg Inflexible body ovoid;ventral surface flattened, dorsal surface convex ;longitudinally ridged; peristome broadly triangular; frontal part of adoral zone lies in flat furrow;nine or more frontal-ventral; five anal; four scattered caudal; macronucleus band like; a micronucleus; contractile vacuoles posterior;fresh or salt water (comparative morphology, Pierson, 1943, Tuffrau, 1960; symbiotic bacteria, fauna Fremiet, 1952; marine species, Borror , 1962).

Description of Species

E.eurystomous is elongated ellipsoid in shape. Length of *E.eurystomus* is about 133 –98 um (Average 115.42) and width is about 105 –67.2 um (Average 90.3).Nine front ventral cirri are present. Five transverse cirri are present .Three caudal cirri are present. Peristome wide and deep, peristome depression is sigmoid. Macronucleus is 3-shaped and micronucleus is round in shape and present at anterior right side of macronucleus. One contractile vacuole is present. At the dorsal side six dorsal ridges are present. It is generally found in fresh water and brackish water but present species is found in soil of Godavari basin.

Classification-Cilliates

Kigdom: Protozoa goldfuss,1818,Rown,1858
Subkingdom: Biciliata
Infrakingdom: Alveolata Cavalier & Smith,1991
Phylum: CiliophoraDoflein, 1901,Copeland,1956
Subphylum: Intramacronucleata Lynn,1996
Class: Spirotrichea Butschi,1889
Subclass:Hypotrichia Stein, 1859
Order: Euplotina small & Lynn,1985
Suborder: Euplotina Small & Lynn,!985
Family: Euplotidae Ehreberg,18
Genus: ***Euplotes*** Ehrenber,1830
Species: ***E.eurystomus*** Wrzesnioweski,1870

Ventral view *of E.eurystomous* ***Dorsal view of E.eurystomous***

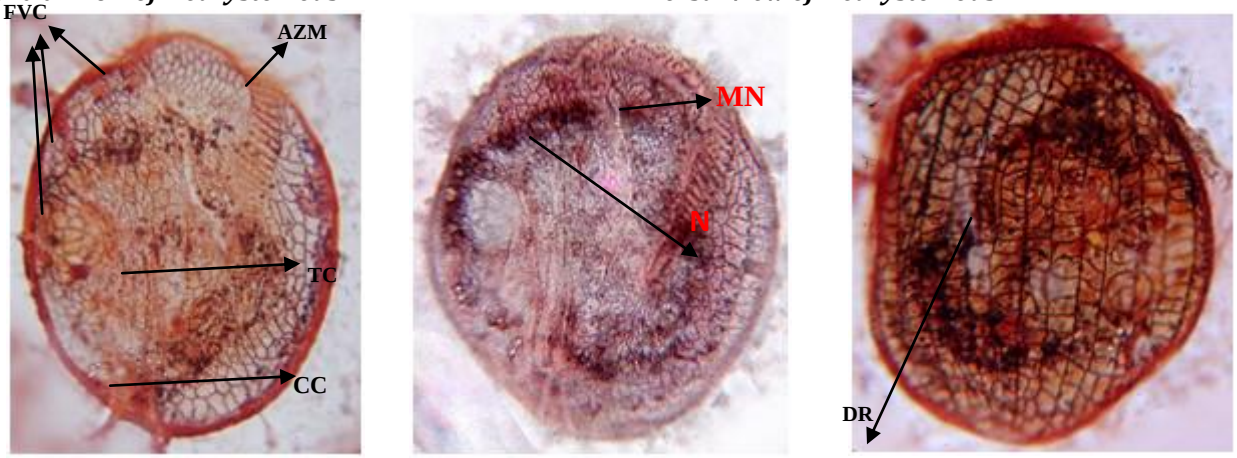

Fig. 1: E. eurystomous *(FVC-front ventral cirri, AZM- Adronalzonalmembrane, TC-Transverse cirri, CC-Caudal cirri, N-3 Shape Macronucleus, MN-Micronucleus, DR-Dorsal ridges. After the comparison of the species with other species of this genus, it is concluded that the present species is E.eurystomus (Wrzesnioweski,1870) redescribed here.*

Table 1: Comparision of the present species with the species of Genus Euplote

Particulars	***E.affines Dujardin (1841)***	***E.eurystomousWrzesniowski (1870)***	***E. muscicola Kahl,(1932)***	***E. woodruffi Gaw,(1939)***	***E. aediculatus Peirson (1968)***	***E.patella Mullar, (1986).***	***E. eurystomous Shaikh(2006)***	***Present species***
Body shape	Small ovoid	ellipsoid	Ovoid	Ovoid	Elliptical	Sub circular or Elliptical	Ovoid or ellipsoid	elongated ellipsoid
Body dimension	40-70$_{um}$ long	138 um by70 um	55.7-68.3µm by33.6-45.3µm	140-90µm	132 um by 84 um	91 um by 52 um	105-170 um by 80-110um	115.4um by 90.3 um
Cirri	9FV,5T,4C	9FV,5T,4C	10FV,5T,4C	9FV,5T,4C	9FV,5T,4C	9FV,5T,4C	9FV,5T,4C	9FV,5T,4C
Peristome	Narrow	Wide and Deep	Narrow	Wide	Narrow	Narrow	Wide	Wide and Deep
Peristomal plate	Long narrow	Broad and triangular	Broad and long	Small	Long triangular	Small triangular	Broad and triangular	triangular
AZM	2/3 of body length	½ of body length	2/3 of body length	2/3 of body length	2/3 of body length	½ of body length	2/3 of body length	½ of body length
Macronucleus	Slight C shape	3 shape nucleus	3 shape nucleus	T shape nucleus	C shape with flattened part	C form band	3 shape nucleus	3 shape nucleus
Micronucleus	Spherical anterior left	Spherical anterior left	Spherical anterior left	Spherical anterior left	Spherical anterior left	Spherical anterior left	Spherical anterior left	Spherical anterior left
Habitat	Fresh and Brackish water	Fresh and Brackish water	Fresh water	Fresh and Brackish water	Fresh and Brackish water	Fresh and Brackish water	Fresh water	Soil sample

Present species has 9 frontoventral, 5 transverse, and 4 caudal cirri present which are similar to *E.patella, E.eurystomus, E.woodruffi, E.adeculatus* and *E.affinis* which also have 9 frontoventrals, 5 transverse and 4 caudal cirri. It also matches with the *E. eurystomus* reported by Glidden, 1996 and Shaikh, 2006 while Curds (1974) reported *E.affinis* with 10 front ventral and 3 caudal cirri and *E.parkein.sp* with 8 front ventral. In present species, peristome is wide with broadly triangular peristome plate which resembles *E. eurystomus* described by Wrzesnioweski, 1870 and Shaikh, 2006. *E. woodruffi* also has wide peristome but small peristome plate. This species differ from *E. patella, E.aediculatus, E.affines, E.moebius*i, as they all have narrow peristome with narrow small triangular or long triangular peristomeplate while *E. moebiusi* has broad and long peristome plate. This species differs from the *E. afffinis* (Bick 1972) which has peristome without peristome plate and also differs from *E. moebiusi* and *E. affinis* described by Curds (1974), which has narrow peristome with long peristome plate.

In present species AZM extends ½ of the body length which is similar to *E. patella,* and *E. eurystomus* while differ from *E. affines* (1841), *E. moebiusi* (1932), *E. woodruffi* (1939*), E.aediculatus* (1968) and *E.eurystomus*, Shaikh (2006) in which the AZM is 2/3 of the body length.AZM covers 35-40 membranelles in the present species (1870*) E.eurystomus* AZM covers about 50-65 membranelles. Macronucleus in present species is '3' shape, resembling with *E. eurystomus* (1870), *E. moebiusi* ,(1932) and *E. eurystomus* (2006) Shaikh also reported '3' shape macronucleus but differ from *E. affines, E. patella, E. adeculatus*, which is having 'C' shape nucleus and *E. woodruffi* having 'T' shape nucleus. In present species micronucleus is spherical, anterior at left margin which is similar to all the previous species of *Euplotes*. Though the species resembles with *E.eurystomus*, their AZM are dissimilar (i.e. in previous (1870) species they are 50-60, in present one i.e. 35-40) and other previous workers did not specify the no of AZM.The present species is compared with all the species of genus *Euplotes*and found *E.eurystomus*. When body dimensions are compared, present species is very close to *E.eurystomus* (Wrzesniowesk, 1870) and Shaikh 2006 hence it is considered as *E.eurystomus* and redescribed here.

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RESEARCH ARTICLE

Effect of folic acid antagonist methotrexate on seminal vesicle of Indian palm Squirrel *Funambulus pennanti* (Wroughton)

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ABSTRACT

The toxic effect of Methotrexate on seminal vesicle have been studied by intramuscularly injecting low dose of 3 mg/ kgBW /day and 6 mg/kg BW/day for 15 days to adult male squirrel (*Funambulus pennanti*) during the breeding period January. For comparing the effects the saline treated vehicle was injected same amount of saline and was maintained for the same duration. Reduction in the size of secretory alveoli of seminal vesicle due to remarkable increase in the fibro-muscular coat, loss of secretory capacity all suggest toxic effect of MTX on seminal vesicle.

Key words: Methotrexate, toxic effects, Seminal vesicle.

INTRODUCTION

Methotrexate (Rheumatrex) is a medicine that is used to treat Rheumatoid arthritis (RA), psoriatic arthritis, Reiter's syndrome and other conditions. Aside from its antineoplastic activity, Methotrexate has also been used with benefit in the therapy of common skin disease psoriasis (Mcdonald, 1981). Additionally Methotrexate inhibits cell mediated immune reaction and is employed as an immunosuppressive agent, for example, in allogenic bone marrow and organ transplantation and for the treatment of dermatomyositis, rheumatoid arthritis, Wegener granulomatosis and Crohn's disease (Messmann and Allegra, 2001; Feagan *et al.,* 1995, Felig and Frohman 2001, Prasad *et al.,* 1996). Methotrexate was formerly known as amethopterin, is an antimetabolite drug used in treatment of cancer and autoimmune diseases. The present study embodies: Histopathological changes undergone by seminal vesicle.

MATERAILS AND METHOD

In all three sets of experiments using low and high-doses of Methotrexate (MTX) were performed for the present study for the duration of 15 days (Tables 1& 2). Animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the organs were excised seminal vesicle was used for histological studies.

Table 1: Experimental Design for Low Dose Methotrexate treatment

Number of animals and sex	Treatment	Dose mg/kg BW	Route	Duration
3 males (Experimental)	Methotrexate	3 mg daily	I.M.	15 days
3 males (Control)	Saline	E.V.	I.M.	15 days

Table 2: Experimental Design for High Dose Methotrexate treatment

Number of animals and sex	Treatment	Dose mg/kg BW	Route	Duration
3 males (Experimental)	Methotrexate	6 mg daily	I.M.	15 days
3 males (Control)	Saline	E.V.	I.M.	15 days

Abbreviations: E. V. = Equal volume, I. M. = Intra muscular, B W = Body weight

RESULT AND DISCUSSION

Histological Studies

Seminal vesicle fixed in Bouin's fluid for 24hrs and preserved in 70% alcohol. The tissues were dehydrated by passing through graded series of alcohol, cleared in xylol and after embedding in paraffin, blocks were prepared and serial sections were cut at various thicknesses between 5µ to 8µ. For routine histological study the sections were stained with Ehrlich's haematoxylin and counter-stained with eosin. Measurements when necessary were taken with the help of an occular micrometer calibrated to a stage micrometer. The photomicrographs were taken with the help of a Carl Zeiss camera attached to the microscope and enlarged to the required size.

Vehicle Treated Control

The seminal vesicle of the control squirrel was composed of a large number of acini embedded within the fibro muscular connective tissues. The acini were lined by tall columnar epithelial cells containing a prominent basal nucleus (fig. 1).

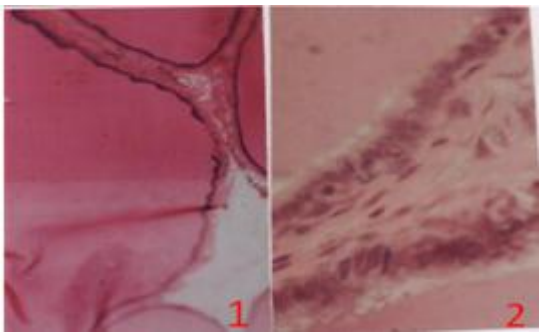

Fig. 1 and 2 Vehicle treated control

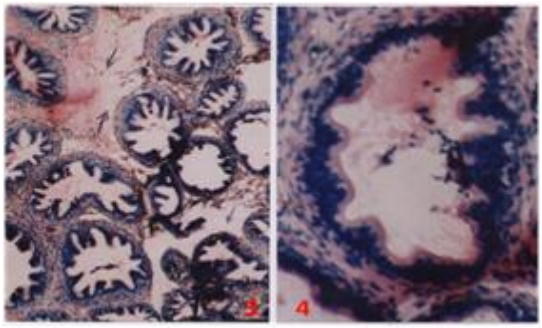

Fig. 3 and 4 Low dose 3 mg/KgBW

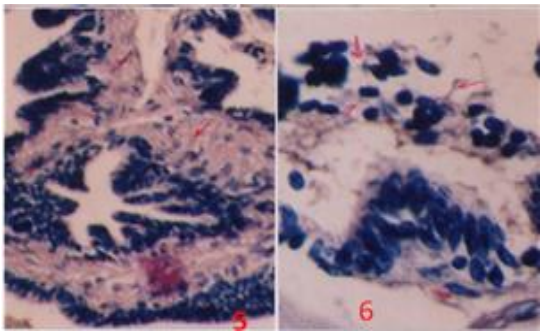

Fig. 5 and 6 High dose 6 mg/KgBW

Few basal cells, almost rounded in shape and basal in position were also observed between the columnar epithelial cells (fig. 1). Large numbers of dense secretory granules were visible in the apical cytoplasm. The lumen of acini was filled with the darkly stained secretory material. Lamina propria surrounding the epithelial cells was comprised of cellular connective tissues containing some smooth muscles rich in elastic fibers (fig. 2).

Low Dose Treatment (3mg/kg BW MTX for 15 days)

Histopathological study

An enormous increase in the fibro-muscular connective tissue in between the secretory tubules have resulted into remarkable reduction in there, inter tubular connective tissue was heavily lost at some places with large vacuole or streaks of cellular tissues. Each tubule was lined by secretory epithelium, which was either club-shaped or thrown into medium-sized, finger like projections, epithelium appeared partially damaged, due to the irregular placement of nuclei. The loss of secretory activity was evident by emptiness of most of the tubules (figs. 3 & 4).

High Dose Treatment (6mg/kg BW/day MTX for 15 days)

The nuclei of the connective tissue were reduced in number and randomly distributed. The secretory epithelium was thrown into crypt like structure and was highly vacuolated the nuclei were also randomly distributed due to extensive vacuolation of the cytoplasm. Almost all tubules showed loss of secretion (figs. 5 and 6).

There was significant reduction in the acinar size due to enormous increase in the intertubular connective tissues. Prevalence of vacuolation in the supranuclear region of regressed secretory epithelium directly suggested the depletion of secretory activity and hence scanty secretion in the lumen. The high dose 6 mg/kg BW/day for 15 days resulted into further increase in the intertubular connective tissue reducing further the size of secretory acini. The epithelial damage was more pronounced due to loss of secretory granules from the supra as well as infra-nuclear region and the total loss of secretion. The epithelial lining appeared disrupted and disorganized, the nuclei were scattered hapzardly with much spaces between them. These sloughed off nuclei were found to be intermingled with the dry flocculent secretion in the centre some tubules showed heavy deposits of such nuclei. The epithelium lining the tubule was manifested towards lumen, partially obliterating the lumen. Thus the enlargements of dense fibroblast cells following both the treatments were suggestive of alteration in the function of. Similar alterations in the sex accessory glands have been reported only by Takeda et al., 1985 that too on prostate, therefore this is the first kind of study which has evaluated the toxic effects of MTX on seminal vesicle which are dose, duration and androgen dependent.

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RESEARCH ARTICLE

Occurrence of Coccidian parasites in Sheep in Omerga region

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ABSTRACT

Coccidian infections were studied in the Omerga region during the period of six months. 127 fecal samples of sheep were collected from different villages. Total samples were examined out of which 92 Samples are positive and there percentage is 72.45 %. The relative prevalence of the sheep was analyzed.

Keywords: Protozoa, coccidia, fecal samples, sheep, oocysts.

INTRODUCTION

Coccidiosis is an economically important disease which is caused by unicellular protozoa, *Eimeria*; with worldwide distribution (Chartier and Paraud 2012, Kheirandish *et al*., 2012). It continues to be a serious threat to animal health and results in lowered productivity due to the associated morbidity, mortality and cost of treatment and control measures (Agyei, 2004). All ages of sheep are susceptible to *Eimeria* infection but lambs are most severely affected by clinical coccidiosis and disease outbreak (Khan *et al*., 2011).The disease is caused by single cellparasite of the genus Eimeria. Infection of sheep with coccidia occurs through ingestion of sporulated oocysts along with water, soil and contaminated with fecal matter. In the small intestine sporulated oocysts release sporozoites which infect intestinal epithelium. The prevalence of coccidiosis species has been recorded in sheep in many countries of the world. Fifteen species of*Eimeria*has been considered that infect sheep, these species found in the intestines of infected sheep (Reginsson and Richter, 1997). Therefore, the objective of the present study is to investigate the factors affecting in prevalence and parasite load of *Eimeria* species of sheep. This effort is an initial survey to gain information that has not been previously reported from Omerga region.

MATERIALS AND METHODS

Study area: A total of 179 samples were collected from various villages in Omerga region.

Fecal sample collection: Fecal samples were collected from the domestic sheeps from Omerga region, which were chosen randomly and each sample was packed in a separate plastic bag, labelled properly and put it in a cooler box at 4°C until examined.

Fecal examination technique

Fecal samples were microscopically examined individually for the search of coccidian oocysts. Faecal samples resulted positive were dissolved in distilled water for centrifugation for 5-6 minutes at 3000 rpm. Then using flotation technique with saturated Sodium chloride solution as flotation solution for the presence of oocysts. After the centrifugation sample were dissolved in a 2.5 % $K_2Cr_2O_7$, solution and maintained in the dark and at the room temperature to allow sporulation of the oocysts. Oocysts were daily checked for sporulation for 3-5 days after submerging the faecal sample in 2.5% $K_2Cr_2O_7$, following which samples were stored at 4°C.Sporulated oocysts were observedusing light microscope with a 100× oil immersion objectiveand images were takenusing a Sony cyber shot dsc-wx 200 digital camera.

RESULTS

Domestic animals are usually infected with many parasites; the knowledge of which is essential for management of the infections. Coccidian parasites are important in domestic animals because they affect their intestinal tract. Coccidian species are host specific and species specific. In coccidiosis some *Eimeria* species are pathogenic and some are non-pathogenic. Thus, these parasites should be considered among those diseases responsible for health and production of sheep. (Mokhtaria *et al*., 2015)However in a study, Gomez *et al*., 1996, have reported transient diarrhea in highly infected mouflon in central Spain.

Since coccidiosis has a great impact on sheep industry, identification, current prevalence of *Eimeria* species will help to minimize the economic losses in the sheep industry. The prevalence of *Eimeria* species in this study was 72.45%. It is within the infection range in different countries. The prevalence rate of *Eimeria* species in India Singh was (1963) 71 %, Bawazir (1980) 34.48 %, Nikam (1983) 34.24 % and More (2011) 24.57%.

In other country Saudi Arabia, was 41% (Toulah F.H. 2007), Iran 16.7% (Yakhchali 2008), Tanzania 93% (Kusiluka 1996), Senegal 94% (Vercruysse J. 1982), Pakistan 51.61% (Asif, 2008), 80% in South Australia (Ocallaghan 1986) and 85% in goats at north of Jordan (Abo-shehada M.N. 2003). Coccidial infection in small ruminants has been reported worldwide (Chhabara and Pandey, 1991; Maingi and Munyua, 1994; Skirnisson, 2007; Kimbita *et al.,* 2009; Gadahi *et al.,* 2009; Wang *et al*., 2010).

In the present study, the prevalence of coccidial infection was 72.45% in sheep in Omerga. Our results are similar to those reported in northeastern china, Zimbabwe and Turkey (Chhabara and Pandey, 1991; Galip K, 2004; Yakhchali and Golami, 2008; Wang *et al*., 2010), but different from that reported in Iran, Pakistan and India(Yakhchali and Golami, 2008; Gadahi *et al*.,2009),which revealed a prevalence of 19.2, 27.77% and 24.57 %respectively.

In this study highest prevalence of coccidian infection in month of July and lowest in month of November. This study suggests that the rate of infection with gastrointestinal parasites in domestic sheep was high rate in comparing with previous studies. Infection is highest in rainy season than winter.

Table : Showing the monthwise prevalence of coccidian infection insheeps in Omerga Region during the period July 2013- December 2014

Sr. no.	Period	No. of Sample		% of Prevalence
		No. of Sample Examined	No. of Sample Positive	
1	July	22	18	81.82%
2	Aug	25	19	76.00%
3	Sep	19	14	73.68%
4	Oct	21	15	71.43%
5	Nov	21	13	61.91%
6	Dec	20	14	70.00%
	Total	**127**	**92**	**72.45%**

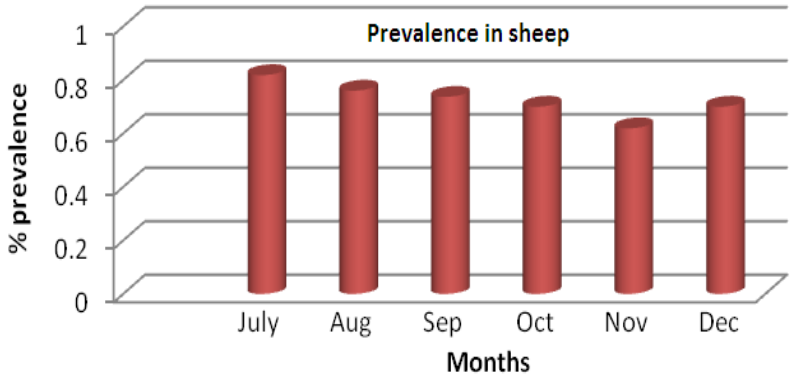

Fig.1 : Showing the monthwise prevalence of coccidian infection insheeps in Omerga Region during the period July 2013- December 2014

CONCLUSION

In conclusion, the present survey revealed that prevalence of coccidian infection in Omerga region is significantly high. Knowledge of the prevalence of coccidiosis and current *Eimeria* species will help to minimize the economic losses in the sheep industry, evaluate infection potential and control programs, especially for lambs. These results also provide relevant "base-line" data for assessing the effectiveness of future control strategies against coccidiosis in sheep.

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RESEARCH ARTICLE

On a new species of the genus *Uncibilocularies* Southwell, 1925, from (Cestoda: Onchobothridae) Ratnagiri, (MS) West coast of India

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ABSTRACT

One new cestode species *U. latkanai* from *Trygonzugei* at Ratnagiri (M.S.) West Coast of India is described. *Uncibilocularies latkanai* differs from all the known species of the Genus in having scolex triangular, tapering at both the ends and bothridia are present. Mature segments are elongated in shape and five to six times longer than broad. Testes 47-50, genital pores submarginal, vitellaria granular like strip. Cirrus pouch alternate.

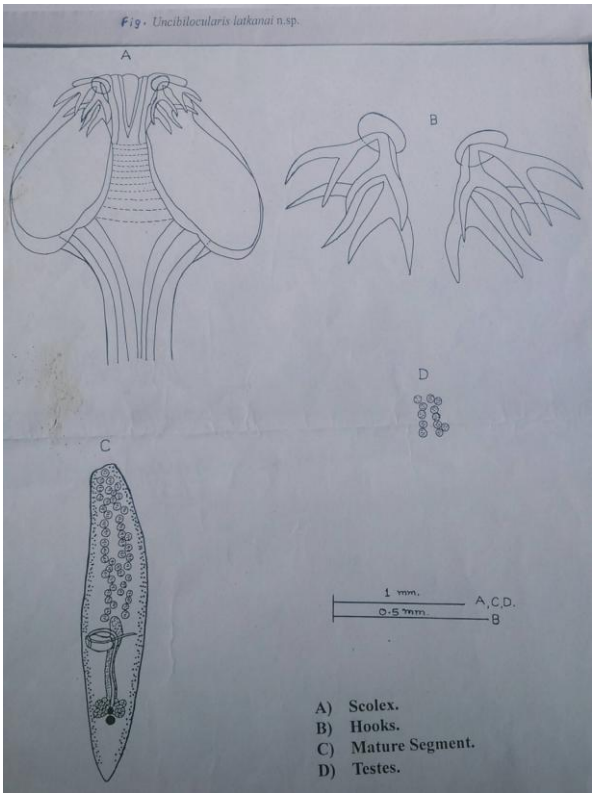
Keywords: *Uncibilocularies*, *Trygonzugei*, *U. latkanai*, parasite, host.

INTRODUCTION

Uncibilocularies was established by Southwell (1925) as its type species *U. trygonis* in *Trygonwalga* and *T.sephen*. He again reported in 1927 *U. mandleyi* in *Hemigaleus balfouri* Subhapradha (1959) reported *U. indica* from *Chiloscyl liumgriseum* in India. Deshmukh and Shinde (1975) reported *U. aurangabadensis* from Stromateus sp. In India, Shinde and Chincholikar (1975) described *U. ratnagiriensis* and *U. southwelli* from *Trygon* species in India. Later on Deshmukh (1977) reported three new sp. *U. thapari* from *T. sephen, U. shindei* from *T. zugei* and *U. somnathi* from *pteroplatamicrura* at veraval, Jadhav and Shinde (1981) described*U. veravalensis*, Jadhav, Shinde and Phad added*U. bombayensis* from *T. sephen*. Later on Jadhavetal (1989) described two new species of the same genus. *U. indiana*and *U. shashtri* from marine fishes; West coast of India. Fifteen marine fishes *Trygonzugei* were dissected at marine Biological Laboratory Ratnagiri, out of which nine fishes were infected and 12 cestodes were recovered.

DESCRIPTION

The scolex is almost triangular tapering at both the ends with four sessile bothridia. Scolex.1.69-1.72 x 1.31-1.87 mm in size.Bothridia larger measures 1.417-1.533 x 0.36-0.62 mm in size. Loculi and accessory suckers are absent. Each bothridia anteriorly bears a pair of bifurcated hooks, inner hook measures 0.34-0.35 x 0.034-0.039 in size. Outer hook measures 0.39-0.40 x 0.034 x 0.043 in size. Each hook has prongs and one



handle. Each prong measures 0.252 x 0.024mm in size. The outer prong measures 0.214-0.218 x 0.024-0.039mm in size. The outer pair of hooks measures 0.219-0.296 x 0.024-0.034 in size. Neck long 0.155-0.175 in length and 0.218-0.277 in breadth.

Mature segments longer than broad measures 2.306 x 2.431 in length and 0.236-0.476 in breadth. Testes oval to round in shape 47-50 in number lies in anterior half of the segment. Cirrus pouch is middle of the segment measures 0.519 x 0.113 in size sub-marginal cirrus long without spines measures 0.147 x 0.011 in size.vas deferens is thin and straight measures 0.159 x 0.005 in size and is opposite to the genital pore. Vagina 0.47-0.51 x 0.022mm in size. *Receptaculum seminis* measures 0.193 x 0.034 in size. Vagina and cirrus pouch opens through oval common genital pore 0.193 x 0.204 in size. Vagina ends in shell gland measures 0.079 x 0.068 in size. Ovary bilobed finger like with 3-4 aciniootype lies in between two ovaries measures 0.170 x 0.170 in diameter. Vitellaria granular uterus longer and broader 0.056-0.090 in size.

Host : *Trygonzugei*
Location : Spiral Valve
Locality : BhagvatiRatnagiri (M.S.) India.

DISCUSSION

The present worm under discussion is having scolex triangular absence of the accessory suckers,absence of tubercle, presence of neck, mature segments are enlongated tapering at both ends. Testes are 47-50 in number. Genital pore sub-marginal alternate ovary bilobed, vitellaria granular.

The present parasite differs from *U. trygonis* in the shape of scolex almost triangular as against square, Testes 47-50 as against 30-40.

From *U. indica* number of testes 47-50 as against 56-60, genital pore anterior to one third as against middle.

From *U. aurangabadensis*scolex almost triangular as against quadrangular presence of neck as against absence of neck.

From *U. ratnagirensis*scolex triangular as against square. Testes 47-50 as against 144.

From *U. southwelli* shape of scolex triangular as against rounded. Testes 47-50 as against 220-230.

From *U. thapari*scolex almost triangular as against rounded. 47-50 as against 25-28.

From *U. shindei*scolex rounded, Testes 40-45, position of genital pore middle.

From *U. somnathi*scolex quadrangular.

From *U. veravalensis*scolex round to oval.Testes 75-80, cirrus spinose.

From *U. bombayensis*scolex circular common genital pore marginal.

From *U. indiana*scolex oval. Tubercle on inner prong as against absent.

From *U. shashtri*scolex broad narrow anteriorly and broad posteriorly. Testes 55-60. Cirrus pouchspinose, vagina posterior to cirrus pouch. Uterus coiled.

CONCLUSION

Because of the varied characters, it is regarded as a new species*U.latkanai*from *T. zugei*at Ratnagiri. Nomenclature is done with the specific name of authors mother Late LatkanbaiBabulalPawar, who inspired and help me for education.

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RESEARCH ARTICLE

Taxonomic studies of mammalian Tapeworm *Avitellina ali n*. Sp. from *Capra hircus* at Dhule, MS, India

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ABSTRACT

The present investigation deals with taxonomic studies of mammalian tapeworm of genus *Avitellina*, viz. *A. ali n. sp.* collected from the host *Capra hircus*at Dhule (M.S.) India. The present worm comes closer to all the known species to the genus *Avitellina* in general topography of organ but differs due to scolex medium, globular, suckers large, oval, neck long, mature segment 27 and half times broader than long, testes oval ,medium, 6-10 in number, outer column 1-2, inner column 2-3 testes, cirrus pouch medium, cylindrical, vas deferens thin, medium, ovary medium, oval, vagina posterior to cirrus pouch, vitelline gland absent, genital pore irregularly alternate, gravid segments show one par uterine organ, containing eggs.

Keywords: *Avitellina ali* n. sp., Mammalian tapeworm, *Capra hircus.*

INTRODUCTION

The genus *Avitellina*was erected by Gough (1911), as a type species *A. centripunctata*, (Rivolta, 1874) in *Ovisaries*in Europe. Later on 8 species are added under this genus i.e *A. chalmersi* Woodland(1927), *A. goughi* Woodland (1927), *A. lahorea* Woodland (1927), *A. sudanea* Woodland (1927), *A. tatia* Bhalerao (1936), *A. woodland* Bhalerao (1936), *A. hircusae* Kale, 2005 and *A. singhii* Shinde, 2013. The present form collected from Dhule (M.S.) India.

MATERIAL AND MATERIALS

The survey of *Capra hircus* were made at Dhule for Cestode infection in the month of January 2009. Four Cestodes were collected from the intestine of *Capra hircus*. All the worms are flattened preserved in 4% formalin, stained with Harris Haematoxyline, passed through various alcoholic grades, cleared in Xylol, mounted in DPX and whole mount slide were prepared for anatomical studies, drawing were made with the help of camera lucida and microphotographs were taken by digital camera.

RESULT AND DISCUSSION

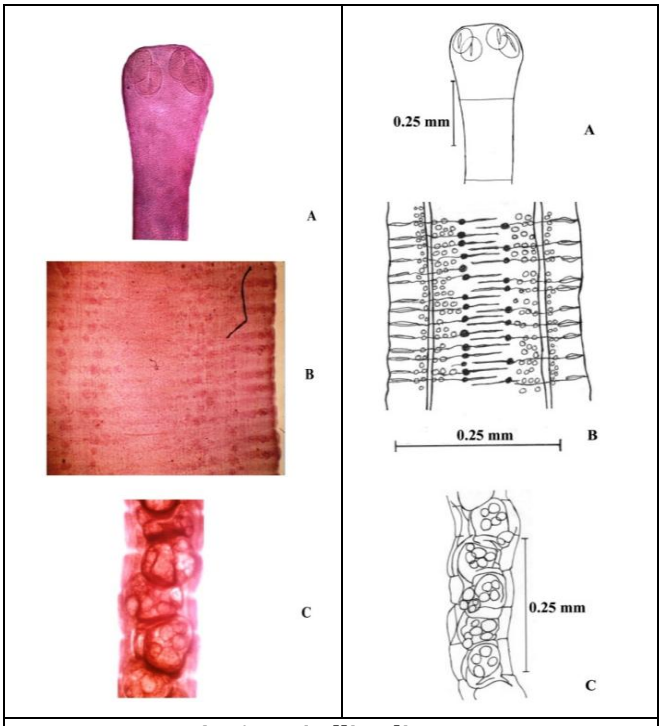

Fig. 1: *Avitellin ali* n. sp.
A-Scolex; B- Mature Segments, C- Gravid segements

Description (**Based on four specimens**):

The worms were long, muscular with numerous proglottides. Scolex medium, muscular, globular, broad anteriorly, narrow posteriorly, without rostellum and rostellar hooks, measures 0.226 - 0.233 x 0.216 - 0.250; four prominent suckers, large, muscular, oval, arranged in two lateral pairs, slightly touching each other, measure 0.086 - 0.093 x 0.066 - 0.070; neck long, measures 0.244 - 0.246 x 0.146 - 0.160.

The mature segment with indistinct segmentation, weak musculature, 27 and half times broader than long, acraspedote, with uniform lateral margin, measure 0.009 - 0.011 x 0.285 - 0.314; testes 6-10 in number, appear in two lateral fields, each field divided into two lateral groups by excretory canal and nerve trunks on both lateral side of ovary, outer column 1-2 testes, inner column 2-3 testes, testes oval ,medium, measure 0.009 - 0.011 x 0.006 - 0.011; cirrus pouch medium, cylindrical, elongated, situated at margins of the segment, transversely placed, measures 0.042 - 0.048 x 0.006 - 0.009; cirrus thin, straight or slightly curved, measures 0.045 - 0.051 x 0.003; vas deferens thin, medium, runs straight, measures 0.026 - 0.029 x 0.003.

The ovary is medium, single, oval, measures 0.009 - 0.011 x 0.006 - 0.011; vagina thin, posterior to cirrus pouch, runs towards the ovary, measures 0.100 - 0.102 x 0.003; vitelline gland absent; genital pore medium, irregularly alternate, measures 0.003; oval; gravid segments broader than long, indistinctly segmented, measure 0.123 - 0.140 x 0.213 - 0.233; segment show one par uterine organ, sac like, containing 5-6 eggs, measures 0.333 - 0.340 x 0.212 - 0.219; eggs are small, round and measure 0.040 - 0.050 in diameter.

The present worm, agrees in all the characters with the genus *Avitellina*, in general topography of organ but after going through the literature, the worm under discussion, comes closer to *A. lahorea*and differs in having scolex small, vas deferens coiled, ovary small, spherical, vagina ventral to cirrus pouch, par uterine organ snail shaped and reported from *Bosindicus* and *Ovisaries.*

The present cestode, differs from *A. centripunctata*in having scolex rounded, testis 12-16 in numbers, vagina dorsal to cirrus pouch, par uterine organ pyriform and reported from and *Ovisaries.*

The present worm, differs from *A. chalmersi*in having scolex rounded testis 12-18 in numbers and reported from and *Ovisaries.*

The present cestode, differs from *A. goughi*in having scolex rounded, testis 10-20 in numbers, vagina ventrally or dorsally to cirrus pouch, par uterine organ resembles a bunch of bananas and reported from and *Bosindicus* and *Ovisaries.*

The present tapeworm differs from *A. sudanea,* in having testes numbers 11-12, par uterine organ kidney shapedand reported from *Ovisaries.*

The present worm differs from *A. tatia,* in having testes numbers 16-24, vagina ventral to cirrus pouch and par uterine organ pear shaped.

The present cestode, differs from *A. woodlandi,* in the number of testes 8-14 and reported from *Ovisaries*.

The present parasite, differs from *A. hircusae*in the shape of scolex oval, in the number of testes 14 and ovary rosette shaped.

The worm under discussion differs from *A. singhii* in the shape of scolex quadrangular, in the number of testes 35-40 and par uterine oval in shaped.

These characters are valid enough, to erect a new species, for these worms and hence the name *A. ali* n. sp. is proposed, after Dr. Syed Mehdi Ali, Ex-Professor and Head, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad; who has contributed so much, to our knowledge of Helminthology

TAXONOMIC SUMMARY

Type species	:	*Avitellinali* n. sp.
Host	:	*Capra hircus*(Linnaeus, 1758)
Habitat	:	Small intestine
Locality	:	Dhule, Dist. Dhule, M.S., India
Holotype and Paratype	:	Deposited in the Helminthology Research Lab. Department of Zoology, Nanasaheb Y. N. Chavan College, Chalisgaon, Dist. Jalgaon (M.S.)
Date of collection	:	11thFebruary, 2009.

A KEY TO THE SPECIES OF GENUS *AVITELLINA* GOUGH, 1911

External column of testes 0-1 testes ……**1**
External column of testes 1-2 testes ……**2**
External column of testes 2-3 or more testes ……**3**

1. Outer column 1 testes and inner column 2-4 testes, par uterine organs snail – shaped……*A. lahorea* Woodland, 1927
2. Outer column 1-2 testes and inner column 2-3 testes, par uterine organs sac- like ……..…..***A. ali* n. sp.**
   Outer column 1-2 testes and inner column 3-5 testes, par uterine organs sac- like……..*A. woodlandi*Bhalerao, 1936
   Outer column 1-2 testes and inner column 5-5 testes , par uterine organs kidney shaped……..*A. sudanea* Woodland, 1927
   Outer column 2 testes and inner column 5-5 testes, par uterine organs saclike……..*A. hircusae*Kale, et al., 2005
3. External column 2-3 testes and internal column 4-6 testes ……..*A. centripunctata;* Gough, 1911
   External column 2-3 testes and internal column 3-7 testes, par uterine organs large, bananas bunch like……*A. goughi;* Woodland, 1927
   External column 3-4 testes, Par uterine organs oval or pear ……**4**
4. Internal column 3-4 testes,Par uterine organs oval……..*A. chalmersi;* Woodland, 1927
   Internal column 5-8 testes,Par uterine organs pear shaped……..*A. tatia;* Bhalerao, 1936

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RESEARCH ARTICLE

Morphological studies of cestode parasites and its impact on intestine of *Capra hircus* in Chalisgaon region

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Acknowlegement
The author is thankful to Dr. A.T. Kalse, N.Y.N.C. College, Chalisgaon for providing facilities and encouragement.

ABSTRACT

Parasitism is the association in which host and parasite are ecologically interrelated. *Capra hircus* is dominant species of Indian goat. *Capra hircus* has richest source of nutrional value**.** Caestodes are the endoparasites which infect the intestine of *Capra hircus.* Their infection causes parasitic diseases and also causes economic losses. Caestode parasites have greater diversity and their species have cosmopolitan distribution. The effect of these parasites is depend on the number of parasites occur in intestine of *Capra hircus*. Morphological studies of Caestode parasites is more beneficial to identify various species of parasites and their classification and external form. It also provide information about diseases they cause and also their occurance in particular region. This information will help to plan for preventive control measures.

Keywords: Parasitism**,** Caestodes, *Capra hircus,* endoparasites, economic, cosmopolitan, Taxonomical, nutritional, diversity.

INTRODUCTION

Cestode parasites have parasitic mode of life due to nutritional needs, shelter, lack of hormones. Cestode parasites are the tapeworms which infect almost all the vertebrates. Domestic goat *Capra hircus* L. is economically beneficial to human population but caestode parasites occur in their intestine causes considerable damage. The genus Stilesia was observed by Ralli *et al*. (1893), from *Ovis aries*, in Europe, Asia and Africa as *Stilesia globipunctata*. *Stilesia vittata* reported by Ralliet (1896), from *Camelus dromedaries* from Africa and India. Later on *Stilesia hepatica* was added to this genus, by Wolffhugel (1903), from Sheep and Goat, in East Africa and India. *Stilesia okapi* observed as a new species, of this genus by Leiper, 1936, from Okapi in Africa, is regarded by Baer (1950), as a variety of *S. globipunctata*. The system of classification is based on the " Advances in the zoology of Tapeworms" 1950-1970 by Wardle *et al*. (1974) and "Systema Helminthum" vol. II by Yamaguti (1959). As far as many researchers concentrating on tapeworm studies but investing the biology of tapeworms parasitizing in Capra hircus L. as an urgent necessity.

MATERIALS AND METHODS

The various steps require for taxonomical studies of Cestode parasites in intestine of Capra hircus are as follows.

Collection of Cestode parasites : Number of small intestines collected from domestic goat, Capra hircus L. at Chalisgaon. The auther has collected and cover maximum places in Chalisgaon region.

Processing of Caestode parasites :

a)Flattening : The collected tapeworms were cut into appropriate size pieces for sake of convenience. Then they were flattened by using two glass slides and ties with the help of thread with gentle pressing.

b) Preservation : The flattened tapeworms preserved in freshly prepared 4% formalin.

c) Staining : The preserved worms were kept in water for some time and then stained with Harris Haematoxylin.

d) Preparation of permanent slides : The stained Cestodes were passed through various alcoholic grades, cleared in xylol, mounted in D.P.X. and whole mount slides were prepared for further anatomical studies.

e) Drawings and measurements : The drawings are made with the help of microphotography unit and all measurements are recorded in millimeters.

f) Observation and Identification : It reveals that these tapeworms are described as a new species of the genus Stilesia. Identification is based on critical observations with related species from simillar ecological niche.

RESULTS

The following table indicate different species of Stilesia railliet. All these species have different morphological charecters. Their locality and their host is also different. Various new species of Stilesia also observed which infect the simillar host Capra hircus.

Systematic position of Parasite : *Stilesia shindei*

Class : Eucestoda
Order : Anoplocephalidea
Family :Thysanosomidae
Genus : Stilesia

The Genus Stilesia was observed by Railliet, 1893 as a type species *Stilesia globipunctata* from *Ovis aris*. *Stilesia vittata* from *Cameles dromedaries*, *Stilesia hepatica* from *Buffelus caffer* and *Silesia leiperi* from *Ovis bharal*. Later on various species are added to this genus by different researchers and they are identified and characterized by morphological studies but *Stilesia shindei* has distinct morphological charecters as squarish scolex and definet more body segmentation.

Table 1: Comparative chart showing the character of the genus Stilesia Railliet, 1893

Character	S. globipunctata	S. vittata	S. hepatica	S. leiperi	S. shindei
Scolex	Small, rounded	--------	-------	Circular	Squarish
Segment	Segment distinct	Segment not distinct	Broder than long	Broder than long	22-24 times broader than long
Testis	4-7 in number	5-9 in number	6-7 in number	5-6 in number	9-12 in number
Ovary	Somewhat/ globular	Rounded larger	Small, compact, oval	Medium, circular	Medium, rounded
Genital pore	Irregularly alternate	In anterior half	In the middle	In anterior half	Unilateral, irregularly alternate
Host	*Ovis aries*	*Camelus dremedaries*	*Buffelus caffer*	*Ovis bharal*	*Capra hircus*
Locality	Europe, Africa, Asia	East Africa	Asia, Africa	Aurangabad, M.S., India	Chalisgaon, M.S., India

The infection of *Stilesia shindei* to intestine of *Capra hircus* is more. The severity of infection is depend on the number of parasite. Their infection also indicate seasonal variation. The main clinical symptoms occur in Capra hircus are weight loss, reduced food intake, diarrhea, poor growth and reduced yield.

CONCLUSION

The parasitic diseases causes increase mortality rate and greater economic losses also. Hence to avoid severe infection preventive control measures should be applied related with immunological aspect.

The name *Stilesia shindei* n.sp. is proposed in honor of Prof. G.B. Shinde, grand research guide and Ex – registrar Dr. B.A.M. University Aurangabad who has contributed a lot of knowledge of Cestodology.

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RESEARCH ARTICLE

A Morphometric study of Baya Weaver (*Plocerousphillipinus Passeriformes*) in Chalisgaon Tehsil Dist- Jalgaon, India

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Dhande Abhishek R, Patil Sushan K and Bhavsar KR (2015) A Morphometric study of Baya Weaver (*Plocerousphillipinus Passeriformes*) in Chalisgaon Tehsil Dist- Jalgaon, India, *International J. of Life Sciences*, Special issue, A3 : 104.-106.

ABSTRACT

The Baya weaver is known for its beautiful and delicate nests hanging on various platforms. An attempt was made to study morphometry of Baya weaver hanging from various places in Chalisgaon tehsil. Seven variables namely suspension, nest length, brood chamber, nest depth, threshold, etc. taken and weight were measured of both complete and incomplete nest. The statistical analysis by one-way ANOVA showed that complete nest differed insignificantly [$p<0.05$] from that of incomplete ones. A total of 33 nests (7 Complete and 26 incomplete) were studied.

Keywords: *Ploceous phillipus*, Morphometry,

INTRODUCTION

Baya weaver (*Ploceous phillipus*) is a found across whole India and Southeast Asia. This bird is found in grasslands and scrub forests and is also associated with open cultivation. Three subspecies are mainly inhibiting in India, *Phillipus* found throughout India, *Burmacus*found eastward in southeast India and *Travancorensis* in southwest India. This bird has been known for their ranging retort shaped nest. The nest are construct from fine fibers of leaves and the nest colonies are usually found on thorny trees or palm fronds, often these nest are built near water or hanging over water making difficult for predators to reach the nest.

Earlier studies on the breeding biology of the Baya weaver have only recorded coconut palms as nesting platforms on the west coast of India, except for rare instances of nesting on exposed overhanging power lines or telecommunication wires (Ambedkar, 1970; Betts, 1952; Davis, 1971; Kirkpatrick, 1952;). The current work is an attempt to study the morphometric characteristics of 'Baya weaver nests of the electric powerlines in Chalisgaon tehsil. The current work is an attempt to study the morphometric characteristics of 'Baya weaver nests of the electric power lines in Chalisgaon tehsil.

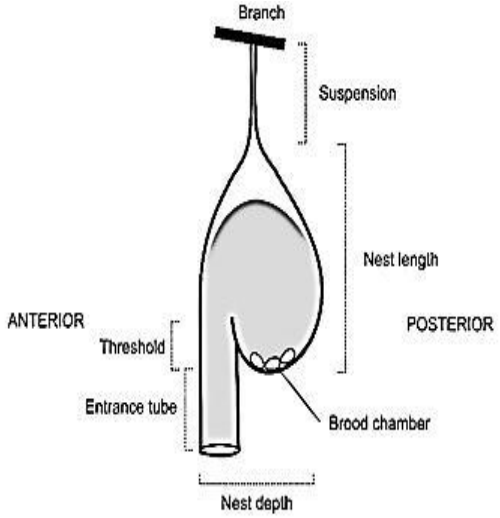
The Bayaweaver is a sexually dimorphic sparrow like bird; the adult male differs from sparrow in having brown streaks, thick bill and short rounded tail; during breeding season it acquires golden yellow plumage on the breast and head whereas female is more drab.

MATERIALS AND METHODS

Study area:

The study was conducted during February & April 2013, Chalisgaon tehsil District Jalgaon of Maharashtra, India. The study area is agricultural lands along the river Girna. The vegetation is dominated grass and scrubs. The prominent plant species found in this area are *Azadirachta indica, Ficus religiosa, Tamarandus indicus, Acacia Arabica, Ficus benghalensis, Prozopisjulifera* and *Jatropa glandulifera*. Also there are agricultural cultivation of sugarcane, cotton, groundnut, Banana, pulses and other cereals.

A number of colonies were observed on electric power lines in agriculture fields. After the breeding season the nests were abandoned by the birds, some of these abandoned nests were collected from the fields and measurements were taken in the laboratory. On the basis of the presence or absence of the entrance tube, the nests were grouped in two categories namely complete and incomplete nests. Total weight of each nest was weighed by an electronic balance with ±0.01g accuracy.



For Parameters measurement (after Quader S. 2006)

RESULT AND DISCUSSION

During the study, totally 07 complete and 26 Incomplete nests were collected and their morphometric measurements are given in table 1 and

Table 1: Complete Nest Morphomerty of various parameters

Complete Nest	Nest 15	Nest 27	Nest 23	Nest 29	Nest 30	Nest 31	Nest 32
Suspension	45	56	130	125	10	88	180
Nest length	490	364	226	425	248	498	310
Brood chamber	60	56	98	67	60	67	69
Nest depth	132	110	130	153	100	142	145
Threshold	65	40	67	50	28	48	39
Entrance Tube	65	48	66	55	45	64	60
Weight	72.2	37.4	58.6	47.5	20.4	41.1	26.9

Table 2: Incomplete Nest Morphomerty of various parameters

Incomplete	Nest 1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	19	20	21	22	28	24	25	26	33
Suspension	110	67	75	114	27	25	50	85	60	49	67	76	54	205	48	28	230	90	17	57	60	110	60	10	34	48
Nest length	210	243	234	204	170	140	155	220	132	170	128	218	330	250	261	188	149	230	217	173	190	260	234	250	278	220
Brood chamber	73	73	75	70	45	64	66	78	70	75	70	65	70	75	78	74	62	85	71	94	54	56	71	78	68	78
Nest depth	98	115	88	90	148	118	87	138	73	110	62	110	175	123	123	80	90	126	90	110	52	145	85	117	113	92
Threshold	59	56	75	48	55	56	68	58	78	67	62	67	56	60	68	65	58	64	50	76	66	58	58	50	66	65
Entrance Tube	68	57	65	50	45	38	56	52	75	57	68	56	60	55	74	72	56	60	64	67	72	51	51	59	69	62
Weight	45.8	54.7	27.6	25.1	41.2	27.5	16.4	64.3	13.2	43.4	13.1	33.3	99.4	56.1	78.2	40.3	16.1	70	54.8	26.3	9.7	52.5	42.3	49.8	54.3	51.6

Table 3: P values for test of significance between complete & incomplete nests Statistically significant (One-way ANOVA; $p<0.05$)

Parameter	Value	Significant/ Insignificant
Suspension	0.32362	Insignificant
Nest length	0.99787	Insignificant
Brood chamber	0.36339	Insignificant
Nest depth	0.70769	Insignificant
Threshold	0.70502	Insignificant
Entrance Tube	0.29453	Insignificant
Weight	0.14991	Insignificant

2.One-way Analysis of Variance (ANOVA) of above parameters resulted in *p* values which were statistically insignificant (Table no. 3). Further studies are needed to compare the complete and incomplete nest hanging from natural and manmade platforms.

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RESEARCH ARTICLE

A Case of Albinism in Common Wolf Snake *Lycodon Aulicus* (Linnaeus, 1758) from Chalisgaon Tehsil, Dist. Jalgaon, Maharashtra

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Thombre R and Dhande Abhishek R (2015) A Case of Albinism in Common Wolf Snake *Lycodon Aulicus* (Linnaeus, 1758) from Chalisgaon Tehsil, Dist. Jalgaon, Maharashtra, *International J. of Life Sciences*, Special issue, A3: 107-108.

ABSTRACT

Through this manuscript, a case of albinism in common wolf snake *Lycodon aulicus* is documented. Given albino individual was spotted/found in Patna village of Chalisgaon Tehsil in 2014. This manuscript also discusses the reasons of albinism and previous cases of albinism in *Lycodon aulicus* in Maharashtra.

Keywords: Albinism, *Lycodon aulicus* , Common Wolf snake, Chalisgaon, Maharashtra

INTRODUCTION

The phenomenon in which the melanin pigment on the body is absent is termed as albinism. It may be partial or totally depending on the extend pigment is absent. Albinism is a genetic disorder which is caused by single mutation which actually stops the formation of tyrosinase, an enzyme that changes tyrosine into a compound that eventually gives rise to melanin (Singh & Mohnot, 2009).

The albinism in snakes has been reported in many species from India and other parts of the world. The albinism in snakes for example in countries other than India, have been reported in *Storeriaoccipitomaculata* (Walkins-Colwell, 2002), *Lampropelties triangulum* (Mitchell & Mcgranahan, 2004); *Coluber logisimis, Coronella austriaca* and *Tropidonotus natrix* (Boulenger, 2000). In India albinism has been described in *Python molurus* (Lahiri, 1955), *Eryx conicus* (Whitaker, 1971); *Naja naja* (Kumar, 1988), *Oliodon arnensis, Coleognathus helena* (Vyas, 2012), Macropisthodon *plumbicolor* (Sayyad, 2012; Hoshing *et al*., 2013), *Gongylophisconicus* (Jadhav & Mahabal, 2012)and in *Gryptotyphlops acutus* (Nivalkar *et al*., 2012).

In case of *Lycodon aulicus* albinism is reported in Maharashtra at two instances. In the first instance Hoshing *et al*. (2013) discussed an albino *Lycodon aulicus* with pink body and dark red bands. In another instance Bhutkar and Mahabal (2014) described an albino *Lycodon aulicus* with purple blue body, white cross bar with unusual patterns, pink patches on sides, eyes pinkish red suggesting it as total albino.

Fig. 1: An Partial Albino wolf snake *Lycodon aulicus* from Chalisgaon

Fig. 1: A Wolf Snake *Lycodon aulicus* with normal colouration

DISCUSSION

Through this note we describe another case of albinism in *Lycodon aulicus.* The *Lycodon aulicus,* commonly known as The Common Wolf Snake is a non-venomous snake from Family Colubridae. It is distributed in Sri Lanka, Indian subcontinent, Maldives, Myanmar, Indo-China, Malaya, Indonesia, Philippines (Daniel, 2002). Generally the colour of *Lycodon aulicus* is brown with transverse white bands with some dark brown spots on the bands. The first author spotted the snake at around 6 PM on January 1st, 2008 at the house of Mr. Anil Patil, Harigiribaba Nagar area of village Patna, tehsil Chalisgaon, and district Jalgaon. The surrounding area is an agro-ecosystem with sparse patches of dry and scrub vegetation and is near to Patnadevi Forest; the snake was photographed and released into the wild. In this case of albinism, the snake observed was having pink coloration, white cross bands with light brown spots on body and black eyes which clearly indicated that this albino is partial. Its length was 50cm approximately. Though it is very difficult for an albino individual of any speciesto survive in the wild as they are detected easily by their predators, the length of this individual suggests that albinos can also survive up till adulthood in the wild.

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RESEARCH ARTICLE

Exploitation of green biomass for the preparation of leaf protein concentrates

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ABSTRACT

The available food resources are always remaining to be constrained for the ever increasing population. Hence the non-conventional food resourced is to be considered in the coming year. The present study includesgreen foliages from 7 plants to prepare leaf protein concentrate viz., Berseem (*Trifoliuma lexandrium*L.), *Alysicarpus vaginalis* L. var. stocksii., *Alternanthera paronychioides* St. Hil., Cabbage (*Brassica oleracea* L. var. capitata), Radish (*Raphanus sativus*L.), Adulsa (*Adhatoda vasica* Nees.), and Bauchi (*Psoralia corylifolia* L.). The selected plants were fractionated and subjected to prepare LPC as suggested by Pirie (1971). The maximum yield of juice and fiber are reported in green foliages of *Brassica oleracea* L. (550 ml/Kg) and *Alysicarpus vaginalis* L.(540 g/Kg) respectively. Maximum dry weight of juice and DPJ was reported in green foliage of *Adhatoda vasica* Nees. (120 and 70.22 g respectively) Maximum dry weight of fiber was reported in green foliage of *Alysicarpus vaginalis* L. (305 g). Maximum dry weight of LPC was reported in green foliages of *Psoralia corylifolia* L. (80.52 g).

Keywords: leaf protein concentrates, non-conventional food resources, DPJ, LPC, fiber, biomass.

INTRODUCTION

In our country huge amount of green biomass are available in the form of weeds, fodder crops and by-products of the vegetable material. The utilization of this material can be enhanced for the benefit of human being by the extracting available protein in this green biomass. To extract proteins from green leaves numerous technologies have been developed over last 50 years. For this purpose, a technique of fractionation has been proposed by Pirie from United Kingdom. This technique of fractionation recommended by Pirie (1942) has now become popular as "Green Crop Fractionation (GCF)" which involves the separation of proteins from the indigestible fibrous material of leaves.

The process of GCF is basically consists of mechanical operations of grinding and pressing, which enable the fresh green leafy foliages to be separated into two fractions; a protein rich juice and the pressed crop residue

(PCR). For this purpose the crop is macerated with IBP pulper (Davys and Pirie, 1969a)or with simple grinder mixer and then pressed with IBP press (Davys *et al*.,1969b) or by hand pressing method. After thorough maceration of the crop, almost all cells in it are damaged and the proteins get liberated in the juice expressed after pressing the pulp. After releasing the juice a fibrous material left behind called as pressed crop which is also nutritionally sufficient to the cattle. The juice or leaf extract contain, along with the proteins, sugars, lipids, vitamins and other soluble components of the cell protoplasm. When this fraction is either heated or acidified, a green curd is produced due to the precipitation of protein. This protein rich curd is referred as leaf protein concentrates (LPC). The LPC can be separated from remaining part of the juice, known as deproteinized juice (DPJ) by filtration through a simple cotton or muslin cloth. Thus, the process of GCF results into four fractions i.e. Leaf extract or juice, Pressed crop residue (PCR) or Fiber, Leaf protein concentrate (LPC), Deproteinized juice (DPJ). All these four products can be used in different ways as suggested by several workers.

First suggestion on the use of protein extracted from leaves was made by Ereky (1927). Slade (1937) argued that the use of protein from grass was more economical than meat obtained after feeding the grass to animals. Chibnall (1939) and his co-workers provided information on the quantity and properties of protein in leaves.

Pirie (1942) put forth the real potential of leaf protein (LP) and its use as human food in during II World War. After two decades of war several workers took interest in leaf protein (Davies *et al*., 1952; Carpenter *et al*., 1954; Mcdonald, 1954; Tilley *et al*., 1954; Anandaswamy and Date, 1956; Cowlishaw *et al.,* 1956; Raymond and Harris, 1957; Guha, 1960; and Chayen *et al.,* 1961).

Byers (1961) evaluated the extractability of leaf protein from leaves of 60 tropical spp. growing in Ghana. His results showed that some common weeds offered promising results, yielding more protein than the leaves of many crops with good quality. Devi *et al*. (1964) studied the isolation and composition of leaf protein from certain species of Indian flora and reported that some of the proteins isolated are potentially useful for supplementation of cereal diets deficient in lysine and methionine. Carlsson and Clarke (1983) studied the suitability of *Atriplexhortensis* L. as a source of leaf protein concentrate and showed that it could be utilized for the preparation of LPC as it was found superior to *Spinaceaoleracea* L. The cultivation of berseem for the measurement of LP shown that it could give a yield of 700kg per hectare extractable protein in 140 days (Mungikar, 1974; Tekale, 1975; Mungikar *et al*., 1978; Mungikar *et al*., 1976b; Patil and Mungikar, 1992). The yield of extractable protein from leaves taken at the time of harvest of the edible part (root, tuber) from brassicas, beet root, turnip and radish ranged between 76 to 171kg/ha (Tekale, 1975; Deshmukh*et al*., 1974; Tekale and Joshi,1976; Giri and Nagpal, 1984; Giri *et al*., 1983). Leafy vegetables were also found suitable for producing good quality of leaf protein concentrates (Mungikar and Ajaykumar, 1995).

MATERIALS AND METHODS

Selection of plants: For the present work green foliages from 7 plants were selected to prepare leaf protein concentrate viz., Berseem (*Trifolium alexandrium*L.), *Alysicarpus vaginalis* L. var. stocksii*., Alternanthera paronychioides* St. Hil., Cabbage (*Brassica oleracea* L. var. capitata), Radish (*Raphanus sativus* L.), Adulsa (*Adhato davasica* Nees.), and Bauchi (*Psoralia corylifolia* L.). These plant material were authentified at Department of Botany, RTM Nagpur University, Nagpur. These plants were collected from different places. Berseem was collected from Walu Sangopan Kendra, Nagpur. Bauchi was collected from Krishi Vidyapith Campus, Nagpur. Cabbage and Radish were collected from local vegetable market. *Alternanthera*, *Alysicarpus,* and *Adhatoda* were collected from RTM Nagpur University campus, Nagpur.

Preparation of leaf protein concentrates: The selected plants were fractionated and subjected to prepare LPC as suggested by Pirie (1971). These plants were first washed well with water and pulped with grinder mixer. The juice was then expressed by hand pressing method. The amount of juice and fiber obtained per Kg of green foliages was recorded. The juice obtained was employed for the preparation of LPC. LPC was prepared by heat coagulation method.

Heat coagulation method: For this purpose, a sample of 100 ml juice was slowly added to 20 ml boiling water with continuous stirring, as a result proteins in juice coagulated resulting into green colour curd called as leaf protein concentrate (LPC). During whole process the temperature was maintained at 95°C. This

heated juice was then filtered through preweighed Whatmann filter paper No.1. During filtration the yellowish filtrate was obtained which is called as deproteinized leaf juice (DPJ) or whey. The green coloured curd (LPC) along with filter paper and DPJ was dried at 55°C in hot air oven. The amount of the dried LPC and DPJ was recorded per Kg of fresh green foliages.

RESULT AND DISCUSSION

I. Fresh weight of fiber and amount of juice from different plants obtained during green crop fractionation.

The yield of juice and fibers of various plants obtained during GCF are summarized and shown in table 1 and fig. 1a, 1b respectively. The maximum yield of juice and fiber are reported in green foliages of *Brassica oleracea*L. (550 ml/Kg) and *Alysicarpus vaginalis* L.(540 g/Kg) respectively, whereas, the minimum yield of juice and fiber are reported in green foliages of *Adhatoda vasica* Nees. (217 ml/Kg) and *Brassica oleracea* L. (250 g/Kg) respectively.

During present investigation 7 plants were considered for fractionation. From these, two are vegetable crops (radish and cabbage), a fodder crop (berseem) and four wild plants (*Adhatoda vasica* Nees., *Psoralia corylifolia* L., *Alysicarpus vaginalis* L., and *Alternanthera paronychioides* St. Hil.). The green foliages (leaves and tender stem) of these plants were employed for the preparation of LPC. Though the foliages of these wild plants are not used as source of vegetables, however, the young shoot part of *Psoralia corylifolia* L., *Alysicarpus vaginalis* L., and *Alternanthera paronychioides* St. Hil. were generally consumed by animals and *Adhatoda vasica* Nees.is well known for its medicinal value. A large variation in both juice as well as fiber content was observed due to their time ofharvesting and vegetative growth. The coefficient of variation of the obtained values of juice and fiber was 35.60 and 30.81% respectively.

PROCESS OF GREEN CROP FRACTIONATION (GCF)

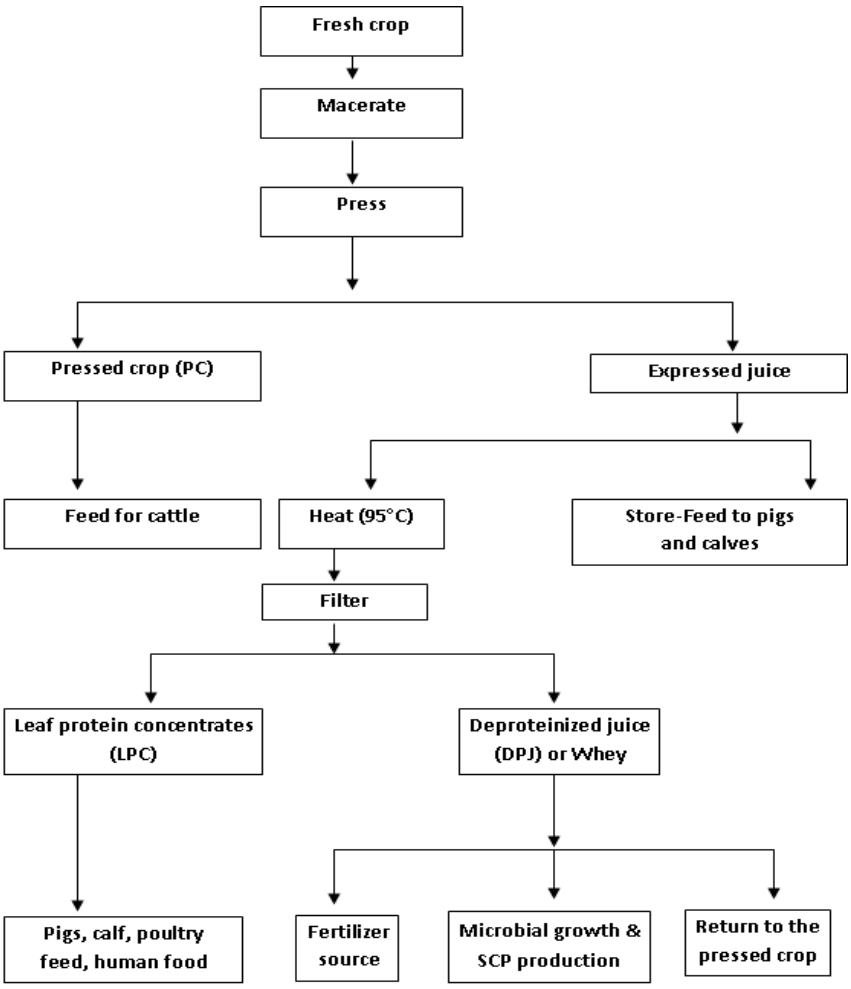

Table 1: Fresh weight of fiber and amount of juice from various plants obtained during green crop fractionation.

Sr. No.	Name of the plant's	Yield of wet fractions of plant materials.	
		Juice (ml / Kg)	Fiber (g / Kg)
1	*Alysicarpus vaginalis* L.	265	540
2	*Trifolium alexandrium* L.	390	354
3	*Alternanthera paronychioides*St. Hil.	407	343
4	*Raphanus sativus* L.	520	258
5	*Brassica oleracea* L.	550	250
6	*Psoralia corylifolia* L.	252	494
7	*Adhatoda vasica* Nees.	217	508
-	Mean	372	392
-	Std. Deviation	132	121
-	Std. Error	50.00	45.70
-	Coefficient of variation	35.60%	30.81%

Table 2: Dry weight of various fractions of plants obtained during green crop fractionation.

Sr. No.	Name of the plant's	Dry weight of fractions, g / Kg of fresh green foliages.			
		Juice	**Fiber**	**LPC**	**DPJ**
1	*Alysicarpus vaginalis* L.	118.00	305.00	71.15	38.95
2	*Trifolium alexandrium* L.	62.75	88.00	31.64	23.36
3	*Alternanthera paronychioides* St. Hil.	115.00	94.74	72.41	31.42
4	*Raphanus sativus* L.	53.40	80.00	20.83	17.83
5	*Brassica oleracea* L.	62.23	68.34	21.51	36.33
6	*Psoralia corylifolia* L.	117.24	160.30	80.52	30.53
7	*Adhatoda vasica* Nees.	120.00	175.00	42.42	70.22
	Mean	92.70	139.00	48.60	35.50
	Std. Deviation	31.20	84.00	25.60	16.90
	Std. Error	11.80	31.80	9.67	6.40
	Coefficient of variation	33.71%	60.53%	52.58%	47.65%

The fresh green foliages from all these plants were fractionated for the measurement of the yield of juice (fresh and dried), fiber (fresh and dried), LPC (dried) and DPJ (dried) per unit weight of foliage. The fractionation of foliages was undertaken using mechanical mixer for pulping and hand pressing method was employed for expressing juice. To justify the usefulness of wild plants in green crop fractionation process, the yields of the wild plants have been compared with *Brassica oleracea*L. since this plant have been extensively utilized for the green crop fractionation process by various workers.

The result obtained showed that the extractability of juice and fiber varied from species to species. The results also showed that the quantity of juice is inversely proportional to the quantity of fiber; if the one fraction was more than other would be low or vice versa. As far as yield of expressed juice is concerned, all the wild plants have relatively low extractability rate than that of *Brassica oleracea*L. (table 1). The variation in extractability of juice might be due to nature of the foliages and their moisture content.

Patil and Salve (2000) has been reported the yield of juice for radish as 584ml/ Kg, (fresh wt. basis) respectively. Gogle (2000) mentioned the yield of juice from cabbage and radish as 628 and 711ml/Kg fresh material, respectively. However, in present study, the yield of juice from cabbage and radish was found 550

and 520ml/Kg respectively. This variation in extractability of juice might be due to equipment used for expressing the juice, stage of harvesting and also because of difference in region. In present study the juice was expressed by hand press method.

Yield of fiber from all wild plants are comparatively higher than that of *Brassica oleracea*L. (table 1). Bhande and Mungikar (1990) reported the yield of fiber from lucerne as 426g/Kg (fresh wt. basis), whereas in present study it ranged from 250 to 540g/Kg, fresh material. The yields of fiber from various crops are depends upon their maturity level and also it varied from specie to species.

II. Dry weight of various fractions of plants obtained during green crop fractionation (g/Kg, fresh green foliages).

The results obtained for the various dried fractions are represented and illustrated in table 2 and fig. 2 respectively. Maximum dry weight of juice and DPJ was reported in green foliage of *Adhatoda vasica* Nees. (120 and 70.22 g respectively) and minimum in green foliage of *Raphanus sativus* L. (53.4 and 17.83g respectively). Maximum dry weight of fiber was reported in green foliage of *Alysicarpus vaginalis* L. (305 g) and minimum in *Brassica oleracea* L. (68.34 g). Maximum dry weight of LPC was reported in green foliages of *Psoralia corylifolia* L. (80.52 g) and minimum in *Raphanus sativus* L. (20.83 g).

The yield of various fractions from wild plants was compared with *Brassica oleracea*L. to check their usefulness in green crop fractionation process. The present results revealed that the dry weight of juice, fiber and LPC of all wild plants are relatively higher than that of *Brassica oleracea*L. The higher yield of LPC in wild plants may be attributed to high crude protein content in the juice extracted from their foliages. In general, leguminous plants gave better yield of LPC. However, the yield of DPJ from *Alysicarpus vaginalis* L. and *Adhatoda vasica* Nees. was found higher than that of *Brassica oleracea*L., but it was lower in *Alternanthera paronychioides* St. Hil. and *Psoralia corylifolia* L.

Several workers have been reported the yield of LPC from radish as 40.9 g/Kg (Patil and Salve, 2000), 23.77 g/Kg (Gogle, 2000) and 20.86 g/Kg (Madhekar, 2008). Gogle (2000) have been reported the yield of LPC from cabbage as 43.94 g/Kg. While, in present investigation radish and cabbage showed LPC yield as 20.83 and 21.51 g/Kg respectively. This variation in LPC yield might be due to equipment, regional difference and the duration of processing. Singh (1969) suggested that a plant can be selected for the preparation of LPC, when the yield of dry LPC exceed 10 g/Kg fresh green foliage and the resulting LPC contains more than 5% nitrogen. In this view all plants species which is chosen in present study could be considered suitable for the preparation of LPC.

CONCLUSION

The present results revealed that the yield of expressed juice from all the wild plants was relatively low as compared to *Brassica oleracea*L.; among all these wild plants *Adhatoda vasica* Nees. yielded minimum amount of juice. However all wild plants show comparatively more yield of fiber than that of *Brassica oleracea*L.

Similarly the dry weight of juice, fiber, LPC and DPJ of all wild plants are relatively higher than that of *Brassica oleracea*L. except *Alternanthera paronychioides* St. Hil. and *Psoralia corylifolia* L. which showed lower yield of dry DPJ.

Thus from the present study it is concluded that the green biomass can be successfully used for the extraction of protein and the preparation of leaf protein concentrate. However, the studies regarding its nutritional aspects are to be considered in future.

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RESEARCH ARTICLE

Studies on effect of Judicious integrated doses of nitrogen fertilizer and biofertilizer on yield performance of hybrid *Napier* grass (CV.RBN-9)

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ABSTRACT

In recent times fertilizers are responsible for 50 percent increase in crop yield. Due to progressive intensification of agriculture and production of high yielding varieties; fertilizers consumption has increased very much accounting to 23.6 metric tonnes of nutrients every year through crop removal. Over use in certain potential areas and sub optimum use in larger areas are crucial issues; and indiscriminate use of chemical fertilizer is creating lots of problems especially soil degradation and pollution. Therefore, emphases should be to reduce the use of inorganic fertilizers and to improve fertilizer use efficiency. Hence, a strategy for integrated nutrient supply is evolved by judicious combination of chemical fertilizers, organic manures and biofertilizers. Therefore, attempts were made during present study to observe the effect of integrated fertilizer dose (urea + biofertilizers) on productivity of popular forage crop hybrid Napier (cv.RBN-9). Study also includes investigation on percentage increase in the yield of forage crop and saving of nitrogenous fertilizer due to use of biofertilizer.

Keywords: Hybrid Napier, Nitrogen fertilizer, Biofertilizer, Integrated dose, Yield.

INTRODUCTION

Fertilizer used to supply N, P and K play crucial role in plant production. Proper soil and crop husbandry linked up with input of chemical fertilizer is a common practice to push up and stabilize yield of crop plants (Wasnik, 1992; Umesha and Purushottam, 1996; Singh et al., 1998 Jha et al,, 2013). In recent time fertilizers are responsible for 50 percent increase in crop yield. Due to progressive intensification of agriculture and production of high yielding varieties; fertilizer consumption has increased very much accounting to 23.6 metric tonnes of nutrients every year through crop removal. Over use in certain potential areas and suboptimum use in large areas are crucial issues; and indiscriminate use of chemical fertilizer is creating lots of problems essentially soil degradation and pollution.

Therefore, emphasis should be to reduce the use of inorganic fertilizers and to improve fertilizers use efficiency. Hence, come the integrated concept of nutrient supply, where efficient use of chemical, organic and biological source is practiced (Surekha and Rao, 1995). Use of inorganic fertilizers has become esential part of the crop production and a balance form of fertilizer use is always a prerequisite to obtain higher yield. However, these fertilizers are costly and also pollute the environment, hence a strategy for integrated nutrient supply is evolved by using judicious combination of chemical fertilizer, organic manure and biofertilizer (Panwar et al., 2001). A combine effect of chemical fertilizer along with biofertilizer was studied by several workers (Mohan and Pradhan, 2001; Gautam and Pant, 2002; Mahajan et al., 2002; Dubey et al., 2014). Hence, attempts were made during present study to observe the effect of integrated fertilizer dose (nitrogenous fertilizer along with biofertilizers) on productivity of forage crop Hybrid Napier (cv. RBN9). This study also includes investigation on percentage increase in the yield of fodder crop and saving of nitrogenous fertilizer due to the use of biofertilizer.

MATERIALS AND METHODS

During present investigation, the fodder crop Hybrid Napier (cv. RBN9) recommended by Mahatma Phule Krushi Vidyapith Rahuri, Maharashtra was selected for treatment with integrated dose of nitrogenous fertilizer and biofertilizers. The fodder crop was cultivated at Maharashtra Sheli va Mendhi Vikas Prakshetra, Bilakhed, Chalisgaon (MS) during summer season in 2000-2001. The soil was analysed by government soil analyzing laboratory, Jalgaon (2000) of its nutrient content before sowing. The soil was poor in phosphorous, moderate in nitrogen and potash with a normal pH 7.8.

A piece of land measuring about 360 sq. m. (15m x 24m) was prepared by ploughing and cross ploughing while preparing the land compost prepared on farm was added at the rate 3000 kg/ ha. The land was then divided into 24 plots each with an area of 15 sq m for sowing the crop. The plots were arranged in randomized block design. The crop sown in 45/60 cm apart in rows by hand. All crops were raised under irrigated condition. The seed rates were used as per the recommendations. Nitrogenous fertilizer was used in the form of urea while biofertilizer Azospirillum.

Crop received eight fertilizers treatment through urea and biofertilizers alone or in combination were N0, N60, N120, N180, N240 i.e. 0, 60,120, 180, 240 kg/ha. BF (biofertilizer alone), Bf + N60 and Bf+N120 kg/ha. The plot which did not received fertilizer were treated as control plot. The biofertilizers were used at a rate of 2 kg./ha. Fifty percent of the dose of fertilizer nitrogen was applied as basal dose and remaining half after a month of crop growth, while biofertilizer (Bf) were applied directly to the seeds at a rate of 2 kg/ha the crop were cultivated under irrigated condition and the use of insecticide and pesticide were evolved. The crop were harvested from three replica every time at preforming stage from the net size of plot harvested was 13.72 m2. The weight of the green fodder obtained from each plot was measured and the samples of green fodder were immediately brought to the laboratory for analysis. The sample were chopped into 2 to 3cm pieces and dried in an electric oven at 75± 5^0c till constant weight for dry matter (DM) determination. Dried sample were ground to a fine powder and are used for estimation of crude protein (CP). Nitrogen (N) content was determined in duplicate by Microkjeldahl method (Bailey, 1967). The value of crude protein (CP) was expressed as N x 6.25.

RESULT AND DISCUSSION

RBN-9 variety of hybrid Napier grass cultivated during the field trial responded satisfactory to fertilizer nitrogen (N) application which produced succulence in plant with lushness in the foliage. Biofertilizer (Azospirillum) alone elicited significant increase in yield over the control. When the biofertilizer (inoculation) was integrated with fertilizer application, the fodder yield increased progressively with an increase in dose of nitrogen as was observed by George et al (1998) and Mishra, etal (2008).

Table 1: Details of the cultivation practices and harvesting of Hybrid Napier (cv.RBN-9) grass.

Crop	Cultivar	Duration	Seed rate (Slips/ha)	No of harvest	Fertilizer treatment (kg/ha)
Hybrid Napier	RBN-9	20 March 2000 to 13 July 2000	25000 slips	1 cut + 1 regrowth	N0, N60, N120, N180, N240, Bf, N60+Bf, N120+Bf

Table 2: Effect of integrated fertilizer dose on the yields of green fodder, dry matter and crude protein from hybrid Napier (cv. RBN-9) Duration 20 March 2000 to 13 July 2000

Date of Harvest	Type of cut and age of the crop (in days)	Fertilizer treatment (Kg/ha)	Green Fodder		Yield (Kg/ha)		
			% DM	N% of DM	Green fodder	Dry matter	Crude protein
5 June 2000	1 cut (78)	N0	21.0	1.65	43916	9222	951
		N60	18.0	1.72	47360	8524	916
		N120	19.5	1.77	49513	9655	1068
		N180	21.5	1.78	50590	10876	1210
		N240	21.0	1.82	54465	11437	1300
		Bf	22.0	1.68	45208	9945	1044
		N60+Bf	18.0	1.74	51235	9222	1002
		N120+Bf	22.0	1.80	52742	11603	1305
13 July 2000	1 regrowth (38)	N0	20.0	1.62	42805	8561	866
		N60	19.0	1.70	45735	8689	923
		N120	20.0	1.75	48524	9704	1061
		N180	21.0	1.80	49325	10358	1165
		N240	22.0	1.80	53400	11748	1321
		Bf	21.0	1.70	43890	9216	979
		N60+Bf	19.0	1.75	50660	9625	1052
		N120+Bf	21.5	1.78	51246	11017	1225
	Total in 116 days	N0			86721	17783	1817
		N60			93095	17213	1839
		N120			98037	19359	2129
		N180			99915	21234	2375
		N240			107865	23185	2621
		Bf			89098	19161	2023
		N60+Bf			101895	18847	2054
		N120+Bf			103988	22620	2530
C.D.(P= 0.05) F value					5912	2411	128
	Replicate				8.09**	NS	9.14**
	Treatment				14.26**	7.57**	51.46**

*Significant, ** Highly significant NS – non significant

Table 3: Effect of Integrated fertilizer doses on the yields from Hybrid Napier (cv.RBN-9)

Treatment	Green fodder yield (kg/ha)	% increase in yield		Dry matter yield (kg/ha)	Crude protein yield (kg/ha)
		Over control	Over respective N level		
N0	86721	-	-	17783	1817
N60	93095	07	-	17213	1839
N120	98037	13	-	19359	2129
N180	99915	15	-	21234	2375
N240	107865	24	-	23185	2621
BF	89098	02	02	19161	2023
N60+Bf	101895	17	09	18847	2054
N120+Bf	103988	19	06	22620	2530

At the first cut, which was harvested 78 days after sowing, dry matter (DM) content was between 18 to 24 percent. At the regrowth cut taken 38 days after first cut, the foliage on control plots had 20 percent dry matter (DM) which showed significant change due to other treatment, but amendment with nitrogen increased N content in foliage from 1.65 to 1.82 percent at the first cut, while from 1.62 to 1.80 percent

at regrowth cut (Table 2) was noticed. In the two harvests taken in 116 days, the crop yielded 86721, 17783 and 1817 kg/ha green fodder, dry matter and crude protein respectively, without fertilizer treatment. Application of nitrogen significantly increased the yields to as high as 107865, 23185 and 2621 kh/ha for GF, DM and CP respectively.

Biofertilizers (BF) alone yielded 89098, 19161 and 2003 kg/ha green fodder, dry matter and crude protein respectively. The results were comparable to those reported by Biswas et al (2001). The yield gradually increased with fertilizer nitrogen (N120 + BF) to 103988, 22620 and 2530 kg/ha respectively in total 116 days. The value of 'F' also indicates significant effect of fertilizer application (Table-2). Effect of integrated fertilizers dose on percent increase in yield over control and respective nitrogen level is given in Table 3. Increase in yield over congtrol ranged from 2 to 24 percent while in maximum 24 percent increase in the yield was observed on plots treated with 240 kg N/ha in 116 days and minimum 02 percent increase in the yield due to biofertilizer (BF) was reported. The results were comparable with these reported by Pisal et al. (1991). Patel et al. (1992) and Biswas et al. (2001). The biofertilizer alone treated plots shown minimum 02 percent increase in yield over respective nitrogen level while maximum 09 percent increase in yield was recorded in plots treated with N60+BF in 116 days.

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RESEARCH ARTICLE

Phytochemical screening, antimicrobial and pesticidal activity in root, stem, and leaves extract of *Parthenium hysterophorus*

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ABSTRACT

The present research work was carried out to study the phytochemical screening, antimicrobial and pesticidal activity in root,stem and leaves extract of *Parthenium hysterophorus.* This study was conducted during 2013 to 2015. The extraction was done by using soxhlet apparatus and studied the phytochemical screening for alkaloids, cholesterols, flavinoids, terpenoids, diterpines, saponins, phenol resins, tannins and cardiac glycosides. In antimicrobial activity it was concluded that *P. hysterophorus* shows highest antimicrobial activity 12.66 mm in *Lactobacillus sporogense* and least antimicrobial activity recorded in stem extract in Staphylococcus aureus measured 3 mm. and pesticidal activity against *Tribolium* sp.and *Oryzaephilus* sp. And in pesticidal activity the l extract of p. Extract of *P. hysterophorus* shows significant lethal effect against *Tribolium sp.* and *Oryzaephilus sp.* The 50% lethal effect shows at a 6% conc. in a root and stem extracts. While in a root extract it's at 8% concentration.

Keywords: Antimicrobial, phytochemical, pesticidal, soxhlet.

INTRODUCTION

Parthenium is commonly called as congress grass, carrot weed, bitter weed, gajar grassetc. At present it occupies almost all part of India. It is native of sub tropics of north and south America & it accidently introduced in Indian subcontinent. It is an annual herb with a deep tap root system and an erect stem that becomes woody with age. As it matures, the plant develops many branches in its top half and may eventually reach a height of two meters. Plant produces a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of (Sukanya *et al*., 2009). It's leaves are pale green, deeply lobed & covered within soft hairs. Small creamy white flowers occur on the tips of numerous stems. Each flower contains four to five black seeds that are wedge-shaped, two millimeters long with two thin, white scales. It weed normally germinates in spring and early summer, produces flowers and seeds throughout its life and dies around late autumn. However, with suitable conditions (rain,

available moisture, mild temperatures), Parthenium weed can grow and produce flowers at any time of the year. In summer, plants can flower and set seeds within four weeks of germination, particularly if stressed. Parthenium weed is a vigorous species that colonizes weak pastures with sparse ground cover. It will readily colonise disturbed, bare areas along roadsides and heavily stocked areas around yards and watering points. It can also colonise brigalow, gidgee and softwood scrub soils. It's presence reduces the reliability of improved pasture establishment and reduces pasture production potential. The contact with plant or the pollen can cause serious allergic reactions such as dermatitis and hay fever. his weed is known to cause many health hazards which have now reached epidemic proportions. Agriculturists are concerned about *P. hysterophorus* affecting food and fodder crops, since the pollen and dust of this weed elicit allergic contact dermatitis in humans. Parthenin the active compound present in *Parthenium hysterophorus* is known to show activity against termites, cockroaches (Tilak, 1977). As well as migratory grasshoppers, *Melanoplus sanguinipes* (Picman *et al*., 1981). Antimicrobial compounds of plant origin may found in plant stems, roots, leaves, bark, flowers, or fruits (Beuchat *et al*., 1994). The present study focus on phytochemical screening, antimicrobial activity and pesticidal activity in ethanol extract of root, stem, and leaves.

MATERIALS AND METHODS

This study was conducted in Department of Botany, B.P. Arts, S.M.A. Science and K.K.C. Commerce College Chalisgaon (M.S.) India.We determined the phytochemical screening, antimicrobial and pesticidal activity in root, stem, and leaves extract of *Parthenium hysterophorus.*

Collection and preservation of plant samples: Fresh roots, stem and leaves of *Parthenium hysterophorus* were collected in the month of August 2014 from local area of Chalisgaon town. These samples were dried for one day to eliminate surface moisture. Thenroots, stem and leaves packed into envelop and kept in oven at 55^{o}c temperature until dried. Driedroots, stem and leaves were grinded separately in a mortarto obtained powder which was then kept in a plastic bags for further use.

Preparation of extract: 20gm of leaves, stem and seeds powders were extracted with 140 ml of solvent (Ethanol) for 24 hr. by using sox late apparatus. Extracts were used for different tests.

Preliminary phytochemical analysis: Preliminary phytochemical screening of Parthenium was done following the standard procedures adapted by the various workers. (Daniel, 1991; Harborne,1998; Kokate *et al*., 2004).

Qualitative phytochemical study: Qualitative phytochemical study done on root, stem and leaves extract of Parthenium hysterophorus. The test done by using standard protocols. Qualitative analysis done for phytochemicals such as alkaloids, cholesterols, flavonoids, terpenoids, diterpines, saponins, phenol resins, tannins and cardiac glycosides.

Antimicrobial activity: Four different species of bacteria will be used in this study to explore the effectiveness of root, stem and leaves ethanol extract of P. hysterophorus on inhibition of growth; the bacteria chosen for this study are both of Gram positive and Gram negative bacteria. The four bacterial species, which would be used in this study are*: Escherichia coli, Pseudomonas auriginosa, Lactobacillus sporogense* and *Staphylococcus aurius.* The experiment method employed for this investigation was the agar well Diffusion Assay method, it was chosen because it was the easiest and the simplest method to use.

Pesticidal activity: Insect rearing- The target insect *Tribolium sp.* and *Oryzaephilus sp.* was reared by using standard laboratory method. Both pests were reared on wheat flour in separate flask. Healthy adult were separated time to time from dead insect and kept in special flask. The optimum temperature was 30^{0}c.Theextract were mixed in to diet of insect. And control was used as water. Each concentration of extract mixed into diet and kept in diet and kept in different flask. Each flask contain ten adult insect The flask stored at 25-30^{o}c and 70-75 % relative humidity with 16; 8 photoperiod. Adult mortality period was observed after 48 hour.

RESULTS

The present study carried out on the *P. hysterorus* revealed the presence of medicinal active constituents. The phytochemical active compounds of *P. hysterorus*

were qualitatively analyzed for stem, roots and leaves separately and the results are presented in Table1 In these screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides, phenols shows different types of results indifferent plant parts extract These phytochemicals have certain medicinal value against chronic diseases. The ethanolic extract of leaves, stem and roots contains Alkaloids, chlolesterol, flavonoid, terpenoid, saponine, resins, tannineand cardiac glycoside. Whereas root extract of *Parthenium hysterophorus* does not contain phenols.

Table 1: Preliminary Phytochemicals Screening*Parthenium hysterophorus*

Sr.No.	Phytochemicals	Extract of ***Parthenium hysterophorus***			Control
		Roots	Stem	Leaves	
1.	Alkaloids	+	+	+	-
2.	Cholesterol	+	+	+	-
3.	Flavonoids	+	+	+	-
4.	Diterpines	+	+	+	-
5.	Saponines	+	+	+	-
6.	Phenols.	-	+	+	-
7.	Resins	+	+	+	-
8.	Tannins	+	+	+	-
9.	Fixed oil	+	+	+	-
10.	Cardiac glycoside	+	+	+	-

Table 2: Antimicrobial activity in *Parthenium hysterophorus.*

Sr.No.	Bacterial strains	Ethanol extract of ***Parthenium hysterophorus*** ***(Average zone of inhibition in mm)***			Control
		Root	Stem	Leaves	
1.	*Escherichia coli*	2.66	05	5.33	00
2.	*Pseudomonas auriginosa*	03	04	06	00
3.	*Lactobacillus sporogense*	12.66	6.66	3.33	00
4.	*Staphylococcus aureus*	09	03	3.33	00

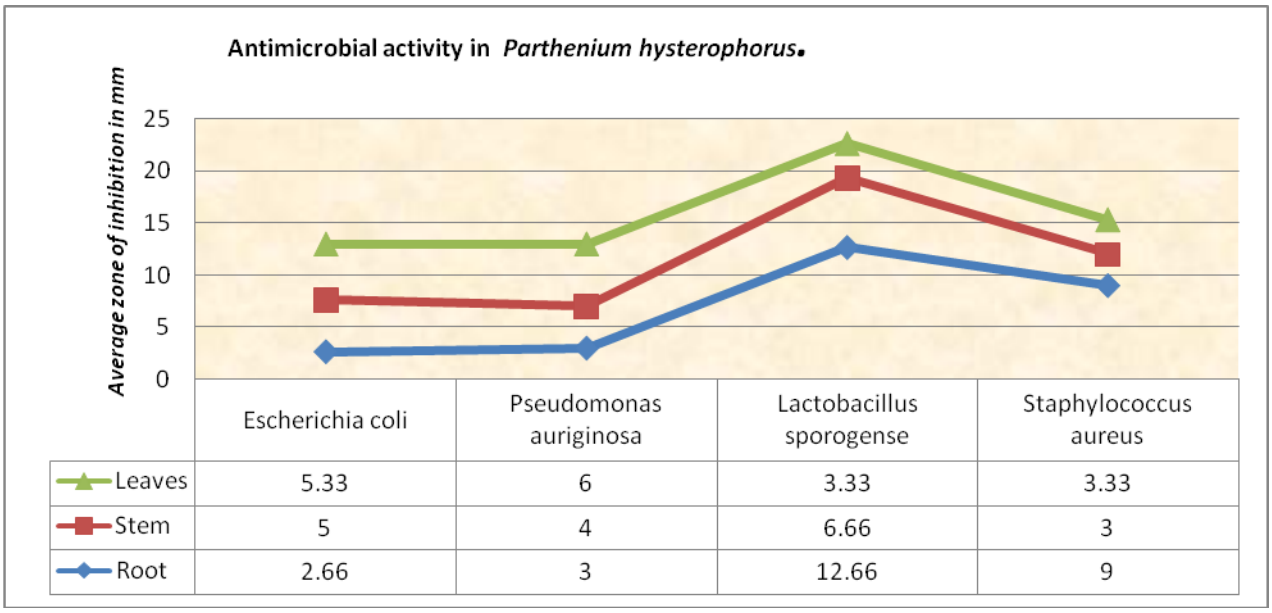

Table 3: Pesticidal activity in *Parthenium hysterophorus*

Sr.No	Sample	Conc.	Mortality % after 48 hrs.		
1)	Control		*Tribolium sp.*	*Oryzaephilus sp.*	*Control*
2)	Root	2	02	20	02
		4	02	20	02
		6	06	80	02
		8	60	80	02
		10	100	100	02
3)	Stem	2	00	60	02
		4	40	60	02
		6	80	100	02
		8	100	100	02
		10	100	100	02
4)	Leaves	2	02	20	02
		4	20	20	02
		04	50	20	02
		8	100	80	02
		10	100	100	01

Results obtained in antimicrobial activity that the tested *P. hysterophorus* plants Root, stem and leaves extracts possess potential antibacterial activity against *E. coli, Pseudomonas auriginosa ,Lactobacillus sporogense* and *Staphylococcus aureus.* (Table 2) When these extract were checked by agar well diffusion method, the root extract of *P. hysterophorus* shows highest antimicrobial activity 12.66 mmin *Lactobacillus sporogense* and least antimicrobial activity recorded in stem extract in Staphylococcus aureus measured 3mm. The Gram negative bacteria *Escherichia coli, Pseudomonas auriginosa* shows resistance against root, stem and leaves extract of Parthenium. But Gram positive bacteria *Staphylococcus aureus, Lactobacillus sporogense* are susceptible against root, stem and leaves extract of Parthenium. The root extract of Parthenium shows higher antimicrobial against *Staphylococcus aureus* and *Lactobacillus sporogens* 12.66 and 09mm respectively.

In pesticidal activity the ethanol extract of p. Extract of *P. hysterophorus* shows significant lethal effect against *Tribolium sp.* and *Oryzaephilus sp.* The 50% lethal effect shows at a 6% conc. in a root and stem extracts. While in a root extract it's at 8% concentration.

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RESEARCH ARTICLE

Ameliorative role of leaf extract of *Pithecollobium dulce* on chloramphenicol induced hematological changes in *Mus musculus*

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ABSTRACT

Leaf extract of *Pithecollobium dulce* was evaluated for its protective effect against chloramphenicol induces hematological changes like aplastic- anaemia, leucocytosis, thrombocytosis, etc. The dose of chloramphenicol i. e. 500 mg/1 kg. body weight of mice for was administered orally for 14 days. On day 15 hematological parameters like Haemoglobin level (Hb), red blood cell (RBC) count, white blood cells (WBC) count, blood platelets (thrombocytes) count, differential WBC count were declined in toxin group except the lymphocytes level was increased. But the methanolic leaf extract of *Pithecollo biumdulce* showed significantly increase in RBC count, total WBC count, platelets count and some leucocytes. While decrease in Haemoglobin (Hb) level, neutrophils etc. The result indicates that the leaf extract of *Pithecollo biumdulce* significantly recovered the altered values of blood cells due to chloramphenicol induced toxicity in bone marrow as well as haematological parameters.

Keywords: Chloramphenicol, *Pithecollobium dulce*, aplastic anaemia, haematological changes.

INTRODUCTION

Chloramphenicol is an antibiotic useful for treatment of a number of bacterial infections. This includes meningitis, plague, cholera, and typhoid fever. It is recommended when safer antibiotics cannot be used. However, it leads to toxic effect to blood parameters like aplastic anaemia (Rich *et al*., 1950), leukemia (Shu *et al*., 1987), bone marrow suppression due to direct toxic effect of the drug on human mitochon- dria (Yunis, 1989). The anaemia is fully reversible once the drug is stopped and does not predict future development of aplastic anaemia. Studies in mice have suggested existing marrow damage may compound any marrow damage resulting from the toxic effects of chloramphenicol (Morley, 1976).

Pithecollobium dulce has been commonly used for fencing and tanning, as fodder for feed and pods for food. Infusions of different parts of *P. dulce* have been traditionally used to treat diseases, for example, skin of the stem is used for dysentery, leaves for intestinal disorders and seeds for ulcers (Zapesochnaya *et al*., 1980; Rzedowski and Rzedowsk ,1985).

MATERIALS AND METHODS

Plant material and authentication: The fresh leaves were collected from the local area of Kalyan, Maharashtra, India and washed the leaves in order to wash the dust particles. The plant was identified at BLATTER HERBARIUM, ST. Xavier's college, Mumbai-400001.

Preparation of extraction: The leaves were dried in sun for 7 days continuously and made powder form. The powdered mass of leaves was defatted with petroleum ether (60^0 to 80^0 c). It is followed by extraction with methanol (95 % v/v) for about 18 hour by using soxhlet apparatus. The extract was filtered and the filtrate was concentrated under reduced pressure using rotary evaporator to obtain the extract as solid as residues. The extraction value (% w/w) of methanol was 18 (Sugumaran *et al*., 2008).

Experimental animals- Mice: Swiss albino mice of both sexes weighing from 30-35 gm were used for the study, obtained from Haffkins Institute, Parel (E), Mumbai-400012. The house was maintained at 28±2o c and exposed to 10-12 hours of day light and a relative humidity of 30-70 %. The animals were provided with drinking water ad libitum and fed on commercially available feed supplied by Amrut feed.

Chloramphenicol palmitate: It was procured from Mehta pharmaceutical limited, 315, Jankicentre, plot no. 29, Shah Industrial Estate, Off. Veera Desai road, Andheri (W), Mumbai, India. It was kept below room temperature.

Experimental design

Group I (6 mice) were used as control

Group II (6 mice) received 500 mg/kg chloramphenicol.

Group III (6 mice) received 200 mg/kg leaf extract of *Pithecollobium dulce.*

Group IV (6 mice) received 500 mg/kg chloramphenicol as well as 200 mg/kg leaf extract of *Pithecollo biumdulce.*

Blood sample collection and analysis

Blood sample was collected by puncture of retro-orbital vein and put the blood in EDTA vial for all haematological analysis like (RBC) count was done using the methods by Dacie and Lewis (2001); Antai *et al*. (2009) Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with Neubauer counting chamber under a light microscope. Sahli'shemoglobinometer was employed for estimation of hemoglobin (Hb) content of the blood. WBC count, differential WBC count and thrombocyte count were done as per thestandard method (Schalm *et al*., 1975).

Statistical evaluation: All the values were expressed as mean ± SD for 6 mice. Statistical significance ofdifferences between the control and experimental groups was assessed by Analysis of variance (ANOVA) two ways without replication. The value of probability less than 5 % ($P < 0.05$) was considered statistically significant.

RESULTS

Table 1 shows the effect of leaf extract of *Pithecollo biumdulce* on hematological parameters of mice like Hb, RBC, WBC and thrombocytes count. While table 2 shows differential WBC count. Hb, RBC, WBC, thrombocytes, eosinophils, lymphocytes and monocytes were significantly increased ($P< 0.05$), while neutrophils value was decreased in prophylactic group of mice fed with chloramphenicol as well as leaf extract of *Pithecollobium dulce*. There was significant difference in Total WBC count as well as neutrophils and monocytes.

Table 1: Haematological observations after treatment chloramphenicol and recovery with the help of leaf extract of *Pithecollobium dulce in* mice.

	Hbgm %	**RBC × 10^6**	**TLC × 10^3**	**Platelets × 10^5**
GroupI (Control)	14.58±0.55	6.5 ± 0.18	8.55±	7.68 ± 0.79
Group II -chloramphenicol	14.22±0.9	6.4 ± 0.48	7.78±	7.26 ± 1.37
Group III- LE of P.D.	14.25±1.87	7.83 ± 0.28	10.45±	6.85 ± 0.78
Group IVchloramphenicol+LE of P.D.	16.08±0.56	7.2 ± 0.57	9.48±	7.6 ± 0.71

Table 2: Haematological observations after treatment chloramphenicol and recovery with the help of leaf extract of *Pithecollobium dulce in* mice.

	Neutrophils%	**Eosinophils%**	**Lymphocytes %**	**Monocytes %**
GroupI (Control)	36.83 ± 5.47	0.66 ± 0.48	61 ± 6.1	1.16±0.37
Group II -chloramphenicol	31.83 ± 5.43	0.5 ± 0.5	67 ± 5.5	1±0
Group III- LE of P.D.	32.33 ± 7.15	1.33 ± 1.79	65.17 ± 8.03	1.17±0.69
Group IV chloramphenicol+ LE of P.D.	23.67 ± 3.4	3 ± 2	72.33 ± 4.1	1±0

P values < 0.05 by 'f' test. The values are expressed as Mean ± SE from 6 rats in eachgroup.
LE of P.D. means leaf extract of *Pithecollobium dulce.*

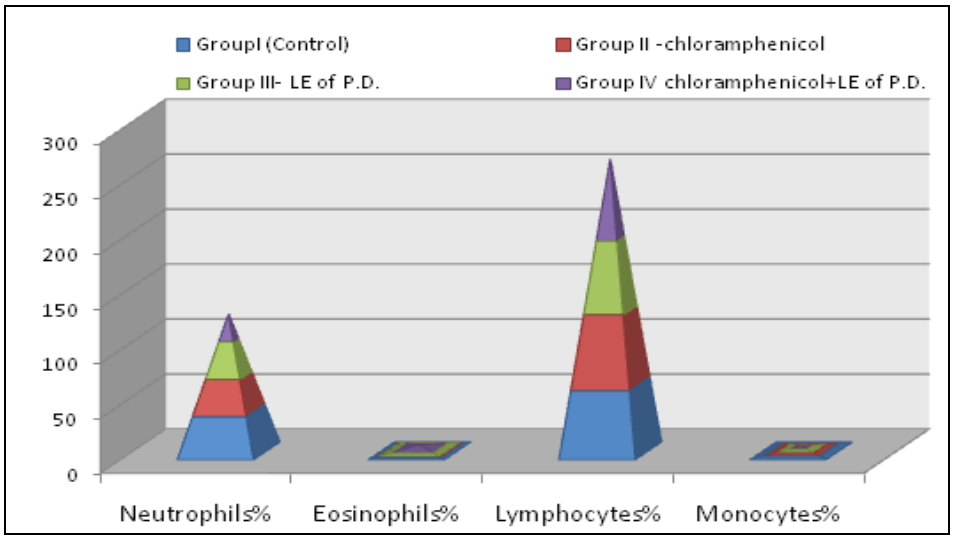

Fig. 1: Effect of methanol extract of *Pithecollobium dulce* leaves on haematological parameters of mice with chloramphenicol induced haemato toxicity.

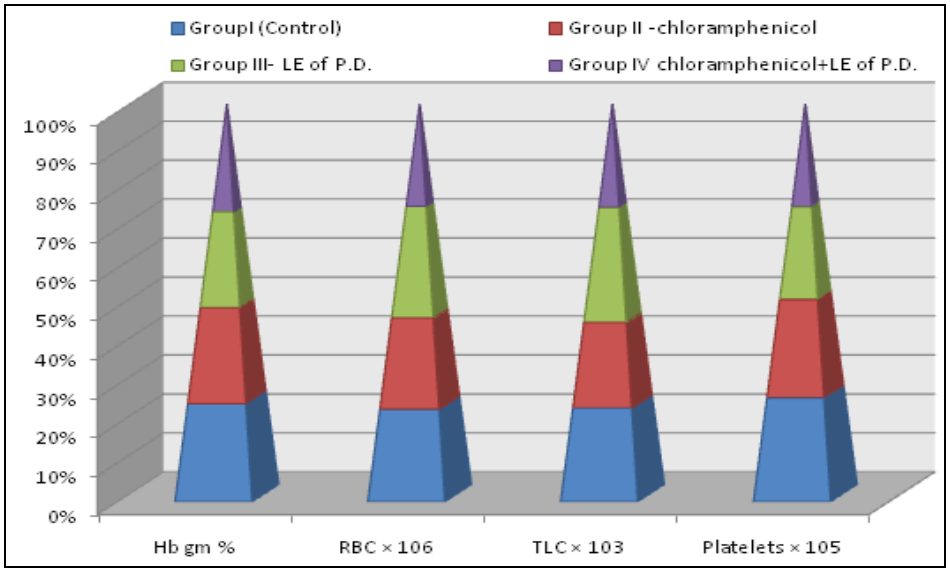

Fig. 2– Effect of methanol extract of *Pithecollobium dulce* leaves on haematological parameters of mice with chloramphenicol induced haemato toxicity.

Medicinal drugs or chemicals induced anaemia (Lewis *et al*., 2002) other haematological disorders, tissue injuries etc. have been known. In the present study it was found that the leaf extract of *Pithecollo biumdulce* recovered the hematological variations induced by chloram- phenicol. It was observed that the treatment of leaf extract of *Pithecollo biumdulce* significantly reduced the tissue damaged in bone marrow that leads to decrease the number of erythrocytes, variations in the leucocytes and thrombocytes.

The results showed that RBC, Hb level were significantly higher in the leaf extract of *Pithecollo biumdulce* feed groups and compared these values with control, toxin and prophylactic group of mice fed with chloramphenicol as well as leaf extract of *Pithecollo biumdulce.* This suggests that the flower extract contain agents that induced the production of red blood cells or enhances erythropoiesis by bone the marrow.

Total leucocyte count increased in only in prophylactic group of mice fed with chloram- phenicol as well as leaf extract of *Pithecollo biumdulce.* Increase in total WBC count may be beneficial as they are vital in the body's defence mechanism (Gilani *et al*., 2000).

The platelets level was also increased except the group III.When study separately the differential WBCs count, neutrophil value was not much recovered as compare to group I (control). The eosinophil value was elevated much more as compare to other groups. The level of lympho- cytes was increased in all the groups II, III and IV as compare to group I. The value of monocytes was near about similar means it was not affected by chloramphenicol as well as leaf extract of *Pithecollo biumdulce.* It means the chloram- phenicol + leaf extract of *Pithecollo biumdulce* group exhibited markchanges in the haematolo- gical parameters indicating that when chloram- phenicol with extract diet fed mice has been ameliorated.

Scientists continue to investigate that when drug entered in body the free radicals are formed which are damaging the bone marrow. But antioxidants present in the leaf extract of *Pithecollo biumdulce* that act as defense system against free radicals.

In conclusion, *Pithecollo biumdulce* methanolic extract of leaves given to mice orally at the dose of 200 mg/kg body weight improved full blood count in mice. (Simplicio *et al*., 1996) The extract should be taken be with cautiondespite of its antioxidant and hematopoietic effect.

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RESEARCH ARTICLE

Diversity of Chlorophyta in Freshwater Lakes of Sangli (MS) India

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ABSTRACT

Study of algal diversity is an important aspect of environmental studies. Algae are present in almost all aquatic habitats. There are very few reports of study of algal diversity from Sangli District of Maharashtra state. However, these studies were carried out in very limited localities. Therefore the present study is important from collection of database of algal diversity in this District. Algal taxa of Sangli District were studied during the present investigation, carried out between the years 2009-2012. For this study, 4 water tanks *Viz.* Chandoli (CH), Morna (MR), Siddhewadi (SD) and Basappawadi (BS) was selected from different talukas of the District having varied rainfall. The precincts of Chandoli and Morna receives comparatively high rainfall (over 1300 mm/year); while Siddhewadi and Basappwadi areas receive comparatively low rainfall (about 300mm/year). In all 51 Chlorophytes were recorded, which includes benthic, planktonic, floating and some epiphytic species.

Key Words: Diversity, Freshwater Algae, Lakes, Sangli

INTRODUCTION

Division Chlorophyta includes a diverse assemblage of photosynthetic organisms commonly known as green algae. The organisms can be unicellular, multicellular, coenocytic (more than one nucleus in a cell) or colonial representatives. They are one of the pioneer species in aquatic food web. During present study 51 chlorophyceae were recorded from different locations. The algae were identified by using various available resources such as reference books, web facilities, Manuals, etc.

MATERIALS AND METHODS

Algae stored initially in a bucket, jar, bottle or plastic bag, with some water from the collecting site. After collecting most algae were kept alive for short periods (a day or two). For long-term storage, specimens were preserved in formalin. Even with preservation, examination of fresh material was sometimes essential for an accurate determination. Motile algae examined with flagella and other delicate structures remain intact.

The phytoplankton samples were collected by using plankton net (200 mesh). The 50 liters of water was filtered through plankton net to get 50ml of sample. Some macroalgae were collected directly with the help of hand. The collections were done frequently at intervals of month. Identification was made with help of standard texts, keys and monographs given by Prescott (1951), Sarode and Kamat (1984), Cox (1996), Prasad and Shrivastava (2005), Prasad and Misra (1992), Philipose (1967), Rath and Adhikary (2005) and publications appeared in journals from time to time. Motile algae particularly examined while flagella and other delicate structures were intact. The morphological studies of specimens were done by using Magnius Research Microscope (Model No. MLM 2051) and Photomicrographs were made with attached Digital camera (C 2000).

RESULTS AND DISCUSSION

Division: Chlorophyta
Class: Chlorophyceae
Order: Volvocales
Family: Polyblepharidaceae

1) ***Chlamydomon asdinobryonii***G. M. Smith
Prescott, 1951, P 70, Pl 1, Fig. 5

Cells ovoid to pyriform, without an anterior papilla, chloroplast disc-shaped to hemispherical, lying either at the base of the cell or along the lateral wall; pigment-spot lacking.

Locality: Siddhewadi, Chandoli
Coll. No. and Date: SD- 01 (26/06/2010), CH-101 (25/08/2010)

2) ***Phacotus lenticularis***(Ehr.) Stein.
Prescott, 1954, P 32, Fig. 23(a)

Envelope composed of 2 overlapping pieces, the seam showing when the vegetative cell is seen from the side. This genus is relatively rare, but is often abundant in collections. It occurs in two localities.

Locality: Basappawadi, Morna
Coll. No. and Date: BS- 110 (21/06/09), MR- 07 (04/03/2010)

3) ***Gonium pectorale*** Muller
Prescott, 1951, P 75, Pl 1, Fig. 21

Colony of 8 ellipsoidal, subspherical cells closely arranged in a flat, quadrangular plate,usually with 4 inner cells, bordered by a series of 12 marginal ones which have their anterior ends projected outward and parallel with the plane of the colony, the inner cells directed at right angles to the plane. Cells inclosed, by individual sheaths, which are connected to neighboring sheaths by very short processes.

Locality: Siddhewadi, Basappawadi
Coll. No. and Date: SD-78 (25/06/2010), BS-12 (20/04/2011)

4) ***Pandorina morum*** (Muell.)Bory.
Prescott, 1954, P 28, Fig. 14
Prescott, 1951, P 75, Pl 1, Fig. 23

Colony spheroidal; cells pear-shaped, crowded together with broad ends all directed outwardly. Cells are pear-shaped and often are more compactly arranged. A tumbling colony in which pear-shaped cells are closely compacted within a spheroidal or oval gelatinous sheath. Colonies are to be seen in which all individuals have divided to form each a daughter colony. Cells pyriform, crowded. More frequent among dense growths of algae in shallows,especially in water rich in nitrogenous matter.

Locality: Siddhewadi, Basappawadi
Coll. No. and Date: SD-78 (25/06/2010), BS-11 (05/10/2011)

5) ***Eudorinaelegans***Ehr.
Prescott, 1954, P 29, Fig. 17
Rath and Adhikary, 2005, P 51, Pl, 16, Fig. 119

All cells of the same size within the colony. Cells have a tendency to arrange themselves in transverse bands. Occurs along with *Volvox*.Colonies spherical, ellipsoidal in shape, colonial envelope smooth in outline, colonies of 16 or 32, rarely 64, cells arranged in distinct tiers with a confluent double layered gelatinous envelope, cells equal in size, mature cells spherical or ovoid in shape with a single radially striated cup-shaped cells.

Locality: Basappawadi, Siddhewadi
Coll. No. and Date: BS-14 (24/05/2010), SD-78 (25/06/2010)

6) ***Volvox globator*** L.
Prescott, 1951, P 78, Pl 2, Fig. 5

Large, monoecious, spherical, gelatinous colonies containing many ovoid cells.Cells with conspicuous protoplasmic interconnections; with 1 parietal plate-like chloroplast and a pigment-spot in each cell, and with 2-6 small contractile vacuoles in the anterior region below the point of flagellar attachment. Individual sheaths of the cells conspicuous and not confluent with the colonial mucilage, clearly visible in surface view of the colony, the sheaths 5-8-sided from mutual compression. Coenobium commonly containing daughter colonies; sexual colony with eggs,

each enclosed by a wide gelatinous sheath; Zygotes with thick walls exteriorly decorated with wart-like, blunt spines.
Locality: Siddhewadi, Basappawadi,
Coll. No. and Date: SD-14 (24/05/2010), BS-60 (22/02/2011)

Family: Sphaerellaceae
7) ***Stephanosphaera pluvialis Cohn***.
Prescott, 1954, P 29, Fig. 16
Prescott, 1951, P 81, Pl 46 Fig. 26

Cells fusiform with sharply pointed lateral processes or extensions of the protoplast.Oval colony forming a median band. The cells commonly show 2 pyrenoids. A colony of 4-8 ovoid cells with branched protoplasmic extensions, arranged in a median circumferential band within an oblate-spheroid colonial mucilage. Cells free from, and some distance from,one another, not connected by protoplasmic extensions. Flagella 2,lateral, near the anterior end of cell; chloroplast parietal.
Locality: Morna, Basappawadi
Coll. No. and Date: MR-13 (04/03/2009), BS- 05 (25/06/2010)

8) ***Haematococcus lacustris***(Girod.)Rostaf.
Prescott, 1954, P 30, Fig. 19

Protoplast at a considerable distance within the cell wall and connected to it by fine, radiating processes; cells with a mass of red pigment often present in the center of the protoplast. Swimming cell showing protoplast with radiating processes; cysts are brick-red in color.
Locality: Basappawadi, Morna
Coll. No. and Date: BS- 05 (25/06/2009), MR- 108 (25/08/2011)

Order: Tetrasporales
Family: Palmallaceae
9) ***Gloeocystis major*** Gerneck ex Lemmermann
Prescott, 1954, P 41 Fig. 41c
Prescott, 1951, P 84, Pl 52, Fig. 9, 10

Cells enclosed by concentric layers of mucilage (individual cell sheaths distinct).Cells ovoid, in colonies of 4, inclosed by a wide, lamellate sheath in which groups of individual sare surrounded by concentric layers;Chloroplast massive, completely covering the wall.
Locality: Siddhewadi, Morna
Coll. No. and Date: SD-144 (24/05/2010), MR-108 (25/08/2011)

Family: Tetrasporaceae
10) ***Tetraspora lacustris Lemmermann***
Prescott, 1951, P 88, Pl 5, Fig. 11

Thallus a free-floating, spherical and irregularly shaped, microscopic gelatinous colony containing relatively few spherical cells, the long pseudocilia clearly seen. Cells arranged in groups of four. This species is microscopic and apparently free-floating at all stages.
Locality: Chandoli, Morna
Coll. No. and Date: CH- 101 (25/08/2010), MR - 47 (09/04/2011)

Order: Ulotrichales
Suborder: Ulotrichineae
Family: Ulotrichaceae
11) ***Ulothrix zonata*** (Weber & Mohr) Kuetz.
Prescott, 1951, P 97, Pl 6, Fig. 14

Filaments attached, usually long and stout, variable in diameter in the same plant mass. Cells Short, or elongate-cylindric, slightly swollen with constrictions at the cross walls.Cell walls thick, especially near the base of the filament.Chloroplast a complete circular band in the mid region of the cell, with several pyrenoids.
Locality: Morna, Chandoli
Coll. No. and Date: MR- 38 (04/03/2010), CH- 101 (25/08/2010)

Order: Microsporales
Family: Microsporaceae
12) ***Microspora loefgrenii***(Nordst.) Lag.Prescott, 1954, P Fig. 172 Prescott, 1951, P 107, Pl 9, Fig. 2

Cells without haematochrome; chloroplast a perforated and padded sheet or a branched, beaded ribbon. The simple, unbranched filaments have chloroplasts that vary greatly in respect to the degree with which they cover the wall. The ends of the filaments where the line of separation having occurred in the midregion of the cell, which forms characteristic H-shaped pieces.Walls thick, sections evident in the midregion of the cell.Cells short-cylindric, rectangular, as long as broad or a little longer.Chloroplast a loose net,covering nearly all of the cell wall.
Locality: Chandoli, Siddhewadi
Coll. No. and Date: CH- 101 (25/08/2010), SD- 39 (20/04/2011)

Order: Chaetophorales
Family: Chaetophoraceae
13) ***Stigeoclonium nanum Koetz***. Prescott, 1951, P 116, Pl 9, Fig. 7, 8

Thallus composed of short-tufted filaments, the branches arising alternately and tapering to blunt points. Cells of the branches scarcely smaller than those of main axis, prostrate portion of plant is expansive, pseudoparenchymatous, becoming filamentous; the cells subglobose and giving rise to vertical branches, species forms green film on submerged plants.

Locality: Chandoli, Morna

Coll.No. and Date: CH- 44 (10/05/2010), MR- 89 (14/06/2010)

14) ***Chaetophoraattenuata***Hazen

Prescott, 1951, P 118, Pl 13, Figs. 4, 5

Forming attached, firm, gelatinous globules, having radiating, nearly parallel, erect branches from numerous basal, rhizoidal processes. Filaments dichotomously branched, ending in pointed, setiferous cells; branches loose and evenly developed from main axis, much elongated.

Locality: Basappawadi, Morna

Coll.No. and Date: BS- 45 (24/09/2010), MR- 89 (14/05/2011)

15) ***Draparnaldia acuta***(C. A. Ag.) Kuetz.

Prescott, 1951, P 120, Pl 15, Fig. 1

Main axis of thallus bearing horizontal branches, from which opposite or whorled fascicles of branches arises, branchlets crowded to acuminate in outline with an apparent rachis that extends beyond the other branches of fascile. Cells of main axis and primary branchesswollen, chloroplast about V the length the cell.

Locality: Siddhewadi, Chandoli

Coll.No. and Date: SD- 40 (24/05/2010), CH- 44 (10/09/2010)

16) ***Aphanochaete repens***A. Braun; Prescott, 1951, P 125, Pl 17, Figs. 2, 3Prescott, 1954, P 104, Fig. 161

Filaments creeping, cells irregularly inflated. Setae long and very slender, wide at the base. It is most common species occurring frequently with other algae. Filaments prostrate, creeping on larger filamentous algae. Very common reeping on the walls of large filamentous algae. The simple setae, with their swollen bases extending from the cell wall.

Locality: Basappawadi, Chandoli

Coll.No.and Date: BS- 48 (24/09/2010), CH- 173 (15/10/2010)

Family: Coleochaetaceae

17) ***Coleochaete orbicularis*** Pringsheim

Prescott, 1951, P129, Pl 18 Figs.3, 5

Prescott, 1954, P 97, Fig. 150

Thallus forming a regular, circular monostromatic disc of branching filaments radiating from common center and adjoining laterally.Cells quadrangular, oogonia ovoid. It is common on submerged plants. Plant a cluster of short, erect filaments (it is branched but sometimes appears unbranched when young; form attached discs).

Locality: Morna, Basappawadi

Coll.No. and Date: MR- 50 (14/05/2010), BS- 45 (24/09/2010)

Family: Trentepohliaceae

18) ***Trentepohlia iolithus***(L.) Wallworth; Prescott, 1951, P 134, Pl 19, Figs.4-8; Prescott, 1954, P 109, Fig.171a

Plants golden-red, forming a compact felt on moist rocks. Basal filaments composed of fusiform cells. Branches possessing cylindrical cells and ending in bluntly rounded apices. Cell walls roughened clearly lamellated. Sporangia globose and terminal. Cells orange or golden-red because of haematochrome; plants aerial on trees and rocks; chloroplast dense and indeterminate of shape.

Locality: Basappawadi, Morna

Coll.No. and Date: BS- 99 (24/04/2009), MR- 49 (12/12/2009)

Order: Cladophorales

Family: Cladophoraceae

19) ***Cladophora profunda*** var ***nordstedtiana*** Brand

Prescott, 1951, P 139, Pl 22, Figs. 1-4

Thallus composed of attached, irregularly and much branched filaments growing from a prostrate, colorless, rhizoidal portion. Basal branches directed downward and ending in colorless rhizoid-like cells; upper branches irregular in arrangement, often entangled and interlocked to form snarled tufts, Cells irregularly inflated or sub-cylindric, long in the main axis. Walls of cells encrusted or merely roughened with lime and sometimes with iron deposits, giving a rust-colored appearance to older plants.

Locality: Siddhewadi, Morna

Coll. No. and Date: SD- 42 (10/01/2010), MR- 21 (25/06/2011)

Order: Oedogoniales

Family: Oedogoniaceae

20) ***Bulbochaete crassa*** Pringsheim

Prescott, 1951, P149, Pl 25, Fig. 8

Nannandrous; gynandrosporous. Vegetative cells long. Oogonia sub-depressed, globose. Oospores depressed-globose with scrobiculate outer wall.Male

filaments on the oogonia; stipe wide and long.Occurs on grass in the marshy end of lakes. It is similar to *B. insignis* but differ in presence of nannandria.
Locality: Chandoli, Morna
Coll. No. and Date: CH- 41 (12/06/2011), MR-21 (25/07/2011)

21)*Bulbochaete insignis* Pringsheim
Prescott, 1951, P 150, Pl 26, Figs. 4-6
Prescott, 1954, P 122, Fig. 195a
Nannandrous; gynandrosporous, vegetative cells long.Oogonia depressed globose. Oospores globose; outer spore wall with high thick denticulate coast (margin); Male filaments on the oogonia. It found commonly in two lakes attached to other aquatic plants. Setae bulb-like at the base.
Locality: Chandoli, Morna
Coll. No. and Date: CH-41 (12/07/2010), MR- 111 (21/09/2011)

22)*Oedogonium spurium* Hirn Prescott, 1951, P 188, Pl 37, Figs. 4-5
Macrandrous; monoecious. Vegetative cells capitulate, oogonia solitary; subglobose; operculate; oospores depressed-globose; sometimes filling the oogonia; wall smooth.
Locality: Morna, Chandoli,
Coll. No. and Date: MR- 35 (10/08/2009), CH- 101 (25/08/2010)
Order: Ulvales (As per Prescott 1954)
Family: Ulvaceae

23) *Enteromorpha intestinalis*(L.) Neesa
Rath and Adhikary, 2005, P 60, Pl 8, Fig. 29
Plant up to 25 cm height, attached to the substratum by a basal rhizoidal portion and later floating when torn away from the substrate; deep green to yellowish green; fronds clavate, more or less compressed, with apices perforated and found gradually increasing in width; branched, branches and branchlets shortly club-shaped, inflated towards the apex, cells in surface rounded, polygonal, irregularly arranged throughout thallus, cell contents granular with one nucleus and a parietal chloroplast.
Locality: Morna, Chandoli
Coll. No. and Date: MR- 46 (04/03/2010), CH- 101 (25/08/2010)

Order: Chlorococcales
Family: Chlorococcaceae
24)*Chlorococcum humicola* (Nag.) Rabenhorst
Philipose, 1967, P 73, Fig. 3
Prescott, 1954, P 42, Fig. 42 Prescott, 1951, P 212, Pl 45, Fig. 12
Cells spherical, solitary or number of cells crowded together to form a stratum,Chloroplast a hollow sphere with a lateral notch and a single pyrenoid. Chloroplast covering almost the entire wall; cells variable in size within the colonial mucilage. This species is luxuriantly represented forming green films in association with *Scenedesmus* and *Euglena.*
Locality: Chandoli, Basappawadi
Coll.No. and Date: CH- 155 (14/04/2010), BS- 90, (12/05/2010)

Family: Characiaceae
25)*Characiumangustum*A. Braun Philipose, 1967, P 84, Fig.10
Cells straight and lanceolate with short hyaline apical beak, stalk short and thick witha colourless disc-shaped basal thickening.
Locality: Morna, Basappawadi
Coll.No. and Date: MR- 89 (14/04/2010), BS- 90, (12/05/2010)

Family: Hydrodictyaceae

26) *Hydrodictyon reticulatum* (L.) Lag.
Prescott, 1951, P 219, Pl 47, Fig. 1
Prescott, 1954, P 54, Fig. 69
Thallus macroscopic composed of cylindrical cells which are adjoined at their ends to form a cylindrical net with 5- or 6-sided meshes; chloroplast at first a parietal plate with a single pyrenoid, later becoming a reticulum covering the entire wall and containing many pyrenoids; cells multinucleate. Cells 1 cm long when fully enlarged, forming a net up to 2 m.in length; chloroplast a much diffused reticulum, light yellow-green color in the plant mass,especially at maturity. Cells cylindrical, one cell attached by 2 others at the end walls repeatedly to form a network. This is the familiar "water-net" which often grows in such dense mats in two lakes, as a troublesome weed. Each cell of the net in turn produces a new cylindrical net of small cells. The nets are of macroscopic size.
Locality: Morna, Siddhewadi
Coll.No. and Date: MR- 29 (25/05/2010), SD- 167 (30/06/2010)

27)*Pediastrum duplex* Meyen Philipose, 1967, P 121, Fig. 43 (a); Prescott, 1951, P 223, Pl 48, Fig. 4
Colony 32 celled and circular to oval with medium-sized perforations between cells.Cells H-shaped with ends of processes round. Cell membrane covered with serially-arranged granules. The walls smooth, with

lens-shaped spaces between the inner cells, which are quadrate, the outer margin concave; peripheral cells quadrate, the outer margin extended into2 tapering, blunt-tipped processes, distance between processes of one cell about one-half the distance between processes of adjacent cells.

Locality: Chandoli, Siddhewadi

Coll. No. and Date: CH -24 (25/06/2010), SD- 105 (08/11/2010)

BS-23(12/10/2010),MR-108 (25/08/2011)

28)*Sorastrum americanum* (Bohlin) Schmidle Prescott, 1951, P 228, Pl 50, Fig. 8

A free-floating spherical colony of 32 heart-shaped cells with the outer free walls emarginate and furnished at each of the 4 angles with a long, stout, outwardly directed spine; cells narrowed toward the base and attached to the center of the colony by a short, stalk cylindrical adjoining the sides of other stalks in such a way as to form a central hollow sphere.

Locality: Morna, Siddhewadi

Coll. No. and Date: MR- 219 (25/06/2010), SD- 167 (30/05/2011)

Family: Coelastraceae

29)*Coelastrumcambricum*var.***Intermedium*** (Bohlin) G. S. West Rath and Adhikary, 2005, P 52, Pl 16, Fig. 122

Colonies spherical, 32 celled, cells spherical and thickened at the poles, 10-12 sided when seen from the apex, connected to each other by 4-6 short gelatinous flat projections,interspaces between cells circular to triangular, outer face of the external cells being subspherical and gradually arched, outstanding projections blunt and rounded and not truncate,interspaces between cells more or less triangular.

Locality: Siddhewadi, Chandoli

Coll. No. and Date: SD- 78 (29/09/2010), CH- 106 (20/12/2011)

Family: Oocystaceae

30)*Chlorella vulgaris* Beijerinck

Philipose, 1967, P 173, Fig. 82(d)

Prescott, 1951, P 237, Pl 53, Fig. 13

Alga free living.Cells solitary in small colonies, spherical and with a thin cell membrane. Chloroplast parietal, cup-shaped and with a pyrenoid which is indistinct. Cells spherical scattered among other algae.

Locality: Morna, Siddhewadi

Coll. No. and Date: MR- 15 (25/06/2010), SD- 19 (29/09/2010)

31)*Dictyosphaerium pulchellum* Wood

Philipose, 1967, P 199, Fig. 110(a)

Prescott, 1951, P 238, Pl 51, Figs. 5-7

Colonies spherical more than 64 cells.Cells ovoid with single parietal cup shaped chloroplast having single pyrenoid. Cells arranged in series of 4 on dichotomously branched threads, inclosed in mucilage.

Locality: Chandoli, Siddhewadi

Coll. No. and Date: CH- 28 (21/05/2011), SD- 167 (30/06/2011)

32) *Oocystis borgei* Snow

Philipose, 1967, P 183, Fig. 93, Prescott, 1951, P 243, Pl 51, Fig. 10

Cells broadly ellipsoid, with rounded ends. Poles not thickened. Colonies 4 celled the enclosing envelope round and narrow. Unicellular or crowded in groups of 2-8, inclosed by the old mother cell wall; ellipsoid cells with the poles broadly rounded and smooth; chloroplast single with parietal plate, each with a pyrenoid.

Locality: Morna, Chandoli

Coll. No. and Date: MR- 16 (25/06/2010), CH-106 (20/12/2011)

33) Ankistrodesmus spiralis (Turner) Lemmermann

Philipose, 1967, P 210, Fig. 121(e)Prescott, 1951, P 254, Pl 56, Figs. 11, 12

Cells acicular with acute apices; in colonies of usually 4 cells spirally twisted round one another in the median region, but free at the ends. Chloroplast single and without a pyrenoid.

Locality: Siddhewadi, Morna

Coll. No. and Date: MR- 84 (08/09/2010)

34) *Closterium lunula* var ***massartii*** (Wildem.) Krieg. Prasad and Misra, 1992, P 111,112, Pl 15, Fig. 8

Cell large, about 6 times longer than broad, more or less straight, outer margin more convex than inner and shows 60 degrees of arc, cell gradually and gently attenuated to slightly truncate spices; cell wall smooth; chloroplast with 7 ridges and numerous scattered pyrenoids.

Locality: Basappawadi, Morna

Coll. No. and date BS - 23 (12/10/2010), MR - 108 (25/08/2011)

35) *Cosmariumbiretum*Breb.

Prasad and Misra, 1992, P 154, Pl 23, Fig. 19

Cells rather small, a little longer than broad, very deeply constricted, sinus narrowly linear with slightly dilated extremity; semicells sub rectangular with

somewhat convex sides and apex; cell wall with granules arranged in indistinct curved vertical series; each semicell with an axile chloroplast and two pyrenoids.

Locality: Chandoli, Siddhewadi

Coll. No. and date CH - 56 (15/04/2010), SD - 157 (18/03/2011)

36) *Cylindrocystis subpyramidata* *W.et* G.s. West

Prasad and Misra, 1992, P 89, Pl 15, Fig. 11

Cells cylindrical, about 1-5 times longer than broad, slightly constricted in middle, cell apices, sub pyramidal with rounded ends; chloroplast sub-stellate with one large pyrenoid in each semicell.

Locality: Basappawadi, Siddhewadi

Coll. No. and date BS - 53 (14/08/2009), SD - 157 (18/03/2011)

37) *Micrasterias foliacea Bail*.

Prasad and Misra, 1992, P 141, Pl 20, Fig. 6

Cells small, united in filaments by inter-locking of polar lobes, rectangular in outline,deeply constricted, sinus narrowly linear, semicells 5-lobed, basal part of polar lobes with subparallel sides, upper part greatly expanded and anvil shaped with an excavation in the median portion, base of excavation exhibits 2 asymmetrically produced spines of unequal length, polar and lateral angles uncinate, lateral lobes asymmetrical, superior lobes divergent,inferior horizontally disposed, incisions simple and sub-acuminate, the ultimate lobelets with truncate-emarginate apices; cell wall smooth.

Locality: Basappawadi, Chandoli

Coll. No. and date BS - 53 (14/08/2009), CH - 68 (10/01/2010)

38) *Euastrum ansatum* Ehr.

Prasad and Misra, 1992, P 133, Pl 19, Fig. 7

Cell small, twice as long as broad, deeply constricted, sinus narrowly linear with somewhat dilated extremity; semicell spyramidate with broadly rounded basal angles, lower part of sides convex, upper part slightly concave, apex rotundo-truncate with a fairly deep incision, each semicells with one slight and one preeminent protuberance and two across the centre; cell wall with punctations arranged in indistinct vertical series.

Locality: Chandoli, Basappawadi

Coll. No. and date CH - 68 (10/05/2009), BS - 60 (22/02/2011)

39) *Pleurotaenium ehrenberghii* (Breb.) de Bary

Prasad and Misra, 1992, P 124, Pl 18, Figs. 9, 10

Cells fairly large, longer than broad, sub-cylindrical, slightly constricted at the base;semicells cylindrical, gently attenuated from base towards apex; basal inflation small with one undulation; apex with a ring of tubercles; cell wall minutely punctate.

Locality: Siddhewadi, Morna

Coll. No. and date SD - 54 (25/06/2009), MR - 59 (04/03/2010)

40) *Staurastrum gracile* Ralfs forma Iyengar *et.* Vimala Bai.

Prasad and Misra, 1992, P 197, Pl 25, Figs. 14, 18

Cell small, longer than broad with slight constriction in the form of an acute notch; semi cell slightly broad towards the faintly convex apex, upper angles produced into more or less horizontally disposed long processes tipped with 3 minute spines and showing many concentric series of denticulations; top view triangular; chloroplast axial with one pyrenoid in each semicell.

Locality: Chandoli, Siddhewadi

Coll. No. and date CH- 68 (10/01/2010), SD- 57 (18/03/2011)

41) *Xanthidium cristatum* var. ***uncinatum*** Hass.

Prescott, 1954, P 77, Fig. 115

Apex of semicell furnished with prominent spines; facial protuberance one large low swelling, the wall thickened here and often pitted or punctuate. Cells, compressed so that they are narrow, when seen from top. There is a facial swelling in the center of the semicell and all angles bear stout spines or short arms that are tipped with spines. Granules present on the wall.

Locality: Morna, Siddhewadi

Coll. No. and date MR - 59 (04/06/2010), SD - 37 (22/03/2010)

42) *Kirchneriella lunaris* (Kirch.) Moebius

Prescott, 1951, P 258, Pl 58, Fig. 2

Philipose, 1967, P 222,223, Fig. 131

Colony composed of numerous cells arranged in groups of 4 within a close, gelatinous envelope; cells flat, strongly curved crescents with rather obtuse points; chloroplast covering the convex wall. Cells irregularly arranged within the envelope, flattened and crescent-shaped with pointed ends and about twice as long as broad. Chloroplast nearly filling the cell and with a single pyrenoid.

Locality: Morna, Siddhewadi

Coll.No.&Date:MR-49(12/12/2009),SD-179 (29/09/2010)

Sub-family: Tetraedronoideae(Philipose,1967)

43) ***Tetraedron pentaedricum*** W. *et* G. S. West Philipose, 1967, P 151, Fig. 65(a-b) Prescott, 1951, P 268, Pl 60, Figs. 21-23

Cells small, irregularly five-lobed with four lobes in one plane and the fifth at an angle to the former.Corners somewhat acute, each with a short slightly curved spine. Angles sharply rounded, the apex of each lobe furnished with a sharp spine.

Locality: Chandoli, Siddhewadi

Coll. No. and Date: CH - 18 (21/06/2010), SD - 167 (30/05/2011)

Family: Scenedesmaceae

44) ***Scenedesmus abudans*** (Kirch.) Chodat Philipose, 1967, P 278, Fig. 184 a-d

Colonies usually 4 celled and arranged in a linear series. Cells ovoid to oblong-ovoid.External cells with one median lateral spine from the outer face in addition to spines from thefour corners of the colony. Internal cells without spines. It is present at bottom of pool.

Locality: Morna, Chandoli

Coll. No. and Date: MR- 88 (10/01/2010), CH-26 (12/07/2010)

45) ***Actinastrum gracilimum*** G. M. Smith Prescott, 1951, P 281, Pl 64, Fig. 5 Philipose, 1967, P 318

Cells cylindrical, with very slightly narrowed to abruptly truncate poles, forming colonies of individuals with the long axes of the cells radiating in all planes from a common center. Cells 7-10 times long than broad. Chloroplast single, parietal and laminate and without pyrenoid.

Locality: Siddhewadi, Morna

Coll. No. and Date: SD- 19 (29/09/2010), MR- 21 (25/06/2011)

46) ***Crucigenia quadrata*** Morren Prescott, 1951, P 285, Pl 65, Fig. 10 Philipose, 1967, P 241, Fig. 152

Colonies free-floating, consisting of a circular plate of 4 triangular cells, crucially arranged with small central space, the outer free wall of the cells broadly convex, the lateral walls straight, adjoined throughout their length with neighboring cells and converging toward the center of the colony; walls with knob-like projections; chloroplasts parietal discs 4 in a cell; pyrenoids not always present; multiple quadrate colonies formed by the close arrangement of component quartets. Cells, with rounded corners.

Locality: Chandoli, Siddhewadi

Coll. No. and Date: CH- 126 (12/07/2010), SD-167 (30/05/2011)

Order: Vaucheriales (Heterosiphonales)

Family: Vaucheriaceae

47) ***Vaucheria sessilis*** (Vauch.) De Candolle Prescott, 1951, P 294, Pl 68, Fig. 5 Prescott, 1954, P 124, Fig. 199 b

Aquatic; filaments somewhat slender, with irregular branching; monoecious; oogonia usually in pairs, ovoid to subglobose with the pore in a short beak and directed obliquely upward; antheridia on a short pedicel between 2 oogonia, either straight or circinate, but usually with the opening directed toward the pore of an oogonium; oospore with a 3-layered membrane, filling the oogonium. Filaments not dichotomously branched; without constrictions.

Locality: Morna, Siddhewadi

Coll. No. and Date: MR - 102 (20/07/2010), SD - 64 (27/06/2011)

Order: Zygnematales

Family: Zygnemataceae

48) ***Mougeotiaviridis*** (Kuetz.) Wittrock; Prescott, 1951, P 306, Pl 71, Figs. 8-10

Filaments slender, becoming geniculate in conjugation; cells long; chloroplast a broad plate extending the full length of the cell with 4-6 pyrenoids. Zygospores formed in the tube,dividing both gametangia; quadrate, the sides concave, corners retuse; median spore wall smooth and colorless.

Locality: Chandoli, Morna

Coll. No. and date CH -34 (25/09/2009), MR -47 (09/04/2011)

49) ***Spirogyra weberi*** Kuetz.Prescott, 1951, P 322, Pl 76, Figs. 8-10

Filaments of long cells, with replicate end walls; chloroplast solitary, broad, making 3 to $6^1/_2$ turns. Conjugation by tubes from both gametangia; fertile cells cylindric.Zygospores cylindric-ovate; median spore wall smooth and brown. The genus seems always to be mixed with other filamentous algae. Long, slender cells and the long ovate spores are distinctive features, Entangled among other algae.

Locality: Siddhewadi, Morna

Coll. No. and date SD -37 (22/04/2010), MR -47 (09/06/2010)

50) ***Zygnema insigne*** (Hass.) Kuetz. Prescott, 1951, P 325, Pl 78, Fig. 11

Vegetative cells 2 times longer than width; fertile cells cylindrical, inflated on one side only.Zygospores

formed in one of the gametangia; subglobose; median wall brown and smooth.

Locality: Chandoli, Morna

Coll. No. and date CH -136 (25/08/2011), MR -108 (25/08/2011)

51)*Zygnema micropunctatum* Transeau Prescott, 1951, P 325, Pl 78, Fig. 12

Vegetative cells long; fertile cells not inflated. Zygospores formed in the tube;depressed-globose, compressed at right angles to the conjugation tube; median wall yellow-brown and minutely punctate.

Locality: Chandoli, Siddhewadi

Coll. No. and date CH -36 (22/08/2009), SD -116 (24/08/2010)

Bhosale *et al.,* (2010b) studied algae of Sangli, Satara and Kolhapur District and reported 116 species of Chlorophyceae during rainy season. Dhumal *et al.,* (2010) recorded 57 species of phytoplankton from Divad lake of Satara District and 19 species filamentous algae from Rajewadi lake in Sangli District. They have also reported *Ankistrodesmus septatus* from Mayani lake of Satara District. Patil, (2010) reported 22 species belonging to 14 genera of Chlorophyceae from Bhambarde lake, 20 species of 13 genera from Siddhewadi lake, 17 species belonging to 11 genera from Borgaon lake and 24 species belonging to 16 genera from Birnal lake in Sangli District. Lohar D. N. and S. L. Korekar (2013) and (2012) also reported 46 Chlorophyta species in two lakes of Sangli District. Mahajan (2011) reported diversity of desmids at Jalgaon, North Maharashtra. In the absence of earlier literature (Bhosale *et al.,* 2010c; Dhumal *et al.,* 2010 and Patil, 2010) *Ankistrodesmus spiralis* appears to be the first record from Sangli District.

CONCLUSION

During present study, the algal taxa belonging to class Chlorophyceae were observed at all the 4 selected tanks. In all 51 species belonging to 49 genera were recorded. It is observed that only one alga i.e. *Pediastrum duplex* was present at all locations. 4 algal taxa were recorded at three sites, 45 algal taxa at two locations and 1 alga *(Ankistrodesmus spiralis)* was recorded only at Morna. It is also revealed from the table that maximum species of algae (32) were recorded at Morna while minimum (13) were recorded at Basappawadi.

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RESEARCH ARTICLE

Diversity of *Ipomoea* (Convolvulaceae) in some of the regions of Maharashtra

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ABSTRACT

The present study deals with genus *Ipomoea* of family Convolvulaceaefrom various regions of Maharashtra state. A total of 17 species of the genus have been collected from various localities of state Maharashtra on the collections made between 2013 and 2015 from different parts. The present paper illustrates the diversity and morphology of the species of *Ipomoea,* which are separated from each other on the basis of their morphological characters.

Keywords: Diversity, *Ipomoea*, Convolvulaceae, Maharashtra.

INTRODUCTION

The family Convolvulaceae is known as morning glory family. About 2000 species of 58 genera are distributed overall the world, mainly in the tropics and subtropics region (Staple and Yang, 1998). More than one third of the species are included into major genera *Ipomoea* and *Convolvulus* (Conquist, 1988). Genus *Ipomoea* represented by 650 species distributed worldwide (Mabberley, 1997).

In India family Convolvulaceae is represented 20 genera and 158 species occurring chiefly in the southern and western India Over60 species of *Ipomoea* are reported in India (Oudhia, 2001). As well it is widely distributed and occurring especially in damp places of Gujarat, Bihar, West Bengal, Chhattisgarh, Maharashtra, Western Ghats, Goa and Karnataka. Many investigators have worked on various topics of *Ipomoea,* biodiversity and taxonomy of tropical plant Calcutta (Sivdasan and Mathue, 1998), climbers of taluka Modasa, Dist. Sabarkantha (Gujrat) India (Jangid and Sharma, 2011), foliar anatomy of some uninvestigated species of Convolvulaceae (Tayadeand Patil,2012), leaf anatomical studies in some species Convolvulaceae (Tayade and Patil,2012), karyotype analysis in some south Indian Convolvulaceae (Sampathkumar, 1970), taxonomic significance of karyotypology in *Ipomoea* species (Sinha and Sharma, 1992), leaf architecture in some Convolvulaceae (Inamdar and Shenoy, 1981), radial secondary growth, formation of successive cambia and their products in *I. hederifolia* (Rajput*et al*., 2008), structure, distribution, development and taxonomic importance of stomata in some *Ipomoea* L. (Leela and Rao, 1994) and epidermal studies in *Ipomoea* (Singh *et al.*,1974).

The family Convolvulaceae is best known in temperate regions for its weedy representatives (e.g. *Calystegia, Convolvulus*). *I. batata*s is the world's second most important root which is used as food stuff (Simpson and Ogorzaly, 1995). The present paper highlights the diversity and taxonomic status of genus *Ipomoea* in some of the regions of state Maharashtra.

Table1: Showing total number of species of*Ipomoea* L. in the World, India and Maharashtra state.

Name of the genus	Number of Species in the world	No. of species in India	Number of species in Maharashtra
Ipomoea L.	650	60	17

MATERIALS AND METHODS

Plant explorations were carried out in different seasons of the year at some selected sites in State of Maharashtra. This paper is based on the collection of genus*Ipomoea* from family Convolvulaceae in particular between 2013 and 2015. The collection trips to distant places were of the duration of 2-3 days. In between, brief trips of day's durations were executed along or in the company of one or more helpers. In this way, it was possible to raise the collections from the different parts of the state. In the first year the collections were massive and in the subsequent years they reduced to solitary specimen.

The specimens were carried to the laboratory in the polythene bags or in plant press depending upon the length of trip and distance of the place of collection. The plants collected were pressed and prepared herbarium sheets. These specimens were identified with the help taxonomist and taxonomic literature of family Convolvulaceae.

A BRIEF TAXONOMIC DESCRIPTION

1. *Ipomoeaaqatica* Forst.
Marshyor aquatic herbs; stem is hollow, spongy; flowerspurplish-pink; capsuleglobose.
Occurrence: Abundant on marshy situations in lake of village DeolgaonTah. Armori,
Dist. - Gadchiroli
Distribution: Throughout India, Pakistan, Ceylon, China, Malaya,Tropical Africa andAustralia
Common name: Naelani Vel, Swamp Cabbage Nadishak

2. *Ipomoea pentaphylla* L.
Perennial glabrous twiner; stem much branched; flowerspurple; capsuleovoid.
Occurrence: Cultivated in gardens as showy plant, Chalisgaon.
Distribution: Deccan peninsula with Ceylon, Tropical Asia, Africa, Australia and America.
Common name: Gandivel, Garvel.

3. *Ipomoea carnea*ssp. *Fistulosa* (Mart. ex Choisy)
Erect or ascending suffruticoses hrubs; flowers purplish-pink, sometime white; capsule globose.
Occurrence: Commonin Maharashtra along the ditches and pond sides
Distribution: Native of America and introduced in Asia and other continents.
Common name: Beshram

4. *Ipomoea eriocarpa* R. Br.
Annual, slender, hispid herbs; flowers pink; capsule globose or ovoid.
Occurrence: Commonlygrows as weed along the road sides at Chalisgaon.
Distribution: India, Ceylon, Afghanistan and Tropics of old world.
Common name: Ranbhovaari, Maalghanti

5. *Ipomoea nil* (Linn.) Roth**.**
Twining herbs; flowers bluish-purple; capsule ovoid, sepals hirsute-villous basally and with a slender tail-like appendage
Occurrence: Common in jungles of Gadchiroli district.
Distribution: Throughout in India.
Common name: Kaladana, Nilvel, Nilpushpa
5.5

6. *Ipomoea hederifolia* L.
Scandant herbs; stem weak; flower scrimson or orange yellow; capsule ovoid.
Occurrence: Seen as wild species throughout India, observed on road side in between Armori and Brahmapuri (Dist. -Chandrapur)
Distribution: Introduced from America, throughout India.
Common name: Scarlet morning glory, scarlet creeper, star *Ipomoea*, LalPungli

7. *Ipomoea pandurata*
Prostratly growing, herbs; flowers white creamy with pink throat; capsule ovoid.
Occurrence: Common in Chalisgaon, found growing in

open places.
Distribution: Native to North America, Florida, west to Texas, Kansas and Michigan.
Common name: Wild Potato Vine, Man of the earth

8. *Ipomoea ochracea* (Lindl.) G. Don.
Twinning, herbs; flowers white creamy; capsule ovoid.
Occurrence: Common in jungles of Tah. Armori,
Distribution: It is native to parts of Africa, Asia, and certain Pacific Islands
Common name: Pungli, Vad Fudardi.

9. *Ipomoea pes-tigridis L.*
Twinning patentlyhirsute herbs; flowers pink; capsule ovoid.
Occurrence: Common at Gadchiroli, Armori, Brahmapuri and throughout the Vidharbha region
Distribution: Throughout India, Pakistan, Ceylon, Burma, Malaya, China, Polynesia and Tropical Africa.
Common name: Vagpadi, Bowervel, Chokhbhilai

10. *Ipomoea quamoclit L.*
Twiningg labrous herbs; flowers deep-red; capsule ovoid.
Occurrence: Commonly cultivated as a showy plant in overall Maharashtra, as well found as weed growing on fences along road sides in Maharashtra
Distribution: Native of Tropical America usually cultivated in India.
Common name: Kamini, Ganesh Vel

11. *Ipomoea sinuate (Jacq.)*
Prostrate hardy twining; hairy; flowers white with pink throat; capsule globose
Occurrence: Chalisgaon, B.P. Arts, S.M.A. Sci. & K.K.C. Com. College, campus.
Distribution: West India, Ceylon, Tropical America, Australia.
Common name: Snakevine, Alamo Vine, Noyau Vine

12. *Ipomoea plebian R. Br.*
Weak twiner, glabrous herbs; flowers white small
Occurrence: Found at Chalisgaon near J.J. Anna Tower and in forest of patnadevi.
Distribution: Oregon, north-eastern New South Wales, northern Australia, Malesia.
Common name: Bell Vine; Vine, Bell; Vellvine

13. *Ipomoea indica*L.
Twiner weak, glabrous herbs; flowerspale-purple
Occurrence: Common at North Maharashtra University canteen, Jalgaon (M.S.)
Distribution: India, Pakistan, Ceylon, Burma and Japan
Common name: Bhamardi, Ghagula, Gariya.

14. *Ipomoea turpethum*(L.)*R. Br.*
Twiner weak, glabrous herbs; flowers white- cream colored
Occurrence: Common at road sides of Armori – Brahmapuri (M. S.)
Distribution: Endemic to India. It is found in North Circars and Deccan region up to 3000 ft.
Common name: Turpeth, Pithori, Nakpatra, Nishut, Nishoth, Shetvad, Nishotar, Tend

15. *Ipomoea parasitica*(kunth) G. Don
Trailing, climber on bushes, velvety; yellow throated blue with purplish tinge flowers; capsule is globose
Occurrence: Collected from jungles of Chikhaldara (Amravati) M.S.
Distribution: It is native to the American continents, but is well naturalized as an escape from cultivation in many parts of the world, including India. Mexico through Central and South America
Common name: Yellow throated morning glory

16. *Ipomoea alba* L.
Glabrescent, stout twiner; flowers white; capsules ovoid-globose.
Occurrence: Chalisgaon, B.P. Arts, S.M.A. Sci. & K.K.C. Com. College campus & Nagpur.
Distribution: Native of Tropical America
Common name: Ganbhowra

17. *Ipomoea triloba* L.
Slender, creeper, twiner; flower spurple; capsule globose.
Occurrence: Grown as food stuff in Maharashtra.
Distribution: Native of America India, Pan Tropical.
Common name: Shakkaria, Ratalu.

RESULT

The Maharashtra has rich vegetation and distribution of *Ipomoea* in the state is quite diverse because of its varied and favorable climatic conditions. Many floras were made in state as it has diversity. Members of this genus are dispersed everywhere, found wild as well cultivated in gardens, hotels and at home in villages. A total of 17 species of *Ipomoea* has

been collected from the various regions of Maharashtra statewhichis2.62%oftheworld reported species. In India the 60 species reported account for 9.23% of the world *Ipomoea*.

DISCUSSION

The present study was conducted on the genus *Ipomoea*, comprising of 17 species (Fig.1). Most of the species are climbers; twiners excepta few suffruticoses hurbs. All these species have been distinguished on the basis of habit, habitat and morphology ofstems, leaves, inflorescence, pedicel, corolla, capsule and seeds. Shimpale (2012) has added *Ipomoea ochracea*from Pateshwar hill ranges of Western Ghats in Satara district of Maharashtra state and *Ipomoea Parasitica, Ipomoea tenuipes(2014)*recorded from Paithan, Aurangabad.

Subsequently *Ipomoea mombassana* Vatke (Biju*et al.*, 1998); *Ipomoea.parasitica*(Kunth) Don (Biju, 2002) and *Ipomoea. Ochracea*(Lindl.) G.Don (Shimpale*et al.,* 2012) has beenadded to Indian flora, bringing the total numberinto 63 species in India.

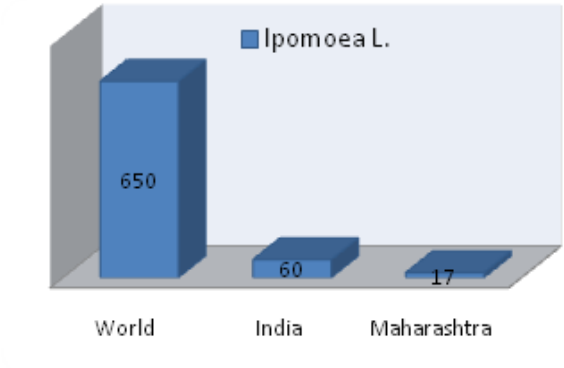

Fig.1: Bar graph showing number of species of *Ipomoea*inthe world,India and Maharashtra.

CONCLUSION

It is concluded that, as we started exploring the various sites in the state in between 2013 and 2015 so for now we had collected about 17 species which is about 2.62 % of the world flora and 28.33 % of the Indian flora.The present study shows that, the dietary supplementation of *Spirulina* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita* and improved the haematological parameters like RBC count and haemoglobin content significantlyas the percent dose of *Spirulina platensis* was increased in a short period of time.

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RESEARCH ARTICLE

Biopesticidal effect of aqueous exctract of leaves of *Argemone maxicana* on *Callosobruchus chinensis* (l) for the protection of *Phaseolusa conitifolius* grains

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ABSTRACT

Extract of leaves of the *Argemone maxicana* was tested against the infestation of *Callosobruchus chinensis* for the protection of the grains *Phaseolusa conitipholeus*. Aqueous extract was found to be very effective in protecting the damage caused by *Callosobruchus chinensis* to the grains of *Phaseolusa conitipholeus*. Loss of weight 3.7%and the loss of protein contents in the total grains of *Phaseolusa conitipholeus*2.6% were effectively reduced in grains treated with at 1.2 ml/kg. Dose concentration at the rate of 25 gms *Phaseolusa conitifolius grains*. Aqueous extract of leaves *of Argemone maxicana* was effective so it can be used for the protection of the *Phaseolusa conitifolius* and other pulses grains during storage.

Keywords: *Argemone maxicana, Callosobruchus chinensis, Phaseolusa conitifolius.*

INTRODUCTION

Phaseolusa conitifolius has high nutritional value in pulses and is cultivated all over the world. The numbers of pulses seeds suffer a great damage and loss during storage due to *Callosobruchus chinensis* attack. Among the insect pests attacking store products, pulse beetle *Callosobruchus chinensis,* (Coleoptera, Bruchidae,) is a serious pest.

Use of chemical pesticides has given rise to many serious problems caused by their residues the need for effective, biodegradable pesticides with greater selectivity search for new types of insecticides, Botanical preparations have long been used for protection of stored grains produces by small scale farmers in India, Various plant by-products have been tried recently with a good degree of success as protectants against a number of stored grain insect pests (Gill and Lewis, 1971), e.g. the parts of Neem, Tulsi, clustered apple leaves Nirgudi tree have been used extensively. Application of the plant originated pesticides causes multiple growth and reproductive abnormalities in insect's pest (Jaiswal and Srivastava, 1992). The biopestiside of plant origin also have the potential to reduce the population of insect in succeeding generation (Raju *et. al.,* 1990) thus they form an ideal component of ecological pest management program.

MATERIALS AND METHODS

Rearing of *Callosobruchus chinensis:*

Non infected grains of the *Phaseolusa conitifolius* were purchased from the local market shop and were screened for the damaged and infected grains if any. The non-infected grains were washed in clean water and were dried at 45 ^{0}C in the oven to kill if any infective stage of the pest exists in the grains. The dry grains were placed in the clean glass bottle. The cap of the bottle was perforated for the ventilation. Wet muslin cloth was tied at the mouth of the bottle before capping to maintain the humidity for the survival of the pest.

Callosobruchus chinensis were separated from the infected grains. Ten males and ten females were released in the plastic jar containing 500 grams of the grains and were allowed to grow. The adult females laid the eggs on the grains. Beetles when died were removed and the culture was maintained for experimental work. The young adults began to emerged out from the grains, the adults hatched, were used for the experimentation and some were released in the fresh pulses again to maintain the stock culture.

Collection and Preparation of extract:

Fresh leaves of *Argemone maxicana* were collected from different parts of natural habitats at Dr. Babasaheb Ambedkar Marathwada University campus Aurangabad and washed with tap water and were dried in the shade at laboratory. The dried leaves were pulverized in the grinder and the powder was screened with cotton cloth than stored in the airtight polyethylene bag. Ten grams of the dried powder was soaked in100 ml. distilled water for 24 hours. Then the mixture was filtered by Whatman filter paper and the filtrate was collected and was directly used with different concentration.

Grains used:

25 grams of the disinfected pulse grains were taken in each of the plastic jar. One jar was maintained as control and others were labeled and used for experimentation.0.4 ml. 0.8 ml 1.2 ml 1.6 ml 2.0 ml. extract were added in each plastic jar containing 25 gms grains. The dead adults were removed and the data as given in the tables was collected and mentioned in the table, it shows the protective role of the aqueous extract of the leaves of *Argemone maxicana* on grains of *Phaseolusa conitifolius* against the *Callosobruchus chinensis*.

RESULTS

Phaseolusa conitifolius is the common pulse used as sprouted grains for several preparations of breakfast and meals in the country. It is highly susceptible to the infestation of the bruchids, *Callosobruchus chinensis* and *Callosobruchus maculates*. General observations show that *Phaseolusaconitifolius* seeds if remains for few months in the house are heavily attacked by these pests and makes them unfit for eating. *Callosobruchus chinensis* occurs commonly in most of the pulses.

The female lays eggs and glues on the grains. The number of eggs glued on single grain varies from one to five. The female lays eggs for about 8 to 10 days. The tiny larva that hatches out penetrates in the grains and grows within the grains. They eat the grains from inside and make them hollow and then pupate. The pupa can be removed from the grain. After about 28 days, the adult emerges out from the grains. Table shows the effect of aqueous extracts of *leaves of Argemone maxicana* on the mortality of adult *Callosobruchus chinensis* and their life cycle stages. The mortality rate was high and the number of eggs laid,

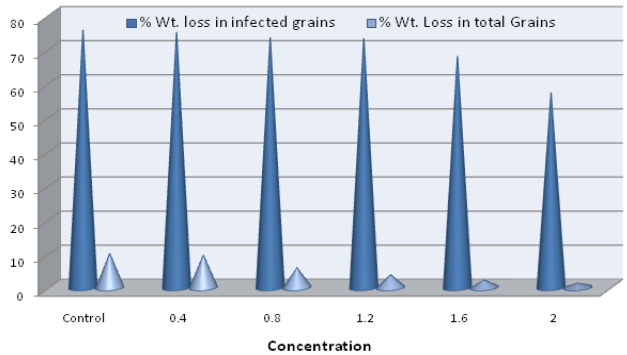

Fig. 1: Effect of aqueous extract of *Argemone maxicana* leaves on the wight loss in grains of *Phaseolus aconitifolius* by the stored grain pest, *C. chinensis*

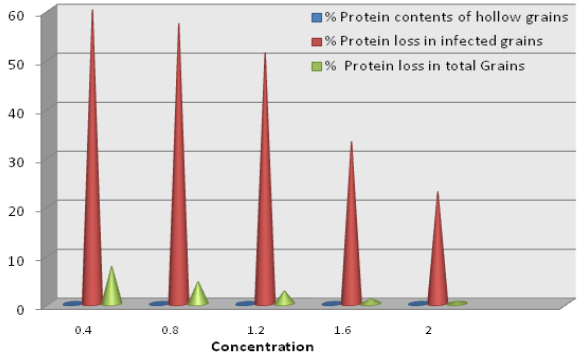

Fig. 2: Effect of aqueous extract of *Argemone maxicana* leaves on the protein loss in grains of *Phaseolus aconitifolius* by the stored grain pest, *C. chinensis.*

grains infested and adults emerged were highly reduced with the increase in the concentration of the extract as shown in the tables. There was no infestation and emergence of adults in the concentrations of 1.2 ml per Kg grains and above 2.0 ml per Kg grains. The mortality rate, egg laying and hatching of the adults was minimized in the aqueous extracts of *Argemone maxicana*.

Caswell (1981) reported that over 130 plants and plant products have been shown to have insecticidal activity against stored product pests. Many of the plants which farmers use as protectants have a strong smell which, it is supposed, repels or kills insects. Other workers have previously reported that plant powders reduce oviposition by bruchids under laboratory conditions. The plants include neem kernel powder (Sowunmi and Akinnusi, 1983) and Kurunegaladasia *Tridax procumbens* (L.) with C. *maculatus* (Bhaduri *et al.,*1985), and custard apple *Annona squamosa*L. seed powder with C. *chinensis* (Ali *et al.*1983). Among bruchids, *Callosobruchus chinensis*is a major cosmopolitan pest that causes serious damage to pulses in storage condition. Gugar and Yadav (1978) reported that 55-69% weight loss and 45.6 - 66.3%protein loss by infestation of pulse beetle on chickpea.

Our study show that the effect of aqueous extracts of leaves of *Argemone maxicana* on the weight loss of infected and total grains, and protein loss in infected and total grains of *Phaseolusa conitifolius*. Percent weight loss in infected and total grains was highly reduced inlow concentrations of the extracts of leaves of *Argemone maxicana*. The whole grains of *Phaseolusa conitifolius* were found to have 24.87 percent proteins while the infected hollow grains had reduced proteins contents on exposure to extracts of leaves of *Argemone maxicana* while percent protein loss in total grains at the said concentrations was highly reduced indicating the saving of the proteins against the damage by *Callosobruchus chinensis*. Protein loss was also minimized in different concentrations of aqueous extracts as given in the tables.

CONCLUSION

The results of the study have confirmed that the beetle, C. *chinensis,* can be effectively controlled by admixing *Argemone maxicana* leaf extract with 25 gm of *Phaseolusa conitifolius* seeds for at most 6 months. The use of plants extract should be encouraged in small farm storage, as the expenditure of these plants originated bio-pestiside are low and easily available when compared with synthetic insecticides. Additionally, further seeds would be available for use as food and for sale by the farmer because grain infestation would be reduced. Consumers would also get additional value for their currency.Thus, the present investigations indicate that plants derivatives might be useful as insect pest control agents for marketable use. To diminish the severe damage caused by insect pests, the traditional use of plant products, proved to be highly effective against stored product insects. Application of plant products to grain seeds for storage is an economical and valuable technique, and its easy adaptability will give additional advantages leading to acceptances of this knowledge by farmers. A study to improve the effectiveness of plants derivatives as insecticides will profitable to agricultural sectors of developing countries, as these material are not only of low cost, but also have less environmental impact. Therefore, from the above study it may be concluded that the *Argemone maxicana* leaf extract can be effectively used as grain protectants and thereby reduce the residual of toxic chemicals in our food stuff as well as in the environment.

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RESEARCH ARTICLE

Submerged aquatic Hyphomycetes *Canalisporium* from Madhya Pradesh

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ABSTRACT

The present paper deals with four species of freshwater submerged aquatic Hyphomycetes, encountered in foam samples collected from Khandwa District of Madhya Pradesh, Viz *Canalisporium caribense (*Hol.-Jech and Mercado) Nawawi and Kuthub. *Canalisporium exiguum Canalisporium pallidum* and *Canalisporium pulchrum,* Nawawi and Kuthubutheen are being reported for the first time from Madhya Pradesh. The data collected provide information on the biodiversity and Geographical distribution of these fungi from freshwater habitats of India, apart from description and illustrations. This data will assist in the compilation of freshwater fungal biodiversity of India.

Keywords: Submerged aquatic hyphomycetes, Foam, Madhya Pradesh.

INTRODUCTION

Submerged aquatic hyphomycetes were first addressed by Ingold (1975), growing on submerged decaying plant material. Most of the species are found on wood litter blocked by rocks in fast flowing streams or babbling brooks. They colonize stem, branches and leaves, fall on the water. It plays an indispensable role of plant substrates in various ecosystems, but aquatic Fungi have been relatively neglected group until the last 4 decades. They play an important role in freshwater food webs as organic matter decomposers and contributors to nutrients cycling, as symbionts with plants, some of the many important roles play by freshwater fungi that help to maintain the energy balance of the ecosystems (Barlocher, 1992a). The aquatic fungi which typically decompose leaf litter and wood with a hyphal network are the polyphyletic group known as "aquatic hyphomycetes". With the aid of an array of extracellular enzymes, aquatic fungi are able to degrade most of the polymeric substances in leaves (hemicelluloses, cellulose, starch, pectin and to some extent lignin; Krauss *et al*., 2011). The present paper deal with the 4 species of submerged aquatic lignicolous fungi encountered on the decaying wood and foam.

MATERIALS AND METHODS

Approximately 10 ml of Foam formed due to the fast flowing turbulent water at study area were collected at morning and evening time. Samples were made with a ladle and placed in clean wide mouthed plastic bottles and kept

for 24 hours to enable the foam to dissolve. It was fixed by adding FAA (Formaldehyde + Acetic Acid + Alcohol prepared as 90 ml. of 70 % Alcohol + 5 ml. Formaldehyde + 5 ml. Acetic Acid) to yield 5% foam solution. The samples were brought to laboratory and examined under low power or high power of a microscope to detect the conidia. The slides were made permanent by using double cover glass method given by Volkmann-Kohlmeyer and Kohlmeyer (1996).

TAXONOMIC ACCOUNT

1)*Canalisporium caribense* (Hol.-Jech and Mercado, 1984) Nawawi and Kuthub (1989) (Fig.1) *Berkleasmium caribense* Hol.-Jech and Mercado(1984).
Mycelium: septate C**onidiophores:** micronematous, fasciculate, simple or sparsely branched, smooth, hyaline, up to 25 µm long, 2-3.5 µm wide **Conidia**: acrogenous, solitary, broadly ellipsoidal, obpyriform to subglobose, flattened, muriform, olivaceous, brown, reddish brown to dark brown, smooth, with dark, thick-walled transverse and longitudinal septa, basal cell cuneiform, thin- walled, sub hyaline to light brown. Cell lumen connected by narrow canals, flattened, with a single, straight or slightly curved column of vertical septa and 3-5 transverse septa, pale brown to brown septa darkly pigmented, 9-11 cells per conidium, 18-28 x 13-15 x 5-8 µm wide.
Habitat: Conidia in foam samples; (Bakhatgarh) Narmada River, 10 July 2011; (Siloda) Chhoti Abna River, 20 Aug. 2012; BAFK-55; Leg., D. K. Patil .
Distribution in India: Karnataka: (Sridhar *et al*., 2011); **Maharashtra:** (Patil *et al*., 2014).

2) *Canalisporium exiguum* Goh *et al*. 1998
(Fig. 2)
Sporodochia: on natural substratum punctiform, minute, scattered, granular, black, glistening, up to 140 µm in diameter **Mycelium:** immersed, branched, septate, subhyaline to pale brown, 1.5-3 µm wide, smooth. **Conidiophores:** micronematous or semi-macronematous, fasciculate, simple or sparsely branched, smooth, hyaline to subhyaline, up to 30 µm long 2-3.5 µm wide. **Conidia:** acrogenous, solitary, flattened, one cell thick, smooth, thick walled, broadly ellipsoidal, to obovoid in surface view, cylindrical to clavate in lateral view, pale olivaceous brown to pale pinkish brown, muriform, comprising of a single straight to slightly curved column of vertical septa and 2-4 rows of transverse septa, slightly constricted at the septa; septa becoming progressively darker with conidial maturity, cell lumen connected by narrow canal, basal cell subhyaline to pale brown, cuneiform Conidia are 18-32 x 12-15 µm 2-4 µm wide with thinner wall.
Habitat: Conidia in foam samples; (Gutighat) Tapi River, 2 Sept. 2012; BAFK-56; Leg., D. K. Patil
Distribution in India: Maharashtra: (Borse *et al*., 2008).

3) *Canalisporium pallidum* Goh *et al*. (1998) (Fig. 3)
Sporodochia: on natural substrate punctiform, scattered, granular, dark grey, up to 200 µm wide. **Mycelium**: mostly immersed in the substrate, composed of branched, septate, sub-hyaline, 1.5-2.5 µm wide, smooth hyphae. **Conidiophores**: micronematous or semi-macronematous, mononematous, fasciculate, simple or sparsely branched, smooth hyaline or sub-hyaline, up to 25 µm long and 2-3 µm wide. **Conidiogenous cells:** integrated, terminal, determinate, cylindrical or slightly vesiculate. Conidial succession rhexolytic. **Conidia:** 25-39 x 16-22 x 8-10 µm acrogenous, solitary, one cell thick and flattened, smooth, more or less ellipsoidal or obovoid in surface view, slightly curved, cylindrical or broadly clavate in lateral view, pale olivaceous very olivaceous brown, muriform, mostly with a slightly curved single column of vertical septa and 4-5 rows of transverse septa, occasionally 1-2 vertical septa, septa unpigmented, thin and canal clearly visible, basal cell cuneiform, sub hyaline, 2.5-3.5 µm wide, thin walled.
Habitat: Conidia in foam samples; (Puran pura) Bhandaria River, 2 Sept. 2012; BAFK-57; Leg., D. K. Patil
Distribution in India: Maharashtra: (Patil *et al*., 2014) .

4) *Canalisporium pulchrum* (Hol. Jeck, and Mercado²) Nawawi and Kuthubutheen, (1989) (Fig. 4)
=*Berkleasmium pulchrum* Hol.-Jeck, and Mercado, (1984).
Mycelium: immersed, composed of branched, septate, smooth, sub-hyaline to pale brown. **Conidiophores:** micronematous or semi-macronematous, mononematous, fasciculate, simple or sometime branched, septate, hyaline to very pale brown, smooth, up to 25 µm long, 2-4 µm wide. Conidial secession rhexolytic. **Conidia:** solitary, acrogenous, sub-globose, complanate, one cell thick, flattened, broadly ellipsoidal or pyriform in surface view, narrowly ellipsoidal to date in lateral view, muriform, with 4-6

1. ***Canalisporium caribense (***Hol.-Jech and Mercado) Nawawi and Kuthub.
2. ***Canalisporium exiguum*** Goh *et al*.
3. ***Canalisporium pallidum*** Goh *et al*.
4. ***Canalisporium pulchrum*** (Hol. Jeck, and Mercado) Nawawi and Kuthubutheen.

rows of transverse septa and 2 straight columns of vertical septa, slightly constricted at the septa, smooth. Apical row of cells darker than the basal rows, dark and thick banded at the septa, canal in the septa obscured by dark pigmentation, supported by a cuneiform three thin walled, pale, small basal cell in a row, visible septal canal, 25-50 x 17.5-35 µm wide.

Habitat: Conidia in foam samples; (Kharuwa) Kharkuli River; 2 Sept. 2013; BAFK-58; Leg., D. K. Patil
Distribution in India: Maharashtra: (Borse *et al*., 2008); **Andhra Pradesh**: (Vasant Rao, 1986.

DISCUSSION

Fungi are an important component of biodiversity in aquatic environment the planktonic taxa is an integral part of food chain .They play an important role in fresh water food web as organic decomposers and contributors to nutrient cycling ,as symbionts with plants aquatic fungi are important for industrial and pharmaceutical use.

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RESEARCH ARTICLE

Phytogeographical elements and analytical aspects of flora of Patnadevi forest, Chalisgaon Taluka, (MS), India

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ABSTRACT

Phytogeographical elements are fundamental for understanding flora of any area. Geographical elements are the real base for subdividing flora into different floral elements. It is clear that floristic work is incomplete without an account of floral elements of an area. There are various types of floral elements occur in Patnadevi forest. These floral elements are such as Indian, Indo-Malayan, Tropical African - Indian, Indo-Malayan Chinese, Tropical Asian, Cosmopolitan, Indo-Malayan-Australian, Pantropics, Paleotropics, Exotic, Mediterranian, Perso - Arabian and Sudano-Deccanianin decresing order and their percentage is also different. Introduction of Exotic elements and their occuranceis more and it is alarming signal to the natural flora. Analysis of a flora of Panadevi forest in such a way indicate status of a forest and such type of analysis is also essential for origin and development of a flora.

Keywords: Floral elements, Patnadevi forest, Mediterranian, exotic, alarming signal.

INTRODUCTION

Forests are the main components on the earth hence they act as life supporting system. But since last few decades the biodiversity of these forests are disappearing at alarming rate. Many important plants are threatened and becoming rare and even some are on the verge of extinction. To stop exploitation, appropriate strategy for conservation and sustainable utilization of plant resources is urgent. To launch any policies and programme for conservation purpose, detailed assessment of floristic diversity at three steps generic, species and ecosystem is essential.

The study area of Patnadevi forest is located on Satmala ranges of Sahyadries, a part of northern Western ghats. Western ghats is one of the well known world's biodiversity hotspots. The present study site is within the Western Ghats of India, hence the present work is an attempt to know the status of vegetation in Patnadevi forest.

MATERIALS AND METHODS

Field work: The study area was demarcated with the help of map. Patnadevi forest area is 6355.39 hectare about 64 sq. km and it is divided in to 18 compartments. Quadrats of 10x10m. were laid down randomly in each of the compartment of forest area, so that the quadrat represents almost all species in the area. Altogether 64 quadrats were laid for the trees and shrubs in the entire area studied. Thus the sampling was done for a total area 64 sq. km. in the forests. All species covered by the quadrats were recorded. Commonly followed methods for floristics are sampling technique has been adopted. We have followed the random sampling technique.

Laboratory work: Extensive periodic floristic survey was carried out for entire study area. Various life forms were collected. Specimens were sorted out into respective species and then genera and families. All the families are arranged according to Bentham and Hooker's system of classification. Collected plant species identified by using related literature "Flora of Presidency of Bombay ", vol I, II, and III. (Cooke, 1958), Flora of Maharashtra state Dicotyledons Vol 1 (Singh and Karthikeyan, 2000), Flora of Maharashtra state Dicotyledons Vol. 2 (Singh *et al*., 2001), Flora of Maharashtra state Monocotyledons (Sharma *et al*., 1996), Flora of Marathwada (Naik, 1998) and other available literature.

RESULTS

A critical study of the distribution of various species in Patnadevi forest shows that in all 637 plant species are recorded out of which 260 species are reported first time. Total 13 floral elements are observed in study area.

Table 1 : Number and Percentage of Floral elements of Patnadevi Forest.

Sr. No.	Floral Elements	Number	Percentage
1	Indian	188	29.51
2	Indo-Malayan	161	25.27
3	Tropical African - Indian	75	11.77
4	Indo - Malayan - Chinese	41	6.43
5	Tropical Asian	38	5.96
6	Pantropics	28	4.39
7	Cosmopolitan	24	3.76
8	Indo-Malayan-Australian	23	3.61
9	Exotics	21	3.29
10	Paleotropics	14	2.19
11	Mediterranian	9	1.41
12	Perso-arabian	8	1.25
13	Sudano-deccanian	7	1.09

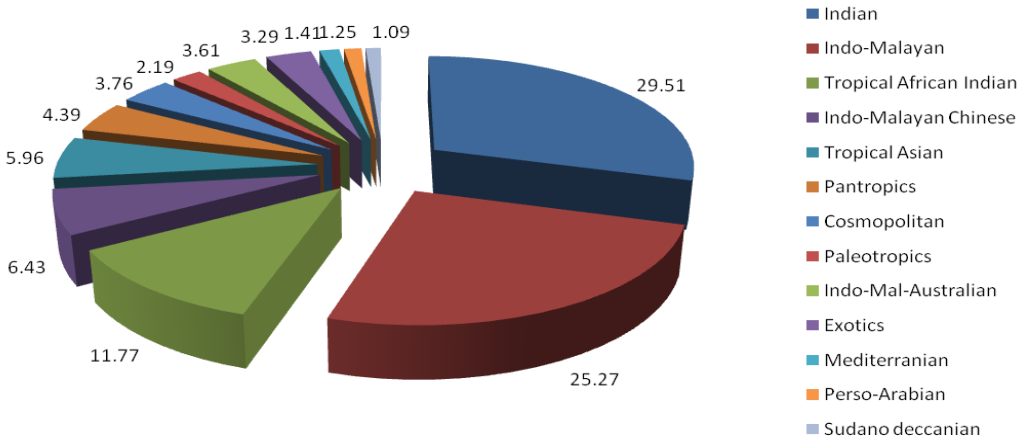

Fig. 1: Floral Elements

Various exotic plant species have naturalized in the area along with natural flora. The chief centers of origin are Mexico, South America and South Africa. The common exotic plants are *Adansoniadigitata* L., *Alternathera pungens* Kunth*, Alysicarpus bupleurifolius* (L). DC*., Ancistrocladus heyneanus* Wall, *Argemone mexicana* L*, Cassia tora* L*, Cassia occidentalis* L, *Cardiospermum helicabum* L*., Casuarina equisetifolia* L*. Ceropegia hirsuta* Wight & Arn*., Chrysanthemum americanum* L*., Clerodendrum multiflorum* (Burm.f.) O. Ktze*.,. Euphorbia heterophylla* L*., Jatrophacurcus* L., *Jatropha gossipyfolia* L., *Lantana camara* L., *Parthenium hysterophorus* L*., Parkinsonia aculeata* L., *Tridax procumbens* L*., Vaccaria pyramidata* Medik., *Zornia gibbosa* span. etc.

Wulff (1943) considered that, the geographical elements are fundamental for understanding flora of study area. Meher -Homji and Misra (1973) explained that origin and development of a flora is based on analysis of given flora of study area. It is clear that no floristic work is complete without an account of floral elements of an area. From the table 1, it will be seen that, the Indian constitutes the largest number (29.51%) followed by Indo-Malayan elements. (25.27%), Tropical African Indian constitute(11.77%) , Indo - Malayan - Chinese (6.43%), Tropical Asian (5.96%),Pantropics (4.39%),Cosmopolitan (3.76%), Indo -Malayan - Australian (3.61%),Exotics (3.29%), Paleotropics (2.19%), Mediterranian (1.41%), Perso-arabian (1.25%), and Sudano-deccanian (1.09%) Introduction of exotic elements and their occurance is more (3.29%) is alarming signal to the natural flora.

CONCLUSION

The most common floral element is the Indian (29.51%) followed by Indo - Malayan (25.27%). Study area is also represented by other floral elements like Tropical - African - Indian, Indo-Malayan-Chinese, Tropical Asian, Pantropics, Cosmopolitan, Indo-Malayan- Australian, Exotics, Paleotropics, Mediterranian, Perso- Arabian and Sudano- Deccanian in decreasing order.

Indian floral elements are most dominant 29.51% but introduction of other floral elements in proportion with Indian floral elements and also exotic elements & their occurrence in study area has threatened the natural flora. For restoration of plant diversity in future, reorientation of local indigenous community is necessary. Hence to maintain natural flora of Patnadevi forest preventive control measures should taken into consideration.

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RESEARCH ARTICLE

Digitization of leguminosae tree plants from Kinwat and Mahur forest ranges of Nanded district in Maharashtra

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ABSTRACT

Kinwat and Mahur taluka of Nanded districts has old heritage of medicinal plants and herbal medicine. Forest is rich in biodiversity and consists of rare medicinal plants. Biogeographical condition such as altitude, soil type and average rain fall make the vegetation diversity in this area. The local tribes Andh, Gond, Kolam, Naikde and pradhan use forest resources for their day to day life. In the present research 27 leguminosae plants were identified through the quadrate method from the study area. Digitization was carried out using morphological information of the plant, taxonomically important photographs and ethnomedicinal uses along with other information related to the plant. Hyperlink computer technique was used while doing the digitization. The present digitization of leguminosae tree plant will gives instant global access to the plants information and easy to maintain and process this information.

Keyword: Digitization, leguminosae plants, Kinwat and Mahur forest.

INTRODUCTION

Kinwat and Mahur taluka of Nanded districts has old heritage of medicinal plants and herbal medicine. Forest is rich in biodiversity and consists of rare medicinal plants. Biogeographical condition such as altitude, soil type and average rain fall make the vegetation diversity in this area.

The Kinwat taluka geographically situated at 19^0 25′ to 19^0 55 North latitude to 77^0 51 to 78^0 19 East longitude and covering about 57,800 hectares under forest which is about 27.25 % percentage of total area (Ghorband and Biradar, 2012). Mahur taluka is geographically situated between $19^0$49' to 19^0 83' North latitude and $77^0$91' to 77^0 55' East longitude. The total geographical area of taluka is 52,160 hectares out of which 14397.39 hectares land area coveredwith forest and 37762.61 hectares are non-forested area (Vijigiri and Bembrekar, 2015). Both these region has rich biodiversity because of the environmental condition. The present work deals with the digitization of the leguminosae tree plants diversity in the Kinwat and Mahur forest ranges.

The use of electronic communication and dominance of the Internet has created a major change in the way information is presented and stored. Taxonomically important set of digital photograph of plants in their natural habitat can be supplemented with the taxonomical description and other information related to the species. This digitized information of the plants is very useful for the researcher.

MATERIAL AND METHODS

Leguminosae tree plants survey was carried out in the Kinwat and Mahur forest ranges of Nanded district using quadrate method. 100 m × 100 m quadrate was taken for survey of the leguminosae tree plants in the study area (Misra, 1968). While taken the quadrate the maximum diverse area of the forest was taken in the account.

Digitization was carried out using ethnomedicinal uses, morphological information of the plants along with other importance were also summarized in the present study. Hyperlink computer technique was used while doing the digitization (Dalitz and Homeie, 2004; Schmidt, *et al*., 2009). Nikon Coolpix P510 camera was used for the digital photograph of the plants. The characters of the plants which were taken in the consideration are Scientific Name of the plant, its classification, distribution and GPS location from the study area. In addition to this common names like English, Hindi, Marathi, Sanskrit were given. It also provides plant morphology which includes habit, root, stem, leaves, flower, fruit, seeds, flowering and fruiting periods and economic importance of the species (Anonymous, 1948; Kirtikar & Basu, 1975; Nadkarni & Nadkarni, 1976; Naik, 1998; Yadav & Sardesai, 2000).

RESULTS AND DISCUSSION

Total 27 leguminosae tree plants were occurred in 45 quadrate of the study area. Out of this 27 leguminosae plants, 09 were belonging to the Papilionaceae, 6 were belonging to the caesalpinaceae and 12 were belonging to the family mimosaceae.

Albizia leback (L.) Benth.

Buteamonosperma (L.) Taub

Caesalpiniapulcherrima(L.)Sw.

Cassia fistula L.

Sennasiamea (Lam.) Irwin

Dalbergia sissoo DC.

Delonixregia (Hook.) Raf.

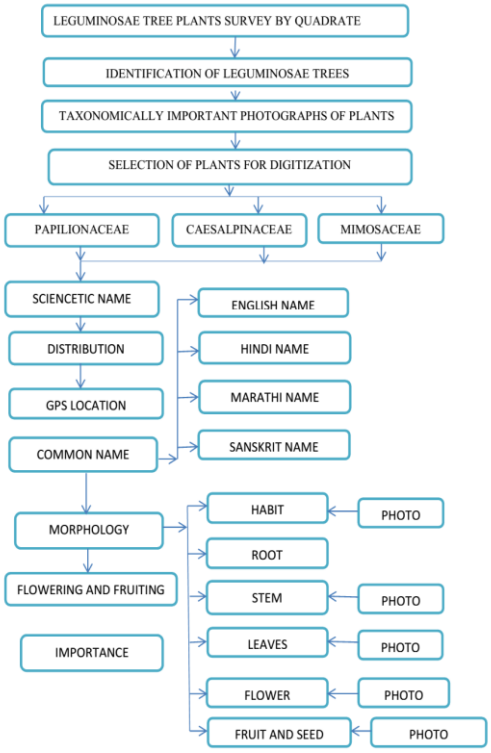
*Erythrinasuberosa*Roxb.,

Gliricidiasepium (Jacq.) Walp.

Peltophorumpterocarpum (DC)

Pongamiapinnata (L.) Pierre

Prosopisjuliflora (Sw.) DC



Leguminosae tree plants flower Photographs from the study area

Plants belonging to these families were *Buteamonosperma* (Lam.) Taub. , *Dalbergia lanceolaria* subsp. *Paniculata* (Roxb.) Thoth., *Dalbergia sissoo* DC., *Desmodium oojeinense* (Roxb.) H.Ohashi, *Erythrina suberosa* Roxb., *Gliricidia sepium* (Jacq.) Walp., *Pongamia pinnata* (L.) Pierre, *Pterocarpusm arssupium* Roxb., *Peltophorum pterocarpum* (DC.) K.Heyne, *Bauhinia recemosa* Lam., *Caesalpinia pulcherrima* (L.)Sw., *Cassia fistula* L., *Sennasiamea* (Lam.) H. S. Irwin & Barnaby, *Delonixregia* (Hook.)Raf.,*Tamarindus indica* L., *Acacia farnesiana* (L.) Willd., *Acacia catechu* (L.f.) Willd., *Acacia leucophloea* (Roxb.) Willd., *Acacia chundra* (Rottler) Willd., *Acacia nilotica* (L.) Delile, *Albizialeback* (L.) Benth., *Albizia procera* (Roxb.) Benth., *Albizia julibrissin* Durazz., *Albizia saman* (Jacq.) Merr., *Leucaena latisiliqua* (L.) Gillis & Stearn, *Pithecello biumdulce* (Roxb.)Benth. and *Prosopis juliflora* (Sw.) DC.

Taxonomically important plant photographs of these plants were taken like photographs of habit, stem, leaves, flower, fruits and seeds. These photographs were supplemented with the scientific name of the plants, common name including English name, Hindi name, Marathi name, Sanskrit name, classification of the plants, distribution, Habit, morphological description of the plants, economic importance, flowering and fruiting month and GPS location from the study area. All these character were taken into consideration while doing the digitization of the leguminosae tree plants from the Kinwat and Mahur forest ranges of the Nanded district.

All these collected information and photographs was used to prepare the digital data about the leguminosae plants found in the Kinwat and Mahur forest ranges. This information documented in the PowerPoint which linked with each other by the Hyperlink computer technique. It results into the digitized information system of the leguminosae plants. In this digitization work total 27 leguminosae plants form the study were documented. This digitization work can be summarized as per the tree diagram shown below.

CONCLUSION

This digitization work was useful for the leguminosae tree plants identification, and teaching taxon recognition. It also provided the useful information related to the species. It also provides the ecological information of the species. It will improve the collection and preservation of the plant specimen. It gives global access to the plants information and easy to process this information. This digitization technology can open the new research opportunity.

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RESEARCH ARTICLE

Hematological Profile of Sickle Cell Anemic Subjects from Gadchiroli District, Maharashtra

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ABSTRACT

After Thallassemia sickle cell anemia is the major hemoglobinopathy in India. It is common autosomal recessive disease due to single nucleotide substitution (GTG-GAG). Observational study was conducted to determine the hematological values of sickle cell subjects and patients from April 2010 to April 2012. 27 carrier subjects (heterozygous Hb-AS) and 24 sufferers (homozygous -HB-SS) were studied at steady state. Our study shown Reduction in level of RBC, MCV, Hemocrit, MCH, low level of platelet found in the patients with Hb-SS pattern as compared to the Hb-AS subjects. MCHC found normal with increased level of RDW% presenting the symptoms of crisis, the mean hemoglobin found significantly lower in SS patient as compared to Hb-AS subjects this difference is because of presence of HB- A in sickle cell traits which causes less number of RBCs to undergo sickling and further hemolysis, while in Hb-SS subjects promotes sickling and increased rate of RBC destruction leading to profound anaemia. Total WBC count and differential leukocyte count showed normal to elevated in both Hb-AS subjects, as well as Hb-SS subjects.

Key words: Hemoglobin, Sickle cell anemia, Complete blood count, Gadchiroli District.

INTRODUCTION

Sickle cell anaemia is known to the medical world since the discovery of this entity (Herrick, 1910) a Chicago cardiologist. He first provided the formal description of sickle cell anemia, he reports that the blood smear of a dental student at the Chicago College of Dental Surgery contains pear shaped and elongated forms. Herrick (1910) report led not only to the recognition of hundreds of abnormalities of hemoglobin synthesis but, also to a series of remarkable scientific advances involving protein Chemistry, Cell Biology, Physiology, and Genetics. The discovery of hemoglobin- S (Hb-S) Pauling *et al.* (1949) was the first demonstrated the production of an abnormal protein could be the cause of a genetic

disorder**.** In India it is the second most dominated haemoglobinopathy after Thallassemia, it is most common in central and southern part of India. Genetically it is an autosomal recessive disorder characterized by the substitution of valine for glutamic acid at position 6 in the β-Globin chain (Hb-S). This results in a solubility problem in deoxygenated state and upon deoxygenation the affected RBCs changes from biconcave discoidal cell to crescent of sickle shaped cell (Evans and Mohandas, 1987)**.**

This major haemoglobinopathy occurs in both homozygous and heterozygous state, red cell contain both normal adult hemoglobin (Hb-A) and the variant, because they rarely have phenotypic expression of clinical significance, heterozygous is said to have the trait for that abnormality, e, g. sickle cell trait. In the homozygous state, Hb-A is totally lacking, and clinical manifestation is of variable severity; individuals so have the anemia called sickle cell anemia. Gadchiroli is a newly carved district, of Maharashtra with a major population of Gond and Madia tribes being most backward and Naxlite hit, the district lag behind in healthcare facility from rest of the region. Though various aspects of SCA are studied aspects with reference to subjects from Gadchiroli district of Maharashtra, are unclear.

MATERIAL AND METHODS

The study conducted in Gadchiroli district, of Maharashtra with a major population of Gond and Madia tribes. The district is having 45 PHC's distributed in Gadchiroli-3, Korchi-2, Kurkheda-3, Wadsa- 3, Armori-4, Dhanora-5, Chamorshi-6, Etapalli-3, Mulchera-3, Bhamragarh-3, Aheri-5, and Sironcha-5. Fifty one SCA subjects visiting different PHCs of district during April 2010 to April 2012 were studied. 27 carrier subjects (heterozygous - HbAS) and 24 sufferer subjects (homozygous - HbSS) proceeded for the complete blood count.

About 3-5 ml of blood sample from both heterozygous and homozygous subjects was made in two vials for the haematological evaluation with the help of concerned staff member with due permission. Blood collected by vein puncture, CBC is done using the Blood Cell Counter following parameters studied. Red Blood Cell Count (RBC), Mean Corpuscular Volume (MCV), Red Cell Distribution width (RDW %), Hemocrit (PCV), Total Platelet Count (PLT), Mean Platelet Volume (MPV), White Blood Corpuscles (WBCS), Hemoglobin (HGB), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Lymphocyte Percentage. (LYM %), Total Granulocyte Percentage (GRA%).

RESULTS AND DISCUSSION

A complete blood count (CBC) gives important information about the kinds and numbers of cells in the blood, especially red blood cells , white blood cells , and platelets. A CBC helps in provide baseline haematological values in sickle cell disease patients that can be used in monitoring the status and management of sickle cell anemia patients. The count suggested that Sickle cell disease is a genetic abnormality primarily, involving the hemoglobin and red cell. The white blood cells and platelets are also affected by the mutation.

Table 1: Showing Hematological Profile of Hb-AS patients (Carrier, N = 27)

Sr. No.	Parameters	Mean	S.D.	SEM
1	**RBC**	3.6337	± 1.3203	0.2541
2	**MCV**	76.589	± 10.497	2.02
3	**RDW %**	17.341	± 2.276	0.438
4	**HCT**	26.444	± 7.958	1.532
5	**PLT**	217.93	± 101.46	19.53
6	**MCHC**	34.969	± 1.294	0.249
7	**MPV**	7.619	± 0.914	0.176
8	**WBC**	7.089	± 2.769	0.533
9	**HGB**	10.33	± 2.511	0.483
10	**MCH**	25.785	± 5.776	1.12
11	**LYM%**	49.026	± 15.505	2.984
12	**GRA%**	40.726	± 16.419	3.16

Table 2: Showing Hematological Profile of Hb-SS patients (Diseased, N =24)

Sr.No.	Parameters	Mean	S.D.	SEM
1	**RBC**	1.954	± 0.4602	0.0939
2	**MCV**	72.658	±11.467	2.341
3	**RDW %**	20.079	± 4.350	0.888
4	**HCT**	15.329	± 3.469	0.708
5	**PLT**	149	± 56.17	11.47
6	**MCHC**	37.063	± 3.825	0.781
7	**MPV**	7.604	± 1.094	0.223
8	**WBC**	4.587	± 2.573	0.525
9	**HGB**	5.454	± 1.016	0.207
10	**MCH**	24.921	± 3.212	0.656
11	**LYM%**	48.183	± 13.038	2.661
12	**GRA%**	41.621	± 11.935	2.436

In these findings we studied, 27 carriers Hb-AS and 24 sufferer Hb-SS subjects for complete blood count. Red Blood Cell count, (Hb-AS – 3.637± 1.3203, Hb-SS – 1.954 ± 0.4602, $p < 0.0001$) MCV (Hb-AS – 76.589 ± 10.497, Hb-SS – 72.658 ± 14.558 , $p < 0.0001$) and Hemocrit (Hb-AS – 26.444 ± 7.958, Hb-SS- 15.329 ± 3.469 % $p < 0.0001$), differed statistically showing decreased level of hematological indices Davies *et al.,* (1983) reported the same, but Powars *et al.,* (1980) reported no correlation between above parameters. Our findings showed decreased level of RBC, MCV, and Hemocrit (PCV), Kar *et al.,* (1986) observed lower mean corpuscular volume. Balgir (2000) found low MCV (60-113 ft) Roy *et al.,* (1996), Rao *et al.* (2012) also observed low level of above parameters and supports to our studies Akuzawa *et al.* (1989) also reported the low level of above parameters indicating hemolytic anaemia and pathological erythrocytes. In our studies observed MCH was low in Hb-SS patients as compared to Hb-AS (Hb-AS-25.785 ± 5.776, SS- 24.921 ± 3.212) with MCHC (Hb-AS-34.959 ± 1.294, Hb-SS-37.063 ± 3.825) and increased red Cell Distribution Width (AS- 7.341 ± 2.276, SS-20.079 ± 4.350%) Low MCH and MCHC reported by Kar *et al.,* (1986) and Balgir, (2000) (low MCH in India it ranges from 0.25-0.42g/dl) Rao *et al.,* (2012) reported low level of MCH and MCHC increase in percentage of red cell distribution width reported by Schweiger, (1981), Webster and Castro, (1986) and Sayed and Tawfik (1994).

Total platelet count and mean platelet volume (Total platelet count in Hb-AS- 217.93 ± 0.914, Hb-SS- 149 ± 56.17 and Mean platelet volume Hb-AS – 7.619 ± 0.914 and Hb-SS- 7.604 ±1.094) shown little difference in the observation total platelet count and mean platelet count and mean platelet count found normal in Hb-AS subjects whereas in diseased (Hb-SS) patients it was little decreased in mean of total platelet count and mean platelet count Ibanga, (2006) found significant rise in platelet count in steady state patients 224+/- 46.3X10(9) L when compared with others of 196.6 +/- 39.3X10(9) L ($p < 0.05$) and platelet fall during Vasoocclusive crises to 140.6+/- 36.3 X (10)/L ($p<0.05$), Haut (1973) studied platelets survival recovery, the result indicated that during the stable period platelets function is normal and that platelets survival is normal or greater than normal. Variation in platelet activity and function also studied by Henstell *et al.,* (1965), Noroha *et al.,* (2007), Elderdery *et al.,* (2011).

The mean difference in hemoglobin level of sickle cell anaemia subjects found in trait (Hb-AS-10.33 ± 2.511 and Hb-SS- 5.454 **±** 1.016) is significantly higher than Hb-SS subjects of ($p<0.0001$) this difference is because of the presence of one normal Hb-A in sickle cell trait subjects which causes less number of RBCs to undergo sickling and further hemolysis in Hb-AS subjects while in diseased subjects both Hb-S promotes sickling and increased rate of RBC destruction leading to profound anaemia. The mean hemoglobin in Hb-AS female was significantly lower than male Hb-AS subjects may be because of average Hemoglobin levels are normally 1.5 gm% lower in females as compared to males. This may be also because of low socioeconomic status, in previous studied similar observation were noted by Serjeant *et al.,* (1968) they found 18.33%) subjects with Hb of ≥10 gm%, (46.67%) patients in the range of 8-9.9

gm% and (31.67%) subjects in the range of 6-7.9 gm% while (3.3%) subjects with Hb below 6 gm% of the total 60 Hb-SS subjects studied Kar *et al.,* (1986) found mean Hb of 8.73 ± 1.69 gm% (range 3.9 – 13.5 gm%) amongst 131 Hb-SS subjects studied. Kar and Devi (1997) reported 6-10 g/dl mean with which they thrived well Davies *et al.,* (1983) found 7.1 – 9.2 g/dl and Rao *et al.,* (2012) reported low hemoglobin in 33 subjects. From observation it is evident that normal WBCs in Hb-AS and Hb-SS patients (Hb-AS – 7.089 ± 0.533 and 4.587 ± 0.525), with difference in Lymphocyte percent and Granulocyte percentage (Lym%, in Hb-AS-49.26 ± 15.505 %, Hb-SS - 48.183 **±** 13.038% and Gra%, in Hb-AS- 40.726 ± 16.419 % in Hb-SS- 41.612 ± 11.935%). The rate of chronic haemolysis associated with sickle cell anaemia subjects could account for all these disturbed values. In view of Sherwood *et al.* (1987) there is a blunted response to erythropoietin secretion in sickle cell anaemia, hence the rate of increase is not proportional to the degree of anaemia. Morris *et al.*, (1991) pointed out that it may be due to right shifted haemoglobin dissociation curve seen in sickle cell disease. Similarly lower values were obtained by Diggs (1965) in his hematological studies in sickle cell disease found that people in stable condition do not have any significant hematological deviation. The studied patients also did not show much deviation from normal in stable condition. This study provides hematologic reference ranges for sickle cell disease patients compared with normal controls in the district of Gadchiroli. It is our hope clinics involved in supervision of sickle cell anaemia patients would become more up to date and make use of the result in this study in their practice ranges for sickle cell disease patients compared with normal controls here. The study would make expert involved in inspecting sickle cell anaemia subjects to become more knowledgeable and help them make use of these findings in their skill to manage this genetic menace in the region.

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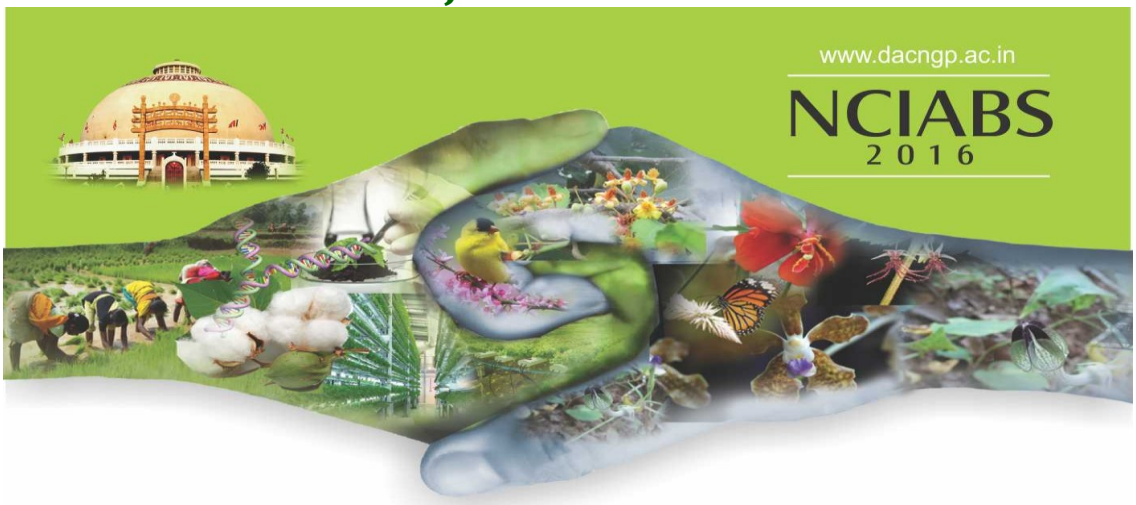

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