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**Number of Research Paper Published**  
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Sr. No.	Name of Teacher	Department	Number of paper Published					Total
			2016-2017	2017 - 2018	2018 - 2019	2019-2020	2020-2021	
1	Mr. N.D.Gorghate	Zoology			1		1	2
2	DR.C.V.Bisen	Chemistry						
3	DR.S.M.Akare	Botany						
4	Mr.R.N.Huse	Chemistry				2		2
5	Ms. Vishakha Wagh	Commerce						
6	Dr.V.K.Sangode	Zoology				1	1	2
<b>Total</b>			0	0	1	3	2	<b>6</b>

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**Number of Research Paper Published**  
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Sr. No.	Name of Teacher	Department	Number of paper Published					Total
			2016-2017	2017 - 2018	2018 - 2019	2019-2020	2020-2021	
1	Mr. N.D.Gorghate	Zoology	1		2	1	2	6
2	DR.C.V.Bisen	Chemistry				2		2
3	DR.S.M.Akare	Botany		1	2			3
4	Mr.R.N.Huse	Chemistry				2		2
5	Ms. Vishakha Wagh	Commerce			1			1
6	Dr.V.K.Sangode	Zoology		1		1	1	3
<b>Total</b>			1	2	5	6	3	<b>17</b>

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# Light and Transmission Electron Microscopic Study of Olfactory Organ of Spotted Snakehead, *Channa punctata* (Bloch)

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Received July 27, 2020; Revised August 25, 2020; Accepted September 29, 2020

## Cite This Paper in the following Citation Styles

(a): [1] Prakash Ghodeswar, Nilesh Gorghate, Suresh Masram, "Light and Transmission Electron Microscopic Study of Olfactory Organ of Spotted Snakehead, *Channa punctata* (Bloch)," *Advances in Zoology and Botany*, Vol. 8, No. 6, pp. 483 - 490, 2020. DOI: 10.13189/azb.2020.080602.

(b): Prakash Ghodeswar, Nilesh Gorghate, Suresh Masram (2020). *Light and Transmission Electron Microscopic Study of Olfactory Organ of Spotted Snakehead, Channa punctata (Bloch)*. *Advances in Zoology and Botany*, 8(6), 483 - 490. DOI: 10.13189/azb.2020.080602.

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**Abstract** The olfactory organ of *Channa punctata* (Bloch) has been studied under light microscope and transmission electron microscope. The olfactory apparatus of *C. punctata* comprises of olfactory rosette, olfactory nerve, and olfactory bulb. Paired, oval shaped olfactory rosette present in the olfactory chamber on fish rostrum. Olfactory chamber opens externally via an anterior inlet and a posterior nostril outlet. Olfactory epithelium comprises of sensory and non-sensory regions. Sensory region is at basal part and non-sensory region is at proximal area of olfactory lamellae. In olfactory lamellae, sensory epithelium consists of olfactory receptor cells, supporting cells, basal cells, goblet cells, mucous cell and white cell. Olfactory receptor cells are of two types microvillous and ciliated. In *C. punctata*, olfactory bulb is sessile and is attached to the telencephalon. Cells of olfactory bulb are organized in four concentric layers. Outer layer is olfactory nerve layer formed by axons of olfactory receptor cells. Inner to it is glomerular layer where axons of olfactory receptor cells synapse with dendrites of mitral cells. Next towards deeper part is mitral cell layer which comprises larger multipolar mitral cells. Central core is formed by granular cell layer with small granular cells.

**Keywords** *Channa punctata*, Olfactory Rosette, Olfactory Bulb, Olfactory Epithelium

## 1. Introduction

The olfactory system brings about responses to a mass of different stimuli vital for the interaction of an organism with its surrounding environment as well as with congener. The stimuli are identified by receptor cells with odorant receptors and the information is transmitted to the olfactory bulb [1]. Number of investigators has been earlier described the structure of the olfactory organ in the teleostean fishes. The teleostean olfactory organ exhibits various variations due to differences in habit and habitat. The structure of the olfactory epithelium in fishes have been investigated by several researchers revealing remarkable diversity exist regarding the shape, number, and arrangement of the olfactory lamellae, the distribution of sensory and non-sensory epithelium as well as variations in olfactory receptor cells in different teleosts [2-7]. Surface ultrastructure of olfactory epithelium although studied in *C. punctata* [4], cellular ultrastructure of olfactory epithelium and olfactory bulb is not yet explored. The present study is an attempt to examine the histology with the help of light microscope (LM) and transmission electron microscopic (TEM) structure of the olfactory epithelium and olfactory bulb of spotted snake head *C. punctata*.

## 2. Materials and Methods

### Collection of Fishes

Adult *C. punctata* fish of both sexes weighing about 200 gm- 250 gm are collected from the natural water bodies in and around Nagpur city. They were brought to the laboratory and acclimatized in the well aerated glass aquaria for 15 days. Fishes were cared and treated in accordance with protocol of Institutional Animal Ethics Committee (IAEC), Post Graduate Teaching Department of Zoology, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (Registration no. 478/01/a/CPC SEA).

### Light Microscopy

For Histological studies, the fishes were anesthetized with 2-phenoxyethanol and sacrificed. Olfactory organs were dissected out and fixed in aqueous Bouin's fixative for 24 hrs. Tissues fixed in the Bouin's fixative were transferred to 70% ethanol and dehydrated in ascending graded series of ethanol, cleared in xylene and embedded in paraffin wax. Sections of olfactory rosette and olfactory bulb were cut on rotary microtome (model- RMT-30) at 8µm thickness in transverse as well as in lateral planes and stained with hematoxyline and eosine (double staining), and Niss'l staining technique [8].

### Transmission Electron Microscopy

The fish were anesthetized with 2-phenoxy ethanol. The olfactory rosette was perfused with 0.1M phosphate buffer (PB) (pH 7.4). The rosettes were then carefully dissected out and primarily fixed in cold 2.5% glutaraldehyde containing 2% paraformaldehyde in 0.1M PB (pH 7.4). The tissues were washed in PB and post fixed in 1% osmium tetroxide for 2 hrs at 4<sup>0</sup>C. After post fixation, the tissues were washed in PB, dehydrated through ascending grades of ethanol, cleared in toluene and embedded in epoxy resin. Semi-thin sections of 1µm were stained with toluidine blue and examined by light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and observed under transmission electron microscope (TEM-Morgani) at Sophisticated Advanced Instrument Facility (SAIF), All India Institutes of Medical Sciences, New Delhi (India).

## 3. Result

Olfactory system of *C. punctata* comprises of paired olfactory rosette, olfactory nerve, and olfactory bulb (Fig. 1a and 1b). In *C. punctata*, olfactory rosette are paired and are situated dorso-laterally on the snout anterior to the eyes. Each organ present in the olfactory chambers that has two

separate apertures through which water enters and leaves. Olfactory rosettes of *C. punctata* have numerous lamellae in parallel arrangement running in rostro-caudal direction (Fig.2a). These lamellae's acquire fibers from their proximal end and extend into olfactory nerve (Fig. 2a). Olfactory nerve is long and caudally connected to the olfactory bulb (Fig.2a). Olfactory nerve runs posterior and peripherally penetrates the olfactory bulb (Fig. 1a). In *C. punctata*, sensory region is at basal and middle part and non sensory region is at proximal area of olfactory lamellae (Fig.2a and 2b). Sensory epithelium consists of olfactory receptor cell (ORCs). Ciliated olfactory receptor cells (cORCs) which are columnar and bipolar cells bearing a cell body. A long dendrite with 7-8 long cilia arises towards outer side. cORCs bears elongated nucleus with denser nucleoplasm and nucleoli. Long axonal process arises from cyton and runs toward the basal lamina (Fig. 3). Microvillous olfactory receptor cells (mORCs) are also columnar but without cilia. mORCs have round nucleus and have olfactory knob (Fig. 5). Basal cells are small, oval in shape with a prominent nucleus lying in deeper part of the epithelium just above the basal lamina (Fig. 4 and 6). In basal cell, nucleus is quite large and occupies maximum area leaving very little space for cytoplasm. Mucous cells are large, oval glandular cells, found along with sensory and non-sensory epithelium (Fig. 5). Supporting cells are elliptical to columnar in shape without dendrite and axonal process (Fig. 4). Supporting cell show rounded nucleus and dense nucleoplasm (Fig 4.). Associated with nucleus, endoplasmic reticulum is observed (Fig.6)

In *C. punctata*, goblet cells are clearly identified (Fig.3). These cells are filled with Golgi complex and rough endoplasmic reticulum. White cell is seen in the olfactory epithelium of *C. punctata* (Fig.5). The sessile olfactory bulb shows four concentric layers. Olfactory Nerve Layer (ONL) is formed of axons of ORCs of *C. punctata*. Underneath ONL, glomerular layer (GL) manifest synapses of axons of ORCs with dendrites of mitral cells (MC) (Fig.9). MC are large sized cells with darkly stained nucleus which in turn form mitral cell layer (MCL) towards inner side (Fig.10). MC are multipolar cells and clearly show numerous dendrites arising from its cyton towards GL. Nucleus of mitral cell is triangular with dense nucleoplasm and nucleoli (Fig.10). In MCL, along with mitral cells smaller glial cells too are observed (Fig.11). In the centre, densely packed smaller granular cells (GC), are present which form granular cell layer (GCL) (Fig.7). GC are bipolar cells and its nucleus occupies maximum part of cell body (Fig.10 and 11). GCL along with granular cell show glial cells (Fig.11).

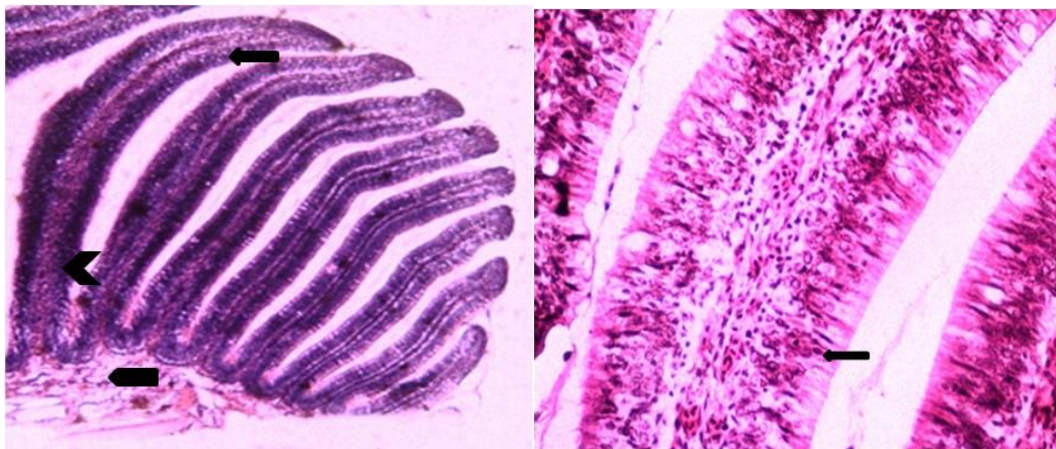
In the olfactory bulb of *C. punctata*, on ventro-medial and dorso-medial side, giant cells of the NT have been identified (Fig.8). NT cells exhibit intense Niss'l staining (Fig.8).



1a

1b

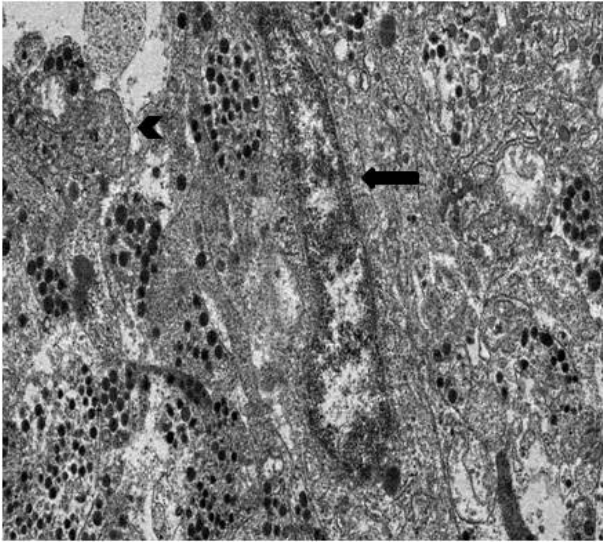
**Figure 1.** (a and b) Dissecting head of *Channa punctata* showing olfactory rosette OR (Arrow), olfactory nerve ON (Arrow head), and sessile olfactory bulb OB (Pentagon)



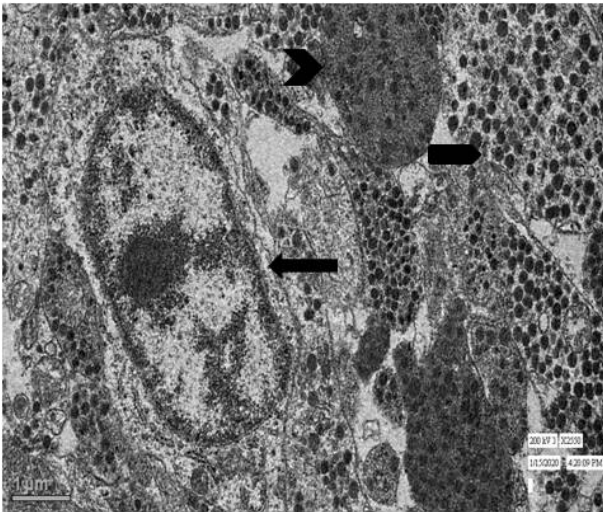
2a

2b

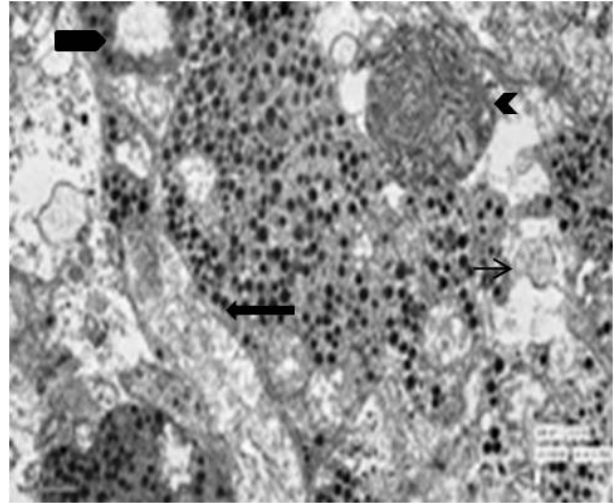
**Figure 2.** a) TS of Olfactory lamellae of *C. punctata* showing sensory epithelium (Arrowhead), non sensory epithelium (Arrow), and fibers (pentagon) : HE 100 X b) TS of sensory olfactory epithelium of *C. punctata* showing olfactory receptor neurons (arrow) HE 400 X



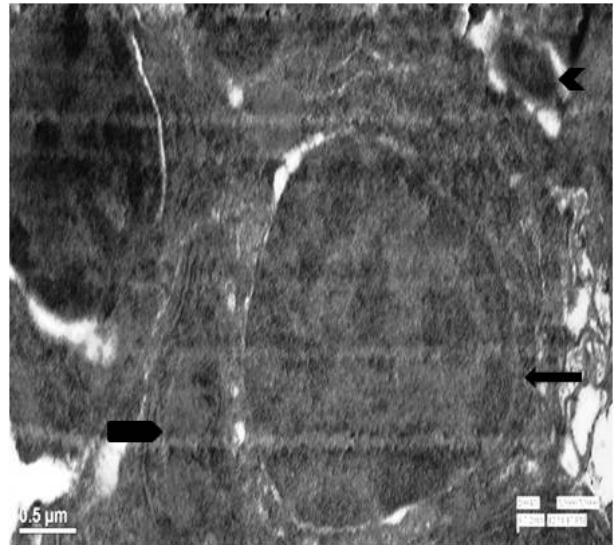
**Figure 3.** TEM photograph of sensory epithelium showing ciliated olfactory receptor cell cORC (arrow), and goblet cell (arrowhead) 18.1 KX



**Figure 4.** TEM photograph of sensory epithelium showing basal cell BC (arrow), supporting cell SC (arrow head), and mucous secreting cells (pentagon) 14.5 KX

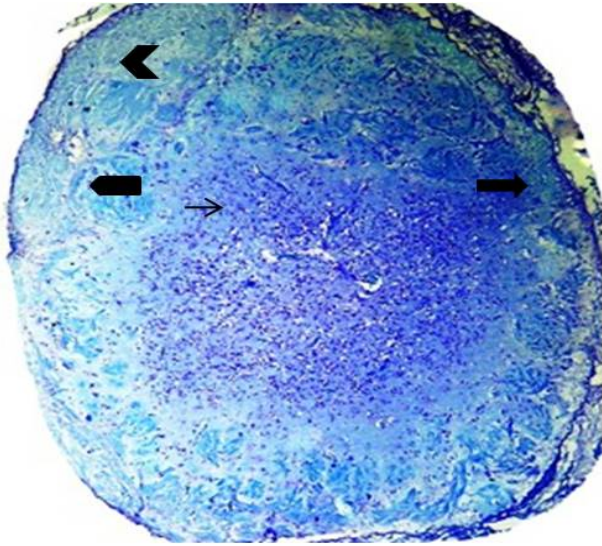


**Figure 5.** TEM photograph of sensory epithelium showing Microvillous olfactory receptor cells mORC (arrow), Golgi apparatus (arrow head), White cell (pentagon), and cistern (thin arrow) 14.5 KX

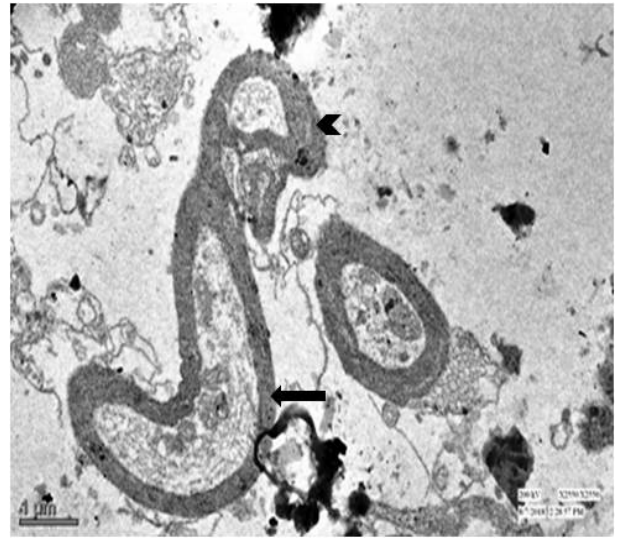


**Figure 6.** TEM photograph of sensory epithelium showing large nucleus of basal cell (arrow), chromatin lump (arrow head), and rough endoplasmic reticulum RER (pentagon) 4.5 KX

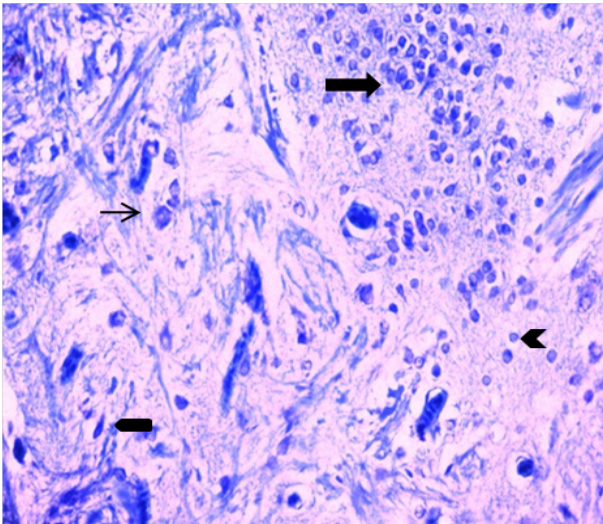




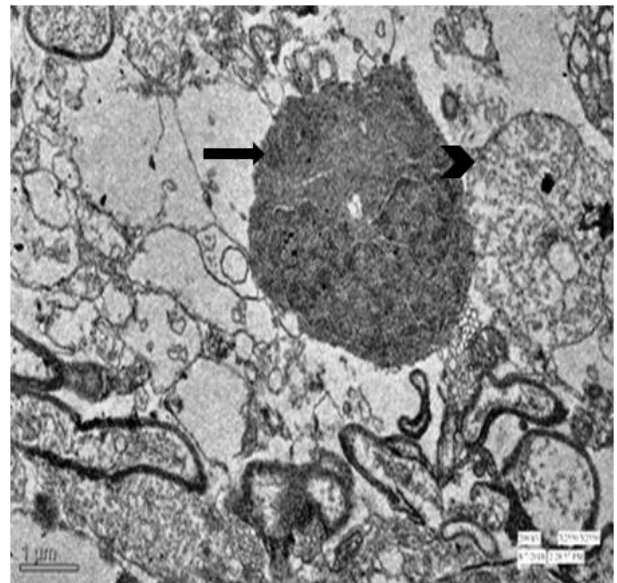
**Figure 7.** Transverse section of the olfactory bulb of *C. punctata* showing olfactory nerve layer ONL (arrow), Glomerular layer GL (arrow head), granular cell layer GCL (pentagon), and mitral cell layer MCL (thin arrow), Kluver and Barrera, 100X



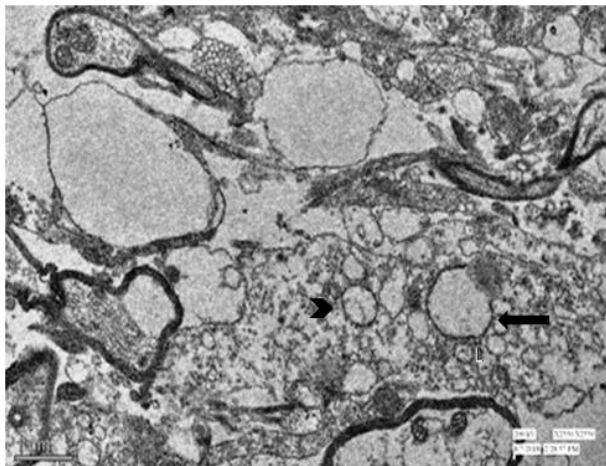
**Figure 9.** TEM photograph of olfactory bulb showing glomerular layer where axons of outer nerve layer ONL (arrow), synapse with dendrites of mitral cell (arrow head). 10 KX



**Figure 8.** Transverse section of the olfactory bulb of *C. punctata* showing mitral cells MC (arrow), granular cells GC (arrow head), Glial cells (pentagon), and bigger sized neurons of nervous terminalis NT (thin arrow), Kluver and Barrera, 400X



**Figure 10.** TEM photograph of olfactory bulb showing Mitral cell MC (arrow) and Glomerular cell GC (arrow head). 30 KX



**Figure 11.** TEM photograph of olfactory bulb showing granular cell (arrow) and Glial cell (arrow head). 15.5KX

#### 4. Discussion

The olfactory apparatus is one of the important chemosensory organs of fish [9]. This organ analyzes the chemical nature of the surroundings environment by reception of different chemical cues. In *C. punctata*, olfactory rosette is oval in shape peculiarly do not show central raphe in olfactory rosette. Similar structure is also observed in *Channa striata* [10] and *Channa gachua* [11]. In *C. punctata*, olfactory rosette is paired and situated dorso-laterally on the snout anterior to the eyes. Each organ present in the olfactory chambers that has two separate apertures through which water enters and leaves. Olfactory rosettes of *C. punctata* have numerous lamellae in parallel arrangement running in rostro-caudal direction. In *Labeo rohita* [5], *N. notopterus* [11] and in other teleosts [12] olfactory lamellae are radiating outward from central raphe. These lamellae acquire fibers from their proximal end and extend into olfactory nerve. In *C. punctata*, olfactory epithelium is folded. Each lamella is crescentic in shape and bears linguiform process along its concave margin as in *Catla catla* [13] and in *Puntius sarana* [14]. Folding on the olfactory epithelium of lamellae increases the surface area of epithelium as well as sensitivity and efficiency of olfactory system [2,11,14,15]. These perspectives are supported by our study as olfactory rosette in *C. punctata* is multi lamellar rosette which may provide more surface area for binding of odorants and sensory activity.

In *C. punctata*, sensory region is at basal and middle part and non sensory region is at proximal area of olfactory lamellae as in *C. striata* [10] and *C. gachua* [11]. However, in the cyprinid *L. rohita*, sensory region at the middle of lamella and non sensory region is at the proximal and basal region of lamella [16]. In *Rhodeus amarus*, sensory region is at the base and middle of lamella and non sensory region at the proximal end [17]. In *Heteropneustes fossilis*, sensory region occupies middle of the lamellae [18].

In *C. punctata*, sensory epithelium consists of olfactory receptor cell (ORCs). Ciliated olfactory receptor cells (cORCs) which are columnar and bipolar cells bearing a cell body. cORCs bears elongated nucleus with denser nucleoplasm and nucleoli. Long axonal process arises from cyton and runs toward the basal lamina. Microvillous olfactory receptor cells (mORCs) are also columnar but without cilia. mORCs have round shape nucleus and have olfactory knob. cORCs and mORCs in sensory epithelium of olfactory rosette demonstrated in several teleosts [10,16,19 20, 21]. Between the basal and supporting cells, white cells and goblet cells are seen in the middle of olfactory epithelium in *C. punctata*, similar findings are observed in *H. fossilis* [22] and *C. striata* [10]. cORCs ontogenetically precedes the mORCs [23], but according to some, cORCs and mORCs develop from identical stem cells [24,25]. Dense gathering of Golgi complex in receptor cells in *C. punctata* indicates secretory nature of receptor neurons. Presence of stimulatory neuropeptide, GnRH in the olfactory receptor cells and their projections to the olfactory bulb is revealed in *Cirrhinus mrigala* [26] suggesting the role of olfactory receptor cells in transduction of environmental cues and further transmitting it through brain-pituitary-gonadal axis. In the present study, ORCs are also characterized in *C. punctata* which may have a role in signal transduction in further areas of brain. Similar findings are explained by [10,21,27].

Basal cells are small, oval in shape with a prominent nucleus lying in deeper part of the epithelium just above the basal lamina. In basal cell, nucleus is quite large and occupies maximum area leaving very little space for cytoplasm. Mucous cells are large, oval glandular cells, found along with sensory and non-sensory epithelium. Supporting cells are elliptical to columnar in shape without dendrite and axonal process. Supporting cell show rounded nucleus and dense nucleoplasm. Associated with nucleus, endoplasmic reticulum is observed. Besides ER, Golgi complex with its parallel arranged cisternae is observed. Presence of large number of vesicles and Golgi complex suggest the secretory nature of f supporting cell. Similar findings reported by [10,15,20,28]. Mitochondria could not be observed indicating slow metabolic activities in supporting cells.

In *C. punctata*, olfactory epithelium shows goblet cells filled with Golgi complex and rough endoplasmic reticulum. Secretion of goblet cells helps in facilitating the odorant removal [10, 29]. *C. punctata* is a bottom dweller fish prefer to live in the mud. Secretion of goblet cell may help in decreasing the friction of water in the chamber as well as protecting the epithelium from coming in contact with the hazardous material to some extent [22]. White cell is reported in the neuroepithelium of rainbow trout [30], and in olfactory epithelium of *H. fossilis* [20]. Similar cell is seen in the olfactory epithelium of *C. punctata*. Basal cells in the fish under study are present towards the basal

region just above the basal lamina. These cells work as stem cells for regeneration of lost or damaged non-sensory and goblet cells [24, 25]. These cells regenerate the olfactory epithelium and are characterized by short life span according to [12, 19]. The ORCs may be replaced throughout the life by these progenitor basal cells. This view is supported by presence of rough endoplasmic reticulum in the cytoplasm of these cells.

ONL is formed of axons of ORCs of *C. punctata* as in *L. rohita* [31], *H. fossilis* [18], *N. notopterus* [11]. GL is area where fibers of ONL synapse with dendrites of MCs. Glomeruli of GL are histologically distinct units that serve as the basic modules in the information processing and as a relay station to several higher brain areas [32].

Multipolar, large sized MCs with triangular nucleus form MCL. Axons of mitral cells originate in the basal part of the soma, become myelinated after some distance [33] and project in the medial and lateral olfactory tracts [34]. They terminate on various telencephalic areas [35].

In the olfactory bulb of *C. punctata* on ventro-medial and dorso-medial side, giant cells of the NT have been identified. These ganglion cells are also noted in *Ictalurus punctatus* [36], *C. batrachus* [37], *C. mrigala* [26], *L. rohita* [16]. NT axons travel caudally via medial olfactory tract (MOT) and project to the retina, various brain areas and pineal organ suggesting their role in reproductive behavior, conduction of sex related olfactory stimuli and modulation of visual inputs to the brain [26,38].

## 5. Conclusions

In *C. punctata*, the olfactory organs are placed in the snout and are paired. The olfactory organs include olfactory rosette, olfactory nerve and olfactory bulb. Olfactory rosette has olfactory lamellae arranged in parallel fashion. The olfactory rosette is oval in shape peculiarly do not show central raphe in olfactory rosette. In TEM study, sensory region of the olfactory epithelium shows cORCs with bipolar neurons. Nucleus of ORCs is elongated. In the cell body, rough endoplasmic reticulum, and mitochondria are aggregated at the apical region. Mucous secreting goblet cells are distributed in the surface area of both sensory and non sensory regions of olfactory epithelium. The olfactory bulb is sessile. Olfactory nerve is long and caudally connected to olfactory bulb. Olfactory bulb concentrically possesses four layers, ONL, GL, MCL and GCL.

## Acknowledgements

Authors feel grateful to the Department of Zoology Rashtrasant Tukadoji Maharaj Nagpur University Campus, Nagpur and All India Institute of Medical Science, New Delhi for providing facilities of transmission electron microscopy to pursue this work.

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# Assessment of water quality status of Chichtola Lake in Gondia District of Maharashtra State, India

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## Manuscript Details

Received :25.11.2020

Accepted: 13.12.2020

Published: 16.12.2020

Available online on <https://www.irjse.in>

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

## Cite this article as:

Gorghate Nilesh D, Raut Mahendra B and Ingale Prashant P. Assessment of water quality status of Chichtola Lake in Gondia District of Maharashtra State, India, *Int. Res. Journal of Science & Engineering*, 2020, Volume 8(6): 235-240.



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## Abstract

Wetlands, the vigorous water filled inland aquatic systems perform variety of functions like provide irrigation, fisheries and recreation resource etc., Assessment of water quality is an important criterion for determining the suitability of water for irrigation, fishing and drinking purpose. The present study deals with the seasonal physicochemical investigation of water of the Chichtola lake, district Gondia of Maharashtra State during the year 2018-20. The physic-chemical parameters such as Temperature, pH, Conductivity, Transparency, Dissolved Oxygen DO, CO<sub>2</sub>, Biological Oxygen Demand BOD, Chemical Oxygen Demand COD, Phosphate and Nitrate were studied at three sampling sites of the lake during the study period. The analysis of various parameters carried out by using standard methods (APHA and NEERI). Regular monitoring of water quality parameters can help to conserve freshwater ecosystem.

**Keywords:** Chichtola lake, Conservation, Freshwater ecosystem

## 1. Introduction

Water contamination is becoming the most serious threats to human health. It has been estimated that about 80% of all the diseases in mankind are due to one or another unhealthy aspects of water. Contamination of lakes and other reservoirs is seen as one of the commonly occurring phenomenon in almost all developing nation, especially urban ones, due to demographic expansion coupled with lack of civic amenities results in hitting these natural water reservoirs very hard. Majority of the urban and rural lakes have vanished due to this human neglect and the others which could sustain this

pressure, present non-potable water or are not able to meet human requirements [1,2,3].

Conservation of Biodiversity has emerged as key environmental concerns of the day [4]. Water is the most abundant and most useful compound in the world and hence it is called "Jeevan" in Sanskrit or life. Life is not possible without water, the 2/3<sup>rd</sup> mass of our body is water and 70% surface of the earth is covered by water [5]. Water of good quality is required for living organisms. The quality of water is described according to its physical, chemical and biological parameters. The water quality assessments are used to detect the effects of pollution on the water quality. Changes in the water quality are reflected in the biotic community structure. Biological production in any aquatic body gives direct correlation with its physicochemical status which can be used as trophic status and fisheries resources potential [6]. The physical and chemical parameters exert their influence both, individually and collectively and their interaction creates a biotic environment, which ultimately conditions the origin, development and finally succession of the biotic communities [7].

Present study deals with a Chichtola lake which is situated in Gondia district of Maharashtra State, India. The lake is situated on the periphery of Nagzira Wildlife

Sanctuary near Chichtola village at coordinates N 21.202600° and E 80.098697°. In the present study the attempt was made to analyze the physicochemical properties (Temperature, pH, Conductivity, Transparency, DO, CO<sub>2</sub>, BOD, COD, Phosphate and Nitrate) from 3 different sites of Chichtola lake to understand the status of water quality from the month of October 2018 to September 2020.

## 2. Materials and Method

Eastern site of the lake has named as site I (S1) where anthropogenic activities like washed cloths, bullock cart and other vehicles cleaning, dirt from washed cloths, idol immersion and animal washing activities were commonly seen at this site. The western side of the lake has named as site II (S2) of the lake. Minimum human activities and disturbances were seen at S2. Site III (S3) of Chichtola lake is at northern side towards the catchment of the lake. The water samples were collected fortnightly in clean glass bottles of various sizes from the water surface of study sites.

In the present study sampling programme were started in the month of October 2018 to September 2020. Sampling was done in the morning hours from 8.30 am to 10.00 am.



Fig 1- Google Map of Chichtola Lake

Water sample were collected from three sites of the lake in fresh unsullied plastic bottles and brought to the laboratory for analysis of physico-chemical parameters by standard methods.

The parameters like temperature, pH and conductivity were measured on the spot during the study with the help of water analysis kit Systronics model-371 at the sampling sites. For the dissolved oxygen, the water sample was taken in 300 ml. capacity of BOD bottle and fixed the DO on the spot. Measurement of transparency was done by Secchi disc. The results were calculated as per the standard formulas and methods suggested by APHA [8] NEERI [9,10].

### 3. Results and Discussion

In the present study water quality assessment of Chichtola lake were analyzed. The mean with standard error value of all physico-chemical parameters of water sample collected from all three sampling sites are presented in table 1 and table 2. The temperature at all the sampling sites range between  $21.59 \pm 0.39$  to  $28.07 \pm 1.10$ . Similar observations reported by Punam [11] with lowest water temperature  $24.66 \pm 1.23$  during winter season and highest water temperature was  $30.99 \pm 3.75$  during summer in Chandpur lake of district Bhandara, Maharashtra. In the current investigation pH value of all sites under study were slightly alkaline throughout study period which ranges from  $7.10 \pm 0.05$  –  $7.78 \pm 0.08$ . Similar observations were reported by Bhaskar [12] with minimum pH value  $7.10 \pm 0.88$  during winter season and maximum  $7.84 \pm 0.43$  during summer season in Shionibandh lake of district Bhandara, Maharashtra. During the present study the conductivity values were differ from  $0.18 \pm 0.01$  during winter season and  $0.34 \pm 0.01$  during summer season. Acharjee et al., [13] also observed the similar observations in Dighali Lake of Assam. In the present investigation minimum transparency was observed during monsoon season however maximum transparency was recorded during summer season at all sites. Average transparency value

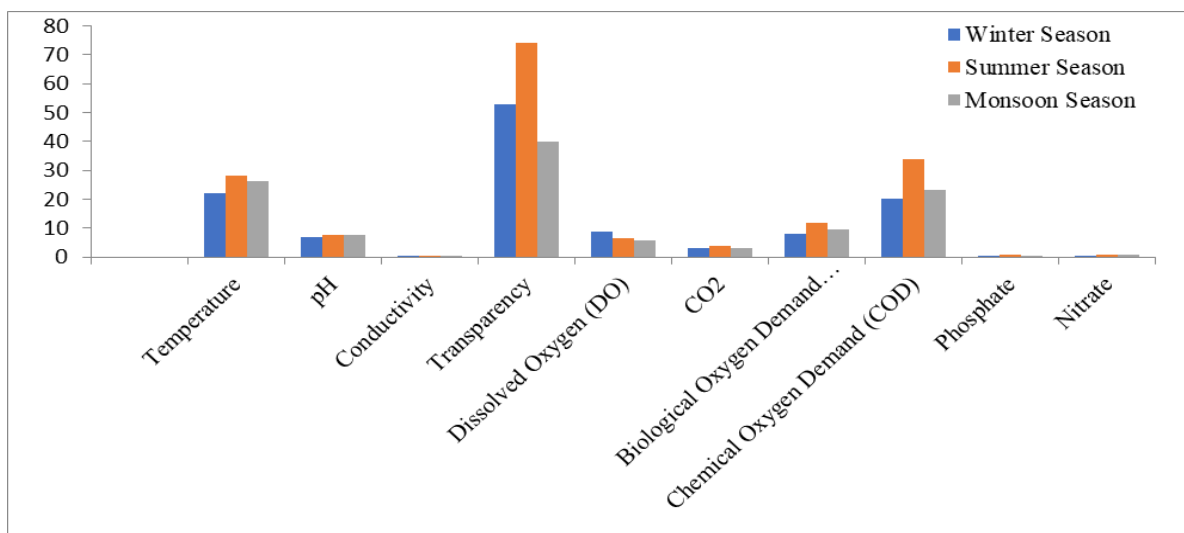
fluctuates from  $74.26 \pm 2.61$  during summer season to  $39.79 \pm 1.63$  during Monsoon season. During the study period the minimum mean values of free Carbon dioxide ( $\text{CO}_2$ ) differ from  $3.03 \pm 0.11$  during Monsoon season to  $3.93 \pm 0.16$  during summer season. Koli et al., [14] observed the  $\text{CO}_2$  ranged in between 1.89 to 5.98 mg/lit. The minimum  $\text{CO}_2$  observed in monsoon and maximum was during summer season in Tulashi tank, Kolhapur district. During the study period, the minimum mean values of BOD differ from  $8.20 \pm 0.42$  during winter season and maximum  $12.72 \pm 0.24$  during summer season. Higher BOD values in summer may be due to organic load from some agricultural activities at the mouth of the lake towards its catchment and reduced water flow. Udayashankara et al., [15] observed the BOD from Lingambudhi lake water ranged in between 5.9 to 25.9 mg/lit. Corroborative results presented by Khiradkar et al. [16] from Labhansarad Dam in Warora Taluka of Chandrapur District, Maharashtra State, India. During the study period the mean values of COD varied from  $21.15 \pm 0.44$  during winter season to  $34.72 \pm 1.19$  during summer season. In the present investigation the maximum value of COD was recorded during the summer season from Site I, it might be due to the domestic and agricultural and other anthropogenic activities from nearby areas. During the study period the mean values of Phosphate was differ from  $0.35 \pm 0.02$  during winter season to  $0.92 \pm 0.03$  during summer season. In the present investigation the lower value were recorded during winter season might be due to rapid utilization by aquatic plants and also due to assimilation by phytoplankton while summer maximum may be due to low water level and inflow of agricultural runoff from summer paddy cultivation in some patches at the catchment area. The lower values of Nitrates were recorded during the winter season at all sites whereas the higher values of Nitrates were recorded during monsoon seasons. During study period the mean values of Nitrate were varied from  $0.57 \pm 0.05$  during winter season to  $1.00 \pm 0.03$  during monsoon season. Analogous findings by Ingale et al [17] from Bhiwapur lake.

**Table 1-** Seasonal Mean Variations of Physico-chemical Parameters in Chichtola Lake in the Year 2018-2019.

S. N.	Parameters	Winter Season (Oct- Nov-Dec- Jan)	Summer Season (Feb- March- April- May)	Monsoon Season (June- July- Aug- Sept)
1	Temperature	22.00±0.49	28.07±1.10	26.21±0.72
2	pH	7.10±0.05	7.49±0.10	7.67±0.08
3	Conductivity	0.18±0.01	0.32±0.01	0.23±0.01
4	Transparency	52.98±1.01	74.24±2.61	39.79±1.63
5	Dissolved Oxygen (DO)	8.70±0.18	6.52±0.20	5.64±0.13
6	CO <sub>2</sub>	3.12±0.13	3.87±0.16	3.03±0.11
7	Biological Oxygen Demand (BOD)	8.20±0.42	11.83±0.30	9.39±0.26
8	Chemical Oxygen Demand (COD)	20.15±0.44	33.89±1.25	23.18±1.37
9	Phosphate	0.35±0.02	0.88±0.02	0.48±0.01
10	Nitrate	0.63±0.05	0.77±0.05	1.00±0.03

**Table 2-** Seasonal Mean Variations of Physico-chemical Parameters in Chichtola Lake in the Year 2019-2020.

S. N.	Parameters	Winter Season (Oct- Nov-Dec- Jan)	Summer Season (Feb- March- April- May)	Monsoon Season (June- July- Aug- Sept)
1	Temperature	21.59±0.39	27.59±1.12	26.09±0.66
2	pH	7.12±0.05	7.61±0.09	7.78±0.08
3	Conductivity	0.20±0.01	0.34±0.01	0.24±0.01
4	Transparency	53.00±1.01	74.26±2.61	41.84±1.55
5	Dissolved Oxygen (DO)	8.98±0.19	6.88±0.24	6.07±0.14
6	CO <sub>2</sub>	3.16±0.13	3.93±0.16	3.08±0.12
7	Biological Oxygen Demand (BOD)	9.37±0.33	12.72±0.24	9.31±0.27
8	Chemical Oxygen Demand (COD)	20.63±0.45	34.72±1.19	24.15±1.40
9	Phosphate	0.37±0.02	0.92±0.03	0.53±0.01
10	Nitrate	0.57±0.05	0.79±0.05	0.96±0.03

**Fig. 1-** Graph showing seasonal fluctuation of Physico-chemical Parameters in Chichtola Lake in the Year 2018-2019.



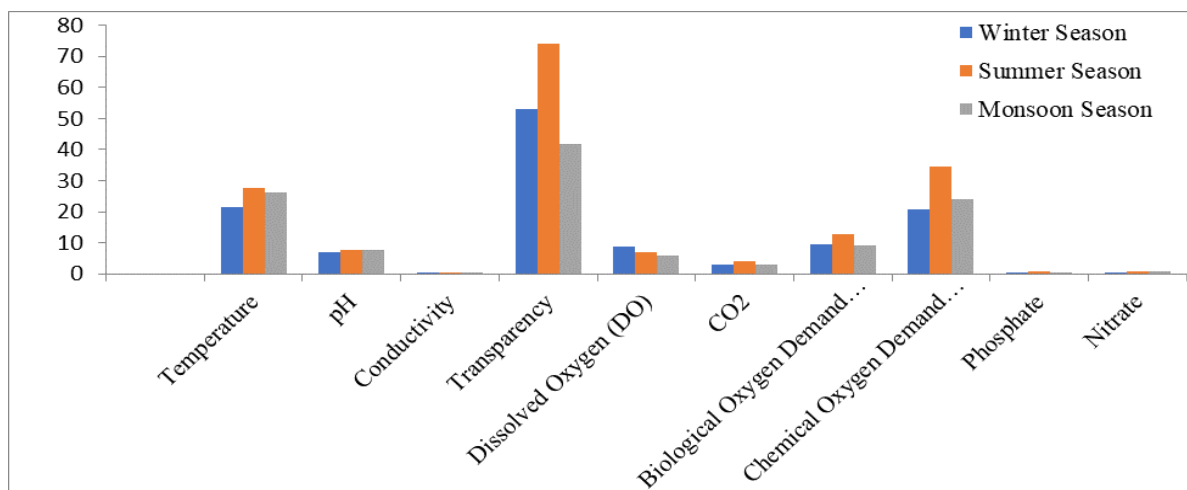


Fig. 2- Graph showing seasonal fluctuation of Physico-chemical Parameters in Chichtola Lake in the Year 2019-2020.

## 4. Conclusion

The Chichtola lake is most important for migratory birds in winter season and lake ecosystems can affect both fauna and flora. Site I of the lake has little polluted due to contamination by human and animal interventions, religious rituals and all anthropogenic activities. Overall the physico-chemical characteristics of lake show good quality water. Biodiversity contributes both.

### Conflict of interest

No conflict of interest influenced in this research.

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## KHAIRBANDHA DAM : A POTENTIAL HOTSPOT OF AVIFAUNAL DIVERSITY AND ITS SOCIOECONOMIC IMPACT ON LOCAL COMMUNITIES IN GONDIA DISTRICT, MAHARASTRA

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(Received 12 October 2019, Revised 23 March 2020, Accepted 12 April 2020)

**ABSTRACT :** Khairbandha Dam is rich in biodiversity and harbors wide variety of local birds and water fowls due to abundant food availability throughout the year in the form of insects, worms and weeds located in Gondia District. Field surveys were carried out in early morning and evening hours in order to observe the avifaunal biodiversity of in and around Khairbandha dam. The village tanks are primarily the source of irrigation. However, they also serve other multitude of functions. The ecosystem services rendered by the village tanks cannot be ignored. Khairbandha tank is considered as a major source of traditional irrigation not only for the poor living in Taluka Khairbandha village of district Gondia peoples, but also for wildlife enthusiast as it is considered as hotspot for the endangered migratory avifauna. The tanks obtain water from a range of sources such as rain-fed, river-fed, and rain-fed cascades. The khairbandha Tank is a part of our study area is due to its unique topographic characteristics as if it is surrounded by gradually undulating terrain mountain range and the impermeable rocky substratum. Our Survey indicates that the recorded avifauna diversity 120 birds belonging to 60 families .

Based upon the preliminary data socioeconomic aspect and ecological significance can be derived from this manmade reservoirs so their conservation and restoration is considered as the utmost urge to society and to common peoples. The village tanks are important for maintaining the groundwater level apart from these it can provide ecosystem services include habitat for the unique flora and avifauna.

**Key words :** Khairbandha tank, avifaunal diversity, ecosystem, conservation.

### INTRODUCTION

Khairbandhan Tank is located at latitude of 21°07'N and longitude 79°07'E considered as the fourth tank of Lakes of Gondia located in district Gondia of the state of Maharashtra, located at geographical centre of India lies on the Deccan plateau of the Indian Peninsula and has a mean altitude of 310.5 meters above sea level. Out of more than 9,000 bird species of the world, the Indian subcontinent contains 1,300 species or over 13% of the world's bird species (Grimmet *et al*, 2004):

The earlier studies on birds were undertaken by investigators like Newton *et al* (1986) and Ghosal (1995) listed birds of Kanha tiger reserve, Osmatston (1922) studied birds from Pachmarhi, Yardi *et al* (2004) reported birds from Salim Ali Lake, Aurangabad, Wadatkar and Kasambe (2002) studied birds of Pohara-Malkhed forest reserve, while Kulkarni *et al* (2005) studied birds in and around Nanded city of Maharashtra. Therefore, in this

context, in order to assess biodiversity of birds, the present investigation was undertaken to prepare a check list of avifaunal diversity of Futala Lake, Nagpur.

Almost all the species mentioned in the checklist were photographed. The study area was visited in morning, afternoon and evening time when the birds are most active. The scientific and local names were ascertained based on the key of Manakadan and Pittie (2001). A check list is prepared as per Abdulali (1981) and Gaikwad *et al* (1997).

### MATERIALS AND METHODS

In an attempt to record the avifaunal diversity regular surveys were conducted by visiting khairbandha tank. To check avifaunal diversity regular field visit was done. A visual encounter survey was conducted for direct count of the birds by walking along the bank of the site. The observation of the birds was carried out at early morning and evening hours by using field binocular Celestron®



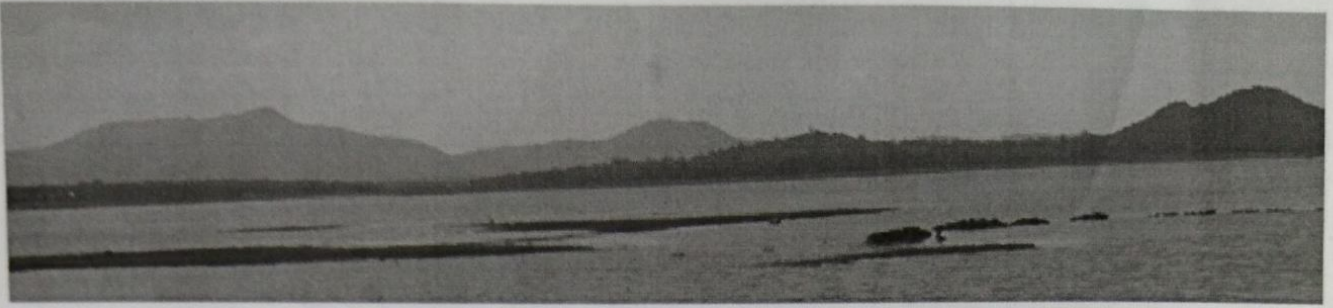


Fig.1 : An panoramic view of Khairbandha dam : A potential hotspot for avifaunal study.

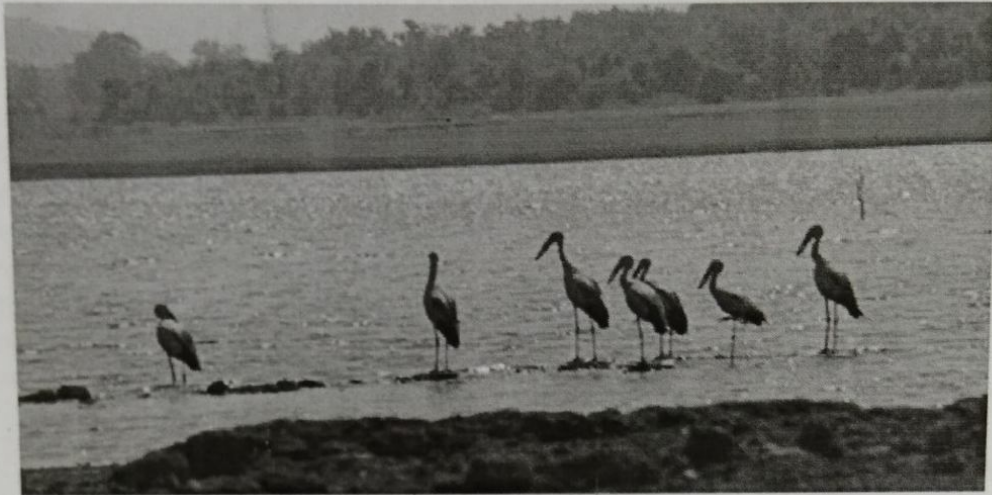


Fig. 2 : Flock of Open Bill stork.



Fig. 3 : Flock of red napped Ibis.

10 × 50 during the day time depending on the light conditions and suitable photographs were taken using Sony DSLR Camera Alpha 57.

#### OBSERVATION AND RESULTS

Our survey indicates that the recorded avifauna diversity enlist the record birds belonging to 60 families which includes Jungle crow, House Crow, Indian Treepie, Ashy woodswallow, Spangled drongo, White Bellied Drongo, Bronzed drongo, Black Drongo, Ashy drongo, Eurasian Golden Oriole, Jungle Myna, Bank Myna, Common Myna, Common Starling, Asian pied Starling, Rosy starling, Brahminy starling, Grey headed Starling

belonging to Sturnidae family, House sparrow, Yellow throated Sparrow, Black Breasted Weaver, Streaked Weaver, Baya weaver belonging to Passarinidae family, Red Munia, Green Munia, White Thorated Munia, White Rumped Munia, Black Thorated Munia, Spotted Munia belonging to Astrlididae, Common Rose Finch, Crimson browed Finch belonging to Fringelididae, Grey Necked Bunting, Ortolan Bunting, White Capped Bunting, Striolated Bunting, Red-headed Bunting, Black headed Bunting belonging to Amberizididae family has been reported. As a single representative family genus Oriental White eye has also been recorded in the survey. Among



sun birds purple rumped Sunbird, small sunbird, Purple sunbird, Little spider hunter, Streaked Sunbird, other representative family includes Desididae Thick billed flowerpecker, Tickell's Flower pecker, Plain Flowerpecker, Fire Breasted Flowerpecker. Spotted Creeper is only representative belongs to family Cythrididae. Other residential birds includes Black Kite, Baya, Weaver Bird, Common Myna Parrot, Common Hoopoe, Asian Koel, Black Shouldered Kite, Pariah Kite, Eagle, Golden Backed Woodpecker, Paddy Field Pipit, Red- Vented Bulbul, Indian Rock Owl, Common Swallow, Small Minivet, Shama, Bay-Backed Shrike, Wood Shrike, Black Naped Monarch Flycatcher, Small Minivet, Tailor-Bird, Scarlet Minivet, Honey Buzzard, Shikra. Rainfall in such water bodies act as "trigger" factor and greatly affects the availability of food just not only by replenishing swamps, producing edible aquatic plants for the birds to eat, but increasing anthropogenic activities, rising cement jungles, depletion of avifaunal habitat are causing hawk to avifaunal ecosystem and may lead to the destruction of this valuable stopover, if not managed properly. Our study will be helpful to obtain comprehensive information on breeding areas of residential birds while staging and wintering areas of migrants that are globally important for the protection of migratory birds. Hence such urban wetlands should be prioritized and its conservation values should be highlighted. A total of 60 family and 109 bird species was recorded in the study area which simply indicates a potential need of an hour to keep eyes on this hotspot of nature to conserve ecosystem for future.

### DISCUSSION

Chinchkhede and Kedar (2012) were counted 59 species of birds of which 45 were resident, 08 were winter migrants, 04 were local migrants and 02 were found to be summer migrants in Sringar lake, near Navegaon national park. With the fluctuation in weather decrease in species richness observed as it changes from colder to warmer. The minimum diversity was recorded in the months of monsoon due to heavy rain, increased flow of water, non availability of food and return of migratory

birds. In Nagpur Ambazari lake, Kedar (2012) was observed 135 species of birds out of which 105 species were recorded as resident, 17 species were resident migrant and 13 were winter migrant. In Bamanwada Lake of Rajura, Chilke (2012) was recorded 58 species of birds belonging to 9 orders and 29 families.

### ACKNOWLEDGEMENT

Author is indebted to all local peoples, who open heartedly made cooperation during survey in the field during study. We are thankful to Mr. Ganesh Madavi and Mr. Abhay Nagpure for extending field help to approach study location as if it is remotely situated. Author is also thankful to the Principal Dr. A. S. Dwivedi Sir of M.B Patel College of Art Commerce and Science, Sadak Arjuni.

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**TRUSS NETWORK SYSTEM AND DNA BARCODING AS PROMISING TOOLS FOR IDENTIFICATION, CONSERVATION AND MANAGEMENT PRACTICES IN FISHERIES: A REVIEW**

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**Abstract**

Freshwater fisheries in India have a great role for its nutritional value. Its general practice that, in market table size fishes were brought for trading from one region to another as per their availability. The concept of stock is usually separates the population into groups having different growth rates as well as reproductive similarities. Morphometric transformations between stocks of a species can be recognized as important tool for evaluation, conservation and management the population. Truss Network System (TNS) technique uses geometric morphometrics for variation in shapes. This can incorporated with the morphological character based scientific identification on primary level and secondly by DNA barcoding. This may help in preparation of conservation and management strategy for endangered fish species.

**Keywords:** Aquaculture, Conservation, TNS and DNA barcoding

**Introduction**

India is one among the 17 mega 'biodiversity hotspots' which contributes 60-70% of the world's biological resources. In India out of a total of 2,500 species of fish, 930 are in freshwaters and belonging to 326 genera, 99 families and 20 orders (Khobragade, 2016). Wetlands of India, estimated to be 58.2 million hectares, are important repositories of aquatic biodiversity of which Maharashtra has 284942 ha (Prasad *et al.*, 2002). River floodplains as well as other terrestrial water bodies are important for fish biodiversity and productivity (Pander, Mueller and Geist, 2015). It is estimated that 1,26,000 described species rely on freshwater habitats, including species of fishes, molluscs, reptiles, insects, plants, and mammals. In the aquatic ecosystem freshwater fishes comprise almost 45% of all fishes and freshwater molluscs about 25% of all molluscs (IUCN 2014).

Freshwater aquaculture has important role in the Indian economy and along with this it improves the nutritional level of rural people by several means (Pawar and Pawar, 2012). It is important in many aspects like food, employment, living standard and economical condition of the associated people (Waghmare and Baile, 2017). But in the rural region there is regular practice to capture juvenile or small size fishes. This results in indirect threat to the native fishes for their survival. Awareness can overcome the situation by educating them the proper management strategy. Fishermen traditional knowledge is moderate and fine to identify fishes locally.

The freshwater aquaculture is important in many aspects like food, employment, economy and living standard of the people associated with it. India is second-largest contributor to global aquaculture production in the world next to China (FAO, 2012). Fishes are rich source of protein, micronutrients and essential fatty acids. The involvement of fish as a food depends on availability, access and cultural and personal preferences (Beveridge *et al.*, 2013). Likewise Murrels (snakehead) and catfishes are economically important species having great potential for aquaculture and capture fisheries throughout southern and southeastern Asia (Haniffa, 2009).

The aquatic resources have huge potential towards the economic welfare of the nation. Taxonomy plays important role for understanding biodiversity and its conservation of species and subspecies. (Jena and Gopalakrishnan, 2012). For species identifications require observations skill and trainings. This become very easy now a days with the help of modern genetic techniques. Barcode identification may be most important in laboratory conditions, for the identification of derived products, so as to make faster detection of new species and for species tracing when used with nextgen sequencing (Fischer, 2013).

Freshwater fishes are expected to exhibit greater levels of genetic differentiation and population subdivision than marine species, due to the isolating nature of freshwater resources like lakes and rivers as well as small effective population sizes (John, Peter and Gopalakrishnan, 2013). Fish diversity are always subjected to severe anthropogenic stress which results in habitat degradation.

### Identification

On the basis of morphological characteristics fishes are identified traditionally. Fishes exhibits peculiar characters like the scales, lateral line, fins, fin rays, position of eyes, ratio of head to body, length and width etc. This help to categorize fishes into similar groups and taxon prepared. Now a days it become easier with most promising tool i.e. TNS providing Truss dimensions, which include components of body length and depth along the longitudinal axis and secondly DNA barcoding.

### TNS

Morphological characteristics have been universally used in fisheries to measure discreteness and relationships among various taxonomic studies (Turan, 1999). Use of TNS as a character set enforces systematic coverage across the form and can be given geometrical interpretations (Strauss and Bookstein, 1982). Truss network measurements are a sequences of distances calculated among landmarks which is forming a regular pattern of connected quadrilaterals or cells across the body form (Rawat *et al.*, 2017). It has been evolved as operational technique in stock conservation and management. The set of TNS dimensions i.e. criss-cross pattern sideways the body simplifies the detection of variances in shape in sloping, longitudinal and vertical directions (Park *et al.*, 2015).



### **DNA bar-coding**

DNA bar-coding has been recognized due to its efficacy in species identification. This method is used for fast and precise species identifications by aiming the analysis on a short standardized segment of the genome (Muchlisin *et al.*, 2013). Barcoding is the use of a standardized short region of DNA to confirm species identity, which is the CO1 region of mitochondrial DNA, typically for fish, with the generation of openly accessible and vastly comparable data (Fischer, 2013). It can be used to allocate a biological specimen to a species.

The approach of DNA based taxon identification can be used to identify fishes, fish eggs and larvae fish product as well as in resolving taxonomic doubt including discovery of new species (Jena and Gopalakrishnan, 2012). Genetic barcoding offers potential to identify animal species quickly from a small but unique, DNA sequence. Majority of unknown species in animal taxa with few taxonomic specialists, this may become the predominant method of discovering new species. (Pimm *et al.*, 2014).

In recent years, mtDNA, has been widely applied in systematics, population genetics and conservation biology of animals because of its fast evolution (John, Peter and Gopalakrishnan, 2013). Use of DNA sequence could impact the genetic structure of fish populations. It may contribute to the fragmentation of the genetic structure of fish communities (Khedkar *et al.*, 2014). DNA barcoding can play a very significant role in assessment and conservation of biodiversity prioritizing conservation strategies (Trivedi *et al.*, 2016).

### **Conservation management**

Excess and uncontrolled fishing practices or selected fishing in the lakes and river are undergoing in the areas of water bodies. Conservation of lake and pond ecosystems can be achieved by avoiding contamination and erosion (Bobdey, 2014). Fish biodiversity is the most treasured but minimum appreciated resource and there is a need of continuous monitoring and inventorization (Khobragade, 2016).

In aquacultural practices conservation of freshwater fauna, particularly the conservation of fishes is less accentuated (Anderson and Maldonado-Ocampo, 2011). Habitat degradation and overexploitation in fisheries could affect conservation aspects.

### **Summary and Conclusion**

Freshwater fishes are major and locally available source of food with good nutrition value. Sustainable utilization of the valuable biological resources is crucial need of today. Beside anthropogenic activities human interest in the aquatic resources is the major factor responsible for the destruction of biodiversity. The conservation of fish diversity requires strong efforts by good knowledge of identification, importance of biodiversity, conservation and scientific management practices with the supervision of expert. This can be achieved with using latest technological innovations. In this genetic tools like DNA barcoding has outstanding importance. Which ensure genetic database and help to achieve sustainable use of biodiversity. In TNS categorizing intraspecific stocks or units of a species having unique morphological characters

enables a better management species and ensures preservations of the resources. Designing the best aquaculture management and conservation practices for future can be done if needed for endangered species of fishes from the IUCN data.

#### **Acknowledgement:**

Thanks to the Head, Department of Zoology, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur for providing research facilities.

#### **Recommendations**

- ❖ Scientists, stakeholders and end users plays important role in fish conservation and management. Communication and their link is required to be strengthen for development of user-friendly fish identification tools.
- ❖ Training of taxonomic community may ensure stability in nomenclature and development of reliable diagnostic data in digital format at public domain.
- ❖ Research funding and infrastructure are the backbone and needs to be strengthened so as to prepare sustainable and conservational strategies by considering early life history stages of fishes.
- ❖ Collaboration and strong network of taxonomists with integration of methodological approaches will increase accuracy and cover large area in less duration.

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# Small period electromagnet undulator and effect of finite conductor width variation

Cite as: AIP Conference Proceedings 2142, 110036 (2019); <https://doi.org/10.1063/1.5122496>  
Published Online: 29 August 2019

Vijay Huse, Ramdas Huse, and Bramh Prakash



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# Small Period Electromagnet Undulator and Effect of Finite Conductor Width Variation

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**Abstract:** In this paper we study the design characteristics of the electromagnet undulator with analytical derived expressions. In electromagnet undulator a copper conductor sheet runs through ferromagnetic laminations in alternating directions. A current in copper sheet produces a periodic magnetic field. The effect of copper sheet is important for the magnetic field of the undulator. In our calculation we examine the important influence of finite spread copper conductor width on undulator radiation.

## INTRODUCTION

In recent year there exists interest in free electron lasers as a high power coherent radiation source to produce electromagnetic radiation at a desired wavelength. In such device a relativistic electron beam propagates along a transverse periodic magnetic field and transfers energy to a co-properties laser under appropriate resonance condition. The radiation wavelength depends on the undulator period and the energy of the relativistic electron beam for a fixed undulator gap. For short wavelength radiation, a highly relativistic electron beam and a short period undulator or both are required. For desktop FEL systems, electron beam energy on the order of MeV is required with short undulator periods in mm range. The undulator field may be either magnetostatic or electromagnetic electromagnet undulator or rf undulator. The magnetostatic undulator is fabricated by permanent magnet arranged according to Halbach rule. The electromagnet undulators are fabricated from a stack of ferromagnetic laminating cover. A conducting strip of copper foil runs between the laminations in alternating directions. A current flow through the conducting strip and a periodic magnetic field created in the gap. Also several configurations of rf undulators are reported for free electron laser applications with sub-GeV electron beam. In this paper we discuss the design consideration of an electromagnet undulator which has been reported in free electron laser experiments. In this scheme, a copper conducting strip runs between the ferromagnetic laminations in alternating directions. Two identical similar structures separated by an air gap define the undulator. The magnetic flux density is determined by the electric current and level of side leakage flux between the ferromagnetic regions.

## ELECTROMAGNET UNDULATOR RADIATION

The field of an small period electromagnet undulator is expressed as, at  $y=0$ ,

$$B_y = -\frac{2\mu_0 I}{\pi h} \frac{\sin(\pi h / \lambda_u)}{\sinh(\pi \delta / \lambda_u)} \sin(k_u z) \quad (1)$$

Where  $\mu_0$  is the permeability,  $I$  is the winding current,  $\delta$  is the undulator gap,  $h$  is the thickness of the copper, and  $\lambda_u$  is the period of the electromagnet undulator. Eq. (1) represent the field of electromagnet undulator, when is uniform along the undulator length. Now, Eq. (1) can be re-written as,

$$B_y = -B_0 b(h) \sin(k_u z) \quad (2)$$

Where  $B_0 = \frac{2\mu_0 I}{\lambda_u} \frac{1}{\sinh(\pi\delta/\lambda_u)}$  and  $b(h) = \frac{\sin(k_u h/2)}{k_u h/2}$

The trajectory of the electron is determined through Lorentz equation, this gives,

$$x = \frac{Kc}{\gamma\Omega_u} b(h) \sin(\Omega_u t) \quad (3)$$

where,  $K = \frac{eB_0}{m_0 c \Omega_u}$ ,  $\Omega_u = k_u c$ . The longitudinal electron trajectory  $\beta_z$  is determined from the consideration that  $\gamma$  is conserved, this gives,

$$\beta_z = \beta^* - \frac{K^2}{4\gamma^2} b^2(h) \cos(2\Omega_u t) \quad (4)$$

$$z = \beta^* ct - \frac{K^2 c}{8\gamma^2 \Omega_u} b^2(h) \sin(2\Omega_u t) \quad (5)$$

Where,  $\beta^* = 1 - \frac{1}{2\gamma^2} \left( 1 + \frac{K^2 b^2(h)}{2} \right)$

The radiation emitted by an electron moving in this electromagnet undulator is evaluated by Lienard-Wiechert potential. The L.W. potential is energy radiated per unit solid angle per unit frequency interval is given by,

$$\frac{d^2 I}{d\omega d\Omega} = \frac{e^2 \omega^2}{4\pi^2 c} \left| \int_0^\infty dt \left[ \hat{n} \times (\hat{n} \times \vec{\beta}) \right] \exp \left\{ i\omega \left( t - \frac{\hat{n} \cdot r}{c} \right) \right\} \right|^2 \quad (6)$$

Solving Eq. (6) we write the intensity as,

$$\frac{d^2 I}{d\omega d\Omega} = \frac{e^2 \omega^2 T^2}{4\pi^2 c} |T_x|^2 [\sin c(\nu/2)]^2 \quad (7)$$

where  $T_x = -\frac{Kb(h)}{2\gamma} [J_{m+1}(0, \xi) + J_{m-1}(0, \xi)]$ ,  $\xi = -\frac{K^2 \omega}{8\gamma^2 \Omega_u} b^2(h)$ ,  $\nu = 2N\pi \left( \frac{\omega}{\omega_1} - m \right)$  and  $\omega_1 = \frac{2\gamma^2 \Omega_u}{1 + \frac{K^2}{2} b^2(h)}$

Using  $\omega = m\omega_1$ , Eq. (6) can be written as,

$$\frac{d^2 I}{d\omega d\Omega} = \frac{4e^2 N^2 \gamma^2}{c} \frac{\frac{K^2}{4} b^2(h)}{\left( 1 + \frac{K^2}{2} b^2(h) \right)^2} [JJ]^2 (\sin c(\nu/2))^2 \quad (8)$$

where  $[JJ] = [J_{m+1}(0, -\xi_1) + J_{m-1}(0, -\xi_1)]$ ,  $\xi_1 = \frac{1}{4} \frac{K^2 b^2(h)}{1 + \frac{K^2}{2} b^2(h)}$ ,  $m = 1$

Eq. (8) is characterized by another dimension line shape function  $\text{sinc}^2(k_u h/2)$  where  $h$  is the copper width where current flows. This expression gives an important insight into the physics of electromagnet undulator radiation. A random variation in the size of the copper width can bring important modification to the radiated spectrum. We consider  $h = h_0 + \delta h$  where  $h_0$  is the resonant width of the copper conductor,  $\delta h$  is the width detuning. Consider width distribution modeled by a Gaussian distribution,

$$f(h) = \frac{1}{\sqrt{2\pi}\sigma_h} \exp\left(\frac{-\varepsilon_d^2}{2\sigma_h^2}\right) \quad (9)$$

Where  $\varepsilon_d = \delta h / h_0$ ,  $\sigma_h$  is the root mean square value of the conductor width variation. Using Eq. (8), we re-write Eq. (7) as,

$$\frac{d^2 I}{d\omega d\Omega} = \frac{4e^2 N^2 \gamma^2}{c} I(\omega) (\sin c(\nu/2))^2 \quad (10)$$

$$I(\omega) = \frac{\frac{K^2}{4} s(h)}{\left(1 + \frac{K^2}{2} s(h)\right)^2} [JJ]^2, \quad \xi_1 = \frac{\frac{K^2}{4} s(h)}{1 + \frac{K^2}{2} s(h)}$$

$$s(h) = \int_{-\infty}^{\infty} \frac{\sin^2(k_u h / 2)}{(k_u h / 2)^2} \frac{1}{\sqrt{2\pi}\sigma_h} \exp\left(\frac{-\varepsilon_d^2}{2\sigma_h^2}\right) d\varepsilon_d \quad (11)$$

Using the equation  $\sin^2(v/2) = 2 \int_0^1 dt(1-t)e^{ivt}$

$$\text{Eq. (11) is simplified to } s(h) = 2 \int_{-\infty}^{\infty} dt(1-t) \cos(bt) \exp(-a^2 t^2) \quad a = \frac{b^2 \sigma_h^2}{2}, \quad b = k_u h_0 \quad (12)$$

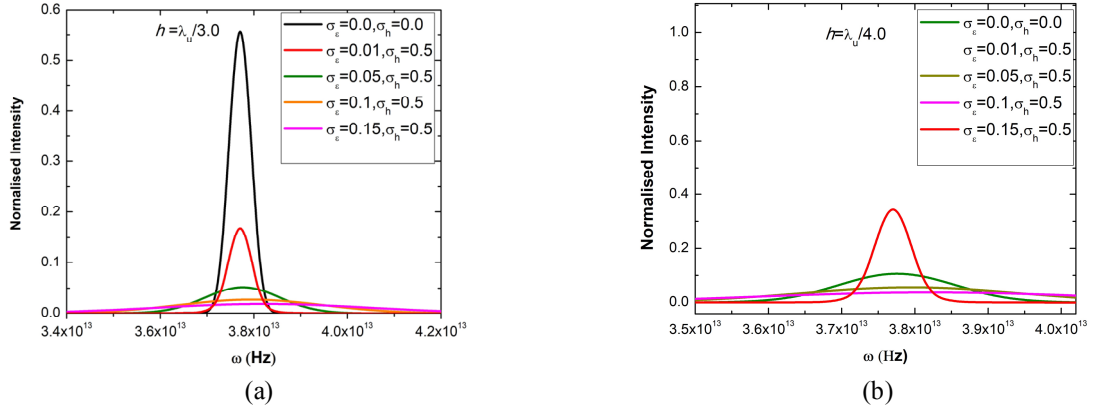
Eq. (12) is evaluated to read

$$s(h) = 1.0 - 0.33\bar{h}^2(1 + \sigma_h^2) + 0.266\bar{h}^4\sigma_h^2, \quad \bar{h} = \pi h / \lambda_u \quad (13)$$

## RESULTS & DISCUSSION

Eq. (12-13) accounts for the conductor width variation with a Gaussian width distribution. The analytical results have been obtained for two optimization models as shown in (Fig. 1) the intensity decrease in the  $h = \frac{\lambda_u}{3}$  in more in

the  $h = \frac{\lambda_u}{4}$  with  $\sigma_h$ .



**FIGURE 1.** (a) Normalised intensity versus frequency for  $h = \frac{\lambda_u}{3}$  and (b)  $h = \frac{\lambda_u}{4}$  with different energy parameter.

In Huse et al., it is derived that  $h = \frac{\lambda_u}{3}$  where it is assumed that the ferromagnetic core has infinite permeability.

In a recent model this limitation has been removed and the calculations are revisited for finite permeability approximation. The finite permeability calculation yields the conductor width as  $h = \frac{\lambda_u}{4}$ . These two calculations have important contributions. The former calculation resembles the hybrid undulator where as the later calculation resembles the permanent undulator geometry.

Let,  $s(v) = [\sin c(v/2)]^2$  by the effect of energy distribution the detuning parameter is written as,  $v = v_0 + \delta v_\varepsilon$

Where  $v_0 = v(\gamma = \gamma_0)$  and having energy distribution in the beam then spectral line shape distribution is given by,

$$s(v) = \int_{-\infty}^{\infty} d\varepsilon \left[ \frac{\sin(v/2)}{(v/2)} \right]^2 f(\varepsilon) \quad (14)$$



For Gaussian type distribution we can write  $f(\varepsilon) = \frac{1}{\sqrt{2\pi}\sigma_\varepsilon} \exp\left(\frac{-\varepsilon^2}{2\sigma_\varepsilon^2}\right)$

Where  $\sigma_\varepsilon$  is the r.m.s relative energy spread and  $\varepsilon = \frac{\delta\gamma}{\gamma}$ ,  $\delta\gamma = \gamma - \gamma_0$  where  $\gamma_0$  being the nominal energy of the electron beam.

## CONCLUSION

We successfully introduced the modifications in the radiation wavelength and intensity due to random error with Gaussian distribution function with analytical and tractable solution. The conductor width variation with a Gaussian width distribution of two optimization models in which the intensity decrease in the  $h = \lambda_u/3$  is more in the  $h = \lambda_u/4$  with  $\sigma_h$ .

## ACKNOWLEDGMENT

Author is thankful to Dr. Jeevakhan Hussain, NITTR, Bhopal, India for useful discussion on electromagnetic undulator.

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Cite as: AIP Conference Proceedings 2142, 110035 (2019); <https://doi.org/10.1063/1.5122495>  
Published Online: 29 August 2019

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# Beat Frequency Undulator Radiation in Electromagnetic Undulator

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**Abstract:** The spectrum undulator radiation of relativistic electron beam in a beat frequency undulator is analytically investigated and analyzed. The field configuration consists of two circularly polarized electromagnetic wave wigglers propagating collinearly in backward to the travelling relativistic electron beam. The electron motion consists of oscillations at the two wiggler frequencies and the beat frequency of the two wiggler frequencies. As a consequence there are higher harmonics emission on axis which is absent in the case of one frequency circularly polarized electromagnetic wave wiggler or magnetostatic helical wiggler scheme.

## INTRODUCTION

In recent years there exists interest in advanced design of wigglers and undulator for development of compact light source and free electron laser facilities [1]. The undulator radiation is tunable over a broad electromagnetic spectrum from microwave to x-ray range either by tuning the undulator gap or by tuning the relativistic beam energy. The polarization tunability of the undulator radiation is another important desirable figure of merit of the undulator quality that depends on the undulator field configuration. The particular choice of the undulator and associated technology ensures the user to optimize the desired spectral range and spectral characteristics. The planar undulator scheme produces radiation at a resonant wavelength and its odd harmonics on-axis. The circularly polarized helical undulator field is characterized by emission of radiation at the fundamental on axis. During the last several years the undulator technology has been improved substantially and number of new design concept have been formulated and proposed. The APPLE and DELTA structure are adjustable phase undulators [2-4] that produces radiation with variable polarization. The spectral features of two frequency undulator and undulator radiation has been reported [5-8]. The spectral properties of the two-frequency undulator radiation is characterized by emission at its two primary frequencies along with emission at sum–difference frequencies resulting from mutual interference. Alternatively works on bi-harmonic undulator have been reported for gain enhancement at a select higher harmonic [9-11]. In bi-harmonic undulator scheme, the on-axis magnetic field oscillates with same polarization but the auxiliary magnetic field is an integer multiples of primary undulator periods. This allows to superpose the radiations at a suitable higher harmonic resulting in a higher gain. All these schemes are based on the permanent magnet undulator technology and has its limitations in design of the short period undulator due to finite magnet size, bulky and complicated massive mechanical structure requirement. On the other hand free electron laser based on the electromagnetic wave wiggler has been studied [12]. In this paper we propose and investigated two frequency field configurations with two circularly polarized electromagnetic wave wigglers. The scheme is a field combination of two circularly polarized electromagnetic wave wiggler in a backward direction to the propagating relativistic electron beam. The scheme is analogous to the two frequency undulator [7] scheme is a unique advantageous scheme. The two frequency undulator based on permanent undulator magnet scheme was proposed but never fabricated due to complicated magnet structures complex spectral properties. The scheme is rich in harmonics due to electron motion modulating and generating sidebands that corresponds to the sum and difference frequencies of the two frequencies. In our scheme the sum frequency modulation of the electron motion disappears and electron motion modulation occurs at the beat frequency of the two frequencies thereby decreasing the harmonic content of the spectrum.

## UNDULATOR RADIATION

We consider the electron motion of a relativistic electron moving with a velocity in a field configuration consisting of two collinearly backward propagating circularly polarized electromagnetic wave wigglers. The magnetic field of the two-frequency wiggler is,

$$\begin{aligned}\vec{B}_{u_1}(z, t) &= B_{u_1} \left[ \hat{x} \cos(k_{u_1} z + \omega_{u_1} t) - \hat{y} \sin(k_{u_1} z + \omega_{u_1} t) \right] \\ \vec{B}_{u_2}(z, t) &= B_{u_2} \left[ \hat{x} \cos(k_{u_2} z + \omega_{u_2} t) - \hat{y} \sin(k_{u_2} z + \omega_{u_2} t) \right] \\ \vec{E}_{u_1}(z, t) &= \frac{\omega_{u_1}}{k_{u_1} c} B_{u_1} \left[ \hat{x} \sin(k_{u_1} z + \omega_{u_1} t) + \hat{y} \cos(k_{u_1} z + \omega_{u_1} t) \right] \\ \vec{E}_{u_2}(z, t) &= \frac{\omega_{u_2}}{k_{u_2} c} B_{u_2} \left[ \hat{x} \sin(k_{u_2} z + \omega_{u_2} t) + \hat{y} \cos(k_{u_2} z + \omega_{u_2} t) \right]\end{aligned}\quad (1)$$

In Eq.(1)  $B_{ui}$  denote the amplitude of the two wiggler fields,  $(\omega_{ui}, k_{ui})$  describe the frequency and the wave numbers of the two electromagnetic wave undulators,  $i = 1, 2$ .  $\hat{x}$ ,  $\hat{y}$  are the unit vectors. In cartesian coordinates assuming the  $\gamma$  is constant then electron motion is described by the Lorentz force and specified by the following equation,

$$\begin{aligned}\frac{d\beta_x}{dt} &= - \left( \frac{\Omega_{u_1}}{k_{u_1} c} (k_{u_1} v_z + \omega_{u_1}) \sin(k_{u_1} z + \omega_{u_1} t) + \frac{\Omega_{u_2}}{k_{u_2} c} (k_{u_2} v_z + \omega_{u_2}) \sin(k_{u_2} z + \omega_{u_2} t) \right) \\ \frac{d\beta_y}{dt} &= - \left( \frac{\Omega_{u_1}}{k_{u_1} c} (k_{u_1} v_z + \omega_{u_1}) \cos(k_{u_1} z + \omega_{u_1} t) + \frac{\Omega_{u_2}}{k_{u_2} c} (k_{u_2} v_z + \omega_{u_2}) \cos(k_{u_2} z + \omega_{u_2} t) \right)\end{aligned}\quad (2)$$

Where  $\Omega_{u_{1,2}} = \frac{eB_{u_{1,2}}}{m\gamma c}$ ,  $e$  is the charge on electron and the velocity component from Eq. (2) reads,

$$\begin{aligned}\beta_x &= \frac{K_1}{\gamma} \cos(\Omega_1 t) + \frac{K_2 \Omega_2}{\gamma} \cos(\Omega_2 t) \\ \beta_y &= - \left[ \frac{K_1 \Omega_1}{\gamma} \sin(\Omega_1 t) + \frac{K_2 \Omega_2}{\gamma} \sin(\Omega_2 t) \right]\end{aligned}\quad (3)$$

$K_1 = \frac{eB_{u_1} \lambda_{u_1}}{2\pi m c^2}$ ,  $K_2 = \frac{eB_{u_2} \lambda_{u_2}}{2\pi m c^2}$ ,  $\Omega_1 = k_{u_1} v_z + \omega_{u_1}$ ,  $\Omega_2 = k_{u_2} v_z + \omega_{u_2}$ . The longitudinal velocity component is calculated

from energy conservation rule i.e.  $\beta_z^2 = 1 - \frac{1}{\gamma^2} - [\beta_x^2 + \beta_y^2]$  and reads,

$$\beta_z^2 = \left\{ \begin{aligned} &1 - \frac{1}{\gamma^2} - \frac{K_1^2 \Omega_1^2}{\gamma^2} \cos^2(\Omega_1 t) - \frac{K_2^2 \Omega_2^2}{\gamma^2} \cos^2(\Omega_2 t) - \frac{2K_1 K_2 \Omega_1 \Omega_2}{\gamma^2} \cos(\Omega_1 t) \cos(\Omega_2 t) \\ &- \frac{K_1^2 \Omega_1^2}{\gamma^2} \sin^2(\Omega_1 t) - \frac{K_2^2 \Omega_2^2}{\gamma^2} \sin^2(\Omega_2 t) - \frac{2K_1 K_2 \Omega_1 \Omega_2}{\gamma^2} \sin(\Omega_1 t) \sin(\Omega_2 t) \end{aligned} \right\} \quad (4)$$

Denoting  $\beta^* = 1 - \frac{1}{2\gamma^2} (1 + K_1^2 \Omega_1^2 + K_2^2 \Omega_2^2)$ , we rewrite Eq. (4) as,

$$\beta_z = \beta^* - \frac{K_1 K_2 \Omega_1 \Omega_2}{\gamma^2} \cos(\Delta\Omega t), \quad \Delta\Omega = \Omega_1 - \Omega_2 \quad (5)$$

$\Delta\Omega$  is the beat frequency of two collinearly propagating electromagnetic wave wiggler. The energy radiated per unit solid angle unit frequency interval by a single electron with the above electron motion can be analytically calculated from the Lienard-Wiechert formula [6, 7],

$$\frac{d^2 I}{d\omega d\Omega} = \frac{e^2}{4\pi^2 c} \left| \omega \int_0^T \left\{ \hat{n} \times (\hat{n} \times \vec{\beta}) \right\} \exp \left[ i\omega \left( t - \frac{\hat{n} \cdot \vec{r}}{c} \right) \right] dt \right|^2 \quad (6)$$



We obtain the on axis components as follows,

$$\begin{aligned}
T^x &= \frac{-K_1\Omega_1}{2\gamma} J_{m^+} J_n(0) J_p(\xi_5) - \frac{K_2\Omega_2}{2\gamma} J_{n^+} J_m(0) J_p(\xi_5) \\
T^y &= \frac{K_1\Omega_1}{2i\gamma} J_{m^-} J_n(0) J_p(\xi_5) + \frac{K_2\Omega_2}{2i\gamma} J_{n^-} J_m(0) J_p(\xi_5) \\
T^z &= 0
\end{aligned} \tag{7}$$

Where,

$$\xi_5 = \frac{-K_1 K_2 \omega \Delta \Omega}{\gamma^2 \Delta \Omega} \quad J_{m^\pm} = J_{m+1}(0) \pm J_{m-1}(0) \quad J_{n^\pm} = J_{n+1}(0) \pm J_{n-1}(0)$$

Defining  $H(\omega) = T e^{i\nu/2} \frac{\sin(\nu T/2)}{(\nu T/2)}$ ,  $\nu = \left( \frac{\omega(1 + K_1^2 \Omega_1^2 + K_2^2 \Omega_2^2)}{2\gamma^2} - \{m\Omega_1 + n\Omega_2 + p\Delta\Omega\} \right) T$ , we get ,

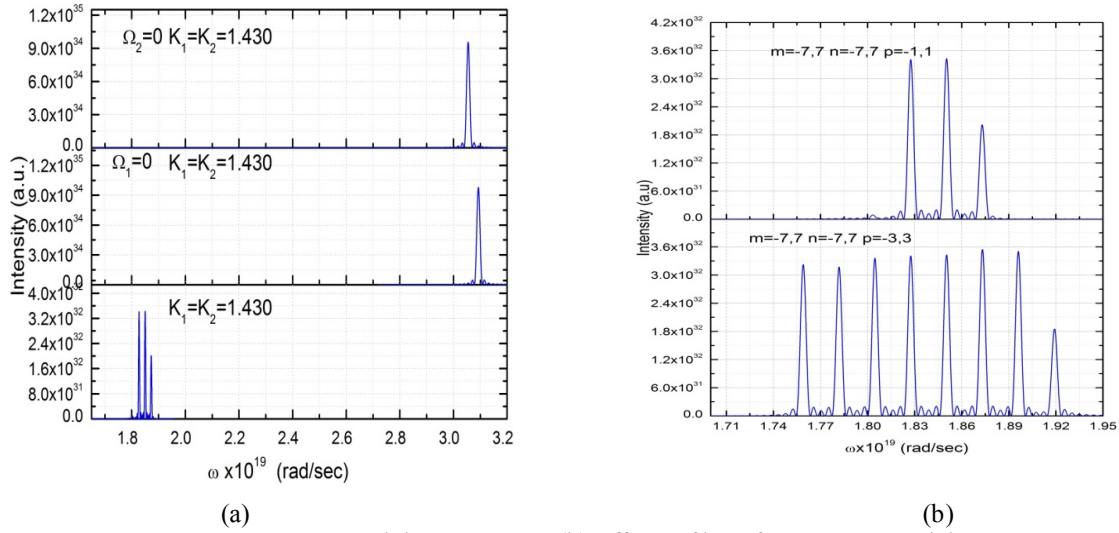
$$\left. \frac{d^2 I}{d\omega d\Omega} \right|_{\omega=0} = \frac{e^2 T^2}{4\pi^2 c} \sum_{m,n,p} \omega_{m,n,p}^2 \left[ |T^x|^2 + |T^y|^2 \right] \sin^2(\nu/2) \tag{8}$$

## RESULT AND DISCUSSIONS

In this paper we have investigated electron motion and spectral properties of undulator radiation with a field configuration that consist of two electromagnetic wave wigglers. It is assumed that two circularly polarized electromagnetic waves collinearly propagate backward to the forward electron motion.

An analytical computation of the Lienard-Wiechert potential is carried out in the far field approximation and the results of the present scheme is evaluated for comparison to permanent magnet based two frequency undulator spectroscopy [7]. One of the main contributions of the present analysis is that the two electromagnetic wave wiggler gives rise to the longitudinal velocity modulation of the electron motion at the beat frequency of the two wigglers. As a consequence the resonance condition is read,  $\omega = \{2\gamma^2(m\Omega_1 + n\Omega_2 + p\Delta\Omega) / (1 + K_1^2 \Omega_1^2 + K_2^2 \Omega_2^2)\}$ .

In the presence of single circularly polarized wave wiggler, the electron longitudinal motion is constant thereby emitting only fundamental on-axis. In the case, when electron undergoes transverse oscillations in the presence of two collinear propagating electromagnetic wave wigglers, the spectroscopic structure of the undulator spectrum gets modified in response to modulating the electron longitudinal velocity at  $\Omega_1, \Omega_2, \Delta\Omega = \Omega_1 - \Omega_2$  and to their mutual interference. The velocity modulation due to finite  $\Delta\Omega$  give rise to a form factor  $J_p(\xi_5)$  that modifies the spectrum. The modification appears through growth of sidebands both left and right of the fundamental, hence increases the frequency band of the emission. The locations of these sidebands occur at the beat frequency and its harmonics. The number of the harmonics that will appear will be decided by the two electromagnetic wave field strengths. In order to quantitatively appreciate the analytical derived results, we consider two Ti:Sapphire lasers at  $800\text{nm}$  and  $810\text{nm}$  [19,20]. Assuming that both laser gives a peak power of  $40\text{TW}$ , we calculate  $K_1 = K_2 = 1.43$  thus we have,  $\Omega_1 = 4.65 \times 10^{15} \text{rad/sec}$ ,  $\Omega_2 = 4.71 \times 10^{15} \text{rad/sec}$ ,  $\Delta\Omega = 0.06 \times 10^{15} \text{rad/sec}$ . This results the emission of fundamental at  $\omega(m=1, n=0, p=0) = 1.827 \times 10^{19} \text{rad/sec}$  (FIGURE 1 (a) and (b)) and  $\omega(m=0, n=1, p=0) = 1.85 \times 10^{19} \text{rad/sec}$ . Considering that the fundamental will have further contribution due to electron oscillation at  $\Delta\Omega$  as its harmonics, the emission at the fundamental will contain a peak at  $\Omega_1 - \Delta\Omega$  ( $p=-1$ ) and  $\Omega_2 + \Delta\Omega$  ( $p=1$ ) at  $\omega = 1.80 \times 10^{19} \text{rad/sec}$  and  $\omega = 1.87 \times 10^{19} \text{rad/sec}$  respectively. Consider the high harmonics, the emission of the radiation will found at  $\omega(m=1, n=1, p=0) = 3.67 \times 10^{19} \text{rad/sec}$  and will have contributions from the beat frequency harmonics.



**FIGURE 1(a)** Beat Frequency Undulator spectrum (b) Effects of beat frequency on undulator spectrum.

## CONCLUSION

We have successfully introduced the conceptual design of a beat frequency undulator scheme involving two circularly polarized electromagnetic wave wigglers. In comparison in the present proposal the electron motion corresponding to only beat frequency modulation, eliminates the sum frequency modulation and its harmonics on the radiated spectrum. The frequency band is controlled by the beat frequency and by a single form factor. The beat frequency undulator with electromagnetic wigglers offers a more versatile design option of a two frequency scheme at x-ray operating wavelength.

## ACKNOWLEDGMENT

The author is very thankful to Dr. Jeevakhan Hussain, NITTTR, Bhopal, India for very useful tips on electromagnetic undulator.

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## AVIFAUNAL DIVERSITY OF FUTALA LAKE, NAGPUR

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(Accepted 27 October 2018)

**ABSTRACT :** Field surveys were carried out in early morning and evening hours in order to observe the avifaunal biodiversity of in and around Futala Lake Nagpur. Futala Lake is rich in biodiversity and harbors a wide variety of local birds and waterfowls due to abundant food availability throughout the year in the form of insects, worms, and weeds. Variations in food availability in different seasons affected the avifaunal diversity in the study area. This habitat attracted 34 bird species, belongs to 15 different orders and among waterfowls geese and ducks of the varied number along with migratory birds.

**Key words :** Avifauna, diversity.

### INTRODUCTION

Nagpur city is located at the latitude of 21°07'N and longitude 79°07'E considered as the winter capital of the state of Maharashtra, located at the geographical center of India lies on the Deccan plateau of the Indian Peninsula and has a mean altitude of 310.5 meters above sea level. Futala Lake is situated at the latitude of 21° 09' 11.74" N and longitude 79° 02' 32.77" E. The catchment area of the lake is 0.40 sq. km. Futala Lake built by King Bhosle dates back centuries. Out of more than 9,000 bird species of the world, the Indian subcontinent contains 1,300 species or over 13% of the world's bird species (Grimmet *et al*, 2004).

The earlier studies on birds were undertaken by investigators like Newton *et al* (1986) and Ghosal (1995) listed birds of Kanha tiger reserve, Osmatston (1922) studied birds from Pachmarhi, Yardi *et al* (2004) reported birds from Salim Ali Lake, Aurangabad, Wadatkar and Kasambe (2002) studied birds of Pohara-Malkhed forest reserve, while Kulkarni *et al* (2005) studied birds in and around Nanded City of Maharashtra. Therefore in this context, in order to assess the biodiversity of birds, the present investigation was undertaken to prepare a checklist of avifaunal diversity of Futala Lake, Nagpur.

### MATERIALS AND METHODS

#### Survey and site selection

A survey for observation and counting of the birds were carried out near study area in the day time depending on the conditions (Namgail *et al*, 2009). The birds were

observed from a safe distance by using a field binocular (10X50, Olympus made). The birds inside the lake, on the shore as well as on bushes and trees surrounding the lake were observed. For identification and confirmation of the species of birds, Keys suggested by Ali (2002), A Field Guide, "Birds of the Indian Subcontinent" by Grimmett (1999) are adopted.

Almost all the species mentioned in the checklist were photographed. The study area was visited in the morning, afternoon and evening time when the birds are most active. The scientific and local names were ascertained based on the key of Manakadan and Pittie (2001). A check list is prepared as per Abdulali (1981) and Gaikwad *et al* (1997).

### RESULTS AND DISCUSSION

During the above survey period a total of 30 bird species were documented (Table 1) belongs to 12 orders, 14 families and 30 genera. Columbidae family represents the maximum (5) number of birds followed by Accipitridae (2) and Phasianidae (2) Strigidae (3). Among the orders Passeriformes represent maximum (43) number of birds followed by Ciconiformes (8) and Falconiformes and Columbiformes (7 bird species); Piciformes and Coraciiformes represent 5 birds each; Charadriiformes consist of 4 birds; Cuculiformes and Strigiformes consist of 3 birds each; Anseriformes, Apodiformes, Gruiformes, Psittaciformes, Pelicaniformes, and Galliformes represents 2 birds each. The least (1) number of bird species represented by Podicipediformes (Table 1). Due to the variability in lakeshore vegetation lead to the formation of microhabitats becomes a niche for many

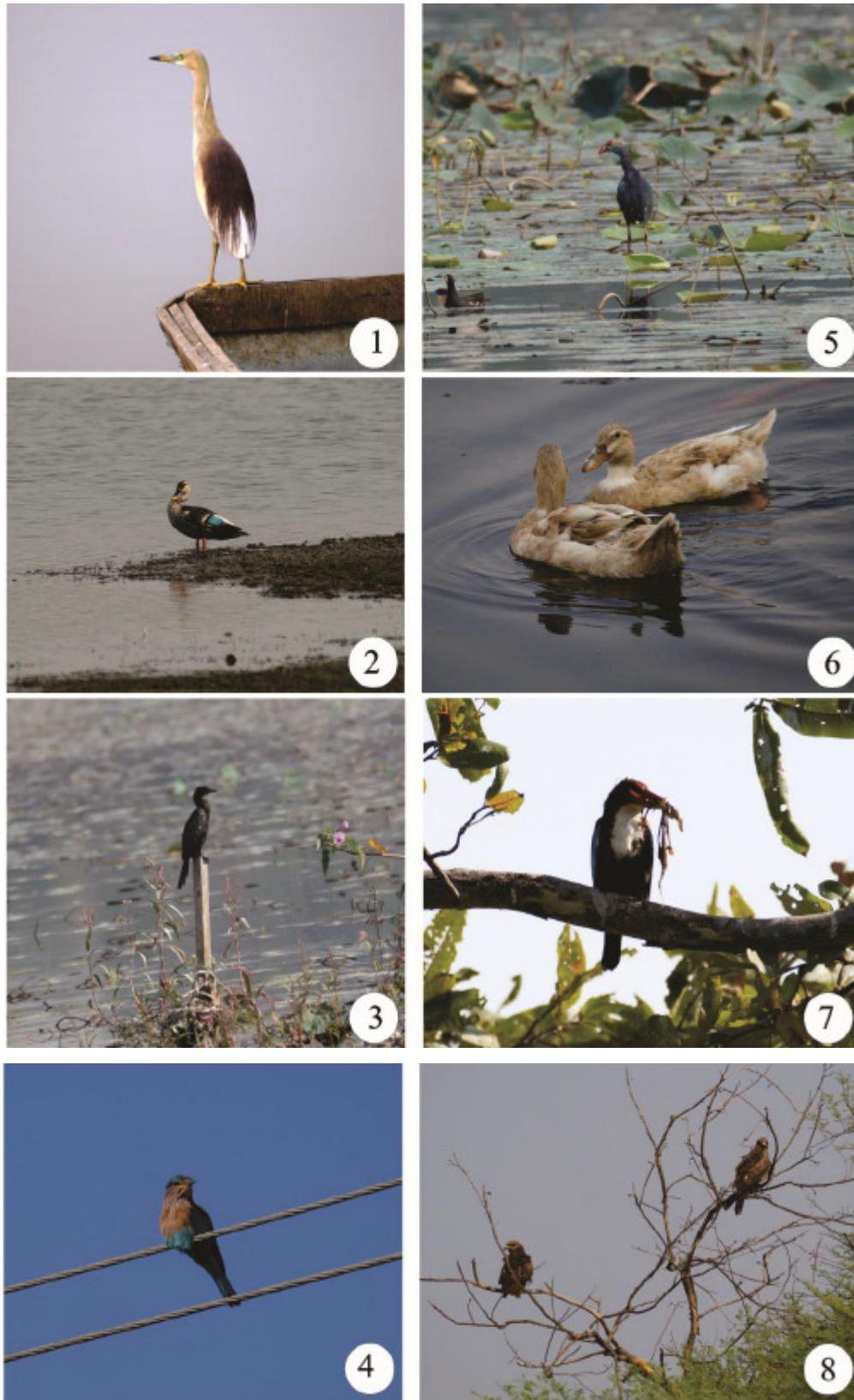


Fig. 1 : Map of Futala Lake .

dabbling ducks including the black duck, the grey teal, the chestnut teal and the shovelers including pink-eared duck. These microhabitats provide the niche required for diversifies fauna both micro and macroorganisms. Futala Lake provides a suitable location as a stopover site to the water whistling duck and grass whistling ducks and waders.

Rainfall in such water bodies act as “trigger” factor and greatly affects the availability of food just not only by replenishing swamps, producing edible aquatic plants for the birds to eat. But increasing anthropogenic activities, rising cement jungles, depletion of avifaunal habitat are causing hawk to avifaunal ecosystem and may lead to the destruction of this valuable stopover if not





**Plate 1 :** 1. Indian Pond Heron, *Ardeola grayii*; 2. Spotted Billed Duck, *Anas poecilorhyncha*; 3. Little Cormorant, *Microcarbo niger*; 4. Indian Roller, *Coracias benghalensis*; 5. Purple Swamphen, *Porphyrio porphyrio*; 6. Garganey, *Spatula querquedula*; 7. White Throated Kingfisher, *Halcyon smyrnensis*; 8. Greater spotted Eagle, *Clanga clanga*.

**Table 1 :** Checklist of common bird found in Futala Lake, Maharashtra.

S. No	Order	Family	Common Name	Scientific Name
1	Galliformes	Phasianidae	Indian Peafowl	<i>Pavo cristatus</i>
			Red Spurfowl	<i>Galloperdix spadicea</i>
2	Anseriformes	Anatidae	Lesser whistling duck	<i>Dendrocygna javanica</i>
			Indian Spot-billed Duck	<i>Anas poecilorhyncha</i>
3	Podicipediformes	Podicipedidae	Little Grebe	<i>Tachybaptus ruficollis</i>
4	Falconiformes	Falconidae	Peregrine Falcon	<i>Falco peregrinus</i>
		Accipitridae	Brahminy Kite	<i>Haliastur indus</i>
			Black Kite	<i>Milvus migrans</i>
5	Columbiformes	Columbidae	Common Pigeon	<i>Columba livia</i>
			Laughing Dove	<i>Stigmatopelia senegalensis</i>
			Spotted Dove	<i>Stigmatopelia chinensis</i>
			Red Collared Dove	<i>Streptopelia tranquebarica</i>
			Eurasian Collared Dove	<i>Streptopelia decaocto</i>
7	Psittaciformes	Psittacidae	Rose-ringed Parakeet	<i>Psittacula krameri</i>
			Plum-headed Parakeet	<i>Psittacula cyanocephala</i>
8	Strigiformes	Strigidae	Barn Fish Owl	<i>Ketupa zeylonensis</i>
			Brown Wood Owl	<i>Strix leptogrammica</i>
			Spotted Owlet	<i>Athene brama</i>
9	Apodiformes	Apodidae	Asian Palm Swift	<i>Cypsiurus balasiensis</i>
			Little Swift	<i>Apus affinis</i>
10	Upupiformies	Upupidae	Common Hoopoe	<i>Upupa epops</i>
11	Coraciformes	Coraciidae	Indian Roller	<i>Coracias benghalensis</i>
		Alcedinidae	Common Kingfisher	<i>Alcedo atthis</i>
12	Passeriformes	Aegithinidae	Common Iora	<i>Aegithina tiphia</i>
		Corvidae	House Crow	<i>Corvus splendens</i>
			Indian Jungle Crow	<i>Corvus culminatus</i>
13	Anhingidae	Suliformes	Darter	<i>Anhinga melanogaster</i>
14	Ardeidae	Pelecaniformes	Cattle Egret	<i>Bubulcus ibis</i>
			Purple Heron	<i>Ardea purpurea</i>
15	Phalacrocoracidae	Suliformes	Indian Cormorant	<i>Phalacrocorax fuscicollis</i>
			Little Cormorant	<i>Phalacrocorax niger</i>

managed properly. Our study will be helpful to obtain comprehensive information on breeding areas of residential birds while staging and wintering areas of migrants that are globally important for the protection of migratory birds. Hence such urban wetlands should be prioritized and its conservation values should be highlighted.

#### ACKNOWLEDGEMENT

Author highly acknowledges supports and necessary help taken by research colleagues Mr. J. Kirsan from Department of Zoology for their field assistance during

field visit.

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Research Article

## Microwave Assisted Synthesis, Characterization and Anti-Tubercular Activity of 4-Quinolyhydrazone

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### ABSTRACT

A series of 4-quinolyhydrazone derivatives was synthesized by reaction of 4-quinolyhydrazine and various substituted carboxaldehyde out of that most of the derivatives show significant antitubercular properties. The microwave assisted organic synthesis was applied to synthesize a series of 4-quinolyhydrazone derivatives. The characterizations of newly synthesized derivatives were done by modern analytical techniques like digital melting point apparatus, IR, NMR and mass spectroscopy.

**Keywords:** Mycobacterium tuberculosis, Hydrazone, Quinoline, Carboxaldehyde.

**Article Info:** Received 11 July 2019; Review Completed 17 August 2019; Accepted 21 August 2019; Available online 15 Sep 2019



### Cite this article as:

Bisen CV, Patle MR, Rahangdale PK, Microwave Assisted Synthesis, Characterization and Anti-Tubercular Activity of 4-Quinolyhydrazone, Journal of Drug Delivery and Therapeutics. 2019; 9(5):95-97  
<http://dx.doi.org/10.22270/jddt.v9i5.3425>

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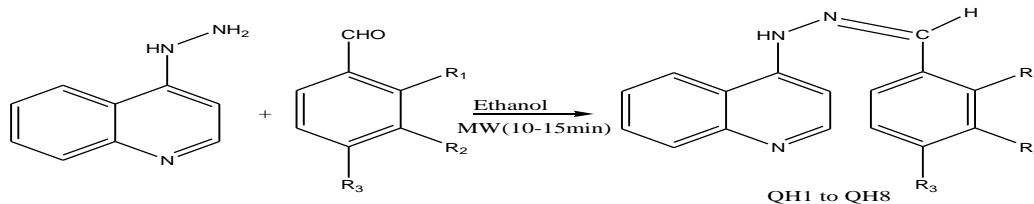
### Introduction:

Tuberculosis (TB) is one of the most predominant infections in human beings and it has considerable contribution towards illness and death all around the world. Tuberculosis is caused by mycobacterium tuberculosis [1]. From previous research it is well known that quinolone is an important heterocyclic nucleus found in many natural as well as synthetic products having wide variety of pharmacological activities such as anti-TB [2], tyrokinase PDGF-RKT inhibiting agent [3], anticancer [4], antibacterial [5] and anti-inflammatory [6]. The physicochemical study data of quinolone derivatives shows the potential antitubercular activity [7]. The literature study of some 4-quinolyhydrazone derivatives unveiled significant activity (MIC=12.5-3.12 µg/ml) when compared to first line drugs such as ethambutol (MIC=3.12 µg/ml) [8]. With reference to

this, in the search of new antituberculosis agents we proposed the synthesis of some quinolyhydrazones containing 4-hydrazinylquinoline moiety which was designed by using molecular modeling methods [9]. From literature survey it is observed that quinolyhydrazone moiety are pharmacologically very active, shows the activities like anti-inflammatory, antimicrobial and antitubercular [2]. The latest development in the field of organic chemistry is the microwave assisted organic synthesis (MAOS) [10], [11] which provides short reaction time and economic use of reagents through green approach [12].

### Experimental:

The synthetic route for the preparation of 4-quinolyhydrazone derivatives QH1 to QH8 is summarized in scheme 1 as below.



**Scheme 1:** 4-quinolyhydrazone, corresponding carboxaldehydes, Ethanol, Microwave (MW) 10-15 min.



A Mixture of 4-quinolyldiazine (1 equivalent), and carboxaldehyde (1 equivalent) in absolute ethanol was irradiated with temperature assisted microwave oven at 180W for 10-15 min with intermittence. All the Chemicals used are of AR grade from Merck, India.

The completion of reaction is monitored by TLC. After conformation of completion of reaction by TLC the reaction mixture is cooled and diluted with water, so that respective hydrazones precipitated out from the reaction mixture. The

product obtained was purified with column chromatography by using ethyl acetate and n-hexane to yield expected hydrazine derivatives. The purified derivatives were recrystallized using suitable organic solvent.

The molecules under study are subjected to in-silico studies by using Datawarrior software for calculation of properties cLogP, cLogS, Total surface area, drug likeness and drug score to evaluate the therapeutic properties are summarized in table 1 as below.

Table 1

Hydrazones	Molecule Formula	Total Molecular weight	cLogS	H-acceptors	H-Donors	Total Surface Area	Drug likeness
QH1	C <sub>16</sub> H <sub>13</sub> N <sub>3</sub>	247.3	-4.385	3	1	179.6	-2.0672
QH2	C <sub>16</sub> H <sub>12</sub> ClN <sub>3</sub>	281.745	-5.121	3	1	195.0	-0.2838
QH3	C <sub>16</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>	292.297	-4.845	6	1	203.3	-9.0516
QH4	C <sub>16</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>	292.297	-4.845	6	1	203.3	-7.0604
QH5	C <sub>16</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>	292.297	-4.845	6	1	203.3	-11.868
QH6	C <sub>17</sub> H <sub>15</sub> N <sub>3</sub> O	277.326	-4.403	4	1	201.89	-1.2243
QH7	C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O	263.299	-4.089	4	2	185.98	-1.3401
QH8	C <sub>17</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>	291.309	-4.398	5	5	203.73	-6.1263

Furthermore the characterizations of hydrazine derivatives were established on the basis of spectral data analysis.

The synthesized 4-quinolyldiazine derivatives with their percentage yield, melting point, clogP, drug score and biological activities are summarized in table 2 as below.

Table: 2

Sr. No.	Hydrazones	Substitutions	% Yield	MP (°C)	clogP	Drug Score	Biological Activity
1	QH1	R <sub>1</sub> =R <sub>2</sub> =R <sub>3</sub> =H	71	223-224	3.8788	0.39297	0.799
2	QH2	R <sub>3</sub> =Cl, R <sub>1</sub> =R <sub>2</sub> =H	78	226-227	4.4848	0.41254	0.786
3	QH3	R <sub>1</sub> =NO <sub>2</sub> , R <sub>2</sub> =R <sub>3</sub> =H	81	250-252	2.9572	0.34878	0.780
4	QH4	R <sub>2</sub> =NO <sub>2</sub> , R <sub>1</sub> =R <sub>3</sub> =H	83	275-278	2.9572	0.34904	0.790
5	QH5	R <sub>3</sub> =NO <sub>2</sub> , R <sub>1</sub> =R <sub>2</sub> =H	89	216-218	2.9572	0.34874	0.786
6	QH6	R <sub>3</sub> =OCH <sub>3</sub> , R <sub>1</sub> =R <sub>2</sub> =H	76	146-148	3.8088	0.43145	0.786
7	QH7	R <sub>3</sub> =OH, R <sub>1</sub> =R <sub>2</sub> =H	84	220-221	3.5331	0.45577	0.812
8	QH8	R <sub>3</sub> =COOH, R <sub>1</sub> =R <sub>2</sub> =H	80	221-223	3.3639	0.36450	0.816

#### Spectral characterization of 4-quinolyldiazine derivatives:

##### (Z)-2-benzylidene-1-(quinoline-4yl) hydrazine

###### (QH1-C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>):

<sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8.18(s,1H), 7.41-8.04(d,4H), 2.5(s,1H), 7.39(t,1H), 7.44(d,1H), 7.51(t,1H), 7.59(d,1H). <sup>13</sup>C NMR -141, 150.3, 128, 128.9, 130.2, 125.8, 121.0. IR cm<sup>-1</sup>-3070(CH str), 1640(C=N str), 3320(NH str) MS m/z-247(100%), 248(18.4%).

##### (Z)-2-(4-chlorobenzylidene)-1-(quinoline-4yl)hydrazine

(QH2-C<sub>16</sub>H<sub>12</sub>ClN<sub>3</sub>): <sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8.17(d,1H), 8.04(d,1H), 8.50(d,1H), 7.30-7.60(m,4H), 3.95(s,1H). <sup>13</sup>C NMR -143.1, 150, 132.1, 136.2, 129, 146.9, 121, 126.2 IR cm<sup>-1</sup>-3070(CH str), 1640(C=N str), 3320(NH str), 782(C-Cl) MS m/z-281(100%), 283(32.2%).

##### (Z)-2-(2-nitrobenzylidene)-1-(quinoline-4yl)hydrazine

###### (QH3-C<sub>16</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>):

<sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8.25(s,1H), 3.99(s,1H), 7.5-8.5(d,2H), 7.40-7.60, 8.60(s,1H). <sup>13</sup>C NMR -148.1, 144.2, 149.9, 151.1, 147.5, 149.3, 121.3, 126.1 IR cm<sup>-1</sup>-3070(CH str), 1640(C=N str), 3320(NH str), 1380(Ar-NO<sub>2</sub>) MS m/z-292(100%), 293(17.5%).

##### (Z)-2-(3-nitrobenzylidene)-1-(quinoline-4yl)hydrazine

###### (QH4-C<sub>16</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>):

<sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8.20(s,1H), 3.99(s,1H), 1.49-8.05(d,2H), (8.5(s,1h) <sup>13</sup>C NMR -148.6, 143, 149.2, 129.3, 121, 150.3, 121.6 IR cm<sup>-1</sup>-3070(CH str), 1640(C=N str), 3320(NH str), 1421(Ar-NO<sub>2</sub> str) MS m/z-292(100%), 293(17.5%).

##### (Z)-2-(4-nitrobenzylidene)-1-(quinoline-4yl)hydrazine

###### (QH5-C<sub>16</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>):

<sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8.0(s,1H), 8.01(t,2H), 7.99(t,2H), 8.54(d,1H), 7.49-8.05(d,2H), 6.41(d,1H) <sup>13</sup>C NMR -150, 142.9, 121.3, 125.8, 128.8, 130, 130.2, 147.5 IR cm<sup>-1</sup>-3070(CH str), 1640(C=N str), 3320(NH str), 1489(Ar-NO<sub>2</sub> str) MS m/z-292(100%), 293(17.5%).

##### (Z)-2-(4-methoxybenzylidene)-1-(quinoline-4yl)hydrazine

(QH6-C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O): <sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8(s,1H), 3.71(s,3H), 6.71(t,2H), 7.54(t,2H), 3.99(s,1H), 7.67(d,1H), 7.40(t,1h), 7.60(t,1H), 8.11(d,1H) <sup>13</sup>C NMR -60.1, 162.9, 114, 126.2, 130.4, 115.2, 129.3, 125.4, 121.5, 143.2, 147.6 IR cm<sup>-1</sup>-3070(CH str), 1640(C=N str), 3320(NH str), 1266(Ar-OCH<sub>3</sub>) MS m/z-277(100%), 278(19.5%).

**(Z)-2-(4-hydroxybenzylidene)-1-(quinoline-4-yl)hydrazine****(QH7-C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O):**

<sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8(s,1H), 10.48(s,1H), 3.99(s,1H), 6.50(d,1H), 8.69(d,1H), 7.67(d,1H), 7.40(t,1H), 7.60(t,1H), 8.11(d,1H) <sup>13</sup>C NMR -160.7, 115.9, 143, 121, 125.5, 126.3, 130.3, 148.9 IR cm<sup>-1</sup> -3070(CH str), 1640(C=N str), 3320(NH str), 1216(Ar-OH) MS m/z-263(100%), 264(17.5%).

**(Z)-2-(4-formylbenzylidene)-1-(quinoline-4-yl)hydrazine****(QH8-C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>):**

<sup>1</sup>H NMR (DMSO, δ ppm, TMS) 8(s,1H), 13.26(s,1H), 3.99(s,1H), 8.10(t,2H), 7.68(t,2H), 7.67(d,1H), 7.40(t,1H), 7.60(t,1H), 8.11(d,1H) <sup>13</sup>C NMR -170.2, 131.8, 130.4, 129.2, 143, 150.1, 112.4, 129.3, 125.6, 130.4 IR cm<sup>-1</sup> -3070(CH str), 1640(C=N str), 3320(NH str), 1714(Ar-COOH) MS m/z-291(100%), 292(19.6%).

**Results and Discussion:**

The series of 4-quinolylhydrazone derivatives from QH1 to QH8 had been synthesized using microwave assisted synthesis. Most of them show a good MIC value when compared with the first line drug Ethambutol with a very significant antitubercular activity. The TLC plate used is coated with alumina, column chromatography on silica gel (60-120mesh) was applied when required. <sup>1</sup>H NMR spectra were recorded on VARIAN NMR spectrophotometer operating at 300MHz, TMS is used as internal standard. IR spectrum recorded on Shimadzu IR Affinity-1S and mass spectra were recorded using water Micromass Q-ToF Mic.

**Conclusion:**

The series of 4-quinolylhydrazone based novel antitubercular agents were synthesized and studied by advanced and sophisticated instruments. As well as, these compounds are also subjected to in-silico studies for their therapeutic studies. These compounds shows drug score in the range of 0.34 to 0.45 and biological activity of about 0.80 against mycobacterium tuberculosis.

In this study it is observed that the hydrazone moiety having para substitution with withdrawing groups in benzylidene ring shows significant antitubercular activity studied by SAR study. In future this can be extended to synthesize more new derivatives and to study their practical application as potential antitubercular drugs.

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**COMPARATIVE STUDY OF OLFACTORY ORGANS OF *CHANNA PUNCTATA* (BLOCH) AND *HETEROPNEUSTES FOSSILIS* (BLOCH)**

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**ABSTRACT**

The present study is an attempt to examine the histology with the help of light microscope (LM) of the olfactory epithelium and olfactory bulb of spotted snakehead *Channa punctata* and *Heteropneustes fossilis*. The olfactory organ of teleost consists of olfactory rosette, olfactory nerve, and olfactory bulb. In *C. punctata*, paired and oval shaped olfactory rosette present in the olfactory chamber on fish rostrum. Olfactory chamber opens externally via an anterior inlet and a posterior nostril outlet depending on presence of olfactory receptor neurons olfactory lamellae has two regions, sensory and non-sensory. A sensory region has olfactory receptor neurons. These neurons send their axons along olfactory nerve to olfactory bulb which work as relay centre. Olfactory bulb sends signal to telencephalon via olfactory tract. Olfactory bulb histologically shows four concentric layer, olfactory nerve layer, glomerular layer, mitral cell layer, and granular cell layer. In *C. punctata*, olfactory lamellae run parallel to each other in antero-postero direction and olfactory rosette lack central raphe. *H. fossilis* has central raphe in olfactory rosette and olfactory lamellae runs outwardly from distinguish central raphe. Location of sensory region on olfactory epithelium also differ in *C. punctata* and *H. fossilis*. Sessile and pedunculated type of olfactory bulb present in *C. punctata* and *H. fossilis* respectively but histologically olfactory bulb of both teleost shows similar structure.

**KEYWORDS:** *Channa punctata*, *Heteropneustes fossilis*, Olfactory Rosette, Olfactory Bulb, Olfactory Epithelium.

## INTRODUCTION

Snakehead, *Channa punctata* is highly esteemed as food. This fish is quite frequent in shallow and deep parts of rivers, tanks or pools with or without aquatic vegetation. The tenacity of the fish is very great and if taken out of water, it can survive for a long time. All the three species of *Channa* bear a pair of folded sac like outgrowth of pharynx called pharyngeal diverticulum to store air. These are lined by highly vascular respiratory epithelium and act as accessory respiratory organs. They can, therefore live for quite some time outside water, and hence grouped among live fishes. Snakeheads as they are commonly called as, are acclaimed all over the country for their flavour, medicinal and recuperative values (Chakraborty, 2006).

Another valuable fish is *Heteropneustes fossilis* which is also highly esteemed as food and occupies foremost place among live catfishes. The fish is locally called as “Singhee”. *Heteropneustes fossilis* is a tropical freshwater fish which attains an adult size of 18-30 cm. long and weighs about 40-80gm.

Various functions of the body including reproduction are controlled by endocrine system and nervous system. In both the systems, brain plays a vital role. Brain regulates the activities of pituitary gland through hypothalamus. Brain receives the stimuli through vision and olfaction. Impulses generated through these stimuli are then conveyed to the appropriate nuclei in the hypothalamus which in response secrete neuropeptides and neurohormones and regulate the secretion of pituitary. Olfactory system consists of olfactory rosette, olfactory tract, olfactory bulb, Olfactory bulb acts as the first relay station receiving primary olfactory nerve input in fish and other vertebrates (Farbman, 1994). Posteriorly it extends as olfactory tract. Each olfactory tract in turn has Lateral olfactory tract (LOT) and Medial olfactory tract (MOT) terminating on telencephalon. Signals arising from each of these tracts are transmitted to respective target areas in the telencephalon and diencephalon which are referred to as secondary olfactory areas.

## MATERIALS AND METHODS

Fishes were collected from natural habitat all around the Nagpur City; they were acclimatized in small ponds. Fishes were treated as per the guidelines of Institutional Animal Ethics Committee (IAEC), Post Graduate Teaching Department of Zoology, RTM Nagpur University, Nagpur (Registration no.- 478/01/a/CPCSEA). Matured fishes

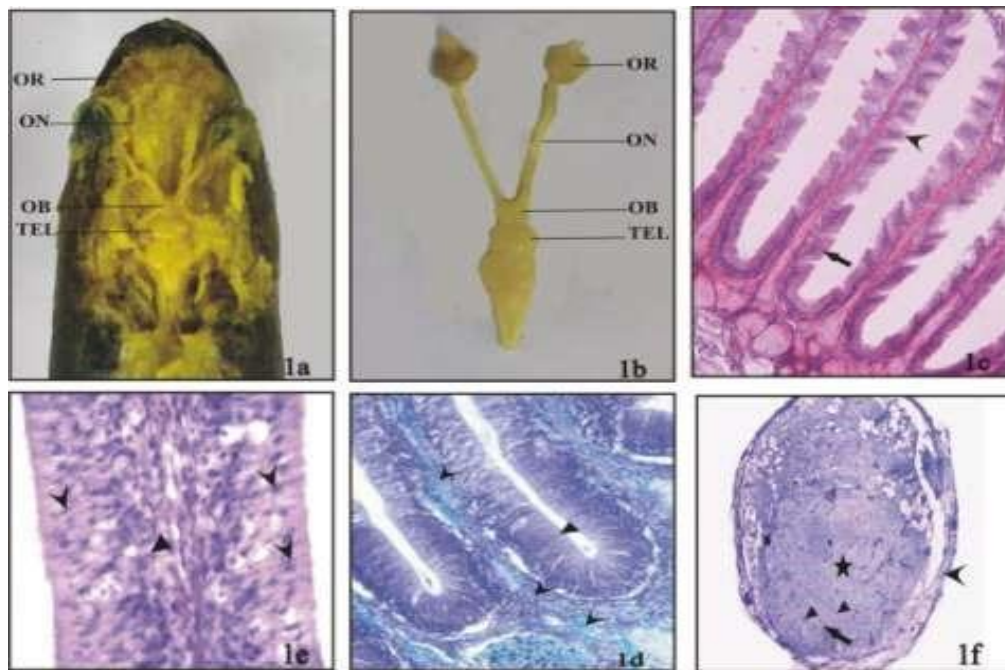


were selected with body weight ranging from 500 to 700 gm. in case of *C. punctata* and 100 to 125 gm. in case of *H. fossilis*. The fishes were anesthetized and olfactory organ, were dissected out and immediately fixed in Bouin's fixative, embedded in paraffin wax and cut at 8 to 10  $\mu$ m in transverse planes. Sections were stained by HE double staining technique and Nissl staining method (Kluver and Barrera, 1953).

## RESULTS

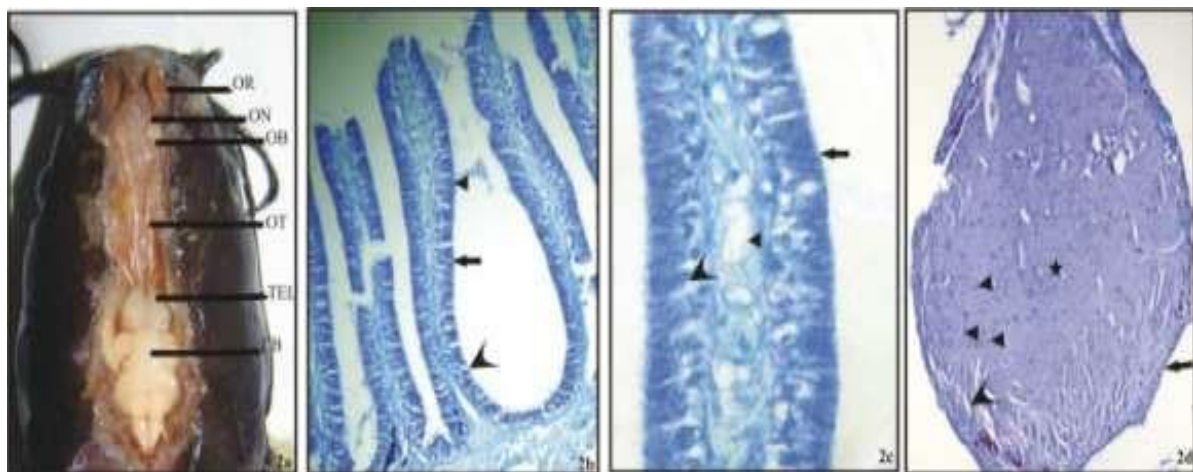
In *C. punctata*, a paired olfactory rosette situated in olfactory chamber (Figure 1a). Olfactory rosette comprises of several olfactory lamellae running in rostro-caudal direction. Olfactory epithelium of olfactory lamellae have sensory region with olfactory receptor neurons (ORNs) and non-sensory region without ORNs. In *C. punctata*, sensory region present at the proximal and basal part of olfactory lamellae and non-sensory region lies at middle and distal part of olfactory lamellae (Figure 1c). ORNs are elongated cells and have dendrite towards the outer side (Figure 1d, 1e). Towards inner side, ORNs send the axon. These axons collectively form olfactory nerve which runs posteriorly and meets with olfactory bulb (Figure 1a, 1b). *C. punctata* has long olfactory nerve (Figure 1a, 1b). Snakehead *C. punctata* have sessile type of olfactory bulb as it is attached with telencephalon (Figure 1a, 1b). Axons of ORNs form outer olfactory nerve layer (ONL) (Figure 1f). Inner to ONL, glomerular layer (GL) with synapses of axons of ORNs and mitral cells present (Figure 1f). GL towards inner side has mitral cell layer (MCL) and central granular cell layer (GCL) (Figure 1f).

In *H. fossilis*, a paired elongated olfactory rosette lies in olfactory chamber (Figure 2a). Olfactory lamellae radiated outwardly from central raphe which is absent in *C. punctata*. In *H. fossilis*, sensory region lies at middle portion as ORNs present in this region (Figure 2b, 2c). ORNs send their axons towards olfactory bulb. Olfactory bulb in *H. fossilis* is of pedunculated type as it remains in proximity of olfactory rosette. Axons of ORNs form ONL which is outermost layer followed by GL, MCL and central GCL as in *C. punctata* (Figure 2d). From olfactory bulb fibers arise and form long olfactory tract which run towards the telencephalon (Figure 2a).



**Fig. 1. Olfactory organs of *C. punctata*.**

**1a:** Dissecting head of *C. punctata* showing olfactory rosette (OR), olfactory nerve (ON), olfactory bulb (OB) and telencephalon (TEL). **1b:** ex-situ olfactory rosette (OR), olfactory nerve (ON), olfactory bulb (OB) and telencephalon (TEL) of *C. punctata*. **1c:** Olfactory epithelium of *C. punctata* showing sensory epithelium (Arrow), non- sensory epithelium (Arrow head), HE staining, 40 X. **1d:** Olfactory epithelium of *C. punctata* showing ORNs (Triangle), which sends their axons (Arrow head) to olfactory bulb, KB staining, 40 X. **1e:** Olfactory epithelium of *C. punctata* showing ORNs (Arrow head), and basal cells (Triangle), HE staining, 40 X. **1f:** Olfactory bulb of *C. punctata* showing olfactory nerve layer (Arrow head), glomerular layer (Arrow), mitral cell layer (Triangle) and granular cell layer (Star), KB staining, 40 X.



**Fig. 2. Olfactory organs of *H. fossilis*.**

**2a:** Dissecting head of *H. fossilis* showing olfactory rosette (OR), olfactory nerve (ON), olfactory bulb (OB) and cerebellum (CB). **2b:** Olfactory epithelium of *H. fossilis* showing sensory region at middle part (Arrow), non-sensory region at distal part (Arrow head), HE staining. 10X, **2c:** Olfactory epithelium of *H. fossilis* showing ORNs (Arrow), Basal cell (Arrow head) and axonal fibers (Triangle), KB staining, 40 X. **2d:** Olfactory bulb of *H. fossilis* showing olfactory nerve layer (Arrow), glomerular layer (Arrow head), Mitral cells of MCL (Triangle) and granular cells of GCL (Star), KB staining, 40 X.

## DISCUSSION

Sensory region is at the proximal end and basal regions of lamellae in *C. punctata* as in *N. notopterus* (Baile et al., 2008) while in *L. rohita*, sensory region occupies middle of the lamellae and non-sensory region is at the proximal and distal regions on either side of sensory region of lamellae (Bhute et al., 2007). Similar arrangement of sensory region over olfactory epithelium found in *H. fossilis*.

In *C. punctata*, olfactory bulb is attached to the telencephalon and is sessile. Lamellae run parallel to central raphe and are arranged in rostro-caudal direction. Sessile olfactory bulb is reported in *Salmon* (Evans et al., 1998), *Carassius*, *Ictalurus* and *Gnthonemus* (Nieuwenhuys, 1998). Olfactory bulb is pedunculated in *H. fossilis*. It is in the proximity of the olfactory rosette and caudally connected to the telencephalon by long olfactory nerve. Such type of olfactory bulb is found in *Salmo*, *Anguilla* and *Gasterosteus* (Nieuwenhuys, 1998), *Labeo rohita* (Bhute et al., 2007), *Notopterus notopterus* (Baile et al., 2008). Interestingly olfactory bulb is altogether absent in moray, *Muraena undulate* (Kapoor and Ojha, 1972).

As in other teleosts, olfactory bulb comprises of four concentric layers; Olfactory nerve layer (ONL), Glomerular layer (GL), Mitral cell layer (MCL) and Granular cell layer (GCL) from superficial to the deep in *C. punctata* and *H. fossilis*. ONL axons march inward and synapse with the dendrite of mitral cell in glomerular layer (GL) (Ichikawa, 1976; Kosaka and Hama, 1982; Oka, 1983). In *Oncorhynchus*, the glomerular layer appears to contain nine discrete terminal fields, each of which receives convergent input from all rosettes in the olfactory epithelium (Riddle and Oakaley, 1992). Inner to the GL, mitral cell layer (MCL) containing comparatively larger mitral cells is present. Distal dendrites of mitral cells synapse with fibers of olfactory nerve while proximal dendritic shaft make contact with granule cell dendrites (Ichikawa, 1976; Oka, 1983). Axons of mitral cells originate in the basal part of the soma, become myelinated after some distance (Kosaka and Hama, 1982) and projected in the medial and lateral olfactory tract (Fujita et al., 1988). There are some slight, but significant morphological differences between medially and laterally located mitral cells (Fujita et al., 1984) that might be correlated with different physiological properties (Satou et al., 1983). Centralmost area is occupied by granular cell forming granular cell layer (GCL). Same and identical arrangement of different cell layers as described above is found both in *C. punctata* and *H. fossilis*.

## CONCLUSION

Snakehead, *C. punctata* has rostro-caudally running olfactory lamellae while olfactory lamellae radiating outward from central raphe. Olfactory bulb is sessile and pedunculated in *C. punctata* and *H. fossilis* respectively. Sensory region is at base of olfactory lamellae in *C. punctata*. In *H. fossilis*, sensory region occupies central part of olfactory lamellae. *C. punctata* has long olfactory nerve. Catfish, *H. fossilis* has short olfactory nerve and long olfactory tract.

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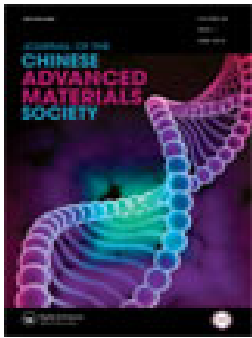


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## Comprehensive hydrobiological status of Bhiwapur Lake of Maharashtra, India: an environmental aspect

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To cite this article: Prashant P. Ingale, Atul D. Bobdey & Nilesh D. Gorghate (2018): Comprehensive hydrobiological status of Bhiwapur Lake of Maharashtra, India: an environmental aspect, Journal of the Chinese Advanced Materials Society, DOI: [10.1080/22243682.2018.1537005](https://doi.org/10.1080/22243682.2018.1537005)

To link to this article: <https://doi.org/10.1080/22243682.2018.1537005>



Published online: 29 Nov 2018.



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# Comprehensive hydrobiological status of Bhiwapur Lake of Maharashtra, India: an environmental aspect

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## ABSTRACT

The study of physico-chemical parameters like water temperature, conductivity, TDS, turbidity, pH, chloride, D.O., free CO<sub>2</sub>, BOD, phosphate, nitrate, and ammonia, were analyzed. The Pearson's coefficient of correlation between physico-chemical and zooplankton were additionally calculated. The zooplankton records nineteen species belonging to four groups and Nauplius larvae. This study found month wise, season wise and annual variation of physico-chemical and biological assessment of Bhiwapur lake water. The high rate of anthropogenic pollution indicates the lake goes toward eutrophication and it may results severe water issue in the study region in near future.

## ARTICLE HISTORY

Received 3 April 2018  
Revised 12 October 2018  
Accepted 13 October 2018

## KEYWORDS

Bhiwapur Lake; physico-chemical;  
Zooplankton; pollution

## 1. Introduction

Water goes to be the foremost agenda and it's entirely the foremost offer of economy in twenty second century. Among the H<sub>2</sub>O resources of the world like ponds, rivers, lakes, reservoirs, wetlands, etc. are very important sources to boot this helps to stay up very cheap water level so they furnish water for all the population among the full year for drinking functions. The H<sub>2</sub>O resources distributions is unequal throughout the globe and conjointly the H<sub>2</sub>O facilitation is becoming boggle day by day in thought of population augmentation and varied human activities. Among the absence of H<sub>2</sub>O resources, groundwater is exploited to satisfy the demand exerted by varied sectors. The variation among the standard of soil water in response to native geologic syntax and anthropogenetic factors vindicate the analysis of the quality of groundwater for any functions additionally as that for human consumption.[1] The advantage of economic and environmental that square measure come back through from the water.[2] The physico-chemical and biological standing in natural science purpose of browse with relevancy elevation of space, fisheries, agriculture and regular domestic uses of water.[3] In India, lakes, reservoirs, rivers, and ponds are used for domestic functions and in agriculture. Higher quality of water is delineated by its physical, chemical and biological characteristics. In fact, the total of the aquatic life in any

water body is ruled by the interaction of variety of physical and chemical conditions.[4] Zooplanktons are basic constituents of water, food webs and contribute suggestively to aquatic efficiency in recent ecological unit. They are additionally wonderful bioindicators to assess the pollution of any recent body and condition of the lake and biological diversity are network like to just about all components of the system.[5] Throughout the extreme events continues looking is required the hydro-biological standing of aquatic ecosystems, that occur scarcely but could having the potent result on fauna and flora.[6]

Hence, by keeping the aforesaid view in our mind now we are demonstrating the major physicochemical parameters along with the presence of zooplankton records in Bhiwapur Lake of Maharashtra, India during July 2012–June 2013.

## **2. Material and methods**

Bhiwapur Lake is located at three district border area in Bhiwapur tassels such as, Nagpur, Bhandara and Chandrapur. Bhiwapur is a tehsil, situated east from Nagpur at 74 km having  $79^{\circ}$ ,  $31'04.78''$ E latitude and  $20^{\circ}45'40.77''$ N longitude. A selection of five sampling stations for the collection of water samples in the interpretation of human activities observed along the lake. All the stations selected on the basis of mode of pollution keeping long distance from each others such as, stations A, B, C, D, and E.

### **2.1. Station A**

This station is present towards the east sight of the Bhiwapur Lake. Most of rural population situated on the bank of lake so that there are daily activities like bathing, washing of cloth, observed more of this station and also some part of this station use for the catching fishes by fisherman.

### **2.2 Station B**

The station located on North sight of the Bhiwapur Lake. Most of wastewater directly coming from village Inflowing inside the lake during rainy season. Greatly increase organic matter input to a water body. The sewage activity occurred near this station. Some locality also disturbs lake water quality by dumping domestic waste. The flow of waste water from the sewage channel to the tunnel varies according to the season.

### **2.3 Station C**

This sampling station situated Southwest sight near the Bhima Devi temple, daily Non-veg market above this station and also the outflow of lake. Human activity by dumping waste material disturbs the water quality during Bhima Devi Yatra.



## 2.4 Station D

This station present in between Station B and Station C toward West sight of lake. Here cattle activity and also sewage activity occurred only summer period during survey and sampling at this station.

## 2.5 Station E

This is central station of the lake. This station is Untouchable directly from human interference. It is deep zone area.

Present study was conducted from July 2012 to June 2013. For the analysis, samples were collected during the early morning between 8.30 to 11.00 a.m. in four time of every month at regular interval. Temperature, Conductivity, Total dissolved solid, Turbidity, pH, Dissolve oxygen was analyzed in the field by means of ELIKO makes a digital water analysis kit and Chloride, Biochemical oxygen demand, Phosphate, Nitrate and Ammonia periodically tested by using standard methods given by APHA and NEERI.[7,8]

Zooplankton analysis using collected zooplankton samples was preserved in 4% formaldehyde. The preserved zooplankton samples were diluted to 80 ml with distilled water for their taxonomic study and numerical estimation. For the quantitative study of zooplankton, a Sedgwick Rafter Counting Cell was used to implement the technique delineated by [9]. All the zooplankton in the counting chamber was observed and identified using standard keys [10–12] and counted under a compound microscope.

## 2.6 Statistical analysis of data

The statistic is concerned with the study of random variables and its help in reaching a logical decision from the state of uncertainty. Water quality and biological parameters were calculated station wise monthly four times, season wise and year wise. The data used for these analyses were the averages of the four measurements made in each season for one two years of study. There were variations in each particular parameter across the stations as per seasons and years. Parameter Values were calculated like mean, standard error, Pearson's correlation coefficient with the help of SalStat2 offline Software. Pearson's correlation coefficient between variables significance level of  $p$ -value at  $\leq 0.05$  works well. Studies were conducted between one year and average were taken of different seasons with all the corresponding physico-chemical parameters and biological parameters.

Simple of Pearson's co-efficient correlation:

$$r = \frac{n(\sum xy) - (\sum x) \sum y}{\sqrt{[n \sum x^2 - (\sum x)^2] [n \sum y^2 - (\sum y)^2]}}$$

Where;

$r$  = co-efficient of correlation

$n$  = number of pairs of score

$\Sigma XY$  = sum of the product of paired scores

$\Sigma X$  = sum of X scores

$\Sigma Y$  = sum of Y scores

$\Sigma X^2$  = sum of squared X scores

$\Sigma Y^2$  = sum of squared Y scores

Correlation may be a technique for work the connection between two quantitative, continuous variables, for instance, age and pressure level.

### 3. Result and discussion

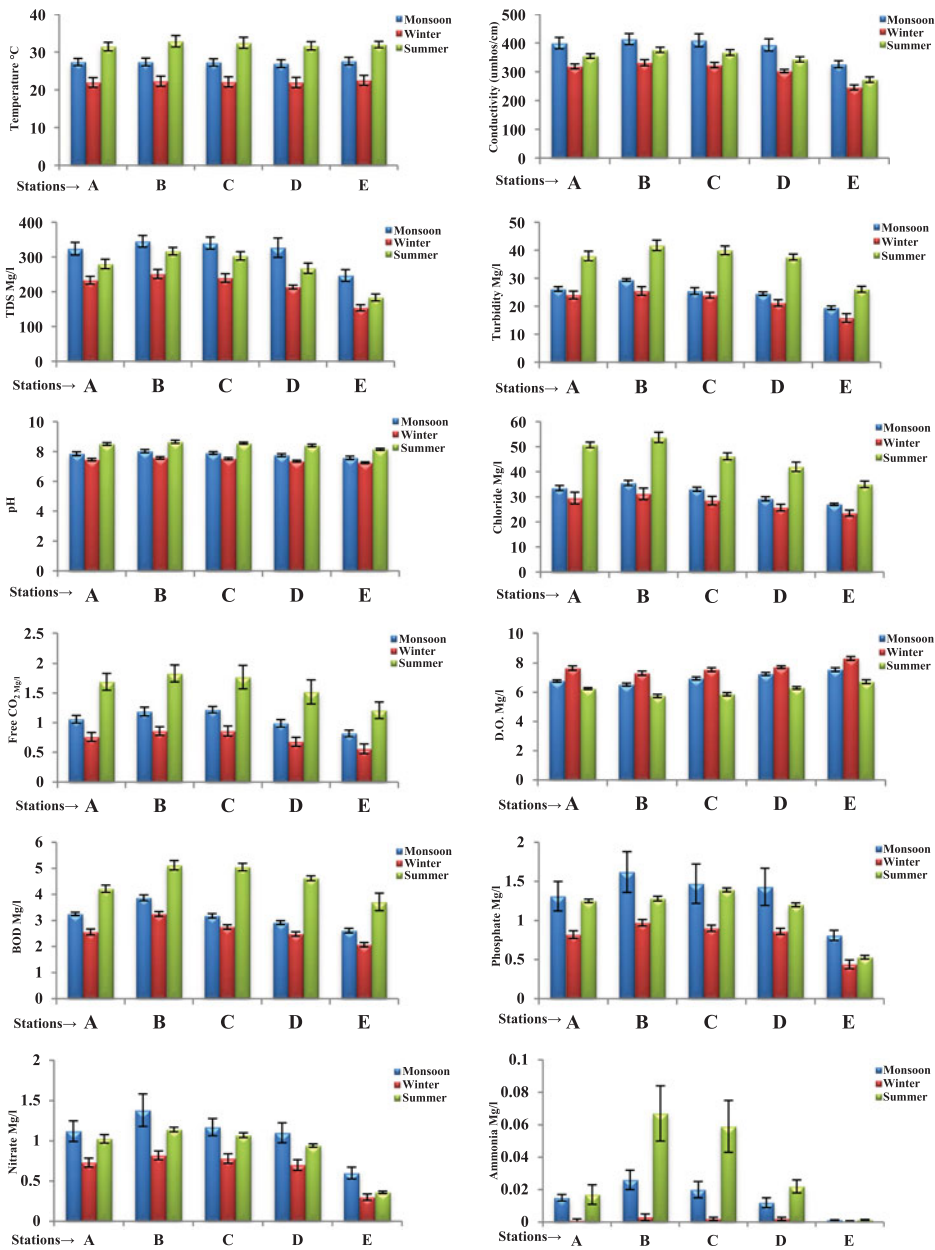
Water temperature is one of the significant parameters, since it encourages the growth and distribution of flora and fauna. Temperature is great important factor for aquatic ecosystem. Water temperature ( $21.9 \pm 1.3^\circ\text{C}$ ) was recorded minimum during the winter season at Station-D while the maximum ( $32.8 \pm 1.5^\circ\text{C}$ ) during the summer season at Station-B (data portrayed in the [Figure 1](#)). The maximum value may be due to higher ambient temperature, intense solar radiation and minimum may be due to low ambient temperature in cold season directly related to water temperature. The present findings are accordance with [13,14].

Electrical conductivity in the water is due to salt present in water and current produced by them. Conductivity was observed lowest during the winter season ( $245.5 \pm 8.5 \mu\text{S}/\text{cm}$ ) at a Station-E and highest concentration was recorded during the monsoon season ( $413.7 \pm 19.01 \mu\text{S}/\text{cm}$ ) at Station-B (data rendered on [Table 1](#) and [Figure 1](#)). The maximum value may be due to inflow during rainy seasons, it's a content domestic waste, sewage content and minimum may be due to expression of low ionic substance and decreased in total dissolved solid range responsible for decreasing the value conductivity in the winter season at station. Similar phenomenon might be responsible for the recorded by [15,16].

Total dissolved solids denote mainly the various kinds of mineral present in the water. Total dissolved solids concentration was observed lowest during the winter season ( $153.7 \pm 8.72 \text{mg}/\text{l}$ ) at a Station-E and highest concentration was recorded during the monsoon season ( $345.2 \pm 16.6 \text{mg}/\text{l}$ ) at Station-B (data portrayed in the [Figure 1](#)). The maximum value may be due to inflow in the lake, Slaughter-house wastes, and decaying matter from the catchment and minimum may be due to lower temperature

**Table 1.** Table showing standard methods given by APHA and NEERI.

Sr. No.	Parameters	Methods	Reference
1.	Water temperature	Water analysis kit	Standard method
2.	Conductivity	Water analysis kit	Standard method
3.	Total dissolved solid	Water analysis kit	Standard method
4.	Turbidity	Nephelometry	[7,8]
5.	pH	Water analysis kit	[7,8]
6.	Chloride	Titrometric	[7,8]
7.	Free CO <sub>2</sub>	Winkler's Azide	[7,8]
8.	Dissolved oxygen	Water analysis kit	[7,8]
9.	Biochemical oxygen demand	Titrometric	[7,8]
10.	Phosphate	Titrometric	[7,8]
11.	Nitrates	Spectrophotometry	[7,8]
12.	Ammonia	Spectrophotometry	[7,8]



**Figure 1.** Physico-chemical parameters seasonal variation in Bhiwapur Lake.

and high water level. Similar findings have been recorded by [17]. TDS was within the permissible level of WHO.

Turbidity of water is due to the presence of suspended substance such as clay, silts, and excellently divided organic and inorganic matter. Turbidity ( $15.86 \pm 1.50$  NTU) was observed minimum during the winter season at the Station-E while maximum ( $41.77 \pm 1.84$  NTU) during the summer season Station-B (data portrayed in Figure 1). The maximum values may be due to low water levels, higher temperature, silt, clay, suspended particles and minimum may be due to settlement of silt, clay. The present

findings are amply supported by [18]. The value of turbidity above the permissible limit accordance with BIS. pH is the term used universally to express the intensity of acid or alkaline condition of a solution. Most of the biological processes and biochemical reactions are pH dependent. pH value ( $7.25 \pm 0.06$ ) was observed minimum during the winter season at Station-E, while the maximum value ( $8.65 \pm 0.10$ ) during the summer season Station-B (data rendered on [Figure 1](#)). The maximum value may be due to increased carbonates and bicarbonate in water and minimum may be due to low turbidity, total dissolved solid and lower water temperature. Similar finding recorded by [19,20]. The pH of the Bhiwapur lake is within the permissible limits of [21–23] for drinking, recreation, agricultural and aquatic life water use (6.5–8.5/9).

Chloride anion is generally present in natural waters. Chloride concentration ( $23.5 \pm 1.19$  mg/l) was observed minimum during the winter season at the Station-E while the maximum value ( $53.7 \pm 2.01$  mg/l) during the summer season Station-B (data rendered on [Figure 1](#)). The maximum value may be due to temperature and consequent evaporation of water from the water body, cattle activity, Human activity and minimum might be due to dilution effect and renewal of water mass alters summer stagnation. Similar trend noticed by [24].

The free  $\text{CO}_2$  is one of the essential parameters of the aquatic ecosystem. The value ( $0.56 \pm 0.08$  mg/l) was observed minimum during the winter season at the Station-E and highest value ( $1.83 \pm 0.144$  mg/l) during the season the summer season at Station-B (data portrayed in the [Figure 1](#)). The maximum value may be due to organic matter input of Slaughterhouse wastes, and anthropogenic activities and minimum may be due to increased aquatic vegetation, lower temperature. These findings corroborated with [20].

Dissolved oxygen is important parameters in water quality assessment and it is reflected on biotic factors in the water D.O. is essential to the metabolism of all aerobic aquatic organisms. The ( $8.32 \pm 0.13$  mg/l) was observed maximum during the winter season at the Station-E and minimum ( $5.75 \pm 0.11$  mg/l) during the summer season at Station-B (data rendered on [Figure 1](#)). The maximum value may be due to lower temperature and minimum may be due to sewage input and manure due to decomposition can reduce D.O level. This study is in accordance with studies by [3,25]. The value of DO within permissible limit accordance with BIS.

Biochemical oxygen demand (BOD) is a topmost parameter that designates the amount of water pollution by the oxidizable organic matter BOD. The value ( $2.07 \pm 0.08$  mg/l) was observed minimum during the winter season at the Station-E while the maximum value ( $5.12 \pm 0.17$  mg/l) during the summer season Station-B (data portrayed in the [Figure 1](#)). The maximum value may be due to higher temperature, decomposition and minimum may be due to lower temperature. Analogous finding recorded by [26]. The value of BOD within permissible limit accordance with BIS.

Phosphorous is measured to be the most important element among the nutrients accountable for eutrophication of an aquatic body, as it is the chief originating factor. Phosphate concentration ( $0.44 \pm 0.05$  mg/l) was observed lowest during the winter season at the Station-E and highest ( $1.62 \pm 0.26$  mg/l) during the monsoon season at Station-B (data rendered on [Figure 1](#)). The maximum value may be due to inflow of



rain water, agricultural runoff, sewage waste, cattle activity domestic waste, Slaughterhouse wastes, anthropogenic activities like cloth washing, bathing, and minimum may be due to uptake for luxuriant growth of macrophytes. This range agrees with the range recorded by [27]. The value of phosphorous above the permissible limit accordance with BIS.

The most chemically stable form of nitrogen is Nitrate. Nitrates are contributing to fresh water through the discharge of sewage, industrial wastes and runoff from agricultural fields. Nitrate concentration ( $1.38 \pm 0.20$  mg/l) was observed maximum during the monsoon season at Station-B while minimum concentration ( $0.30 \pm 0.03$  mg/l) during the winter season at the Station-E (data rendered on Figure 1). The maximum value may be due to the inflow of the lake, domestic waste, agricultural runoff from the catchment area, wastewater containing detergent and minimum may be due to high D.O level. This range is in accordance with the value recorded by [28]. The value of nitrates within permissible limit accordance with BIS.

The most vital source of ammonia in aquatic body is the ammonification of organic matter. Ammonia concentration ( $0.067 \pm 0.01$  mg/l) was observed maximum during the summer season at Station-2 while minimum concentration ( $0.0005 \pm 0.0003$  mg/l) during the winter season at all stations (data portrayed in the Figure 1). The maximum value may be due to The maximum value may be due to inflow of sewage, organic, domestic waste and minimum may be due to low pH due to conversion of ammonia into ammonium ions (which are much less toxic than the gaseous form) decreases its toxicity [29] have observed the findings correlating with these results of the present study.

### 3.1. Zooplanktons

In this study, four major groups of zooplankton were recorded such as rotifera, cladocera, copepoda, ostracoda, and nauplius larvae. Rotifera dominant over all groups recorded 10 species, followed by cladocera and copepoda, 4 species, respectively, and 1 species of ostracoda.

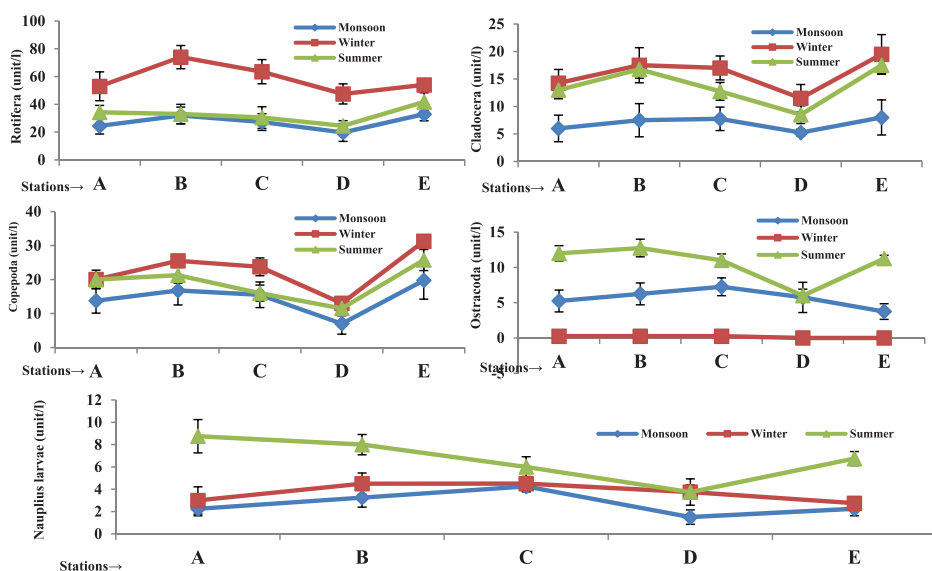
In all the station rotifera, cladocera, and copepoda maximum diversity observed during winter season followed by summer and monsoon and the maximum diversity of ostracoda and nauplius larvae during summer followed by monsoon and minimum during winter. The maximum population recorded at Station B and E (data portrayed in the Table 2 and Figure 2). Similar work observed by [30].

The rotifers, cladocerans, and copepods population density was minimum during the monsoon season and maximum during the winter season followed by summer

**Table 2.** Pearson coefficient of correlation of physico-chemical parameters and zooplankton during July 2012–June 2013.

Parameters → zooplanktons↓	W.T.	Con.	TDS	Tur.	pH	Cl	F.CO <sub>2</sub>	DO	BOD	PO <sub>4</sub>	NO <sub>3</sub>	Am.
Rotifera	0.95	-0.05	-0.013	0.034	0.025	0.28	0.19	-0.24	0.31	-0.13	-0.05	0.038
Cladocera	0.90	-0.37	-0.34	-0.30	-0.07	-0.05	-0.12	0.098	-0.02	-0.45	-0.38	0.07
Copepoda	0.79	-0.48	-0.46	-0.42	-0.21	-0.12	-0.25	0.23	-0.19	-0.58	-0.50	-0.11
Ostracoda	0.60	0.49	0.51	0.53	0.7	0.73	0.69	-0.67	0.61	0.38	0.46	0.66
Nauplius larvea	0.60	0.51	0.52	0.55	0.72	0.75	0.71	-0.69	0.64	0.40	0.48	0.68

Pearson's correlation coefficient between variables significant level of  $p$ -value at  $\leq 0.05$ .



**Figure 2.** Zooplankton seasonal variation in Bhiwapur Lake.

season at all stations of the lake during the study period. The ostracoda and nauplius larvae show minimum population during the winter season and maximum during summer followed by the monsoon season. Same argument had also support to our findings by [5,31,32].

### 3.2. Coefficient of correlation

Pearson's correlation coefficient between zooplankton and physico-chemical parameters such as: Rotifera shows positive with Water Temperature, Turbidity, pH, Chloride, Free CO<sub>2</sub>, BOD, Ammonia and negative with conductivity, TDS, D.O., Phosphate, and Nitrate. Cladocera positive with Water Temperature, D.O., Ammonia and negative with Conductivity, TDS, Turbidity, pH, Chloride, Free CO<sub>2</sub>, BOD, Phosphate, Nitrate. Copepoda positive with Water Temperature, D.O., Ammonia and negative with Conductivity, TDS, Turbidity, pH, Chloride, Free CO<sub>2</sub>, BOD, Phosphate, Nitrate. Ostracoda shows positive with all parameters except Dissolved oxygen. It shows the negative relation. Nauplius larvae shows positive with all parameters except dissolved oxygen, it shows the negative relation (data portrayed in the Table 2). Such a correlation of physicochemical parameters has also been exhibited by several workers like [33–36].

## 4. Conclusion

The physico-chemical parameters fluctuate monthly and seasonally throughout the study period while the zooplankton population mustn't be affected in close to future. This successively ought to guarantee sensible fish production. The physico-chemical condition of Bhiwapur Lake is probably going to be modified because of domestic wastes, shoughterhouse wastes, anthropogenic activities and commercial effluents

dumping into the water. This work is incredibly vital, because of the rising environmental pollution with the time, there's an excellent chance for lake waters to extend their pollution levels nonetheless to return. Therefore, it's most significant to observe consistently the importance of water in Bhiwapur Lake with reference to their hydrobiology for aquatic ecosystem as well as for sustainable well-fare of human beings. Presence of assorted varieties of zooplankton and its availableness throughout the year assure to sensible ecological condition of the lake. The condition of the wetlands is an alarming situation for country and unchecked flow of sewage and factory effluents being the main reasons of pollution.

## Acknowledgements

Authors gratefully acknowledges funding support rendered by R. T. M. Nagpur University, Nagpur for giving Ph. D. research scholar fellowship. Also giving special thanks to Dr. Pravin Charde, Principal, Sevadal Mahila Mahavidyalaya, giving positive direction in the present investigation and kind help during the research work and Dr. Trimurti Lambat provided constant encouragement given.

## Disclosure statement

No potential conflict of interest was disclosed by the authors.

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## ISOLATION AND IDENTIFICATION OF SOIL MYCOFLORA IN AGRICULTURAL FIELD OF SADAK ARJUNI OF GONDIA DISTRICT (MS)

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### ABSTRACT:

Soil samples were collected from different locations of Sadak Arjuni of Gondia District during the months of February 2014 to January 2015 in three intervals. The samples collected in two zones viz. rhizoplane and rhizosphere. The collected samples were inoculated in Potato Dextrose Agar (PDA) and CzapekDox Agar (CDA) medium supplemented by antibiotics such as penicillin and Streptomycin by using Serial dilution method and soil plate method. A total of 230 colonies were isolated. About 19 species belonging to 7 genera of fungi were isolated and identified while 21 strains were unidentified. Identification and characterization of the soil mycoflora were made with the help of authentic manuals of soil fungi. Maximum number of fungal colonies belonged to Ascomycotina and *Deutero mycotina* (191) and few to zygomycotina (18). Among the isolates *Aspergillus flavus*, *A. fumigatus*, *A. nidularis*, *A. niger*, *A. terreus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Rhizopusstolonifer* and *Trichoderma viridae* were predominant. The percentile contribution of the mycoflora was graphically and statistically analyzed.

**Keywords:** Microfungi, Culture Media, Isolation, Fungal Diversity.

### INTRODUCTION:

Soils are extremely complex structures with many constituents playing diverse functions mainly due to the activity of soil organisms (Chiang and Soudi, 1994). Soil microflora plays an essential role in evaluation of soil conditions and in stimulating plant growth (Kiran *et al.*, 1999). Microorganisms are helpful in increasing the soil fertility and plant development as they are included in several biochemical transformation and mineralization activities in soils. Method of cultivation and crop management practices found to have greater influence on the activity of soil microflora (Mc.Gill *et al.*, 1980). Continuous use of chemical fertilizers over a long period affecting soil microflora and thereby indirectly affect biological properties of soil leading to soil degradation (Manickam *et al.*, 1972). There is a massive microbial flora present in the earth and they are found in all types of soils (Mukherji *et al.*, 2006). These microbes may interact with the plants resulting sometimes in useful effect and other times in harmful consequences. Fungi are an important component of the soil microflora and are present in the form of mycelium, rhizomorphs or as spores. They play an important role in soils and plant nutrition. Saprophytic fungi are able to live on dead and decaying organic matter. They secrete a varied number of enzymes that attack effectively any organic material and convert it into simple soluble forms, which are readily available to higher plants. Due to the degradative activities fungi play an important role in recycling organic waste in

environment. Unfortunately their degradative ability also results in the undesirable growth of fungi that destroy useful materials (Aina *et al.*, 2011). Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examinations and biochemical and physiological characterization (Aina *et al.*, 2011).

The species richness of a fungal community and relative abundance of individual species have been considered as measures of functional activities of the group in the particular habitat (Kjoller and Struwe, 1982). Fungi, bacteria and actinomycetes colonize diverse habitats and substrates and play a substantial role in plant health and productivity besides producing diseases. The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem.

### Rhizosphere

The rhizosphere is a micro-ecological zone in direct closeness of plant roots. It is functionally defined as the particulate matter and microorganisms that adhere to roots after being moderately shaken in water. The theoretical extent of the rhizosphere is dependent on the zone of influence of the plant roots and associated microorganisms. The rhizosphere is a metabolically busier, faster moving, more competitive environment than the surrounding soil.



### Rhizoplane

The rhizoplane is the region around the root epidermis and outer cortex where soil particles, bacterial and fungal hyphae adhere. The functional definition is that after the roots have been shaken rapidly in water the remaining microorganisms and soil particles left are considered as belonging to the region of rhizoplane.

There are more numbers of microbes present in the rhizoplane than in the rhizosphere. The diversity of the fungal population is determined by counting the number of colony forming units (CFUs). By spreading the extracted soil microorganisms across agar and counting the number of independent clusters of microorganisms the CFUs were determined. Micro-organisms are abundant where the reliability of the root is compromised. Hence rhizoplane microorganisms tend to be found on older ones rather than younger roots.

### STUDY SITE AND LOCATION:

Sadak Arjuni of Gondia district (MS) located at 21.10°N 80.15°E. It has an average elevation of 256 metres (843 feet). It is located near the Maharashtra Chhattisgarh border on Mumbai-Kolkata National Highway 6. The temperature ranges from 15 - 42°C. Red Sandy soils and Laterite soils are the major soil types existing.

The climate is characterized by a hot summer, well distributed rainfall during the south-west monsoon season and generally dry weather during the rest of the year. Farmers take up first crop of Paddy with monsoon rainfall and a second of Wheat, Gram, Linseed, Sunflower and many more crops with irrigation in Rabi Season.

### METHOD AND MATERIAL:

#### Nutrient Medium Used:

Potato Dextrose Agar (PDA) and CzapekDox Agar (CDA) medium used for isolation of fungi. The pH of the medium was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi.

#### Collection of Soil Samples:

The soil samples were collected from Six different crop fields from Six different locations of Sadak Arjuni. The samples were collected between the months of February 2014 to January 2015 in three intervals. Majority of fungi are microscopic and show huge variation in different sites of collection and at different depths. Therefore soils were collected from a depth of 15cm and are kept in sterilized Ziploc polyethylene bags. Each sample bag was labeled properly by indicating the site of collection, time, date and place of collection. The

collected soil samples along with locations showed in Table:1.

#### Isolation of fungi from the soil samples:

The soil microfungi were isolated by two methods, Soil Dilution and Soil Plate method on Potato Dextrose Agar and Czapek, sDox Agar media.

#### Soil Dilution Plate Method (Waksman, 1922):

1gm of soil sample was suspended in 10ml of double distilled water to make microbial suspensions ( $10^{-1}$  to  $10^{-5}$ ). Dilution of  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  were used to isolate fungi. 1 ml of microbial suspension of each concentration were added to sterile Petri dishes (triplicate of each dilution) containing 15 ml of sterile Potato Dextrose Agar or Czapek, sDox Agar medium. One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth and incubated at  $28 \pm 2^\circ\text{C}$  in dark. The plates were observed everyday up to 4-7 days. Fungi were easily isolated because they formed surface colonies that were well dispersed (Fig: 2), particularly at higher dilutions.

#### Soil Plate Method (Warcup, 1950):

Almost 0.005g of soil was dispersed on the bottom of a sterile petri dish and molten cooled ( $40-45^\circ\text{C}$ ) agar medium (PDA) and (CZA) was added, which was then rotated gently to scatter the soil particles in the medium. The Petri dishes were then incubated at  $28 \pm 2^\circ\text{C}$  in dark for 4-5 days. One isolate of each fungal genus from each soil sample were selected at random for further subculturing and experiments. The subcultures were maintained on Potato Dextrose Agar.

#### Inoculating Techniques:

The working benches in the laboratory were thoroughly sterilized by swapped with 70% alcohol, and also a burning blue flame was allowed to sterilize the surrounding air before the inoculation proper. The conical flasks were corked tightly with cotton wool and the Petri dishes were fully autoclaved (Aina *et al.*, 2011).

#### Identification of the Soil Fungi:

Generally identification of the fungal species is based on morphological characteristics of the colony and microscopic examinations (Diba *et al.*, 2007). The colony growth which includes length and width of the colony, the presence or absence of aerial mycelium, the color, wrinkles furrows and any other pigment production were the macro morphological characters evaluated. Although molecular methods continue to improve and become more rapidly available, microscopy and culture remain commonly



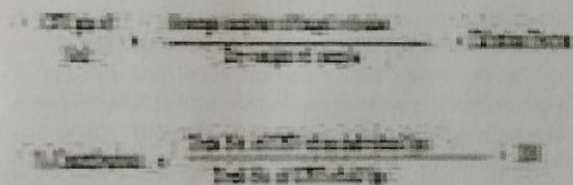
used and essential tools for identification of fungal species (Diba *et al.*, 2007). The fungi were identified with the help of standard procedure and relevant literature (Gilman, 2001; Nagamani *et al.*, 2006).

#### Staining Technique for Fungi:

Inoculating needles were flamed over the burning Bunsen burner. Then using the needle, a small portion of the growth on the culture plate was transferred into the drop of lacto phenol in cotton blue on the slide. The specimen was teased carefully using inoculating wire loops to avoid squashing and over-crowding of the mycelium (Aina *et al.*, 2011). The specimen is observed under the microscope for microscopic identification (Fig: 4).

#### Statistical Analysis:

Population density expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factors. The CFU and Percent Contribution of each isolate was calculated by –



#### RESULT AND DISCUSSION:

Soil microorganisms act as vital determinants of plant community variety and productivity (Wardle *et al.*, 2004). The environmental factors such as the soil pH, moisture, temperature, organic carbon and nitrogen play an important role in the distribution of mycoflora (Gaddeyya *et al.*, 2012). These are the main factors affecting the fungal population and diversity. The soil mycoflora in six different rice fields viz., Sadak Arjuni, Wadegaon, Kohmara, Sawangi, Saundad and Tidka were observed. Soil Dilution Plate and Soil Plate methods were used for the isolation of fungi during the present investigation.

During a period of 12 months the total number of fungal colonies isolated on Petri plates containing PDA medium were 229. As stated earlier Soil dilution plate and Soil plate method were employed for the isolation of fungi during the present investigation. A greater number of species and colonies were isolated on soil plates than on dilution plates and further the total number of species isolated decreased with increased dilutions of the samples. The purification of the culture (Fig: 3) was

done either by single spore isolation or by culturing of the hyphal tips and was transferred to fresh agar slants of CDA medium. Most of the fungal forms which sporulate heavily were abundant on dilution plates.

Fungi act as major decomposers of dead organic matter and contribute significantly in recycling of nutrients in natural and modified ecosystems (Gadd, 2004). Altogether six soil samples from six different locations were examined for fungal diversity. The study resulted the presence of 19 species of fungi were identified and characterized from PDA plates (Table: 2). the maximum fungal species belonged to Ascomycotina and Deuteromycotina (191 colonies) and Zygomycotina (18 colonies) and 21 colonies were left unknown on the plates containing PDA medium (Table: 2)

PDA medium is the most frequently used culture media and was stated to be the best media for mycelia growth by several workers worked with it earlier (Maheshwari *et al.*, 1999; Saha *et al.*, 2008), due to its simple formulation and potential to support wide range of fungal growth. CDA medium preferred for pure culture to minimize the contamination of *Rhizopusstolonifer*. Characterization of the isolates up to genus level and to the species level was made based on the macromorphological (Colony characters) and micro-morphological characters by using authentic manuals of soil fungi.

Our findings are in accordance with the results of Noor Zaman *et al.*, (2012) as the microbial analysis of different samples in rainfed areas of Punjab, Pakistan. They isolated genera like *Aspergillus*, *Alternaria*, *Curvularia*, *Fusarium*, *Penicillium* and *Rhizopus*. Similar genera were isolated during our investigation. These findings were similar to those isolated by Rasheed *et al.*, (2004). *Aspergillus* species particularly like *A. flavus* and *A. niger*, *Penicillium* and *Rhizopus* were isolated only from the soil where as *Alternaria alternata*, *Curvularia lunata* and *Fusarium* species were obtained from both soil and plant parts. Hence it is considered that isolation of soil samples yielded more fungal species than from plants (Noor Zaman *et al.*, 2012).

In our investigation among the obtained fungal isolates the genera *Aspergillus*, *Fusarium*, *Rhizopus* and *Penicillium* were dominant on media used (Tables: 2). The most common isolates among them viz., *A. candidus*, *A. flavus*, *A. fumigatus*, *A. granulosis*, *A. nidulans*, *A. niger*, *A. ochrasius*, *A. terreus*, *Curvularia clavata*, *C. lunata*, *C. lunata*,



*Fusarium oxysporium*, *F. solani*, *Penicillium chrysogenum*, *P. digitatum*, *Rhizopus stolonifer*, *R. oryzae*, *Trichoderma hamatum*, *T. viride* were isolated and characterized.

The percent contribution of different soil mycoflora of all the six Rice fields was evaluated. The fungi were mostly observed in the months of June to October as it has been reported that the diversity of fungal population occurred during the monsoon season where the soil moisture was significantly high (Bissett & Parkinson, 1979; Deka & Mishra, 1984).

Isolation of fungal species from soil samples by repeated screening and plating on starch agar medium by Ratnasri *et al.*, 2014 yielded *Aspergillus fumigatus* and *A. niger* along with other fungal species. *Fusarium solani* was isolated from the soils of infected fields and showed 100% frequency (Javed *et al.*, 2008). Recent study on Soil Microflora in National Parks in Gujarat yielded fungal species like *Aspergillus niger* and *Fusarium* species (Megha *et al.*, 2015). The conservation of diversity of mycoflora in agricultural fields becomes very essential for the development of sustainable agriculture (Gaddeyya *et al.*, 2012). Natural and anthropogenic disturbances can alter the species composition or may have negative effect on species diversity of the decomposer fungi (Dong *et al.*, 2004). These changes may directly or indirectly affect the vital functions of the soil such as decomposition and mineralization and may result in disturbances. Graphical representation of percent contribution of fungal species diversity in various paddy fields on the media PDA is represented in (Graph:1).

The studies on fungal diversity and percentile contribution and periodic occurrence of soil mycoflora are useful for farmers, agronomists, researchers and microbiologists for future activities in the view of conservation of soil ecosystem, conservation of soil microbial diversity and sustainable agriculture (Gaddeyya *et al.*, 2012).

#### CONCLUSION

In the present study the soil sample of six different paddy fields of viz; Sadak Arjuni, Wadegaon, Kohmara, Sawangi, Saundad and Tidka studied for detecting the fungal diversity. The greater fungal population was observed mostly in the monsoon season as the soil moisture was high. Among the isolates *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichoderma* were dominant in all agricultural fields of all areas mentioned due to high sporulation and production of bacterial antibiotics from the

*Penicillium* species and production of different types of toxins from the *Aspergillus* species may prevent the growth of other fungal species. This study is an effort to understand the soil microbial diversity in the agricultural fields of Sadak Arjuni as soil microflora not only plays an important role in decomposition and contribute to biogeochemical cycling but also are responsible for the prevalence of diseases in the crop fields.

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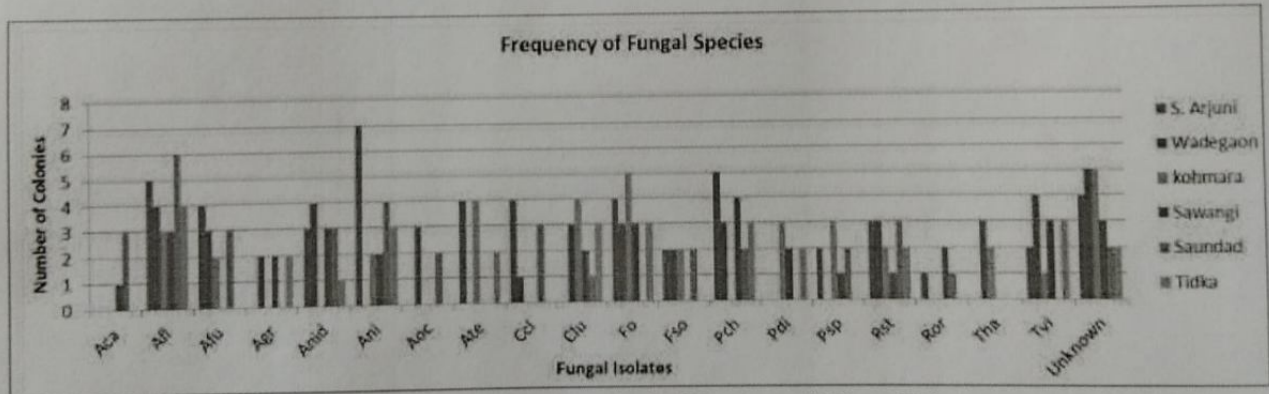
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Sample No.	Agricultural field	Place
1	Paddy	SadakArjuni
2	Paddy	Wadegaon
3	Paddy	Kohmara
4	Paddy	Sawangji
5	Paddy	Saundad
6	Paddy	Tidka

Table 1: Agricultural soil samples collected from different places in SadakArjuni Tehsil

Sr. No.	Rice Fields	Total No. of Colonies	Average Number of Individual Colonies																			
			Aspergillus								Curvularia		Fusarium		Penicillium		Phoma	Rhizopus		Trichoderma		Unknown
			Aca	Afl	Afu	Agr	Anid	Ani	Aoc	Ate	Ccl	Clu	Fo	Fso	Pch	Pdi	Psp	Rst	Ror	Tha	Tvi	
1	S. Arjuni	50		5	4		3	7		4	4		4	2	5		2	3	1		2	4
2	Wadegaon	43		4	3	2	4		3		1	3	3	2	3		3			3	4	5
3	Kohmara	38		3	2			2		4						3	3	2		2	1	5
4	Sawangji	32	1	3		2	3	2				2	3		4	2	1	1	2		3	3
5	Saundad	37	3	6	3		3	4	2		3	1		2	2		2	3	1			2
6	Tidka	30		4		2	1	3		2		3	3		3	2		2			3	2
Total		230	4	25	12	6	14	18	5	10	8	13	18	8	17	7	8	14	4	5	13	21
% Contribution			1.7	10.8	5.2	2.6	6.08	7.8	2.1	4.3	3.4	5.6	7.8	3.4	7.3	3.04	3.4	6.08	1.7	2.1	5.6	9.1

Table 2: Frequency of Mycoflora in different Agricultural Fields as on Potato Dextrose Agar Medium



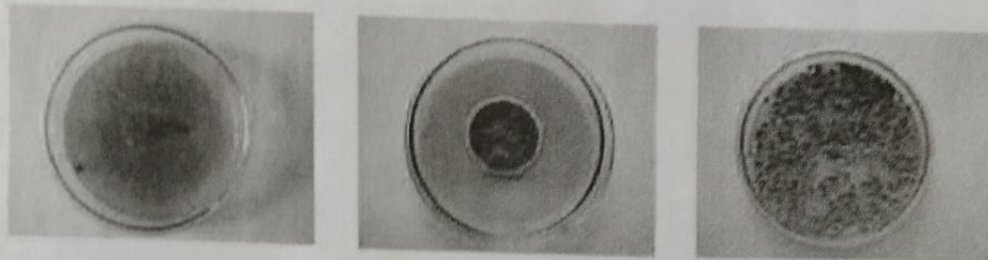
Graph 1: Frequency of Fungal species in different crop fields on PDA

Aca:-*Aspergillus candidus*, Afl:-*A. flavus*, Afu:-*A. fumigatus*, Agr:-*A. granulosis*, Anid:-*A. nidulans*, Ani:-*A. niger*, Ate:-*A. terreus*, Afu:-*Curvularia clavata*, Afu:-*C. lunata*, Afu:-*C. lunata*, Afu:-*Fusarium oxysporium*, Afu:-*F. solani*, Afu:-*Penicillium Chrysogenum*, Pch:-*P. chrysogenum*, Pdi:-*P. digitatum*, Rst:-*Rhizopus stolonifer*, Ror:-*R. oryzae*, Tha:-*Trichoderma hamatum*, Tvi:-*T. viride*

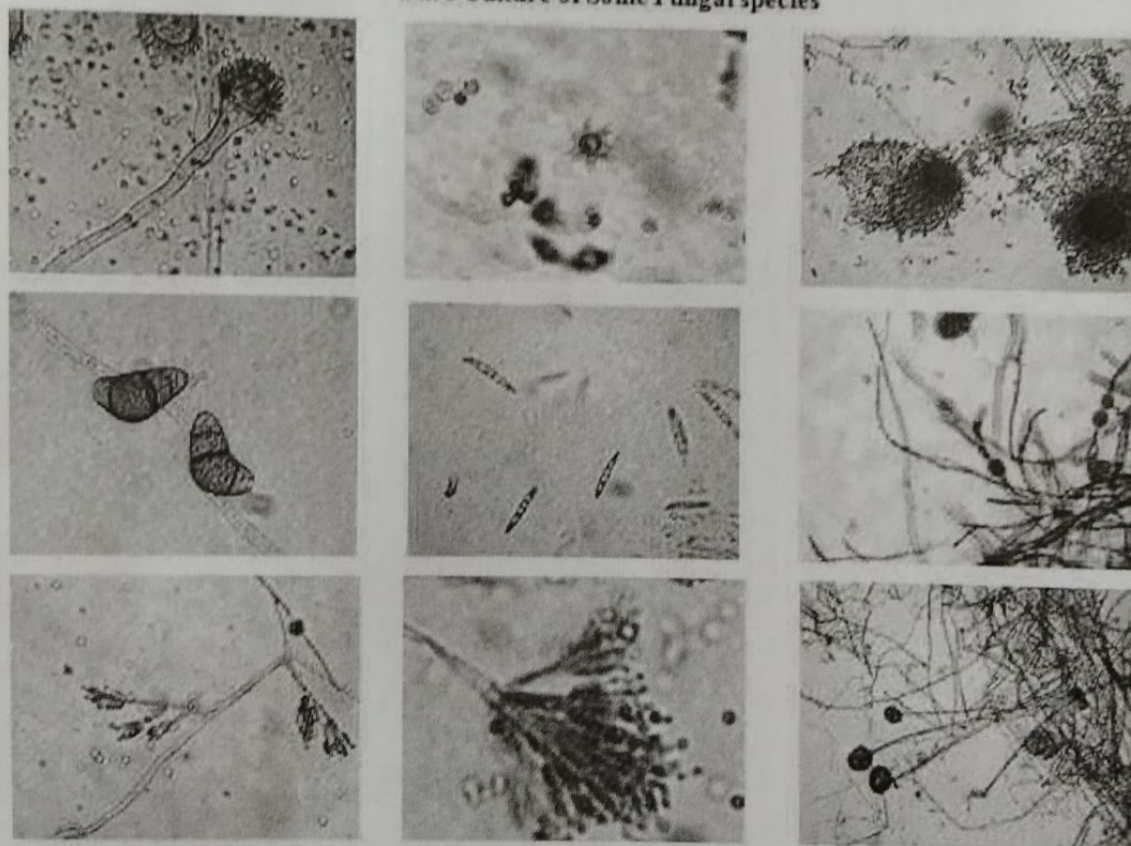




**Fungi Isolated by Soil Dilution Method**



**Pure Culture of Some Fungal species**



**Fig.4: Some of the isolated fungal species**

# FLORAL BIOLOGY OF 'WILD SNAKE ROOT' (*RAUVOLFIA TETRAPHYLLA* L.)

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**Abstract :** *Rauvolfia tetraphylla* L. (Apocynaceae) is a small perennial woody shrub with huge medicinal properties flowers more or less throughout the year. Flowers are small, white to creamy white born on apical portion of branches in umbellate cyme. They are hermaphrodite with small floral tube comprising nectar deep at the base of the corolla tube and introse anthers form cone around the apex of style head where pollens are deposited. Anthesis occurs during 06:30 to 08:30 h and anther dehisced between 07:30 to 09:00 h on the day of anthesis. Flowers survive for one day and are self-incompatible. Pollination is mainly brought about by insects (entomophily).

**Keywords:** Anthesis, self-incompatible, *Rauvolfia tetraphylla*

## INTRODUCTION

*Rauvolfia tetraphylla* L. is commonly known as Wild snake root, Devil-pepper or Still tree belongs to family Apocynaceae, growing as a perennial woody shrub. It has been cultivated as an ornamental and medicinal plant in India (Farooqi and Sreeramu, 2001). The plant has various significances and it is extensively used by Indians as a substitute or adulterant of *R. serpentina*.

*Rauvolfia tetraphylla* L. have huge therapeutic properties due to presence of about 30 alkaloids in the root, stem, leaves and fruits. Out of them Reserpine is pharmacologically highly effective. Also reported alkaloids are ajmalicine, reserpinine, sarpagine, deserpidine, rescinnamine, serpentine, ajmalidine, alloyohimbine, chandrine, corynathine, iscajmaline, neo-ajmaline, papaverine, reserpoxidine, serpinine, thambine and yohimbine (Farooqi and Sreeramu 2001; Mukherjee 2004).

Due to the huge medicinal properties of the plants, it is important to study the floral biology which will be supportive for hybridization programme and the information of floral biology is a necessity in assessing overall reproductive potential of the species.

## MATERIAL AND METHODS

**Study site-** Study was conducted in small field located in Sadak Arjuni of Gondia district of Maharashtra (21°10'N 80°15'E, 256m msl). Temperature ranges from 30 – 45°C in Summer and 12 – 30°C in winter. Sadak Arjuni receive 1296mm average annual rainfall and relative humidity highest during rainy season. A field was prepared by planting saplings of about 2- months old collected from nursery stock of Manas Ayurveda Nagpur.

**Flower morphology-** Position of flower in inflorescence, structure of flowers, structure of separate floral parts, position of nectary were studied. Twenty flowers (n=5 per plant) were used for this study and observed with Olympus stereoscopic microscope. They were dissected to locate anthers, stigma, ovary and ovules. Photomicrographs were taken using Canon Digital Camera.

**Flowering phenology-** The plant species under study were visited on each day. The flower phenology was determined by visual observations commenced at the beginning of flowering and continued until fruiting (Mark and Francoise, 2005). The initiation of anthesis, anther dehiscence, nectar production and termination of flower, flowering period were noted. Dates of mediocre and peak flowering were observed by visual observations. Longevity of flower determined by recognizing the time of opening and shedding.



**Pollen Productivity-** The total number of anthers per flower was counted and a single anther was randomly selected to estimate the total pollen number per stamen. To determine pollen productivity undehisced mature anthers from the flower buds were collected from different plants. Pollen productivity per flower was determined by simple method (Nair and Rastogi, 1963).

The undehisced mature anthers were crushed in 5ml of 50% glycerine in a graduated test tube. The plastic dropper was standardized and the pollen per drop were counted by adding one drop of suspension on a slide and covered by a cover glass. From this the mean pollen production per flower was calculated.

**Pollen morphology-** Pollen morphology studied by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The pollen grains were acetolysed by the method of Erdtman (1960). Acetolysed pollens for light microscopic observations were stored in glycerine and those for SEM examination were stored in absolute alcohol. For LM study acetolysed pollen grains mounted on glycerine jelly and observed under the light microscope. The size of the pollen grains was measured by using standard calibrated ocular micrometer.

For SEM analysis, acetolysed pollen grains were placed directly to stubs with double sided adhesive tape and sputter coated with gold, photomicrographs were taken using Scanning Electron Microscope (Carl Zeiss EVO 18).

**Pollen-Ovule Ratio-** The pollen ovule ratio was calculated dividing the average number of pollen grains produced per flower by the number of ovules in the flower (Cruden, 1977). Ovule number was achieved from dissection of ovaries under stereoscopic microscope.

#### **Pollen Viability**

**Stainability in 1% Acetocarmine-** Mature but undehisced anthers were squashed in a drop 1% Acetocarmine stain and incubated for 5 min. The slides were observed under microscope, all deeply/completely stained pollen grains were considered viable and unstained were non-viable (Qureshi *et al.*, 2009). Duration of pollen viability determined by repeated the same procedure from 0600, 0800, 0900, 1000, 1200, 1400 and 1600hr.

**In-vitro pollen germination-** We follow sitting drop method for *in-vitro* pollen germination study. Fresh pollen grains from undehisced but mature anthers were squashed in different concentration of Sucrose i.e. 1%, 2%, 3%, 4%, 5% to 20% along with 200 ppm of Boric acid (Shivanna and Rangaswamy, 1993). The prepared slides incubated for 24 hrs in humid chamber (made by keeping moist filter paper in covered petriplates) and observed for germinated pollen grains.

**Stigma Receptivity-** The receptive stigmas were appeared to be wet, shining and turgid when observed through hand lens (10X). Subsequently a definite period of time, it became dry and blackish in colour, representing the loss of receptivity. Also stigma receptivity was estimated through peroxidase activity by using a 3% H<sub>2</sub>O<sub>2</sub> solution and examined under a stereoscope (Dafni and Maues, 1998; Etcheverry, 2005).

Further the receptivity and duration of stigma were determined by confining the activity of non-specific esterases (Mattsson *et al.*, 1974), and peroxidases (Galen *et al.*, 1985), at one day before and on the day of anthesis (flower opening).

**Artificial pollination-**Artificial pollinations viz, autogamy, geitonogamy and xenogamy were carried out to determine the type of pollination took place in plant. Experiment conducted at 07:00 to 08:00 h followed by bagging during peak flowering season. After time for fruit set passed, bags were opened and percentage fruit set and number of fruits and seeds recorded.

## **RESULTS**

**Plant morphology-** Plant is perennial, small woody shrub belonging to family Apocyanaceae. It is grows nearly 2m (6 feet) in height. Stem is dichotomously branched. Leaves are sub-sessile, arranged in verticillate phyllotaxy having of 3–4 leaves at each node. The shape of leaf lamina is varying from ovate, narrowly

ovate to oblong. Leaves become membranous, base broadly cuneate to round with acute or obtuse apex (Fig. 1A).

**Floral morphology and pollination mechanism-** Inflorescence born on terminal and axillary position of branches and comprises of 4 - 10 flowers in umbellate cyme. Flowers are small, pedicillate, complete and hermaphrodite with small floral tube comprising nectar deep at the base of the corolla tube. Petals white in colour, corolla tube urceolate, 3–4 mm long, hairy inside at distal half and lobes ovate or suborbicular. Five epipetalous stamens inserted at corolla throat. Anther introse form cone around the apex of style head where pollens are deposited (Fig. 2-A,B). Anther dehisces longitudinally. The Ovary is superior with bicarpellary, syncarpous, bilocular with 2 ovules in each locule (Fig. 2C). Ovaries connate, style filiform, stigma is wet, papillate and dumbel shaped/capitat. Nectar secretion was started about one hour before the opening of flower and produces continuously throughout anthesis. Drupes subglobose, 5–10 mm in diameter, glabrous, connate. 2 Seeds per fruit. Flowering almost throughout the year but peak flowering occurs during the months of early March to late April and middle of June to middle of August.

For estimation of the amount of nectar, flowers were randomly selected from different plants and bagged just before opening to prevent floral visits. They were excised at hourly intervals (N=15) and the amount of nectar was determined using calibrated microcapillary tubes. Anthesis and anther dehiscence was observed in the field using hand lens, following the method of Reddi & Janaki Bai (1981) and Mathur & Mohan Ram (1986).

As the corolla tube short, the flowers of *R. tetraphylla* offers both nectar and pollens to the pollinators it shows generalized mode of pollination and took place by insects (Entomophily).

**Floral phenology-** Flowering in *R. tetraphylla* occurred almost throughout the year under climatic conditions of Sadak Arjuni. But peak flowering occurred during early March to late April and middle of June to middle of August when ambient temperature ranges from 31.4°C to 39.7°C maximum and 20.2°C to 27.8°C minimum. The flower buds take 10-15 days from initiation to full bloom. Mediocre flowering persisted for about four weeks from last week of June to last week of July when maximum and minimum temperatures ranged between 29.2°C - 37.3°C and 20.5°C - 28.9°C, respectively. From the month of June rainy season starts in study area, plants gets sufficient moisture and humidity so it is most favorable period for sprouting of new buds as well as flowering of *R. tetraphylla*. But due to the heavy rainfall during July and August, soil becomes more moistened, that affects the plant and leads to the decline of flowering which was started from end of August. During this period plants were seen with an abundant number of fruit. Number of flowers per plant reduced greatly after September.

Flowers started opening in the early morning during 06:30-08:30 h when ambient temperature fluctuated between 21.2° – 30.4°C (Table 1). Anthers dehiscence occurred just after the anthesis at 07:30 – 09:00 h. The longevity of flower is 1-day.

**Pollen:Ovule ratio-** A flower of *R. tetraphylla*, have 5 anthers and single ovary. Each anther contained an average number of 460 pollen grains, therefore single flower has an average number of pollen is 2300 (n = 20). Pollens are round and circular/spherical in shape having tricolpate aperture (Fig. 1-E,F,G). They are smaller in size with 41.83µm in diameter. The ovary has average 4 ovules (n = 20) (Fig. 2C), so the pollen-ovule ratio is 575:1.

**Pollen viability-** The maximum viability of pollen grains of *R. tetraphylla* observed at the time of anthesis to six hours after anthesis (ranges from 81 to 86%). After that it declined and virtually low after 8 hours and very low after 12 hours (Fig.1B-C).

**In-Vitro pollen germination-** *In vitro* pollen germination (Fig.1D) was detected from 5% to 10% sucrose supplemented with 200 ppm boric acid. But maximum germination found in 6% sucrose + 200 ppm boric acid. Although the percentage of pollen grain germination was very low.

**Stigma receptivity-** Stigma remained receptive almost 1-hour before the opening of flowers and became fully receptive for about six hours after the anthesis. Thereafter, receptivity of stigma declined. Stigmatic receptivity also determined by location of enzyme activity on surface of stigma – 1. Before anthesis (one day before opening of flower) and 2. After anthesis (on the day of opening of flower).

**I. Esterase activity-** Esterase activity was confined by the presence of a brown precipitate uniformly spread across the surface of stigmas of pistils excised from flowers before anthesis (Fig. 2E). It also detected in style. Enzyme esterase activity found to be more in stigma and style after anthesis produced more intense dark brown colour (Fig. 2E-F).

**II. Peroxidase activity-** Peroxidase activity was detected as a yellowish-orange precipitate in the papillar cells of stigmas of flowers. Result indicated that Peroxidase activity found more in stigma after anthesis. No reaction was detected either in the sub-papillar cells or in the styles of these flowers (Fig. 2G-H).

**Floral rewards-** The flowers of *R. tetraphylla* (Wild Snake Root) offered both nectar and pollen to the visitors. Nectar secretion started 1-hour before the anthesis (05:30 - 06:30 h) and it oozes continuously in minute quantity all over a day.

**Artificial pollination-** Various artificial pollination experiments were performed for determination of type of pollination (Cross/Self-pollination) favoured in flowers (Table 2). Three types of pollination treatment given to flowers  $n = 50$  in each case. 50 marked open/natural pollinated flowers carefully observed for percent fruit set. 74% fruit set was detected in natural pollination (open-pollination), however 58% fruit set was observed through manual geitonogamy and 82% fruit set from xenogamous pollinations and there is only 4% fruit-set by autogamous self-pollination.

The fruit development took 22-28 days for attaining ripeness after fertilization. Each fruit commonly contains two seeds. The seed germination percentage in natural habitat was approximately 25-30%.

## DISCUSSION AND CONCLUSION

The flowering in *Rauwolfia tetraphylla* occurred throughout the year but timing of blooming and anthesis were varied in summer and winter season. This due to factors like photoperiod, light intensity, temperature, moisture supply including ambient humidity, soil moisture and nutrient supply. Similar findings also reported by Sihag (1982).

The pollen production and availability of pollen to the receptive stigma is an indispensable necessity for achievement of pollination. We observed average number of 2300 pollen grains per flower. But our result fluctuated from the previous study on *R. tetraphylla* done by Subbu *et al.*, (2008) they reported 9280 pollen grain per flower. Single ovary contained 2 ovules per flower under the climatic conditions of Sadak Arjuni (Gondia district). But there were initially 4 ovules found in flower, 2 in each locule (Ovary bilocular). 2 ovules get aborted (1 from each locule) during the developmental stages by unknown cause and hence ovules number reduces to 2 (1 in each locule) (Fig. 2C-D). Abortion of ovules started after 3-4 days of fertilization. Similar result also found in Sarpagandha (*R. serpentina*) by Sihag and Wadhawa (2011).

Pollen germination is the first significant morphogenetic event in the pollen in the direction of accomplishing its release of male gametes in the embryo sac. The stigma provides an appropriate site for pollen germination, however studies on in vivo are not easily possible because of the obstacles involving in pistillate tissue (Biswas and Mondal, 2014). In *R. tetraphylla* maximum pollen germination occurred in 6% sucrose + 200 ppm boric acid solution.

Stigma receptivity is the capability of stigma to support the viable and compatible pollen to germinate. The time and duration of stigma receptivity should be accompanied for successful breeding of crops (Stone *et al.*, 1995). In general at the time of anthesis, the stigma is receptive and may lose for one, two or several days (Shivanna *et al.*, 1997; Kalinganire *et al.*, 2000). Here highest receptivity of stigma was found from an anthesis time to 6 hrs after the anthesis, afterwards it became declined. It is also confirmed by



peroxidase and esterase enzyme activity. Both peroxidase and esterase enzyme activity higher at the day of anthesis which indicates that stigma become most receptive.

Flowers of *R. tetraphylla* offered pollen and nectar to pollinators. Nectar secretion started one hour before the time of anthesis and oozed in minute quantity throughout a day. Generally, the nectar secretion depends on the physiological state of the plant (Huber, 1956). But even in healthy and well-nourished plants, nectar production shows a marked autonomous rhythm that corresponds to the periodicity of the pollination process.

Viability of pollen grains and stigmatic receptivity were highest at the time of anthesis i.e, both pollen grains and stigma mature at the same time. But Autogamy could not take place because self-pollen unable to fertilized ovary. This indicated that there is self-incompatibility found in flower and due to this self-pollination prevented and cross pollination permitted. Similar results also reported in *R. micrantha* (Kullooli and Sreekala, 2009). From the observation and results it noticed that the plants favour geitonogamy but autogamy occurred very rarely. While observing of stigma of bagged flowers it seemed that pollen germination was very less on the surface of stigma. So this may be one of reason for inhibiting self-pollination.

From above observation it is concluded that, cross-pollination (xenogamy) boosted the quantity of fruit-set in *Rauvolfia tetraphylla*. Also this study has provided valuable information on the floral biology and mode of pollination of *R. tetraphylla*.

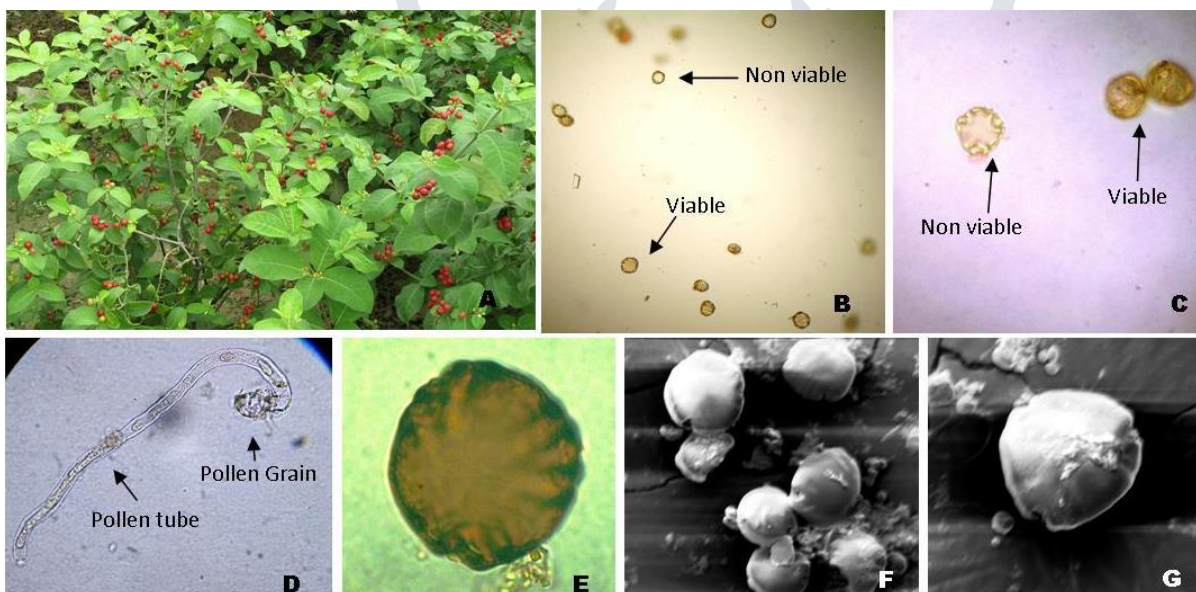
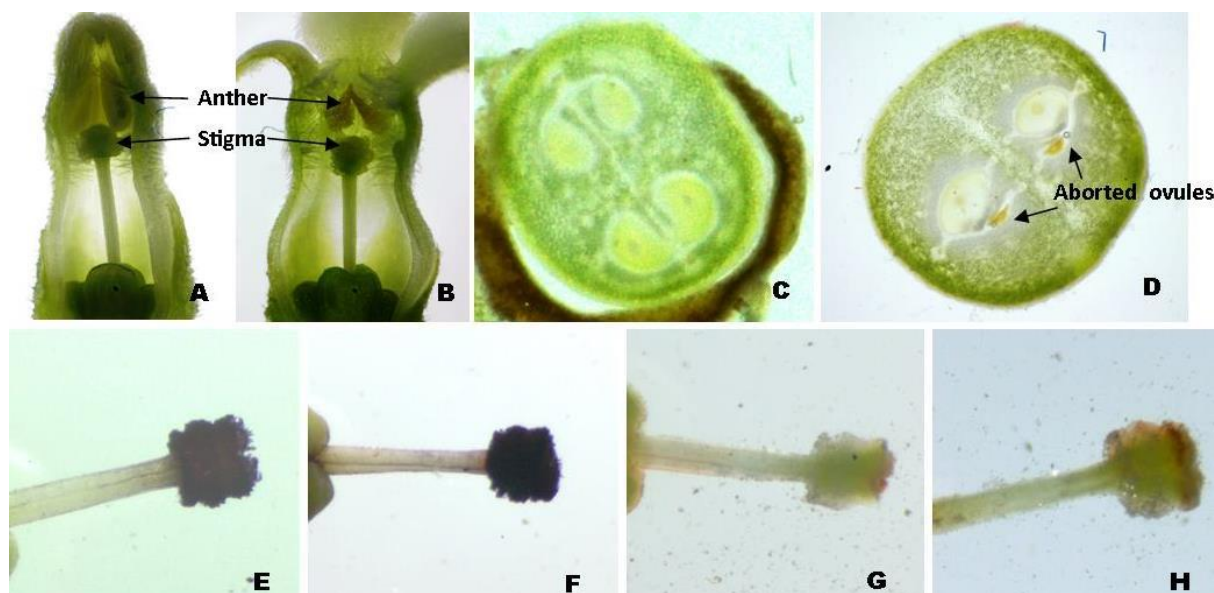


Fig.1-A. Plants with full bloom and fruits, B-C. Pollen viability by 1% Acetocarmine stain, dark red stained pollens are viable, poorly stained (light coloured) - Nonviable D. Germinated pollen grain, E. Acetolysed pollen grain, F-G. Scanning Electron Micrograph of pollen grain



**Fig. 2- A-B.** Longitudinal Section of flower showing position of anthers and stigma - A. Unopened, B. Opened flower, **C-D.** Transverse Section of Ovary - C. Ovary showing 4 ovules D. Ovary showing 2 healthy and 2 aborted ovules, **E-H.** Enzyme activity on stigma - Esterase activity on stigma: E. One day before anthesis, F. On the day of Anthesis, Peroxidase activity on stigma: G. One day before anthesis, H. On the day of Anthesis

**Table 1- Floral characters of *Rauwolfia tetraphylla***

S.N.	Floral Characters	Observations
1	Flowering period	Throughout the year but peak during - March to April and middle of June to middle of August
2	Number of inflorescences on a plant	30 - 94
3	Number of flowers in an inflorescence	4 - 10
4	Flower type	Pentamerous, hermaphrodite, actinomorphic
5	Flower colour	White to Creamy White
6	Flower opening time (Anthesis)	06:30 - 08:30hr
7	Nectar	2 - 3 $\mu$ l
8	Nectar secretion	Started 1 hour before the time of anthesis and secreted continuously in minute quantity throughout a day
9	Number of anthers/flower	5
10	Anther dehiscence mode	Longitudinal slit
11	Anther dehiscence time	07:30 - 09:00 hr
12	Average no of pollens/anther	460
13	Mean no. of pollen grains/flower	2300
14	Number of ovaries/flower	1
15	Mean no. of ovules/flower	4
16	Pollen /ovule ratio	575:1
17	Pollen shape	Round and circular/spherical
18	Pollen aperture	Tricolpate



19	Pollen size	41.83 µm in diameter
20	Stigma type	Wet, calyptriform and papillate type
21	Stigma receptivity	78 - 83 %
22	Pollen viability	81 - 86%
23	Fruit type	Drupe
24	Longevity of flower	1 day

**Table 2- Details of pollination treatments, percentage of fruit set and number of seeds per fruit**

S.N.	Treatment	Total Number of fruit set	Fruit set percent	No. of seeds per fruit
1	Autogamous self pollination*	2	4%	2
2	Geitonogamy (pollen of different flower of same plant)*	29	58%	2
3	Xenogamy (pollen of different flower of another plant)*	41	82%	2
4	Open (Natural) pollination*	37	74%	2

\* (n = 50)

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# Self Help Group- An Instrument to Rural Women for Sustainable Development

## (A study of Bhandara District of Maharashtra State in India)

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### Abstract

A buzzword in India today is Women Empowerment. India is committed to the empowerment of women. A woman plays a very vital role in every criteria of development. Sustainable development depends on equal distribution of resources for present and future, can be achieved only through gender equality. Woman empowerment is a key factor for economic growth, social development and environmental sustainability. Self Help Group is a well-known concept. An instrument helps a woman to develop on her own. The more attractive scheme with less effort is Self Help Group. It is a tool to remove poverty and inequality. It also helps in maintaining health and saving environment from degradation. The paper analysis the development of Bhandara district Women through Self Help Group.

Keywords: Women, Empowerment, Sustainable Development, Self Help Group.

### Introduction

Mohammed Yunus, a Bangladeshi social entrepreneur, banker, economist and civil society leader awarded with the Nobel Peace Prize for founding the Grameen Bank and pioneering the concepts of microcredit and microfinance, formed Self Help Group in 1975. SHG were introduced in India in 1986, but came into full force only after 1991. This SHG popularly named **MAHILA BACHAT GUT** is a very powerful tool and a scheme with less finance for rural **women** to remove poverty and improve rural development. Mahila Bachat Gut is a part of Self Help Group (SHG). Mahila a women, Bachat means saving and Gut means a group. Combined together it comes to **WOMENS' SAVING GROUP**.

A Rural Women is a woman who lives in villages or remote area always shares the primary responsibility for food, child and household management very sedately. They also help in environmental Management. In India especially in rural areas women plays a major role as farmers, animal's tenders, and water and fuel collectors.

Self Help Group helps a Women adequately represent herself in the decision making process related to the issues of environment and development at local, national and international levels.

Food, Shelter and Cloth as said are the basic needs of living life for a human being, very truly said, but it's been observed that as the income increase the basic needs also grows. These basic needs are full filled in every manner in every rural area. The need is of living quality life, which includes foundation of well being and opportunities to be recognized. A human nature, as soon as one want or desire is full filled another crops up. Everyone wants to live a standard life, a level of continuation, comfort and wealth been enjoyed by a community, class or individual in everyday life. However, to avail all necessities money is required which can be accumulated by saving. Through SHG a women learns how to save money and were to invest.

### About Bhandara District

Bhandara district is an administrative district in the state of Maharashtra in India. After the formation of Maharashtra in 1960, Bhandara evolved as a district of the state. It included 15 talukas. However, on 1<sup>st</sup> May 1999 Bhandara district was divided in two districts namely Bhandara district and Gondia district

that included seven talukas in former and eight in latter one. The study is restricted to Bhandara district only, comes under the Vidharbh region of Maharashtra State. The district includes seven talukas viz. Bhandara, Mohadi, Pauni, Tumsur, Sakoli, Lakhni, and Lakhandur. Total population of Bhandara district is 11, 35,835. Male 5,73,184, Female 5,62,651, Rural population is 9,60,483, Rural Women population is 4,52,867, Urban population is 1,75,352, Literate population 7,75,494, Rural literate woman population is **1,00,594** . There is 3248 SHGs overall working in these talukas. Rural literate woman in this study taken are those who knows how to read and how to write only.

## Working of SHGs

Self Help Group is Central Government Scheme run by State Government. SHGs are working in democratic manner. Twenty members form a group. Out of these twenty members, two of them selected as representative and one as ‘animator’. The animator selected for the period of two years. The group members meet every week. They discuss about group savings, lending loans, interest charge, bank loan, repayment of loan, social and community programmers’. Small savings are been promoted under SHGs among its members. The saving is kept with the bank in the name of SHG like Vainganga Mahila Bachat Gut or Sharda Mahila Bachat Gut etc.

## Objective of the Study

The study aims at:

1. Examining the performance of Self Help Group in rural areas.
2. Analyzing the Self Help Group as an instrument for women empowerment.
3. Finding out the role of Self Help Group in sustainable development.

**Hypotheses:** In Bhandara district Self Help Group is a powerful tool for sustainable development and it is a practicable system for the improvement in the overall condition of Rural Women in Bhandara District.

## Research Methodology

Primary data been collected from the seven talukas of Bhandara district through a well-designed questionnaire schedule and secondary data has been collected mostly from various government publications, various books and journals. The data thus collected been analyzed properly for specific interpretation. The study covers seven talukas of Bhandara district namely Mohadi, Pauni, Tumsur, Sakoli, Lakhni, and Lakhandur. The data been assembled through random sample of 160 respondents.

## Findings and observations

**Table 1 Source of Information about Self Help Group**

Source of Information	Number of Respondents	Percentage (%)
Newspapers	25	17
NGOs	80	50
Television	15	09
Friends & Relatives	40	24
Total	160	100

It shows that the NGOs are the main source of information about self-help group. It covers up to 50% of respondents for information regarding SHGs. Secondly, friends and relatives played a very vital role in spreading the information regarding SHGs. These relatives and friends are those who first availed and still enjoying the benefits of SHGs. Newspaper played the role providing detail information with the addresses of location to visit. Television on other hand gives flash light information.

**Table 2 Awareness and satisfaction of respondents about performance of SHGs**

Level of awareness and satisfaction	Awareness about SHGs		Satisfaction level of SHGs	
	Number of Respondents	Percentage (%)	Number of Respondents	Percentage (%)
High	110	69	90	56
Moderate	40	25	55	34
Low	10	06	15	10
Total	160	100	160	100

It shows that majorities of the respondents were aware of the functioning of the SHGs and almost two third majorities of the respondents were satisfied with the performance SHGs. This awareness and satisfaction has showed signs of a spatial variation in the area under the study.

Table 3.

**Loan Status****Table 3.1 Loan taken from**

Sources	Number of Respondents	Percentage (%)
From own SHG	76	48
From other SHGs	50	31
Banks	09	06
Mortgage	25	15
Total	160	100

Most of members take loan from their own SHG. This happens with the concern and signature of all members of respective SHGs. Loan can be taken from other SHGs. In this process, one of the members of particular SHG has to barrow loan from her own SHG and lend to the member of other SHG on personal ground/risk. In this case, the interest charged is very less from both sides. Very few people prefer to take loan from bank due to high interest rate. On other hand loan taken through mortgage is more compare to bank. This is to avoid harassment and tension of paying debts.

**Table 3.2 Reason for taking loan**

Sr. No.	Reasons	Number of Respondents	Percentage (%)
01	Business purpose	31	19
02	Paying off old bank loans	32	20
03	Marriage of daughter	13	08
04	Medical treatment	04	03
05	Education	28	17
06	Building house(renovation)	52	33
	Total	160	100

Most of the members take loan for the renovations of their houses. This is done to convert the mud houses into cement one, to be protected from all seasons and to store the farm grains. Loans are also taken mostly for doing small business like for tea stall, vegetables n fruits vendor, live stock business, seasonal flower business etc., and also for paying off old bank loans. Taking loans for marriage purpose depends upon the demand and price inflation. Taking Education loan depends upon the student performance.

**Table 3.3 Regularity in payment of installment for loan**

Sr. No.	Responses	Number of Respondents	Percentage (%)
01	Yes	111	69
02	No	03	03
03	Sometimes late	46	28



At the time of formation of group, it is made clear to every that delay in payment will lead to double amount of penalty. If not able to pay on due date, they should inform the group about the delay and ask for next date, otherwise they will be charged.

**Table 4 Progress after joining SHGs**

Criteria	Responses	Percentage (%)
75% Development	77	48
50% Development	60	38
25% Development	23	14

Some of the members of SHGs stated that though development was there before joining SHGs but the pace of progress was very slow. The reason was governmental paper work. After joining SHG, most of the work became progressive. Sometimes prolonged work-in-progress turned out to be work done with no time.

## Conclusion

Finance or money can solve problems related to financial conditions, the only thing is one must know how to create and use it properly. No doubt, Self Help Group has significantly transformed the way of life and the life style of women in rural areas. Most of the women have become self-employed. Bachat gut programs or say a government scheme has created awareness and knowledge among women. Standard of living is up graded. Beside all the positive outcomes, some negative outcomes was also observed like hesitation in taking self-decision, tradition and orthodox views, lack of proper knowledge about various schemes. Over the entire researcher had observed 65% development in women.

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INVESTIGATION ON POLLINATORS OF SARPAGANDHA (*RAUVOLFIA SERPENTINA*) FROM  
SADAK-ARJUNI OF GONDIA DISTRICT (M.S.), INDIA

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**ABSTRACT**

Pollination, the transfer of pollen grains from anther to the stigma of flower is a vital step in the sexual reproduction of flowering plants. The majority of flowering plants depend on animals for the transfer of pollen. *Rauvolfia serpentina* (Linn.) Benth., ex Kurz, is an important medicinal plant, hence it is essential to study pollination effects. Scan sampling method were carried out to notice the insect pollinator diversity from 7.00am to 5.00pm. Visiting frequency of pollinators and their behaviour were studied by focal sampling. Total 15 species of pollinators observed during study belonging to – Lepidoptera, Hymenoptera, Passeriformes, Coleoptera and Diptera. Out of these lepidopteran insects found to be common due to high visitation rate.

**Keywords:** Pollination, Pollinators, Medicinal plants, Diversity

**INTRODUCTION**

The transfer of pollen grain from anther to stigma of flower is called as pollination. Pollination is the first step for sexual reproduction in plants. The flowers purpose is to result in sexual reproduction. On the basis of types of agent involved, there are two types of pollination, abiotic and biotic. Pollination is facilitated without the involvement of other organisms denotes to abiotic pollination. Only 10% of flowering plants are pollinated without animal support. Pollination is intervened the involvement of other organisms refers to biotic pollination.

Plants depend on pollen vectors, such as wind, insects and birds, to transport their pollen to another individual. Several insects carried out pollination are – bees, butterflies, ants and flies (Wilson, 1999). These visitors must be attracted to the same species repeatedly to bring about pollination. For this the visitor must be attracted, collect pollen accidentally by brushing floral parts, or purposefully collect pollen to take back to a nest, and then visit another flower of the same species and brush up against the stigma, effecting pollination. Flowers attract pollinators by providing ample nectar of the right composition, and by advertising this nectar by deep shape and recognizable floral patterns, by providing excess pollen as food, or by providing shelter or a place to raise and feed young - or by at least looking as if they do (Faegri and van der Pijl 1971).

For study we have selected *Rauvolfia serpentina* (Linn.) Benth., ex Kurz (Sarpagandha) plant belonging to family Apocyanaceae. The plants have enormous medicinal properties. Various parts of this plant are used to treat human ailments (Farooq 2005; Ebadi 2007) in ayurvedic medicine. A large number of alkaloids have been isolated from *R. serpentina* and other species of *Rauvolfia* – Reserpine. Reserpine, deserpidine, deserpideine, serpentine, serpentinine, ajmaline, ajmalinine and rauwolfinine.

This type of study has got definite economic value because the pollination of Sarpagandha (*Rauvolfia serpentina*) depends upon insect pollinators.

**MATERIAL AND METHODS**

**Study Sites**

A study was conducted at self-maintained small field situated in Sadak Arjuni town of Gondia district of Maharashtra state from October 2015 to September 2017. Sadak Arjuni is located at 21.10°N 80.15°E. It has an average elevation of 256 metres (843 feet). It is located near the Maharashtra-Chhattisgarh border on Mumbai - Kolkata National Highway 6. The major crop of this area is rice, that's why Gondia is called as 'Rice City'. The climate in this area remains dry and hot throughout the year with moderate rainfall from June to middle of October months. The experimental field located in close vicinity to residential area and paddy field.

**Flower Morphology**

The structure of flowers, their position in the inflorescence and morphology of separate floral parts were assessed.

**Flower Phenology**

It was determined by visual observations commenced at the beginning of flowering and continued until fruiting (Mark and Francoise, 2005). The time of anthesis initiation and termination in flowers, flowering period of inflorescence and entire flowering period were observed.



**Pollination Syndrome**

Pollination syndrome study included the colour, form, shape, size, pollen tract, odour or scent of flower to attract different pollinators.

**Pollinators visiting frequency**

Observation of pollinators visiting frequency was conducted by scan sampling method. (Martin and Bateson, 1993).

**RESULTS**

Plants were grown in self-maintained small field in Sadak Arjuni town of Gondia district (MS). After the commencement of flowering observed regularly for insects and other visitors visited to flowers and carried out the process of pollination.

**DESCRIPTION OF PLANT**

Sarpagandha is small, erect and perennial shrub, ranges from 60 – 90cm. in height. Whorled phyllotaxy, 3 leaves at each node and about 7- 19cm long, lanceolate, acute or acuminate, glabrous.

Inflorescence is compact corymbose cymes arises from terminal and axillary in position. Flowers are small, pedicillate, fragrance-free, complete and hermaphrodite. Five fused, dark red, glabrous sepals. Petals 5, fused (gamopetalous condition) forming a long corolla tube which is inflated in the middle and white to pink in colour. The length of Corolla tube ranges from 13.5 to 22.2 mm & 2 to 3 mm in breadth / diameter. Nectar secretion takes place from nectary's found at a base of corolla tube. Stamens 5, epipetalous are enclosed within the enlarged portion of the corolla tube. Carpels 2, fused (syncarpous), with filiform style and large bifid stigma; bilocular ovary with two ovules in each locule.

**FLOWER PHENOLOGY**

The flowering in *R. serpentina* takes place throughout the year at climatic conditions of Sadak Arjuni, but inflorescence bears maximum flowers during the end of May to middle of July and from February to April. Flowers open early in the morning between 5.30 – 6.00 hr in summer season & 6.30 – 7.00hr in winter season. The flowers of Sarpagandha are Protogynous prevents the self-pollination and hence favours cross pollination.

**Table 1: List of Flower Visitors species**

Sr. No.	Species	Order	Pollinator/Nectar gatherer/Non-pollinator
1	<i>Papilio demoleus</i>	Lepidoptera	Pollinator + Nectar gatherer
2	<i>Papilio polytes</i>	Lepidoptera	Pollinator + Nectar gatherer
3	<i>Catopsila pyranthe</i>	Lepidoptera	Pollinator + Nectar gatherer
4	<i>Catopsila pomona</i>	Lepidoptera	Pollinator + Nectar gatherer
5	<i>Borbo cinnara</i>	Lepidoptera	Pollinator + Nectar gatherer
6	<i>Eurema hecabe</i>	Lepidoptera	Pollinator + Nectar gatherer
7	<i>Junonia lemonius</i>	Lepidoptera	Non-pollinator
8	<i>Junonia almanac</i>	Lepidoptera	Non-pollinator
9	<i>Amegilla</i> spp.	Hymenoptera	Pollinator + Nectar gatherer
10	<i>Xylocopa fenestrata</i>	Hymenoptera	Pollinator + Nectar gatherer
11	<i>Camponotus</i> spp.	Hymenoptera	Pollinator
12	<i>Cinnyris asiaticus</i>	Passeriformes	Pollinator
13	<i>Cinnyris venustus</i>	Passeriformes	Pollinator
14	<i>Chrysomya megacephala</i>	Diptera	Non-pollinator
15	Bettles	Coleoptera	Non-pollinator

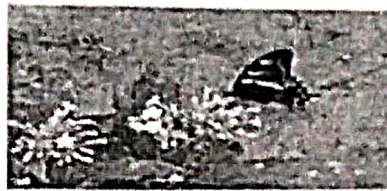
**Table 2: Percentage of Flower Visitors species**

Sr. No.	Taxon	Species	Percentage
1	Lepidoptera	8	53.33%
2	Hymenoptera	3	20.00%
3	Passeriformes	2	13.33%
4	Coleoptera	1	6.67%
5	Diptera	1	6.67%
	Total	15	100





1. *Papilio demoleus*



2. *Papilio polytes*



3. *Catopsila pyranthe*



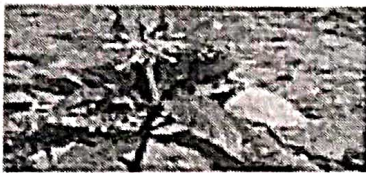
4. *Catopsila pomona*



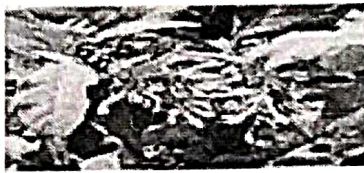
4a. *Catopsila pomona*



5. *Borbo cinnara*



6. *Eurema hecabe*



7. *Junonia lemonius*



8. *Junonia almana*



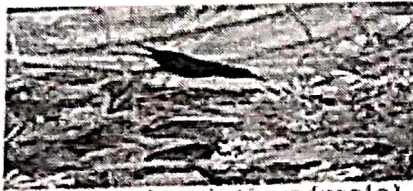
9. *Amegilla* spp.



9a. *Amegilla* spp.



10. *Xylocopa fenestrata*



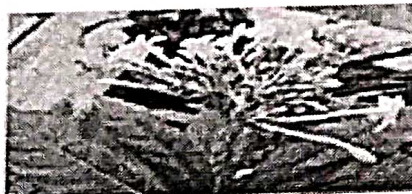
11. *Cinnyrus asiaticus* (male)



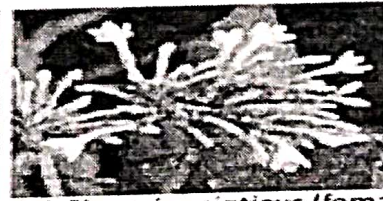
11a. *Chrysomya megacephala*



12. *Cinnyrus venustus*



13. *Camphonia* spp.



14. *Cinnyrus asiaticus* (female)



15. Beetle

During the study it has been found that lepidopteran (Butterflies) species were mostly common pollinators of Sarpagandha. They were abundantly found during 7.00hr to 4.00hr. Sunbirds are also act as pollinators, occurs during the months of July to February also few hymenopteran species were reported during 7.00 to 5.00hr of the day. Maximum numbers of pollinators found during 9.00 to 3.00 hr of the day. Total 15 species of flower visitors were found, out of these 11 species acted as pollinators while 4 species were non-pollinators (Table-1).

## DISCUSSION

Total 15 species of flower visitors like insect, bees, butterflies, birds, etc. were found during the study but not all of them act as pollinators, some of them visited for nectar while some for pollen or both. Certain characters are develops in combination by flowers including shape, size, colour, odour/scent, quantity of nectar, location and type of pollen to attract animal pollinators. Hence only Specific visitors like insects, butterflies, ants, birds, etc. attracted towards the specific flowers depends on the trait produce by the plants.

The flowers of *R. serpentina* (Sarpagandha) have highly narrow and long tubular corolla which makes them a perfect representative of psychophilous mode of pollination rejecting all other syndromes were reported by Barrows (1976); Schemske (1976); Faegri & van der Pijl (1979); Suzuki et al. (1987); Sihag & Kaur (1997). We have also found similar type of results. The numbers of animal visitors were found but among them butterflies are in rank first position followed by bees, similar type of result reported by Wadhawa and Sihag (2012).



It was reported that the birds are attracted towards the red coloured and odourless flowers but we found birds specially sunbirds acts as pollinators of Sarpagandha. We observed that the highest visiting frequency of pollinators during the 9.00 – 3.00hr while Pollobi and Kalita (2013) found during 8.00 – 12.00hr.

Hardwicke (2003) and Faheem et al. (2004) found that the environmental factors such temperature and humidity affecting the insect pollinators and similar type of result also found in our study, during the hot days (middle of April to first week of June) of summer there is great decline in number of pollinators.

## CONCLUSION

From the above study it is concluded that Butterflies (Lepidoptera) were most effective pollinators of *Rauvolfia serpentina* due to their high visited frequency, percentage of occurrence and abundance. This investigation may serve as precursor for further research on topic like pollination ecology and conservation of pollinators.

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## Preliminary Study of Some Physico-Chemical Parameters In Labhansarad Dam In Warora Taluka of Chandrapur District, Maharashtra State, India

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### Abstract:

The habitats of freshwater such as reservoirs, dam, lakes, ponds and tanks hold almighty assurance as dominantly source of drinking water and irrigation. The today's need of mankind is the lively ecological status of freshwater bodies. Parameters like Temperature, pH, conductivity, dissolved oxygen were analyzed to find out the physico-chemical nature of the dam water in Labhansarad dam. The above parameters monthly noted and calculations showing monthly values in the table and graphs.

**Keywords:** Almighty, ecological, Labhasard dam.

### Introduction

The fresh water resources distributions is unequal throughout the global and the fresh water facilitation is becoming boggle day by day in consideration of population augmentation and sundry human activities. In the absence of fresh water resources, groundwater is exploited to meet the demand exerted by various sectors. The variation in the quality of wetland water in response to local geologic syntax and anthropogenic factors vindicate the evaluation of the quality of groundwater for any purposes including that for human consumption (Annapoorna H., and Janardhanab M.R., 2015). The advantage of economic and environmental that are achieve from the water (Picini and Harper, 2016). The physico-chemical and biological status in limnological point of view with respect to elevation of area, fisheries, agriculture and regular domestic uses of water (Ingale, 2016). The increasing anthropogenic activities in the contiguous catchment caused increased inflow of unprocessed sewage and solid wastes to the lake. It also indicates that the oligotrophic water bodies are slowly changing as mesotrophic and in future may be change as eutrophic (Shiddamallayya and Pratima 2008) hence we all have to word of honor for responsibility consequently.

### Materials and Methods:

Labhansarad dam located in Warora Taluka of Chandrapur District, Maharashtra State, India. It is situated in the remote area from the localities. Town Kotha at West direction 1 kilometer, at the north 1.5 Km Mahalgaon, town Sumthana 1.5km towards the East- South direction and town Lonar 1.5km towards South direction from the location of Labhansarad dam. The study carried out for the Six month during February 2016 to July 2016 for analysis the some

physico-chemical parameters such as, temperature, conductivity, total dissolved solid, turbidity, pH, dissolved oxygen will be performed at the site using portable water analysis kit using methods prescribed by APHA (1985) and NEERI (1986).

### Results and Discussion:

#### Temperature:

Water temperature is the most important physico-chemical factor for the hydrobiological studies point of view. In the months of May, maximum atmospheric temperature and moderate pollutants in dam water is due to low quantity of water may be responsible for increasing in the values of temperature at Station -B. In the month of February minimum atmospheric temperature and high level of dam water might be responsible for minimum value of the water temperature at stations D, C, A, and E respectively. The similar line investigations reported by Sharma and Walia (2014).

#### Conductivity:

It depend upon the ionic status of water. Ionic status of water determines the conductivity of water. The maximum value of conductivity recorded in the month of July might be due to inflow carries surface runoff from agricultural area of the dam at station- B, containing decaying organic matter, and may be due to the higher total dissolved solids. While minimum in the month of February may be due to expression of low ionic substance and decreased in total dissolved solid range responsible for decreasing the value conductivity at station - E. Same argument had also support to our findings recorded by Ajayan and Naik (2014) Ingale *et al.*, (2015).

#### Total dissolved solid:

Total dissolved solids are resolute as the residue remains left after evaporation of filtered sample. The maximum TDS observed in the month of July

may be due to surface runoff, due to surface inflow to the dam at station B, cloth washing, bathing activities and cattle activities (at station A, C and D respectively) generate inorganic matter into the dam. Minimum TDS value noted in the month of February might be due to the lower temperature and high water level. Analogous findings recorded by Pawale and Lokhande (2012) and Choudhary *et al.*, (2014).

**Turbidity:**

Turbidity, the optical material goods given to the water by suspended solids, disturbs human observation visually. The maximum turbidity noted in the month of July at station B, might be due to the inflow gathering clay, slits and suspended particles into the dam water also decaying vegetation, high planktonic growth was responsible for the turbidity at station - B, A, C and D. The minimum turbidity in the month of February at station E, may be due to settlement of silt, clay and suspended particles at station - E. The values are corroborated with the study of Saxena (2012).

**pH:**

The opinion that nearly neutral pH of water is synchronized by the carbon dioxide and bicarbonates. The hydrogen ion concentration of

natural water is a vital factor which is associated with all life processes and also influences the immigration of an aquatic macro fauna in the water bodies (Hutchinson 1957). The maximum pH noted in the month of May and June, might be due to the increased carbonates and bicarbonate in dam water at station B. Minimum pH noted in the month of February and April, may be due to total dissolved solid, low turbidity and lower water temperature at station - E. Similar findings recorded by Bobdey *et al.*, (2014) in Bhiwapur Lake, Dist. Nagpur (M.S) India.

**Dissolved oxygen:**

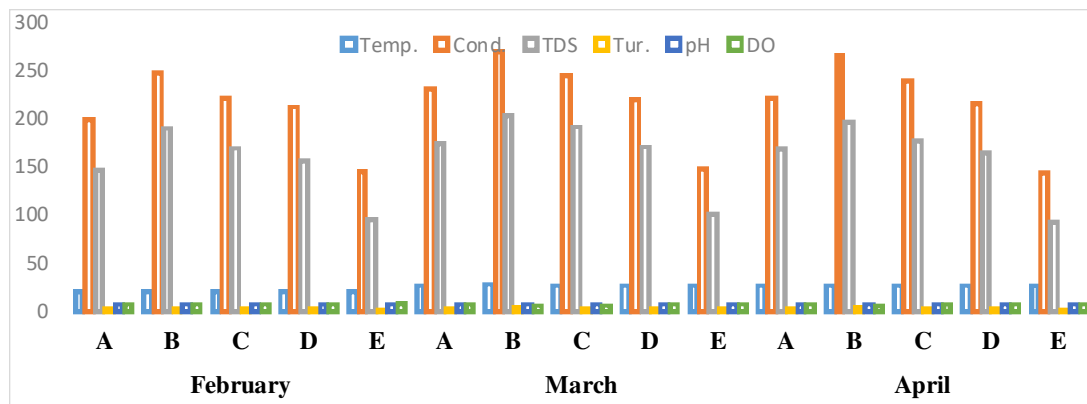
Dissolved oxygen determines the distribution of aquatic organisms. The maximum dissolved oxygen recorded in the month of February, may be due to the circulation of cold water as well as higher solubility of oxygen at the temperature at station - E. The minimum DO noted in the month of May might be due to the lower water level demand for oxygen, higher temperature responsible to enhance microbial activities and decomposition can reduce D.O level at station - B, C, A and D. The present findings amply supported by Shyam and Khatri (2015) and Ingale (2016).

**Table no.1:** Table showing Monthly Variation of some physico-chemical parameters in Labhansarad Dam during 2016-2017.

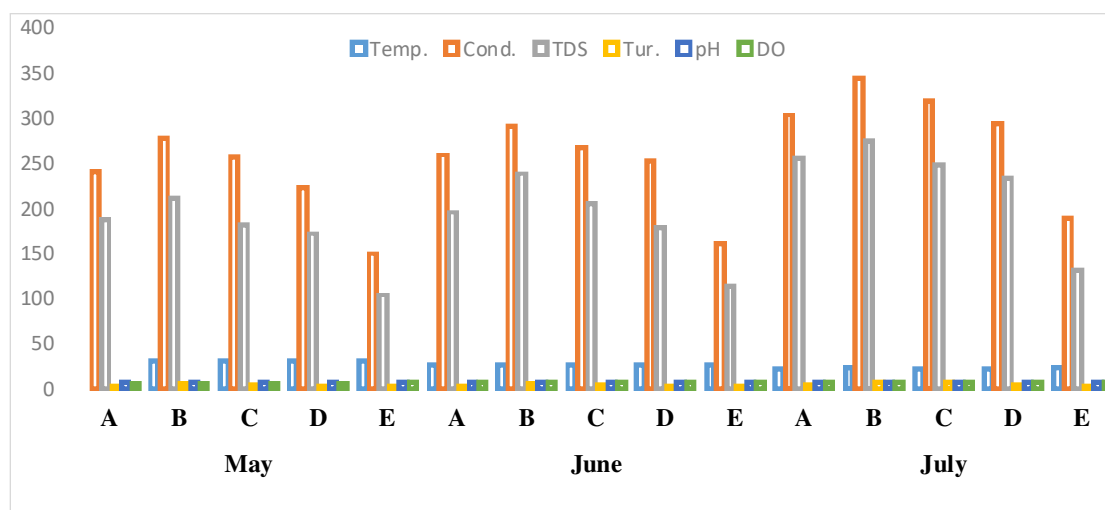
Sr. no.	Monts → Parameters ↓	February					March					April				
		Stations ↓					Stations ↓					Stations ↓				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
1	Temperature	20	20.5	20.1	20.2	20.4	27.4	28	27.2	27.1	27.5	26.8	27.5	26.7	26.4	26.8
2	Conductivity	200	247	221	213	145	230	269	245	220	148	222	265	240	216	143
3	TDS	146	190	169	157	96	175	203	191	171	101	168	197	177	165	93
4	Turbidity	2	4	3	2	1	2	5	4	3	2	2	5	4	3	1
5	pH	7.3	7.4	7.3	7.2	7.1	7.3	7.4	7.4	7.3	7.2	7.2	7.4	7.4	7.2	7.1
6	DO	7.2	6.9	7.2	7.6	8.2	7	6.5	6.7	6.9	7.5	7.1	6.7	6.9	7	7.6

**Table no.1:** Table showing Monthly Variation of some physico-chemical parameters in Labhansarad Dam during 2016-2017.

Sr. no.	Months → Parameters ↓	May					June					July				
		Stations ↓					Stations ↓					Stations ↓				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
1	Temperature	30.6	31.6	31	30.9	31.1	26.4	27.1	26.8	26.3	26.9	23.2	23.9	23.4	23.2	23.5
2	Conductivity	241	278	257	224	150	259	291	268	253	162	304	344	319	295	189
3	TDS	187	211	182	173	105	196	239	206	179	114	255	275	249	234	132
4	Turbidity	3	6	5	3	3	4	6	5	4	3	5	8	7	5	4
5	pH	7.5	7.7	7.6	7.5	7.3	7.3	7.6	7.5	7.4	7.2	7.3	7.5	7.4	7.3	7.2
6	DO	6.8	6.1	6.3	6.8	7.1	7.2	6.9	7	7.3	7.6	7.3	7.1	7.2	7.4	7.7



**Fig.1:** Graph showing monthly variation of some physico-chemical parameters at all stations in Labhansarad Dam during Feb- April 2016.



**Fig.2:** Graph Showing monthly variation of some physico-chemical parameters at all stations in Labhansarad Dam during May-July 2016.

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**BIO-EFFICACY OF *PONGAMIA PINNATA* ON LARVAL GROWTH AND MORTALITY OF TOBACCO CATERPILLAR, *SPODOPTERA LITURA* FABRICIUS**

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**Received-27-07-2017**

**Accepted-15-11-17**

**Published-31-12-2017**

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**ABSTRACT:** A methanolic extract from the leaves of *Pongamia pinnata* (L.) was tested for its insecticidal property on the third instar larvae of *Spodoptera litura* (F.) under laboratory conditions. Different concentration of the plant extract were used for leaf disc test to observe the effect on feeding, growth and the survival rate of the test insect at different stages of the development. The maximum mean percent mortality was observed in 50% dose concentration. The present results showed that single application of 50% solution caused up to 96.66% mortality. This concentration is the highest limit dosage, especially in order to observe possible phytotoxicity shows a potential biopesticide to check the mortality of polyphagous pest *Spodoptera litura*.

**KEY WORDS:** *Pongamia pinnata*, leaf extract, *Spodoptera litura*, survivability

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### **INTRODUCTION**

*Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) is polyphagous pest and has about 120 host species belonging to 44 families. In India, it feed on 74 species of cultivated crops and some wild plants. It has been reported as an increasingly important pest during the rainy seasons causing heavy yield loss throughout India<sup>2</sup>. It is an indigenous pest of a variety of crops in South Asia and was found to cause 26-100 per cent yield loss in ground nut<sup>3</sup>. Among the polyphagous pests, the tobacco caterpillar, *S. litura* has emerged as a serious and dominant pest causing enormous losses to crops like pulses, cotton, oil seeds, vegetables, tobacco, cauliflower, castor, banana, groundnut, mulberry, etc. The fully grown caterpillars of the tobacco cutworm, *S. litura* are most voracious feeders.

The widespread use of chemical pesticides has resulted in problems including health hazards to human being and domestic animals, development of pesticide resistance by pest, pest outbreak, etc. The broad spectrum action of many synthetic pesticides may also cause adverse environmental effects by harming beneficial organisms such as natural enemies and pollinators. Plants are known to possess toxins including phytotoxins. The phytotoxins protect the economically important crops from pests and pathogens all over the world. Plant derived insecticides are reported to have the ability to influence the proportion of various biochemical components (carbohydrates, lipids, proteins etc.) in the body of insects, disturbing the internal metabolism of the insect, causing their reduced activity or mortality.

*Pongamia pinnata* Linnaeus (Fabaceae) is a medium-sized glabrous tree distributed along the coasts and river banks in India and Myanmar. Locally known as Karanja, is a mangrove plant belonging to the family, Fabaceae. It is a medium size glabrous tree with a short bole and attaining a height of round 18 m and its habitat is in the littoral regions of South East Asia, Australia and Fiji. The leaves, flowers, seeds and stem bark of *P. pinnata* are known to have karanjin<sup>6,7</sup>. Literature shows presence of flavonoids, especially furano flavonoids, quercetin, amino acids, fatty acids and triterpenoids in *P. pinnata*<sup>8,9</sup>. Hence, the present investigation was conducted to study the effect of leaf extract of *P. pinnata* against the larval growth, development and survivability of tobacco caterpillar, *Spodoptera litura*.

## **MATERIALS AND METHODS**

### **Maintenance of insect culture**

The egg masses were collected from the field, brought to the laboratory and incubated. On hatching the first instars were released on host the plant castor, *Ricinus communis* in the laboratory and reared at the temperature 25 - 28°C and 70% relative humidity.

### **Plant Extract**

The leaf of *P. pinnata* were collected from the premises of Centre for Sericulture and Biological Pest Management Research, and brought to the laboratory and shed dried and further grinded in mixer to make powder. The methanolic extracts were

obtained by using Sauxlet Extraction Method.

### **Preparation of doses**

The four different concentrations were prepared from the stock. The stock solution was considered 1% solution and other concentrations were prepared by diluting the stock solution with distilled water such as 0.50%, 0.25% and 0.125%. Distilled water considered as Control for all the respective four concentrations.

### **Treatment**

The 500 third instars larvae were selected and collected from the stock culture for each concentration. The larvae was kept in individually in plastic cups for the treatment, such three replicate was done. The larvae were treated with extract of *Pongamia pinnata* 1%, 0.5%, 0.25%, 0.125%, and control (distilled water) were spread by smearing 1ul solution on the piece of leaf of host plant and fed to the larvae individually in the plastic cup. The larval weight, growth and mortality were recorded and calculated by using Abbott's formula.

## **RESULTS AND DISCUSSION**

During the present study all the treated group of larvae showed the symptoms like inhibiting growth, slow feeding activity, oozing of fluid from the mouth and blackening of thorax, reduction in mobility due to retraction of legs and inhibition of moulting. The larvae treated with 1% concentration could not reach up to the pupal stage and though larvae treated with lower concentrations reached up to pupal stage and

transformed into malformed pupae. Similar results were found when larvae of *S. litura* treated with extract of azadirachtin reported by<sup>15</sup>.

Sahayaraj and Nirupa in (2006) also observed the mortality, root extract of *Pongamia murex* highly reduced the pupal weight compared to the leaves and fruit extracts. When they compared to the control pupal weight, the treated pupal weight was less than the control, which suggested that *P. murex* has a unique phyto-chemical profile. The reduction of weight and shriveling of pupa in all the treatments might be related to the loss of large quantities of body fluids before pupation. Crude extracts also affect the detoxification enzyme levels of *S. litura*<sup>17</sup>, which might be the reason for the reduced pupal weight observed. This finding directly supports the present results that with

increasing dose concentration, occurs reduction in weight of treated larvae and metamorphic changes also get disturbed. It was observed that there appears to be a biologically significant interaction between dose various dose concentration with the percent larval mortality.

In the present study the effect of extract of *Pongamia pinnata* was also recorded reduced in the growth of the larvae, resulting decreased body weight after an interval of 24 hours of the treatment. The larvae treated with 1% concentrations died and larvae treated with 0.50 and 0.25 and 0.125 % concentration showed weight reduction within 48 hours as compared to control. Significant reduction in body weight observed with 0.50% treated larvae. Though all the treated larvae reached up to fifth instar stage and formed malformed pupa (Table 1).

**Table 1. Effect of *Pongamia pinnata* extract on larval weight *Spodoptera litura***

<i>P. pinnata</i> extract Conc.	Weight of Larva (g)						
	Initial	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
<b>1%</b>	0.055±0.042	0.042 ±0.01	All dead	-	-	-	-
<b>0.50%</b>	0.056±0.035	0.042±0.02	0.174±0.014	0.35±0.043	0.549±0.060	0.595±0.034	0.654±0.048
<b>0.25%</b>	0.052±0.001	0.040±0.01	0.213±0.008	0.386±0.021	0.773±0.036	0.807±0.039	1.023±0.022
<b>0.125%</b>	0.058±0.002	0.052±0.02	0.209±0.014	0.447±0.035	0.773±0.049	0.831±0.048	1.065±0.068
<b>Control</b>	0.045±0.001	0.049±0.02	0.236±0.010	0.453±0.028	0.782±0.035	0.864±0.035	1.134±0.042



**Table 2. Effect of *Pongamia extract* on larval mortality of *Spodoptera litura***

<i>P. pinnata</i> extract Conc.	No. of larvae dead / (%)							
	No. of larvae taken	24hrs	48hrs	72hrs	96hrs	120hrs	Adults	Total Mortality (%)
1%	30	28/ (93.33)	2/ (100)	-	-	-	0	100
0.50%	30	26/ (86.66)	2 / (93.33)	1/ (96.66)	-	1/ (100)	0	100
0.25%	30	16/ (53.33)	3/ (63.33)	-	-	-	11	63.33
0.125%	30	07/ (23.33)	1/ (26.66)	-	-	-	22	26.66
Control	30	-	-	-	-	-	30	-

In (1998) Sahayaraj and Paulraj evaluated the effect of relative toxicity of some plant extracts to *Spodoptera litura* such as antifeedent action of Neem seed kernel extracts and its commercial formulation have been found to be effective insecticide against many insect pests and can be integrate them in Integrated Pest Management programme (Schmutterer., 1990, 1988; Gupta and Sharma., 1998). In the present study also showed that single application of high dosage (1% solution) of *P. pinnata*, caused up to 100% mortality after 48 hrs of treatment due the toxicity of *P. pinnata*, increases the dose of extracts concentration, it increases the rate of mortality (Table 2).

The results obtained from the present finding clearly indicates that the application methanolic extract of *P. pinnata* reduce the population of *S. litura* and minimize the infestation to crop. The eco-friendly active principle present in this plant, therefore it will be recommended to farmers used as integrated pest management in the field.

### ACKNOWLEDGEMENT

The Authors are grateful to UGC New Delhi for financial assistance to pursue Research Work under funding M.A.N.F. Scheme.

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