ICV 2014: 30.06
Standardized Value: 3.31

ISSN: 2320-7817 | e-ISSN: 2320-964X
Volume 3, Issue 4, December, 2015

INTERNATIONAL JOURNAL OF
LIFE SCIENCES

AN INTERNATIONAL PEER REVIEWED OPEN ACCESS JOURNAL

STATUTORY WARNING

Articles, data, figures, scientific contents and its interpretation and authenticity reported by author(s) and published in IJLSCI are the exclusive views of author(s). The editorial board, IJLSCI is not responsible for any controversies arising out of them.
In case of any plagiarism found, author(s) will have to face its consequences.

All enquiries and manuscript should be directed to

The Managing editor IJLSCI
46, Guruvandan, Jawahar Nagar,
VMV Road, Amravati -444604,
(MS) India

Contact: +91-942 077 5527
+91-997 055 9438
Email: editor@ijlsci.in
editorijlsci@gmail.com
web:www.ijlsci.in

About IJLSCI

International Journal of Life Sciences (IJLSCI) is a peer-reviewed, open access journal; it publishes original articles in all areas of Biology and Life Sciences. Authors are encouraged to submit complete unpublished and original works, which are not under review in any other journals. The scopes of the journal include, but limited to the following topic areas: Biology, Anatomy, Botany, Cytology, Genetics, Microbiology, Physiology, Zoology, Ecology, Environmental Science, Hydrobiology, Neurobiology, Developmental Biology, Immunology, Molecular Biology, Biochemistry, Biophysics, and Biotechnology, Genomics and Proteomics. It is an open access journal, which publishes original research articles, reviews and short communication in all areas of life sciences and at the interface of related disciplines.

TYPES OF RESEARCH PAPER

Original Articles:
These should describe new and carefully confirmed findings, and experimental methods should be given in sufficient detail for others to verify the work. The length of an original article is the minimum required to describe and interpret the work clearly.

Short Communications:
Short communication also contain abstract. The list of references should not exceed 15. The presentation of Short Communications should contain headings such as Introduction, Materials and Methods, Results and Discussion, etc.

Reviews Articles:
Submissions of review articles and perspectives covering topics of current interest are welcome and encouraged. Publication Frequency: Quarterly (published in March, June, September & December).

The journal is published in print as well as online version which is free access and downloads.

This Journal is circulated / Distributed Through Subscription only

Submission of Paper: Submission of paper is encouraged by e-mail to editorijlsci@gmail.com
Subscription Plan: Rs. 2500 /- or 150 US$ (Individual Annual); Rs. 3500/- or 250 US$ (Institutional)
Life Membership: Rs. 10000/- or 550 US$ (Individual) Rs. 15000/- or 850 US$ (Institutional)
Fellow Membership: Rs. 15000/- or 850 US$ (Individual) Rs. 25000/- or 1700 US$ (Institutional)

Publisher and Owner: **Dr. Arvind Chavhan,** Published from IJLSCI, 46, Guruvandan, Jawahar Nagar, VMV Road Amravati -444604, India

Printed at IJLSCI, 46, Guruvandan, Jawahar Nagar, VMV Road Amravati -444604, India

Managing Editor: Dr.Arvind Chavhan,Assistant Professor & Head, Department of Zoology, D.B. College, Bhokar, Nanded, India

Indexing:

Index Copernicus, OCLCWorldCat, Directory of Science INNOSPACE (SJIF), ROAD, OAJI, AcademicKeys, CiteFactor, J-index.net, Scholar Steer, ResearchBible, Google Scholar, DAIJ , DRIJ, DIIF, SIS, ISI, ASI.

All Articles are freely accessible on the website of the International Journal of Life Sciences http://www.ijlsci.in As well as some of the Indexing agencies website via., oaji.net, research bible, Cite factors, ISI, etc.

IJLSCI

ISSN:2320-7817(p) | 2320-964X(o)
Volume 3, Issue 4, December 2015

INTERNATIONAL JOURNAL OF LIFE SCIENCES

Contents

IJLSCI

ISSN:2320-7817(p) | 2320-964X(o)

INTERNATIONAL JOURNAL OF LIFE SCIENCES

Editorial Board

RESEARCH ARTICLE

Assessment of disease intensity and isolates characterization of blast disease (*Pyricularia oryzae* CAV.) from South West of Ethiopia

Mebratu Gebremariam Asfaha[1], Thangavel Selvaraj[1]*and Getaneh Woldeab[2]

[1]Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo University, Ambo, Post Box No: 19, Ethiopia, East Africa.

[2]Department of Pathology, Ambo Plant Protection Research Center (APPRC), Ambo, Ethiopia, East Africa.
*Corresponding author: Email: tselvaraj_1956@yahoo.com: Mobile Number: +251-913073294.

Manuscript details:

Received: 23.10.2015
Revised: 20.11.2015
Accepted: 06.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Mebratu Gebremariam Asfaha, Thangavel Selvaraj and Getaneh Woldeab (2015) Assessment of disease intensity and isolates characterization of blast disease (*Pyricularia oryzae* CAV.) from South West of Ethiopia. *International J. of Life Sciences*, 3(4): 271-286.

ABSTRACT

Blast (*Pyricularia oryzae* Cav.) disease is one of the most important biotic constraints to rice production in South West of Ethiopia. The present study was conducted to determine the intensity of the blast disease and their isolates characterization in upland rice cultivated fields of South West of SNNPRS of Ethiopia. The assessment of rice blast was carried out in 90 farmers' fields in three districts during the main cropping season from May to October 2013, in the major upland rice growing areas of Kaffa, Benchi-Maji and Sheka zones in South West of Ethiopia. The results of the assessment revealed that the incidence and severity of the disease vary from low to high on the rice fields depending on the agro-ecological and cultivars differences. Rice blast was observed in all assessed locations at variable levels. The incidence of rice blast in six different localities varied from 42.01 to 85.69%. The highest mean incidence of rice blast was recorded in Otuwa locality (85.69%) and the lowest incidence recorded in Argoba locality (42.01%). The overall mean incidence of six localities in three districts of the South west of Ethiopia (SNNPRS) reached 65.68%. Likewise, blast severity showed similar trend as that of incidence in all six localities. The highest severity was recorded in Otuwa locality with range of 8.88 - 88.8 % and the mean severity values of 55.7% while the lowest severity was recorded in Argoba locality (33.62%). The overall mean severity of the six localities in three districts of the South west SNNPRS reached 47.15%. The entire assessed three districts showed 100 % blast disease prevalence. Rice blast, *Po* isolates were characterized and identified based on their growth parameters into six isolates such as *Po*12, *Po*28, *Po*41, Po55, *Po*72 and *Po*85. Among the four culture media (oat meal agar, rice flour agar, malt extract agar and potato dextrose agar), the *Po* isolates were grown on optimum growth and good sporulation in oat meal agar

followed by rice flour agar. The optimum temperature and pH of the growth of the *Po* isolates were at 30°C and 6.5, in almost all isolates, respectively. It could be concluded that the rice blast was the most important disease of rice cultivars in South West (SNNPRS) of Ethiopia. Further studies could be conducted to evaluate the upland rice cultivars resistance against the isolated blast (*Po* isolates) pathogens under greenhouse and natural environmental conditions.

Key words: Rice, blast, *Pyricularia oryzae*, Assessment, Disease Intensity, *Po* isolates, Characterization.

INTRODUCTION

Rice (*Oryzae sativa* L.) belongs to the family Poaceae, is one of the main cereal food crops in most part of Africa, however, the rice cultivation and utilization as a food crop in Ethiopia is very recent phenomenon (Traore *et al.,* 2006; Abdu *et al.*, 2013). It is recognized as an important strategic food security crop and as a crucial element in the staple food economies of Sub Saharan Africa (Abdu *et al.*, 2013). It is also the most rapidly growing source of food crop in Africa, and significant importance to food security and food self-sufficiency in an increasing number of low-income food deficit countries (FAO, 2012). Rice cultivation in Ethiopia has begun at Fogera (Amara Region) and Gambella plains in the early 1970's (EIAR/ FRG II, 2012). It is reported that the potential rice production area in Ethiopia, which is estimated to be about thirty million hectares (MoARD, 2010). The number of farmers engaged in rice production has increased from about 53,302 ha in 2006 to about 284,868 ha in 2009. Similarly, the area allocated has increased from about 18,527 ha in 2006 to about 155,886 ha in 2009 along with production increase from about 42,825 tons in 2006 to about 498,332 tons in 2009. There is an increased trend in area allocation and production of rice in Ethiopia. A total of 4,98,332 tons of rice was produced in the country and out of these 92,562 tons production was produced in Southern Nation and National People of Regional States (SNNPRS) and out of these 90,953 tons production was produced in Kaffa, Benchi Maji and Sheka zones. The rice average yield in Ethiopia is 2.7 tons/ha in upland rain-fed and 3.2 tons/ha in lowland areas (EIAR/ FRG II, 2012).

The major problems in rice production around the world are biotic and abiotic stresses against rice crops (Ou, 1985). One of the biotic stresses in rice crop is blast disease, which caused by a filamentous, ascomycetous fungus, *Pyricularia oryzae Cav*. It is an infectious fungal disease, which is distributed worldwide and prevailing in more than 85 countries of the world ((Jamal *et al.,* 2012) and also as one of the most important disease infecting rice plants in African countries (WARDA, 2004). The disease is a significant problem in temperate regions including Ethiopia and can be found in areas such as irrigated lowland and upland. *Pyricularia oryzae* is otherwise known as rice blast, rice seedling blight, and rotten-neck blast, pitting disease, leaf blast, node blast, panicle blast, collar blast and Johnson's spot (NDPRB, 2011). Blast can be found on the rice plant from the seedling stage to maturity. The leaf blast phase occurs between the seedling and late tillering stages (Couch and Kohn, 2002). Rice blast causes economically significant crop losses annually and causes yield loss as high as 70–80% when pre-disposition factors favor epidemic development (Piotti *et al*., 2005). Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses in these areas ranging from 50 to 90 % of the expected crop (Jamal *et al* ., 2012).

In Ethiopia, blast disease has been recorded on rice in Amhara Region, since 1985 and also in SNNPRS (Abraham, 2008). This disease is one of the major constraints to intensification of rice cultivation. Assessment of the incidence and severity of plant disease is important to determine the disease intensity and status of the disease throughout a studies area in order to prioritize research. Despite the frequent occurrence of severe epidemics of the rice blast disease, there is no detailed information with regards to the rice blast disease intensity against *P. oryzae* pathogen in Ethiopia. *In vitro* condition, these fungi was isolated and identified and also inoculum multiplied on the specific culture media with optimum temperature and hydrogen – ion concentration (pH) (Meena, 2005). But some reports on the effect of culture media, temperature and pH on radial growth as well as on the morphological characters of the pathogen showed contradictory findings. Characterization of *P. oryzae* isolates will be needed in order to determination of cultural (medium), physiological (temperature and pH) and the morphological studies (growth and sporulation) of the native isolates. With this regard it could be vital to conduct the assessment of the rice disease intensity in the South West SNNPRS of Ethiopia and also to identify the domains where rice blast disease may become constraint to the productivity. Therefore, the present study was conducted to determine the intensity of the blast disease and its isolate characterization in upland rice cultivated fields of South west of SNNPRS, Ethiopia.

MATERIALS AND METHODS

Description of the study areas

The assessment of rice blast intensity was carried out in 90 farmers' rice cultivated fields of Kaffa, Bench-Maji and Sheka Zones in SNNPRS South west of Ethiopia during the main cropping season from May to October 2013. The experimental rice research plots at Bonga Agricultural Research Center and one Private Sector Farm from Guraferda district were also included in the assessment.

Table 1 : Study areas environmental conditions.

Zone	District	Altitude range (m)	Longitude (°E)	Latitude (°N)	Temperature °C		Mean annual rainfall(mm)
					min	max	
Kaffa	Gimbo	500 -1950	36° 0′ 0″	7° 15′ 0″	16.7°C	24°C	1710
Benchi-maji	Guraferda	814-1995	34°88' - 36°14'	5°33'-7°.21'	10°C	30°C	1200
Sheka	Yeki	800-1400	35°31'-35°35'	7°24'-7°52'	15.5°C	28.5°C	2298

$$\text{Prevalence (\%)} = \frac{\text{Number of fields affected by the disease}}{\text{Total number of fields assessed}} \text{ X } 100$$

The incidence of rice blast was calculated using the number of infected plants and expressed as a percentage of the total number of plants assessed (Jamal *et al*., 2011).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \text{ X } 100$$

The severity of the disease was examined visually on the whole plants within the quadrants and recorded as the percentage of plant parts (tissue) affected (percentage of blast infection of the plant).

$$\text{Percent severity index (PSI)} = \frac{\text{Sum of numerical rating}}{\text{No. of plants rated * max score of scale}} \text{ X } 100$$

The assessment of disease intensity was conducted at Kaffa zone, Gimbo district (Argoba and Choba localities), Benchi - Maji zone, Guraferda district (Otuwa, Berji and Kuja localities) and at Sheka zone, Yeki district (Alamo locality). A total of 6 localities, two from Kaffa zone, three from Bench-Maji zone, and one from Sheka zone were assessed for rice blast intensity. The description of the study areas altitude, longitude, latitude, temperature and mean annual rainfall is given in table 1:

Assessment of blast disease intensity

The survey was conducted using simple random sampling method, within at 2-3 km intervals on rice fields along the main and accessible road sides. The rice blast incidence and severity were recorded along the two diagonal 'X' fashion of the fields at five random spots using $1m^2$ quadrants and used to calculate the average values. Totally, 90 farmer's rice fields were surveyed at critical growth stage of the crop (tillering) during which the blast symptoms reached its maximum severity level. From each locality, 15 farmer's rice fields were selected. The prevalence of the disease was calculated using the number of fields affected by the disease divided by the total number of fields assessed and expressed in percentage. Scoring scale of blast disease under field condition was rated according to the International Rice Research Institute (IRRI) scale of 1-9 (0 = No lesions; 1 = Small brown speaks of pin point size or large brown speck without speculating centre; 2 = Small round dish to slightly elongated necrotic grey spots about 1-2 mm in diameter with distinct brown margin lesions are mostly found on lower leaves; 3 = Lesion type is same as in scale 2, but significant number of lesion are one on upper leaves; 4 = Typical susceptible blast lesion, 3 mm or longer infecting lesions on 2% of leaf area;5 = Typical blast lesion infecting 2-10% of the leaf area; 6 = Typical blast lesion infecting 11-25% of the leaf area; 7 = Typical blast lesion infecting 26-50% of the leaf area; 8 = Typical blast lesion infecting 51-75% of the leaf area; 9 = More than 75% leaf area affected) (IRRI, 1996; 2009). Data on geographical information (longitude, latitude, and altitude) of each field were recorded using GPS (Trex Legend GPS system, Garmin).

Diseased plant sample collection

Blast infected rice leaf samples at vegetative stage (tillering 3rd critical growth stage) were collected from farmer's fields at an altitude ranges between 1107 and 1423 m. a. s. l. Infected leaves were cut from the mother plant and placed in an envelope, which were labeled with all necessary informations including the name of the region, zone, district, localities, cultivars, GPS data and date of collection. Samples were kept in refrigerator at 4^oC until the surveys in all the districts were finalized. Then after, samples were preserved in ice box and transported to Ambo University Plant Pathology Laboratory for pathogen identification and characterization.

Isolation, purification and identification of rice blast isolates

The oat meal agar (40 g of rolled oats, 5 g of sucrose, and 16 g of agar and 1000 ml of distilled water), and potato dextrose agar media (200g of peeled potatoes, 20 g of dextrose, and 20 g of agar and 1000 ml of distilled water) were used for the isolation of blast pathogen. Diseased leaves of rice cultivars infected with pathogen were cut into small pieces (less than 1.0 cm in size) around the area showing the blast lesion including the edge of the lesion and were surface sterilized with 1% sodium hypochlorite for 1 min. followed by 3 washes with sterile distilled water. Then the plant pieces were placed in Petri dishes lined with moist filter papers and it was incubated at 25 ^0C for 24 h to encouraged sporulation. After incubation, these infected leaf pieces were examined under stereo-dissecting microscope. Abundant pathogen growth and sporulation were observed from in and around the lesions with grey, dense and bushy appearance. A sterile moistened needle was used to pick out some conidia by brushing the needle across the sporulating lesion. The conidia were placed on oat meal agar and potato dextrose agar media plates containing streptomycin (WARDA, 2004).

Plates were incubated at 25°C for about 7-10 days with 12 h darkness and 12 h light. The identity of *P. oryzae* was verified by checking the conidia under light microscope (WARDA 2004). Identification of the pathogen was carried out according to the cultural and morphological characteristics as described by Agrawal *et al*., (1989) and Mew and Gonzales, (2002). The blast isolates designated as *Po* denotes *Pyricularia oryzae* and number denotes representative of sites based on Meena, (2005).

Isolates of the blast pathogen were isolated and purified using single spore (mono conidial) technique (Hoang Dinh *et al.,* 1999). Water agar medium (30 g of agar, 1000 ml of distilled water, 40 mg streptomycin, 5 g of sugar and 2 g of yeast extract) was used for the purpose of single conidial isolation Mono-conidial cultures were isolated from the field blast pathogen isolates, which were derived by streaking a loopful of conidial suspension across a water agar plates in a "W" pattern, thus spreading the conidia. A guideline can be drawn on the undersurface of the plate. Following 24 h incubation at 25^0C, germinating conidia can be easily picked up and sub cultured on to a fresh oat meal agar and potato dextrose agar media plates amended with streptomycin using a fine scalpel.

Characterization of the rice blast isolates

Evaluation of different culture media for growth of P. oryzae isolates

The four culture media viz. oat meal agar media (40 g of rolled oats, 5 g of sucrose, 16 g of agar and 1000 ml of distilled water), rice flour agar media (15 g of complete rice flour, 4 g of yeast extract,15g of agar and 1000 ml of distilled water), malt extract agar media (35.5 g of malt extract agar, and 1000 ml of distilled water) and potato dextrose agar media (200g of peeled potatoes, 20 g of dextrose, 20 g of agar and 1000 ml of distilled water) were used to compare the growth rate of *P. oryzae* isolates after 10 days inoculation (Meena, 2005). From the margin of actively growing of *P. oryzae* isolates; 6 mm diameter mycelia discs of the 14 day old cultures of different *P. oryzae* isolates were inoculated on the middle of the Petri plates and three replications were maintained for each media. The inoculated Petri plates were kept at 30°C. The colony diameter of the growth of each isolate was measured after 10^{th} day of the incubation period and the growth was calculated in mm with the help of a scale. The different colony characters like pigmentation, color of mycelia, surface texture, margin, mycelial growth, sporulation and size, shape and septation of conidia were recorded in all four media by visual and microscopic observations (Meena, 2005). The sporulation capacity of each isolate on different media was assessed by microscopic observations. A loopful of culture was transferred to a clean slide and mixed well with lactophenol and a cover slip was placed on it. The rate of sporulation was recorded in microscopic fields at 40X (Excellent >30; Good- 20-30; Fair-10-20; Poor-<10 and Nil-0) (Meena, 2005). Six *P. oryzae* isolates were identified. All the six isolates of *P. oryzae* were multiplied on OMA for 14 days and spores were collected. The length and width of 10 spores were measured under high power objective (40 X) for each isolate using a micrometer. The average size and shape of conidia (length and width) was determined using ocular and stage micrometer. Number of septa and color were also recorded (Chipili *et al*., 2004).

Effect of temperature on growth of blast isolates

The effect of temperature levels on growth of only selected isolates of *P. oryzae isolates* were grown on potato dextrose agar media. Mycelial discs of the 10 day old culture of *P. oryzae isolates* (6 mm diameter discs) were placed on the middle of PDA Petri plates and incubated at seven different temperatures level i.e., 15, 20, 25, 30, 35, 40 and 45^0C. The experiment was laid out in Completely Randomized Design (CRD) with three replications. After five days of incubation, the colony diameter of each isolate was measured in mm (Getachew *et al.,* 2013).

Effect of pH on the mycelial growth of blast isolates

The growth of the pathogen was also measured in terms of mycelial dry weight. The effect of pH on the growth of the pathogen was studied by the method of Meena, (2005) using potato dextrose broth media. Potato dextrose broth was prepared in 250 ml Erlenmeyer flask, each containing 30 ml broth basal medium. The pH of the broth was adjusted to 3, 3.5, 4, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.with a help of digital pH meter using 0.1 N HCL and 0.1 N NaOH. Reaction of the medium was adjusted to the desired pH by using di-hydrogen phosphate citric acid buffer according to the method of Vogel (1951). Then the medium was sterilized in autoclave at 120°C for 15 min. From 10 day old culture of *P. oryzae* (6 mm diameter discs) were cut and inoculated into 30 ml basal medium in 250 ml flask and incubated at 30°C. The experiment was laid out in Completely Randomized Design with three replications. After five days of incubation at 30 ^{0}C, the dry weight of the mycelium of each isolate was recorded. After the given incubation period, the mycelial mat of the pathogen was removed and collected in pre-weighed what man's filter paper No 42 and the filter papers with mycelial mat were dried at 60^0 C for 6 days in electric oven. After drying, the filter papers with mycelium were re-weighed. The mycelial dry weight per culture was determined by subtracting the weight of filter paper from the weight of filter paper + mycelial mat (Meena, 2005).

Pathogenicity test for P. oryzae isolates

Once the pathogen was isolated and identified, the pathogenicity test of the pathogen was necessarily for verification, that the fungus was the real cause of the rice blast disease or not. The pathogenicity of all six purified isolates of *P. oryzae* was confirmed by Koch's postulates by the method of Chevalier *et al.,* (1991) under greenhouse conditions at Ambo Plant Protection Research Center Ambo, Ethiopia. The greenhouse soil was prepared from field soil (sandy loam) : sand mixture (3:1), respectively and the soil was autoclaved at 121°C for 2 h. Disinfected viable seeds of Guraferda local rice cultivars were sown in autoclaved sterilized soil using 6 cm diameter pots with 5 plants per pot. The plants were inoculated after germination, at the age of 3-4 leaves and the seedlings in each pot was sprayed with 40–50 ml of spore suspension adjusted to 10^5 spores/ml with the help of haemocytometer. Atomizer sprayer was rinsed with 95% Ethanol and then washed with sterile distilled water and used for spraying (Hans *et al.,* 2003). Inoculated pots were placed on a rotating rack and were sprayed all plants simultaneously while rotating the rack to ensure even coverage of the leaves. The conidial suspensions were sprayed on to the rice seedlings until runoff while water was used for spraying the control treatment. The plants were placed inside the dew chamber at 25°C for 24h and then transferred them to a greenhouse bench at 25-30°C. The severe symptoms were observed on inoculated plants. The symptoms of the lesions that appeared on the leaves of the inoculated plants were observed and compared similar to the symptoms described on the naturally infected plants from the field. Periodical observations were made for the development of symptoms on the leaves starting 7 days after inoculation. Experiments were conducted in CRD with three replications. The fungus was re isolated from the artificially inoculated rice seedlings leaves showing typical *P. oryzae* symptoms and the culture obtained was compared with the original culture and was found to be similar for its morphology and colony characters.

Statistical analysis

The prevalence, incidence and severity data were analysed by using the descriptive statistical analysis (means) over district / localities and altitudinal ranges. Analysis of data on disease parameters were subjected to perform by statistical analysis using SAS software version 9.0 used in Complete Randomized Design with three replications. Also charts using of Excel were drawn and comparison of means were conducted with Duncan's multiple range tests at 5 %

statistical probability level to examine mean statistical differences among treatments.

RESULTS AND DISCUSSION

Assessment of blast disease intensity

During the survey, the rice blast was observed on 100% of all the 90 rice cultivated inspected fields at variable levels (Table 2). The results of the assessment revealed that the intensity (incidence and severity) of the disease vary from slight to high intensity depending on the agro-ecological and cultivars differences. The incidence of rice blast in six different localities varied from 42.01 to 85.69%. The highest mean incidence of rice blast was recorded in Otuwa (85.69%) followed by Berji (75.5 %), while the lowest was recorded in Argoba locality (42.01%).The overall mean incidence for the six localities of three districts of South west of Ethiopia (SNNPRS) was reached 65.68% (Table 2). Likewise, blast severity also showed similar trend as that of incidence in the six localities. The highest severity was recorded in Otuwa locality with range of 8.88 - 88.8 % and mean severity values of 55.7%. This was followed by Berji locality, with a range of 22.22 - 84.4% and mean severity of 55.4% while the lowest severity was recorded in Argoba locality with range of 2.22-73.33% and means severity values of 33.62%. The overall mean severity for the six localities in three districts of the South west SNNPRS reached 47.15% (Table 2). The assessment of blast disease showed that the disease incidence and severity were varied from field to field and locality to locality due to the different geographical and environmental conditions prevailing in each locality. All the assessed six localities were exhibited 100 % blast disease prevalence. This indicated that the rice blast disease was occurred over all areas found in selected localities.

Among the fields inspected, 100 % of the fields assessed were found at altitudes below 1500 m. a. s. l. (lowlands agro ecology). The prevalence of the blast disease was recorded at the lowland elevation agro ecology zone (1107-1423 m. a. s. l.). In a similar way, at the altitude of 1107 - 1290 m. a. s. l., the incidence of the rice blast disease recorded 85.6, 75.5 and 48.4% and severity of the rice blast disease was 55.7, 55.4 and 48.4%. At the altitude of 1327-1423 m. a. s. l., the incidence of the rice blast disease were 66.5, 54.6 and 42.01% and severity of the rice blast disease were 47.7, 42.07 and 33.6%. This result showed that blast severity and incidence was increased with decreased altitude range but lower severity and incidence was observed with increased altitude (Table 3). The higher incidence and severity was recorded in Guraferda district but the lowest incidence and severity was recorded in Gimbo district. WARDA, (2004) reported that the incidence/severity of rice blast varies across different locations and cultivars in different years and blast was prevalent and severe in the rice plants at the vegetative stage. Nutsugah (1997) and Nutsugah and Twumasi (2001) identified the disease as a serious threat to rice production in Ghana. The incidence and severity of blast across rice growing areas in Ghana have been surveyed and areas of low - high blast incidence were identified Nutsugah and Twumasi (2001).

Prevalence, incidence and severity of blast disease across rice cultivars

The assessment of incidence and severity of blast disease revealed that varied depends on cultivars. The most dominant cultivar grown by the farmers in the South west of Ethiopia was Guraferda local but the other cultivars were recently introduced which were grown in few farmers fields, private sector farm and Bonga Agricultural Research Center (Table 4). During the assessment, cultivars such as Guraferda local, Getachew, Hidassie, Tana, Andassa and Suparica-1 were scored highest incidence and severity of blast disease whereas cultivars such as Nerica -3, Nerica -4, Nerica -12 and Kokit were scored lowest incidence and severity (Table 4).

Table 2: Prevalence, incidence and severity of blast disease in rice cultivated fields at six localities of South west Ethiopia.

Blast survey areas			No. of fields inspected	Infected fields	Prevalence (%)	Incidence (%)		Severity (%)	
Zone	District	Localities				Range	mean	Range	mean
B.Maji	Guraferda	Otuwa	15	15	100	41.48-100	85.69	8.88-88.88	55.70
		Berji	15	15	100	34.6-100	75.5	22.22-84.44	55.40
		Kuja	15	15	100	30.56-100	69.8	13.33-84.44	48.44
Sheka	Yeki	Alamo	15	15	100	0.606-100	54.61	2.22-91.11	42.07
Kaffa	Gimbo	Choba	15	15	100	0-100	66.5	0-82.22	47.70
		Argaoba	15	15	100	1.85-100	42.01	2.22-73.33	33.62
Total			90	90					
Mean			15	15	100	18.18-100	65.68	8.28-81.85	47.15

Table 3: Prevalence, incidence and severity of blast disease in rice cultivated fields across altitude range in districts.

Altitude range (m)	No. of fields inspected	Infected fields	Prevalence (%)	Incidence (%)	Severity (%)
1107-1223	15	15	100	85.69	55.70
1163-1277	15	15	100	75.5	55.40
1144-1290	15	15	100	69.8	48.44
1327-1405	15	15	100	54.61	42.07
1367-1389	15	15	100	66.5	47.70
1387-1423	15	15	100	42.01	33.62
Mean	15	15	100	65.68	47.15

Table 4: Prevalence, incidence and severity of blast disease in rice cultivars fields.

Cultivars	Fields inspected	Prevalence (%)	Incidence (%)	Severity (%)
Kokit	1	100	24.6	3.33
Hidassie	1	100	91	78.34
Suparica-1	1	100	83.3	73.4
Nerica-3	3	100	20.7	2.6
Nerica-4	12	100	23	2.6
Andassa	1	100	87.6	60.2
Tana	1	100	92.2	75.4
Getachew	1	100	94.6	80.2
fofi3737	1	100	74	47.03
fofi3730	1	100	81.6	54.2
Guraferda local	76	100	96.7	86.3
Nerica 12	1	100	18.9	2.2
Mean			65.68	47.15

CHARACTERIZATION OF THE RICE BLAST ISOLATES

Evaluation of different culture media on sporulation of blast isolates

Sporulation of each *P. oryzae* isolate on 14th day of different media showed significant differences between isolates. Totally six *Po* isolates (*Po*12, *Po* 28, *Po* 41, *Po* 55, *Po* 72 and *Po* 85) were identified from 90 blast samples based on growth characteristics of the test pathogen and geographical location of the origin of the isolate. All the six *Po* isolates were observed good sporulation on media of oat meal agar (OMA) and also good sporulation was observed on rice flour agar (RFA) for the following four isolates *Po* 12, *Po* 28, *Po* 41, and *Po* 85. The fair sporulation was observed on RFA media for the isolates of *Po* 55, and *Po* 72 and on potato dextrose agar (PDA) media for the isolates of *Po* 41, *Po* 72 and *Po* 85. The poor sporulation was recorded on PDA media on isolates of *Po* 12 and *Po* 55 and malt extract agar (MEA) media on isolates of *Po* 28, *Po* 41, *Po* 55, *Po* 72 and *Po* 85, respectively. Similar results were also reported by Afshana *et al*., (2011) and Bandyopadhyay *et al*., (2009) found that the OMA media was suitable and good for sporulation of blast isolates. Also, Gopal *et al*., (2012) reported that among the different media used, OMA media was found to be the best for sporulation of the blast isolates from both rice and finger millet. In the present study also among the four medias, OMA media was the good sporulation of all the six *Po* isolates viz., *Po* 12, *Po* 28, *Po* 41, *Po* 55, *Po* 72 and *Po* 85.

Colony growth diameter of rice blast isolates on different culture media

The results revealed that there is a considerable variation among the colony diameter of the *P. oryzae* isolates on different solid medias (Table 5). The mean of radial growth of different isolates on different solid media, the OMA and RFA wee optimum for all the cultures of *Po* isolates. The radial growth of five *Po* isolates viz., *Po* 12 (88 mm), *Po* 72 (85.3 mm), *Po 55* (83.6 mm), *Po* 28(83.3 mm) and *Po* 41(82mm) on the 10thday showed significantly highest on OMA media followed by isolate *Po* 85 (80.3 mm). On RFA media, the highest colony growth diameter was recorded significantly in *Po* 12 isolate (86 mm) followed by the isolates of *Po* 28 (77.6 mm), *Po* 41(77 mm), *Po* 72 (76 mm) and *Po* 55 (75.6mm), whereas the *Po* 85 (55.6mm) isolate showed the least radial growth. On PDA media, the highest colony growth diameter was recorded significantly only in *Po* 85 (75.6 mm) isolate followed by the isolate of *Po* 28 (61.3 mm) whereas *Po* 55 isolate showed the least radial growth (55.3 mm). On MEA media, the highest colony growth diameter was observed significantly on isolates of *Po* 85 (59 mm) and *Po* 28 (53.3 mm) followed by the isolates of *Po* 41 (50mm) and *Po* 72 (49.6 mm) while the least growth was observed on isolates of *Po* 12 (44 mm) and *Po* 55 (44 mm). Among the four media, the maximum radial growth of the isolate was observed in OMA (88 mm) of isolate *Po* 12 followed by RFA (86 mm) of isolate *Po* 12 and the least radial growth was observed in MEA (59 mm**)** of isolate *Po* 85. Similarly, Kulkarni (1973) reported that among the solid and liquid medias, OMA was found to be good for growth of the *P. oryzae* isolates. Similar results were also reported by Afshana *et al*., (2011**)** that the OMA was suitable for growth of *P. oryzae*. The present study results were also supported by Gopal *et al*., (2012) among the different media used, OMA was found to be the best for mycelial growth of the isolates of *P. oryzae* from rice and finger millet crop plants.

Colony characteristics of Po isolates on the different culture media

Significant variation among the colony characteristics of the different isolates were observed in culture plates which were collected from different localities. All the six *Po* isolates grown on four different media were observed the mycelial color, margin, pigmentation, surface texture and growth. On the OMA medium, the isolate *Po* 12 colony color was dark gray and the light gray color in the isolate *Po* 85 and off white color in isolates *Po* 12 and *Po* 72 and grayish

Table 5 Colony diameter of *Po* isolates *on* different solid medias

Isolate	Mean Colony diameter(mm) 10 DAI				Mean
	Media				
	PDA	**OMA**	**RFA**	**MEA**	
Po 12	52 E	88 A	86 A	44 C	67.5AB
Po 28	61.3B	83.3AB	77.6B	53.3 AB	68.87A
Po 41	53.6D	82 AB	77 B	50 B C	65.65B
Po 55	47.6F	83.6AB	75.6B	44 C	62.7C
Po 72	55.3C	85.3AB	76 B	49.6 BC	66.55AB
Po 85	75.6A	80.3B	55.6C	59A	67.62AB
Mean	57.56C	83.75A	74.63B	49.98D	66.48
LSD(0.05)	0.93	6.90	7.95	6.87	
CV(%)	0.91	4.63	5.98	7.73	

Means followed by the same letters are not significantly different at the 5 % level by DMRT.

Table 6 Colony characteristics of *Po* isolates on different solid media

Isolates	**Media**	**Colony characters**					
		Colony color	Margin	Pigmentation	Surface texture	Mycelial growth*	Sporulation
Po 12	PDA	Grey	Irregular	black`	cottony	Medium growth	Poor
	OMA	Dark gray	Entire	black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	black	Velvety, thick	Good growth	Good
	MEA	olive gray	Irregular	Dark brown	cottony	Poor growth	Poor
Po 28	PDA	Grey	Entire	black`	cottony	Medium growth	Fair
	OMA	off white	Entire	black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	black	Velvety, thick	Good growth	Good
	MEA	olive gray	Irregular	Dark brown	cottony	Medium growth	Poor
Po 41	PDA	Grey	Entire	black`	cottony	Medium growth	Fair
	OMA	Grayish Black	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Good growth	Good
	MEA	olive gray	Irregular	Dark brown	Velvety, thick	Medium growth	Poor
Po 55	PDA	Grey	Irregular	black`	cottony	Poor growth	Poor
	OMA	Grayish Black	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Good growth	Fair
	MEA	olive gray	Irregular	Dark brown	cottony	Poor growth	Poor
Po 72	PDA	Light greyish	Irregular	black`	cottony	Medium growth	Fair
	OMA	off white	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Good growth	Fair
	MEA	olive gray	Irregular	Dark brown	Velvety, thick	Poor growth	Poor
Po 85	PDA	Grey	Entire	black`	cottony	Good growth	Fair
	OMA	Grayish Black	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Medium growth	Good
	MEA	olive gray	Irregular	Dark brown	Velvety, thick	Medium growth	Poor

*Growth (mean colony diameter)

Good = 75-90 mm

Moderate = 56-75 mm

Low/poor = <56 mm (Narendra, 2006).

black color in the other three isolates, *Po* 41, *Po* 55 and *Po* 85 were noticed. Colony of all the isolates margins were entire and the pigmentation was black, velvety in surface texture and good in growth. On the RFA medium, the colony of all the isolates were light gray in color, entire in margin, black in pigmentation, velvety and thick in surface texture and good in growth. On the MEA media, the colony of all the isolates were olive gray in color, irregular in margin, and dark brown in pigmentation. The isolates of *Po* 12, *Po* 28 and *Po* 55 were cottony surface texture whereas the other three isolates (*Po* 41, *Po* 72 and *Po* 85) were velvety and thick in surface texture. The colony of isolates, *Po* 28, *Po* 41 and *Po* 55 were medium in growth whereas the other isolates (*Po* 12, *Po* 55 and *Po* 72) were poor in growth. On the PDA media, the colony of the five isolates (*Po* 12, *Po* 28, *Po* 41, *Po* 55 and *Po* 85) wee gray in color whereas the isolate *Po* 85 was light gray. The colony margin of the isolates of *Po* 12, *Po* 55 and *Po* 72 were irregular while the other three isolates, *Po*28, *Po*41 and *Po*85 margins were regular. All the isolates pigmentations were observed black in color and the surface texture were noticed cottony. The isolate, *Po*85 was observed good growth and the isolates of *Po* 12, *Po* 28, *Po* 41, *Po* 55 and *Po* 72 were medium in growth whereas the isolate *Po*72 was poor in growth (Table 6).

Similarly, Meena (2005) also reported that the colony characteristics of *Po* isolates on OMA media showed greyish black and entire colony margin and also showed both irregular and entire colony margins of some isolates on PDA medium. Bandyopadhyay, (2009) was reported that the OMA media produced off-white, good and regular mycelial growth. These results also agreed with Gopal *et al*, (2012) in rice blast isolates of the color of colony showed grey and black grey for the finger millet isolates on OMA medium. The present study results were also supported by Arunkumar and Singh (1995) that the *Po* isolates showed best performance in OMA for the rate of colony growth. Agarwal *et al*., (1989) and Vanaraj, (2013) stated that the colony color of *Po* isolates were appeared grey on PDA medium. Mew and Misra (1994) reported that the colonies of *Po* isolates on PDA medium showed blackish in pigmentation. Also in this present study, the pigmentation color was observed black. Mew and Gonzales, (*2002)* indicated that the *P. oryzae* pathogen colonies on PDA medium grow very slowly and colony on the reverse side of the agar plates were black.

Conidial characteristics of rice Po isolates

The morphological characteristics of six different isolates of *Po* on OMA only were observed. The results showed that all of the conidia in each isolate was pyriform in shape, base rounded, apex narrowed, two-septate, with three celled observed in isolates of *Po* 28, *Po* 55, *Po* 72 and *Po* 85 and one-septate with two celled was observed in isolates of *Po* 12 and *Po* 41. The conidium in each isolate was observed hyaline to pale olive colors. Among the different isolates, the morphological variability in respect to conidia length and width were also observed significantly. The observed conidial length was varied from 14.5-26.5µm. The maximum length of the conidia was recorded in isolates of *Po* 55 (24.73 µm), *Po* 12 (23.5 µm) and *Po* 41 (21.66 µm) followed by isolates of *Po* 28 (19.96 µm) and *Po* 72 (18.93µm) and shortest conidia was observed in *Po* 85 isolate (18.6µm). The observed conidial width was varied from 5.1-8.3 µm. The maximum width of the conidia was observed on the *Po* isolates of *Po* 28 with (7.86 µm) and *Po* 72 (7.59 µm) followed by *Po* 12 (6.56µm) whereas the narrowest width was observed in isolates of *Po* 85 (6.04 µm), *Po* 41 (6.03µm) and *Po* 55 (5.96 µm), respectively (Table 7). Ono and Nakazato, (1958) observed that the size of conidia of *P. oryzae* varied with the culture media. The sizes as well as shape of the spores and colonies of filamentous fungi are the most important factors in fungal identification. The present study results were also supported by the other workers (Mew and Gonzales 2002; Meena 2005 and Afshana *et al.,* 2011).

Table 7: Conidial characteristics of *Po* isolates

Isolate	Conidial characteristics						
	Conidial Size (in µm)				Conidial shape	Conidial color	Septation of conidia
	Length		Width				
	Range	Mean	Range	Mean			
Po 12	21.3-25.1	23.5AB	6.2-7.01	6.56BC	Pyriform	hyaline to pale olive	1-septate ,2 celled
Po 28	17.6-22	19.96BC	7.5-8.3	7.86A	Pyriform	hyaline to pale olive	2-septate,3 celled
Po 41	19.4-23.1	21.66ABC	5.5-7	6.03C	Pyriform	hyaline to pale olive	1-septate ,2 celled
Po 55	22.3-26.5	24.73A	5.1-6.7	5.96C	Pyriform	hyaline to pale olive	2-septate,3 celled
Po 72	16.7-21.3	18.93BC	7.2-8.25	7.59AB	Pyriform	hyaline to pale olive	2-septate,3 celled
Po 85	14.5-21.01	18.60C	5.8-6.5	6.04C	Pyriform	hyaline to pale olive	2-septate, 3 celled
Grand mean		21.23		6.67			
LSD (0.0)%		4.33		1.07			
CV (%)		11.46		9.02			

Table 8:Effect of different temperatures on the mycelial growth of *Po* isolates grown on PDA media

Isolate No.	Mean Colony diameter (mm) at different temperature levels in(^{o}C)							
	15	20	25	30	35	40	45	Mean
Po12	13.33B	27.66AB	44.66C	56ABC	30.66A	5.66B	NG	25.424C
Po 28	16.66AB	27.66AB	38.66D	52C	30.66A	7AB	NG	24.663C
Po 41	18.33A	25.33ABC	54.33A	57.66AB	32.66A	8.66A	NG	28.139A
Po 55	14.33AB	28A	44C	60.33A	32.66A	7AB	NG	26.617B
Po 72	13.66B	22C	50B	55.33BC	30A	6B	NG	25.284C
Po 85	12.66B	24BC	41.66CD	59.33AB	32.66A	6.66B	NG	25.281C
Mean	14.83E	25.77D	45.55B	56.77A	31.55C	6.83F	G	
LSD (0.05%)	3.97	3.48	3.35	4.31	3.88	1.77		
CV (%)	15.07	7.59	4.13	4.27	6.92	14.63		

In a column, means followed by a same letters are not significantly different at the 5 % level by DMRT.

Effect of temperature on growth of *Po* isolates

Temperature is one of the most important physical environmental factors for regulating the growth and reproduction of *P. oryzae* isolates. The effects of different temperatures on the mycelial growth of six *Po* isolates were studied. The results showed that the colony diameter of each *Po* isolates on the 5th day after inoculation on different temperature levels showed significantly differences between different temperatures and isolates. Among the six different isolates, the radial growth of isolates of *Po* 55 (60.33 mm), *Po* 85 (59.33 mm), *Po* 41(57.66 mm) and *Po* 12 (56 mm) on the 5th day after inoculation were significantly highest on the temperature level of 30^{o}C followed by the *Po*72 isolate with 55.33 mm whereas the minimum growth was recorded at *Po* 28 isolate with 52mm (Table 8).

At the temperature level of 25^{o}C, among the six different isolates, *Po* 41 isolate colony radial growth on the 5th DAI was significantly highest (54.33 mm) over the other isolates followed by the *Po* 72 isolate (50mm), whereas the minimum growth was recorded in isolate of *Po* 28 (38.66 mm) (Table 8). On the temperature level of 35^{o}C, the radial growths of all isolates were not observed significantly difference between them. Minimum growth was observed in temperature level of 40^{o}C and 45^{o}C. At the temperature of 40^{o}C the different isolates of radial growth, *Po* 41

Table 9: Effect of different pH levels on the mycelial growth of *Po* isolates grown on PD broth media.

Isolates	Dry mycelium weight (mg) at different pH level									
	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	Mean
Po **12**	6.66A	11ABC	19.33AB	51.66AB	84A	115.33AB	249C	323.33A	206.66B	89.66B	32.66B	108.11B
Po **28**	4.33AB	7.33C	22.66AB	53A	74C	92C	265.33B	304.66B	184.33C	93.33A	28.66B	102.69C
Po 41	6AB	8.66BC	21AB	39.66C	65D	109B	293.33A	281.33C	171D	94A	33.66AB	102.05C
Po 55	4.76AB	13AB	24.33A	47.33C	60F	125.33A	229.33D	309.66B	180C	95.66A	31.33B	101.20C
Po **72**	3.6B	14A	17C	41.66C	63E	100.66BC	284A	318.33A	164.66E	85C	32.33A	102.20C
Po **85**	6.66A	11.66ABC	22AB	54A	80B	115.66AB	293.66A	318A	216.66A	85.66C	38A	112.90A
Mean	5.33K	10.94J	21.05I	47.88G	71F	109.66D	269.11B	309.22A	187.22C	90.55E	32.77H	
LSD	2.35	4.10	3.16	4.89	0	14.85	12.6	7.5	6.26	2.93	5.06	
CV	24.7	21.1	8.45	5.7	0	7.61	2.63	1.36	1.88	1.82	8.68	

In a row, means followed by the same letters are not significantly different at the 5 % level by DMRT.

isolate was significantly highest (8.66 mm) over the other isolates followed by the isolates of *Po*28 (7), *Po*55 (7), *Po* 85 (6.66), *Po* 72 (6.66) and *Po* 12 (5.66 mm) but all the six isolates were not grown on the temperature level at 45°C. The ideal temperature range for the maximum growth of different isolates of *P. oryzae* was observed from 25°C to 30°C temperature levels followed by temperature levels of 35°C and 20°C and the minimum mycelial growth was recorded at the temperature levels of 15°C, 40°C and 45°C. The mycelial growths of different *P.oryzae* isolates were highly suppressed at temperatures of 15°C, 40°C and 45°C. It was observed that after 20°C, the colony growth was increased and attained maximum at 30°C and then start reduced **(**Table 8). Similarly, Jamal *et al*., (2012) and Bahadur *et al.,* (2013**)** also reported that *P. oryzae* isolates grew at 30°C was the optimum temperature for mycelial growth. The present study results were also supported by Meena, (2005) at the temperature of 30°C was the optimum temperature for the mycelia growth of the *Po* isolates. In this study, the growth of all the isolates was decreased drastically at 40°C. Arunkumar and Singh (1995) studied the differential response of *P. oryzae* isolates from rice, finger millet and pearl millet related with temperature and they reported that all the isolates exhibited maximum growth at 30°C. The present study results are also in close agreement with those already reported by Hossain *et al*., (2004) who reported 30°C as optimum temperature for growth of *P. oryzae*.

Effect of pH on the mycelial growth of Po isolates grown on PD broth media

The results revealed that there was a considerable variation among the dry mycelial weight of the different isolates on different pH concentrations. The maximum mean dry mycelial weight was observed at pH 6.5 (309.22 mg) followed by pH 6 (269.11 mg) and pH 7 (187.22 mg). But the least mean dry mycelia weight was recorded at pH 3 (5.33mg), pH 3.5 (10.94mg), pH 4 (21.05), and pH 8 (32.77mg).

The dry mycelial weight at pH 6.5 (323.3-318mg) for isolates of *Po*12, *Po* 85 and *Po* 72 were significantly difference over the mycelial weight for other isolates. The dry mycelial weight (293.7-284mg) at pH 6 for the isolates of *Po* 85, *Po* 41 and *Po* 72 were significantly difference over the mycelial weight for other isolates (Table 9). The dry mycelial weights of *Po* isolates were significantly increased from pH 3.0 to pH 6.5 and which further start too deteriorated. The results of the present study indicated that *Po* isolates prefers pH range of 6.00- 6.50. The pH below six and above seven was observed to be hindering for the growth of *Po* isolates. Therefore, at pH 6.5 almost all *Po* isolates revealed maximum dry mycelial weight; this showed that it is an optimum pH for growth of *Po* isolates (Table 9). The present study results were also supported by Arunkumar and Singh (1995) who obtained the best growth of the *Po* isolates at pH 6.5 and Mijan Hossain (2000) reported that growth of *Po* isolates increased with increase in pH from 3.5 to 6.5. The present study results were strongly supported by Meena (2005) who reported that the growth of all the isolates at pH 6.5 was significantly superior, over other treatments and growth of all the isolates significantly increased from pH 3.0 to pH 6.5 which further started declining and the least growth was observed at pH 8.0.

Pathogenicity test for Po isolates

Pathogenicity test results revealed that the disease symptoms and development of six *Po* isolates on susceptible local cultivar after inoculation with the inoculum of the test *P. oryzae* isolates. The diamond and spindle shaped with gray center and dark brown to necrotic margins were observed on all of the rice seedlings after the 7th day of inoculation. After 7 day of inoculation, 86.66% disease incidence with 63.2 % of average disease severity was recorded in *P. oryzae* inoculated rice plants, whereas the disease was not developed in un inoculated rice plants. The *P. oryzae* isolates were re-isolated from the infected lesions and compared with the original culture and thus Koch's postulates was proved. The re-isolation revealed that the isolated fungi from diseased rice seedlings were found to be identical with those used for artificial inoculation. Although the reaction types showed by local susceptible rice cultivar to *P. oryzae* isolates were similar in both field and greenhouse but the disease severity was more intense in the field of the selected localities. All the isolates were the causative agents for blast disease of rice. Pathogenicity test revealed that all *P. oryzae isolates* were able to infect local susceptible rice.

CONCLUSIONS

The primary aim of this study was to assess the rice blast disease prevalence, incidence and severity on upland rice cultivars in the South west of Ethiopia and to characterize the rice blast pathogen isolates collected from various South West areas of Ethiopia. It was observed in all assessed localities at variable levels. The results of the assessment revealed that the incidence and severity of the blast disease varied from slight to high intensity from field to field and localities to localities depending on the agro-ecological and environmental conditions prevailing in each locality. The different culture media was evaluated based on colony diameter to get suitable media for the growth of the *Po* isolates. The different media was evaluated on their potential of sporulation of *Po* isolates and good sporulation was observed on the medium of oat meal agar for all the six isolates (*Po*12. *Po*28, *Po*41, *Po*55, *Po* 72 and *Po* 85). The ideal temperatures for the maximum growth of six Po isolates were ranged from 25°C to 30°C temperature levels. The dry mycelial weight of *Po* isolates was significantly increased from pH 3.0 to pH 6.5 and which further start too deteriorated. It could be concluded that rice blast was the most important disease of rice cultivars in South West (SNNPRS) of Ethiopia. Suitable media, optimum temperature and pH level preferred to the mycelial growth and sporulation of the *P. oryzae* isolates and maximum mycelial

growth and sporulation was found in oat meal agar medium followed by rice flour agar medium. The best mycelial growth was also found at temperature 30°C and pH 6.5 which was suitable for mycelial growth of *P. oryzae* isolates. Further studies have to be conducted to evaluate their resistance under green house and natural environmental conditions. To privilege rice production in South West (SNNPRS) of Ethiopia, these diseases that occur concurrently on rice could be manage using the resistant cultivars. Farmers and development agents should be trained in the management of rice blast disease by using resistant cultivars.

REFERENCES

Abdu S, Nonglak P, Prapa Sripichitt and Sreewongchai T (2013) Identification of blast-resistant varieties from landrace, improved and wild species of rice. *Thailand. Kasetsart J. (Nat. Sci.)* 47: 1 – 7.

Abraham T (2008) Increasing crop production through improved plant protection - volume 1.proceedings of the 14th annual conference of the protection society of Ethiopia (PPSE), MOARD19-22 December 2006.*Addis Ababa, Ethiopia*. pp 377.

Afshana BD, Shahijahan M, Hussain DS and Snober HB (2011) Morphological variability among various isolates of *Magnaporthe grisea* collected from paddy growing areas of Kashmir. 8(1): 45-56.

Agrawal PC, Mortensen CN and Mathur B (1989) Seed borne diseases and seed health testing of rice. *Technical Bulletin* No.3, Phytopathological paper No. 30, CAB Int. Mycological Ins. (CMI) Kew, Surrey, UK. pp7.

Arunkumar S and Singh RA (1995) Differential response of *Pyricularia grisea* isolates from rice, finger millet and pearl millet to media, temperature, pH and light. *Indian Journal of Mycology and Plant pathology*, 25:238-242.

Bahadur R, Tiwari KS, Netam RS and Tiwari U (2013) Effect of different culture media, carbon source, nitrogen source, temperature and *p*H, Level on the Growth and Sporulation of *Pyricularia grisea* isolate from finger millet. *Research Journal of Agricultural Sciences*, 4(1): 83-86.

Bandyopadhyay J, Kumar RK, Srivastava L and Bhatt RP (2009) Effect of media on growth, sporulation, and production of perithecia of blast pathogen, *Pyricularia grisea.* Department of Genetics and Plant Breeding, India. 2(1): 37-40.

Chevalier M, Yespinasse Y and Renautin S (1991) A microscopic study of the different classes of symptoms coded by the Vf gene in apple for resistance scab (*Venturia inaequalis*), *Plant Pathology*, 40:249-256.

Chipili JK, Twumasi K, Dartey SK, Nutsugah S, Screenivasprasad Y, Sere Y and Dogbe W (2004) Survey of rice blast and varietal screening in Ghana. West Africa Rice Development Association, 01 BP 2551, Bouaké, Côte d.Ivoire. pp 49-60.

Couch BC and Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia*, 94, 683-693.

EIAR/ FRG II (2012) Backing Rice Extension Rightly. FRG II Project Empowering Farmers' Innovation Series No. 4. Ethiopian Institute of Agricultural Research EIAR-JICA, Addis Ababa, Ethiopia . pp 1-5.

FAO (2012) FAO Rice Market Monitor, Trade and Markets Division Food and Agriculture Organization of the United Nations Volume XV - Issue No. 3: Available online at http://www.fao.org/economic/est/publications/rice-publications/rice-market-monitor.

Getachew G, Tesfaye Alemu and Kassahun Tesfaye (2013) First Bio-Innovate Regional Scientific Conference United Nations Conference Centre (UNCC-ECA) Addis Ababa, Ethiopia, 25-27.

Gopal B, Hira KC, Manandhar K, Ram B, Khadka L, Sundar M and Shrestha L (2012) Study on differential response of *Pyricularia grisea* isolates from rice, finger millet and *Panicum* spp. with Local and Alien Media, and their host range. *Nepal Journal of Science and Technology*, 13: 7-14.

Hans Y, Bonos SA, Clarke BB and Meyer WA (2003) Inoculation techniques for selection of gray leaf spot resistance in perennial ryegrass. *USGA Turfgrass and Environmental Research online*, 2:1-9.

Hoang Dinh, Takahito Noda and Pham Van du (1999) Deployment of resistant varieties to blast (*Pyricularia grisea)* in the Mekong Delta. *Omon RICE*, 7: pp 133-134.

Hossain MM, Srikant K and Hegde YR (2004) Physiological and nutritional studies on *Pyricularia grisea*, the causal agent of blast of rice. Department of Plant Pathology, University of Agricultural Sciences, Dharwad India. *Karnataka Journal of Agricultural Sciences*, 17(4): 851-853.

International Rice Research Institute (IRRI) (1996) Standard evaluation system for rice. 4th.ed. IRRI, Manila, Phillipine.

International Rice Research Institute (IRRI) (2009) Rice Policy-World Rice Statistics (WRS). Retrieved May 28, 2010 from. http://www.irri.org/science/ricestat.

Jamal-u-ddin Hajano G, Mubeen A, Lodhi A, Mumtaz A, Pathan L and Qayoom AR (2011) Rice blast-mycoflora, symptomatology and pathogenicity. Sindh Agriculture University Tandojam, 5 (1): 53-63.

Jamal-u-ddin Hajano G, Serwar Shah M, Ali Khanzada, Mubeen Lodhi and Mumtaz Pathan (2012) *In-vitro* evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen, *Magnaporthe oryzae* couch. Sindh Agriculture University, Tandojam, Sindh, Pakistan. *Pak. J. Bot*., 44(5): 1775-1778.

Kulkarni S (1973) Studies on the blast disease of *Eleusine coracana* (L.) Gaertn.(Finger millet or Ragi) in Mysore State. M.Sc. (Agri.) Thesis,U.A.S Bangalore. pp104.

Meena BS (2005) Morphological and molecular variability of rice blast pathogen, *Pyricularia grisea* (Cooke) Sacc.Department of Plant Pathology, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad. Pp 12-54.

Mew TW and Gonzales PA (2002) Handbook of Rice Seed borne Fungi. International Rice Research Institute, Los Banos, Philippines, pp. 83.

Mew TW and Misra JK (1994) A Manual of Rice Seed Health Testing. International Rice Research Institute: Manila, Philippines. pp 83.

Mijan Hossain MD (2000) Studies on blast disease of rice caused by *Pyricularia grisea* (cooke) Sacc. in upland areas. *M.Sc. Thesis,* University of Agricultural Sciences, Dharwad, pp. 53.

MoRAD (Ministry of Agriculture and Rural Development) (2010) National Rice Research and Development Strategy of Ethiopia. Addis Ababa, Ethiopia.

NDPRB (2011) National diagnostic protocol for rice blast on rice caused by *Magnaporthe oryzae* Australian Government Department of Agriculture, Fisheries and Forestry Protocol number 14, 1: 3-7.

Nutsugah SK (1997) Survey of diseases/pests of upland/ hydromorphic rice in Northern Ghana. Proceedings of the SARI Annual In-house Review, March 24.26, 1997, Nyankpala, Ghana.

Nutsugah SK and Twumasi JK (2001) Fungal Diseases of Rice in Ghana: Knowledge and Research Needs. *In*: S. Sreenivasaprasad and R. Johnson, (eds.). Major Fungal Diseases of Rice Recent Advances. Kluwer Academic Publishers. *The Netherlands*, pp. 321.329.

Ono K and Nakazato K (1958) Morphology of the conidia of *Pyricularia* from different host plants produced under different conditions. *Annals of the Phytopathological Society of Japan*, 23: 1 – 2.

Ou SH (1985) Rice Diseases. CAB International Mycological, Institute Kew, Survey, UK.

Piotti E, Rigano MM, Rodino D, Rodolfi M, Castiglione S, Picco AM and Sala F (2005) Genetic structure of *Pyricularia grisea* (Cooke) Sacc. isolates from Italian paddy fields. *Journal of Phytopathology*, 153: 80–86.

Priya Vanaraj, Ramalingam L, Robin Sabariyappan, Saveetha Kandasamy, Sankaralingam Ambalavanan and Rabindran S (2013) Variability in *Pyricularia oryzae* from different rice growing regions of Tamil Nadu, India. *African Journal of Microbiology Research*. 7(26): 3379-3388.

Traoe O, Pinel A, Hébrard E, Dieudomme-Gumedzoe MY, Fargette D, Traore, A and Kanaté G (2006) Occurrence of Resistance –Breaking Isolates of Rice Yellow mottle virus in West and Central Africa. *Plant Dis*., 90: 259-263.

Vogel AI (1951) A Text Book in Organic Quantitative Analysis, Congmcans. Green and Co., London, N. Y., Toronto, pp. 865 – 872.

West Africa Rice Development Association (WARDA) (2004) Rice blast in West Africa: Characterization of pathogen diversity, key screening sites and host resistance. Proceedings of a stakeholder workshop, Project R7552, UK Department for International Development and Crop Protection Programme, *Proceedings Series* no. 3, Vol. 4: pp 123.

RESEARCH ARTICLE

Effect of faba bean (*Vicia faba* L.) genotypes, plant densities and phosphous on productivity, nutrients uptake, soil fertility changes and economics in Central high lands o Ethiopia

Tekle Edossa Kubure[1], Raghavaiah Cherukuri V [1]* Chavhan Arvind[2] and Ibrahim Hamza[1]

[1]Department of Plant sciences, College of Agriculture and Veterinary Sciences, Ambo University, P.O. Box 19, Ambo, Ethiopia, East Africa.
[2]Department of Zoology, D.B. College, Bhokar, SRTM Univerity, Nanded, Maharashtra, India-431801
***Corresponding author**: Professor Cherukuri V Raghavaiah, Tel: 0933907158.
Email: cheruraghav@yahoo.in

Manuscript details:

Received: 23.10.2015
Accepted: 06.12.2015
Published : 30.12.2015

Cite this article as:
Tekle Edossa Kubure, Raghavaiah Cherukuri V Chavhan Arvind and Ibrahim Hamza (2015) Effect of faba bean (*Vicia faba* L.) genotypes, plant densities and phosphous on productivity, nutrients uptake, soil fertility changes and economics in Central high lands o Ethiopia, *International J. of Life Sciences*, 3(4): 287-305.

ABSTRACT

A field experiment was conducted at Ambo University research farm with the objective to determine the optimum P rate and population densities in faba bean (*Vicia faba* l.) genotypes grown on vertisols. The treatments comprised three genotypes (Hachalu, Walki and Local), three spacings (30 cmx7.5 cm, 40 cmx5.0 cm and 60 cmx5.0 cm) and two phosphorus levels (0 kg p_20_5/ha and 46 kg p_20_5/ha,) which were laid out in a split- split plot design with three replications. The results showed that the improved genotype, Walki (3,407 kg/ha) was comparable with Hachalu (3,037 kg/ha) and gave substantially greater seed yield than the local cultivar (2,833 kg/ha). Seeding at 44 plants/m^2 resulted in substantially higher seed and biological yields (3,815 kg/ha and 7,894 kg/ha) than 50 plants/m^2 (3,074 kg/ha and 6,570 kg/ha) and 33 plants/m^2 (2,388 kg/ha and 4,696 kg/ha); although the harvest index was unaltered. Fertilization of faba bean with 46 kg p_20_5/ha resulted in substantial increase in seed (3,531 kg/ha) and biological yields (7,172 kg/ha) over no fertilizer check (2,654 kg/ha seed and 5,602 kg/ha haulm yield). The harvest index tended to improve with p nutrition (49.7) over no phosphorus (47.4). Correlations worked between yield and growth and yield components showed a significant positive relation between seed yield and plant height at different stages, leaf area/plant, leaf area index, biological yield and seed yield/plant. Biomass yield is correlated with leaf area/plant, leaf area index, and plant height. Nutrient (np) removal of genotypes both in seed and haulm has been greater in Walki and Hachalu than in the local cultivar. The n removal in seed and haulm of Walki was 107 kg/ha and 58 kg/ha; and the corresponding removal of Hachalu was 95 kg/ha and 52 kg/ha; while that of the local cultivar was 89 kg/ha and 48 kg/ha. The n uptake in seed and haulm has been greater with 44 plants/m^2 (120 kg/ha and 66 kg/ha) in comparison with 50 plants/m^2 (97 kg/ha and 54 kg/ha)

and 33 plants/m^2 (75 kg/ha and 37 kg/ha). The uptake of p in seed and haulm increased with p application (11.3 kg/ha and 3.6 kg/ha) over no p-application (8.5 kg/ha and 2.7 kg/ha). In protein yield, Walki and Hachalu were better than the local variety. Maintaining 44 plants/m^2 performed better than 50 and 33plants/m^2. Application of 46 kgp_2o_5/ha out yielded no P check. Nutrient dynamics of soil after harvest of crop showed that there was an increase in soil N status ranging from 0.00 to 0.05 percent but a sharp decline in soil P and K contents after the crop harvest due to greater removal by the crop, which ranged from 4.03 to 4.27 ppm of P and 0.15 to 0.01 meq/100g of K. Economic analysis of the genotypes showed that Walki gave the highest net returns (ETB 29,642/ha) followed by Hachalu (ETB 24,827/ha) and the local cultivar (ETB 22,178/ha). Maintaining 44 plants/m^2 gave higher net return (ETB 34,938/ha) than 50 plants/m^2 (ETB 25,309/ha) and 33 plants/m^2 (ETB 16,401/ha). Phosphorus fertilization resulted in a net return of ETB 31,247/ha compared with ETB 21,233/ha obtained with no phosphorus.

Keywords: Faba bean genotypes, plant densities, phosphorus nutrition, yield, nutrient removal, soil fertility changes, economics, vertisols.

INTRODUCTION

Faba bean *(Vicia faba L.)* is one of the major pulse crops grown in the highlands (1800 – 3000 masl) of Ethiopia. The main faba bean global producers are China (1.65 Mt), Ethiopia (0.61 Mt), France (0.44 Mt), Egypt (0.29 Mt) and Australia (0.19 Mt) (FAOSTAT, 2009). The crop takes the largest share of the area under pulses production in Ethiopia. The Central Statistical Agency (CSA, 2013/14) reported that faba bean is planted to 4.34 % (about 538,458.21ha), of the grain crop area with an annual production of about 9,917,002.83 quintals, 3.94 % of the total grain production and yield of 18.42 Qt/ha in Ethiopia. It is grown in several regions of the country with an annual rain fall of 700 – 1000 mm. It is a crop of manifold merits in the economy of the farming communities in the highlands of Ethiopia and serves as a source of food and feed and a valuable and cheap source of protein, apart from playing a significant role in soil fertility restoration in crop rotation through fixation of atmospheric nitrogen. It is mainly produced in Tigray, Gondar, Gojjam, Wollo, Wollega, Shoa and Gamo-Gofa regions of the nation. The export of Ethiopia's faba beans has moved upward since the year 2000 and the major destinations are Sudan, South Africa, Djibouti, Yemen, Russia and USA, though its share in the countries pulse export is small (Amanuel *et al.*, 1993, Newton *et al.,* 2011). Despite the importance, the productivity of the crop is far below the potential and is constrained by several limiting factors.

In Ethiopia, Faba bean is raised by farmers at varied row spacing resulting in dwindled productivity. Plant density is an important component that ultimately determines the yield of crop per unit area; per unit time. In addition to this, being a legume, it needs phosphorus for better root and nodule development, which is often neglected by farmers. The inherent low-yielding potential of the indigenous cultivars is among the most important production constraints (Asfaw *et al*., 1994; Yohannis, 2000). Moreover, diseases like chocolate spot (*Botrytiss fabae*), rust (*Uromyces vicia fabae*) and root rot (*Fusarium solani*) and abiotic stresses like waterlogging have all been identified as

important production constraints (Asfaw *et al*., 1994; Amare ,1990.). Apart this, in vertisols of Ethiopian highlands, phosphorous is fixed and its non-availability is a challenge for better crop growth and development. It is known that Phosphorous nutrition plays a prime role in growth and development of roots and its role in nodulation, dry matter production, N fixation, and protein synthesis of leguminous crops is vital (Haque *et al.; 1986)*, although the crop nutrition for nitrogen is met through rhizobial fixation of atmospheric nitrogen. The need of P for faba bean is high due to energy expenditure in nodule formation (Kopke and Nemecek,2010).Hence a balanced nutrition of legumes gains significance to harvest better yields, specially under rain fed cropping conditions, where rain fall quantum and its distribution controls the total crop production system.

Nitrogen and Phosphorous interact closely in affecting plant maturity. Phosphorous is implicated in speeding up maturity and enhancing root-shoot growth ratio, the formation of glycol-phosphate involved in photosynthesis, respiratory metabolism, apart from being a part of nucleotides (RNA, DNA) and phospholipids of membranes and play a role in energy transfer metabolism (ATP, ADP, AMP, Pyro-phosphates) (Salisbury and Ross, 1992).The high yield potential of faba bean has not been exploited in Ethiopia and the yield in the southwestern Ethiopian highlands is generally low (1.3 ton ha^{-1}. compared to 1.8 ton ha^{-1} world average) (FAOSTAT, 2008). This is largely attributed to raising low yielding local varieties, low soil pH and low P-availability in Nitisols (Agegnehu *et al.*, 2006, Agegnehu and Chilot, 2009). However, faba bean has the capacity to mobilize soil phosphorus by secretion of acids from its rhizosphere, and is therefore of important value in low-input crop rotation systems (Nuruzzaman *et al*., 2005). The yield potential and N fixation has been reported to be high in deep, heavy clay soils (Kopke and N emecek,2010).Besides, there is dearth of information on the optimum plant density to reap better harvests. Therefore, the present investigation was made to evaluate the performance of faba bean genotypes in relation to varied plant densities and phosphorus levels, to know the uptake of N P nutrients, soil fertility changes and economic analysis of these production factors under rain-fed vertisol conditions of central highlands of Ethiopia.

MATERIALS AND METHODS

Description of the study area

The field experiment was conducted under rain-fed condition at Ambo University research farm during the main cropping season of 2014 on vertisol. Ambo is located in the West Shoa Zone of Oromia Regional State, Western Ethiopia, at about 115 km west of Addis Ababa, located within Coordinates: 8°59′N 37°50′E , and an altitude of 2068 m.a.s.l. The seasonal total rain fall of the area during the crop season was 570 mm, with the average minimum and maximum temperature of 9.2 ^{0}C and 27.08 ^{0}C, mean relative humidity of 58.02 % and a mean sun shine hours of 5.62 day^{-1}, respectively. The soil on which the experiment was conducted is characterized by Pellic vertisol (Tesfaye Balemi, 2012). The farm area preceding the current faba bean was a fallow.

Experimental materials

In the current experiment two improved high yielding genotypes of faba bean viz; Hachalu and Walki, which are adapted to the vertisols of the highland areas were compared with a local cultivar. The varieties Hachalu and Walki are recommended for highlands vertisols of Ethiopia (Ambo, Adadi, Arsi, Robe, Sinja and etc.) with altitudes of 1900-2800 m.a.s.l, having a rain fall of 700-1000 mm for planting in mid-June to early July, moderately resistant to chocolate spot and rust, released from HARC /EIAR in 2010 and 2008, respectively. The days to maturity of Hachalu and Walki are 122 – 156 & 133 – 146, respectively. The potential yields of Hachalu & Walki variety were 32- 45 & 24-52 quintal ha^{-1} on research stations and 24–35 & 20-42 quintal ha^{-1} on farmer's field, respectively (EIAR, 2011).

Treatments and design

The treatments consisted of three faba bean genotypes (Hachalu, Walki and a local cultivar) tested as main -plot treatments; three spacing's (30 x 7.5 cm, 40 x 5 cm and 60 x 5 cm) as sub - plot treatments and two phosphorous levels (0 and 46 kg P_20_5 ha^{-1}) assigned as the sub-sub plot treatments, all combined factorial in split-split plot design with three replications.

Analysis of soil and plant

The initial soil samples were collected from randomly selected sites of the experimental plots from a depth of 0- 30 cm prior to cultivation and fertilizer application. The composite soil samples were analyzed for physical and chemical properties, using standard procedures with reference to pH, CEC, organic carbon, total N, available P and K to evaluate the initial nutrient status. After the crop harvest, the soil of each treatment was analyzed for N, P, and K status. The soil physicochemical and plant tissue analysis was carried out at Holetta Agricultural Research Center (HARC), Soil and Plant Tissue Analysis Laboratory. The soil samples were air dried and ground to pass through 0.2 mm sieves for total N. All samples were analyzed following standard procedures. Organic carbon was determined by wet digestion method as described by Walkley and Black (1934).Total N was estimated by Kjeldahl method described by Jackson (1958). Available P in soil was determined by Olsen method (Olsen *et.al*., 1954). Soil texture was analyzed by Bouyoucos hydrometer method (1955) and Soil pH was measured by glass electrode pH meter. The plant samples were analyzed (seed, haulm) for N and P contents to calculate the nutrient uptake treatment wise. Protein content of seed was calculated based on N content of seed.

Field operations

The field was cleared, plowed thoroughly twice by a tractor and harrowed twice to obtain a fine tilth free from weeds. The field was then marked out into 18 plots per replication each of 3 m^2 (1 m x 3 m). Planting was done on 7 July 2014. Two seeds of each genotype were planted per hill at a depth of 2 –3 cm using three spacing's (30 x 7.5 cm, 40 x 5 cm and 60 x 5 cm) to obtain 444,444, 500,000 and 333,333 plants ha^{-1}, respectively. Thinning was carried out two weeks after germination to maintain one plant/hill. The source of phosphorus was Di-ammonium phosphate which was applied pre- planting at the rate of 0 kg P_20_5 and 46 kg P_2O_5 ha^{-1}. Nitrogen was applied uniformly at 18 kg N ha^{-1} as a starter dose with urea and DAP being the sources of N. DAP was applied at the time of sowing.In the current experiment chocolate spot and rust diseases were observed which were managed using a fungicide Mancozeb 80 WP (Dithane M-45), at the rate of 2.5 kg a.i/ha at weekly intervals 3 times as foliar spray. Harvest of the crop was carried out at physiological maturity on 19 November 2014, and further subjected to sun drying to standardize the seed moisture content to 10 percent. Net plots were harvested leaving border rows to determine the per plot yields of beans and haulms.

Observations recorded on crop

The following observations and data were collected on growth, yield and yield components from five randomly selected and tagged representative plants from each net plot.

Nutrient removal

The total uptake of nitrogen and phosphorous was calculated for each treatment employing the formula Nutrient uptake = nutrient concentration (mg $g.dm^{-1}$) x dry biomass weight (mg $plant^{-1}$).

Economic analysis of production factors

Economic analysis (CIMMYT, 1988) of data in relation to different factors of production under test viz; genotypes, plant density and Phosphorus nutrition, was computed in terms of 1. Gross return (ETB ha^{-1}), 2. Net return (ETB ha^{-1}), 3.Cost of Production (ETB ha^{-1}), 4. Benefit**:** Cost ratio (Gross return/Cost of production), 5. Per day Productivity (kg ha^{-1}) (Grain Yield/Crop duration), 6.Return/Birr Investment (Net return/Production cost).

Statistical analysis of data

All the data collected were subjected to the analysis of variance using SAS version 9.1.3 (2009), with Model: $Y_{ijkl} = \mu + r_i + A_j + e_{ij}(a) + B_k + (AB)_{jk} + e_{ijk}(b) + C_l + (CA)_{lj} + (CB)_{lk} + (CAB)_{jkl} + e_{ijkl}$ (c).Where, μ = Population mean, r = replication, A = Main plot, ea = Main plot error, B = Sub plot, eb = Sub plot error, (AB, CA, CB, CAB) = Interaction, C = Sub-sub plot, ec = Sub-sub plot error. Wherever, the treatment showed a significant effect, the Duncan's multiple range test (DMRT) was used for means separation. The treatments were compared for their significance using calculated least significant difference (LSD) values at p = 0.05.

RESULTS AND DISCUSSION

Pre- sowing initial physico-chemical properties of the experimental soil

The pre- planting soil analysis showed that the texture of the soil of the experimental site is dominated by the clay fraction. On the basis of particle size distribution, the soil contains Sand 2.5 %, Silt 22.5 %, and Clay 75 % (Table 1). The soil reaction (pH) of the experimental site is 6.79, which was near neutral. According to FAO (2008), suitable pH range for most crops is between 6.5 and 7.5 in which total N availability is optimum. This indicates suitability of the soil reaction in the experimental site for optimum crop growth and yield (Table 1). The organic carbon content of the soil was 1.17%. According to Tekalign (1991) the soil has low organic carbon content, indicating moderate potential of the soil to supply nitrogen to plants through mineralization of organic carbon. Analysis of soil samples from 0-30 cm depth indicated low (0.070%) level of total N, indicating that the nutrient was not optimum for crop growth, and the available phosphorus content of the soil, is low (5.94 ppm). The K content of soil was 1.63 meq/100g; while the Cation exchange capacity was 1.17 meq/100 g soils.

Crop growth in relation to weather

The meteorological parameters that have profound influence on crop growth in terms of seasonal rain fall, minimum temperature, maximum temperature, relative humidity% and sunshine hours/day are presented in Figure 1. In the present study, faba bean was sown on 7, July 2014 and harvested on 19, November 2014. The maturity period of faba bean ranges from 90 – 220 days depending up on cultivars and climate (Bond *et al.,* 1985). The crop received a seasonal rain fall of 570 mm from June to November with uniform distribution from June through August and tapering towards September through November.

Table 1. Selected physico-chemical properties of the experimental soil before sowing

Physical properties				Chemical properties					
Particle size distribution (%)									
Sand	Silt	Clay	Textural class	pH	OC (%)	CEC (Meq/100g)	Total N (%)	Av. P (ppm)	K (Meq/100g)
2.5	22.5	75	Clay	6.79	1.17	1.17	0.07	5.94	1.63

Table 2: Interaction effect of genotype with P level on pod length (cm) of faba bean.

Genotype	Phosphorus level		Mean
	0 P_2O_5 kg/ha	46 P_2O_5 kg/ha	
Hachalu	6.14	6.24	6.19
Welki	5.59	6.49	6.04
Local	4.56	4.61	4.58
Mean	5.43	5.78	
LSD (0.05)	0.29		

Table5 : Interaction effect of genotype x plant density x phosphorus levels on pod length of faba bean.

Genotype	Pod Length (cm)						
	0 kg $P_2 0_5$/ha			46 kg $P_2 0_5$/ha			
	44 plants/m^2	50 plants/m^2	33 plants/m^2	44 plants/m^2	50 plants/m^2	33 plants/m^2	Mean
Hachalu	6.60	5.67	6.17	5.77	6.33	6.63	6.19
Welki	5.50	5.73	5.53	6.83	6.53	6.10	6.04
Local	4.47	4.40	4.80	4.47	4.43	4.93	4.58
Mean	5.52	5.27	5.50	5.69	5.77	5.89	
LSD (0.05)	0.69						

Kay (1979) reported that the annual rain fall of 650 – 1000 mm evenly distributed is ideal for faba bean. The crop thus had ample opportunity of experiencing adequate moisture supply favorable for better vegetative growth and development. The minimum temperature varied between 6.8 ^{0}C to 11.3 ^{0}C, the maximum being in July and in the minimum in November when the crop is in anthesis and floral development. The maximum temperature oscillated between 25.5 ^{0}C and 29.6^{0}C with maximum in June, lowered in July and August, and gradually escalated from September through November when the crop is in transition from vegetative to reproductive stage. Duke (1981), reported that the optimum temperature for faba bean production range from 18 to 27^{0}C (65 – 85^{0}F).The crop experienced a relative humidity ranging from 44% to 69.9%, peaking in August and tapering towards November gradually. The mean sun shine hours/day hovered between 3.1 and 8.8 in July and November, respectively providing ample opportunity for photosynthetic assimilation of $C0_2$ even through the reproductive period.

Yield and yield components of faba bean Pods plant^{-1}

The local cultivar produced more number of pods (21.6) compared to the improved genotypes that produced 19.2 and 18.6 pods/plant in Hachalu and Walki, respectively. With regard to plant densities, the number of pods/plant did not differ significantly, though 30 x 7.5 cm spacing tended to produce more pods/plant. Faba bean did not respond to phosphorus application in terms of pod number/plant. The pods number/plant is a genetic character and is less influenced by the environment in terms of plant density and P nutrition (Table 6). These results are in agreement with Gemechu *et al.,* (2006) who reported 3 to 15 pods/plant for faba bean genotypes in different geographical regions of Ethiopia. Davood Hashemabadi (2013) also observed increase in plant height, fresh weight, and pod number with 80 kg$P_2 0_5$/ha.

Pod Pod weight/plant (g)

The genotype Hachalu produced highest pod weight/plant (24.3 g) followed by Walki (23.5 g) and the local cultivar (20.9 g) (Table 6). Wider spacing of 60 x 5.0 cm resulted in substantially greater pod weight/plant (24.3 g) than 30 x 7.5 cm (22.9 g) and 40 x 5.0 cm spacing (21.5 g). This indicates that pod weight/plant can be altered by plant spacing. The greater pod weight/plant recorded with low plant density could be attributed to less competition for growth resources like soil moisture, nutrients and sun light as compared to the dense stands. Faba bean exhibited significant response in terms of greater pod weight/plant with application of 46 kg $P_2 0_5$/ha (24.0 g) compared to 21.7 g obtained with no phosphorus. This signifies the beneficial role of phosphorus in improving the pod weight of faba bean .

Seeds pod^{-1}:

Seeds/pod did not vary significantly among the genotypes, while it tended to vary with plant density and phosphorus nutrition (Table 6).

By and large, the seed number/pod of the test genotypes remained at 3. Among the spacings, wider spacing tended to improve the seeds/pod (3.0) as compared with narrow spacings (2.7). On the other hand, phosphorus application tended to improve seeds/pod (3.0) when compared with no phosphorus (2.8). The number of seeds/pod varied distinctly when Hachalu fertilized with 46 kgP_20_5/ha and sown at wider spacing of 60 x 5.0 cm (3.33) than when, sown at 30 x 7.5 cm (2.33) as evident from interaction (Table 7). Interaction effect of genotype, plant density and phosphorus levels on seeds pod^{-1} of faba bean (Table 8) showed that local cultivar was relatively superior (2.94) to Hachalu (2.89) and Welki (2.83). With the application of phosphorus fertilizer and sowing at a spacing of 60 x5.0 cm Hachalu produced more seeds/pod (3.33) than Walki (3) and local cutivar. In general, as the seeds/pod is a genetic character, it is less influenced by either management or P nutrition. These results are in agreement with Gemechu *et al.,* (2006) who reported that seeds pod^{-1} of faba bean genotypes ranged from 2-3.

Number of seeds plant^{-1}

Among the test genotypes, the local cultivar produced significantly more number of seeds/plant (36.6) than the improved genotypes, Hachalu (30.7) and Walki (35.1)(Table 6). This could be due to production of more number of pods/plant in the former and its better adaptation to the environment even under moisture stress conditions than in the latter genotypes. With regards to planting density, low density planting adopting wider spacing (60 x5.0 cm) offered significantly more number of seeds/plant (37.3) in comparision with high density planting with 30 x 7.5 cm (33.1) and 40 x 5.0 cm (32) under rain fed conditions.The crop fertilized with 46 kg P_20_5/ha produced significantly more number of seeds/plant (35.2) than the unfertilized crop (33.1), high lighting the importance of phosphorus nutrition in faba bean. Interaction between genotypes and phosphorus levels showed that the local genotype produced significantly more seeds/plant (39.1) when compared to that produced by Welki (36.1) and Hachalu (30.4) (Table 8).

Seed weight plant^{-1}(g)

Among the genotypes, Hachalu produced maximum seed weight/plant (18.7 g) followed closely by Walki (17.8 g) and the least weight by the local cultivar (16.24 g)(Table 6). This reveals that the partitioning efficiency is better in improved faba bean genotypes than the local traditional cultivar; which is of para amount importance in enhancing seed yields per unit area. The seed weight/plant showed distinct variation in relation to different plant densities. Sparse density (60 x 5.0 cm) resulted in significantly higher seed weight/plant (18.24 g) than dense plant stands of 30 x 7.5 cm (18.0 g) and 40 x5.0 cm (16.52 g). The reduction in seed weight/plant in dense stands could be due to greater inter- plant competition for growth resources like soil moisture, nutrients and sun light. Crop fertilization with 46 kg P_20_5/ha brought about substantial improvement in seed weight/plant (18.42 g) in comparison with un fertilized crop (16.75 g) indicating the role of phosphorus in improving seed weight of faba bean.

Test weight of seed (g)

Among the genotypes, Hachalu recorded substantially greater test seed weight (650 g) compared to Walki (524 g) and the local cultivar which recorded the least weight (344 g)(Table 6). This elucidates the greater assimilatory efficiency of the improved genotypes than that of traditional cultivars. High density of 44 plants/m^2 (508 g) and 50 plants/m^2 (517 g) had seeds of greater weight than low density planting at 33 plants/m^2 (492 g).Phosphorus fertilization at 46 kg P_20_5/ha significantly improved the test seed weight (520 g) over no phosphorus (492 g). These results are in agreement with Gemechu *et al.,* (2006) who found that 1000 seed weight of faba bean genotypes ranged from 249-553 g.

Table 6: Effect of genotypes, plant density and P fertilizer levels on yield and yield components of Faba bean.

Treatments	Yield and yield components of faba bean											
	Pods/plant	Pod Length (cm)	Pod Weight/Plant (g)	No. of Seeds/pod	No. of Seeds/plant	Seed weight/Plant (g)	Seed Yield/plot (g)	1000 Seed wt. (g)	Biological Yield/plot (g)	Seed Yield (kg/ha)	Biological Yield (kg/ha)	Harvest Index (%)
Genotype												
Hachalu	19.17b	6.19a	24.27a	2.89a	30.72b	18.72a	911.11ab	650.06a	1908.4a	3037.0ab	6361.4a	48.17a
Welki	18.61b	6.04a	23.47a	2.83a	35.11a	17.80ab	1022.22a	523.89b	2092.1a	3407.4a	6973.5a	48.42a
Local	21.56a	4.58b	20.90a	2.94a	36.61a	16.24b	850.00b	344.06c	1747.7a	2833.3b	5825.7a	49.10a
Mean	**19.78**	**5.61**	**22.88**	**2.89**	**34.15**	**17.59**	**927.78**	**506**	**1916.07**	**3092.6**	**6386.9**	**48.57**
LSD (0.05 %)	**2.16**	**0.99**	**NS**	**NS**	**6.06**	**2.36**	**169.32**	**34.7**	**NS**	**564.4**	**NS**	**NS**
CV%	**11.79**	**19.19**	**17.25**	**15.08**	**19.18**	**14.52**	**19.72**	**7.41**	**19.92**	**19.72**	**19.92**	**4.58**
Pl. density/m²												
44	20.00a	5.61a	22.87ab	2.72b	33.11b	18.00a	1144.44a	508.89a	2368.25a	3814.8a	7894.2a	48.93a
50	19.50a	5.52a	21.46b	2.94a	32.00b	16.52b	922.22b	517.33a	1971.05b	3074.1b	6570.2b	47.32a
33	19.83a	5.69a	24.31a	3.00a	37.33a	18.24a	716.67c	491.78b	1408.89c	2388.9c	4696.3c	49.45a
Mean	**19.78**	**5.61**	**22.88**	**2.89**	**34.15**	**17.59**	**927.78**	**506**	**1916.07**	**3092.6**	**6386.9**	**48.57**
LSD (0.05 %)	**NS**	**NS**	**1.75**	**0.22**	**2.14**	**1.35**	**50.39**	**16.93**	**110.19**	**167.98**	**367.29**	**NS**
CV%	**10.53**	**8.17**	**10.55**	**10.38**	**8.66**	**10.57**	**7.48**	**4.6**	**7.91**	**7.48**	**7.91**	**9.9**
P_2O_5 (kg/ha)												
0	19.33a	5.43b	21.75b	2.81a	33.07b	16.75b	796.36b	492.16b	1680.51b	2654.3b	5601.7b	47.45a
46	20.22a	5.78a	24.01a	2.96a	35.22a	18.42a	1059.26a	519.82a	2151.62a	3530.9a	7172.1a	49.69a
Mean	**19.78**	**5.61**	**22.88**	**2.89**	**34.15**	**17.59**	**927.78**	**506**	**1916.07**	**3092.6**	**6386.9**	**48.57**
LSD (0.05 %)	**NS**	**0.23**	**2.4**	**0.19**	**1.75**	**1.84**	**75.34**	**17.5**	**139.5**	**251.14**	**465.01**	**NS**
CV%	**11.45**	**7.35**	**18.35**	**11.54**	**8.95**	**18.3**	**14.2**	**6.05**	**12.73**	**14.2**	**12.73**	**11.64**
Interaction												
G x Pl									*	*	*	
G x P		*			*		*			*		
Pl x P												
G x Pl x P		*		*								

Means in same columns followed by the same letter(s) are not significantly different, *. = significant.

Table 7: Interaction effect of genotypes, plant density and phosphorus levels on Seed pod^{-1}of faba bean.

Genotype	0 kg $P_2 0_5$			46 kg $P_2 0_5$			
	44 plants/m^2	50 plants/m^2	33 plants/m^2	44 plants/m^2	50 plants/m^2	33 plants/m^2	Mean
Hachalu	3.00	2.67	3.00	2.33	3.00	3.33	2.89
Walki	2.33	3.00	2.67	3.00	3.00	3.00	2.83
Local	2.67	3.00	3.00	3.00	3.00	3.00	2.94
Mean	2.67	2.89	2.89	2.78	3.00	3.11	
LSD (0.05)	0.41						

Table 8: Interaction effect of genotype with phosphorus levels on number of seeds plant^{-1}of faba bean.

Genotype	Phosphorus level		Mean
	0 kg $P_2 0_5$	46 kg $P_2 0_5$	
Hachalu	31.00	30.40	30.70
Welki	34.10	36.10	35.10
Local	34.10	39.10	36.60
mean	33.07	35.20	
LSD (0.05)	3.15		

Table 9: Interaction effect of genotype with plant density on biological yield of faba bean.

Genotype	Biological Yield (kg/ha)			Mean
	44 plants/m^2	50 plants/m^2	33 plants/m^2	
Hachalu	7915.77	6465.6	4702.74	6361.37
Welki	8367	7630.58	4923.01	6973.53
Local	7399.74	5614.34	4463.11	5825.73
Mean	7894.17	6570.17	4696.29	
LSD (0.05)	400.85			

Biological yield (kg ha^{-1})

The biological yield followed a trend similar to that of seed yield/plot. Improved genotypes of Walki (6973.5 kg/ha) remaining at par with Hachalu (6361.4 kg/ha) produced significantly higher biological yield/plot than that of local variety (5825.7 kg/ha). This shows that improved cultivars have the potential to produce more dry matter than the local genotype. Higher plant densities represented by closer spacing of 30 x 7.5 cm (7894.2 kg/ha) and 40 x 5.0 cm (6570.2 kg/ha) produced superior biological yield kg/ha to that of wider spacing 60 x 5.0 cm (4696.3 kg/ha). Significantly greater biological yield kg/ha has been obtained with the application of 46 kg$P_2 0_5$/ha (7172.1 kg/ha) than that obtained in no phosphorus plots (5601.7 kg/ha). Significant interaction between genotype and spacing on biological yield/ha showed that irrespective of genotype there was reduction in biological yield/ha with reduced plant density. The genotype Walki grown with a spacing of 30 x 7.5 cm produced significantly higher biological yield 8,367 kg/ha than the other genotype spacing combinations. The next best is Hachalu raised with 30 x 7.5 cm spacing of produced 7,915.77 kg/ha and the local cultivar produced 7399.74 kg/ha (Table 9).

Seed yield (kg ha^{-1})

Among the faba bean genotypes, Walki (3407 kg/ha) and Hachalu (3037 kg/ha) gave significantly higher productivity than the local genotype (2833 kg/ha)(Table 6). The percentage yield enhancement of Walki and Hachalu over local chek was 20 and 7.2 %, respectively. The superior performance of Walki could be attributed to more length of pods, greater pod weight/plant, higher seed weight/plant, more seeds/plant, higher test seed weight, ultimately leading to substantial enhancement in seed yield/plot. Bianchi *l.,* (1979) also reported that the number of seeds/pod and seed weight are most stable components and seed weight varies between cultivars and range from 0.1g to 2.4g/seed. Among the plant densities, seeding at 30 x 7.5 cm (44 plants/m^2) resulted in superior seed productivity (3814.8 kg/ha) than that obtained with 40 x 5.0 cm (50 plants/m^2) (3074.1 kg/ha) and 60 x 5.0 cm (33 plants/m^2) (2388.9 kg/ha). Significant interaction effect between genotype x plant density on seed yield revealed that by and large, all the genotypes yielded maximum with 30 x 7.5 cm spacing, closely followed by 40 x 5.0 cm spacing, while their yields significantly dwindled with wider spacing of 60 x 5.0 cm. The genotype Walki seeded at a spacing of 30 x 7.5 cm surpassed (4166.7 kg/ha) the rest of the genotype x spacing combinations in seed productivity (Table 10). The next best was Hachalu grown at 30 x 7.5 cm (3777.8 kg/ha) in terms of productivity. Fertilizing the crop with 46 kg P_20_5/ha resulted in significantly greater seed yield (3531 kg/ha) than that without P fertilizer (2654 kg/ha) in vertisols. Application of 80 kgP_20_5/ha has been reported to give 13 t/ha green pods of faba bean (Davood and Hashemabadi, 2013; Salih, 1986, Balaban and Sepetoglu, 1991; Babiker, 1995). Hamissa (1973), based on results of 31 fertilizer trials (1967-1973) on faba bean concluded that response to phosphorus was high, increasing P from 36 to 72 kg/ha increased yield by 9.8 and 15.7% over control. There was significant interaction between genotype x phosphorus on seed yield, where Walki fertilized with 46 kg P_20_5/ha gave greater productivity (4074 kg/ha) than the rest of the combinations. The next best was Hachalu grown with 46 kg P_20_5/ha (3407 kg/ha).

Table 10. Interaction effect of genotype and plant density on seed yield (kg/ha) of faba bean.

Genotype	Population density			Mean
	44 plants/m^2	50 plants/m^2	33 plants/m^2	
Hachalu	3777.78	2944.44	2388.89	3037.04ab
Walki	4166.67	3555.56	2500	3407.41a
Local	3500	2722.22	2277.78	2833.33b
Mean	3814.81a	3074.07b	2388.89c	
LSD (0.05)	205.76			

Table 11. Interaction effect of genotype with phosphorus on seed yield (kg/ha) of faba bean.

Genotype	Phosphorus level		Mean
	0 P_20_5 kg/ha	46 P_20_5 kg/ha	
Hachalu	2666.67	3407.41	3037
Walki	2740.74	4074.08	3407.4
Local	2555.55	3111.11	2833.33
Mean	2654.3	3530.9	
LSD (0.05)	198.4		

Thus the new genotypes responded better to phosphorus application than the local cultivar (3111 kg/ha) (Table 11). Seed yield of faba bean is a product of number of plants/m^2, number of pod bearing nodes/plant, pods/node, seeds/pod and seed weight (Thompson and Taylor, 1977; Ishag, 1973).

Harvest Index (HI)

The genotypes did not differ in harvest index. Low density planting 60 x 5.0 cm (33 plants/m^2) resulted in higher harvest index (49.45) over high density seeding at 30 x 7.5 cm (44 plants/m^2) (48.93) and 40 x 5.0 cm (50 plants/m^2) (47.32) which gave comparable harvest index. Harvest index was not much altered among the genotypes; though the seed yields exhibited distinct variation; which was due to non-significant variation in biological yield per unit area. The application of phosphorus tended to improve the harvest index (49.69) of faba bean when compared with no P application (47.45) through the variation was not discernible. Application of 50 kgP_20_5 has been reported to enhance nodulation and yield of faba bean in soils having 3.5 and 2.0 ppm P at ICARDA (Murinda and Saxena, 1983).

Correlation between Seed yield, growth and yield components

Correlations between growth, yield and yield components showed a significant positive relation between seed yield and plant height at different stages, leaf area/plant, leaf area index, biological yield and seed yield/plant. Biomass yield was correlated with leaf area/plant, LAI and plant height .

Nitrogen and phosphorus removal of faba bean

The results showed that the uptake of nutrients differed between genotypes and also with spacing (Table 12). In the uptake of phosphorus (mg $plant^{-1}$) the improved genotypes Hachalu (909.27 mg/plant) and Walki (966.29 m/plant) surpassed the uptake by local cultivar (838.72 mg/plant) with 30 x 7.5 cm spacing. Walki genotype proved better than other in total N (mg $plant^{-1}$), when raised with 30 x 7.5 cm spacing closely followed by Hachalu at the same plant density. The local genotype removed relatively less NP nutrients than Walki and Hachalu in the respective spacing. In general, nutrient up-take mainly depends on ability of genotype to extract nutrients and spacing also affected the up-take of nutrient. As faba bean is having naturally biological nitrogen fixing ability, the study showed that the up-take of nitrogen by plant remained greater when sown in dense stands than in sparse stands in all the test genotypes.

Nitrogen removal (kg/ha) in seed

The N content in grain (3.15 %) has been greater than that of stalks (0.82 %). The P content of grain (0.32 ppm) was higher than that in stalks (0.05 ppm). As the nutrient removal is the product of nutrient content in plant tissue and biomass yield, the nutrient removal followed the trend of seed yield and biomass yield in relation to different production factors under investigation (Table 13). The nitrogen removal in seed of different genotypes varied where improved genotype Walki removed maximum (107.33 kg/ha) closely followed by Hachalu (95.67 kg/ha), while the least was removed by local cultivar (89.25 kg/ha).The influence of plant density on N removal by seed showed that higher plant density (44 plants/m^2) removed maximum (120.17 kg/ha) followed by 50 plants/m^2 (96.83 kg/ha) which surpassed low density (33 plants/m^2) (75.25 kg/ha). The N removal is in tandem with the seed yields obtained in different plant densities. Application of 46 kg P_20_5/ha resulted in distinct enhancement in N removal in seed (111.22 kg/ha) over no P application (83.61 kg/ha), suggesting the complementary role played by P in the N removal by the faba bean seed (Table 13). Fageria (2002) also reported beneficiary effect of fertilizer on nutrient uptake, and the removal ranged between 280 – 328 kg/ha for N; 27.7 34 kg/ha for P and 188 – 222 kg/ha for K.

Table 12: Nutrient removal of faba bean genotypes in relation to plant density.

Genotype	Plant density	TN (mg plant^{-1})	P (mg plant^{-1})
Hachalu	44 plants/m^2	14912.1	909.27
	50 plants/m^2	10603.58	646.56
	33 plants/m^2	10885.43	663.75
Walki	44 plants/m^2	15847.13	966.29
	50 plants/m^2	12514.15	763.06
	33 plants/m^2	11973.95	730.12
Local	44 plants/m^2	13755.04	838.72
	50 plants/m^2	9207.52	561.43
	33 plants/m^2	10705.93	652.80

Table 13: Uptake of Nitrogen (kg/ha) in seed of faba bean.

Genotype	Population density			Mean	Population density	P level		Mean
	44 plants/m^2	50 plants/ m^2	33 plants/m^2			0 kg P_2O_5/ha	46 kg P_2O_5/ha	
Hachalu	119	92.75	75.25	95.67	44 pl/m^2	106.16	134.17	120.17
Welki	131.25	112	78.75	107.33	50 pl/m^2	82.83	110.83	96.83
Local	110.25	85.75	71.75	89.25	33 pl/m^2	61.83	88.66	75.25
Mean	120.17	96.83	75.25	97.41		83.61	111.22	97.41

Table 14: Protein yield (kg/ha) of genotypes, plant densities and P nutrition of faba bean.

Genotype	0 kg P_2O_5/ha				46 kg P_2O_5/ha			
	44 plants/m^2	50 plants/m^2	33 plants/m^2	Mean	44 plants/m^2	50 plants/m^2	33 plants/m^2	mean
Hachalu	678.56	503.44	394.00	525.33	809.89	656.67	547.22	671.26
Walki	656.67	591.00	372.11	539.93	985.00	809.89	612.89	802.59
Local	656.67	459.67	394.00	503.44	722.33	612.89	503.44	612.89
mean	663.96	518.04	386.70	522.90	839.07	693.15	554.52	695.58

Protein yield of faba bean

Depending upon the N content of seed the protein content was calculated as: Protein % = N % x 6.25. The protein content varied in relation to genotypes, apart from agronomic practices such as plant density and P fertilizer application. Results in Table 14 showed that improved genotypes with different P levels responded better than the local cultivar in protein content and protein yield, especially Walki genotype performed better than others. Regarding plant density, 44 plants/m^2 recorded higher protein yield than 50 plants /m^2 and 33 plants /m^2. Chavan *et al.* (1989) reported a wide variation in protein content of faba bean (20 – 41%), and winter beans have higher protein content than spring faba beans (Bond *et al.,* 1985). Protein content is influenced by both genetic and environmental factors. Winter and spring types of faba bean showed different compositions of not only protein but also among different amino acid varieties (Ford and Hewitt, 1980).

Phosphorus removal (kg/ha) in seed

The P content of grain (0.32 ppm) was higher than that in stalks (0.05 ppm). The phosphorus removal in seed of different genotypes varied where improved genotype Walki removed the maximum amount (10.9 kg/ha) closely followed by Hachalu (9.72 kg/ha), while the least was removed by the local cultivar (9.07 kg/ha).The influence of plant density on P removal by seed showed that higher plant density (44 plants/m^2) removed the maximum amount (12.21 kg/ha) followed by 50 plants/m^2 (9.84 kg/ha) which surpassed low density (33 plants/m^2) (7.64 kg/ha). The P removal is in tandem with the seed yields obtained in different plant densities. Application of 46 kg P_20_5/ha resulted in distinct enhancement in P removal in seed (11.29 kg/ha) over no P application (8.49 kg/ha) (Table 15). Hill-cottingham and Sarisum (1980) also reported that the fruits of faba bean made dominant contribution for N, P, K, S to the whole plant total. The stems and leaves contained most P, K in mid-July, most N in end July, while Ca, Mg accumulation continued till mid-August, and the greater part of NPKS in harvested bean has been absorbed directly from soil. In faba bean 80% total seed protein is in cotyledon as non-metabolic reserve (Wallace, 1951).

Nitrogen removal (kg/ha) in haulm

The improved genotype Walki removed the maximum N (57.64 kg/ha) followed by Hachalu (51.86 kg/ha), while the least was removed by local cultivar (47.62 kg/ha).The influence of plant density on N removal by haulm showed that higher plant density (44 plants/m^2) removed the maximum (65.95 kg/ha) followed by 50 plants/m^2 (53.87 kg/ha) which surpassed low density (33 plants/m^2) (37.29 kg/ha). The N removal is in tandem with the haulm yields obtained in different plant densities. Application of 46 kg P_20_5/ha resulted in distinct enhancement in N removal in haulm (59.62 kg/ha) over no P application (45.12 kg/ha), suggesting the complementary role played by P in the N removal by the faba bean haulm (Table 16). Removal of nutrients has been reported to increase significantly with increase in dry matter production, while responses varied with genotypes in faba bean (Ihsanullah Daurl *et al.*, 2010), which is in agreement with the present findings.

Table 15: Uptake of Phosphorus (kg/ha) in seed of faba bean.

Genotype	Population density			Mean	Plant density	P level		Mean
	44 plants/m^2	50 plants/m^2	33 plants/m^2			0 kg P_20_5/ha	46 kg P_20_5/ha	
Hachalu	12.09	9.42	7.64	9.72	44 pl/m^2	10.78	13.63	12.21
Welki	13.33	11.38	8.00	10.90	50 pl/m^2	8.41	11.26	9.84
Local	11.20	8.71	7.29	9.07	33 pl/m^2	6.28	9.00	7.64
Mean	12.21	9.84	7.64	9.89		8.49	11.29	9.89

Table 16: Uptake of nitrogen (kg/ha) in haulm of faba bean.

Genotype	Population density			Mean	Population density	P level		Mean
	44 plants/m^2	50 plants/m^2	33 plants/m^2			0 kg P_20_5 /ha	46 kg P_20_5 /ha	
Hachalu	66.27	53.01	36.28	51.86	44 pl/m^2	58.35	73.55	65.95
Walki	70.43	62.57	39.91	57.64	50 pl/m^2	46.96	60.79	53.87
Local	61.13	46.04	35.68	47.62	33 pl/m^2	30.06	44.53	37.29
Mean	65.95	53.87	37.29	52.37		45.12	59.62	52.37

Table 17: Uptake of phosphorus (kg/ha) in haulm of faba bean

Genotype	Population density			Mean	Population density	P level		Mean
	44 plants/m²	50 plants/m²	33 plants/m²			0 kg P_2O_5/ha	46 kg P_2O_5/ha	
Hachalu	4.04	3.23	2.21	3.16	44 pl/m²	3.56	4.48	4.02
Welki	4.29	3.81	2.43	3.51	50 pl/m²	2.86	3.71	3.28
Local	3.73	2.81	2.17	2.90	33 pl/m²	1.83	2.71	2.27
Mean	4.02	3.28	2.27	3.19		2.75	3.63	3.19

Table 17a: Faba bean nutrient removal in seed, haulm and total removal in relation to genotypes, plant densities and P. nutrition.

Genotype	Spacing	P. level (P_2O_5 kg/ha)	Seed yield (kg/ha)	Removal in Seed (kg/ha)		Biomass (kg/ha)	Removal in Halum (kg/ha)		Total up take (seed+haulm) (kg/ha)	
				Ni	P		N	P	N	P
Hachalu	30x7.5cm	0	3444.45	108.5	11.02	7308.2	59.93	3.65	168.43	14.68
	30x7.5cm	46	4111.11	129.5	13.16	8523.33	72.62	4.43	202.12	17.58
	40x5.0cm	0	2555.55	80.5	8.18	5509.27	45.18	2.75	125.68	10.93
	40x5.0cm	46	3333.33	105	10.67	7421.93	60.86	3.71	165.86	14.38
	60x5.0cm	0	2000	63	6.4	4080.91	28.91	1.76	91.91	8.16
	60x5.0cm	46	2777.78	87.5	8.89	5324.58	43.66	2.66	131.16	11.55
Welki	30x7.5cm	0	3333.33	105	10.67	7198.58	59.03	3.6	164.03	14.27
	30x7.5cm	46	5000	157.5	16	9535.42	81.83	4.99	239.33	20.99
	40x5.0cm	0	3000	94.5	9.6	7061.07	57.9	3.53	152.4	13.13
	40x5.0cm	46	4111.11	129.5	13.16	8200.09	67.24	4.1	196.74	17.26
	60x5.0cm	0	1888.89	59.5	6.04	3742.51	29.78	1.82	89.28	7.86
	60x5.0cm	46	3111.11	98	9.96	6103.51	50.05	3.05	148.05	13.01
Local	30x7.5cm	0	3333.33	105	10.67	6839.82	56.09	3.42	161.09	14.09
	30x7.5cm	46	3666.67	115.5	11.73	7959.67	66.18	4.04	181.68	15.77
	40x5.0cm	0	2333.33	73.5	7.47	4611.53	37.81	2.31	111.31	9.77
	40x5.0cm	46	3111.11	98	9.96	6617.16	54.26	3.31	152.26	13.26
	60x5.0cm	0	2000	63	6.4	4063.27	31.5	1.92	94.5	8.32
	60x5.0cm	46	2555.56	80.5	8.18	4862.96	39.88	2.43	120.38	10.61

Phosphorus removal (kg/ha) in haulm

The phosphorus removal in haulm of different genotypes varied where improved genotype, Walki removed the maximum amount (3.51 kg/ha) followed by Hachalu (3.16 kg/ha), while the least was removed by local cultivar (2.90 kg/ha). Balban and Sepetoglu (1991) reported variability in faba bean genotypes for nutrient uptake and their response. The influence of plant density on P removal by haulm showed that higher plant density (44 plants/m²) removed maximum (4.02 kg/ha) followed by 50 plants/m² (3.28 kg/ha), which surpassed low density (33 plants/m²) (2.27 kg/ha). Application of 46 kg P_2O_5/ha resulted in distinct enhancement in P removal in haulm (3.63 kg/ha) over no P application (2.75 kg/ha) (Tables 17,17a).

Table 18: Selected Chemical properties of the experimental soil after crop harvest:

Treatment			Nutrient in soil at harvest					
Genotype	Spacing	P level (kg $P_2 0_5$/ha)	TN (%)	Av P (ppm)	K (Meq/100g)	Change over the initial (+/-)		
						N	P	K
Hachalu	30 x7.5 cm	0	0.08	1.75	1.53	0.01	-4.19	-0.10
	30 x7.5 cm	46	0.10	1.86	1.58	0.03	-4.08	-0.05
	40 x5.0 cm	0	0.07	1.74	1.55	0.00	-4.20	-0.08
	40 x5.0 cm	46	0.12	1.85	1.59	0.05	-4.09	-0.04
	60 x5.0 cm	0	0.08	1.80	1.56	0.01	-4.14	-0.07
	60 x5.0 cm	46	0.09	1.84	1.59	0.02	-4.10	-0.04
Walki	30 x7.5 cm	0	0.09	1.71	1.58	0.02	-4.23	-0.05
	30 x7.5 cm	46	0.11	1.91	1.61	0.04	-4.03	-0.02
	40 x5.0 cm	0	0.07	1.72	1.53	0.00	-4.22	-0.10
	40 x5.0 cm	46	0.09	1.89	1.62	0.02	-4.05	-0.01
	60 x5.0 cm	0	0.07	1.77	1.52	0.00	-4.17	-0.11
	60 x5.0 cm	46	0.08	1.87	1.55	0.01	-4.07	-0.08
Local	30 x7.5 cm	0	0.07	1.68	1.53	0.00	-4.26	-0.10
	30 x7.5 cm	46	0.09	1.80	1.58	0.02	-4.14	-0.05
	40 x5.0 cm	0	0.07	1.69	1.48	0.00	-4.25	-0.15
	40 x5.0 cm	46	0.09	1.81	1.53	0.02	-4.13	-0.10
	60 x5.0 cm	0	0.07	1.67	1.49	0.00	-4.27	-0.14
	60 x5.0 cm	46	0.09	1.78	1.51	0.02	-4.16	-0.12
Initial status of soil nutrients			0.070	5.94	1.63			

Post-harvest chemical properties of the Soil

The soil chemical properties analysis indicated a change from initial level at post-harvest stage. There has been an increase in % TN, while % P and % K contents decreased due to more uptake by the crop. Genotypes responded differentially based on plant density and Phosphorus levels in nutrient removal from soil (Table 18). There was an increase in total nitrogen content of soil ranging from 0.00 to 0.005% which could be due to the contribution of rhizobial fixation of atmospheric nitrogen in root nodules of faba bean apart from contribution from soil mineralization. However, the available phosphorus content of soil decreased sharply ranging from 4.03 to 4.27 ppm as compared with the initial status; indicating large removal of P by the legume crop. The available potassium content of soil too showed a declining trend as compared with initial status which ranged between 0.15 to 0.01 meq/100g, indicating marginal decline.

Economic analysis of production factors

The results of economic analysis showed that Walki recorded the highest net return (ETB 29,641.92 than Hachalu (24,826.72) while the local variety accrued the least return (ETB 22,178.62). The corresponding benefit**:** cost ratios of Walki, Hachalu and local variety were

Table 19. Economic analysis of faba bean as influenced by genotype, plant density and phosphorus level.

Treatments		Grain yield (kg ha^{-1})	Dry biomass yield of Faba bean (t ha^{-1})	Gross return (Birr ha^{-1})	Cost of Production (Birr ha^{-1})	Net return (Birr ha^{-1})	Benefit : cost ratio (Birr) (GR/PC)	Per day productivity (GY/CD) kg/ha	Return/Birr Investment (NR/PC)
Genotype	Hachalu	3037.0	6.36	39481.00	14654.28	24826.72	2.69	23.36	1.69
	Welki	3407.4	6.97	44296.20	14654.28	29641.92	3.02	26.21	2.02
	Local	2833.3	5.83	36832.90	14654.28	22178.62	2.51	21.79	1.51
Plant density/m^2	44	3814.8	7.89	49592.40	14654.28	34938.12	3.38	29.34	2.38
	50	3074.1	6.57	39963.30	14654.28	25309.02	2.73	23.65	1.73
	33	2388.9	4.69	31055.70	14654.28	16401.42	2.12	18.38	1.12
P. Nutrition (Kg P_20_5/ha)	0	2654.3	5.60	34505.90	13272.50	21233.40	2.60	20.42	1.60
	46	3530.9	7.17	45901.70	14654.28	31247.42	3.13	27.16	2.13

Where, GY= Grain yield, CD= Crop duration, NR= Net return, PC= Production cost, GR= Gross return.

3.02, 2.69 and 2.51, respectively. Maintaining 44 plants/m^2 resulted in higher net return (ETB 34,938.12/ha) than 50 plants/m^2 (ETB 25,309.02/ha) and the lowest with 33 plants/ m^2 (ETB 16,401.42/ha), the benefit: cost ratios being 3.38, 2.73 and 2.12, respectively (Table 19).Application of 46 kg P_20_5/ha resulted in net return of ETB 31,247/ha in comparison with ETB 21,233/ha obtained without P application. The benefit: cost ratios of growing faba bean with and without P were 3.13 and 2.60, respectively. The per day productivity of genotypes varied distinctly where Welki (26.21 kg/ha) surpassed Hachalu (23.36 kg/ha) and local cultivar (21.79 kg/ha) indicating that improved genotypes possess greater production efficiency than traditional types. The net return/Birr investment was 2.02, 1.69 and 1.51 for Walki, Hachalu and local cultivar, respectively. Seeding the crop at 44 plants/ m^2 density offered higher per day productivity (29.34 kg/ha) than that obtained from 50 plants/ m^2 (23.65 kg/ha) and 33 plants/ m^2 (18.38 kg/ha), with the respective net return/Birr investment being 2.38, 1.73 and 1.12. Fertilizing the crop with 46 kg P_20_5/ha resulted in higher per day productivity (27.16 kg/ha) than that with no P application (20.42 kg/ha) and accruing a net return/Birr investment of 2.13 and 1.60, respectively.

CONCLUSIONS

From the foregoing account it could be inferred that the improved genotype Walki (3407 kg/ha) remaining comparable with Hachalu (3037 kg/ha) gave substantially greater seed yield than the local cultivar (2833 kg/ha) .Nutrient (NP) removal of genotypes both in seed and haulm has been greater in Walki and Hachalu than that in local cultivar. The N removal in seed and haulm of Walki was 107 kg/ha and 58 kg/ha, and the corresponding removal of Hachalu being 95 kg/ha and 52 kg/ha; while that of local cultivar was 89 kg/ha and 48 kg/ha, respectively. The P removal in seed and haulm of Walki was 10.9 kg/ha and 3.5 kg/ha, respectively. While the corresponding values for Hachalu were 9.72 kg/ha and 3.16 kg/ha; whereas the removal by the local cultivar was 9.1 kg/ha and 2.9 kg/ha. Nutrient dynamics of soil after harvest of crop showed that there has been an increase in soil N status ranging from 0.00 to 0.05 percent ,and a sharp decline in soil P and K contents after the crop harvest due to greater removal by the crop, which ranged from 4.03 to 4.27 ppm of P and 0.01 to 0.15 meq/100g of K. The NP uptake of faba bean was found to be greater in dense stands(44plants/m2) in comparison with sparse stands regardless of genotype. Phosphorus fertilization at 46 kg P_20_5/ha brought about significant increase in yield components resulting in substantial increase in seed yield (3531 kg/ha) and biological yield (7172 kg/ha) over no fertilizer check (2654 kg/ha seed and 5602 kg/ha haulm yield) besides more harvest index and greater uptake of N and P. Economic analysis of genotypes showed that Walki accrued the highest net returns (ETB 29,642/ha) followed by Hachalu (ETB 24,827/ha) and the local cultivar (ETB 22,178/ha), with the corresponding Benefit: cost ratios of 3.02, 2.69 and 2.51, respectively. Maintaining 44 plants/m^2 resulted in higher net return (ETB 34,938/ha) than 50 plants/m^2 (ETB 25,309/ha) and 33 plants/m^2 (ETB 16,401/ha), with the corresponding Benefit**:** cost ratios of 3.38, 2.73 and 2.12. P application resulted in a net return of ETB 31,247/ha compared with ETB 21,233/ha obtained from no phosphorus treatment, with the respective Benefit**:** cost ratios of 3.13 and 2.60. Application of phosphorus increased the residual N content of soil after crop harvest; while the depletion of soil P and K was more in unfertilized plots.

REFERENCES

Agegnehu G and Chilot Y (2009) Integrated nutrient management in faba bean and wheat on Nitisols. Research Report No. 78. EIAR, Addis Ababa, Ethiopia.

Agegnehu G, Ghizaw A and Sinebo W (2006) Yield performance and land-use efficiency of barley

and faba bean mixed cropping in Ethiopian highlands. European Journal of Agronomy 25: 202–207.

Amanuel Gorfu,Tanner DG, Assefa Taa, Duga Debele (1993) Observation on wheat and barley-based cropping sequences trials conducted for eight years in South-eastern Ethiopia. Paper presented at the 8th Regional Workshop for Eastern, Central and Southern Africa, 7-20 June 1993, Kampala, Uganda.

Amare G (1990) Evaluation of Faba bean (*Vicia fabae L.*) production packages on Farmers fields in Arsi Administrative Region Ethiopia. M.Sc. Thesis, Haramaya University of Agriculture, Haramaya, Ethiopia.

Asfaw Tilaye, Tesfaye Getachew and Beyene Demtsu (1994) Genetics and breeding of faba bean. P.97-121. In: Asfaw Tilaye *et al.,* (Eds.) Cool-season food legumes of Ethiopia. Proc. First National cool-season food legumes review conference, 16-20 December 1993, Addis Abeba, Ethiopia. ICARDA/IAR. ICARDA. Syria.

Babiker EE,Elsheikh Osman AG and El-Tinay AH (1995) Effect of nitrogen fixation, nitrogen fertilization and viral infection on yield, tannin and protein contents and In vitro protein digestibility of faba bean. *Plant Foods Hum Nutr*., 47: 257-263.

Balaban M and Sepetoğlu H (1991) Growth, nutrients uptake and grain yield in faba bean genotypes under various plant densities. *J. Ege Univ*., 26(3): 181-197.

Bond DA, Lawes DA, Hawtin GC, Saxena MC and Stephens JS (1985) Faba Bean (*Vicia faba L.*). In: R.J. Summerfield and E.H. Roberts (eds.), Grain Legume Crops. William Collins Sons Co. Ltd. 8 Grafton Street, London, WIX 3LA, UK.pp199-265

Bouyoucos GH (1951) Reclamation of the hydrometer for making mechanical analysis of soils. Agron. J. 43: 434-438.

Central Statistical Agency (CSA) (2013/14) Agricultural sample survey. Report on area and production for major crops (private peasant holdings, meher season). Addis Ababa, Ethiopia.

Chavan JK, Kute KS and Kadam SS (1989) In: CRC Hand Book of World legumes..D.D .Salunkhe and S.S. kadam (eds) ,Boka Raton, Florida, USA:CRC Press. Pp 223-245

Davood Hashemabadi (2013) Phosphorus fertilizers effect on the yield and yield components of faba bean (*Vicia faba L.*) Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran.

Duke JA (1981) Handbook of legumes of world economic importance. Plenum Press, New York. pp. 199-265.

Ethiopian Institute of Agricultural Research (EIAR) (2011) Faba bean producing manual. Holetta Agricultural Research Center. Addis Ababa, Ethiopia.

Fageria NK (2002) Nutrient management for sustainable dry bean production in the tropics. *Communications in Soil Sci. and Plant Analysis*, 33: 1537-1575.

FAO (2008) Fertilizer and plant nutrition bulletin: Guide to laboratory establishment for plant nutrient analysis. Rome, Italy. p203.

FAOSTAT (2008) http://faostat.fao.org/site/567/default.aspx#ancor.

FAOSTAT (2009) Food and Agriculture Organization
http://faostat.fao.org/site/567/default.aspx
United Nations field bean (*Vicia faba L.*var. minor). C.R. Seances Acad. Agric. Fr., 66: 757-770.

Ford JE and Dewitt D (1980) Protein quality in Sorghum and field bean (*Vicia faba L*) as measured as biological and microbiological assays. In vicia faba: feeding value, processing and Viruses. Pp. 125-140 Ed. By D.A.Bond; Martinus Nijhoff, The Hague.

Gemechu Keneni, Mussa Jarso and Tezera Wolabu (2006) Faba bean (*Vicia faba L*) Genetics and Breeding Research in Ethiopia: A Review. In: Ali Kemal, Kenneni Gemechu, Ahmed Seid, Malhatra Rajendra, Beniwal Surendra, Makkouk Khaled, Halila MH,(eds). Food and Forage legumes of Ethiopia: Progress and prospects. Proceedings of the workshop on Food and Forage Legume, 22-26 September 2003. Addis Ababa, Ethiopia.

Hamissa MR (1973) Fertilizer requirements for Broad beans and Lentils Improvement and Production of Field crops, First FAO/SIDA Seminar for Plant Scientists from Africa and Near East, Cairo, Egypt, Sept.

Haque *et al*. (1986) Improved management of vertisols for sustainable crop-livestock production in Ethiopia. 4. Nutrient Management. Plant Science Division Working Document 13. ILCA, Addis Ababa., Ethiopia.

Hill-Cottingham DG and Sansum LL (1980) Seasonal changes in the major nutrient content of Vicia

faba plants. Communs. Soil Science, pl. Anal. 11: 517-524.

Ihsanullah Daur, Hasan Sepetoğlu, Khan Bahadar Marwat and Mithat Nuri Geverek (2010) Nutrient Removal, Performance Of Growth and Yield Of Faba Bean (*Vicia faba L.*) Department of Agronomy, NWFP Agricultural University Peshawar, Pakistan. Department of Field Crops, Ege University, 35100 Bornova-İzmir, Turkey, Department of Weed Science, NWFP Agricultural University Peshawar, Pakistan.an (*Vicia faba L.*) II d production of dry matter.

Ishag HM (1973) Physiology of seed yield in field beans (*Vicia faba L.*) J. Agric. Sci. Camb. 80: 191-199.

Kay D (1979) Crop and Product Digest No. 3 -Food legumes. London: Tropical Products Institute. UK. p.26-47. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Kopke U and Nemecek T (2010) Ecological services of faba bean. Field Crops Research, 115: 217-233.

Murinda MV and Saxena MC (1983, 1985) Agronomy of faba beans, lentils and chickpeas. p. 229-244. In: M.C. Saxena and S. Verma (eds.), Proceedings of the International Workshop on Faba Beans, Kabuli Chickpeas and Lentils in the 1980s. ICARDA, 16-20 May, 1983. Aleppo, Syria.

Newton Z, Ann C and Rowland M (2011) Grain legume impacts on soil biological processes in sub-Saharan Africa. *African Journal of Plant sciences*, 5**:** 1-7.

Nuruzzaman M, Lambers H,Bolland MDA and Veneklaas EJ (2005) Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. *Austr. J. Agricult. Res.* ***56:*** 1041-1047.

Olsen SR andDean LA (1965) Phosphorous. In C. A. Black (ed). Methods of Soil analysis, Agronomy No. 9, Am. Soc. Agron. Madisen, Wisconsin, USA.pp1044-1046

Salih FA, Ali AM and Elmubarak AA (1986) Effect of phosphorus application and time of harvest on the seed yield and quality of faba bean. *FABIS Newsletter*, 15: 32-35.

Salisbery FB and Ross CW (1992) Plant Physiology. 4 ed. Wadsworth cengage learuing, New Delhi.

Tekalign Tadesse (1991) Soil, plant, water, fertilizer, animal manure and compost analysis. Working Document No. 13. International Livestock Research Center for Africa, Addis Ababa.

Tesfaye Balemi T (2012) Effect of integrated use of cattle manure and inorganic fertilizers on tuber yield of potato in Ethiopia . *Journal of Soil Science and Plant Nutrition*, 12 (2): 257-265

Thompson R and Taylor H (1977) Yield components and cultivars sowing date and density in faba bean. Ann. Appl. Biol. 86: 313-320.

Wallace I (1951) The diagnosis of mineral deficiencies in plants, HMSO, London.

Walkley A and Black CA (1934) An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci*., 63:251-263.

Yohannis D (2000) Faba bean (*Vicia faba L.*) in Ethiopia.

RESEARCH ARTICLE

Differential productivity response of rain fed sorghum (*Sorghum bicolor* L.) genotypes in relation to graded levels of nitrogen in Kellem Wollega zone of Ethiopia, East Africa

Sheleme Kaba Shamme, Raghavaiah Cherukuri V*, Tesfaye Balemi and Ibrahim Hamza

Department of Plant Sciences, College of Agriculture and Veterinary Sciences, P.O. Box 19, Ambo University, Ambo, Ethiopia, East Africa.

*Corresponding author: Cherukuri V. Raghavaiah; Email: cheruraghav@yahoo.in;
Mobile No: 00251-933907158.

Manuscript details:

Received: 29.10.2015
Revised: 20.11.2015
Accepted: 20.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Sheleme Kaba Shamme, Raghavaiah Cherukuri V, Tesfaye Balemi and Ibrahim Hamza (2015) Differential productivity response of rain fed sorghum (*Sorghum bicolor* L.) genotypes in relation to graded levels of nitrogen in Kellem Wollega zone of Ethiopia, East Africa, Int. j. of Life Sciences 3(4): 306-316.

ABSTRACT

Field experiment was conducted at Haro Sabu Agricultural Research Center using three sorghum genotypes (Lalo, Chemada and Local) during the rainy cropping season of 2014 with an objective to determine the effect of nitrogen rates on growth and yield parameters of sorghum genotypes. The treatments consisted of factorial combination of four nitrogen rates (0, 46, 92 and 138 kg N ha^{-1}) and three sorghum genotypes (Lalo, Chemada and Local variety) tested in a Randomized Block Design with four replications. The results revealed that there was significant effect of N rates on days to 50% flowering, days to 50% physiological maturity, plant stand, Lodging percentage, leaf area at 90 and 120 DAS, leaf area index, number of green leaves/ plant , biological yield, grain yield, and harvest index. There was significant interaction effect of N rates and sorghum genotypes on most of the parameters studied. Significantly higher grain yield was obtained in response to the application of 92 kg N ha with Lalo genotype, which was comparable with Local variety fertilized with 92kgN/ha. The genotype chamada exhibited response up to 46kgN/ha .The superior productivity performance of genotype Lalo could be attributed to better growth in terms of plant stature, more productive tillers, more assimilatory surface,LAI,1000seed weight, and panicles/plant leading to better harvest index than the other genotypes. Across genotypes, increased N rates delayed flowering, physiological maturity ,increased lodging ,enhanced plant height, tillers /plant, leaf area, panicles /plant, grain yield, harvest index; but showed no discernible influence on leaf number/plant, LAI, and test seed weight.

Key words: Growth, yield, nitrogen rates, Sorghum varieties.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is an important drought tolerant rain fed cereal largely cultivated for food, feed and fodder by subsistence farmers in some African countries Mali, Ghana and Ethiopia. In some parts of the world, it is consumed as staple food and is also used in the production of a variety of by-products like alcohol, edible oil, and sugar. Cereals are the major food crops in Ethiopia, both in terms of the cover and volume of production (CSA, 2006). Sorghum is the fifth most important cereal crop worldwide. In the year 2005, sorghum was grown worldwide on 43,727,353 ha with an output of 58,884,425 metric tons (FAO, 2005). Of the total grain crop production area, 79.46 % (8.1million hectares) was under cereals. Teff, maize, sorghum and wheat share 22.08% (2.2 million hectares), 15.00% (1.5 million hectares), 14.43% (nearly 1.5 million hectares) and 14.35% (nearly 1.5 million hectares) of the grain crop area, respectively (CSA, 2006). Cereals contributed 86.86% (more than 116.2 million quintals) of the grain production. Maize, wheat, Teff and sorghum made up 24.93% (33.4 million quintals), 16.58% (22.2million quintals), 16.26% (21.8 million quintals) and 16.24% (21.7 million quintals) of the grain production in the same order and the Ethiopian national average yield was14.81 kg/ha (CSA, 2006).

Nitrogen is very transitory in the soil, due to its susceptibility to leaching, de nitrification, and volatilization. Over use of N fertilizer can lead to pollution of water streams and may result in soil acidification. The efficient use of applied N should be considered more seriously in the overall nutrient management than any other plant nutrient in order to reduce its negative impact on the environment. In addition, even under the best management practices, 30%-50% of the applied N is lost through different routes (Stevenson, 1985), and hence more fertilizer has to be applied than actually needed by the crop to compensate for the loss. The loss of N not only causes loss to the farmer but also causes hazardous impact on the environment (Kessel *et al.*, 1993; Gosh and Bhat, 1998). High inputs of chemical fertilizer cause environmental pollution (William, 1992). Thus, it calls for a need to optimize the level of N fertilizer to be applied, and the genotypes may have differential response to this major nutrient, especially under rain fed farming situation.

MATERIALS AND METHODS

A field experiment was conducted at Kellem – Wollega zone of Ethiopia to evaluate the effect of nitrogen rates on growth, yield and nitrogen use efficiency of sorghum genotypes during the main cropping season of 2014 at Haro Sabu Agricultural Research Center (HSARC). The center is located in Western Ethiopia ,Oromia Regional state at a distance of 550 km west of Addis Ababa. It lies at an latitude of 8^0 52.51”N,longitude of 35013.18”E and altitude of 1515 m. a. s. l. The center is characterized by a warm humid climate with average minimum and maximum temperature of 14^0C and 30^0C, respectively. The area received an average annual rain fall of 1000 mm with a uni model distribution pattern, most of the rain being received from April to October. The soil type of the experimental site is reddish brown, with a pH of 5.5. The area is characterized by coffee –based crop-live stock mixed farming system in which cultivation of coffee, maize, sorghum, finger millet, haricot bean ,soy bean ,sesame ,banana, mango, sweet potato are practiced.

Sorghum varieties Lalo and chemada and a Local variety, which is adapted to the agro-ecology of the area, were used for the study. Varieties Lalo and Chemada are among the most successful hybrid varieties released by Bako Agricultural Research Centre in 2006 and 2013, respectively. Both varieties have wider adaptability and grow well at altitudes ranging from 1500 to 1900 meters above sea level with annul precipitation of 1100 to 1200 mm. The cultivars need about 199 and 180 days to reach maturity. The seed colors are brownish- red and creamy for Lalo and Chemada, respectively. The potential yield of Lalo

was 48 quintal ha^{-1} at research station and 35 quintal ha^{-1} at farmer's field, and the yield of Chemada was 32 quintal ha^{-1} on station and 25 quintal ha^{-1} at farmer's field. Planting was done on June 7, 2014 and seeds were sown at a row spacing of 75 cm and 15 cm plant spacing . The nitrogen fertilizer source used was urea (46% N) which was applied by drilling in two splits, half of the quantity 14 days after emergence and the remaining half at knee height stage along the rows to ensure that N is evenly distributed.

The treatments consisted of factorial combination of three (3) varieties (Lalo, Chamada and Local) with four N rates (0, 46, 92 , and 138 kg N ha^{-1}). The treatments were arranged in Randomized Complete Block Design with three replications. Each plot was 3 m long and 4.5 m wide with 6 rows. The inside four rows were set aside for data collection to eliminate any border effects. Phosphate fertilizers in the form of triple super phosphate (TSP) at the recommended rate of 100 kg $P_2 0_5$ ha^{-1} were equally applied to all plots by drilling in the rows at the time of planting. Finally, sorghum plants in the central net plot area (9 m^2) were harvested at physiological maturity. The heads were harvested manually using sickle and were separately threshed for each variety.

Days to 50 % sorghum flowering was taken as the time from the date of planting until half of the plant Populations in the plot started to flower. Similarly, days to 90 % physiological maturity was taken as the time from date of planting until 90% of the plant leaves turned yellow and the lower most head started drying .Total leaf area was measured at 60, 90 and 120 DAS, and determined by multiplying leaf length and maximum breadth of leaf adjusted by a correction factor of 0.75 as suggested by McKee (1964). Number of leaves were counted from randomly selected five pre-tagged plants after plant emerge and their averages were taken as the number of leaves $plant^{-1}$ .The Leaf Area Index (LAI) was calculated from five randomly selected plants by dividing the leaf area by its respective ground area at 120 DAS. The heights of five randomly selected pre-tagged plants (cm) were measured at 60, 90 and 120 DAS from the ground level up to the head/tip of the plant. Five pre-tagged randomly selected plants were considered for determination of above ground dry biomass weight by drying in sunlight for ten to twelve days till a constant dry weight was attained. Lodging was recorded at the time of harvest from four middle rows and thousand seed weight (g) of sorghum was counted and weighted using sensitive balance from the bulk of the seeds of sorghum and adjusted to 13.5% moisture level. Number of panicles/plant was also recorded from five pre-tagged randomly selected plants. Number of effective tillers per plant was counted at plant physiological maturity. Grain yield (g/plot or quintal/hectare) was recorded after harvesting the central four rows of the net plot of 3 m x 3 m = 9 m^2. Seed yield was adjusted to 13.5% moisture using moisture tester (Dickey-john) and converted to quintal ha^{-1} for statistical analysis.

$$\text{Adjusted yield} = \text{Actual yield} \times \frac{100\text{-M}}{100\text{-D}}$$

where,
M, is the measured moisture content in grain and D, is the designated moisture content (13.5%).

Harvest index was calculated as:

$$\text{HI}(\%) \frac{SY}{by} \times 100$$

where,
HI is harvest index, SY is seed yield and BY is above ground dry biological yield.

All the data collected were subjected to the analysis of variance (ANOVA) using SAS (2002) version 9.1. Where treatment means are significant, the Tukey test at alpha= 5% was adopted for mean comparison.
The model for randomized complete block design is:-

$$yij = \mu + \tau i + \beta j + \varepsilon ij$$

where:
Yik - An observation in treatment i, and block jμ - the overall mean τi - the effect of treatment iβj - the fixed effect of block jεij = random error.

RESULTS AND DISCUSSION

Effect of Nitrogen rates on phenology of sorghum genotypes

The days to 50% flowering significantly varied with N levels in genotypes and the interaction (P< 0.01) at flowering. Higher levels of nitrogen application tended to delay flowering period, the longest being 141days when 138 kg N ha $^{-1}$ was applied to variety Chemada. Minimum days to flower (124days) were recorded for the control treatment for Lalo variety at 0 kg ha^{-1} followed by the local variety (Table 1). This result is in line with the finding of Getachew (2004) and Mekonnen (2005) who also reported that heading was significantly delayed at the highest N fertilizer rate in wheat and barley crops than at lowest rate respectively. Contrary to the results of the present study, Sewnet (2005) reported early flowering with an increase in the rate of N application in rice. In general, the physiological maturity of sorghum plants was hastened under lower N rates than under the higher rates. Thus, increasing the rates of nitrogen from 0 to 138 kg N ha^{-1} prolonged days to maturity (Table 2). This result is in line with the report of Marschener (1995), Tanaka *et al*. (1995) and Brady and Weil (2002) that N applied in excess than required delayed plant maturity. Consistent with the result of this study, Gobeze (1999) reported that N rates delayed the maturity of sorghum.

Lodging percent

Lodging was significantly influenced by nitrogen fertilizer rates and the genotypes. Increasing the rates of nitrogen increased the lodging of sorghum genotypes across all nitrogen fertilizer rates. The maximum lodging percentage was observed in Lalo, followed by Chemada and the lowest percentage was in Local variety (Table 4). Seyfu (1983) reported that lodging in cereals is considered to be caused by high rates of nitrogen fertilizer application.

Table 1: Interaction effect of nitrogen rates on days to 50% flowering of sorghum genotypes

Nitrogen rates	**Sorghum varieties**			***Mean***
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	124.33c	136.67b	138.00ba	133
46 kg N ha $^{-1}$	124.67c	135.67b	138.33ba	132.89
92 kg N ha $^{-1}$	126.00c	136.33b	139.00ba	138.33
138 kg N ha $^{-1}$	137.00b	136.67b	141.33a	133.55
Mean	128	136.33	139.17	134.45
CV%		1.73		

Table 2: Interaction effect of nitrogen rates on days to 90% physiological maturity of sorghum genotypes

Nitrogen rates	**Sorghum varieties**			***Mean***
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	156.33c	167.67b	170.00ba	164.67
46 kg N ha $^{-1}$	156.67c	168.67b	170.33ba	165.22
92 kg N ha $^{-1}$	158.00c	168.33b	170.33ba	165.55
138 kg N ha $^{-1}$	169.00b	168.67b	173.33a	170.33
Mean	160	168.34	170.99	166.44
CV%		1.45		

Table 3: Interaction effect of nitrogen rates on plant stand plot^{-1} of sorghum genotypes

Nitrogen rates	Sorghum varieties			*Mean*
	Lalo	Local	Chamada	
0 kg N ha^{-1}	65.33ef	69.67abcde	64.33f	68.11
46 kg N ha^{-1}	68.33bcdef	70.67abcd	68dcef	69.00
92 kg N ha^{-1}	70.33abcd	73.67a	69abcdef	69.33
138 kg N ha^{-1}	73ba	72bac	66.67def	70.56
Mean	69.25	71.5	67	69.25
CV%		4.16		

Table 4: Interaction effect nitrogen rates on lodging percentage of sorghum genotypes

Nitrogen rates	Sorghum varieties			*Mean*
	Lalo	Local	Chamada	
0 kg N ha^{-1}	10.65c	8.63d	10.43c	9.9
46 kg N ha^{-1}	12.2b	9.1dc	12.33b	11.21
92 kg N ha^{-1}	12.9b	9.68c	12.59b	11.72
138 kg N ha^{-1}	15.33a	10.63c	13.2b	13.05
Mean	12.77	9.51	12.14	11.47
CV%		5.8		

The maximum plant stand was obtained in Local variety followed by Lalo and Chemada varieties (Table 3). Nitrogen application rates significantly ($P<0.001$) influenced plant height at 90 DAS, but the effect was not significant at 60 DAS and 120 DAS. Sorghum genotypes differed significantly in plant height (Table 5) at different growth stages. Plant height increased linearly with the increase in the rate of nitrogen application. When the rate of nitrogen increased from 0 through 138 kg N ha^{-1}, plant height increased, but the increase was significant up to 46 kgN/ha.

The increase in plant height following increased N application rate indicates maximum vegetative growth of the plants under higher N availability. These results are in agreement with the results obtained by Akbar et al. (1999) who found that plant height in maize increased with increase in N rate. However, Sadeghi and Bahrani (2002) reported that increase in N rate had no significant effect on plant height which could be due to the difference in the population stand, soil fertility status, and the crop varieties used**.**

***Number of green leaves plant*$^{-}$**

Nitrogen rates had no significant influence on leaf number /plant , whereas sorghum genotypes significantly differed in the number of green leaves per plant. There was a significant interaction effect of nitrogen rates and sorghum genotypes on the number of green leaves per plant (Table 6) where the highest number (14.00) was recorded with the application of 46kgN ha^{-1} for Local variety, which was comparable with Chamada fertilized with 92 kg N/ha(14).

Table 5: Effect nitrogen rates on plant height at various stages of sorghum genotypes

Treatment	**Plant height(cm)**		
Nitrogen(N) rates	60DAS	90DAS	120DAS
0 kg N ha^{-1}	34.54b	146.82b	263.33b
46 kg N ha^{-1}	39.39ba	165.77b	275.30ba
92 kg N ha^{-1}	40.72ba	196.30a	284.88a
138 kg N ha^{-1}	41.60a	204.99a	290.90a
Sorghum varieties			
Lalo	43.86a	227.42a	299.73a
Local	38.74ba	158.01b	276.54b
Chamada	34.59b	149.98b	259.53b
CV%	18.06	11.92	7.88

Number of effective tillers/plant

The sorghum genotypes varied distinctly in the number of effective tillers, but the nitrogen fertilizer rates did not show effect on tillers. The number of effective tillers were significantly higher in Local variety (2.18) than Lalo variety (1.2) and Chamada (Table 7).The current result is in agreement with that of Botella *et al*. (1993) who reported that stimulation of tillering with high rates of N application might be due to its positive effect on cytokinin synthesis. Corroborating with the results of this study, Genene (2003) reported higher tillering and maximum survival percentage of tillers with increasing N application in bread wheat.

Total leaf area

There was significant effect of nitrogen rates on leaf area at 90 DAS, but there was no discernible effect at 60 and 120 DAS. Sorghum genotypes significantly ($P<0.01$) differed in leaf area at 60 DAS (Table 8) and 90 DAS, but not at 120 DAS. There was significant interaction between sorghum genotypes and nitrogen fertilizer rates on leaf area at 90 and 120 DAS. The highest total leaf area per plant of 3193.3 cm^2 was obtained from Lalo variety with 46 kg N ha^{-1} which was at par with (2805cm) Chemada variety receiving 92kgN/ha at 90 DAS (Table 9). The highest total leaf area per plant (3694 $cm^{2)}$ was recorded in variety Lalo fertilized with 132kgN/ha, and the lowest leaf area per plant of 2631.9cm^2 was recorded in variety Lalo at 120DAS (Table 10). Increasing nitrogen fertilizer rates did not result in increment of leaf area at all stages in this study. Demir *et al*., (1996) reported that leaf area increased with increasing N levels.

Leaf Area Index (LAI)

The results revealed that there was no discernible effect of nitrogen fertilizer rates and sorghum genotypes on leaf area index. However, nitrogen rates and sorghum genotypes significantly interacted to influence this parameter ($P<0.05$). The highest LAI in Lalo genotype (3.28 cm^2) was recorded with the application of 138 kg N ha^{-1}, while the lowest (2.15cm^2) was recorded from variety Chemada under no N application. Generally, an increasing trend in LAI was observed with increased N application rates (Table 11). The increase in LAI was possibly due to the improved assimilation in plants receiving optimum nitrogenous fertilizers. Similar to this finding, Haghighi et al. (2010) also reported an increasing trend in LAI in maize due to an increase in N fertilizer application rates.

Table 6: Interaction effect of nitrogen rates and sorghum genotypes- on number of green leaves plant.

Nitrogen rates	Sorghum varieties			*Mean*
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	11.67bdc	14.00a	13.67a	13.11
46 kg N ha $^{-1}$	11.33dc	14.00a	12.67bac	12.67
92 kg N ha $^{-1}$	10.67d	13.67a	14.00a	12.78
138 kg N ha $^{-1}$	12.00bdc	13.00ba	11.33dc	12.11
Mean	11.42	13.67	12.92	12.67
CV%		7.33		

Table 7: Effect of nitrogen rates on number of effective tillers plant^{-1} of sorghum genotypes

Treatment	Number of effective tillers per plant
Nitrogen(N) rates	
0 kg N ha $^{-1}$	1.36a
46 kg N ha $^{-1}$	1.47a
92 kg N ha $^{-1}$	1.54a
138 kg N ha $^{-1}$	1.51a
Sorghum varieties	
Lalo	1.20b
Local	2.18a
Chamada	1.03b
CV%	22.46

Table 8: Effect of nitrogen rates on leaf area (cm) of sorghum genotypes at 60 DAS

Treatment	Leaf area
Nitrogen(N) rates	60DAS
0 kg N ha $^{-1}$	884.8a
46 kg N ha $^{-1}$	1028.5a
92 kg N ha $^{-1}$	1092.5a
138 kg N ha $^{-1}$	1054.5a
Sorghum varieties	
Lalo	1192.8a
Local	1005.4ba
Chamada	847.0b
CV%	25.31

Table 9: Interaction effect of nitrogen rates on Leaf area (cm) of sorghum genotypes at 90 DAS.

Nitrogen rates	Sorghum varieties			*Mean*
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	2869.4ba	2485.1bc	2376.1dc	2576.89
46 kg N ha $^{-1}$	3193.8a	2715.3bac	2361.5dc	2756.87
92 kg N ha $^{-1}$	2521.0bc	2728.3bac	2805.0bac	2684.77
138 kg N ha $^{-1}$	2799.0bac	2338.9dc	1955.9d	2364.6
Mean	2845.8	2566.9	2374.63	2595.78
CV%		11.27		

Table 10: Interaction effect of nitrogen rates on Leaf area (cm) of sorghum genotypes at 120DAS.

Nitrogen rates	**Sorghum varieties**			***Mean***
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	3229.8ba	3169.2bac	3204.5ba	3201.17
46 kg N ha $^{-1}$	3092.7bc	2813.6bc	2814.2bc	2906.83
92 kg N ha $^{-1}$	2631.9c	2859.3bc	3229.0ba	2906.73
138 kg N ha $^{-1}$	3694.9a	2950.8bc	2638.2c	3094.63
Mean	3162.33	2948.23	2971.48	3027.34
CV%		10.89		

Table 11: Interaction effect nitrogen rates and sorghum genotypes on Leaf area index

Nitrogen rates	**Sorghum varieties**			***Mean***
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	2.87ba	2.82bac	2.15ba	2.85
46 kg N ha $^{-1}$	2.75bc	2.50bc	2.50bc	2.58
92 kg N ha $^{-1}$	2.34c	2.54bc	2.87ba	2.58
138 kg N ha $^{-1}$	3.28a	2.62bc	2.38bc	2.76
Mean	2.81	2.62	2.65	2.69
CV%		10.88		

Table12: Interaction effect of nitrogen rates and sorghum genotypes on biological yield plot-1 (grams)

Nitrogen rates	**Sorghum varieties**			***Mean***
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	20459a	20131ba	22123a	20904.33
46 kg N ha $^{-1}$	21046a	20529a	20100ba	20558.33
92 kg N ha $^{-1}$	23443a	21784ba	21087a	22104.67
138 kg N ha $^{-1}$	21099ba	20237a	17934b	19756.67
Mean	21511.75	20670.25	20311	20831
CV%		10.01		

Effects of nitrogen rates on yield and yield components of sorghum genotypes

Biological yield

Biological yield of sorghum genotypes was not significantly influenced by nitrogen rates. However, there was significant interaction effect ($P<0.05$) of nitrogen rates and sorghum genotypes on this parameter (Table 12). Biological yield is a function of photosynthetic rate and proportion of the assimilatory surface area. The increase in biological yield with increase in rate of N might be due to better crop growth rate, LAI and accumulation of photo assimilates due to maximum days to maturity of the crop, which ultimately resulted in more biological yield. Biomass yield generally increased with the increase in the rate of nitrogen across the increasing frequency of application. The variety Lalo recorded the highest biomass yield (23443 g plot^{-1}) with the application of 92 kg N ha^{-1} and the lowest biomass yield was obtained from variety chemada (1793 g plot^{-1}) with the application of 138 kg ha^{-1}. This result is, however, in variance with the report of Haftom *et al*. (2009).

Thousand Seed weight

Thousand seed weight is an important yield determining component and reported to be a genetic character that is influenced least by environmental factors (Ashraf *et al*, 1999).

Significantly higher 1000 seed weight (32.25 g) was obtained from variety Lalo than Local variety (22.52 g),while the lowest weight (21.24 g) was obtained from Chemada. Regarding Nitrogen , the highest 1000seed weight was observed with 92 kg N ha^{-1} and lowest with 46kgNha^{-1} and 138kgNha^{-1} (Table13). Melesse (2007) reported no significant effect of the application of different rates of nitrogen fertilizer on 1000 kernel weight of bread wheat.

Panicle number plant^{-1}

Panicle number per plant is one of the yield attributes of sorghum that contribute to grain yield. Crops with higher panicle number could have higher grain yield. Panicle number was significantly (P< 0.01) influenced by the sorghum genotypes, but not by nitrogen rates (Table13). The genotype Lalo produced substantially higher number of panicles /plant (72.6) than local(59) and chamada(50).

Table 13: Effect of nitrogen rates on thousand seed weight and panicle number plant^{-1} of sorghum genotypes

Treatment	**Thousand seed weight(g)**	**Panicle number per plant**
Nitrogen(N) rates		
0 kg N ha $^{-1}$	26.07ba	59.67a
46 kg N ha $^{-1}$	24.17c	61.11a
92 kg N ha $^{-1}$	26.57a	62.89a
138 kg N ha $^{-1}$	24.53bc	59.00a
Sorghum varieties		
Lalo	32.25a	72.67a
Local	22.52b	59.25b
Chamada	21.24b	50.08c
CV%	3.41	9.4

Table 14: Interaction effect of nitrogen rates on grain yield hectare^{-1} (quintal) of sorghum genotypes

Nitrogen rates	**Sorghum varieties**			***Mean***
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	41.63	40.16	24.27	35.35
46 kg N ha $^{-1}$	45.97	41.10	31.54	39.54
92 kg N ha $^{-1}$	47.72	45.27	30.83	41.27
138 kg N ha $^{-1}$	41.87	41.08	28.93	37.29
Mean	44.3	41.9	28.89	38.36
LSD(0.05)		2.82		
CV%		4.13		

Table 15: Interaction effect nitrogen rates on harvest index of sorghum genotypes

Nitrogen rates	**Sorghum varieties**			***Mean***
	Lalo	Local	Chamada	
0 kg N ha $^{-1}$	20.64a	17.11 cb	10.24f	15.99
46 kg N ha $^{-1}$	21.02a	18.12 cd	12.29ef	17.14
92 kg N ha $^{-1}$	22.0a	19.63ab	13.38f	18.34
138 kg N ha $^{-1}$	21.3a	18.97 ed	12.85e	17.71
Mean	21.24	18.46	12.19	17.3
CV%		8.54		

Grain yield

Sorghum grain yield was significantly affected by the nitrogen rates, sorghum varieties and their interaction ($p<0.01$) (Table 14). Significantly greater grain yield was obtained from the variety Lalo with the application of 92 kg N ha^{-1}(47.72quintal ha^{-1}) which was on par with 46kgN/ha(45.9q/ha), and with 92kgN/ha of Local variety (45.2q/ha),and the lowest grain yield was recorded in variety Chemada with no nitrogen application (24.27ql ha^{-1}) (Table14). Thus Lalo and Local varieties responded up to 92kg N/ha , while chamada responded upto 46N/ha. The results of this study are consistent with result of Sage and Pearcy (1987) who reported that a well-balanced supply of N results in higher net assimilation rate and increased grain yield as also observed by Al-Abdulsalam (1997). Corroborating the results of this study, Blankenau *et al*. (2002) reported that proper rate and time of N application are critical for meeting crop needs, and indicated considerable opportunities for improving yields.

Harvest index **(HI)**

The physiological efficiency and ability of a crop for converting the total dry matter into economic yield is known as harvest index (HI). The interaction effect of nitrogen rates and sorghum cultivar on harvest index was highly significant (Table 15). In line with the result obtained by Lawrence (2008) who reported that harvest index in maize increases when nitrogen rates increases. With the increase in the rate of nitrogen application, harvest index increased. This indicates significantly lower biomass partitioning to grain production when N was increased. The lower mean HI values in this experiment for the higher N application might indicate the need for the enhancement of biomass partitioning through genetic improvement. In line with the results of this study, Abdo (2009) reported highest harvest index from treatments with the lower rates of nitrogen application.

CONCLUSIONS

From the foregoing account it could be inferred that among the test genotypes of sorghum , improved Lalo and traditional Local variety exhibited greater response up to 92kgN/ha, while Chamada responded to 46kg N/ha under rain fed conditions of Ethiopia, East Africa. Across genotypes, increased N rates delayed flowering, physiological maturity ,increased lodging ,enhanced plant height, tillers /plant, leaf area, panicles /plant, grain yield, harvest index; but showed no discernible influence on leaf number/plant, LAI, and test seed weight.

REFERENCES

Abdo Woyema (2009) Effect of different rates of nitrogen fertilizer on yield, yield related traits and quality of durum wheat (Triticum turgidum L.var. durum). *Plant Soil,* 257:205–217.

Akbar F, Wahid Akhtar A and Ahmad S (1999) Optimization of method and time of nitrogen application for increased nitrogen use efficiency and yield in maize. *Pakistan Journal of Botany*, 31: 337-34.

Al-Abdul Salam MA (1997) Influence of nitrogen fertilization rates and residual effect of organic manure rates on the growth and yield of wheat. *Arab Gulf Journal of Science Research* 15: 647-660.

Ashraf A, Khaid A and Ali K (1999) Effects of seeding rate and density on growth and yield of rice in saline soil. *Pak. Biol. Sci*., 2(30): 860-862.

Blankenau K, Olfs HW and Kuhlmann H (2002). Strategies to improve the use efficiency of mineral fertilizer nitrogen applied to winter wheat. *Journal Agronomy and Crop. Sci*. 188:146-154.

Botella MA, Cerda AC and Lips SH (1993) Dry matter production, yield and allocation of carbon-14 assimilate by wheat as affected by nitrogen source and salinity. *Agronomy Journal* 35(5):1044-1049.

Brady NC and Weil RR (2008) The nature and properties of soils. *Revised 14th ed*. Pearson Prentice Hall. New Jersey. pp. 358-682.

Centeral Statistical Authority, Agricultural Sample Survey (2006) Report on the area and major

production for major crops Private peasant holdings. Addis Ababa, Ethiopia

Demir K, Yanmaz R, Ozcoban M and Kutuk AC (1996) Effects of different organic fertilizers on yield and nitrate accumulation in spinach. GAP 1. Vegetable Symposium, *Sanliurfa*, Turkey, pp 256-257.

FAO (2005) Scaling soil nutrient balance. Enabling some level application for African realities: FAO fertilizer and plant nutrition. *Bulletin* 15, FAO, Rome.

Genene Gezu (2003) Yield and quality response of bread wheat varieties to rate and time of nitrogen fertilizer application at Kulumsa, Southern Ethiopia. MSc Thesis, Alemaya University, Alemaya Ethiopia.

Getachew Fisseha (2004) Soil characterization and bread wheat (*Triticum aestivum* L.) response and P fertilizatoin. MSc Thesis, Alemaya University, Alemaya, Ethiopia.

Ghosh BC and Bhat R (1998) Environmental hazards of nitrogen loading in wetland rice elds. *Environment Pollut*. 102 Suppl. 1:123-126.

Gobeze Loha (1999) Effect of special arrangement and nitrogen fertilizer application on (*Sorghum binocular* L. Moench) and ground nut (*Arachis hypogeal*) intercrop system in Eastern Ethipopia. MSc Thesis, Alemaya University of Agriculture, Alemaya, Ethiopia.

Haftom Gebretsadik, Mitiku Haile and Yamoah CH (2009) Tillage frequency, soil compaction and N-fertilizer rate effects on yield of tef (*Eragrostis tef (Zucc.) Trotter). Ethiopia Journal of Science* 1(1): 82-94.

Haghighi BJ, Yarmahmodi Z and Alizadeh O (2010) Evaluation of the effects of biological fertilizer on physiological characteristics and yield and its components of corn (Zea mays L.) under drought stress. American Journal of Agricultural Biological Science. 5: 189193. DOI: 10.3844/ajabssp.2010.189.193

Kessel, C. V., Pennock,D. J and Farrel, R.E.. 1993. Seasonal variation in denitrifcation and nitrous oxide evolution at the landscape scale. Soil Sci. Soc. Am. J. 57:988-995.

Lawrence JR, Ketterings QM and Cherney JH (2008) Effect of nitrogen application on yield and quality of corn. *Agronomy Journal*, 100 (1): 73-79.

Marschner H (1995) Mineral Nutrition of Higher Plants. 2nd ed. Academic Press, London.783p. Brady, N. C. and Weil, R.R. 2002. The Nature and Properties of Soils. 13th ed. Person. Education Ltd, USA.621 pp

Mekonnen Asrat (2005) Response and uptake of barley (*Hordem Vulgarae L*.) to different rates of ORG- P and N fertilizer. MSc Thesis, Alemaya University, Alemaya, Ethiopia.

Melesse Harfe (2007) Response of bread wheat (*Triticum aestivum* L.) varieties to N and P fertilizer rates. M.Sc. Thesis, Haramaya University, Haramaya, Ethiopia.

Perry LJ Jr. and Olso RA (1975) Yield and quality of maize and grain sorghum and residues as influenced by nitrogen fertilization. *Agron. J*., 67:816 – 823.

Sadeghi H and Bahrani MJ (2002) Effects of plant density and N rates on morphological characteristics and protein contents of corn. Iranian Journal of Agriculture Science, 33: 403-412.

SAS (Statistical Analysis System), 2002. SAS user guide, statistics SAS Inc. Cary. North Carolina, USA.

Sewnet Ashebir (2005) Effects of nitrogen and seed rates on grain yield components and nitrogen uptake of rain-fed rice (*Oryza sativa*) in Fogera, South Gonder. MSc Thesis, Alemaya University, Alemaya, Ethiopia.

Seyfu Ketema (1983) Study of lodging, floral biology and breeding techniques in tef [*Eragrostis tef* (Zucc) Trotter] PhD Thesis. University of London, London, United Kingdom.

Stevenson FJ (1985) The nitrogen cycle in soil: Global and ecological aspects. In Stevenson, F. J. ed. Cycles of Soils. A Wiley Interscience Publication. Wiley, New York, USA. pp 106-153.

Tanaka K, Kasai Z and Ogawa M (1995) Physiology of ripening. pp.97-118. *In*: T. Matsuo, K. Kumazawa, R. Ishii, K.Ishihara, and H. Hirata, (eds) .*The Science of the Rice Plant*. Vol. 2. Food and Agriculture Policy Research Center, Tokyo.

William PH (1992) The role of fertilizers in environmental pollution. In: Proc. of Inter. Symp. on nutrient management for sustainable productivity. Dept. Soil, Punjab Agric. Univ. Ludhiana, India. pp 195-215.

RESEARCH ARTICLE

Effects of Selected Pollutants on the growth and survival of *Clarias gariepinus* (Burchell, 1822)

Abidemi-Iromini AO[1]* and Kusemiju K[2]

[1]Fisheries and Aquaculture Technology Department, Federal University of Technology, Akure, Nigeria.
[2]Department of Marine Sciences, University of Lagos, Akoka-Yaba, Lagos, Nigeria.
*Corresponding author: attytej@gmail.com

Manuscript details:

Received: 23.10.2015
Accepted: 06.12.2015
Published : 30.12.2015

Editor:

Cite this article as:
Abidemi-Iromini, AO and Kusemiju K (2015) Effects of selected pollutants on the growth and survival of *Clarias gariepinus* (Burchell, 1822). *International J. of Life Sciences*, 3(4): 317-324.

ABSTRACT

Spent engine oil, NPK fertilizer (15:15:15) and sewage effluent pollutants on growth and survival of *Clarias gariepinus* catfish were investigated for 70 days. Juvenile fish of length range 7.5cm to 10.6cm; and weight range 4.00g to 8.20g were used; and fish fed 5% body weight of conventional floating feed on daily basis. Temperature (^{0}C), pH and dissolved oxygen (mg/l), mortality and growth rate were monitored. 35% mortality was experienced in NPK media; and 15% mortality was experienced in control experimental tanks. Specimens in control and NPK tanks survived to end of experimental period (70 days) with highest mean weight control tank (51.9g) and length (9.6cm); NPK specimen had mean weight 35.0g and length 8.4cm. Condition factor 'K' in control tank and NPK tank were: 0.98 and 0.93. Food Conversion Ratio (FCR) of control and NPK tanks were: 2.00 and 1.51. Food Conversion Efficiency (FCE) in control and NPK tanks were: 50.11% and 66.35%. Specific growth rate (SGR) {%/day} in control water tank was 1.47% per day, and NPK was 1.29% per day. Test statistic indicated there was no significant difference in growth/survival rate within surviving specimens of NPK and control environmental tanks.

Keywords: *Clarias gariepinus*, growth, mortality, pollutants, survival.

INTRODUCTION

Clarias gariepinus is one of the most highly valued freshwater fishes of Africa, and among the most highly prized food fishes of West Africa. *C. gariepinus* fish had been used in fundamental research work for the improvement of science of fisheries (Nguyen *et.al.,* 1999). The fish occur in brackish and fresh waters, flood plains, lakes, and creeks of Nigeria.

Adebiyi (1991) reported that pollution in the inland and fresh water areas in Nigeria has been a major hindrance to enhanced fish production, hence the need to control all pollutants in our water bodies. (Otitoloju (2006) reported that Nigeria accounts for more than 8.7 million liters of spent lubricant oil annually, of which about 80 – 90 % is disposed into the environment, causing pollution.

According to International Center for Aquaculture and Aquatic environment, (ICAAE (1998), most fish species are found only in well aerated aquatic water and may not survive any form of pollution in their water bodies. Moreover spillage or erosion of inorganic fertilizer into aquatic environment can result in toxicity (ICAAE, 1998). (Luger and Brown (2004) reported that, treated sewage effluent is one of the most common types of pollution found in urban rivers, and its persistent discharge results in pollution of the freshwater systems which causes reduction in oxygen concentration through microbial overload, and contamination of fishery resources. The objective of this study was to investigate the effect of selected sources of pollution: spent engine oil, inorganic fertilizer, sewage effluent, on the growth and survival of *Clarias gariepinus.*

MATERIALS AND METHODS

Several *Clarias gariepinus* post-juvenile fish of length range between 7.50 – 10.60 cm and weight range between 4.00g – 8.20g were collected from a private aquaculture farm in Egbeda, Lagos; in a 25 liters plastic container with water from the farm; and transported during early hours between 8am – 10am to research laboratory of Marine Sciences Department, University of Lagos. Fish were acclimatized for three days and fed fortified floating feed at 5% body weight in the laboratory environment to allow them adapt to laboratory conditions.

Test chemicals and water supply

Two liters spent engine oil was collected in a mechanic work station in Shomolu, Lagos; NPK (15:15:15) inorganic fertilizer was obtained from a sales outlet in Agege, Lagos; sewage effluents was collected in 50 liters plastic container from the discharge outlet of sewage treatment plant in University of Lagos; and Fresh water used was collected from Marine Sciences laboratory.

Experiment set-up

Experimental tanks A, B, C, and D (Table 1) in plastic material dimension [50x30x34] cm (L x B x H) were set up in replicates for the three pollutants and control experimental condition which were filled with 30 liters fresh water. Spent engine oil (30 ml) was introduced into bioassay tanks for spent engine oil polluted enclosure (tanks A_1, A_2) and 30g NPK fertilizer in 30 litres fresh water (tanks B_1, B_2), 30 liters fresh water control experiment (tanks C_1, C_2), and 30 liters sewage effluents for the sewage effluent condition (tanks D_1, D_2). Ten specimens each of *Clarias gariepinus* specimens of initial length range between 7.50 – 10.60 cm and initial weight range between 4.00g and 8.20g were randomly introduced into the experimental tanks respectively to test the effect of pollutants on the growth and survival of *Clarias gariepinus*. Experimental tanks were covered with 4mm mesh size net to prevent the specimens from jumping out of the tanks and proper aeration of tanks were ensured using Boyu silent air pumps (Model No SA- 1500) with air stones inserted into the end of the tube from the pumps for agitation of the water.

The experiment was carried out for a period of ten weeks; during which specimens were fed 5% body weight fortified pelleted feed (Table 2) daily, every 0900 hours and 1600 hours. Concentrations of pollutants media and fresh water in the experimental tanks were freshened every three days to avoid deterioration. Daily monitoring of the fish specimens were carried out to assess the state of being and behavioral attitude of fish within the pollutant media; and mortality were monitored and recorded daily throughout experimental period. Physico-Chemical parameters of experimental media were

carried out between 0900 hours and 0930 hours weekly. Air and water temperature were obtained using mercury in glass thermometer and Hydrogen – ion concentration were obtained using pH-009IIATC (High Accuracy Pen Type Portable pH meter), Dissolved oxygen was determined by the use of Extech direct reading dissolved oxygen meter.

Growth measurement and monitoring were carried out between 0900 hours and 1000 hours fortnightly throughout the ten weeks experimental period on any five surviving randomly selected specimens from each experimental media. Morphometric measurements on total length (cm), standard length (cm) were carried out using graduated board; and weight (g) gained by specimen were measured using digital measuring scale. Mean length and weight measurement were determined by dividing the sum of the totality of the standard length of the fish by the number of the specimen per tank throughout the experimental period.

Condition factor (K) {equation 1} of survived fish specimens from the experimental media were determined to assess the state of well-being of the fish; Food Conversion Ratio {equation 2} and Food Conversion Efficiency {equation 3} were determined to assess the behavioral disposition of fish to feeding within the pollution media; and Specific Growth Rate (SGR) {equation 4} of survived specimens within experimental tanks were calculated. Statistical package for social sciences (Pasw statistics 18) was used to assess significant difference in growth among the survived fish specimens in the polluted experimental media and control experiments.

$$\text{Condition Factor (K)} = \frac{100W}{L^b} \quad \text{... (Bannioter, 1976) (eqn. 1)}$$

Where K = Condition factor
W = Weight of specimen in cm
L = Length of specimen in cm
b = Regression coefficient

$$\text{Food Conversion Ratio(FCR)} = \frac{\text{Dry food fed (g)}}{\text{Live Weight gained (g)}} \quad \text{...(Goddard,1996) (eqn. 2).}$$

$$\text{Food Conversion Efficiency(FCE)} = \frac{\text{Mean Weight gained}}{\text{Feed in-take}} \times 100 \quad \text{... (Goddard, 1996)(eqn. 3).}$$

$$\text{Specific Growth Rate (SGR)} = \frac{\text{Log final body weight} - \text{Log initial body Weight}}{\text{Time (in days)}} \times 100$$

... (Goddard, 1996) (eqn. 4)

RESULTS

Mean physico-chemical parameters assessed on the experimental media over the period of the experiment indicated a condusive parameters range (Table 1).

Behavioral observations of fish at introduction into the pollutant experimental media and throughout the experimental period show irrational reactions and adaptation to environmental condition in survived fish. Specimens introduced into spent oil (tank A) had erratic movement on introduction with increase in breathing shown by increase in rate of opercula flap. Feeding activity was closed to none due to the polluted environment, and further behavioral observation were: settling down at the

base of the tank with increase panting, hanging and dangling within the environment and incident of blood from the urinary system and followed by death of the fish. Erratic movements were observed in fish exposed to NPK 15:15:15 fertilizer (Tank B), followed by settling down at the bottom of the tank; and increase in respiration observed by increase in rate of opercula flapped. The coloration of fish bleached, and the fish drag around the environment and later adjust to the environmental condition and feeding activity increase with increase in rate of adjustment of the fish and adapted to the environmental condition. Fish subjected to sewage effluents (Tank C) were observed to experience irrational and erratic movement, followed by settling down at the bottom of the tank with increase opercula flap which indicated increased breathing, hanging and dangling. Little or no feeding activity was observed, and specimen death. Specimen in the control experiment (tank D) performed normally with little or no irrational movement and carryout feeding activities.

Table 1: Mean physico-chemical parameters of the experimental media

Tanks	Air temperature (^{0}C)	Water temperature (^{0}C)	pH	Dissolved Oxygen (mg/l)
A	29.80 ± 1.20	28.60 ± 0.70	6.90 ± 0.20	2.80 ± 0.50
B	29.70 ± 1.00	28.50 ± 0.60	6.00 ± 0.60	3.70 ± 0.50
C	29.60 ± 1.20	28.10 ± 0.70	6.30 ± 0.60	3.70 ± 0.50
D	29.70 ± 1.00	28.50 ± 0.30	6.20 ± 0.40	3.50 ± 0.70

Table 2: Survival and Mortality recorded for *Clarias gariepinus* specimens in the experimental tanks

Tanks↓	**Weeks→**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A	A1	1	0	8	0	1	0	0	0	0	0
	A2	10	0	0	0	0	0	0	0	0	0
B	B1	2	1	0	0	1	0	0	0	0	0
	B2	2	1	0	0	0	0	0	0	0	0
C	C1	6	3	0	0	0	1	0	0	0	0
	C2	10	0	0	0	0	0	0	0	0	0
	D2	1	1	1	0	0	0	0	0	0	0

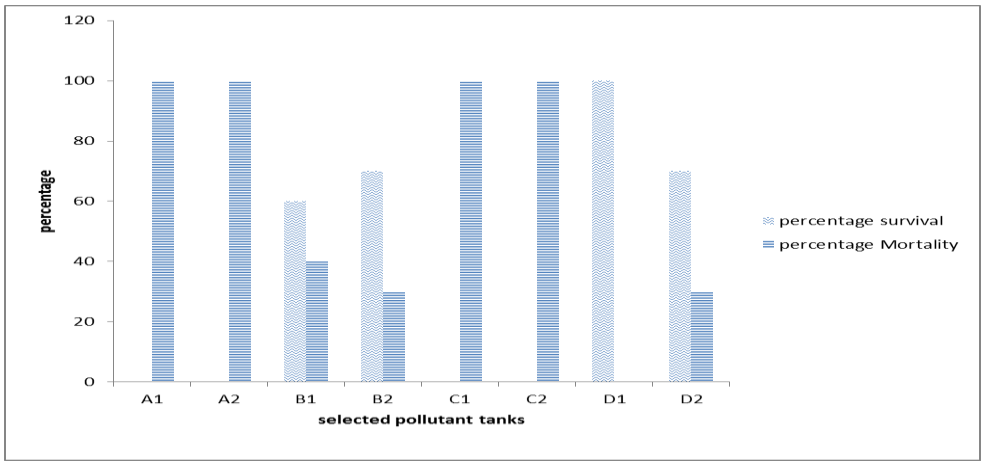


Fig.1: Percentage Mortality/ Survival of *Clarias gariepinus* in Experimental media

Table 3: Mean Weight Gain / Loss of Clarias gariepinus Specimens with Time under Different Environmental Conditions

Treatments	Initial mean weight (g)	Mean weight (g) at 2 weeks	Fortnight mean weight (g) gain/loss	Mean weight at 4 weeks	Fortnight mean weight (g) gain/loss	Mean weight (g) at 6 weeks	Fortnight mean weight (g) gain/loss	Mean weight (g) at 8 weeks	Fortnight mean weight gain/loss	Mean weight (g) at 10 weeks	Fortnight mean weight (g) gain/loss
Spent oil	8.20	10.50	2.30	3.20	-7.30	0.00	0.00	0.00	0.00	0.00	0.00
NPK fertilizer	5.10	10.50	5.40	21.10	10.60	19.90	-1.20	34.64	14.74	40.10	5.46
Sewage effluent	4.00	5.00	1.50	0.00	-1.50	0.00	0.00	0.00	0.00	0.00	0.00
Control	5.30	10.10	4.80	17.40	7.30	25.70	8.30	45.53	19.83	39.20	-6.33

Table 4: Percentage Mean Length, Weight Gain, Food Conversion Ratio, Food Conversion Efficiency, Specific Growth Rate and Condition factor of Clarias gariepinus fish in Different Experimental Media

Treatment	**Initial mean length (cm)**	**Initial mean weight (g)**	**Final mean length (cm)**	**Final mean weight (g)**	**Mean length gained (cm)**	**Mean weight gained (g)**	**% length gained**	**% weight gained**	**Food conversion Ratio (g)**	**Food conversion Efficiency**	**Specific Growth Rate**	**Initial Condition Factor (F)**	**Final Condition Factor (F)**
Spent oil	10.60	8.20	--	--	--	--	--	--	--	--	--	--	--
NPK fertilizer	7.90	5.10	16.30	40.10	8.40	35.00	106.30	686.30	1.5	66.40	1.3	1.03	0.93
Sewage effluent	7.50	4.00	--	--	--	--	--	--	--	--	--	--	--
Control	8.40	5.30	18.00	57.20	9.60	51.90	114.30	979.30	2.0	50.10	1.5	0.89	0.98

Mortality data from the four experimental media indicated that specimens in the spent engine oil (tank A) and sewage effluent (tank C) media suffered highest mortalities within two week of pollutants assessment. Spent engine oil media recorded 55% mortality within two weeks and the remaining 45% mortality before the end of the fifth week of the experiments. There was 95% mortality within two weeks in the sewage effluent media, and the surviving 5% specimen died before the end of the sixth week. Totality of 35% mortality was recorded in the NPK inorganic fertilizer media (tank B) while control experiment (tank D) experienced 15% mortalities throughout the experimental period. Dead specimens were removed from tanks to prevent deterioration of the experimental media and mortalities were recorded (Table 2). Survival rate of fish in the experimental media was 65% for NPK fertilizer media and 85% for the control experiment void of pollutant. Spent engine oil and sewage effluent experimental media had zero survival rates (0%) respectively at the end of the experimental period (Fig. 1).

Table 3 shows the mean weight measurement with time and mean weight gain/loss fortnightly for the experimental specimens. The mean weight gained in survived fish specimens indicated that specimens in the NPK experimental media were the survivors of the three pollutants experimental media; and 35.0 g mean weight gain was recorded from it at the end of the 10 weeks experiments; while mean gain in weight calculated for control experiment was 51.9 g.

Growth Assessment of Survived Clarias gariepinus in Experimental Media

Mean length gained in specimens in control experiment (9.60 cm) was 1.20 cm more than mean length gained in specimens from NPK polluted media (8.40 cm); and percentage mean length and weight gained were indicated in table 4. Fish from control experiment showed a better food conversion ratio (2.0g) to the survivor from the polluted media (1.5g) which yielded higher food conversion efficiency (66.40%) above the control experiment (50.10%). Specific growth rate of survived fish in the control experiment was higher (1.5%) than survived fish in NPK fertilizer polluted environment (1.3%). And Condition factors 'K' of the specimens in the different tanks indicated a range from 0.69 to 1.03 for initial condition factor of the fish in all experimental media, and 0.93 to 0.98 for final condition factor in fish from NPK fertilizer-polluted and control experiments respectively (Table 4).

Result of Statistical Analysis

Chi- square used to analyze significant difference in the survival/mortality of *Clarias gariepinus* fish in control and NPK fertilizer experimental media indicated acceptance of null hypothesis of value: (2.133) at 0.05 significant level; and it was concluded that there was no significant different in the survival / mortality rate between specimens in the control and NPK experimental media respectively. T-test was used to assess level of significant difference in growth rate of survived specimens in control and NPK fertilizer-polluted experimental media; and result obtained indicated the acceptance of the null hypothesis with fortnight growth rates values: (0.881, 0.522, 0.215, 0.314 and 0.107) at 0.05 significant level; and it was concluded that growth rate of specimens in control and NPK experimental media shows no significant difference.

DISCUSSION

Physico-chemical parameters monitored in experimental media indicated fairly conducive aquatic environmental conditions. However, total mortality was experienced in *Clarias gariepinus* specimens exposed to sewage effluent-polluted and spent oil-polluted experimental media; as experienced by (Soyinka and kusemiju, (2004), which reported highest mortality in specimens exposed to diesel oil-polluted water and untreated sewage water. And survival rate of fish in the experimental media was 65% for NPK fertilizer-polluted media and 85% for the control experiment void of pollutant.

Luger and Brown, (2004) reported that, treated sewage effluent is one of the most common types of pollution found in urban rivers in which both the quality and quantity of effluent result in various impacts on the receiving freshwater as well as estuaries and near shore marine environment. Discharge into aquatic systems results in reduction in species diversity and degradation of aquatic environment which can have negative impacts on human health, primarily from bacteriological and other forms of pathogens that survive the biological treatment process and inadequate disinfection of the effluent. Breakdown of ammonium (NH_4^+), into its un-ionised form (NH_3) which is toxic to many forms of aquatic life and increases in relative proportion to NH_4^+ as pH and temperature increase, becoming a serious threat in alkaline conditions (pH > 8) is of ecological implication. Nonetheless, conferment within a small enclosure without access to escape enhanced complete mortality within a short period. This investigation may be the case especially at area of discharge of sewage effluent (introduction area) as well as aquatic environments exposed to constant or continual sewage effluent. (Ajao (1990) in (Soyinka and Kusemiju, (2004), also reported the effects of untreated sewage and sludge in the Lagos lagoon to included aesthetic nuisance, nauseating odor and human health hazard.

Pollution of commercial petroleum fuels (CPF) is one of the environmental constrains that produces aqua-toxicological effects, which affect growth performance and survival; and are deleterious to aquatic life (Safaa and Mohsen, 2011). According to Otitoloju and Okusada (2003), spent oil has negative impacts on aquatic organisms because it acts more as a physical poison forming a barrier on the water surface which reduces the rate of oxygen diffusion into water; and in such event leading to soon used up of available dissolved oxygen by the animals which slowly die from asphyxiation. This supported the experience studied from exposure of *C. gariepinus* to spent oil whereby fish became agitated, imbalanced, gasping for air with increased opercula movement and unable to feed, thereby resulted in total kill of the specimen within a short time. This is because the fish are in a confined environment, but in natural environment, high kill rate will occur at instant of high discharge especially in low mobility organisms, and moving away of fish from such environment will result in lesser impact or survival. This is supported by the report of Soyinka and Kusemiju, (2004) which stated that, in a natural aquatic condition, oil spillage into the aquatic environment might not have high mortality effect due to high mobility rate of organism.

Reduced percentage (35%) mortality experienced in NPK fertilizer-polluted experimental media resulted after the fish experienced changes in behavioral response of physiological condition to the environment pollution, which was observed through the loss of equilibrium, erratic swimming, sudden swimming motion reduced feeding and excessive mucus secretion. Skins of the survivors were observed to have bleached out as they adjusted to the polluted environment. These responses and results obtained indicated similarities to the observed responses of fish under various stress conditions studied by (Erol *et al*. (2010). The result is an indicative that nitrogen fertilizers can increase ammonium concentrations in the water which might positively or negatively affect the ecosystem quality to the benefit or detriment of live aquatic organisms including fish; and the effects for aquatic organisms is ability to movement away from toxic effects of pollutant which may be deadly or quick recovery as reported by (Yaro *et al*. (2005) as supported the observed results of the present study. None-the-less, continuous exposure of *C. gariepinus* to the inorganic fertilizer may continue to impair physiological formation of the fish and death will continue to increase when physiological sustainability is reduced. Prediction of water quality impacts of fertilizer and related land management practices is an essential element of site-specific control

options and for the development of generic approaches for fertilizer control, (Quirós, 1993).

CONCLUSION

Sewage effluents, spent engine oil and NPK fertilizer (15:15:15) were toxic for *Clarias gariepinus*. Impact of sewage effluent and spent engine oil was highest for mortality than the inorganic fertilizer within the confined environment, but likelihood of survival in open water will be high due to means of escape and leaching of toxic with time from the body of the fish. Fish exposed to fertilizers recover quickly when they were moved to freshwater. It is concluded that the sewage effluents, spent engine oil and NPK fertilizer may have toxic potentials in the shallow water and enclosed waters; and therefore environmental impact of release or introduction of such substances should be carefully assessed in areas closed to waterside.

REFERENCES

Adebiyi OF (1991) Integrated Rural fisheries Development Project (Status, Prospects and Constraints). *Paper presented at the national seminar on the dev. of Art. Fish. in Nig.* p.5

Ajao EA (1990) The influence of domestic and industrial effluents on population of sessile and benthic organisms in the Lagos lagoon. *Ph.D Thesis, Uni. of Ib., Nig.* P.413.

Erol C, Sevki K, Halis B, and Ilhan A (2010) Acute Toxicity of Some Agriculture Fertilizers to Rainbow Trout. *Turk. J. of Fisheries and Aqu. Sci., 10:* 19-25

ICAAE (1998) Water quality for Pond Aquaculture. Research and development Series No43. 37pp.

Luger K and Brown C (2010) The Impact of treated Sewage Effluent on urban rivers. An ecological, social and economic perspective. *Ninham Shand consulting Ser.,* www.shands.co.za. p.9

Nguyen LTH, Janssen CR and Volckaert FAM (1991) Susceptibility of embryonic and larval African catfish (*Clarias gariepinus*) to toxicants. *Bull. of Environ. Cont. and Tox.,* 62: 230-236

Otitoloju AA (2006) Joint action toxicity of spent lubrication oil and laundry detergent against Poecilia reticulate (Telostei: Poeciliidae). *Afr. J. of aqu. Sci.,* 31 (1): 125-129

Otitoloju AA and Okusada BO (2003) Impact of waste materials generated by roadside mechanics and panel beaters against gutter-dwelling fish, Poecilica reticulate. *The Zoo. 2*(2): 46-54.

Quiros CF (1993) Genetic Improvement of Celery. In: Kalloo and Bergh (eds.), Vegetable Crops Improvement, *Pergamon*, 37:523-534.

Safaa MS and Mohsen A (2011) Eco-Physiological Impact of Commercial Petroleum fuels on Nile tilapia, Oreochromis niloticus. *In Proceedings of the Ninth International symposium on Tilapia in Aquaculture, Shanghai, China,* 3: 28-38.

Soyinka OO and Kusemiju K (2004) Effects of pollutants on *Sarotherodon melanotheron* (Rupell), *Batanga lebretonis* (Steindachner), *Macrobrachium macrobrachion* (Herklots) and *Clibanarius africanus* (Aurivillius). *J. Sci. Res.* Dev. 9: 39 – 46.

Yaro BI, Lamai SL and Oladimeji AA (2005) The effect of deferent fertilizer treatments on water quality parameters in rice-cum-fish culture systems. *J. of App. Ich., 21*: 399-405.

RESEARCH ARTICLE

Histochemical and histoenzymatic observations on the intestinal epithelium of *Haemonchus contortus* (Nematoda)

Jatinderpal Singh

Department of Zoology, Baring Union Christian College, Batala-143505 (India)
e-mail: doctorjatinder@yahoo.com

Manuscript details:

Received: 26.11.2015
Accepted: 12.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Singh Jatinderpal (2015) Histochemical and histoenzymatic observations on the intestinal epithelium of *Haemonchus contortus* (Nematoda). *International J. of Life Sciences*, 3(4): 325-332.

Acknowledgements: Author is thankful to Prof. (Dr.) Manjit Johal for her guidance, encouragement and constant support for this research work. Author also indebted to the Professor and Head, Department of Zoology, Punjabi University, Patiala and Principal, Baring Union Christian College, Batala for providing necessary facilities to carry out the research.

ABSTRACT

Histochemically, an intense concentration of glycogen, general proteins, lipids, nucleic acids and acid and alkaline phosphatases is seen in the intestinal epithelium of *Haemonchus contortus*. A well developed microvillar border positive for general carbohydrates, -NH_2 bound proteins, lipids, acid phosphatases is present. The microvillar border is totally free from glycogen indicating that the microvilli help in the absorption of simple carbohydrates, which in turn are converted to and stored in the form of glycogen in the intestinal epithelium. The intestine of *H. contortus* does not only act as a place for the transport of absorbed materials but also a tissue of considerable synthetic activity. The presence of proteins and RNA activity indicates that the intestinal protein synthesis pool also distributes a large quantity of protein to the other body organs. A close approximation of intestine with the reproductive organs indicates the trans-membrane flow of nutrients from the former to the later.

Keywords: Intestinal epithelium, microvilli, histochemistry, nematoda, *Haemonchus contortus*.

INTRODUCTION

The nematode *Haemonchus contortus* is a serious pathogenic endoparasite of sheep and other domestic ruminants. Morphologically Nematoda is an exceedingly variable group and there hardly exists any common statement that could be made regarding their histomorphology and histochemistry, which would apply to all forms. Pawlowski (1987) while addressing the 6th International Congress at Brisbane, Australia stated that there is a renewed interest in basic research which can fill the hitherto unexplained gaps. Different histochemical parameters have been

described in the intestinal epithelium of various nematodes by Chitwood and Chitwood (1950), Lee (1960), Bird (1971), Jenkins (1970,1973), Johal *et al.* (1997), Johal and Singh (1998), Anderson (2000) and Sood (2006). Previously, the histo-morphological study on the intestinal epithelium of *H. contortus* was performed by Singh and Johal (2004). The present study describes many histochemical variations in the intestine of *H. contortus*, which can fill the hitherto existing gaps in information regarding this aspect. This histochemical localization of various macromolecules will be of significance to understand the metabolic activities and fundamental functional aspects. It can also form the basis in evolving chemotherapeutic measures against this pathogenic parasite.

MATERIALS AND METHODS

The nematode *Haemonchus contortus* was extracted from the abomasum portion of stomach of sheep (*Ovis aries*). In order to remove debris, the nematode worms were washed in 0.85% NaCl solution. For histochemical studies, the worms were fixed in alcoholic Bouin's fixative and Carnoy's fixative, dehydrated in a graded series of alcohol, cleared in methyl benzoate and embedded in paraffin wax. The sections were cut at 7µm in transverse and longitudinal planes by using rotary microtome. The serial sections arranged on albuminised slides were stained. For the histochemical localization of carbohydrates, glycogen, acid mucopolysaccarides, proteins, lipids, acid phosphatases and alkaline phosphatases the following staining methods were used.

General carbohydrates were studied by Periodic acid Schiff's staining technique (McManus, 1948). Glycogen was detected histochemically by Best's carmine staining (Best, 1906) and acid mucopolysaccharides by Alcian blue (Steedman, 1950). Nucleic acids were detected by Gallocyanin chromalum (Einarson, 1951) and Methyl green pyronin Y (Kurnick, 1955) techniques. For the localization of proteins, Mercuric bromophenol blue staining (Bonhag, 1955) and Ninhydrin Schiff's staining (Yasuma and Ichikawa,1953) were used. The histochemical presence of lipids was detected by Sudan black B staining (McManus, 1946) and Oil red O in isopropanol (Lillie and Ashburn, 1943). For histoenzymatic studies, acid phosphatase paraffin section technique (Ruyter, 1964) and Modified Gomori method for alkaline phosphatese (Fredricsson, 1956) were used. The slides were examined under the microscope and photo micrographed.

RESULTS AND DISCUSSION

The carbohydrate is seen in a diffused form or in a sort of network form in the cytoplasmic region of intestinal epithelium of *Haemonchus contortus.* The intestinal contents and microvillar border also stains pink with periodic acid Schiff's technique indicating the absorption of carbohydrates. The outer covering or basal lamina of intestine contains carbohydrates as one of the main constituent (Fig. 1 and Fig. 2).

A substantial amount of glycogen is aggregated in the intestinal epithelium but the microvillar border is glycogen free (Fig. 3 and Fig. 4). A negligible amount of acid mucopolysaccharides is evidenced at the tips of microvilli (Fig. 8).

In the intestinal region the basal lamina, intestinal epithelium and nuclei reveal a higher concentration of general proteins than the microvillar border. The intestinal contents are also proteinaceous (Fig. 5 and Fig. 6). Proteins with $-NH_2$ group are intensely concentrated in microvillar border and epithelial nuclei than in the cytoplasmic region of the epithelium as evidenced by Ninhydrin Schiff's staining (Fig. 7). In the intestinal region of digestive system, the concentration of proteins in the epithelium is also accompanied by presence of nucleic acids (Fig. 9 and 10). A rich quantity of lipid is seen at the site of terminal web as well as the epithelial cytoplasmic area. Some lipoidal concentration is also observed at the tips of microvilli probably of secretary nature (Fig.11). The rectal glands too are lipoidal in nature (Fig. 12).

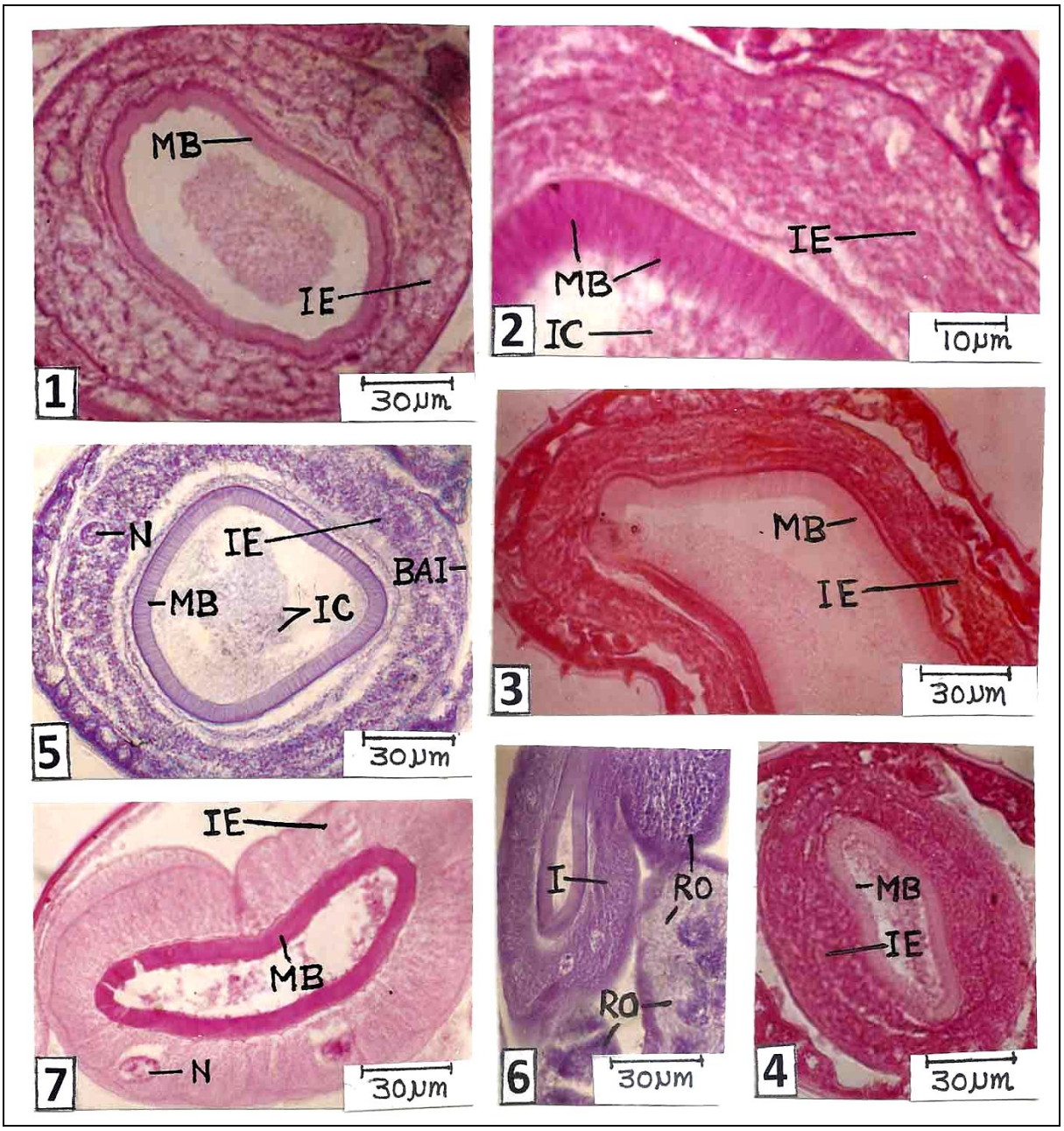


Fig. 1-7: *Haemonchus contortus*

Fig. 1 and 2 : T. S. of intestine showing concentration of general carbohydrates in the intestinal epithelium and microvilli (Periodic acid Schiff's staining); **Fig. 3 and 4 :** T. S. of intestine showing distribution of glycogen (Best's carmine staining); **Fig. 5 :** T. S. of intestine revealing distribution of proteins (Mercuric bromophenol blue staining); **Fig. 6 :** A portion of T. S showing close approximation of intestine and reproductive organs (Mercuric bromophenol blue staining); **Fig. 7:** T. S. of intestine showing distribution of $-NH_2$ proteins (Ninhydrin Schiff's staining).

Abbreviations used: BAI: Basal Lamina of Intestine; I: Intestine; IC: Intestinal Contents; IE: Intestinal Epithelium; MB: Microvillar Border; N: Nucleus; RO: Reproductive Organs.

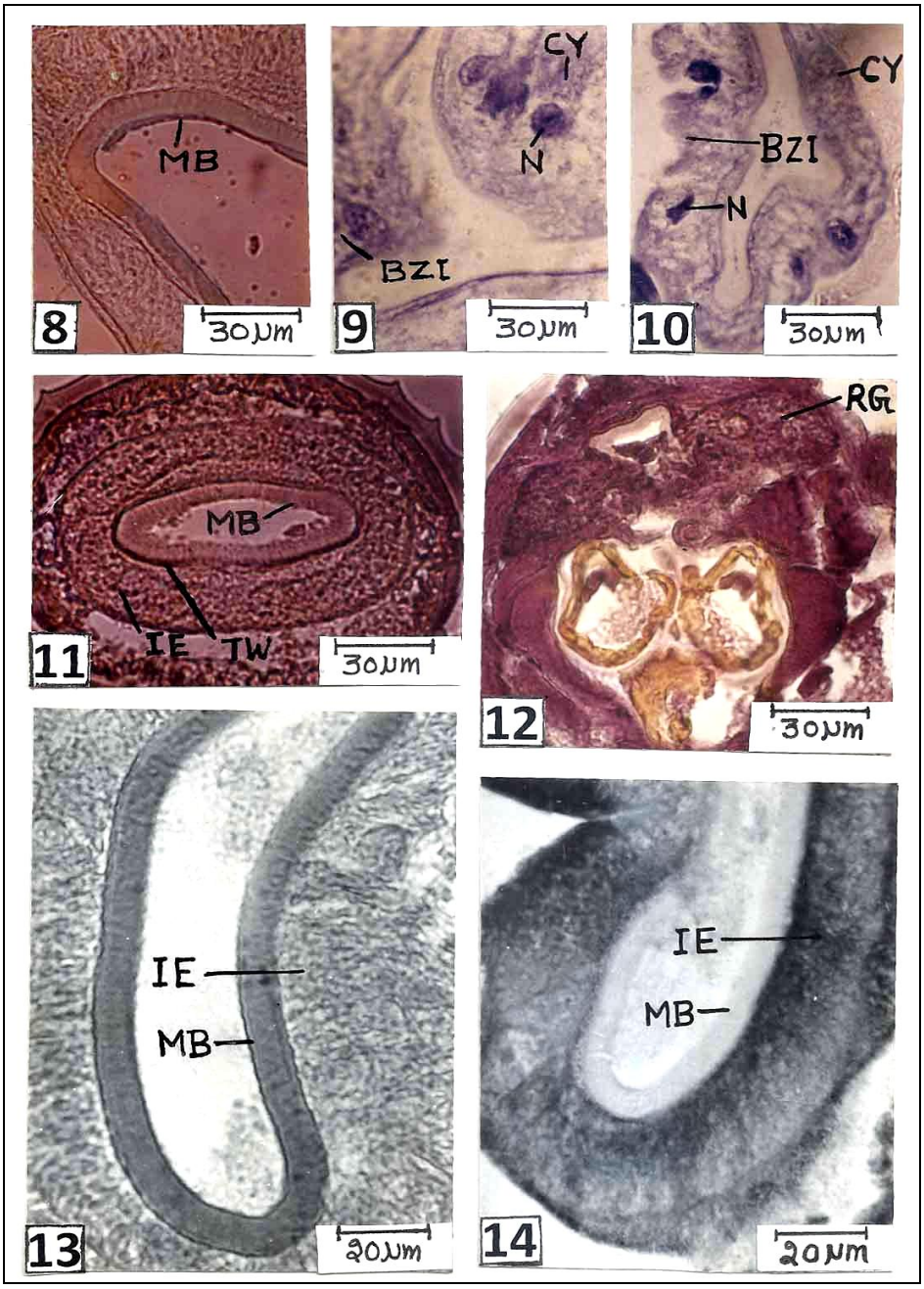


Fig. 8-14 : ***Haemonchus contortus***

Fig. 8: T. S. of intestine showing localization of acid mucopolysaccharides at the tips of microvilli (Alcian blue staining); **Fig. 9 and 10**: A portion of L.S. showing concentration of nucleic acids in the intestine (Gallocyanin chromalum staining); **Fig. 11:** T. S. showing concentration of lipids in the intestine (Sudan black B staining); **Fig. 12:** A portion of T. S. through the cloacal region of male showing distribution of lipids in the rectal glands (Sudan black B staining). **Fig. 13:** A portion of T. S. through the intestine showing presence of acid phosphatase activity in the intestinal epithelium and microvillar border (Acid phosphatase paraffin section technique). **Fig. 14:** A portion of T. S. through the intestine revealing alkaline phosphatase activity in the intestinal epithelium and negative stain for microvillar border (Modified Gomori method).

Abbreviations used: BZI: Basal Zone of Intestinal Epithelium; CY: Cytoplasm; IE: Intestinal Epithelium; MB: Microvillar Border; N: Nucleus; TW: Terminal Web; RG: Rectal glands.

Intense acid phosphatase activity is found in the microvillar border and to some extent in intestinal epithelium (Fig. 13). A substantial amount of alkaline phosphatase is found in the intestinal epithelium, whereas microvillar border is devoid of it (Fig. 14).

Generally, a glycogenous epithelium is the common feature of parasitic nematodes as reported by Von Kemnitz (1912), Enigk (1938) and Von Brand (1938) in different species but the concentration of glycogen content varies depending upon the feeding habit of the various nematodes. In *Haemonchus contortus,* the intestine contains carbohydrates as one of the main constituent. The intestinal epithelium possesses carbohydrates in a diffused form in the cytoplasm and a profuse concentration of glycogen is observed in the intestinal epithelium. Tanaka (1961) while working on *Ascaris lumbricoides* denied the presence of glycogen from the intestinal epithelium. In *Thelastoma bulhoesi,* Lee (1960) described an uneven distribution of glycogen in the intestinal epithelium, being more in the mid region as compared to the anterior and posterior regions. In *Nippostrongylus brasiliensis, Paranisakis kherai, Setaria cervi, Trichinella spiralis* and *Tanqua anomala* an adequate amount of glycogen was observed in the intestinal epithelium by Jamuar (1966), Gupta and Garg (1976), Gupta and Kalia (1978), Takahashi *et al*. (1988) and Kankal (1989) respectively. In the present study on *H. contortus* an intense concentration of glycogen is seen and it is evenly distributed throughout the length of the intestine.

Protein deposits in the form of secretory granules and ribosomes have been reported in the cytoplasm of intestinal cells of *Nippostrongylus brasiliensis* (Jamuar, 1966). In *Paranisakis kherai,* Gupta and Garg (1976) found that the intestinal epithelium contains a moderate quantity of proteins and the chromatin present in the cells is also mercuric bromophenol blue positive. A similar report was given by Gupta and Kalia (1978) for *Setaria cervi.* Proteins bound by both –NH_2 and –SH groups have been detected in the intestinal masses of *Meloidogyne incognita* (Marwah and Khera, 1987). In *Trichuris ovis* and *Oesophagostomum columbianum* appreciable quantities of protein is located in the intestinal epithelium (Johal *et al.* 1997, Johal and Singh 1998). In the present study on *Haemonchus contortus* the intestinal epithelium exhibits a considerable quantity of general proteins as evidenced by Mercuric bromophenol blue technique. In addition, the epithelial nuclei are positive for both general as well as –NH_2 group containing proteins.

Besides these metabolites, some nucleic acid activity is also observed in the intestinal epithelium. Jamuar (1996) has reported that the distribution of RNA in the cytoplasm of intestinal epithelium of *Nippostrongylus brasiliensis* is an indication of protein synthesis at this place. In *Setaria cervi* the basal portion of the intestinal epithelium is rich in RNA content (Gupta and Kalia, 1978). Moderate amount of RNA is also detected in the intestinal epithelium of *Trichuris suis* and *Diplotriaena tricuspis* by Jenkins (1973) and Wajihullah and Ansari (1981) respectively. In *Ancylostoma caninum*, where the presence of proteins is accompanied by RNA activity, Browne *et al.* (1965) have suggested that the cytoplasm of intestinal epithelium acts as a right place for the synthesis of proteins. Von Brand (1952) maintains that the proteins present in the intestinal cells are utilized as a metabolite for the production of energy as well as renewal of protoplasm. In *H. contortus,* the presence of protein and RNA activity indicates that the intestinal protein synthesis pool also distributes a large quantity of protein to the other body organs such as gonads and muscle cells of the body wall, in the latter large amounts of proteins are localized but no nucleic acid activity is evident thus suggesting their dependence for protein on the intestine. It can be inferred that the intestine of *H. contortus* does not only act as a place for transportation of the absorbed material but also a tissue of considerable synthetic activity.

Chitwood and Chitwood (1950) have described that in the intestine of *Cephalobellus papilliger,* the chief constituent of stored food is the lipid. A host of workers such as Lee (1960), Dimitrova (1962), Anya (1964), Jamuar (1966), Reznik (1971), Kankal (1989), Johal *et al.* (1997) and Johal and Singh (1998) have recorded the presence of lipid in the form of granules or fat droplets in the intestinal epithelium of various nematodes. In the present study on *Haemonchus contortus,* appreciable quantities of lipids are found in the intestinal epithelium. The major bulk of lipid seem to be meant for the consumption of reproductive organs lying in close proximity with the intestine, which reveal an enormous quantity of cytoplasmic as well as structural lipid contents. The secretion of some lipoidal enzymes is also indicated in the microvilli. Consequently, *H. contortus* accounts for a tremendous lipoidal activity in it.

The chemical nature of the microvillus border has drawn the attention of many workers for the reason that firstly, like cuticle the microvilli form an interface with the host and secondly, they have a variety of functions. Presence of polysaccharides with 1:2 glycol group and glycogen is reported in the bacillary layer of *Setaria cervi* by Gupta and Kalia (1978). Microvillar border of intestine of *Trichuris ovis* also reveals a tremendous amount of carbohydrate concentration (Johal *et al.*, 1997). In *Haemonchus contortus* too, the microvillar border shows an appreciable quantity of carbohydrates, whereas it is totally free from glycogen. The intestinal contents are also positive for carbohydrates. This indicates that the microvilli helps in the absorption of simple carbohydrates, which in turn are converted to and stored in the form of glycogen in the intestinal epithelium.

The presence of mucopolysaccarides which form a chemical barrier has been reported from the intestinal epithelium and bacillary layer by Gupta and Garg (1976), Wajihullah and Ansari (1981), Marwah and Khera (1987) and Johal and Singh (1998) in *Paranisakis kherai, Diplotriaena tricuspis*, *Meloidogyne incognita* and *Oesophagostomum columbianum* respectively. In present study on *Haemonchus contortus,* very minute quantities of acid mucopolysaccharides are detected at the tips of microvilli. As *Haemonchus* is a blood sucker so its intestine is not exposed to host's gut enzymes.

In *Paranisakis kherai,* Gupta and Garg (1976) reported that the bacillary layer is metabolically rich in general as well as –SH bound proteins. Johal *et al*. (1997) and Johal and Singh (1998) described that the protein forms the main constituent of the microvillar border of the intestine of *Trichuris ovis* and *Oesophagostomum columbianum* respectively. The microvillar border of *H.contortus* too, reveals an intense concentration of $-NH_2$ bound proteins. Considerable amounts of lipids are detected in the microvilli of intestine by a number of previous authors (Gupta and Kalia, 1978; Johal *et al.,* 1997 and Johal and Singh, 1998). In the present study on *H. contortus,* some lipoidal concentration is found at the tips of microvilli which leads to the assumption that some enzyme of lipoidal nature is being released from the microvilli into the lumen of the intestine. A poorly developed terminal web, a cytoplasmic area formed by the fusion of the cores of microvilli at their bases, is observed. The degree of development of terminal web can be related to the kind of fluid which the parasite ingests. Body fluids probably are in a state of assimilation and require less enzymatic activity for their digestion, hence a poorly developed terminal web in *H. contortus.* However a rich quantity of lipid is seen at this site indicating that some enzyme of lipoidal nature is synthesized here.

A highly positive reaction for the activity of acid phosphatase in the intestine of a number of parasitic nematodes is detected by Maki and Yanagisawa (1980a & b). The presence of various hydrolytic enzymes from the intestine of *Trichuris suis, Tetrameres fissispina* and *Haemonchus contortus* is reported by Jenkins (1970), Riley(1973) and Sood and Sehajpal (1978)

respectively. In the present study on *Haemonchus contortus*, both acid as well as alkaline phosphatase activity is seen in the intestinal epithelium. The microvillar border is intensely stained for acid phosphatase activity only, whereas it is negative for alkaline phosphatase.

A characteristic feature of *H. contortus* is that its reproductive organs lie coiled around the intestine and outer wall of the intestine gives an irregular appearance (Singh and Johal, 2004). The uplifting of the basement membrane of the intestine to make a close contact with the reproductive organs indicates the trans-membrane flow of nutrients from the former to the latter. The basal membrane of the intestinal cells probably plays an important role in the rapid uptake kinetics from the intestinal epithelium to the enormous number of developing gametes lying in its vicinity.

REFERENCES

Anderson RC (2000) Nematode Parasites of Vertebrates, 625pp, CABI Publishing.

Anya AO (1964) The distribution of lipids and glycogen in some female oxyurids. *Parasitology,* 54 : 555-566.

Best F (1906) *Z. Wiss. Mirk.* 23 : 319. Cited from : Histochemistry, Theoretical and Applied. A.G. E. Pearse (ed.), J and Churchill, London (1968).

Bird AF (1971) *The Structure of Nematodes*, Academic Press, New York and London. 318pp.

Bonhag PF (1955) *J. Morphol.* 96 : 381. Cited from : The Structure of Nematodes, A. F. Bird (1971), Academic Press, New York and London.

Browne HG, Chowdhary AB and Lipscomb L (1965) Further studies on the ultrastructure and histochemistry of the intestinal wall of *Ancylostoma duodenale*. *J. Parasitol.,* 51: 389-391.

Chitwood BG and Chitwood MB (1950) *An Introduction to Nematology*. University Park Press, Baltimore, Maryland, 334pp.

Dimitrova E (1962) Accumulation and distribution of lipid in *Ascaris suum*. *Izvestiyana Tsentralanta Khelmintogikhna Laboratoriya.,* 15(3): 258-290.

Einarson L (1951) *Acta Pathol. Microbiol. Scand.,* 28: 82. Cited from: The Structure of Nematodes, A. F. Bird (1971), Academic Press, New York and London.

Enigk K (1938) Ein Beitrag zur physologie und zum wirt-Parasitenvehaltnis von *Graphilium strigosum* (Trichostrongylidae, Nematoda). *Z. Parasitenkd.,* 10: 386-414.

Friedricsson B (1956) *Acta Anat.*, 26: 246

Gupta NK and Garg VK (1976) On the taxonomical and histochemical observations on a new nematode of the genus *Paranisakis* Baylis, 1923. *Rivista Di Parassit.*, 37 (2-3): 265-275.

Gupta NK and Kalia DC (1978) Histochemical studies on *Setaria cervi*, a filarioid nematode of veterinary importance, *Rivista Di Parassit.*, 39(1): 55-70.

Johal M, Shivali and Singh J (1997) A study on the midgut of *Trichuris ovis. Current Nematology*, 8(1, 2): 61-66.

Johal M and Singh J (1998) Histochemical study on the intestinal epithelium of *Oesophagostomum columbianum. Journal of Parasitology and Applied Animal Biology,* 7(1): 51-57.

Jamuar MP (1966) Cytochemical and electron microscopic studies on the pharynx and intestinal epithelium of *Nippostrongylus brasiliensis. J. Parasitol.,* 52: 209-232.

Jenkins T (1970) A morphological and histochemical study of *Trichuris suis* (Schrank, 1788) with special reference to the host parasite relationship. *Parasitology*, 61: 357-374.

Jenkins T (1973) Histochemical and fine structure observations of the intestinal epithelium of *Trichuris suis* (Nematoda: Trichuroidea). *Z Parasitenkd*., 42(3): 165-183.

Kankal NC (1989) Histochemistry of *Tanqua anomala*, a nematode parasite of water snake *Tropidonatus piscator*. Rivista Di Parassit., 4(48)(2): 175-184.

Kurnick NB (1955) Stain technology, 30: 213-230. Cited from: *Histological and Histochemical methods,* J. A. Kiernan (ed.), Pergamon Press (1981), Oxford.

Lee DL (1960) The distribution of glycogen and fat in *Thelastoma bulhoesi*, a nematode parasitic in cockroaches. *Parasitology*, 50: 247-259.

Lillie RD and Ashburn IL (1943) Supersaturated solution of fat stains in dilute isopropanol for demonstration of acute fatty degeneration not shown by Herztieimer technique. *Arch. Pathol*., 36: 432.

Maki J and Yanagisawa T (1980a) A comparison of the sites of acid phosphatase activity in an adult filaria, *Setaria* sp. And in some gastrointestinal nematodes. *Parasitology*, 81(3): 603-608.

Maki J and Yanagisawa T (1980b) Acid phosphatase activity demonstrated in the nematodes, *Dirofilaria immitis* and *Angiostrongylus cantonensis* with special reference to the characters and distribution. *Parasitology*, 80: 23-38.

Marwah R and Khera S (1987) Histochemical localization of protein, carbohydrate and lipids in females of *Meloidogyne incognita*. *Rivista Di Parassit*., 46(3): 313-322.

McManus JFA (1948) Cited from: *The Structure of Nematodes*, A. F. Bird, Academic Press, New York and London.

McManus JPA (1946) In: *Histochemistry: Theoretical and Applied*. A.G. Pearse (ed.), J.A. Churchill Ltd., London.

Pawlowski ZS (1987) Intestinal helminthiasis and human health: recent advances and future needs. *International Journal for Parasitology*, 17(1): 159-168.

Reznik GK (1971) Distribution of non-specific carboxylic esterases and fat in the musculo-cutaneous sac and the mid intestine of *Ascaridia galli. Isdatel 'Stvo "Kolos*", Vigis, Moscow, pp 317-321.

Riley J (1973) Histochemical and ultrastructural observations on digestion in *Tetrameres fissispina. International Journal for Parasitology,* 3 (2): 157-164.

Ruyter JHC (1964) *Histochemic*, 3: 52.

Singh, J and Johal, M (2004) Histological study on the intestine of *Haemonchus contortus* (Rud., 1803) *Journal of Parasitology and Applied Animal Biology*, 13 (1, 2): 13-24.

Sood ML (2006) Histochemical, biochemical and immunological studies in *Haemonchus contortus* (Nematoda: Trichostrongyloidea)- an Indian Perspective. *Journal of Parasitic Diseases,* 30(1): 4-15.

Sood ML and Sehajpal K (1978) Morphological, histochemical and biochemical study on the gut of *Haemonchus contortus* (Rud., 1802). *Z. Parasitenkd.,* 56: 267-273.

Steedman HF (1950) Alcian blue 8 G.S.: a new stain for mucin. *Quart. J. Micro.Sci*., 91: 477.

Takahashi Y, Uno T, Furuki J, Yamada S and Araki, T (1988) The morphology of *Trichinella spiralis*: ultrastructural study of the midgut and hindgut of the muscle larvae. *Parasitol. Res.,* 75: 19-27.

Tanaka Y (1961) Histochemical study on tissue of *Ascaris lumbricoides* with special reference to direstive organs. *J. Tokyo Medical College,* 19 (2): 1499-1570.

Von Brand T (1938) Physiological observations on a larval Eustrongylid VII, Influence of respiratory poison uypon the aerobic gaseous metabolism. *Journal of Parasitology,* 31: 381-393.

Von Brand T (1952) *Cytochemical Physiology of endoparasitic animals.* Academic Press, New York and London. 272 pp.

Von Kemnitz G (1912) Die Morphologie des. Stoffwechsels bei *Ascaris lumbricoides. Arch. Zell forsch., 7: 463-603.*

Wajihullah D and Ansari JA (1981) Histochemical studies on *Diplotriaena tricuspis* (Nematoda: Diplotriaenidae). *Indian Journal of Helminthology,* 33(2): 95-98.

Yasuma A and Itchikawa T (1953) J. Lab. Clin. Med., 41: 296, *Histochemical Techniques*, Bancroft, J.D. (1975).

RESEARCH ARTICLE

Production of silver nanoparticles synthesis of *Couroupita guianensis* plant extract against human pathogen and evaluations of antioxidant properties

Sivakumar T*, Rathimeena T, Shankar and Ponmanickam P

Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi-626124, Tamilnadu, India.
*Corresponding author E-mail: sivasadhana@yahoo.co.in

Manuscript details:

Received: 03.11.2015
Accepted: 10.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Sivakumar T, Rathimeena T, Shankar and Ponmanickam P (2015) Production of silver nanoparticles synthesis of *Couroupita guianensis* plant extract against human pathogen and evaluations of antioxidant properties, *International J. of Life Sciences*, 3(4): 333-340.

Acknowledgements:
The facilities provided by the Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamilnadu are gratefully acknowledged.

ABSTRACT

Couroupita guianensis, Silver nanoparticles synthesis of Plant extract were confirmed by UV-vis and FTIR. Further it was tested against various pathogenic microorganism and followed by antioxidant properties. The *Couropita guinanensis* flower extract mediated nanoparticles showed absorbance peaks at 318--323nm region in the spectral analysis.Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1631.95 cm−1. At concentration of plant extract with silver nanoparticles. The (12 mm) clear inhibitory zone appeared around 100µl against *Pseudomonas aeroginosa* MTCC 2453after incubation for 24h followed by *Bacillus subtilis* MTCC121 (23 mm), *Staphylococcus aureus*MTCC96 (13 mm), *Klebsiella pneumonia* MTCC109 (15 mm) and *E.coli* MTCC 912 (20 mm).In *Couroupita guianensis* total antioxidant was found to increase with increase in concentration in standard, plant extract and $AgNO_3$.

Keywords: *Couroupita guianensis,* FTIR, UV and Antioxidant.

INTRODUCTION

The new field of nanotechnology has become a major thrust in scientific research. It has adapted itself to various field of science and technology including physics, chemistry, etc. It is expanding and continues to change the way we perceive and execute things and has a pronounced effect on therapeutics and shaping the ever evolving society and influencing our daily lives (Chakraborty *et al*., 2011). These nanoparticles due to their targeted action increase the efficacy of the drug. Their small size gives them an edge while evading the immune responses and also gives them the ability to cross relatively impermeable membranes (Uchegbu and Schatzlein, 2012).

Nanoparticles result in significantly low toxicity on adoption of this technique, it can be used for encapsulation of drug molecules. Further research in the field of nanomedicine with respect to AgNPs is going on worldwide. Bacterial strains, both gram positive and gram negative, have been employed in the non-enzymatic production of AgNPs through the interaction of silver ions with the organic compounds present on the bacterial cells. For example, *Lactobacillus*, *Enterococcus*, *Pediococcus pentosaceus* and *Enterococcus faecium* reduce silver ions in alkaline conditions (Ahmad *et al*. 2013). AgNPs synthesized by *Plectonema boryanum* precipitates spherical AgNPs of size 200 nm. *Bacillus subtilis* yields AgNPs of 5–60nm on microwave irradiation. Bio-reduced diamine silver complexes of *Corynebacterium* strain, SH09, results in silver nanoparticles of size ranging between 10 and 15 nm (Bhattacharjee *et al*. 2005). *Spirulina platensis* is also used for the extra cellular synthesis of nanoparticles. AgNPs of size7–16nm and gold nanoparticles of size 6–10nm are obtained at optimum conditions, i.e.37 ◦C, and 120h and pH5.6 (Sintubin *et al*. 2009).

Fungi have been immensely used for the green synthesis of nanoparticles. AgNPs are known to be excellent antimicrobial and anti-inflammatory agents and are thus used to enhance wound healing. Compared to bacteria, fungi have been known to secrete much higher amounts of bioactive substances and so fungi are considered more suitable for large-scale production. *Fusarium oxysporum* synthesizes bioactive substance extracellularly by reducing silver nitrate. The process includes stabilization of AgNPs in a solution with the help of protein secreted by the fungal strain and the metal ions produced are reduced by nitrate-depend reductase and quinines huttle.

The AgNPs thus produced are tested for their bactericidal effect against *S.aureus* on cotton and silk cloth (Fu *et al*. 2006). Algae are employed for the synthesis of nanoparticles which reduces the Ag+ ions by means of proteins released by them and these proteins reduce the nanoparticles and help in maintaining AgNP's stability. In *Chorella vulgaris*, the proteins in the extract have dual function of Ag+ ion reduction, and shape controlled synthesis of NPs. The Ag nano plates are obtained at room temperature. Reduction of Ag+ ions is done by the hydroxyl groups in Tyr residues and carboxyl groups in Asp/Glu residues. It is responsible for the isotropic growth of Ag nano plates which yields rod-like particles with a mean length of 44nm and width of 16–24nm. By way of this background we are reported that the edifice of silver nanoparticles synthesis of *Couroupita guianensis* plant extract effective against human pathogen and its antioxidant properties.

MATERIALS AND METHODS

Collection of Plant Materials

Couroupita guianensis are evergreen trees, native to tropical northern South America, southern Caribbean and also India. Its flowers are orange, scarlet and pink in colour, and form large bunches. Floral parts of *C. guianensis* was collected from Sorimuthu Ayyanar Koil , Pabanasam, Thirunelveli District, Tamil Nadu, India (8º 39' N and 77 º 20' E) with elevation of 1500 m above sea level.

Plant Extract Preparation

The fruit pulp (white in color which converts into blue to brown within minutes) was collected for the synthesis of nanoparticles (Torresdey, 2003).

Preparation of 1mM Aqueous Solution of Silver Nitrate

0.017gm of Silver Nitrate ($AgNO_3$) was added to the 100 ml of distilled water and the solution was stirred well continuously until the silver nitrate is dissolved. This 1mM Silver Nitrate solution stored in brown bottle at 4° C for further use for the synthesis of Silver Nanoparticles from *Couroupita guianensis.*

Synthesis of AgNPs

1mM aqueous solution of silver nitrate (Himedia, Mumbai) was prepared for synthesis of silver nanoparticles. For the synthesis of AgNPs, two boiling tubes were taken, one containing 10ml of 1Mm AgNO3 solution as control and the second containing 9ml of 1mM silver nitrate solution and 1ml of plant leaf extract as test solution. These were incubated at room temperature for 1-2 hours. The color change of the leaf extracts from pale yellow to dark brown was checked periodically. The silver nanoparticles were confirmed by color changes and qualitatively characterized by UV-Visible spectrophotometer.

Characterization of silver nanoparticles

a) UV-visible spectroscopy:

Synthesis of silver nanoparticles by reducing, the respective metal ion solution with leaves extract may be easily observed by UV- Vis spectroscopy.

b) FT-IR chemical analysis:

The interaction of Ag-NPs obtained with PEG and gluconic acid products by reduction of sugar compound were confirmed by FT-IR spectra.

c) Antibacterial Activity:

The synthesized AgNPs was evaluated against human pathogenic bacteria (*Pseudomonas aeroginosa* MTCC 2453, *Bacillus subtilis*MTCC121, *Staphylococcus aureus*MTCC96, *Klebsiella pneumonia*MTCC109 and *E.coli* MTCC 912) using the agar well diffusion method (Perez *et al*. 1990).

d) Determination of total antioxidant capacity (TAC):

Total antioxidant activity of sulfated polysaccharides from seaweeds will be determined according to the method (Prieto *et al*. 1999).

e) DPPH radical scavenging assay:

The free radical scavenging activity of sulfated polysaccharides from seaweeds were measured by the 1-1-Diphenyl-2-picryl-hydrazyl (DPPH) following method (Blois, 1958).

f) Hydroxyl radical scavenging assay:

Hydroxyl radical scavenging activity will be measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}- Ascorbate EDTA H_2O_2 system. The free radical damage imposed on the substrate, deoxyribose (TBARS) will be measured by the method (Yamaguchi *et al*. 2006).

RESULTS

The *Couropita guinanensis* flower extract mediated nanoparticles showed absorbance peaks at 318--323nm region in the spectral analysis shown in Fig.1.The peaks were stable with time duration also.It indicates that the synthesis of silver nano particles reqires the reduction of α-NADPH to α- $NADP^+$ and the hydroxy quinoline probably acts as electron shuttle tranforming the electron generated during the reduction of nitrate to Ag^+ ions translating them to Ag^0

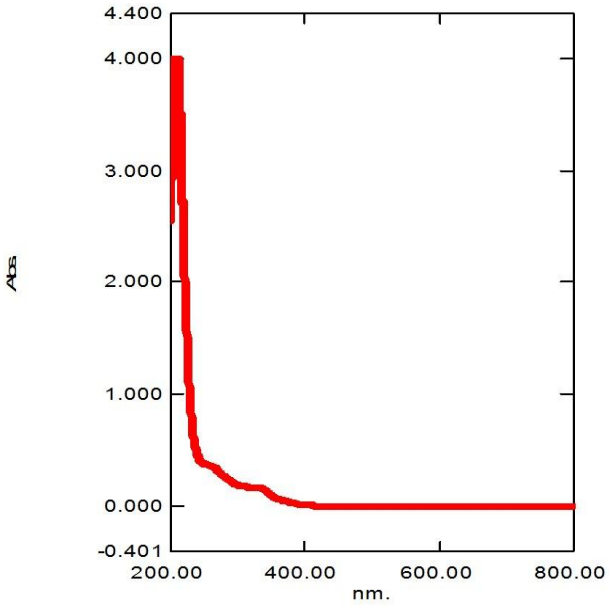


Fig.1: UV-spectrophotometer for $AgNo_3$ Synthesis of *Couroupita guianensis*

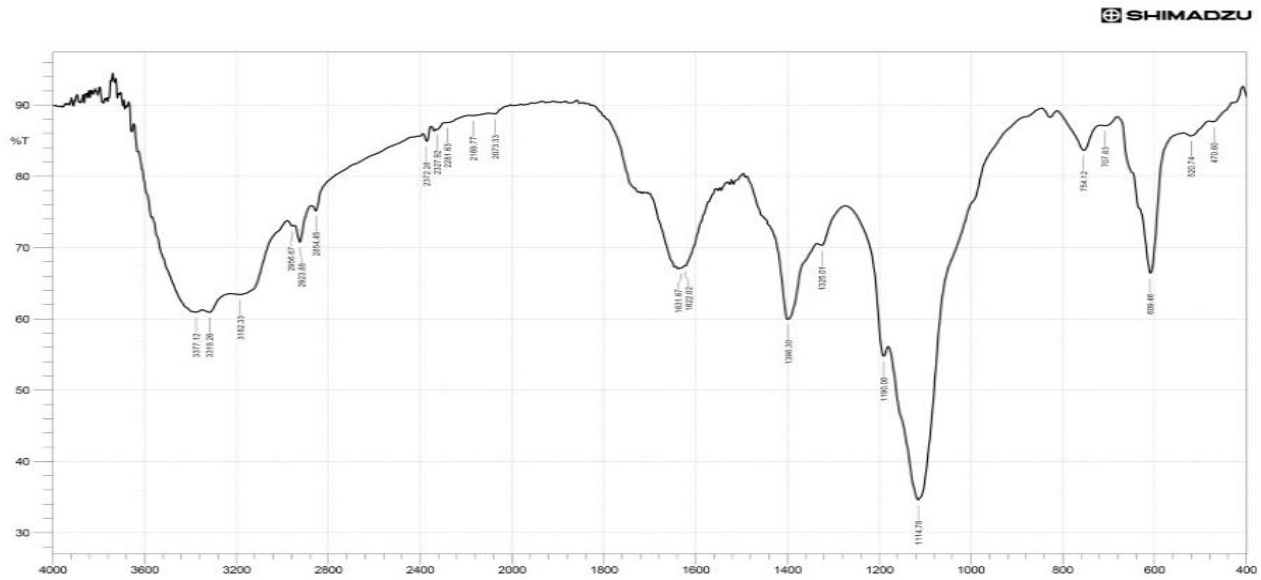


Fig.2: FTIR analysis for $AgNo_3$ synthesis of *Couroupita guianensis*

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1631.95 cm−1 (Fig.2). The stretching vibration of C=C obtained at 1622 and the single absorbance peak located at 1114 cm−1 is assigned to C-O Polyols Further distinct peaks in the region of 2372.28 cm−1 correspond to C≡N stretch for nitrile groups, while 3377.12 and 3319.26 cm−1 corresponds to O-H and N-H stretching vibration. The aromatic C-H stretching vibrations obtained at 2956.67 and 2923.88 cm−1 respectively.

ANTIBACTERIAL STUDIES

The antimicrobial activity of silver nanoparticles *Couroupita guianensis* against various pathogenic organisms including bacteria and yeast was investigated. Compared with the control, the diameters of inhibition zones increased for all the test pathogens. At concentration of plant extract with silver nanoparticles. The (12 mm) clear inhibitory zone appeared around 100µl against *Pseudomonas aeroginosa* MTCC 2453after incubation for 24h followed by *Bacillus subtilis* MTCC121(23mm), *Staphylococcus aureus*MTCC96 (13 mm), *Klebsiella pneumonia* MTCC109 (15 mm) and *E.coli* MTCC 912 (20 mm).(Fig.3. and Table.1)

Total antioxidant properties

Free radical scavenging activity of the silver nanoparticles was assessed by DPPH solution exhibited a deep purple colour with a maximum absorbance at 517nm. The disappearance of purple colour on adding synthesized silver nanoparticles might due to presence of antioxidant in the medium.

In *Couroupita guianensis* total antioxidant was found to increase with increase in concentration in standard, plant extract and $AgNO_3$. $AgNO_3$ showing a minimum activity at 19% at 100µg/ml and maximum activity observed 40% at 500µg/ml followed by the plant extract showing a minimum activity at 10% at 100µg/ml and maximum activity observed 60% at 500µg/ml(Fig.4).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of *Couroupita guianensis flower extract of* silver nanoparticles exhibit higher activity at 15% in600µg/ml, likewise lower activity observed at 200 µg/ml with 3%followed by flower extract showing minimum activity at 200 µg/ml with 10% and also maximum activity observed at 600 µg/ml with 35%(Fig.5).

Table.1: Mean zone of inhibition of synthesized silver nanoparticles from *Couroupita guianensis*

Pathogens	Zone of inhibition (mm)			
	Water	Plant extract	$AgNO_3$	Plant extract with $AgNO_3$
Pseudomonas aeruginosa MTCC 2453	-	-	9	12
Bacillus subtilis MTCC121	-	-	11	23
*Staphylococcus aureus*MTCC96	-	-	7	13
Klebisella pneumoniae MTCC109	-	-	5	15
*E.coli*MTCC912	-	12	-	20

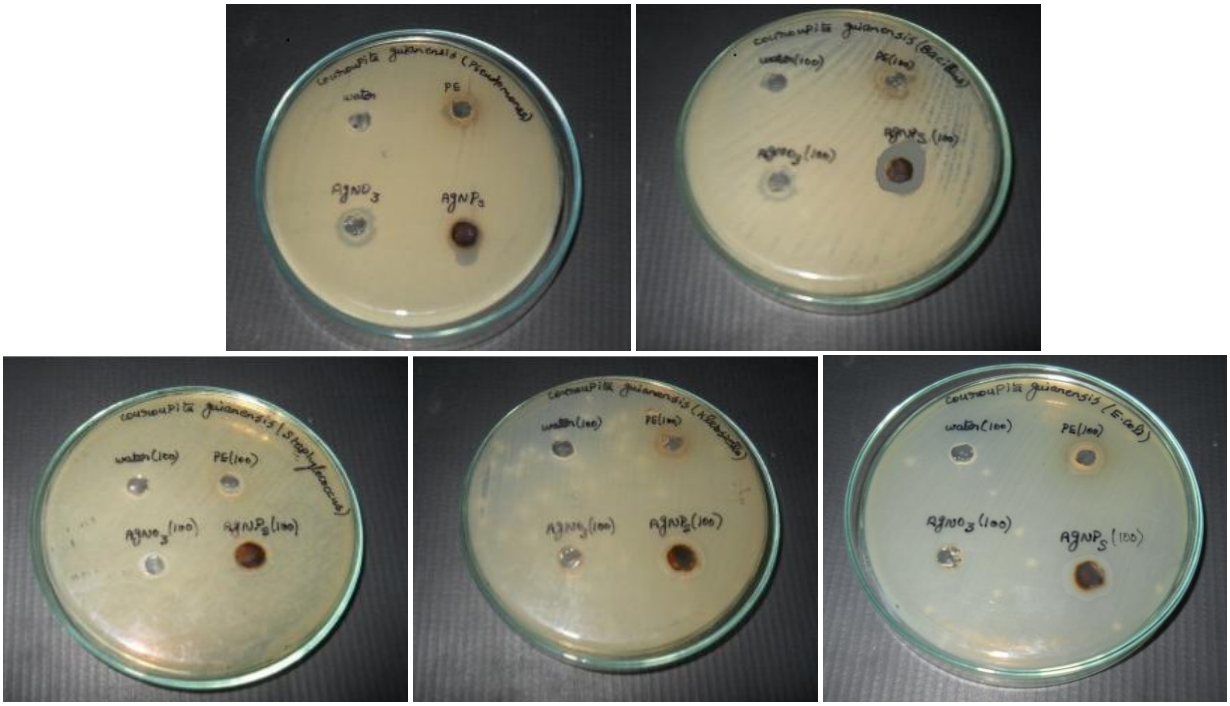


Fig.3: Activity of silver nanoparticles against different microorganisms depicting zones of inhibition of (**A**) Water control. (**B**) Plant extract-positive control (**C**) Silver nanoparticle positive control **(D)**Silver nano particle mediated plant extract synthesis at 100µg.

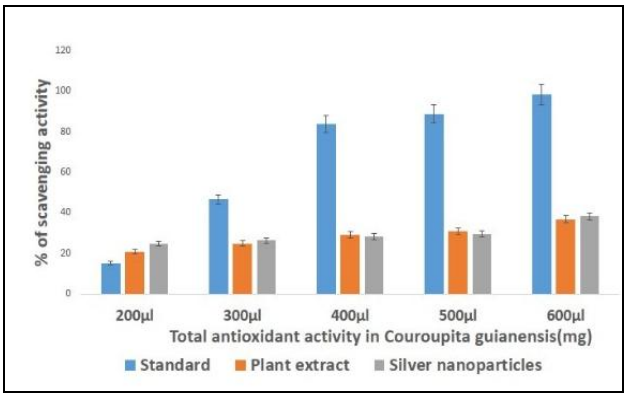


Fig. 4: Total antioxidant activity of *Couroupita guianensis.*

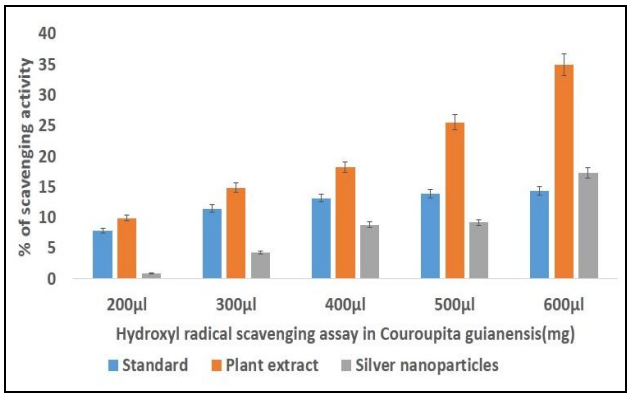


Fig.5: Hydroxyl radical scavenging assay in *Couroupita guianensis*

Hydrogen peroxide scavenging activity

In *Couroupita guianensis* $AgNO_3$ showing a minimum activity at 4% at 200µg/ml and maximum activity observed 9% at 500µg/ml followed by the plant extract showing a minimum activity at 37% at 200µg/ml and maximum activity observed 60% at 600µg/ml(Fig.6).

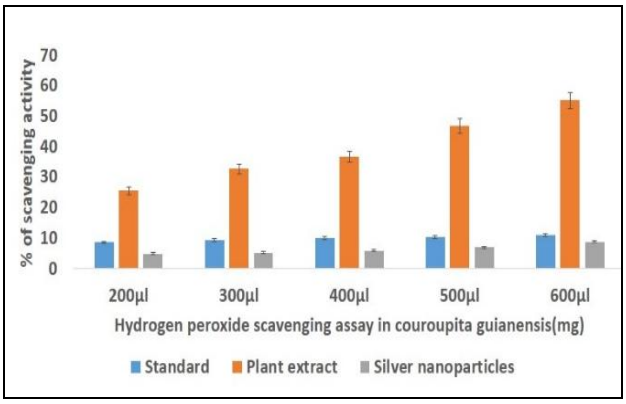


Fig.6. Hydrogen peroxide scavenging assay in *Couroupita guianensis*

DISCUSSION

The present exploration stataed that the *Couropita guinanensis* flower extract, mediated nanoparticles showed the peaks were stable with time duration also.It indicates that the synthesis of silver nano particles reqires the reduction of α-NADPH to α- $NADP^+$ and the hydroxy quinoline probably acts as electron shuttle tranforming the electron generated during the reduction of nitrate to Ag^+ ions convert into Ag^0. This is identical to the characteristics UV-visible spectrum of metallic silver.

Likewise, Bhat *et al*. (2011) stated that the bio reduction was achieved and attributed to the metabolites present in the plant. Synthesis of gold nanoparticles was pronounced when 15 mL of root extract was used for bioreduction, while 10 mL of the same root extract was sufficient for production of silver nanoparticles. Nanoparticles may grow in a process involving rapid bio reduction and strongly influence surface plasmon resonance in the water extract. Similarly it was correlated by *Artemisia nilagirica*, indicating occurrence of a silver band at 340–400 nm followed by Vijayakumar *et al*. (2012).

Couropita guinanensis flower extract mediated nanoparticles determined by the Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1631.95 cm^{-1}. The stretching vibration of C=C obtained at 1622 and the single absorbance peak located at 1114 cm^{-1} is assigned to C-O Polyols and further distinct peaks in the region of 2372.28 cm^{-1} correspond to C≡N stretch for nitrile groups, while 3377.12 and 3319.26 cm^{-1} corresponds to O-H and N-H stretching vibration. The aromatic C-H stretching vibrations obtained at 2956.67 and 2923.88 cm^{-1} respectively. The FTIR spectra of the silver nanoparticles depicted presence of functional groups like C-N, C-O-C, amide linkages and –COO-. These functional were found to play an important role in the capping of nanoparticles and their further stability in aqueous solution.

Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1653.96 cm^{-1} and 1027.44 cm^{-1} in the region of 1000–1800 cm^{-1}. Two absorption peaks located at 1653 cm^{-1} are associated with the stretch vibration of C=C and the single absorbance peak located at 1027 cm^{-1} is assigned to C-N stretching vibrations of amine. Further distinct peaks in the region of 2343.97–2362.27 cm^{-1} correspond to C≡N stretch for nitrile groups, while 3447.86 cm^{-1} corresponds to O-H stretching vibration by Geethalakshmi *et al*. (2012) Therefore, the present study showed same functional groups of silver nanoparticles

In the contemporary study reported that the *Couropita guinanensis*, plant extracts are also effective against selected human pathogenic organisms. In this junction, the antimicrobial activity effect of silver was identified. Hence there are a number of studies in the field of silver nanoparticles by using different type of procedure. *Couropita guinanensis*, silver ion and

silver based compounds are highly toxic to microorganisms showing a strong biocidal effect against microbial species.

These clearly indicate that the enhancement of efficacy was due to the synergistic antibacterial action between antibiotics and silver nanoparticles. Silver nanoparticles facilitate the transport of antibiotics to the cell surface acting as a drug carrier. More recently it is shown that silver chelation prevents unwinding of DNA. Silver nanoparticles are composed of silver atoms. Silver nanoparticles are larger in size than silver ions, which makes them react with more molecules, leading to more antimicrobial activity. In the identical way, the antibacterial effect was more pronounced in Gram-negative bacteria than Gram-positive ones. The antimicrobial activity of colloidal silver particles is influenced by the particle dimensions Silver has long been recognized as having an inhibitory effect on microbes present in medical and industrial processes. The most important application of silver and silver nanoparticles is in medical industry, such as in topical ointments to prevent infection against burns and open wounds by Kaviya *et al*. (2011).

In the present examination, *Couropita guinanensis* was studied for antioxidant properties. DPPH is a stable and well characterized synthetic solid radical for evaluation of antioxidant potential of compounds. The DPPH will be reduced by accepting the hydrogen or electron, the DPPH reducing ability of silver nanoparticles was quantified spectrophotometrically by changing the DPPH color from purple to yellow. Inhibition was found to be high in silver nanoparticles, when compared with gold nanoparticles, which may be due to the facts that silver act as a good oxidant can easily lose electrons. Similar observations with enhanced DPPH scavenging activity by selenium, platinum, silver nanoparticles have been reported by Saikia *et al*. (2010).

In the present analysis *Couropita guinanensis*, shows the superoxide scavenging activity of both the plant extract and AgNPs as determined by the PMS-NBT reduction system. Superoxide (O_2 −) radicals easily react with DNA and protein which necessitate their immediate clearance in living systems. The superoxide radical quenching activity of plant extract and AgNPs was found to be increased with increasing concentrations and the average inhibition was about 40%. Similarly, the superoxides radical inhibition has been reported for platinum and selenium nanoparticles by Ramamurthy *et al*. (2013).The potential superoxide scavenging activity of gold and silver nanoparticles report supported our findings by Pacher *et al*. (2007).

Couropita guinanensis, of H_2O_2 scavenging activity of Plant extract and AgNPs are active in quenching H_2O_2 radicals and the average inhibition was found to be 80 % By the same token, PLAgNPs were as effective as PLFE in quenching H_2O_2 radicals and the average inhibition was found to be 96% as compared to PLFE. In this study, it could be noted that the superoxide radical quenching activity and NO quenching activity of PLAgNPs was 60% and 70% respectively as compared to PLFE which can be explained on the fact that the concentration of phytocompounds responsible for the scavenging activities was higher in the extract than adhered to the nanoparticles. On the other hand, the observed increase in H_2O_2 scavenging activity of PLAgNPs (96%) may be because of the plant condensed tannins present in the extract that are involved in the formation of nanoparticles. Similar observations were made with silver nanoparticles prepared with stem bark of *Shorearox burghii* previously observed by Subramanian *et al* (2013).

CONCLUSION

Silver nanoparticles synthesis of *Couropita guinanensis* exhibited high antioxidant properties.

REFERENCES

Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, and Sastry M (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surface,* 28:313-318.

Bhat R, Deshpande R, Ganachari SV, Huh DS, and Venkataraman A (2011) Photo-irradiated biosynthesis of silver nanoparticles using edible mushroom *Pleurotus florida* and their antibacterial activity studies. *Bioinorganic Chemistry and Applications*, *650979*–650986.

Bhattacharjee RR, Das AK Haldar, D, Si S, Banerjee A and Mandal TK(2005) Peptide assisted synthesis of gold nanoparticles and their self-assembly. *J. Nanosci. Nanotechnol*, 5: 1141-1147.

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature,* 181:1199-1200.

Chakraborty M, Jain S and Rani V (2011) Nanotechnology: emerging tool for diagnostics and therapeutics. *Appl Biochem & Biotechnol,* 165:1178-1187.

Fu M, Li Q, Sun D, He Lu, Deng X, Wang H. and Huang J (2006) Rapid preparation process of silver nanoparticles by bioreduction and their characterizations, *Chin.J.Chem. Eng*, 14:114-117.

Geethalakshmi R., Sarada DVL and Marimuthu P (2012) Evaluation of antimicrobial and antioxidant potentials of *Trianthea decandra* L. *Asian J Biotechnol*, 2:225-231.

Kaviya S, Santhanalakshmi, J, and Viswanathan B (2011) Green synthesis of silver nanoparticles using *Polyalthia longifolia* leaf extract along with D-sorbitol: study of antibacterial activity. *J Nanotechnol*, 1: 1-5.

Pacher P, and Beckman, SJ. Lucas Liaudet (2007) Nitric oxide and peroxy nitrite in health and disease. Physiol. Rev 87:315-424.

Perez C, Pauli M and Bazerque P. (1990). An antibiotic assay by the agar well diffusion method. *Acta Biol. Med. Exp*, 5:113-5.

Prieto PD, Rojas AA and Jordano J (1999) Seed-specific expression Patterns and regulation by AB13 of an unusual late embryo-genesis-abundant gene in sunflower. *Plant Mol Bio*, 39:615-627.

Ramamurthy CH, Padma M, Samadanam IDM, Mareeswaran R, Suyavaran A, Suresh Kumar M, Premkuar K. and Thirunavukkarasu C (2013) The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. *J. Colloid Surface*, 102:808-815.

Saikia JP, Paul S and Samdarshi BK (2010) Nickel oxide nanoparticles: a novel antioxidant. *J. Colloid Surface*, 8:146-151.

Sintubin L, Windt WD, Dick J, Mast J, Ha DVD, Verstraete, W and Boon, N (2009)Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. *Appl Microbial & Biotechnol,* 84(4):741-749.

Subramanian R, Subbramaniyan P and Raj V (2013) Antioxidant activity of the stem bark of *Shorearox burghii* and its silver reducing power. *Springer Plus*, 2:28.

Torresdey JL, Gomez E. and Videa, J (2003) Synthesis of gold nanotriangles and silver nanoparticles using *Alfalfa* sprouts: A natural source for the synthesis of silver nanoparticles. *Langmuir*, 19:1357-1365.

Uchegbu IF and Schatzlein AG (2012) Nanotechnology in drug delivery In: Burger's Medicinal Chemistry, Drug Discovery and Development, seventh ed. Wiley, Hoboken pp 234-243.

Vijayakumar M, Priya K, Nancy FT, Noorlidah A and Ahmed ABA (2012) Biosynthesis, characterization and anti-bacterial effect of plant mediated silver nanoparticles using *Artemisia nilagirica*. *Indus Crops Prod*, 41:235-240.

Yamaguchi H, Yamaguchi M. and Adachi M (2006) Specific-detection of alkaline phosphate activity in individual species of marine phytoplankton. *Plankton Benthos Res*, 14:214-217.

RESEARCH ARTICLE

Thermal power station effluent induced biochemical changes in the blood of freshwater fish, *Labeo rohita*

Deshpande Anant[1*] and Zade Suresh[2]

[1]Department of Zoology, Chintamani College of Science, Pombhurna, Dist. Chandrapur, Gondwana University, Gadchiroli, MS, India
[2]PGTD Department of Zoology, R.T.M. Nagpur University, Nagpur, MS, India
*Corresponding author, Email: anant_sanjay@yahoo.com

Manuscript details:

Received: 08.11.2015
Revised : 28.11.215
Accepted: 17.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Deshpande Anant and Zade Suresh (2015) Thermal power station effluent induced biochemical changes in the blood of freshwater fish, *Labeo rohita*, *International J. of Life Sciences*, 3(4): 341-350.

ABSTRACT

The hazardous effect of heavy metal pollution from the water body on serum biochemistry of the freshwater fish, *Labeo rohita* (Rohu) was studied in this investigation. The aim of this study was to evaluate whether the waste effluent from thermal power station (TPS) were pose any hazardous changes in the blood biochemistry of common carp, *Labeo rohita* (rohu). The heavy metals like As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu were observed in water body adjacent to thermal power station in varying quantities that indicates the presence of heavy metal in water body. The biochemical changes in blood serum monitored through total protein, albumen, creatinine, total cholesterol and enzyme AST and ALT tests, were showed elevation in concentration which might be due to damage of the liver, kidney, and other tissues in the state of stress caused by exposure to metals.

Keywords: Heavy metals, water pollution, TPS, blood biochemistry, rohu.

INTRODUCTION

The main purpose of any industrial development is to provide an opportunity for better living and an employment to the people residing the area. Though industrial development produces more employment it is also responsible for the degradation of the environment by introducing various pollutants into the atmosphere which produces air, water and land pollution. Hence now there is need to protect the environment from these harmful effects at any possible limits. In recent years the energy demand has been increased so rapidly which is being largely met by using fossil fuel. The increasing demand for energy is the one of the

challenges that faces the development of the country (Bashar, 2010). Thermal power plants are the main source of energy production in India where the energy is produced by using coal as a fossil fuel. Coal is largely composed of organic and some inorganic components such as including trace elements which have been cited as possible cause of health and environmental effects. Due to coal combustion a significant quantity and variety of trace elements are transformed into surrounding environment by various pathways.

In natural systems even a low concentration of heavy metals and trace elements can have beneficiary or harmful effect on aquatic biota. During recent years the environment is being contaminated with wide range of pollutants that includes heavy metals, trace metals, pesticides released from various domestic, industrial and other manmade activities, which are having harmful effect on ecological balance of the recipient environment.

Heavy metal contamination has been reported in aquatic organisms (Adham *et al*., 2002 and Olojo *et al*., 2005) and trace metal contaminations are important due to their potential toxicity for the environment and human beings (Gueu *et al*., 2007; Lee *et al*., 2007; Adams *et al*., 2008; Vinodhini and Narayanan, 2008). Heavy metals includes both essential and non essential elements that have a particular significance in ecotoxicology, as they are highly persistent and all have the potential to be toxic to living organisms (Storelli *et al*., 2005).

Major pollutants released by coal based power generation include sulphur, carbon and nitrogen compounds, heavy metals and fly ash. Coal operated thermal power plant can be a source of pollution, because ash derived from burning of coal containing heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) can contaminate water, presenting a potential hazard to the environment (Kanungo and Mahapatra, 2000).

Fly ash is a fine residue resulting from the burning of coal which is discharged into the surrounding environment either by dry or wet method. Chemically fly ash consists of Si, Al, Mg, Ca, K, Ti, and Fe in greater proportion with many trace elements such as V, Mn, Cr, Cu, Ni, As, Pb, Cd, and less quantity of various potential toxic elements. Chemical composition study of fly ash shows mostly the presence of four major elements Al, Si, Fe and Ca in the fly ash. Other metals such as K, Mg, Ba, Co, Cd, Zn, Mo, Pb etc. are present in traces. Though in the traces, compared to original coal, most of the elements are enriched in the fly ash, giving birth to the growing environmental concerns in the disposal and utilization in environment due to release of trace heavy metals.

According to Gupta *et al*. (2002) and Mehra *et al*. (1998) the major part of fly ash is disposed off in unmanaged landfills or lagoons which lead to environmental pollution through fly ash erosion and leachate generation along with metal contamination of surface and ground water resources and hencecan transfer these contaminants into the food chain.

Singh *et al*. (2004), Praharaj *et al*. (2002), Suresh *et al*. (1998) and Ramachandra *et al*. (2012) studied leaching of trace elements in coal ashes from Bokaro Thermal Power Station, Kharagpur, Vijayawada Thermal Power Station (VTPS), Andhra Pradesh and Yellur and surrounding villages closer to a thermal power plant in Udupi district, Karnataka State. They reported that nearly every naturally occurring element is likely to be present in coal and these get entertained in the resultant coal ash.

Chakraborty and Mukherjee (2009) studied the bioaccumulation of heavy metals like Fe, Zn, Cu, Mo, B, Si, Al, Cr, Pb, Cd, Hg and As in aquatic, terrestrial and algal species in the vicinity of thermal power station in fly ash contaminated areas in Uttar Pradesh. Studies of trace elements and the elements presents in fly ash are

distributed into traction of the fly ashes based on volatilization temperature (Bahor *et al*., 1981).

Fish are located at the end of the aquatic food chain and are the inhabitants that cannot escape from the detrimental effects of these pollutants which may accumulate metals and pass them to human beings through food, causing acute and chronic diseases (Al – Yousuf *et al*., 2000; Vosyliene and Jankaite, 2006; and Farombi *et al*., 2007).

Biochemical biomarkers like glucose, total protein, creatinin, total cholesterol and enzymes like AST and ALT are frequently used as an indicator of the general state of health and early warning of stress in fish under stressful conditions (Barnhorn and Van-Vuren 2004; Abou El-Naga *et al*. (2005) and Osman *et al*. 2010). The pollutants interferes in the metabolic pathway and affects carboxyl, amino, sulphhydryl, phosphate and other groups of the molecules which result in the damage of enzyme systems by blocking active sites, immobilization of essential metabolites, modification of membrane structure and its permeability (Martinez-Porchas *et al*., 2011).

MATERIALS AND METHODS

A. Study site:

This study was conducted at pond in the vicinity of thermal power station (TPS) located at Koradi village of Dist. Nagpur during 2010 to 2012.

B. Collection of water sample for heavy metal analysis:

The water samples were collected from the pond of TPS for the heavy metal analysis and were further processed as, 5 ml of concentrated HNO_3 was added to a 50 ml of water sample to digest all the organic matter and to get the clear solution. The digested and cleared water samples were filtered using Whatman filter paper and made upto original 50 ml volume and injected into Inductively Coupled Plasma Atomic Emission Spectrometer (ICP - AES) for metal estimation.

C. Sampling and collection of the fishes:

The fishes, *Labeo rohita* (Rohu) were sampled with fishing net with the help of fishermen. These fishes were scrutinized. Below aged and diseased fishes were discarded and released into pond, only healthy and about 2 year old fished were kept for experimentation in container filled with pond water.

D. Collection of blood from fishes:

The blood sample was collected by puncturing the caudal fin with the help of syringe and needle.

E. Biochemical assays:

The biochemical tests for total protein, total cholesterol, creatinine, albumin and enzyme assays viz. AST and ALT were carried out by using standard techniques.

Each biochemical parameter was assessed in triplicate in both control and experimental fish. The blood samples from both control and experimental fish were centrifuged to separate the serum sample and the separated serum samples were then processed for biochemical and enzyme assays like liver and kidney function tests. For biomolecules, test like total proteins, total cholesterol, albumin and creatinine were carried out by using standard method for respective blood parameters.

i. **SGPT:** The activity of enzyme, Serum Glutamate Pyruvic Transaminase (SGPT) was observed by 2-4 DNPH method (Reitman and Frankle, 1957),
ii. **SGOT:** The activity of enzyme, Serum Glutamate Oxaloacetic Transaminase (SGOT) was observed by 2-4 DNPH method (Reitman and Frankle, 1957),
iii. **Total Protein:** Total Protein was estimated by Biuret method (Rosenthal *et al.,* 1956),
iv. **Albumin:** Albumin was estimated by Bromocresol green method (Spencer and Prince, 1977),

v. **Creatinine:** Creatinine was estimated by Alkaline picrate method (Rock *et al.,*1987)**,**

vi. **Total Cholesterol:** Total Cholesterol was estimated by CHOD-PAP method (Allain *et al.,* 1974).

Statistical Analysis

All the results were subjected to statistical analysis to evaluate the authenticity of the results.

RESULTS

I. Heavy Metals in Pond Water

The concentration of heavy metals in pond water from TPS was shown in Table 01 shows presence of As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu in varying quantities. From the above data it is clear that the pond water is contaminated with heavy metals in different concentrations.

II. Biochemical Assays

During the present study various biochemical assays were performed to know the status of fish from the pond in the vicinity of TPS, where TPS effluent gets released. The findings of biochemical assays are indicated in the Table 02.

In this study significant differences were observed in biochemical parameters like total protein, total cholesterol, albumin, creatinine and serum enzyme (SGPT, SGOT) between control and exposed group except in globulin value which was found to be insignificant throughout the study period.

i. Serum SGPT and SGOT

The ALT and AST activity increased in TPS effluent exposed fish when compared with the control fish. The highest elevation in ALT activity was measured during May-Aug 2012 (90.84 ± 5.908 IU/L) and least during Jan. - Apr. 2011 (16.98 ± 2.661 IU/L), though the highest AST activity was measured in Sept. - Dec. 2010 (421.388 ± 23.174 IU/L) and least during May-Aug 2012 (174.842 ± 12.432 IU/L) in TPS effluent exposed fish.

ii. Total Protein

In the present study, total protein concentration in the serum of TPS effluent exposed fish was found to be increased as compared to control fish blood sample. It was observed to be higher during Sept. - Dec. 2011 (2.959 ± 0.175 g/dl) and lower in May – Aug. 2012 (2.250 ± 0.172 g/dl).

iii. Albumin

In the present study, it was observed that the serum albumin accounts more in the serum of exposed fish than the control. The elevation in serum albumin was observed in Sep. – Dec. 2011 (2.668 ± 0.301 g/dl) while it showed decline in Jan. – Apr. 2011 (1.950 ± 0.186 g/dl).

iv. Globulin

In the present study, the globulin concentration in the serum shows same trend as that of protein and albumin. The globulin concentration in the serum of effluent exposed fish was found to be highest during Jan. – Apr. 2011 (0.751 ± 0.304 g/dl) while it lowest during Sep. – Dec. 2010 (0.345 ± 0.166 g/dl).

v. Sreum Creatinine

In the present study, TPS effluent exposed fish showed increased serum creatinine than the control value. More serum creatinine was found in May – Aug. 2012 (0.785 ± 0.109 g/dl) and less in Jan. – Apr. 2011 (0.225 ± 0.050 g/dl).

vi. Total Cholesterol

In the present study, cholesterol level in the serum of TPS effluent exposed fish was generally found to be elevated as compared to that of the control value. Highest increase was observed during May-Aug 2011 (182.71 ± 4.612 mg/dl) while lowest values were observed during Jan. – Apr. 2012 (133.623 ± 38.813mg/dl).

Table 1: Shows concentration of heavy metals (in ppm) in water of TPS pond during the year September 2010 to August 2012. (all values are expressed in mean ± S.D.)

Metals	Sept.- Dec. 2010	Jan.-Apr. 2011	May-Aug. 2011	Sept.-Dec. 2011	Jan.- Apr. 2012	May - Aug. 2012
Al	0.42 ±0.042	0.357±0.102	0.364±0.125	0.346±0.059	0.309±0.039	0.304±0.014
As	0.215±0.021	0.166±0.017	0.171±0.042	0.185±0.017	0.146±0.040	0.125±0.022
Cd	0.096±0.010	0.238±0.052	0.095±0.006	0.085±0.013	0.090±0.009	0.089±0.008
Co	0.250±0.094	0.287±0.224	0.168±0.043	0.247±0.050	0.228±0.069	0.292±0.012
Cr	0.815±0.147	0.630±0.268	0.553±0.170	0.856±0.058	0.773±0.086	0.937±0.063
Cu	0.815±0.093	0.701±0.168	0.731±0.129	0.874±0.036	0.751±0.198	0.925±0.043
Fe	3.247±0.500	3.067±0.107	4.891±0.782	7.522±1.217	7.921±1.341	7.422±1.692
Mn	0.927±0.128	1.070±0.096	0.840±0.053	0.953±0.108	0.775±0.106	0.677±0.125
Ni	0.923±0.045	0.844±0.048	0.771±0.092	0.931±0.053	0.737±0.101	0.822±0.104
Pb	0.090±0.002	0.077±0.009	0.085±0.006	0.086±0.016	0.084±0.015	0.091±0.006
Zn	3.317±0.584	2.795±0.453	2.764±0.411	4.261±0.694	4.761±0.364	5.017±0.825

Table 2: Shows biochemical changes in serum of freshwater fish, *L. rohita* exposed to TPS effluent. (Values are expressed in Mean ± SD)

		SGPT	SGOT	TP	Albumin	Globulin	Creatinine	Cholesterol
Sept-Dec 2010	**C**	21.713 ± 1.216	336.466 ± 31.628	1.678 ± 0.678	1.211 ± 0.220	0.298 ± 0.146	0.193 ± 0.078	144.35 ± 10.453
	E	27.957 * ± 5.799	421.388 * ± 23.174	2.298 * ± 0.239	1.952 ± 0.238	0.345≠ ± 0.166	0.246≠ ± 0.058	150.565 ≠ ± 16.623
Jan-Apr 2011	**C**	15.4 ± 2.544	180.255 ± 12.264	1.558 ± 0.213	0.908 ± 0.059	0.65 ± 0.210	0.136 ± 0.027	132.16 ± 18.341
	E	16.98 * ± 2.661	231.87 * ± 21.187	2.701 * ± 0.171	1.950 ± 0.186	0.751 * ± 0.304	0.225 ± 0.050	142.226 ≠ ± 27.326
May-Aug 2011	**C**	22.915 ± 3.013	241.407 ± 16.75	2.15 ± 0.148	1.733 ± 0.221	0.228 ± 0.0624	0.208 ± 0.064	117.425 ± 8.602
	E	31.07 * ± 4.352	298.215 * ± 12.029	2.715 * ± 0.155	2.032 ± 0.114	0.633 * ± 0.253	0.42 ± 0.093	182.71 * ± 4.612
Sept-Dec 2011	**C**	25.018 ± 4.141	280.933 ± 13.835	4.365 ± 0.650	1.81 ± 0.176	0.34 ± 0.115	0.237 ± 0.0585	151.335 ± 11.551
	E	28.231 ≠ ± 1.728	331.065 * ± 9.431	2.959 * ± 0.175	2.668 ± 0.301	0.415 * ± 0.250	0.357 ≠ ± 0.046	173.458 * ± 13.876
Jan-Apr 2012	**C**	33.34 ± 5.601	183.757 ± 12.307	1.96 ± 0.196	1.65 ± 0.147	0.22 ± 0.077	0.191 ± 0.033	121.991 ± 9.239
	E	68.823 * ± 3.342	233.153 * ± 51.939	2.282 * ± 0.179	2.046 ± 0392	0.456 * ± 0.259	0.398 ± 0.084	133.623 ≠ ± 38.813
May-Aug 2012	**C**	34.068 ± 5.891	162.25 ± 7.404	1.9 ± 0.130	1.673 ± 0.217	0.31 ± 0.098	0.508 ± 0.179	136.971 ± 6.550
	E	90.84 * ± 5.908	174.842 * ± 12.423	2.250 * ± 0.172	2.623 ± 0.134	0.373 * ± 0.213	0.785 ≠ ± 0.109	164.476 * ± 23.435

SGPT = IU/L, SGOT= IU/L, Creatinine=mg/dl, TP (Total Protein)=g/dL, Albumin=g/dL, Globulin=g/dL, Cholesterol=mg/dl. C-Control, E-Experimental. Values represented by (*) significantly differ from control ($p<0.05$) and (≠) are non significant when compared with control.

DISCUSSION

The presence of heavy metals in any water body is important for carrying out various metabolic activities of living organisms but once they reach their maximum level, they serve as environmental pollutants that produce hazardous effects on the aquatic organisms.

In the present study, the water indicates presence of As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu in varying quantities when analyzed through ICP-AES. The heavy metal concentration values in water samples from TPS pond were found within permissible limit as per guidelines of APHA (2005) and USEPA (1986). Though the heavy metals concentrations were within permissible range they may cause threat to aquatic life in long run.

The present findings for heavy metal concentrations in water is supported by the observations of Thorat and Charde (2013) who had studied the physico-chemical properties of Kanhan river water receiving fly ash disposed waste water of Khaperkheda Thermal Power Station, Nagpur and had reported the concentrations of copper, cadmium, zinc, lead, mercury and arsenic metals were within normal range. Similar results were also reported by Junshum *et al.* (2007) when they studied water quality assessment in reservoirs and wastewater treatment system of the Mae Moh power plant, Thailand where the heavy metal concentration did not exceed both the surface water quality standards and the industrial effluent standards of Thailand.

The presence of pollutants in aquatic environment exerts its effect at cellular or molecular level which results in a significant change in biochemical responses and for monitoring of aquatic environment analysis of biochemical methods offer as an important biomarkers (Authman *et al.*, 2013b). So, analyses of serum constituents have been proved to be useful in the detection and diagnosis of metabolic disturbance and disease processes (Elghobashy *et al.*, 2001). Heavy metals are some of the most-active polluting substances as they can cause serious impairment to circulatory, metabolic, physiological, and even structural systems when high concentrations are present in aquatic ecosystems (Yang and Chen, 2003).

The kidney is one of the major target organs for environmental contaminants such as heavy metals, and they are important organs for metabolic waste excretion and heavy metal elimination in fish (Yang and Chen, 2003). Kidney function tests such as serum creatinine, uric acid and urea can be used as a rough index of the glomerular filtration rate where low values of creatinine, uric acid and urea have no significance but increasing values indicate the presence of disturbances in the kidney (Elghobashy *et al.*, 2001).

Serum enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) are considered to be important serum markers to investigate the health of animal species in concern. In addition, two major aminotransferases, AST and ALT, are the most significant enzymes involved in protein and amino acid metabolism (Folmar, 1993; Zikic *et al.*, 2001). Likewise, the other serum biomarkers such as glucose, triglyceride, total protein, and urea are commonly used to detect health of animals. Therefore, it was emphasized that measurement of serum biochemical parameters can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicants (Zikic *et al.*, 2001).During the present study, ALT and AST activity increased in TPS effluent exposed fish as compared with the control.

Transaminases like AST and ALT has a significant role in protein and amino acid metabolism and they may release into the blood following tissue damage and dysfunction. Singh and Reddy (1990), demonstrated that 0.25 mg Cu/L caused significant increases in serum AST and ALT

activities in *Heteropneustes fossilis* with increases in exposure period. According to Oluah (1999) AST activity in *Clarias albopunctatus* increased significantly when exposed to 0.15 mg/L Zn and Hg for 21 days. Also Zikic *et al.* (2001) showed that plasma AST and ALT activities increased in Cd-exposed fish *Carassius auratus gibelio*. From the current study it was indicated that release of these transaminases into the blood circulation might occur due to damage of the liver, kidney, and other tissues in the state of stress caused by exposure to metals. It was suggested that serum enzymes such as ALP, AST, and ALT could be used as sensitive biomarkers in ecotoxicology to provide an early warning of potentially hazardous alterations in contaminated aquatic organisms (Levesque *et al.*, 2002; Vaglio and Landriscina, 1999, De La Torre *et al.*, 2000).

Cholesterol concentrations in the serum of TPS effluent exposed fish generally increased as compared to control. The present data is supported by other researchers showing increased serum cholesterol concentrations in metal-exposed fishes (Yang and Chen, 2003; Singh and Reddy, 1990; Canli, 1995). The cholesterol is an essential structural component of membranes and the precursor of all steroid hormones, its concentration may increase due to the liver and kidney failure causing the release of cholesterol into the blood. Exposure of fish to TPS effluent seems to elevate the level of serum cholesterol probably due to stress they caused by the toxicants. Heavy metals are known to have hazardous effects on cell structure, especially on the membranes. Therefore, increase in cholesterol may be a better indication of environmental stress.

In the present investigation, *Labeo rohita* collected from TPS pond showed an increase in serum creatinine. This may be attributed to the action of heavy metals and other pollutants on the glomerular filtration rate which causes pathological changes of the kidney (Oikari and Soivio, 1977). These recorded results are in agreement with that of Elghobashy *et al.* (2001) who observed increase in kidney functions in *Oreochromis niloticus* collected from the River Nile and some Egyptian lakes due to heavy metal pollution. Yang and Chen (2003) found significantly higher concentrations of creatinine in serum of intoxicated carp after 28 days of gallium (intermetallic elements) exposure. Zaki *et al.* (2009) observed a pronounced elevation of creatinine level in grey mullet after three weeks of exposure to 0.25 ppm of cadmium chloride and attributed it to kidney injury.

Hadi *et al.* (2009) reported that, creatinine and uric acid are biomarkers for muscle and purine metabolism, liver damage and kidney function. The rise in the creatinine of *Labeo rohita* collected from TPS pond may be attributed to heavy metals and other pollutants which affect the muscle metabolism. Hadi *et al.* (2009) reported that the increase of creatinine level might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism.

Serum albumin and globulin have been used as indicators of healthy status of the fish and considered as important indicators for the effect of pollutants in fish (Tayel *et al.*, 2007 and Mohammad *et al.*, 2013). Protein is also one of the important biochemical parameters which have been used to understand the general state of health and biological mechanism of metabolism under stress. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. So, due to this, proteins in liver degrade and the serum protein level increase.
The toxicity of these elements is due to their ability to cause, oxidative damage to living tissues. Toxic heavy metal can cause dermatological diseases, skin cancer and internal cancers (liver, kidney, lung and bladder), cardiovascular disease, diabetes, and anaemia, as well as reproductive, developmental, immunological and neurological affects in the human body. Prolonged exposure to water pollutants even in very low concentrations have been reported to induce morphological,

histological and biochemical alterations in the tissues which may critically influence fish quality.

REFERENCES

Abou El-Naga EH, El-Moselhy KM and MA Hamed (2005) Toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish *Mugil seheli*. *Egyptian J. Aquat. Res.,* 31(2): 60–71.Adams, R.H., Guzmán Osorio, F.J.. and J. Zavala Cruz (2008). Water repellency in oil contaminated sandy and clayey soils. *Int. J. Environ. Sci. Tech.,* 5 (4):445-454.

Adham Khadiga G, Hameed Sherifa S, Ibrahim H, Saleh M and A Ramadan (2002) Impaired functions of Nile tilapia, *Oreochromis niloticus* from polluted waters. *Acta Hydrochem. et Hydrobiol.*, 29: 278-288.

Allain CC, Poon IS, Chan CHG, Richmond W and PC Fu (1974) Enzymatic determination of serum total cholesterol. *Clin. Chem.,* 20: 470-471.

Al-Yousuf MH, El-Shahawi MS and Al-Ghais SM (2000) Trace metals in liver, skin and muscle of Lethrimus lentjan fish species in relation to body length and sex. *Sci. Total Environ.,*256: 87 – 94.

APHA, AWWA, WPCF (2005). *Standard methods for examination of water and wastewater* (21st Eds: Andrew D. Eaton; Clesceri L.S; Rice E. W; Greenberg A.E.). The Edition AM. Publ. Health Association, Washington, D.C.

Authman MMN, Ibrahim SA, El-Kasheif MA and Gaber HS (2013b) Heavy metals pollution and their effects on gills and liver of the Nile Catfish *Clarias gariepinus* inhabiting El-Rahawy Drain, Egypt. *Global Veterinaria,* 10(2): 103-115.

Bahor MP, Mclaren Niece RJ and Pedersen HC (1981) Coal ash disposal Manual, Second edition, *Electric Power Research Institute, Final Report* (CS-2049).

Barnhorn IEJ and Van-Vuren JHJ (2004) The use of different enzymes in feral freshwater fish as a tool for the assessment of water pollution in South Africa. *Ecotoxicol. Environ. Saf.*, 59:180–5.

Bashar Al Smadi (2010) Water management and reuse opportunities in a thermal power plant in Jordan. *Afr.J.Biotechnol.,* 9 (29):4607-4613.

Canli M (1995) Effects of mercury, chromium, and nickel on some blood parameters in the carp *Cyprinus carpio*. *Turk. J.Zool.*,19:305–311.

Chakraborty R and Mukhergee A (2009) Mutagenicity and genotoxicity of coal flyash water leachate. *Ecotoxico. Env. Monit. Assess.,* 184:1581-1592.

De la Torre FR, Salibian A and Ferrari L (2000) Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. *Environ. Poll.,* 109:277-282.

Elghobashy HA, Zaghloul KH and Metwally MAA (2001) Effect of some water pollutants on the *Nile tilapia*, *Oreochromis niloticus* collected from the River Nile and some Egyptian lakes. *Egypt. J. Aquat. Biol. Fish.*, 5(4): 251-279.

Farombi EO, Adelowo OA and YR Ajimoko (2007) Biomarkers for oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria Ogun River. *Int. J. Environ. Res. Pub. Health,* 4(2): 158-165.

Folmar LC (1993) Effects of chemical contaminants on blood chemistry of teleost fish: A bibliography and synopsis of selected effects. *Environ. Toxicol. Chem.* 12:337–375.

Gueu S, Yao B, Adouby K and Ado G (2007) Kinetics and thermodynamics study of lead adsorption on to activated carbons from coconut and seed hull of the palm tree. *Int. J. Environ. Sci. Tech.,* 4 (1):11-17.

Gupta DK, Rai UN, Tripathi RD and Inouhe M (2002) Impacts of fly ash on soil and plant responses. *J.Plant Res.,* 115(6): 401-409.

Hadi A, Shokr A and Alwan S (2009) Effects of aluminum on the biochemical parameters of fresh water fish *Tilapia zillii*. *Journal of Science and Its Applications,* 3(1): 33-41.

Junshum P, Menasveta P and Traichaiyaporn S (2007) Water quality assessment in reservoirs and wastewater treatment system of the Mae Moh power plant, Thailand. *Journal of Agriculture and Social Sciences,* 3(3):91-94.

Kanungo SB and Mohapatra R (2000) Leaching behavior of various trace metals in aqueousmedium from two fly ash samples. *J Environ. Qual.,* 29(1); 188-196.

Lee CL, Li XD, Zhang G, Li J, Ding AJ and Wang T (2007) Heavy metals and Pb isotopic composition of aerosols in urban and suburban areas of Hong Kong and Guangzhou, South China Evidence of the long-range transport of air contaminants. *Environ. Pollut.,* 41 (2): 432-447.

Levesque HM, Moon TW, Campbell PGC and Hontela A (2002) Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquat Toxicol.,* 60:257–267.

Martinez-Porchas M, Hernandez-Rodriguez M, Davila-Ortiz J, Vila-Cruz V and Ramos-Enriquez JR (2011) A preliminary study about the effect of benzo[α]pyrene (BaP) injection on the thermal behavior and plasmatic parameters of the Nile tilapia (*Oreochromis niloticus* L.) acclimated to different temperatures. *Pan-Am. J. Aqua. Sci.,* 6(1): 76-85.

Mehra A, Farago ME and Banerjee DK (1998) Impact of fly ash from coal fired Power stations in Delhi, with particular reference to metal contamination. *Env. Mon. Asses.,* 50: 15–35.

Mohammad MN, Authman Seham A, Ibrahim Midhat A El-Kasheif and Hanan S Gaber (2013) Heavy Metals Pollution and Their Effects on Gills and Liver of the Nile Catfish *Clarias glariepinus* Inhabiting El-Rahawy Drain, Egypt. *Global Veterinaria,* 10 (2): 103-115.

Oikari A and Nakari J (1985) Ubacute physiological effects of bleached kraft mill effluent (BKME) on the liver of trout, (*Salmo gairdneri*). *Ecotoxicol. Environ. Saf.,* 10:159.

Olojo EAA, Olurin KB, Mbaka G and Oluwemimo AD (2005) Histopathology of gill and liver tissue of the african catfish *Clarias gariepinus* exposed to lead. *African J. Biotechnol.,* 4(1): 117-122.

Oluah NS (1999) Plasma aspartate aminotransferase activity in the catfish *Clarias albopuntatus* exposed to sublethal zinc and mercury. *Bull. Environ. Contam. Toxicol.,* 63:343–349.

Osman AGM, Koutb M and Sayed AED (2010) Use of hematological parameters to assess the efficiency of quince (*Cydonia oblonga*, Miller) leaf extract in alleviation of the effect of ultraviolet—iradiation on African catfish, *Clarias gariepinus* (Burchell, 1822). *J. Photochem. Photobiol. B. Biol.,* 99:1–8.

Praharaj T, Swain SP, Powell MA, Hart BR and Tripathy S (2002) Delineation of groundwater contamination around an ash pond: geochemical and GIS approach. *Environ Int.*, 27(8):631-638.

Ramachandra TV, Bhat Sudarshan P, Durga MM and Gautham K (2012) Impact of indiscriminate disposal of untreated effluents from thermal power plant on water resources. *Indian Journal of Environmental Protection,* 32(9): 705-718.

Reitman S and Frankel S (1957) Colorimetric Composition of glutamic oxaloacetic and glutamic pyruvic transaminases, *Am. J. Clin. Pathol.,* 28: 53-56.

Rock RC, Walker WG and Jennings CD (1987) Nitrogen metabolites and renal function. In: Tietz, N.W., ed. *Fundamentals of clinical chemistry*, 3rd Ed Philadelphia: W.B. Saunders, pp: 669-704.

Rosenthal MW, Fried JW and Schubert J (1956) Method for estimation of protein. *Proc. Soc. Exp. Biol. N.Y.*, 92:331.

Singh B, Kumar S and Kumar M (2004) Leaching study of trace elements from coal ashes: A case study of Bokaro Thermal Power Station "B". *J. Environ Sci. Eng.,* 46(3):203-9.

Singh HS and Reddy TV (1990) Effect of copper sulfate on hematology, blood chemistry, and hepatosomatic index of an Indian catfish, *Heteropneustes fossillis* (Bloch), and its recovery. *Ecotoxicol. Environ. Saf.,* 20:30–35.

Spencer K and Price CP (1977) Influence of reagent quality &reaction condition on the determination of serum albumin by bromocresol greendye binding method. *Ann. Clin. Biochem.,* 14 (2), 105-115.

Storelli MM, Storelli A, D'ddaabbo R, Morano C, Bruno R and Marcotrigiano GO (2005) Trace Elements in loggerhead turtles (*Caretta caretta*) from the Eastern Mediterranean; Overview and Evaluation. *Environmental Pollution*, 135: 163 – 170.

Suresh IV, Padmakar C and Venkata Rao K (1998) Effect of ash pond on ground water quality: a case study. *Env. Manage. Health,* 9(5): 200-208.

Tayel SI, Yacoub AM and Mahmoud SA (2008) Histopathological and haematological responces to fresh water pollution in the Nile catfish *Clarias gariepinus*. *Journal of Egyptian Academic society for Environmental Development*, 9: 43-60.

Thorat PB and Charde VN (2013) Physicochemical Study of Kanhan River Water Receiving Fly Ash Disposal Waste Water of Khaperkheda Thermal Power Station, India. *International Research Journal of Environment Sciences*, 2(9):10-15.

United States Environmental Protection Agency ,USEPA (1986) Quality Criteria for Water. EPA-440/5-86-001, Office of Water Regulations and Standards, Washington DC., USA.

Vaglio A and Landriscina C (1999) Changes in liver enzyme activity in the teleost *Sparus aurata* in

response to cadmium intoxication. *Ecotoxicol. Environ. Saf.,* 43:111–116.

Vinodhini R and Narayanan M (2008) Bioaccumullation of heavy metal in organs of fresh water fish *Cyprius carpio* (Counou camp). *Int. J. Environ. Sci. Tech.,* 5(2): 178- 182.

Vosyliene MZ and Jankaite A (2006) Effect of heavy metal model mixture on rainbow trout biological parameters. *Ekologika,* 4: 12-17.

Yang JL and Chen HC (2003) Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. *Chemosphere,* 53(8): 877-882.

Zaki MS, Mostafa SO, Fawzi OM, Khafagy M and Bayumi FS (2009) Clinicopathological, Biochemical and Microbiological Change on Grey Mullet Exposed to Cadmium Chloride. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 5(1): 20-23.

Zikic RV, Stajn S, Pavlovic Z, Ognjanovic BI and Saicic ZS (2001) Activities of superoxide dismutase and catalase in erytrocyte and plasma transaminases of goldfish (*Carassius auratus* gibelio Bloch.) exposed to cadmium. *Physiol. Res.,* 50:105–111.

RESEARCH ARTICLE

Phytotoxicity of Profenofos 50% EC (Curacron 50 EC) to *Vigna radiata*, L. seedlings: III. Studies on Secondary metabolites and enzymes

Mishra IP, Sabat G and Mohanty BK*

P.G Department of Botany & Biotechnology, Khallikote University(formerly Khallikote Autonomous College) Berhampur-760 001, Odisha, India,
*Corresponding author e-mail: mohantysir57@yahoo.com

Manuscript details:

Received: 20.11.2015
Accepted: 17.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Mishra IP, Sabat G and Mohanty BK (2015) Phytotoxicity of Profenofos 50% EC (curacron 50 EC) to *Vigna radiata*,L. seedlings: III. Studies on Secondary metabolites and enzymes, *International J. of Life Sciences*, 3(4): 351-359.

Acknowledgements:
Authors are thankful to HOD & Principal,KhallikoteAutonomous College, Berhampur for necessary laboratory facilities and encouragement for research activities..

ABSTRACT

Profenofos 50% EC (Curacron 50 EC) is a hazardous pesticide commonly used in agriculture is also an important contaminant of environment. Its presence in biological system has gained importance due to bioaccumulation in food chain. The phyto-toxic effect of profenofos was assessed based on the secondary metabolites like Phenolic Compounds, Flavinoids) and Enzymes of the test species, *Vigna radiata, L*. The concentrations of pesticide chosen were based on EC50 and are in the range of 0.02, 0.05, 0.08, 0.1 and 0.2 % of profenofos. The changes in phenol and flavinoids enzymes (peroxidase and polyphenol oxidase) are not dose dependant but the catalase enzyme activity was profenophos concentration dependant.

Keywords: Profenofos, organophosphate, seedlings, morphology, pigments

INTRODUCTION

Pesticides are playing a pivotal role in meeting the food, cotton fibre and tobacco demand of escalating population and control of vector-borne diseases. However, most of the applied pesticides get dispersed in the environment and affects the health of un-protected agricultural and industrial workers. The three major routes of entry for pesticides include contamination of the skin, lungs and the gut. Exposure to pesticides is one of the most important occupational risks among farmers in developing countries (Wesseling *et al*., 2001; Konradsen *et al*., 2003 and Coronado *et al.* 2004). Occupational exposure to pesticides is of great interest in order to identify the hazards of pesticide use and the establishment of safe methods of pesticide handling. This is

because pesticide misuse in various sectors of the agriculture often has been associated with health problems and environmental contamination worldwide (Soares *et al*., 2003; Mancini *et al*., 2005; Remor *et al*., 2009). Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides, is one of the major reasons for the high incidence of pesticide poisoning in developing countries (Konradsen *et al*., 2003).

Profenofos is the International Organization for Standardization (ISO) approved name for(*RS*)-*O*-4-bromo-2-chlorophenyl *O*-ethyl S-propyl phosphorothioate (IUPAC). The Chemical Abstracts Service (CAS) chemical name for profenofosis *O*-(4-bromo-2-chlorophenyl) *O*-ethyl S-propyl phosphorothioate (CAS No.41198-08-7). It is a broad-spectrum organo-phosphorus insecticide that is used to control insect pests in cotton, maize, sugar beet, soya bean, potato, vegetables and other crops.

Application of organophosphate insecticides inhibits the seed germination seedling growth of *Peniesetum americanum* L. (Siddiqui *et al*., 1999). Like wise use of systemic fungicides produced chlorosis irregular depression at the central marginal portions of saffron leaf (Reyes, 1975), induced sharp decrease in cell division (Coman *et al*., 1990) inhibited seedling growth of pea (John *et al*., 1976). Despite the facts, use of systemic agrochemicals is the need of present time.

MATERIALS AND METHODS

Selection of profenofos concentration

The concentrations of pesticide, Profenofos chosen were, 0.02, 0.05, 0.08.0.1 and 0.2%. A control set was maintained with distilled water only for comparison purpose.

Selection of test seed

The prime pulse seed Vigna *radiata*, var. PDM 139 Samart popularly called as mung commonly used in eastern state of India, particularly Odisha State has been chosen for study. Healthy seeds of *Vigna radiata*, were obtained from OUAT extension Ratnapur, Ganjam for the experimentation. A standard filter paper method was used. Mung seeds (20 per replication) were placed in Petridishes (6'') on filter paper moistened with 10 ml of test solution.

The mung seeds surfaces sterilized with sodium hypochlorite for 10 minutes and were incubated in the dark at 25±2 °C for 7 days in Seed Germinator (REMI- 6C). After seven days (168hours) the following assessments were made on germination (%), Phyto pigments (Chlorophyll a, Chlorophyll b, Total Chlorophyll, Caretonoids and Phaeophytin) following the methods of Arnon (1949), length of seedling roots and shoots (cm) and fresh and dry weight (g) of roots and shoots. The experiment was set in three replicates. Estimation of total phenolic content and total flavonoid content (Malik and Singh, 1980) was done in shoot of the 7 days old seedlings.

The activity of catalase was assayed after the modified method of Kar and Mishra (1976). The shoot/ root sample weighing about 200 mg were homogenized with 10 ml of phosphate buffer pH 7.2 (0.1 M) and was centrifuged at 2^0C for 15 minute at 17000 g in a refrigerated centrifuge. The clear supernatant was taken as the enzyme source. The plant material shoot/ root of 200 mg weight was ground with 0.1 M phosphate buffer PH 7.2 in a pre-chilled mortar and pestle, and the homogenate was centrifuged at 15000 g at 4^0C for 30 min and the aliquot was used as the source of the enzymes.

The enzyme activity of peroxidase was assayed by modified method of Kar and Mishra (1976).The plant material was ground with 0.1 M phosphate buffer PH 7.2 in a pre-chilled mortar and pestle, and the homogenate was centrifuged at 15 000 g at 4^oC for 30 min and the aliquot was used as the source of the enzymes. The activity of polyphenol oxidase was assayed by the method of Kar and Mishra (1976) with slight modification.

The data are expressed as mean values of (n=5) and were analyzed employing Correlation Analysis to determine whether the values were significantly different from control at 0.05P with 4 d.f.

RESULTS

Effect of Profenofos on the secondary metabolite activity of *Vigna radiata* L. seedlings were studied after treatment for seven days at different concentration of profenofos. The secondary metabolite activity of root and shoot of the seedlings were studied and recorded in separate tables and graphs (Table-1 and Fig. 4-5).There is a significant increase in Phenol content of the root and shoots, of *Vigna radiata* L. seedlings treated with different concentrations of Profenofos 50 % EC solution. Phenol content in the controlled roots was 0.396 (A) of g^{-1} fresh wt. It was increased up to 0.472 (A) at 0.01 % of Profenofos 50 % EC then declined to 0.426 (A) at 0.02 % of Profenofos.

The trend of Phenol content in shoot was similar to that of the root of *Vigna radiata* L. Seedlings when treated with different concentrations of Profenofos 50 % EC solution. Phenol content in the control shoots was 0.049 (A) of g^{-1} fresh wt. And it increased up to 0.180 (A) with 0.01 % of Profenofos (an increase of 267.34 %) and then it declined to 0.113 (A) at 0.02 % of Profenofos treatment . However the effect of Profenofos 50 % EC on increasing the phenol content was much more in the roots at all the concentrations with comparision to shoots.

Regression analysis and ANOVA indicates the statistical insignificance and the change in phenol content in root was not profenofos treatment dependant. (Fig. 5 and Table. 1)

Table 1: Correlation Analysis of different parameters observed after Treatment of Profenofos to 07 days old seedlings of *Vigna radiata.* L.

PARAMETER	Correlation Coefficient (r- Value)	d.f	P level	Statistical Significance
Concentration of Profenofos Vs Catalase activity (shoot)	-0.971	5	0.01	Statistically Significant
Concentration of Profenofos Vs Catalase activity (root)	-0.974	5	0.001	Highly Significant
Concentration of Profenofos Vs peroxidase activity (shoot)	-0.208	5	NS	Statistically not Significant
Concentration of Profenofos Vs peroxidase activity (root)	-0.250	5	NS	Statistically not Significant
Concentration of Profenofos Vs polyphenoloxidase activity (shoot)	-0.044	5	NS	Statistically not Significant
Concentration of Profenofos Vs polyphenoloxidase activity (root)	-0.142	5	NS	Statistically not Significant
Concentration of Profenofos Vs phenol activity (shoot)	-0.378	5	NS	Statistically not Significant
Concentration of Profenofos Vs phenol activity (root)	-0.312	5	NS	Statistically not Significant
Concentration of Profenofos Vs flavanoid activity (shoot)	-0.040	5	NS	Statistically not Significant
Concentration of Profenofos Vs flavanoid activity (root)	-0.237	5	NS	Statistically not Significant

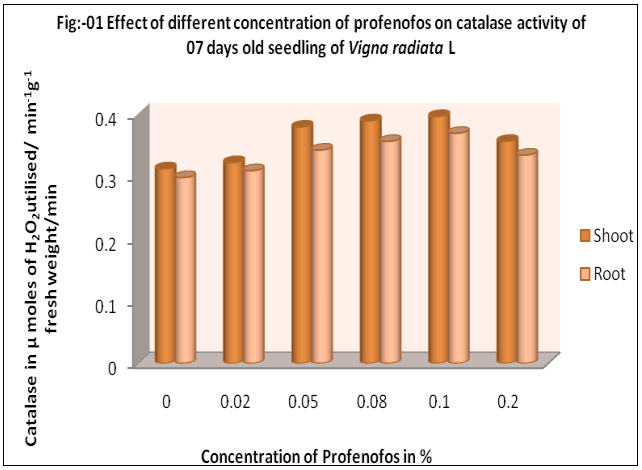


Fig. 1: Effect of different concentration of profenofos on catalase activity pf 07 days old seedling of *Vigna radiate* L.

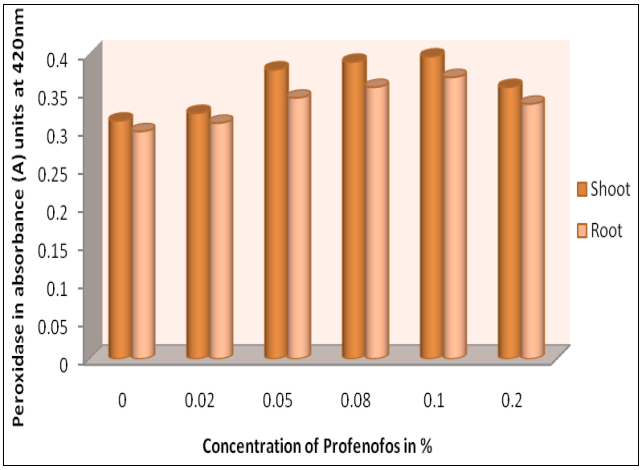


Fig. 2: Effect of different concentration of profenofos on Peroxidase activity of 07 days old seedling of *Vigna radiate* L.

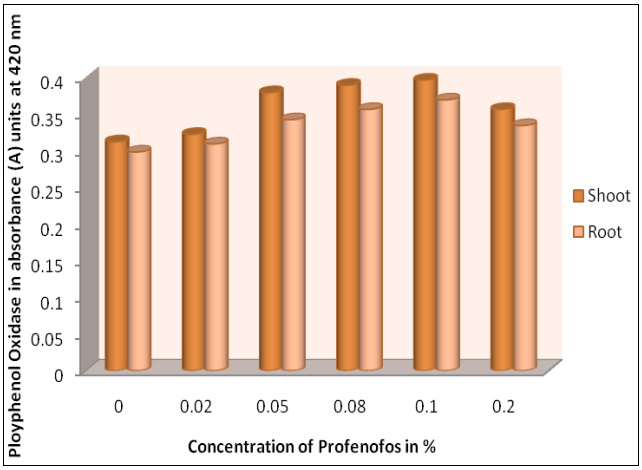


Fig. 3: Effect of different concentration of profenofos on polyphenol oxidase activity of 07 days old seedling of *Vigna radiate* L.

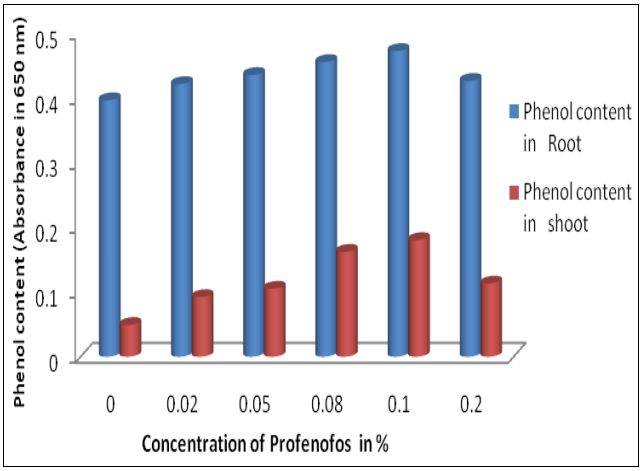


Fig. 4: Effect of different concentration of profenofos on Phenol Content of 07 days old seedling of *Vigna radiate* L.

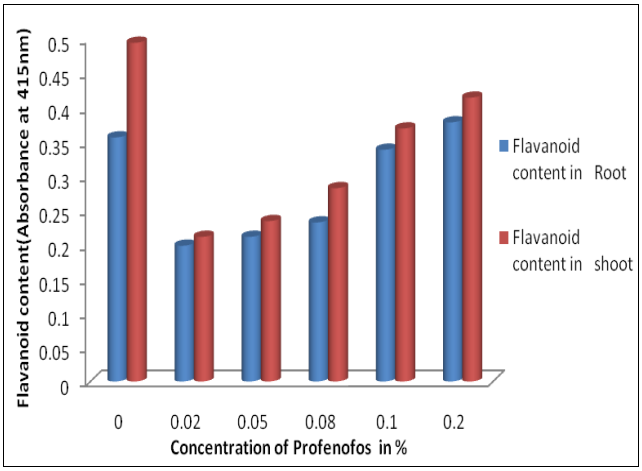


Fig. 5: Effect of different concentration of profenofos on Flavanoid content of 07 days old seedling of *Vigna radiate* L.

There is a significant decrease in Flavanoid content of the root and shoots, of *Vigna radiata* L. Seedlings treated in different concentrations of Profenofos 50 % EC solution. Flavanoid content in the controlled roots were measured in absorbance units was 0.356 (A) of fr wt. And a drastic decline of 0.198 (A) at 0.02 % of Profenofos 50 % EC that is a decrease of 44.39 % over the control value but then the decline was lessened to 0.338 (A) at 0.1 % of Profenofos that is a decrease of 5.06 % over the control value but at 0.02 % of treatment the Flavanoid content increased to 0.378 (A) that is an increase of 6.17 % over the control value.

The trend of Flavanoid content in shoot was nearly similar to that of the root of *Vigna radiata* L. Seedlings when treated in different concentrations of Profenofos 50 % EC solution. Flavanoid content in the controlled shoots were measured in absorbance units was 0.494 (A) of fr wt. and decreased up to 0.211 (A) at 0.01 % of Profenofos 50 % EC that is an decrease of 57.29 % over the control value but then there was a significant recovery up to 0.02 % with 0.414 (A) and an increase of 16.20 % over the control value. Regression analysis and ANOVA indicates the statistical insignificance and the change in flavanoid content in root was not profenofos treatment dependant.

Enzyme Activity:

The Profenofos 50 % EC application to the seedlings of *Vigna radiata* L. increased in the catalase activity of root with the increase of the test chemical from 0.02 % to 0.2 % . There was very significant increase by 210 catalase in µ moles of H_2O_2 utilized / min $^{-1}g^{-1}$ fresh root weight/ min in the enzyme activity and an increase of 840 % at 0.2 % of Profenofos over the control values (Fig,-1).

A similar observation was also noticed with shoot with the application of Profenofos 50 % EC to the seedlings of *Vigna radiata* L. increased in the catalase activity of shoot with the increase of the test chemical from 0.02 % to 0.2 % . There was very significant increase by 270 catalase in µ moles of H_2O_2 utilized / min $^{-1}g^{-1}$ fresh root weight/ min in the enzyme activity and an increase of 771.4 % at 0.2 % of Profenofos over the control values (Fig.1).

Increased activity of peroxidase was observed in root and shoot with increase in Profenofos 50 % EC concentrations (0.02,0.05,0.08,0.1 &0.2 %) At 0.1 % the percent increased was 23.82 in roots and 26.92 in shoot over the control values, but at 0.2 % there was a percent decrease with respect to the treatment at 0.1 % by 12.08 in root and 14.1 in shoot over the control value. (Fig.2).

Polyphenol oxidase which oxidises phenol in the absence of hydrogen peroxide, shows a increase in the activity with application of Profenofos 50 % EC concentrations (0.02,0.05,0.08,0.1 &0.2 %) At 0.1 % the percent increased was 19.19 in roots and 15.31 in shoot over the control values, but at 0.2 % there was a percent decrease with respect to the treatment at 0.1 % by 7.57 in root and 3.15 in shoot over the control value. (Fig. 3)

DISCUSSION

Increase in total phenols at higher concentrations of systemic fungicide provides further insight to the reduction in growth parameters discussed above. Production of phenols in the plants subjected to fungicidal spray is a response of the plants that not only helps them to cope with the resulting chemical stress but at the same time act as protective compound to check the growth of invaded pathogens (Reid *et al*., 1992). It is presumed that increase in total phenols may act as prophylactic measure against pathogens before invasion. On the other hand phytotoxin in the form of phenols have been found to have an adverse affect on germination and growth parameters (Hafeez *et al*., 1988; Macias *et al*., 1992; Berger and Cwick, 1990; Ahmed and Siddiqui, 1995; Siddiqui *et al*., 1997).

Einhellig (1995) proposed that a primary effect of phenolic acid is on the plasma membrane, and this perturbation contributes to a number of physiological effects causing growth reduction. It seems that cultivation of soybean plants in a soil treated with higher concentration of pesticide initiate some kind of abiotic stress (chemical stress) in plants triggering formation of phenolic compounds like iso-flavones (Genistein, diadzein), phenolic acid (elagic, tannic and vanilic acid) and hydroxycinnamic acid derivatives (ferulic acid, *ρ*- hydroxy benzoic acids and *ρ*-caumeric acid). These compounds are potential inhibitors of germination and plant growth (Einhellig *et al*., 1985; Macias *et al*., 1992; Mersie and Singh 1993). Few reports have elucidated the physiological mechanism of phenols induced inhibition on plant growth. Einhellig *et al*., (1985) who proposed that Ferulic and ρ-coumaric acids reduce leaf water potential and stomatal diffusive conductance in sorghum and soybean. Another study by Einhellig (1995) found that a primary effect of phenolic acids is on the plasma membrane, and this perturbation contributes to a number of physiological effects causing growth reduction. High level of ρ-coumaric, Ferulic, Cinnamic and Vanillic acids and Coumarins severely suppressed the photosynthesis of soybean and *Lemna minor* L., (Patterson, 1981; Einhellig, 1986). Three phenolic acids, ρ-Coumaric acid, Ferulic and Vanillic acids, were also reported to severely inhibit photosynthesis and protein synthesis of isolated leaf cells of velvet leaf *Abutilon theophrasti* (Mersie and Singh, 1993). Friend (1977) is of the opinion that these very compounds may act as protective compounds against pest as well.

Increase in total phenolic content and flavanoids in the test species usually indicates some kind of chemical stress produced by the application of insecticide . It has been suggested that plant treated with the chemical pesticide suffer from the chemical stress and phenolic compound produced as a result of the stress may act as a protective compound against pest and disease (Friend, 1977; Siddiqui *et al*., 1997). Stress condition cause abnormal changes in metabolic pathway resulting in production of toxic phenolic compound (Reid *et al.1992*). Phytotoxin in the form of phenolic compound and flavanoids are responsible for limiting cell division, nodulation, respiration, photosynthesis, disruption of cell membrane and reduction in total prtein and carbohydrate content of various plant species (Wilson , 1970; Bernestein and Ogata, 1966; Hafeez *et al.*1988; Siddiqui and Ahmed, 1996, Siddiqui *et al*.,1997). Consequently , the synthesis of carbohydrate, DNA and RNA may also be affected by the application of insecticide specially at higher concentration.

In the present study the shoot and root of green gram seedling showed a significant increase in phenol and flavanoid content with a gradual increase in profenofos concentrations, and our observations are similar to all of the above reports.

Enzyme activity indicates the phyto-toxicity of the stress material. The effect of pesticides on the enzyme activity in plants has been reviewed (Van Assche and Clijsters, 1990).

The activity of catalase increased in the shoot and root of green gram seedling with the increase of concentration of profenofos significant increase of catalase activity to 771.4% and 840.0% in shoot and root of green gram seedlings at 0.2 % profenofos treatment was recorded in present investigation. A highly significant positive correlation ($r=0.974$, $p \leq 0.01$, $d.f=4$) was found between the concentration profenofos and the catalase activity of shoot and root of pigeon pea seedlings.

The stress induced decline in catalase activity has also been reported by Somasekaraiah *et al*., (1992) and Galeogo *et al*., (1996). Accumulation of hydrogen peroxide in higher quantities may be cytotoxic and therefore the hydrogen peroxide formed due to the activity of the superoxide dismutase may be degraded by catalase and peroxidase resulting in the formulation of water

and oxygen (Scott *et al*.,1987). Therefore concurrent increase in catalase a predominant utiliser of hydrogen peroxide may become necessary. The hydrogen peroxide accumulation may result from the increased activities of superoxide dismutase and reduced activities of catalase. The end product, hydrogen perioxide will in turn may be toxic to the green gram seedlings.

Conflicting reports exist on the activities of catalase in plant tissues exposed to stress; Subhadra *et al*., (1991) reported an increase in catalase activity in roots of *Lemna minor* L and *Allium Cepa* L. Whereas Cakmak and Horst (1991) and Luna *et al*., (1994) reported a decrease in catalase activity in roots and leaves respectively. Stress seems to affect the pathway of synthesis of the enzyme and its activity in leaves at seedling stage was drastically inhibited by the metal; catalase being photosensitive needs constant fresh synthesis (Feierbend *et al*., 1992).

Peroxidases are antioxidant enzymes which play a crucial role in plant growth and development, and activities of these enzymes are changed under both abiotic and biotic stress conditions (Doganlar and Atmaca, 2011). Sandalo *et al*., (2001) noticed a decrease in peroxidase activity in pea under the influence of stress. Pesticides causes oxidative stress, probably through an interaction with the anti oxidative defense, disruption of the electron transport chain, or induction of lipid peroxidation. Stimulation of anti oxidant enzyme activity at low stress levels could play a significant role in protecting cells against stress -induced oxidative stress (Scebba *et al*., 2006). Profenofos is a highly toxic, non essential mutable and biodegradable pesticide that undergoes many changes during transfer through different levels of food chain. Pesticides treated seedlings of *phaselous vulgaris* showed reduction or inhibition of the growth of the main axis of the root with a consequent reduction in root length. There was an inverse correlation between cell wall peroxidase and growth . Zinc induced rise in peroxidase was reported in the leaves of *phaseolus vulgaris* (Van Assche *et al*.,1988). The author found a significant correlation between peroxidase induction and the presence of metals like zinc, cadmium and copper in plant tissues. Reddy and Prasad (1992) observed an increased peroxidase activity in *Oryza sativa* treated with different concentration of cadmium. Patro *et al*., (2001) reported that all concentration of the effluents found to have strong effect on the activity of peroxidase in the leaves of *oryza sativa* L. Very low cadmium levels in vitro have shown to stimulate the activities of certain enzymes like peroxidases, acid phosphatases etc. (Ernst 1980, Shah and Dubey 1997).

REFERENCES

Ahmed S and Siddiqui ZS (1995) Effect of topsin fungicide (Methylthiophenate) on chlorophyll, protein and phenolic contents of *Hibiscus esculentus* and *Capsicum annum*. *Pak.J.Bot*., 27:175-178.

Arnon DI (1949) Copper enzyme isolated chloroplast polyphenoloxidase in Beta vulgaries, *Plant physiol*,24,1-15.

Berger S and Cwick K (1990) Selected aspect of adverse nutritional effect of pesticides. *Ernahrung*., 14: 411-415.

Bernestein L and Ogata G (1966) ffect of salinity on nodulation, nitrogen fixation and growth of soybean and alfalfa. *Agron. J*., 58: 201-203.

Cakmak I and Horst WJ (1991) Effect of aluminium on lipid peroxidase activities in root tips of soyabean. (*Glycine max*) *Physiol. Plant,* 83 (3): 463-468.

Coman N, Dordea M and Perseca T (1990) The estimation of alcholor and metaxyl on cell division. *Biologia*, 35: 55-60.

Coronado GD, Thompson B, Strong L, Griffith WC, Islas I (2004) Agricultural task and exposure to organophosphate pesticides among farm workers. *Environ. Health 18 Persp*., 112., 142-147.

Doganlar Z and Atmaca M (2011) influence of airborne pollution on Cd , Z, pb, Cu and as accumulation and physiological parameters of

plant leaves in *Antakya* (Turkey). *Water, Air, Soil Poll*. 214 (4). 509- 523.

Einhellig FA (1986) Mechanisms and modes of action of allelochemicals. In*: The Science of Allelopathy.* (Eds.): P.A. Putnam and C.S. Teng. John Wiley & Sons, New York.

Einhellig FA (1995) *Allelopathy*: *Organisms, Process and Applications*. American Chemical Society. Washington. Pp. 96-116.

Einhellig FA, Muth MS and Schon MK (1985) Effects of allelochemicals on plant-water relationship. In: *The chemistry of Allelopathy.* (Ed.): A.C. Thomson. American Chemical Society, Washington.

Ernst (WHO) (1980) – Biochemical aspects of cadmium in plants. In J D Nriagu, ed. Cadmium in the Environment Part-I. Ecological Cycling, Wiley Interscience, New York, 639-653.

Feierabend J, Schaan C, Hertwig B (1992) Photo inactivation of catalase occurs under both high and low temperature stress conditions and accompanies photoinhibition of photosystem II- *Plant physiol*. 100. 1554-1561.

Friend J (1977) Phenolic substances and plant diseases. *Recent Adv. Phytochem.,* 12: 557.

Gallego SM, Benavides MP and Tomaro ML (1996) Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. *Plant Sci*., 121: 151-159.

Hafeez FY, Aslam Z and Malik KA (1988). Effect of salinity and inoculation on growth,nitrogen fixation ,and nutrient uptake of *Vigna radiate.*(L.)Wiczek. *Plant & soil*, 106:3-8.

John BS and Sisler HD (1976) Effects of ancymidol (a growth retardant) and Triarimol (a fungicide) on the growth, sterols and gibberellins of *Phaseolus vulgaris*. *Plant Physiol*., 57:640-644.

Kar M and Mishra D (1976) Catalase, Peroxidase and Polyphenol oxidase activities during rice leaf senescence. *Plant Physiol*. 57: 315- 319.

Konradsen F, Van der Hoek W, Cole DC, Hutchinson G, Daisley H, Singh S, Eddleston M (2003) Reducing acute poisoning in developing countries-options for restricting the availability of pesticides. *Toxicology,* 192., 249-261.

Luna CM, Gonzalez CA and Trippi VS (1994) Oxidative damage caused by an excess copper in oat leaves. *Plant Cell Physiol*., 35: 11-15.

Macias FA, Galindo JCG and Massanot GM (1992) Potential allelopathic activity of several sesquterpene lactone models. *Phytochem*., 31: 1969-1777.

Malik EP, Singh MB (1980) Plant Enzymology andHittoenzymology,(1st Edn.,), Kalyani Publishers, New Delhi,286

Mancini F, Van Bruggen AHC, Jiggins JLS, Ambatipudi AC, Murphy H (2005) Acute pesticide poisoning among female and male cotton growers in India. *Int. J. Occup. Environ. Health,* 11., 221-232.

Mersie W and Singh M (1993) Phenolic acids affect photosynthesis and protein synthesis by isolated leaf cells of velvet-leaf. *J. Chem. Ecol.,* 19: 1293-1301.

Patro B, Panigrahi SI, Das M and Tripathy S (2001) The effectiveness of industry effluents on chlorophyll degradation and hydrogen peroxide utilisation in rice leaves during senescence. *Asian J. of Microbiol. Biotech and Env . Sc*., 3 (83-190).

Patterson DT (1981) Effects of allelopathic chemicals on growth and physiological responses of soybean (*Glycine max*). *Weed Sci.,* 29: 53-59.

Reddy GN and Prasad MNV (1992) Cadmium induced peroxidase activity and isoenzymes in *Oryza sativa*. *Biochem Arch*. 8 (2): 101-106.

Reid LM, Mather DE, Arnason J, Hamilton T and Bolton RJ (1992) Changes in phenolic constituent in maize silk infected with*Fusarium graminearun*. *Can. J. Bot*.,70:1697-1700.

Remor AP, Totti CC, Moreira DA, Dutra GP, Heuser VD and Boeira JM (2009) Occupational exposure of farm workers to pesticides: Biochemical parameters and evaluation of genotoxicity. *Environ. Int*. 35, 273-278.

Reyes AA (1975) Phytotoxicity of binomial to saffron. *Phytopathology*, 65:1-6.

Sandalo LM, Dalurzo HC, Gomez M, Romero Puertes MC and Del Rio AA (2001) cadmium induced changes in growth and oxidative metabolism of pea plants. *J. Exp. Bot*. 52: 2115-2126.

Scebba F, Arduini I, Ercoli L. and Sebastiani L (2006) Cadmium effects on growh and antioxidant enzymes activities in Miscanthus sinensis, *Biologia plantarum*, 50:688-692.

Scott MD, Meshnick SR and Eaton JW (1987) Superoxide dismutase—rich bacteria, paraoxidal increase in oxidant toxicity. *J. Bio. Chem*, 1987, 262; 3640-3645.

Shah K and Dubey RS (1997) Cadmium alters Phosphate level and suppresses activity of Phosphorolytic enzymes in germinating rice seeds. *J. Agron Crop Sc.* 179(1):35-39. doi: 10.1111/j.1439-037X.1997.tb01145.x

Siddiqui ZS and Ahmed S (1996) Effect of systemic fungicides on seed germination, seedling growth and phenolic content of *Vigna radiata. Pak. J. Bot.,* 28: 191-193.

Siddiqui ZS, Ahmed S and Gulzar S (1997) Effect of topsin-M (Methyl-thiophenate) and Bayleton (Triademifon) on seedling growth, biomass, nodulation and phenolic content of *Sesbania sesban*. Bangladesh J. Bot., 26: 127-130.

Siddiqui ZS, Ahmed S and Shaukat SS (1999) Effect of systemic fungicide (Topsin-M) and insecticide (Dimecron) on germination, seedling growth and phenolic content of *Pennisetum americanum* L. *Pak. J. Biol. Sci*., 2: 182-184.

Soares W, Almeida RMVR, Moro S (2003) Rural work and risk factors associated with pesticide use in Minas Gerais, Brazil. *Cad. Saúde Pública.,* 19., 1117-1127.

Somashekaraiah BV, Padmaja K and Prasad ARK (1992) Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. Physiol. *Planta*. 85: 85-89.

Subhadra AV, Nanda AK, Behera PK and Panda BB (1991) Acceleration of catalase and peroxidase activities in *Lemna minor* L. and Allium cepa L. i8n response to low levels of aquatic mercury,*Environ.Pollut*. 69; 169-197

Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. *Plant Cell Environ.,* 13: 195 – 206.

Van Assche F, Cardianaels C and Clijsters H (1988) Induction of enzyme capacity in plants as a result of heavy metal toxicity. Dose- response relations in *Phaseolus Vulgaris L*. treated with Zinc and Cadmium. *Environ. Pollut*. 52: 103 – 116.

Wesseling C, Aragon A, Castillo L, Corriols M, Chaverri F, de la Cruz E, Keifer M, Monge P, Partanen TJ, Ruepert C, van Wendel de Joode B (2001) Hazardous pesticides in Central America. *Int. J. Occup. Environ. Health*, 7., 287-294.

Wilson JR (1970) Response to salinity in Glycine VI. Some effect of a range of short- term salt stress on the growth, nodulation and nitrogen fixation of Glycine wightii (formerly javanica). *Aust. J. Agric. Res*., 21:571-582.

RESEARCH ARTICLE

Comparative study of macrozoobenthos of Kunghada Bandh lake and Chamorshi lake, tah. Chamorshi, Dist. Gadchiroli, (India)

Tijare RV* and Kunghadkar GE

Department of Zoology, Govt. Institute of Science, Nagpur. R. T. Road, Civil Lines, Nagpur-440001, M. S., India. Email: rvtijare@rediffmail.com

Manuscript details:

Received: 13.10.2015
Revised : 26.11.2015
Accepted: 06.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Tijare RV and Kunghadkar GE (2015) Comparative study of macrozoobenthos of Kunghada Bandh lake and Chamorshi lake, tah. Chamorshi, Dist. Gadchiroli, (India). *International J. of Life Sciences*, 3(4): 360-366.

ABSTRACT

The present study deals with the qualitative and quantitative comparison between macrozoobenthos of Kunghad Bandh lake (20.22˚N - 80.01˚E) and Chamorshi Lake (19.55˚N - 79.52˚E). The collection and analysis of macrozoobenthos were done once in a month during two years i.e. February 2012 to January 2014. Total 19 species of macrozoobenthos were observed in Kunghada Bandh lake and 18 species in Chamorshi Lake belonging from phylum Annelida, Arthropoda and Mollusca. It is concluded that both the Lakes are rich in diversity of macrozoobenthos. Kunghad Bandh lake is shows slightly more diversity and quantity of macrozoobenthos as compare to Chamorshi Lake, due to good quality of water.

Key words: Macrozoobenthos, Chamorshi Lake, Kunghada Bandh, Gadchiroli.

INTRODUCTION

Most of the benthic fauna are the important indicators of water quality which indicates the past and current environmental and ecological status of an aquatic ecosystem (Hynes, 1960; 1962). A macro benthic invertebrate including both adult and larval forms varies according to their sizes (Cummins, 1975). They also act as an agent for the biomonitoring and proved to be very useful bio-indicators (Hofman, 1978). Many workers like Aston, 1973; Osborne *et al*., 1976; Jonason and Lindegard, 1979; Clare and Edwards, 1983 are carried out the work on the studies of benthic fauna in relation to the water quality. Most of the benthic organisms are devoid of backbone and inhibit the bottom substratum by spending almost their entire life to complete their life cycle (Rosenberg and Resh, 1922).

MATERIALS AND METHODS

Kunghad Bandh and Chamorshi Lake are situated at 20.22˚N - 80.01˚E and 19.55˚N - 79.52˚E respectively. Benthic organisms were collected from all five stations in the plastic bucket (white transparent of 5 liter capacity) by using Ekman's dredge and Van-Vin grab. Both the dredge is of medium size i.e. 6" X 6" X 6". The samples were collected monthly for the period of two years (February 2012 to January 2014) and categorized them according to their seasons e.g. 15th February to 15th May-Summer, 15th June to 15th September-Monsoon and 15th October to 15th January-Winter. Samples were collected during 10 am to 12 pm. and analyzed in the same day to avoid any error. The macrozoobenthos were snapped by using "Nikon Camera-Coolpix L29". The identifications or qualitative study were done by using various prescribed keys of Naidu and Shrivastava (1979), Tonapi (1980), Needam (1962) and Thorp (2009). For quantitative analysis, the segregated benthic organisms are counted species wise with naked eye or under binocular microscope. Their density are counted individuals (N) per M^2 and calculated by using the formula-$N/M^2 = n/A \times 10^4$

View of Kunghada Bandh

View of Chamorshi Lake

RESULTS AND DISCUSSION

Total 19 species were observed in Kunghada Bandh lake and 18 species in Chamorshi Lake of macrozoobenthos belonging to the Phylum from Annelida, Arthropoda and Mollusca. Out of 19 species of macrozoobenthos in Kunghada Bandh, 3 were of annelids**-:** i) *Limnodrillus hoffemeistry* of Family-Tubificidae and ii) *Lumbricus variegatus* of Family- Lumbricidae, Order-Haplotaxida and Class-Oligochaeta were observed during the collection of benthic organisms. iii) *Hirudinaria granulosa* of Family-Hirudinidae, Order-Hirudinida, and Class-Hirudinea. 9 species of Arthropods- i) *Hydracarina sp.* of Order-Trombdiformes, Class- Arachnida, ii) *Gelasimus sp.* of Family- Ocypodidae, Class- Arachnida, iii) *Dragonfly nymph* of Sub-order- Anisoptera, and iv) *Damselfly nymph* of Sub-order- Zygoptera, Order- Odonata, v) *Culex larve* and vi) *Anopheles larve* of Family Culicidae, vii) *Tabanus sp.* of Family- Tabanidae, Order- Diptera, viii) *Nepa cinerea* and ix) *Ranatra elongata* of Family-Nepidae, Order- Hemiptera, Class- Insecta and 7 were molluscans- i) *Vivipara bengalensis* of Family- Viviparidae, Order- Archtaenioglossa, ii) *Melanoides striatella* of Family- Tharidae, iii) *Fanus ater* of Family- Pachychilidae, Order-Sorbeoconcha, iv) *Lymnea luteola* of Family-Lymnaeidae, Order- Hygrophila, Class-Gastropoda, v) *Lamellidens marginalis*, vi) *Lamellidens correanus* and vii) *Parreysia corrugata* of Family- Unionidae, Order- Uniondia, Class- Bivalvia. While out of 18 species in Chamorshi Lake, 3 were annelids**-:** i) *Limnodrillus hoffemeistry* of Family-Tubificidae and ii) *Nais communis* of Family- Naididae, Order-Haplotaxida and Class-Oligochaeta were observed during the collection of benthic organisms. iii) *Hirudinaria granulosa* of Family-Hirudinidae, Order-Hirudinida, Class-Hirudinea, 10 species were arthropods**-:** i) *Hydracarina sp.* of Order-Trombdiformes, Class- Arachnida, ii) *Gelasimus sp.* of Family- Ocypodidae, Class- Arachnida, iii) *Dragonfly nymph* of Sub-order- Anisoptera, and iv) *Damselfly nymph* of Sub-order- Zygoptera, Order- Odonata, v) *Chironomous larve* of Family-

Table 1 : Kunghada Bandh - Analysis of macrozoobenthos (N/M^2) observed during February 2012 to Janary 2014 (Average of two years)

Class,Order	**Genus &**	**SUMMER (N/M^2)**				**MONSOON (N/M^2)**				**WINTER (N/M^2)**			
Family	**species**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Class-Oligochaeta	---	**377.78**	**311.11**	**155.56**	**44.44**	**111.11**	**266.67**	**244.44**	**377.78**	**355.56**	**400**	**311.11**	**244.44**
Family-Tubificidae	*Limnodrillus hoffemeistry*	222.22	155.56	88.89	0	44.44	155.56	133.33	200	177.78	222.22	177.78	133.33
Family-Lumbricidae	*Lumbricus variegatus*	155.56	155.56	66.67	44.44	66.67	111.11	111.11	177.78	177.78	177.78	133.33	111.11
Class-Hirudinea	*Hirudinaria granulosa*	**88.89**	**66.67**	**22.22**	**0**	**22.22**	**66.67**	**111.11**	**133.33**	**88.89**	**133.33**	**88.89**	**111.11**
Class-Arachnida	*Hydracarina* sp.	**88.89**	**66.67**	**22.22**	**0**	**44.44**	**88.89**	**111.11**	**111.11**	**133.33**	**155.56**	**111.11**	**111.11**
Class-Crustacea	*Gelasimus* sp.	**44.44**	**22.22**	**0**	**0**	**44.44**	**88.89**	**111.11**	**66.67**	**88.89**	**44.44**	**66.67**	**44.44**
Class-Insecta	---	**444.44**	**266.67**	**177.78**	**22.22**	**244.44**	**333.33**	**711.11**	**666.67**	**533.33**	**555.56**	**400**	**511.11**
Order-Odonata	---	**155.56**	**88.89**	**22.22**	**0**	**111.11**	**133.33**	**222.22**	**244.44**	**200**	**222.22**	**133.33**	**155.56**
Suborder-Anisoptera	*Dragonfly nymphs*	66.67	44.44	0	0	44.44	88.89	111.11	133.33	88.89	133.33	88.89	66.67
Sub.order-Zygoptera	*Damselfly nymphs*	88.89	44.44	22.22	0	66.67	44.44	111.11	111.11	111.11	88.89	44.44	88.89
Ord-Diptera	---	**177.78**	**44.44**	**88.89**	**0**	**66.67**	**200**	**288.89**	**266.67**	**288.89**	**288.89**	**244.44**	**244.44**
Fam-Culicidae	*Culex* larvae	66.67	22.22	44.44	0	22.22	44.44	111.11	88.89	111.11	111.11	88.89	44.44
Fam-Culicidae	*Anopheles* larvae	44.44	0	0	0	44.44	88.89	66.67	111.11	88.89	44.44	66.67	88.89
Fam-Tabanidae	*Tabanus* sp.	66.67	22.22	44.44	0	0	66.67	111.11	66.67	88.89	133.33	88.89	111.11

Table:1: Continued…

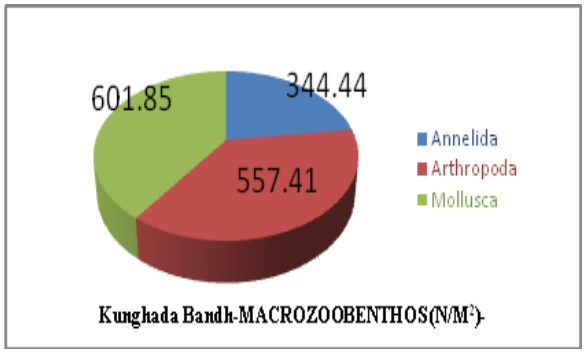
Class,Order	Genus &	SUMMER (N/M^2)				MONSOON (N/M^2)				WINTER (N/M^2)			
Family	**species**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Order-Hemiptera	---	**111.11**	**133.33**	**66.67**	**22.22**	**66.67**	**155.56**	**200**	**155.56**	**44.44**	**44.44**	**22.22**	**111.11**
Fam-Nepidae	*Nepa* sp.	66.67	66.67	44.44	0	44.44	88.89	111.11	66.67	44.44	0	22.22	66.67
Fam-Nepidae	*Ranatra eongata*	44.44	66.67	22.22	22.22	22.22	66.67	88.89	88.89	0	44.44	0	44.44
Class-Gastropoda	---	**488.89**	**288.89**	**155.56**	**44.44**	**133.33**	**355.56**	**444.44**	**533.33**	**577.78**	**466.67**	**488.89**	**444.44**
Family-Viviparidae	*Vivipara bengalensis*	200	133.33	66.67	44.44	0	111.11	155.56	177.78	155.56	111.11	133.33	155.56
Family-Thiaridae	*Melanoides striatella*	88.89	44.44	0	0	44.44	88.89	88.89	133.33	111.11	88.89	133.33	88.89
Family-Lymnaeidae	*Lymnea lutiola*	111.11	66.67	44.44	0	44.44	66.67	111.11	88.89	133.33	88.89	88.89	111.11
Family-Pachilidae	*Fanus ater*	88.89	44.44	44.44	0	44.44	88.89	88.89	133.33	177.78	177.78	133.33	88.89
Cla-Bivalvia	---	**200**	**133.33**	**66.67**	**0**	**200**	**266.67**	**311.11**	**377.78**	**311.11**	**377.78**	**288.89**	**266.67**
Family-Unionidae	*Lamellidens marginalis*	66.67	44.44	22.22	0	44.44	88.89	88.89	133.33	88.89	133.33	88.89	88.89
Family-Unionidae	*Lamellidens correanus*	88.89	44.44	44.44	0	88.89	88.89	88.89	133.33	155.56	111.11	111.11	88.89
Family-Unionidae	*Parreysia corrugata*	44.44	44.44	0	0	66.67	88.89	133.33	111.11	66.67	133.33	88.89	88.89
Total Number of species (N/M^2)=		**1733.3**	**1155.5**	**622.22**	**111.11**	**800**	**1622.2**	**2044.44**	**2266.6**	**2088.8**	**2133.3**	**1755.5**	**1733.3**

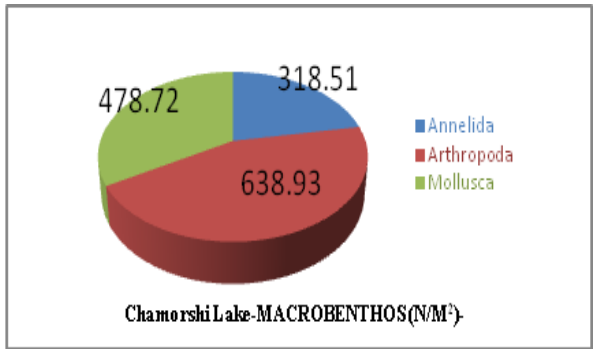
Table 2: Chamorshi Lake - Analysis of macrozoobenthos (N/M^2) observed during February 2012 to January 2014 (Average of two years)

Class,Order	Genus &	SUMMER (N/M^2)				MONSOON (N/M^2)				WINTER (N/M^2)			
Family	**species**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Class-Oligochaeta	---	**355.56**	**244.44**	**133.33**	**44.44**	**155.56**	**222.22**	**266.67**	**377.78**	**355.56**	**333.33**	**222.22**	**266.67**
Family-Tubificidae	*Limnodrillus hoffemeistry*	200	111.11	111.11	22.22	44.44	88.89	111.11	200	200	155.56	133.33	155.56
Fam-Naidiae	*Nais communis*	155.56	133.33	22.22	22.22	111.11	133.33	155.56	177.78	155.56	177.78	88.89	111.11
Class-Hirudinea	*Hirudinaria granulosa*	**44.44**	**66.67**	**22.22**	**0**	**44.44**	**66.67**	**88.89**	**133.33**	**111.11**	**111.11**	**88.89**	**66.67**
Class-Arachnida	*Hydracarina* sp.	**111.11**	**66.67**	**22.22**	**0**	**66.67**	**177.78**	**244.44**	**288.89**	**244.44**	**244.44**	**155.56**	**155.56**
Class-Crustacea	*Gelasimus* sp.	**66.67**	**44.44**	**22.22**	**0**	**0**	**88.89**	**133.33**	**155.56**	**155.56**	**133.33**	**111.11**	**88.89**
Cla-Insecta		**444.44**	**333.33**	**200**	**66.67**	**311.11**	**555.56**	**733.33**	**755.56**	**688.89**	**755.56**	**555.56**	**511.11**
Order-Odonata	**---**	**111.11**	**66.67**	**22.22**	**0**	**88.89**	**155.56**	**200**	**222.22**	**222.22**	**222.22**	**133.33**	**111.11**
Suborder-Anisoptera	*Dragonfly nymphs*	44.44	22.22	22.22	0	44.44	88.89	111.11	133.33	88.89	133.33	88.89	44.44
Suborder-Zygoptera	*Damselfly nymphs*	66.67	44.44	0	0	44.44	66.67	88.89	88.89	133.33	88.89	44.44	66.67
Order-Diptera	---	**222.22**	**155.56**	**111.11**	**22.22**	**111.11**	**244.44**	**355.56**	**400**	**422.22**	**511.11**	**400**	**288.89**
Fam.-Tendipididae	*Chironomous larve*	66.67	44.44	22.22	0	44.44	44.44	88.89	88.89	133.33	177.78	133.33	88.89
Fam-Culicidae	*Culex larvae*	44.44	22.22	0	0	44.44	66.67	133.33	88.89	88.89	133.33	88.89	66.67
Fam-Culicidae	*Anopheles larvae*	22.22	0	44.44	22.22	0	44.44	88.89	133.33	111.11	66.67	88.89	44.44
Fam-Tabanidae	*Tabanus* sp.	88.89	88.89	44.44	0	22.22	88.89	44.44	88.89	88.89	133.33	88.89	88.89

Table 2: continued...

Class,Order	Genus &	SUMMER (N/M^2)				MONSOON (N/M^2)				WINTER (N/M^2)			
Family	**species**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Order-Hemiptera	---	**111.11**	**111.11**	**66.67**	**44.44**	**111.11**	**155.56**	**177.78**	**133.33**	**44.44**	**22.22**	**22.22**	**111.11**
Fam-Nepidae	*Nepa* sp.	66.67	44.44	44.44	0	44.44	66.67	111.11	66.67	44.44	0	22.22	44.44
Fam-Nepidae	*Ranatra elongata*	44.44	66.67	22.22	44.44	66.67	88.89	66.67	66.67	0	22.22	0	66.67
Class-Gastropoda	---	**355.56**	**222.22**	**44.44**	**44.44**	**111.11**	**333.33**	**488.89**	**422.22**	**511.11**	**400**	**400**	**400**
Family-Viviparidae	*Vivipara bengalensis*	111.11	88.89	0	44.44	0	88.89	177.78	133.33	133.33	88.89	88.89	155.56
Faily-Ampullariidae	*Pila globusa*	44.44	22.22	0	0	44.44	66.67	88.89	44.44	88.89	88.89	44.44	88.89
Family-Planorbidae	*Indoplanorbis exustus*	88.89	44.44		0	44.44	88.89	88.89	111.11	133.33	111.11	133.33	66.67
Fam-Tharidae	*Melanoides tuberculata*	111.11	66.67	44.44	0	22.22	88.89	133.33	133.33	155.56	111.11	133.33	88.889
Class-Bivalvia-	*Parreysia corrugata*	**66.67**	**22.22**	**0**	**0**	**44.44**	**88.89**	**111.11**	**111.11**	**66.67**	**111.11**	**88.89**	**44.44**
Total no. of species =		**1377.7**	**933.33**	**444.44**	**155.56**	**711.11**	**1444.4**	**1800**	**2000**	**1977.7**	**1911.1**	**1511.1**	**1444.4**





Chironomidae, vi) *Culex larvae* and vii) *Anopheles larvae* of Family Culicidae, viii) *Tabanus sp.* of Family- Tabanidae, Order- Diptera, ix) *Nepa cinerea* and x) *Ranatra elongata* of Family- Nepidae, Order- Hemiptera, Class- Insecta and 5 species were molluscans- i) *Vivipara bengalensis* of Family- Viviparidae and ii) *Pila globusa* of Family- Ampullariidae, Order- Architaenioglossa, iii) *Indoplanorbis exustus* of Family- Planorbidae, Order- Hygrophila, iv) *Melanoides tuberclata* of Family- Tharidae, Order- Sorbeoconcha, Class- Gastropoda, v) *Parreysia corrugata* of Family- Unionidae, Order- Uniondia, Class- Bivalvia.

In Chamorshi Lake minimum average total macrozoobenthos (727.78 N/M^2) was recorded in summer and maximum average total macrozoobenthos (1711.11 N/M^2) in winter as compared to the annual average total macrozoobenthos (1309.26 N/M^2). In Kunghada Bandh minimum average total macrozoobenthos (905.56 N/M^2) was recorded in summer and maximum average total macrozoobenthos (1927.18 N/M^2) in winter as compared to the annual average total macrozoobenthos (1505.56 N/M^2). The seasonal fluctuation of macrozoobenthos is occurs might be due to quantity of water and depth of water body is generally decreases in summer while increases in monsoon and winter, as more the quantity of water more will be the organisms.

In Kunghada Bandh, percentage of annelids is 29.91%, while 22.18% in Chamorshi Lake. Percentage of arthropods in Kunghada Bandh is 37.07%, while 44.49% in Chamorshi Lake. In Kunghada Bandh, percentage of molluscs is 40.02%, while 33.33% in Chamorshi Lake.

REFERENCES

Aston R J (1973) Tubificids and water quality: a review. *Envi. pollution* 5: 1-10.

Clare P and Edwards RW (1983) The Macro invertebrate fauna of the drainage channels of the Gwent levels, South wales. *Fresh water Biology*, 13: 202-225.

Cummins KW (1975) Macroinvertebrates: Whitton, B. A. (Ed.) *River Ecology*: Blackwell Scientific publication, Oxford London, pp. 725.

Hoffman W (1978) Analysis of animal microfossils from the Grosser Segebergerse (F. R.G.). *Arch. Hydrobiology.,* 82 (1-4): 316-346.

Hynes HBN (1960) The biology of pollute waters. Liverpool, Liverpool University press, 202 pp. in the assessment of water pollution in Sweedish lakes. *Zoon* 1: 125-139.

Hynes HBN (1962) The Invertebrate fauna of a Welch mountain stream. Arch *Hydrobiol*, 57: 344-388.

Jonason PM and Lindegard CH (1979) Zoobenthos and contribution to the metabolism of Shallow lakes. *Arch. Hydrobiology*. Beih. Ergebn. Limnol., 13: 162-180.

Naidu K Vanmala and Shrivastava HN (1979) Some fresh water oligochaetes of Nagpur, India. *Hydrobiologia*, Volume 72, Issue I, Pg: 261-271.

Needam (1962) A guide to study of fresh water biology, Holen day Inc. San Fancisco, 5th ed .

Osborne A, Wanielista P and Yousef AA (1976)Benthic fauna species diversity in six central Florida Lakes in summer. *Hydrobiologia*, 48 (2): 125-129.

Rosenberg DM and Resh WH (1992) Freshwater Biomonitoring and Benthic Macro invertebrates. Cahpman & Hall publications, New York, London. p. 488.

Thorp JH and Covich AH (2009) Ecology and classification of North American Fresh water invertebrates. Elsevier Publication, Academic Press, London, UK.

Tonapi GT (1980) Freshwater animal of India: *An ecological approach*, Oxford and IBH Publishing company, New Delhi pp. 341

RESEARCH ARTICLE

Pharmacological effect on scals of Rasbora elenga

Swamy Meena

Asst. Prof. of Zoology, Government Autonomous Post Graduate College, Chhindwara, M.P. India
E.mail id- smtmswamy@gmail.com

Manuscript details:

Received: 23.10.2015
Revised: 03.11.2015
Accepted: 26.11.2015
Published: 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Swamy Meena (2015) Pharmacological effect on scals of Rasbora elenga. *International J. of Life Sciences*, 3(4): 367-374.

ABSTRACT

In the present study it is observed that the histamine *perse* caused dose dependent significant aggregating effects in the dorsal skin melanophores of *Rasbora elenga*. Higher concentrations of histamine caused more sever aggregating effects of the scale melanophores.The isolated scale melanophores of fish *R.elanga* maintained an intermediate state in physiological solution of 0.7 % NaCl. After this, melanophores are pretreated with a specific H_3 Receptor antagonist thioperamide in the concentration of 2×10^{-6} g/ml. They were later incubated in increasing concentrations of histamine in a logarithmic scale of 1×10^{-6} g/ml to 6.4×10^{-5} g /ml. In the lowest concentration of 1×10^{-6} g/ml of histamine it was observed that thioperamide blocked the melanophore aggregation effect of histamine *per se.* Increase in the concentration of histamine with the blocker thioperamide to 3.2×10^{-5} g /ml the teleost melanophores were not able to aggregate in the presence of the antagonist. After that the melanophores of R.elenga were previously bathed in 0.7% Saline in order to bring the melanophores in the neutral state, where melanophores remained in a state of neither aggregation nor dispersion. When such scale melanophores were incubated with H_3 Agonist immethridine it was observed that immethridine induced physiologically significant melanophore aggregation in all concentration. The intial concentration of immethridine *perse* of 1×10^{-6} g/ml caused the melanophores to decrease in size showing aggregation. Increase in the dose concentration of immethridine *perse* to 6.4×10^{-5} g /ml, induced a complete aggregation of all the melanophores

Keywords: *Histamine, Thioperamide, Immethridine, Melanin granules, Aggregation*

INTRODUCTION

The pigment cells of vertebrates (melanophores/ melanocytes) are specialized type of smooth muscle cells which due to their

intracellular movement of melanin granules , control skin hue. Colour change in vertebrates represents some of the most drametic example of adaption to the environment and a scientific interact in this phenomenon can be traced back to the days of Aristotle. Change in or colour patterns are mediated through the activity of integumentary pigment containing cell called melanophores (Parkers, 1948) Physiological colour changes are due to the motile activities of pigment granules within chromatophores (Fuji, 1969, Bagnara and Hadley, 1973 & Fuji & Oshima, 1986). The findings of the present study are very important from pharmacological characterization point of view, as histaminargic receptorss of all the four sub typewhich have been recentaly discovered have been found to be present on the melanophores of *Rasbora. Elenga.*

In the vertebrates several types of chromatophores can be distinguished and these are classified into five categories. According to the type of pigment they contain:

1. Melanophore (Black or brown)
2. Erythropore (Red)
3. Xanthophore (Yellow)
4. Leucophore (white)

Melanophores are spherical or ellipsoidal bodies with an average diameter of about 0.5 µm. Each melanosomes is surrounded by a limiting membrane. The brown or black pigment in the melanosome have been shown to be melanin's, which are highly polymerized studies these melanophores of a indian fresh water carp *Rasbora elanga* have been investigeted to unviel the mechanism involved i.e. aggregation or dispersion. The melanophores of vertebrates are generally controled either neural or hormonal control exist (Fuji, 1969, Bagnara and Hadley, 1973 & Fuji & Oshima, 1986).

***Chromatophores*:**

The word Chromatophore is derived from the Greek word Chroma = colour, phore = to bear. Chromatophore is integumentary coloured cells which contain pigment that can disperse or concentrate thereby changing the colour of the barers. These cells are located in the skin, scales or even in certain deeper tissues of the body.

1. The chromatophores on the basis of the colour pigment present in the have been classified as Black or brown melanophores, containing melanin granules.
2. White reflecting Iridophore containing guanine rich reflecting platelets.
3. Yellow Xanthophore containing caretenoid vesicles pteridine rich pterinosomes.
4. Red Erythropores, also containing caretenoid vesicles and pterinosomes.

***Melanophores*:**

Melanophores are the black or brown pigments cells present in the skin and scales of fish. The melanophores are the best known of all pigment cells and are perhaps the most important of the cell active in colour change since the skin of fish is different from the other "terrestrial vertebrate."

***Origin*:**

A wide histological observation on the embryonic and adult tisseues of every class of vertebrate has has been brought to bear upon the general problem of melanophores origin. After carefully examination of various theories, it has been confirm that the neural crest is the origin of melanophores (Rawles, 1948; Wild CE Jr 1961; Bagnara & Hadley 1973).

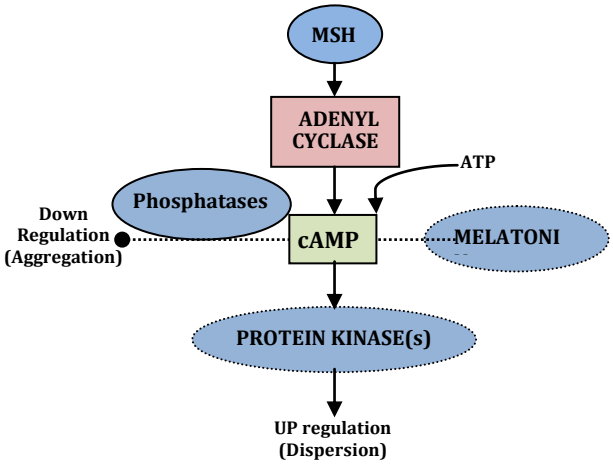
***Morphology*:**

Melanophores are ovoid; astes shaped cells with long dendritic protruding out from a central core, resembling the nerve cells. In fish the melanophores are arranged in definite some in the anterior half of the scales which remain embedded in the skin.

***Melanin and its formation*:**

The melanin pigment is a complex polymer derived from tyrosinase with a high molecular weight and great stability. The synthesis of melanin takes place in specialized vesicles, the melanosomes of melanocyte. The melanin synthesis involve first the oxidation of tyrosinase

to 3, 4 –di hydroxl phenyl alanine and then to dopaquinone. The enzyme concerned is copper containing tyrosinase. Dopaquinone is then polymerized to form melanin which is usually found attached to a protein. . Melanophores transport their pigment in response to extracellular cause, neurotransmitters in the case of fish and hormonal stimuli in the case of frogs. In both cases, melanosomes dispersion is induced by elevation of intracellular cAMP levels, while aggregation is triggered by depression of cAMP. (Reiter, 1985).



Types of Melanophores:

Two distinct types of melanophores are generally marked among vertebrates; these are the dermal melanophores and the epidermal melanophores. The dermal melanophores of fishes are involved in the colour changes. The epidermal cells are elongated and are often referred to as spindle shaped.

***Hormonal control of colour change in fishes*:**
Pigment movment withine the melanophores is a highly specialized phenomenon. The translocation of pigment is displayed either by aggregation or dispersion across the cytoplasm, which is controlled by hormone. (Fuji and novales, 1969; baganara and hadley, 1973; Fuji and Oshima, 1994; Fuji, 2000 and salim and Ali, 2011,2012)

***Neural control of colour change in fishes*:**
Chromatophores responsible for the skin colouration are predominantly under nerves control. The autonomic system has two major components, the sympathetic and parasympathetic. System controls effects or response opposite in nature to the other i.e. one excitatory and the other inhibitory depending on the tissues.

The aggregation nerves fiber have been identified to be adrenergic in nature by histochemical methods, when the melanin aggregation fiberd were concluded to belong to the sympathetic division, an assumption was naturally made that the parasympathetic nerve might be involved in dispersing the melanophores.

Pharmacological control of colour change in fish

***Histamine*:**
An amine causing contraction of muscle in hollow organs and dilution of capillaries released by cells in response to injury in allergic and inflammatory reaction.

Receptor:
An organs which capable to respond to an external stimulus such as light, heat or a drug & transmit a signal to sensory nerve.

There are 5 typse o f receptors present in in-vertebrate animal. These are followin g type
1 histaminergic receptors
2 cholinergic receptors
3 Beta adrenergic receptors
4 Gabaergic receptor
5 adrenergic receptors

The Histamine receptor are a class of G-protein coupled receptors with receptor as their endogeneous ligand. There are four known histaminergic receptor H_1, H_2, H_3 & H_4.

Histamine Agonist:
Histamine agonist is a drug which causes increased activity at one or more of the four histamine subtype.

Histamine receptor agonist are as follows:

H_1 – Receptor Agonist – Pyridyl ethyl amine

H_2– Receptor Agonist - Amthamine

H_3 – Receptor Agonist - Immethridine

H_4 – Histamine Receptor Agonist – VUF8630

Histamine Ligand Antagonist:

H_1 – Receptor Antagonist – Diphendramine

H_2– Receptor Antagonist – Ranitidine

H_3 – Receptor Antagonist – Thioperamide

H_4 – Receptor Antagonist – JNJ7777120

Drugs used for experiment:

1. H_3- Immethridine (Agonist)
2. H_3- Thioperamide (Antgonist)

MATERIALS AND METHODS

The teleostean fish *Rasbora elenga* has been selected for studying the effects of recent new class of histaminergic drugs on its isolated dorsal scale melanophores, in order to find the nature and role of receptors of histaminergic type in controlling skin pigmentation processes. The fish was selected because of its easy availability, sturdy nature as it can be kept live in laboratory conditions for long periods and the fact that its melanophores are excellent model for *in vitro* studies, and no study has been done on them till now. They were caught with the help of fishermen from various water bodies and transported to the laboratory alive and they were kept in glass aquaria containing 100L of dechlorinated tap water. Experiments were performed in the laboratory conditions having ambient temperature of 25-30° C with a pH of 7.2 to 7.4. Prior to the experiments, the fish were allowed to acclimatize to laboratory conditions for 3 days. Diseased, injured, or lethargic fish were removed and only active, uniformly colored fish were used. For the *in vitro* studies, the fish scales were removed in accordance with the method of Spaeth, (1913) which included the removal of 20–25 scales from the dorsolateral region of live *R. elenga* kept in a wet cloth, held loosely. The scales were removed by forceps from the dorsal lateral pigmented area. These were immediately placed in 0.7% normal saline, containing 700 mg of sodium chloride in 100 mL of double distilled water. They were equilibrated in saline medium for 7–10 min with frequent shaking.

The responses of control as well as of those melanophores that were incubated in 10 mL 0.7% fish saline containing various concentrations starting from 1×10^{-6} to 6.4×10^{-5} g/mL of Histamine, Thioperamide (Specifi c H3 antagonist) and Immethridine (Specifi c H3 agonist) *perse*. Responses of the melanophores were measured in accordance with the method of Bhattacharya *et al.*, (1976) based on Hogben and Slome (1931). In this method, actual diameter (length×width with the processes) of 10 randomly selected melanophores from each scale was measured using a Leitz Occulometer calibrated previously with stage micrometer. The value was then multiplied by the unit of the micrometer 15 µm. Thereafter, the arithmetical mean was calculated and this value was then divided by 100 to obtain the values. This was the mean melanophore size index (MMSI).

Ten scales of fish were used in various dishes with each dish having a different concentration of drugs. After a constant incubation (07-10 min) period, the MMSI of ten of such treated melanophores from each concentration was recorded. Thus a set of experiment comprised the measurement of responses of about hundred melanophores.

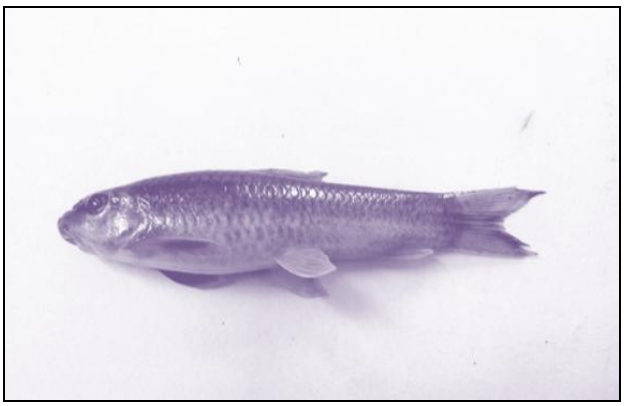


Fig. 1 showing photograph of *Rasbora elanga*

Measurement methods

Individual melanophores were measured with the Ocular-meter (Erma, Japan) in look power microscope and melanophores size index was calculated according to the method of Bhattacharya *et al.* (1976). The observed values have been multiplied by unit of micrometer which was 15µm. Thereafter the mean was calculated and this value was divided by 100 to obtain a value in a digit with three decimal points. This was Mean Melanophores Size Index (MMSI). Statistical analysis of data was conducted according to Cochran – 1967.

Statistical analysis

Statistical data analyses are presented as mean standard error of the mean (SEM) and n = 7, which represents the number of individual experiments conducted with equal numbers of animals. Comparisons were made between treated and control groups by use of Student's t-test. All data were analyzed using GraphPad Prism software (UK). $P < 0.05$ indicates statistically signifi cant difference.

$$(\text{MMSI}) \frac{\text{VD} \times \text{HD}}{100} \text{ X } 15$$

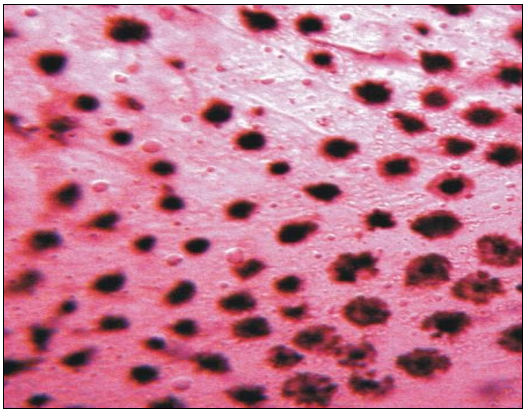
Where,

VD = Vertical diameter

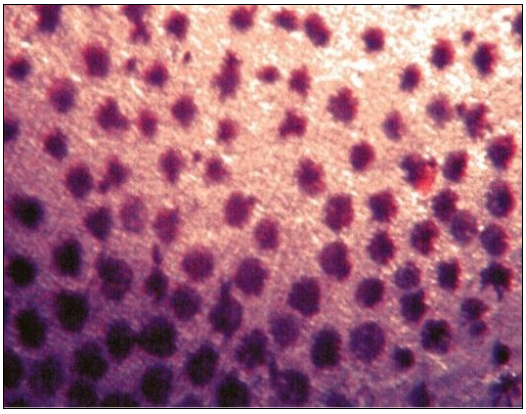
HD = Horizontal diameter

RESULTS AND DISCUSSION

Histamine is an important autacoid biogenic amine present in all biological tissues, and also regarded as a chemical mediator and neurotransmitter on broad spectrum physiological level (Goodman and Gillman, 2006). It is contained in mast cells and basophiles found in all animal and mammalian tissues in both neural and non-neural compartments (Goodman and Gillman, 2006). In the present study histamine *per se* aggregated the dorsal skin melanophores of *R. elenga* in varying doses ranging from 1×10^{-6} g/ml to 6.4×10 10^{-5} g /ml. The MMSI decreased from the control value of 4.532 ± 0.1282 to 0.9257 ± 0.05455 by the highest dose of histamine. The different concentration of histamine used in the present study ranging from 1×10^{-6} g/ml to 6.4×10 10^{-5} g /ml, could gradually and markedly aggregate the scale melanophores, since the MMSI decreased only slightly in lower dose from the control value of 4.532 ± 0.128 to 4.157 ± 0.223 as seen by the first concentration of 1×10^{-6} g/ml of histamine per se. Higher concentrations of histamine caused more sever aggregating effects of the scale melanophores. From these result it becomes clear that histamine *per se* caused dose dependent significant melanin aggregating effects in the melanophores of the fish, *Rasbora elenga* in all concentration used.



Melanin granule aggregation in melanocyte cell (In *Rasbora elenga*)



Melanin granule Dissappearsion in melanocyte cell (In *Rasbora elenga*)

Table 1- Showing the effect of Histamine perse, on the response of *R. elanga* isolated dorsal scale melanophores MMSI.

No. of exp.	Experimental drugs	Dose in µg/ml	MMSI ± SE	P-value
07	Control	**0.7% saline**	4.532 ± 0.128	
07	Histamine Perse	**1×10^{-6}**	4.532± 0.128	0.0016
07		**2×10^{-6}**	3.284± 0.098	0.5446
07		**4×10^{-6}**	2.380 ± 0.072	0.1871
07		**8×10^{-6}**	1.695 ± 0.056	0.0669
07		**1.6×10^{-5}**	1.307 ± 0.033	0.0044
07		**3.2×10^{-5}**	1.106 ± 0.027	0.0016
07		**6.4×10^{-5}**	0.925 ± 0.054	0.0565
07		**Reimmersion in 0.7% saline water**	4.441 ±0.113	0.7793

Table no.2 Showing the effect of H3 antagonist Thioperamide on the response of *R. elanga* melanophore MMSI

No. of exp.	Experimental drugs	Dose in µg/ml	MMSI ± SE	P-value
07	Control	**0.7% saline**	4.680 ± 0.074	
07	Thioperamide Perse (antagonist)	**1×10^{-6}**	3.990 ± 0.038	0.1254
07		**2×10^{-6}**	4.140 ± 0.038	0.1353
07		**4×10^{-6}**	4.847 ± 0.049	0.3476
07		**8×10^{-6}**	4.393 ± 0.015	0.0012
07		**1.6×10^{-5}**	4.006 ± 0.083	0.7962
07		**3.2×10^{-5}**	3.990 ± 0.038	0.1254
07		**6.4×10^{-5}** **Reimmersion in 0.7% saline water**	3.719 ± 0.044 4.659 ± 0.089	0.2343 0.6775

Note :- (concentration of antagonist **2×10^{-6}**, value of MMSI– 4.433 ± 0.052, P -value 0.4048)

Table no.3 Showing the effect of H3 agonist Immethridine on the response of *R. elanga* isolated melanophore. MMSI

No. of exp.	Experimental drugs	Dose in µg/ml	MMSI ± SE	P-value
07	Control	**0.7% saline**	4.543 ± 0.124	
07	Immethridine Perse (Agonist)	**1×10^{-6}**	4.157 ± 0.027	0.002
07		**2×10^{-6}**	3.284 ± 0.098	0.5976
07		**4×10^{-6}**	2.380 ± 0.072	0.2130
07		**8×10^{-6}**	1.695 ± 0.056	0.0779
07		**1.6×10^{-5}**	1.307 ± 0.033	0.0052
07		**3.2×10^{-5}**	1.157 ± 0.037	0.0106
07		**6.4×10^{-5}**	1.524± 0.184	0.3565
07		**Reimmersion in 0.7% saline water**	4.441 ± 0.113	0.8405

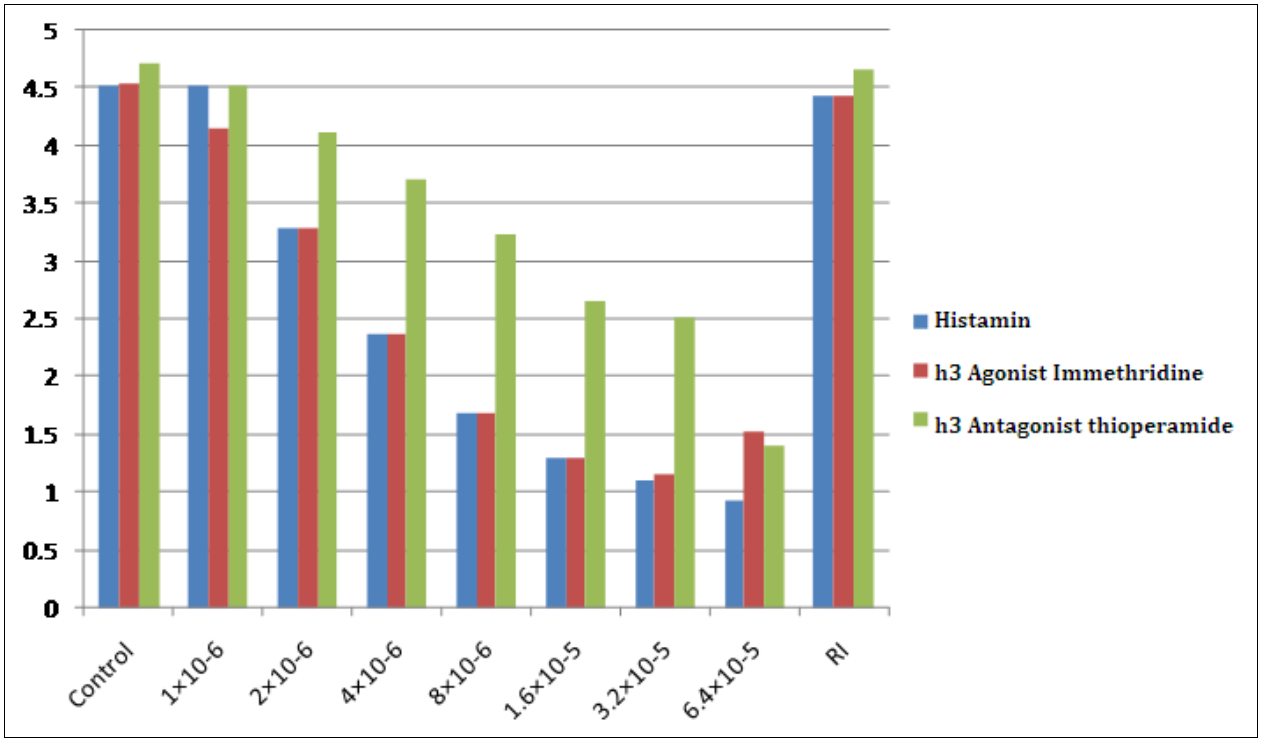


Fig. 2: Showing the effect of Histamine perse, specific H_3 receptor Antagonist Thiperamide, H3 agonist Immethridine on the response of MMSI of *R. elanga* scale skin melanophores

The isolated scale melanophores of fish *R.elanga* maintained an intermediate state in physiological solution of 0.7 % NaCl. After this, melanophores are pretreated with a specific H_3 Receptor antagonist thioperamide in the concentration of 2×10^{-6} g/ml. They were later incubated in increasing concentrations of histamine in a logarithmic scale of 1×10^{-6} g/ml to 6.4×10^{-5} g /ml. In the lowest concentration of 1×10^{-6} g/ml of histamine it was observed that thioperamide blocked the melanophore aggregation effect of histamine *per se.* The MMSI at this stage remained 3.990 $\pm$ 0.0380 which is almost near the MMSI at the control melanopores of 4.680 $\pm$ 0.07464. Increase in the concentration of histamine with the blocker thioperamide to 3.2×10^{-5} g /ml the teleost melanophores were not able to aggregate in the presence of the antagonist and MMSI remained at 3.990 $\pm$ 0.1254. The blocked of the histamine melanophore aggregating effect by thioperamide contined even when the highest concentration of histamine i.e. 6.4µg/ml was employed, where no aggregation was observed and the MMSI recorded as 3.719 $\pm$ 0.014453. In the absence of antagonist thioperamide, the effect of histamine was highly aggregating and the MMSI was 0.9257 $\pm$ 0.0545.

After that the melanophores of R.elenga were previously bathed in 0.7% Saline in order to bring the melanophores in the neutral state, where melanophores remained in a state of neither aggregation nor dispersion. When such scale melanophores were incubated with H_3 Agonist immethridine in the concentrations ranging from 1×10^{-6} g/ml to 6.4×10^{-5} g /ml, it was observed that immethridine induced physiologically significant melanophore aggregation in all concentration.

The intial concentration of immethridine *perse* of 1×10^{-6} g/ml Caused the melanophores to decrease in size showing aggregation and the MMSI at this stage was reduced a control value of

4.543 ± 0.1240 to 4.157 ± 0.027. Increase in the dose concentration of immethridine *perse* to 6.4×10^{-5} g /ml, induced a complete aggregation of all the melanophores thus making the melanophores appear ball like or punctate, where the MMSI was found to be 1.524 ± 0.1844 from a control value of 4.543 ± 0.124 .

It was later found that when the highest concentration i.e. 6.4×10^{-5} g /ml immithridine treated scale melanophores were washed repeatedly with teleost 0.7% saline and re-immersed for 15-20 minutes, the melanophores aggregation effect completely dissappeared and the melanophores returned to their control state of neither aggregation nor dispersion. At this stage of the MMSI of the melanophores had become 4.441 ± 0.1138 which is almost near the control value of 4.543 ± 0.124.

In the present investigation clearly indicated that histamine and immithridine induced the aggregation of melanophores in the fish scale while thioperamide blocked the melanophore aggregation effect of histamine *per se* These data have been considerable significance in relation to the species diversity, which is not only found in genus level of this species.

REFERENCES

Bagnara and Hadley (1973) Chromatophores and colour change.Prentice Hall, Engelewood, Cliffs, New Jersey.

Bagnara and Hadley (1973) Chromatophore movements in teleost fishes.

Bhattacharya *et al.* (1976) Effect of catecholamines on the melanophores of frog *Rana tigrina*. *Ind.J.Expt. Biol*., 14; 486-488.

Fuji R (1969) Chromatophore and Pigment. In : Fish Physiology, 3; 307- 353.(Eds. W.H. Hoar and Randall, D.J.) Academic Press New York.

Fuji and Oshima (1986) Control of Chromatophore movements in teleost fishes. Zool Sci. 3; 13-47.

Hogben and Slome (1931) The pigmentory effector System VI. The Dual Character of endocrine co-ordination in amphibian colour change. Proc. R. Soc. London. 108; 10-53.

Goodman LS and Gilman A (2006) The pharmacological basis of therapeutics. McGraw Hill.

Parkers GH (1948) Animal Colour Changes and their Neurohumours. Cambridge University Press, London.

Rawles (1948) Origin of melanophores and their role in the color patterns in vertebrates. Physiol. Rev. 28; 383-408

Spaeth RA (1913) The Physiology of the chromatophores of fishes. J.Exptl. Zool. 15; 527-585.

Swamy Meena (2014) Studies of Cholinergic and Histaminergic Drugs on Melenophores of a Teleost Fish Rasbora elanga (Ham.) Int. J. of Life Sciences, 2014, Vol. 2(1): Page no. 13-16

Swamy Meena (2014) Effects of histamine and its new H1 and H2 receptor agonists on the isolated scale melanophores of teleost fish, Rasbora elenga. Biosci. Biotech. Res. Comm. Vol.7(1): Page no. 84-88 (2014).

Wild CE Jr (1961) The differentiation of vertebrate pigment cell. Advances in morphogenesis, (1) M. Abercrombie and J. Brachet (Eds. Academic Press, New York, 267-300).

RESEARCH ARTICLE

Gonado-Somatic Index of *Gerres oblongus* (Cuvier) from Mithbav estuary, Sindhudurg district, Maharashtra, India

Yeragi SS and Yeragi SG

Department of Zoology, K.J.Somaiya College of Science, Vidhayavihar Mumbai-400 077
Email : dryeragi@gmail.com

Manuscript details:

Received: 03.09.2015
Revised : 12.11.2015
Accepted: 10.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Yeragi SS and Yeragi SG (2015) Gonado-Somatic Index of *Gerres oblongus* (cuvier) from Mithbav estuary, Sindhudurg district, Maharashtra, India.
International J. of Life Sciences, 3(4): 375-378.

ABSTRACT

Gonado-Somatic Index (GSI) of *Gerres oblongus* (Cuvier) ranged from 0.3 to 1.6 in male and 0.8 to 3.9 in female and seen high peak in the month of June-July in both the sexes. During the present investigation the higher values of GSI were observed from May to July which ranges between o.9 to 1.4 in the first year and 1.1 to 1.6 in second year for males and 1.9 to 3.8 in the first and 2.4 to 3.9 in the second year for females. Therefore, it was noticed that the fish spawned once in a year with one spawning peak in the month of June-July as indicated by the Gonado-Somatic Index values.

Keywords: GSI, Mithbav, *Gerres oblongus*

INTRODUCTION

Gerrres oblongus (Cuvier) are commonly seen on shallow sand flats of Mithbav estuary (L.16^0 20' N.L. 17^0 25'). They are usually seen in small schools. This species gets large and is more slender when adult compared to other silver bellies in the area. This type of body structure enhances the fishermen to take out the entangled fish easily through the mesh of nets. They are available throughout the year of standard length ranged between 5 to 7cm (SL). The caudal fin is very much forked with long lobes. It has greatly expandable mouth. They are bottom as well as column feeders. They are shallow water species. The standard fishing size wise ranging between 4 to 5.5 cm.

Reproductive cycle is the significant stages in the life span of a fish, which in combination with others ensures the continuation of the species. It is directly co-related with the fishery management. The availability of favorable condition in an aquatic environment enhance the life cycle of the fish to recruit more fish population. This species is commercially very much important to fulfil the basic

economic need of the coastal people. It is but natural to understand their spawning time to avoid over fishing for the particular period. It helps to assess the reproductive potential of a population. The present study was under taken of *G.oblongus,* which is a near shore species which penetrate into estuaries to a considerable distance.

MATERIAL AND METHOD:

The live fish samples were collected from Mithbav estuary fortnight for the period of two years. The specimens were brought to the local laboratory and removing the surface moistere with blotting paper, each fish was measured for its total length to the nearest 1mm and weighed to the nearest 0.01gm. Fish were cut open to find out their sex and matarity stages and noticed together with colour, length, and weight of the gonads. The gonads were dissected out and preserved in 4% for maldehyde for further investigation. The fluctuation in the weight of the gonad in relation to weight of the fish was studied to indicate the spawning of the fish, was stanched to indicate the spawning of the fish. The weight of the individual fish was recorded. The gonads were removed carefully and noticed their weight.

The GSI was calculated by using the formula.

$$GSI = \frac{\text{Weight of the Gonad}}{\text{Weight of the fish}} \times 100$$

Fig. 1: *Gerres oblongus* (Cuvier)

RESULTS

To find out the approximately the time of fully matured and spawning period to avoid the fishing in an aquatic environment is always essential to control the fluctuation of fishery. This study will definitely focused toward the awareness of coastal people. The Gonado Somatic Index of male and female *Gerres oblongus* was calculated for different months from Jan.2011 to Dec.2012 as shown in Fig. 2 & 3.

Table 1: Mean Gonado-Somatic Index of males and females of *Gerres oblongus* for the period of two year (2011-2012).

Months 2011	Males	Females	Months 2012	Males	Females
January	0.5	0.8	January	0.6	1.1
February	0.6	0.9	February	0.6	01.3
March	0.7	1.2	March	0.8	1.4
April	0.8	1.7	April	0.9	1.9
May	0.9	1.9	May	1.1	2.04
June	1.3	2.9	June	1.5	3.1
July	01.4	3.8	July	1.6	3.9
August	0.3	1.2	August	0.4	1.4
September	0.4	1.3	September	0.5	1.6
October	0.4	0.8	October	0.5	0.9
November	0.5	0.9	November	0.6	1.1
December	0.5	1.1	December	0.7	1.2

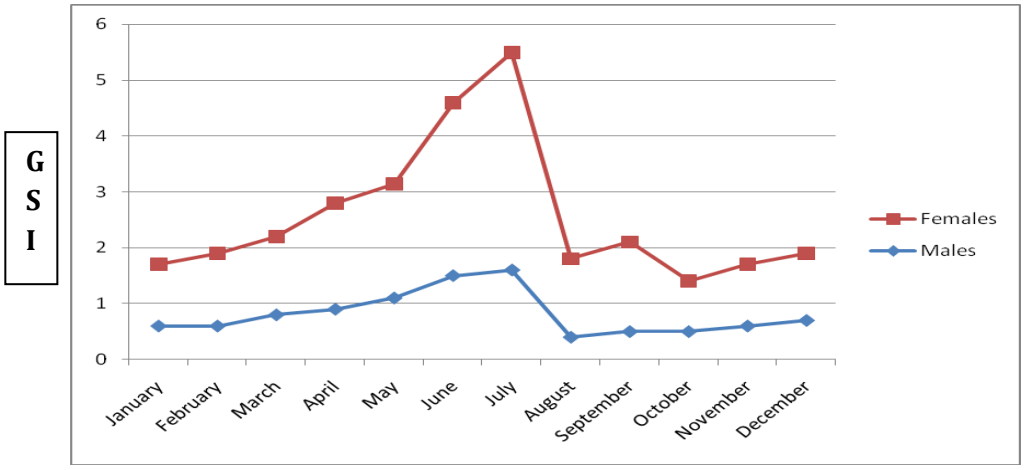


Fig.2. Manth wise Trend in the GSI of G.oblongus (Jan 2011 Dec.2011)

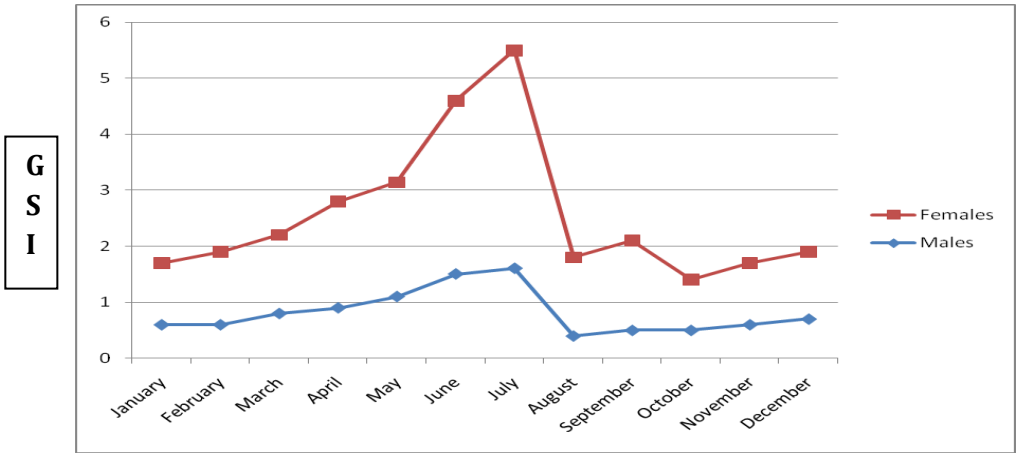


Fig.3 : Month wise in GSI of G.oblongus (Jan 2012-Dec 2012)

It is seen from the table values that the sexual maturity in both male and female run side by side. The weight of the fish was increasing along with the development of gonads. As the gametogenesis begins by releasing sex hormones. The weight of both the gonads and fish increased gradually. The highest weight was found in male 1.4in July 2011 compare to 3.8 in female. The GSI values of both males and females were increased gradually from April to reach a peak during July in both the years of observation. July is considered as the peak spawning period due to sharp fall of GSI values from 1.4 to 0.3 for males and from 3.8 to 1.2 for females during 2011.

On the basis of GSI the annual cycle of reproduction of *G.oblongus* can be divided into three phases. Pre-spawning period ranging between April to July, characterized by high GSI values, spawning period August to September characterized by sharps decrease in the weight of the gonad and post spawning period October to February characterized by gradually increase in the weight of the gonads. After spawring the females can regain the loss of energy by active feeding. The intensity of feeding in female was observed more than males. At maturity the abdomen bellies of females were seen in swollen condition while in males by slight applied pressure to abdomen released out seminal fluid quickly. It is also seen that pre-spawning time the general weight increasing rapidly due to the huge numbers of gamets.

CONCLUSION

The GSI period is known to be reliable index of the breeding season in this fish. The maximum values of GSI denote attainment of peak maturity of gonads while the minimum GSI values indicate peak spawning. The lowest value GSI of both the sexes were seen in August which seems to be the peak spawning moth of *G.oblongus*. An increase of GSI values in an indication of the post-spawning period, when the spawned out individuals recover, and their gonads start the process of gametegenesis under the sex hormones secreted by pituitary gland. After spawning , the females were found in active mode of feeding to restore the used energy in the development of ovaries. Cultivation of *G.oblangue* is likely to be profitable because of the consumer demand. The females were significantly larger than males (yeargai 1999).

REFERENCES

Jayabalan N (1988) Reproductive biology of the pony fish, *Gazza minuta* (Bloch) from porto Novo, East coast of India, *Indian Journal of Marine Science,* 17.51-54.

Sivashanthini K and Abeyrami B (2003) Lenght-weight relationship and relative condition of a silver biddy *Gerres oblongus* (Pisces: perciformes) from the jaffna lagoon, Sri. Lanka. *Indian Journal of Geo-Marine Sciences*, 32(3): 252-254.

Yeragi SG and Yeragi SS (1999) Effect of environmental parameters on haematology of *Gerres filmentosus*. *Bioresearch Journal*, 2 (1):1-4.

RESEARCH ARTICLE

Studies on micromorphology of leaves in some members of genus *piper* linn.

Dassharma Kakoli[1] and *Ravnang Pratik[2*]

[1]Department of Botany, Bhavan's College, Andheri (W) India
[2*]Department of Botany,Savitribai Phule Pune University, Ganeshkhind,Pune-411007
*Corresponding author email: pratikr865@gmail.com

Manuscript details:

Received: 17.10.2015
Revised: .17.11.2015
Accepted: 10.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Dassharma Kakoli and Ravnang Pratik (2015) Studies on micromorphology of leaves in some members of genus *piper* linn. *International J. of Life Sciences*, 3(4): 379-386.

ABSTRACT

Stomata, the vital gate between plant and atmosphere may play a central role in plant vegetation responses to environmental conditions, which have been and are being investigated from molecular and whole plant perspectives, as well as at ecosystem and global levels. Stomatal diversity in foliar epidermis has great value in plant systematic studies. The present work deals with the anatomical investigations with respect to stomatal complex in genus *Piper* belonging to family Piperaceae where an attempt has been made to recognize the taxonomic value of genus *Piper.* Stomatal complex of eight species of genus *Piper* has been studied for the present work. The species are as follows: *P. chaba, P. pedicellosum, P. sylvestre, P. talboti, P. boehmeriaefolium, P. lonchites, P. miniatum, P. galeatum*. Three types of stomata were observed: paratetracytic, staurocytic and anisocytic. All the species show the presence of stomata on the lower epidermis only. *P. galeatum* was noticed with combination of two types of stomata on its epidermis. Rest all the species were found to show single type of stomata. The observations suggest the predominance of paratetracytic stomata followed by and staurocytic and anisocytic type. Detailed study of foliar trichomes in the above mentioned species showed abundance in glandular trichomes. Under glandular trichomes, types of unmodified epidermal cells and modified epidermal cells were found to be persistent. Interestingly, eglandular trichomes of type trichome apex obtuse were also noticed in two species. *P. boehmeriaefolium* was been observed with both glandular as well as eglandular type of trichomes.

Keywords: *Piper*, Stomatal complex, Paratetracytic, trichomes, taxonomic value, Intrageneric classification.

INTRODUCTION

A stoma is a microscopic pore on the surface of land plants. It is surrounded by a pair of specialized epidermal cells called guard cells, which act as a turgor-driven valve that open and close the pores in

response to given environmental conditions. Transpiration via stomata supplies water and minerals to the entire plant system. When a plant encounters adverse environmental conditions, such as drought, a plant hormone called abscisic acid triggers stomata to shut tightly in order to prevent plants from dehydration and wilting. Many workers such as Edeoga (1991), Edeoga and Osawe (1996), Mbagwu and Edeoga (2006), Nwachukwu and Mbagwu (2006) stressed that epidermal and cuticular traits of plants epidermal cells, type and arrangement of stomata, size and shape of trichomes and number of vascular bundles could serve as vital tools in solving taxonomic problems in Angiosperms. Stomatogenesis has long been studied by morphologists, physiologists and taxonomist .The morphology and ontogenies of taxa are important in intrageneric systematics. Diversity in stomata types, even on the same surface of an organ, indicates the weakness in using stomata as a taxonomic character (Pant and Kidwai, 1964). Inspite of diversity, the most frequent stomata type can be used as a taxonomic character. Apart from physiognomic characters, anatomical properties of plant parts are sources for taxonomic inferences in different groups of flowering plants (Edeoga et al., 2007; Guimeraes et al., 2007; Kaplan et al., 2007; Keshavarzi and Zare, 2006). Despite the immense economic importance of the legumes and the physiological importance of the stomatal apertures, reports on the frequency and the structure of the stomata are lacking or incomplete for many species.

Piperaceae are grouped in about 8-12 genera and about 1200- 5000 species; are pantropical in distribution; and confined to the tropical and subtropical portions of the world. It is commonly known s pepper family. these are mostly herbs and shrubs but there are also small trees and woody climbers. The genus *Piper,*Linn., the largest in the fmily Piperaceae, occurs throughout the tropical and subtropical regions of the hemispheres. As conventionally construed, *Piper* is a large pantropical genus. More than 3000 species have been recorded (Rahiman and Nair, 1983), but because of the large number of species, wide distribution, very small, achlamydous and closely aggregated flowers, many unisexual species, and lack of critical phyletic study (Hooker, 1886; Lawrence, 1951), an acceptable species concept could not be established till date. Pipers are generally perennial shrubs bearing adventitious or sometimes epiphytic roots. Leaves are alternate, petiolated and with deciduous stipules. Leaf blade is simple, ovate, lanceolate or elliptic. Leaves are pinnately costateor multicostate. Inflorescence is spike or catkin; oppositifolius. Flowers are usually dioecious; unisexual; sessile; with the peltate or copular bracts. Stamens are 1-4 with 2-celled anthers. Ovary is superior; 1-celled, free with solitary ovule. Fruit is sessile, oblong or globose, pulpy, reen, red, yellow, drupe or berry. Seeds are with thin testa. Pipers are of huge economic and medicinal significance. Boiled stem and leaves of Piper are used as medicine while roots are used to cure stomachache, common cold, etc. the aromatic leaf is a masticatory used with lime, catechu, arecanut and other species, and sometimes with tobacco act as a stimulant, intoxicant, carminative, astringent, aphrodisiac and antiseptic. Leaf juice has fungicidal and nematodicidal properties due to the presence of essential oil. Seeds powdered and given with honey are used in cough, cold and asthma.

There is meager anatomical work carried out in genus *Piper* of piperaceae. Family piperaceae has undergone considerable changes in the circumscription of various taxanomic ranks. Howard (1973) has considered the family as the most difficult family. In spite of all the efforts carried out by the taxonomists, many species could not be placed comfortably in the classification on the basis of available morphological data. A number of species are very intimate with the leaf morphological characters. Hence, in the present study, attempt has been made to recognize the taxonomic value of the stomatal complex and trichomes. Variations in stomata and foliar trichomes of eight species of genus *Piper* has been studied for the present

work. The species are as follows: *P. chaba, P. pedicellosum, P. sylvestre, P. talboti, P. boehmeriaefolium, P. lonchites, P. miniatum, P. galeatum*.

MATERIAL AND METHODS:

Plant material, fresh and preserved, was collected from various states such as Maharashtra, Tamil Nadu, Kerala, Assam, Meghalaya, and West Bengal. Majority of plants were however obtained from Meghalaya. Most of the species are procured through B.S.I., Eastern circle, Shilong and few from C.N.H. and Indian Botanical Garden, Howrah. The identifications were checked with reference to various floras and standard Indian Herbaria. The detailed study of the various species of *Piper* was carried out in the laboratory using both dissecting and compound binocular microscope.

I. To study the stomata, various methods were tried for the mounting of lamina showing the surface layers in surface views.

1) Simple scraping technique employed by Jordell Laboratories in U.K. as described by Metcalfe (1963) was tried.
2) Some pieces of lamina were boiled in 5- 15% concentrated HNO_3 till the leaf showed blister formation on its surface. The lamina was then thoroughly washed and the blisters with epidermis was peeled off.
3) The boiling of the lamina was also tried in 5% NAOH/ KOH and the remaining procedure followed was the same as above.
4) 5% $CuSO_4$ solution was taken, in which some pieces of lamina were boiled for 10-15 minutes. While boiling, two to three ml of concentrated HNO_3 was added. Blisters were formed on the leaf surface. As in the above procedure, the lamina pieces were washed and the blistered epidermal surface was peeled off.

   The peeled epidermis from the above methods was then stained with Safranine or Haematosylin solution and mounted in 80%-85% glycerine.

II. To study the foliar trichomes in several lamina, cleared with sodium hypochlorite solution were observed directly under the compound microscope.

The camera lucida sketches of the stomata and trichomes at x 450 magnification were drawn by using Erma Camera Lucida. Finally, the text figures of the stomata were prepared.

RESULT AND DISCUSSION:

The different types of stomata have been reported on the same surface of an organ in diverse angiospermic families (Tognini,1897; Loftfield, 1921; Sen, 1958; Pant and Kidwai, 1964; Paliwal, 1965;Pant and Mehra, 1965; Inamdar 1969; Inamdar and Patel, 1971; and Bahadur et.al. 1971). Stomata are always restricted to the lower epidermis in *Piper* (Metcalf and Chalk, 1950). Interestingly, a few stomata are also noted on upper epidermis of *Piper leptosachya*, chapm. (Yuncker and Gray, 1934). Most of the stomata are surrounded by a rosette of many epidermal cells, while some are cruciferous in nature (Metcalf and Chalk, 1950). However, stephanocytic type of stomatal apparatus have been found to be characteristic for chloranthaceae, Saururaceae and piperaceae (Baranov, 1987). The epidermal characters of plants in systematic studies in distinguishing certain groups of plants have been used (Stace,1965; Ramayya and Rajgopal, 1968, 1971). The mature stomata of 8 species of *Piper* have been studied. The results obtained indicates the presence of paratetracytic, staurocytic and anisocytic type of stomata. All the eight species showed the stomata at their lower epidermis only. Only one species amongst all was noticed with the combination of two types of stomata.

The types of stomata observed in the studied species of Piper are as follows:

Paratetracytic type: The guard cells elongated and kidney shaped. The epidermal cells are pentagonal, hexagonal and polygonal in shape.

Staurocytic type: The guard cells are kidney shaped in appearance. The epidermal cells are almost in shape, with a rounded cell wall pattern.

Anisocytic type: The guard cells are slightly elongated and kidney shaped in appearance. The epidermal cells are pentagonal and hexagonal in shape.

Paratetracytic and Staurocytic type: The guard cells are large and kidney shaped in appearance. The epidermal cells are larger in size as compared to the lower epidermis. They are pentagonal and also isodiametric in shape having a rounded cell wall pattern.

KEY BASED ON STOMATAL COMPLEX:

Type 1: Paratetracytic type:

P. chaba - Lower epidermis
P. pedicellosum - Lower epidermis
P. sylvestre - Lower epidermis
P. talboti - Lower epidermis

Type 2: Anisocytic type: :

P. boehmeriaefolium - Lower epidermis
P. lonchites - Lower epidermis

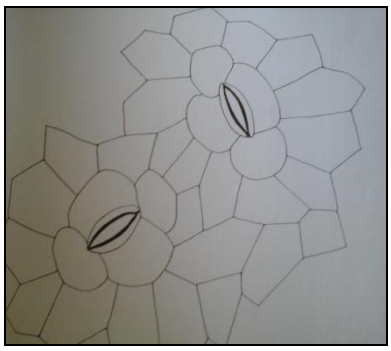
Type 3: staurocytic type:

P. miniatum - Lower epidermis

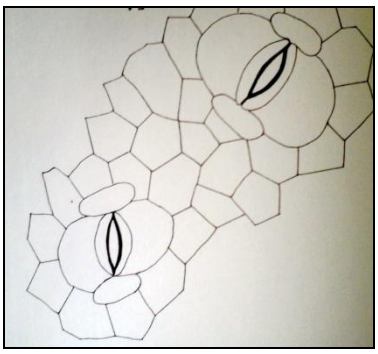
Type 4: paratetracytic and Staurocytic :

P. galeatum - Lower epidermis

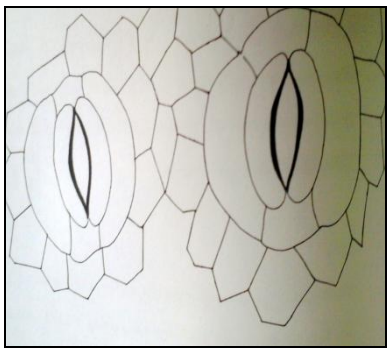
Type 1: Paratetracytic type:



P. chaba

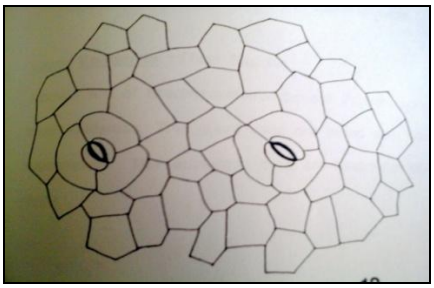


P. pedicellosum

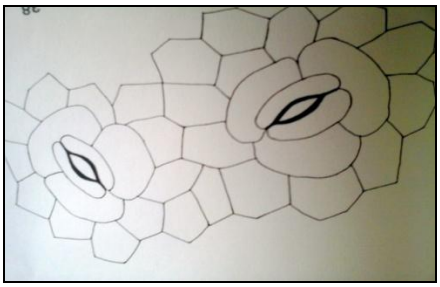


P. sylvestre & P. talboti

Type 2: Anisocytic type:

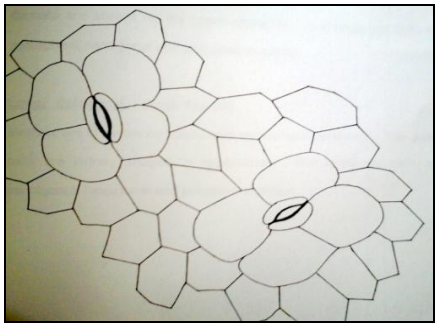


P. boehmeriaefolium



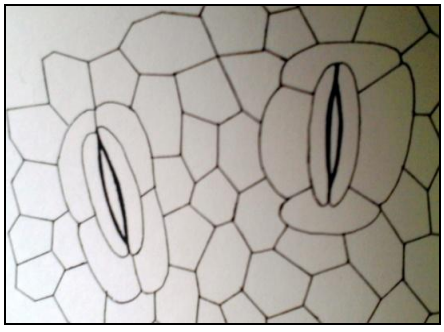
P. lonchites

Type 3: staurocytic type:



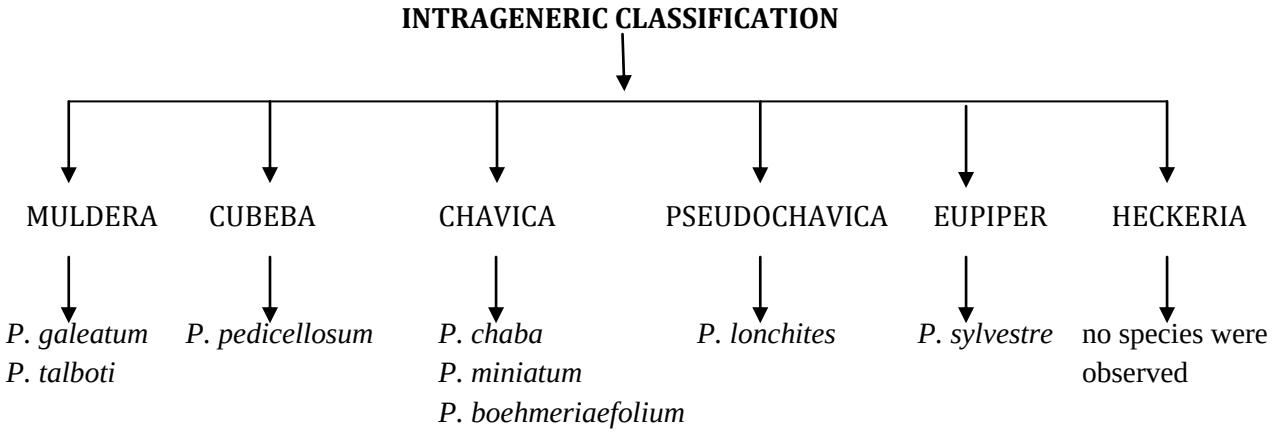
P. miniatum

Type 4: paratetracytic and Staurocytic :



P. galeatum

Intrageneric classification of genus *Piper* given by hooker:



According to intrageneric classification of *Piper* Section 1, Muldera consists of *P. galeatum* and *P. talboti* of which P. galeatum shows paratetracytic and staurocytic types of stomata while in *P. talboti* paratetracytic ype of stomata are observed. Section 2, Cubeba consists of *P. pedicellosum* shows paratetracytic type of stomata. Section 3 Chavica shows maximum species namely *P. chaba, P. miniatum, P. boehmeriaefolium*. *P. chaba* shows paratetracytic type of stomata and *P. miniatum* shows staurocytic type of stomata. In *P. boehmeriae folium* anisocytic type of stomata was observed. *P. lonchoites* categorized under section 4 Pseudochavica shows anisocytic type of stomata. *P. sylvestre* categorized under section 5 Eupiper shows paratetracytic type of stomata. No species were categorized under section 6 Heckeria.

The structure and development of trichomes and glands have been studied in many angiosperm families (DeBary, 1884; Solereder, 1928; Netolitzky, 1932; Cowan, 1950; Farooq, 1963; Pant and Banerji, 1965; Inamdar, 1967, 1968; Inamdar and Patel, 1973; Roe, 1971; Ramayya, 1972; Singh, Jain and Sharma, 1974; Lowell and Lucansky, 1986). Metcalfe and Chalk (1950) have reported 14 eglandular and 5 glandular type of trichomes in Polemoniales. They have also observed glistening pearl glands in Piper sporadically, which are usually filled with protein and oil. Inamdar and Patel (1973) have recorded the structure, ontogeny and classification of trichomes in 40 genera and 112 species of the Polemoniales, and have found eglandular, glandular and eglandular cum glandular types of trichomes. In the present research work, trichomes of all eight Piper species were examined and it has been observed that piper show the presence of either eglandular trichomes or glandular or both. Interestingly, two species of *Piper* were found with both the types of trichomes.

The types of trichomes observed in the studied species of Piper are as follows:

A. EGLANDULAR TRICHOMES:

a) Trichome Apex Obtuse:

i. Trichomes are eglandular, simple and of unicellular, conical type, with an obtuse apex and a rounded base. The outer walls are concave and sinuous in nature. (eg)

ii. Trichomes are long and eglandular, simple and of unicellular type, with almost parallel outer walls showing a slightly obtuse apex and a rounded base. (eg)

B. GLANDULAR TRICHOMES:

a) Unmodified Epidermal Cells:

i. Trichomes are glandular , multicellular and of peltate type. The trichome basal cell is with unmodified epidermal cells and a thickened, polygonal trichome base.

b) Modified Epidermal Cells:

i. Trichomes are glandular , multicellular and of peltate type. The trichome basal cell is with modified radially thickened epidermal cells and a thickened, polygonal trichome base.

KEY BASED ON TRICHOME COMPLEX :

A. EGLANDULAR TRICHOMES:

a) Trichome Apex Obtuse:

- *P. boehmeriaefolium*
- *P. miniatum*

B. GLANDULAR TRICHOMES:

a) Unmodified Epidermal Cells:

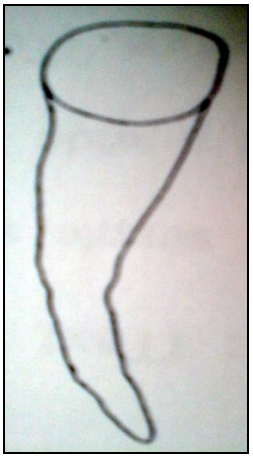
- *P. boehmeriaefolium*
- *P. miniatum*
- *P. chaba*
- *P. galeatum*
- *P. lonchites*
- *P. pedicellosum*
- *P. sylvestre*

b) Modified Epidermal Cells:

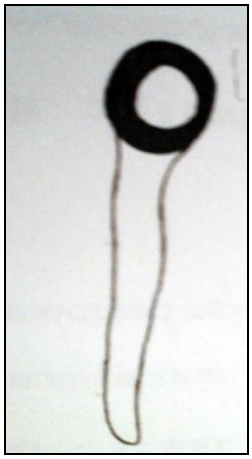
- *P. talboti*

A. EGLANDULAR TRICHOMES:

a. Trichome Apex Obtuse:



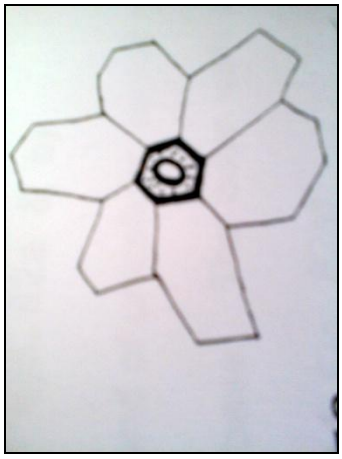
P. boehmeriaefolium



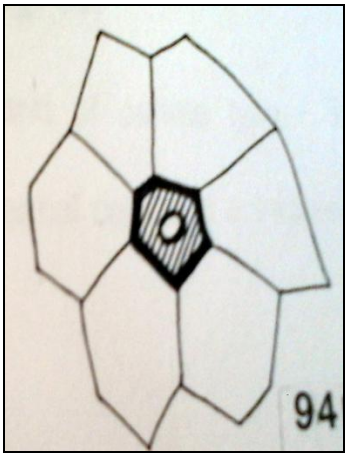
P. miniatum

B. GLANDULAR TRICHOMES:

a.Unmodified Epidermal Cells:



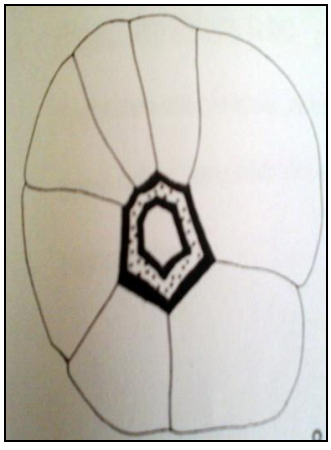
P. boehmeriaefolium



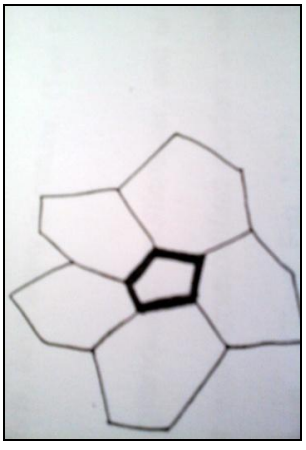
P. miniatum

A. GLANDULAR TRICHOMES:

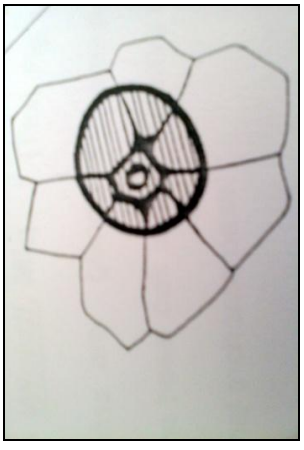
a. Unmodified Epidermal Cells:



P. chaba



P. pedicellosum

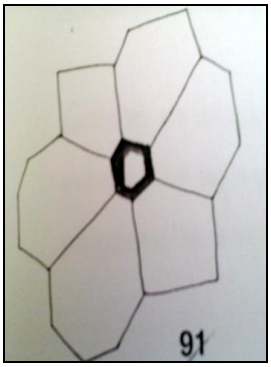


P. sylvestre

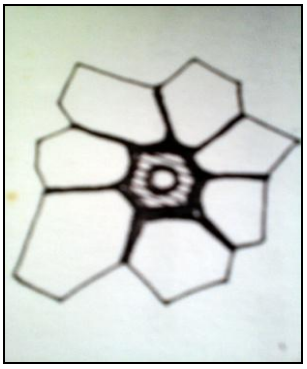
P. galeatum

A. GLANDULAR TRICHOMES:

b. Unmodified Epidermal Cells



P. lonchites



b)Modified epidermal cell: *P. talboti*

CONCLUSION

The present work deals with the laminar anatomical investigations on genus *Piper* of family Piperaceae. 12 species of Piper have been studied in the present work. The family Piperaceae has undergone considerable changes in the circumscription of various taxonomic ranks. From taxonomic point of view, Howard (1973) has considered Piperaceae as the most difficult family. The vegetative morphological characters diagnosed family piperaceae and genus *Piper* to a certain extent, but a large number of species are very intimate by their leaf morphological characters which posed difficulty in determining the group. However, the intrageneric classification of *Piper* (Hooker, 1883) was mainly based on reproductive morphological characters. Hence, an attempt has been made to recognize the taxonomic value of stomatal complex. The leaf anatomical studies is applicable to pharmacognosical importance as well. In the present work, seven species were found to show single type of stomata, however, *P. galeatum* was noticed with combination of two types of stomata. From the intrageneric classification, it has been found that the artificial key to the stomatal complex suggests the predominance of Paratetracytic type of stomata (5 species) followed by staurocytic and anisocytic type of stomata (2 species each). The Paratetracytic types of stomata are also characteristically observed in Section III – Chavica, which covers the majority of the studied species. Detailed study of foliar trichomes have shown abundance of glandular trichomes (unmodified epidermal cells type) in most of the species studied. Hence, the study of the micromorphology of leaves piperaceae members has been concluded to be quite significant taxonomically.

REFERENCES

Bahadur B, Rajagopal T and Ramayya N (1971) Studies on the structure and development variation and distribution of stomata in the Rubiaceae. *Bot. J. Linn. Soc.*, London, 64 : 293-310.

Baranov MA (1987) Stephanocytic type of stomatal apparatus in Anthophyta. *Bot. Zh.* (Leningr.), 72(1): 59-64.

Cowan VM (1950) The Rhododendron leaf: Botany. A study of Epidermal appendages London.

De Bary A (1884) Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns. Oxford: Clarendon Press

Edeoga HO (1991) comparative morphology and leaf epidermis of *Costus afer- C. lucanstanus* (costaceae) complex and its system importance. *Nature Science.,* 24: 1-243.

Edeoga HO, Omosun G, Osuagwu GGE and Emezue OO (2007) Microscopic Anatomy and Histochemistry of Stem And Root of Some *Mimosa* Species, (Leguminosae- Mimosoideae)", *Asian Journal of Plant Sciences*, Vol. 6, No. 4, pp. 688-691.

Farooq M (1963) Trichomes of the flowers of *Utricularia, J. Indian Bot. Soc*., 45: 242 –248.

Guimeraes AC, Kuster RM, Amaral A F, Ferreira J P and Siani A C (2007) "Histological Study of the Leaf and Stem of the Amazonian Medicinal Mistletoe *Cladocolea micrantha* (Loranthaceae)", *International Journal of Botany*, Vol. 3, No. 2, pp. 218-221.

Howard RD (1973) Notes on the Piperaceae of the Lesser Antilles. *J. Arnold Arb*., 54(3) : 377-411.

Inamdar JA (1967) Studies of trichomes of some oleaceae, structure and ontogeny, Proc. *Indian acad. Sci*., 66: 164 –177.

Inamdar JA (1968) Trichomes and nectarines on the floral organ, Beitr. *Bio. Pflanz*., 45: 39-47.

Inamdar JA (1969) Development of stomata in some Solanaceae. *Flora*, 158 : 462- 472.

Inamdar JA and Patel R C (1973) Structure and development of sclereids and stomata in

Ipomoea quamoclit, L. Ceylon J. Sci. (*Biol. Sci*.), 9 : 64-74.

Kaplan A, Hasanoglu A and Ince I A (2007) "Morphological, Anatomical and Palynological Properties of Some Turkish *Veronica* species (Scrophulariaceae)", *International Journal of Botany*, Vol. 3, No. 1, pp. 23-32.

Keshavarzi M and Zare G (2006) "Anatomcial Study of *Salicornieae* Dumort, (Chenopodiaceae Vent.) Native to Iran", *International Journal of Botany*, Vol. 2,No. 3, pp. 278-285.

Lawrence GHM (1951) Taxanomy of vascular plants. The Macmillan Company. New York.

Loftfield J V G (1921) The behavior of stomata. Carneg. Instn. No. 314 : 1-114.

Lowell and Lucansky TW (1986) Vegetative anatomy and morphology of *Ipomoea hederifolia* (convolvulaceae), *Bull. Torrey. Bot. Club*., 113 (4): 382 –397.

Mbagwu FN and Edeoga HO (2006) "Observations on the Vegetative and Floral Morphology of Some *Vigna* Species (Leguminosae-Papilionoideae)" *Pakistan Journal of Biological Sciences,* Vol. 9, No. 9, pp. 1754-1758

Metcafe CR (1963) Comparative anatomy as a modern botanical discipline- with special reference to recent advances in the systematic anatomy of Monocotyledons, *Advancces in Botanical Research* vol 1., pp. 101-147.

Metcalfe CR and Chalk L (1950) Anatomy of Dicotyledons. Vol.-1. Clarendon Press, Oxford.

Netolitzky F (1932) In handbuch der pflazenanatomie (ed. K. Linsbauer) Abt. 1. Teil. 2 Hautgewebe. Band IV. Die pflazenhaare Gebruder Borntraeger, Berlin,.

Nwachukwu CU and FN Mbagwu (2006) "Morphological features in some species of *Indigofera* L. (Leguminosae-Papilionoideae)", *Journal of Fisheries International*, Vol. 2-4, pp. 50-54.

Paliwal GS (1965) The development of stomata in *Basella rubra*, Linn. *Phytomorphology*, 15 : 50-53.

Pant DD and Banerji R (1965) Epidermal structure and development of stomata in some convolvulaceae, Scnckenberg. *Biol.,* 46: 155 – 175.

Pant DD and Kidwai P (1964) On the diversity in the development and organization of stomata in *Phyla nodiflora,* Michx*. Curr. Sci*., 33 : 653- 654.

Rahiman BA and Nair MK (1983) Morphology, cytology and chemical constituents of some Piper species from India. *J. Plant Crops*, 11 (2) : 72-90.

Ramayya N (1972) Classification and phylogeny of trichomes of angiosperm, In: Ghouse AKM & Yunus M (eds.) *Research Trends in Plant Anatomy* , pp. 91–102. Bombay-New Delhi, India: Tata McGraw Hill Publishing Company.

Ramayya N and Rajgopal T (1968) Foliar epidermis as taxanomis aid in the Flora of Hyderabad. *J. Osmania Univ.* (sci), 4; 147-160.

Ramayya N and Rajgopal T (1971) Foliar dermotypes of the Indian Aizoaceae and their use in the identification. *J. Ind. Bot. Soc*., 50 : 355- 362.

Roe KE (1971) Terminology of hairs in genus *Solanum*. *Taxon*, 20: 501-508.

Sen S (1958) Stomatal types in Centrospermae. *Curr. Sci*., 27 : 65-67.

Sing V, Jain DK, Sharma M (1974) Epidermal studies in *Ipomoea* (convolvulaceae). Systematic Anatomy of the Dicotyledons. (Transt. L. A. Boodle and F. E. Fritsch), Oxford Uni. Press, London.

Solereder H & Meyer F J(1928) Systematische Anatomie der Monokotyledonen.-Berlin.

Stace CA (1965) The significance of the leaf epidermis in the taxanomy of combretaceae. 4. The genus *Combretum* in Asia. *Bot. J. Linn. Soc*., 65 : 97- 115.

Tognini P (1897) Contribuzione allo studio della organogenic comnparata delgi. *Stomi. Alt. Inst. Bot. Univ. Pavla*, 4 : 1- 42.

Yunker TG and Gray WD (1934) Anatomy of Hawaiian *Peperomias.* 10; 20 : 19. *Bernice P. Bishop Mus.* (Occasional Papers).

RESEARCH ARTICLE

Physicochemical parameters of Erai dam, Chandrapur (MS) India

Jadhao RG* and Dhage DM

P.G. Department of Zoology, Shri Shivaji Science College, Amravati.(M.S.) India.
*Corresponding author Email: Dr. RG Jadhao, Associate Professor and Head, P.G. Department of Zoology, Shri Shivaji Science College, Amravati.(M.S.) India. | Email: rajusingjadhao@gmail.com

Manuscript details:

Received: 03.09.2015
Revised: 12.10.2015
Accepted: 10.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Jadhao RG & Dhage DM (2015) Physicochemical parameters of Erai dam, Chandrapur (MS) India. *International J. of Life Sciences*, 3(4): 387-391.

ABSTRACT

The study area was visited monthly and water sample were collected from five stations and carried to the laboratory. Standard method were used to determine the concentration, total suspended matter and Physicochemical parameters, such as temperature, pH, conductivity, turbidity, free co_2, dissolved oxygen, chloride, total hardness, calcium hardness, magnesium hardness and total phosphorus were estimated.

Keywords : Physicochemical Parameters, Erai Dam.

INTRODUCTION

Water plays a vital role in human life. The physicochemical parameters are very important in the study of any aquatic environment. For the developmental activities of the region water resource quality from any region is an important aspect, because the rivers, lakes and manmade dam or reservoir are used for water supply to domestic, industrial, agricultural and fish culture. The water quality is describes by its physical, chemical and microbial characteristics. The data collected would create environmental awareness for the local people concerning the status of the dam.

MATERIAL AND METHOD:

Erai dam is situated in Chandrapur District (Maharastra State), at latitude and longitudinal area of the dam was 20.1677381^0 N and 79.3048096^0E respectively. The height 30m(98 ft),Length 1620 m(5310 ft), Dam volume 985 km^3 (236 cu mi), Total copacity193000km^3(46,000 cu mi),Surface area58000 km^3(22000 sq mi)The dam water used for irrigation, thermal power plant, CSTPS and Chandrapur city. The live storage capacity of the Erai dam between R.L. 200.5m to R.L. 207.00m inMm^3 and full original

Map 1: Location map of study site

capacity in the year (1983-85) was 193.003m and SRS survey based capacity in year (2007-08) was 144.796m in Mm^3, the live storage capacity was reduced due to siltation. Reduction rate of capacity in Mm^3 was 48.207. The amount of silt is estimated as the difference between original capacity and the present capacity. The study area was visited monthly and water sample were collected from five stations and carried to the laboratory analysis. Standard method were used to determine concentration, total suspended matter and physicochemical parameters, such as temperature, pH, conductivity, turbidity, free co_2, dissolved oxygen, chloride, total hardness, calcium hardness, magnesium hardness & total phosphorus were estimated by using the standard methods of APHA(198).

RESULTS

The result from data analysis showed that the water quality is suitable for aquatic life. This study involves determination of physicochemical parameters of water, different stations are summarized in table 1.

Variation in temperature affects the biological productivity 20^0 C to 41^0 C temperatures was required range of fish culture. In present investigation shows temperature value range between 22^0 C to 30^0 C which is suitable for the fish culture. Similarly reported earlier by Borse and Bhave (2000). The water was highly turbid in the monsoon season. The turbidity of water body due to the suspended material like salt and clay. Turbidity restrict light penetration, which is directly affect on bioproductivity. Turbidity value was observed during study is between 1 NTU to 6 NTU. So value indicates that dam was suitable for fish and other aquatic culture. The pH ranging from 6.5 to 9.00 before daybreak is most suitable for culture. The present study reveals that pH range between 7 to 9, so the water quality was good for aquatic culture. WHO (1984) and ICMR prescribe these values. In the present investigation conductivity range between 114 to 200 mho/ cm in the summer season the total volume of water decreases as a result, the conductivity increases. The conductivity value of Erai dam was favoring to the biological productivity.

According to Swingle (1967) more than 15mg/l, CO_2 range is detrimental for fishes. Present study showed that the range of CO_2 is between optimum level which is suitable for dam life. Alkalinity and pH are so closely related. High alkalinities are able to shift in pH. The standard level of alkalinity was 20-200 mg/l. Total alkalinity during study period was shows that water quality suitable for aquatic life. D.O. is another vital parameter regulating survival of aquatic life. In present investigation showed that the D.O. range between 3 to 5 mg/l. In the month of December and January the higher range of D.O. documented which good for production. The chloride range was observed high in the summer season. Presence of chloride in the water source is used as indicator of pollution.

The values of calcium and Magnesium hardness never exceeded the standard limit of *WHO (1989)* i.e. 200 mg/l and 100 mg/l respectively. And a positive co-relation shows between calcium magnesium and total hardness Phosphorus is a vital factor for fertility less than 0.5mg/l is unfavorable for fish growth. In the present investigation phosphorus range between 0.10 to 0.70 mg/l which is favorable for fish growth

Temperature is one of the important physical parameter which directly influence some chemical reactions in aquatic ecosystem. In present study period, the temperature of water range between 22^0 C to 30^0C and the lowest temperature were observed in the month of November and December and maximum temperature were observed in the month of May, June and July (2007-08).

Table 1: Annual average of physicochemical parameters of Erai dam

Parameter	Jun-07	Jul-07	Aug. 07	Sept. 07	Oct. 07	Nov 07	Dec . 07	Jan. 07	Feb 07	Mar 07	Apr 07	May 07
Temperature 0C	29	30	27	26	25.5	24.6	22.3	24.7	25.2	26.2	28.2	29.5
Turbidity NTU	3.2	3.8	5.4	2.6	2	1.8	1.4	1.2	0.8	0.2	0.4	0.6
pH	7.8	7.78	7.45	7.58	7.7	7.57	7.79	7.77	7.95	8.16	8.64	8.75
Conductivity (mho/cm)	190	182	114	134	152	179	172	181	182	182	190	200
Free CO2 (mg/l)	0.48	1.14	0.38	1.16	0.4	0.36	0.014	0.4	0.14	1.14	1.22	0.18
Alkalinity (ppm)	90	91	62	60	64	87	92	90	91	88	91	89
Dissolved Oxygen (mg/l)	3.46	4.1	4.28	4.34	4.54	4.3	5.2	5.02	4.78	4.26	3.84	3.8
Chloride (mg/l)	14	15	10	10	10	10	9	13	13	14	13	13
Total Hardness (mg/l)	56	57	44	45	52	59	63	67	67	65	61	54
Calcium Hardness (mg/l)	34	35	32	34	40	43	47	46	47	47	40	33
Magnesium Hardness (mg/l)	22	22	12	11	12	16	16	21	20	18	21	21
Total Phosphorus (mg/l)	0.11	0.12	0.1	0.11	0.52	0.66	0.62	0.59	0.28	0.25	0.18	0.16

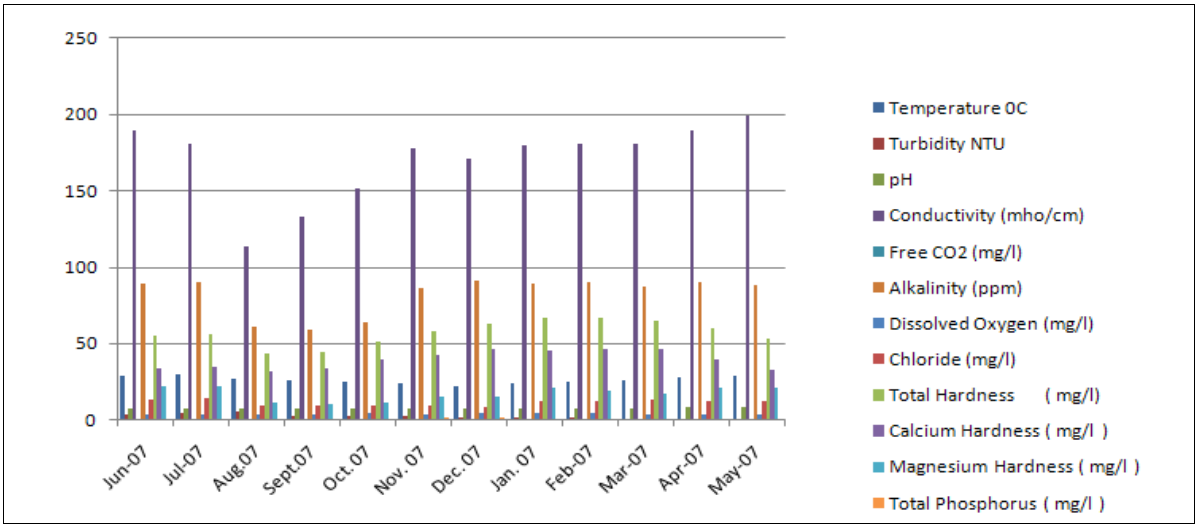


Fig. 1: Annual average of physicochemical parameters of Erai dam

The fresh water are generally alkaline and acidic conditions, The pH observed at various stations range between 7 to 9, highest in the month of May and lowest in the month of October. Conductivity is strongly dependent parameter on temperature. Conductivity can approximately as being directly Proportional to temperature. Also depend upon number of ions present in water. The conductivity of said dam range between the 130 to 200 mho/cm. The free carbon dioxide was observed throughout the investigation period. The concentration varied between 0.014 to 1.22 mg/lit. Its maximum concentration was recorded during April and minimum during December. During the study period, total alkalinity range between 60 ppm to 92 ppm. The maximum value was observed in December and minimum in September. Phenolphthalein alkalinity was significantly absent. Dissolved oxygen is very essential for metabolism of all aquatic organism (Wetzel,1975). The result shows that D.O. ranged between 3 to 5 mg/lit, The lowest D.O. in June and highest in December.

Although the chlorides are not harmful concentration beyond 250 mg/lit. The maximum concentration was observed in July and minimum in December. The chloride value observed between 9 to 15 mg/lit. The total hardness of Erai dam was recorded from 44 to 67 mg/lit. The peak value was observed in January and February and lowest value was in August. Calcium which is utilized in bone building and shell formation, occurred between 30 to 50 mg/lit, while its required level is at 25-80 mg/lit. Maximum calcium observed in December, February and March, while lowest in August.

The magnesium was recorded ranged between the 10 to 25 mg/lit. The peak valued of magnesium hardness was observed in June and July and lowest in September. Phosphorus is a vital factor for fertility less than 0.5mg/lit, is unfavorable for fish growth. In the present study total Phosphorus ranged between 0.10 to 0.70. The maximum value in November and minimum observed in August. Alkalinity and pH are so closely related high alkalinity are able to shift in pH. The general standard level for alkalinity was 20-200 mg/lit are typical of fresh water. Total alkalinity range showed that water quality suitable for aquatic life.

Dissolved oxygen (D.O.) is another vital parameter regulating survival of aquatic life .Rao et.al.(1998) found the D.O. range between 3.7 mg/L to 5.72 mg/L in water ponds and 3.02 mg/L is observed an annual range. Similarly present investigation shows that the D.O. ranged between 3 to 5 mg/L. In the month of December and January the higher range of D. O. documented is good for production. The chloride range was observed high in the summer season. Presence of chloride in the water source is used as an indicator of pollution Koshy and Nayar (1999) evaluated that there was same fluctuation of chloride similarly Shinde (1995) observed its gradually increase from August to May. The Calcium and Magnesium hardness are the two elements which form the most abundant ions in freshwater. The values of Calcium and Magnesium hardness never exceeded the standard limit of WHO (1989) i.e. 200 mg/L and 100 mg/ L respectively. A positive correlation shows between Calcium, Magnesium, and total hardness.

DISCUSSION

The result from data analysis showed that the water quality is suitable for aquatic life. This study involves determination of physical and chemical parameters of water at different points. Many of the physical, chemical and biological characteristics of dams are directly affects by water temperature. A wide range of temperature can occur due to many factors warmer water cannot hold as much oxygen as cooler water, also increase the energy consumption by dam life to greater oxygen used by dam life. Some organism can suffer by internal damage. The 20^0C to 41^0C temperature was required range of fish culture. In present investigation shows temperature value

ranged between 22^{0}C to 30^{0}C which is suitable for the fish culture. Similarly reported earlier by Borse and Bhave (2000 . High water temperature observed in May, similar work was done by (Kumar (1984), Ramesh (1989) and Mridula Mendon (2000).

The water was highly turbid in the monsoon season due to flooding. And because the thermal power plant is located near to the dam which discharge ash in the Erai river, it is clear that from the analysis, that industries have negative impact on water resources. The turbidity of water body due to the suspended material like silt and clay. Turbidity did not permit the light penetration and affect photosynthesis, which is directly affects on bioproductivity. Some fishes are tolerate high ranges of turbidity (Jhingran, 1991). Turbidity was observed during study is between 1 NTU to NTU. So the range of turbidity is in optimum level so it indicates that dam was suitable for fish and other aquatic culture.

The pH ranging from 6.5 to 9.5 before day break is most suitable for culture, while values more than 9.5 are unsuitable in the absence of carbonates Swingle (1967). The present study reveals that pH range between 7 to 9 so the water quality was good for aquatic culture. WHO and ICMR (1999) with in the maximum permissible limit prescribe these values. The present investigation shows that the conductivity range between 114 to 200 mho/cm in the dry season, the total volume of water decreases as a result, the conductivity increases. The conductivity of Erai dam was favoring to the biological productivity.

According to Swingle (1967), CO_2 range more than 15 mg/L is detrimental for fishes. Present study showed that the ranged of CO_2 is between optimum level. It is suitable for dam life. The amount of hydrogen and hydroxyl ions is equal than chemically pure water is neutral. Water is said to be alkaline when the concentration of the hydroxyl ions exceeds that of hydrogen ions.

REFERENCES

APHA (1985) standard methods for the examination of water and waste, New York ,16 th Ed.

Borse SK and Bhave PV (2000) Seasonal temperature variation and their influence on the level of dissolved carbondioxide

Jhingran (1991) Inland Fishes of India adjecent countries-Vol,Oxford and IBH publishing co. pvt. Ltd. New Dehli , 541pp.

Koshy and Nayer (1999) The quality of water usually described according to it's physical,chemical and biological characteristic.

Kumar SR (1984) Studies on the diatrbution of plankton in waters of Mangalore-M.F.Sci thesis univ. agric.,sci.,Banglore, pp231.

Mridala Menon (2008) Seasonal variation in physicochemical charaterstics of water , sediment and soil texture in arid zone Mangroves of Kachcch-Gujrat *J. of Environ. Bio*., 29(5)725-732pp.

Ramesh AM (1989) Distribution of copopodes and copopodies in relation to phytoplankton and hydrography of the coastal waters of Mangalore M.F.Sci thesis univ. agric.,sci.,Banglore ,pp193.

Rao et al. (1998) Limnological studies on fresh water pond of Hyderabad.

Shinde et al. (1995) Limnological studies on Mir Alam lake.

Swingle HS (1967) Standardiztion of chemical analysis for water and muds, *Ibid* 44 (4) :397-442.

WHO (1984) International guideline for drinking water quality volume 1- (Health criteria and other supporting information , Geneva 7pp.

WHO (1989) Safe use of waste water and excreta in agriculture

WHO And ICMR (1999) Workshop on health research management at Chennai.

Wetzel (1975) Book review bolic zone in a majority of the lakes of the world.

SHORT COMMUNICTION

Record of brown paper nautilus *Argonauta hians* Lightfoot, 1786 off Mumbai, northwest coast of India

Sundaram Sujit[1] and Mane Sushant[2]*

[1]Mumbai Research Centre of Central Marine Fisheries Research Institute, 2nd Floor, C.I.F.E old campus, Seven Bungalows, Andheri (W), Versova, Mumbai - 400 061, Maharashtra, India.
[2]Department of Zoology, Wilson College, Chowpaty, Mumbai – 400 007, Maharashtra, India.
*Corresponding author: Email: sushantmane@yahoo.com

Manuscript details:

Received: 26.10.2015
Revised: 20.11.2015
Accepted: 06.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Sundaram Sujit and Mane Sushant (2015) Record of brown paper nautilus *Argonauta hians* Lightfoot, 1786 off Mumbai, northwest coast of India. *International J. of Life Sciences*, 3(4): 392-394

Acknowledgement: The authors are thankful to Dr. K. S. Mohamed, Principal Scientist & Head, Molluscan Fisheries Division, Central Marine Fisheries Res. Institute, Kochi.

ABSTRACT

The brown paper nautilus, *Argonauta hians* Lightfoot, 1786 was recorded for the first time from the northwest coast while investigating the biodiversity of cephalopods from Maharashtra waters. One specimen of the said species was collected as an incidental by-catch in the trawl catch off Mumbai, northwest coast of India.

Keywords: Argonautidae*, Argonauta hians,* Mumbai, northwest coast of India

INTRODUCTION

Cephalopods use a range of mechanisms to counter their body mass and maintain vertical position in the water column, including the use of fins, water jets directed through the funnel and shells with partially evacuated closed chambers such as Nautilus, Spirula and Sepia (Voight *et al*., 1994). The Argonauts (Family: Argonautidae) are free-swimming octopuses of open ocean habitats. The unique characteristic of *Argonauta* is that the female produces a brittle white shell commonly known as a 'paper nautilus', while dwarf males lack a shell. Since the study of Naef (1923), the shell has primarily been considered a receptacle for attachment and brooding of egg strings. The shell has an enlarged web of dorsal arms, functioning as an elaborate egg case. The calcareous structured shell is thin and laterally compressed. The egg case is a single chamber with a flat keel fringed with two rows of tubercles. The lateral sides of the shell have radial ribs. The shell provides protection and is used as a flotation device and as a place to attach their eggs (Beesley *et al*., 1998).

Among the six known species of the monotypic family Argonautidae, *A. argo* is the largest with the female attaining a maximum size of nearly 300 mm shell diameter (Heeger *et al.,* 1992; Finn, 2009). Argonauts exhibited extreme sexual dimorphism in size. The male is dwarf and much smaller than female (Roper *et al.,*1984). The hectocotylus of Argonautidae consists of three parts; a basal spermatophore reservoir, a central section bearing suckers and distally, a long lash like 'penis' (Beesley *et al.,* 1998). During copulation, the hectocotylus detaches and forms an active, autonomous spermatophore carrier remaining in the mantle cavity of the female (Hanlon and Messenger, 1996; Iliffe, 1982). It's assumed that the female argonauts may attain neutral buoyancy by way of pockets of air in their shells (Nixon & Young 2003; Julian and Normani, 2014). Sukhsangchana *et al.* (2009) studied the biology of this species from Andaman Sea. Silas *et al.* (1985) described this species from Indian waters. Vaitheeswaran *et al.* (2014) have recorded the occurrence of this species from the Gulf of Mannar, southeast coast of India. The occurrence of *A. hians* off Mumbai waters, northwest coast of India is reported for the first time.

MATERIALS AND METHODS

A single female specimen of Argonauta was collected on 08-12-14 from the trawl landings at Sasoon Dock, Mumbai, northwest coast of India. The trawlers operated 70-80 km off south of Mumbai at a depth of 40-50 m. The specimen brought to the laboratory for identification and further biological analysis. The dorsal mantle length (DML) was measured using a digital calliper and total body weight (TBW) (± 0.01 g) was determined using an electronic balance after the specimens were dried on blotting paper.

RESULTS AND DISCUSSION

The specimen was identified as *Argonauta hians* based based on the distinctive and unique shell and the characteristics described by Silas *et al.* (1985). The length of the specimen was 61 mm and weighed 105 gm. *A. hians*, is also known as the muddy argonauta and has calcareous, extremely thin and structured shell (Fig. 1, 2 and 3). The paper nautilus has a slender body, narrow head, and unequal arm length. Paper nautilus has eight arms, each arm with two rows of sucker; the number of suckers on the arm is different among species. Dorsal arms in female are with laterally enlarged membrane. Shells vary from white with brownish black tint on the nodules and adjacent ribs to light brown with sooty brown pigmentation over most of the surface of the shell (Voss and Williamson, 1971).

The description given by Thach (2005) agrees with the present specimen. The keel is wide and

Fig.1: *Argonauta hians* Lightfoot, 1786.

bears the characteristic 15 to 23 prominent, large and blunt nodules placed in pairs over the keel. Great variations exist in size and form of the nodules. The brown paper nautilus, *A. hians*, Lightfoot, 1786 is an epipelagic octopod which has been reported from tropical and subtropical oceans (Beesley *et al.,* 1998). *A. hians* is widely distributed in the Indo-Pacific (Thach, 2005; Sukhasangchan and Nabhitabhat, 2007; Sukhsangchan *et al.,* 2009).

The present record of this species is an incidental by-catch in the trawl catch off Mumbai, Northwest coast of India which seems to be the first record from the northwest coast of India. The specimen is deposited in the cephalopod museum collections of the Central Marine Fisheries Research Institute, Mumbai.

REFERENCES

Beesley PL, Ross GJB and Wells A (1998) Mollusca: The Southern Synthesis. Fauna of Australia. Vol. 5, *CSIRO Publishing*, Melbourne, p.451–563.

Finn JK (2009) *Systematics and biology of the argonauts or 'paper nautiluses'* (Cephalopoda: *Argonautidae*). PhD thesis, Department of Zoology, School of Life Sciences, Faculty of Science, Technology and Engineering, La Trobe University, Bundoora, Australia.

Hanlon RT and Messenger JB (1996) Cephalopod Behaviour, *Cambridge University Press*, Cambridge.

Heeger T, Piatkowshi U and Moller H (1992) Predation of jellyfish by the cephalopods *Argonauta argo*. *Mar. Ecol. Prog. Ser.* 88 : 293-296

Iliffe TM (1982) Argonaut: Octopus in a parchment shell. *Sea Frontiers*. 28: 224-228.

Julian K and Normani MD (2014) The Argonaut shell: gas-mediated buoyancy control in a pelagic octopus*. Proc. R. Soc. B.* http://rspb.royalsocietypublishing.org

Naef A (1923) Cephalopoda (systematics). *Fauna and Flora of the Bay of Naples*, Monograph 35, part 1, Vol. 1, pp. 293–917. Translated from German. Jerusalem, Israel: Israel Program for Scientific Translations.

Nixon M and Young JZ (2003) The brains and lives of cephalopods. New York, NY: Oxford University Press. *In:* Packard, A. and Wurtz, M. 1994. An octopus, Ocythoe, with a swim bladder and triple jets. *Phil. Trans. R. Soc. Lond.* B 344, 261–275.

Roper CFE, Sweeney MJ and Nauen CE (1984) FAO species catalogue, Vol.3, Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. *FAO Fish. Synop.,* (125) 3 : 277 pp.

Silas EG, Sarevesan R, Rao KS, Nair KP and Meiyappan MM (1985) Cephalopod Fisheries of India - An introduction to the subject with methodologies adopted for this study. *In:* E. G. Silas (Ed.), Cephalopod bionomics, fisheries and resources of the Exclusive Economic Zone of India. *Bull. Cent. Mar. Fis. Res. Inst.,* 37: 13-37.

Sukhasangchan C and Nabhitabhat J (2007) Embryonic Development of Muddy Paper Nautilus, *Argonauta hians* Lightfoot, 1786, from Andaman Sea, Thailand. *Kasetsart J. Nat. Sci.,* 41: 531-538.

Sukhsangchana C, Meksumpuna S, Nabhitabhata J and Segawac S (2009. Distribution, biology and stomach contents of paper nautilus (*Argonauta hians*) in the Andaman Sea. *Science Asia* Vol 35: 315-319.

Thach NN (2005) *Shells of Vietnam*. 336 pp + 91 color plates.

Vaitheeswaran T, Jayakumar N and Venkataramani VK (2014) A record of brown paper nautilus *Argonauta hians* Lightfoot, 1786 (Family: Argonautidae) (Lamark, 1809), Thoothukudi coast of Gulf of Mannar from India (08º 35' 22.5" N 78º 27' 40.9" E and 08º 31' 91.2"N 78º 25' 32.7"E). *Ind. J. Vet & Anim. Sci. Res.* Vol: 43 (1) 40 – 43.

Voight JR, Portner HO and O'Dor RK (1994) A review of ammonia-mediated buoyancy in squids (Cephalopoda: Teuthoidea). *In: Physiology of cephalopod molluscs. Lifestyle and performance adaption* (Eds: Portner, H. O., R. K. O'Dor and D. L. Macmillan), pp. 193–203. Amsterdam, the Netherlands: Gordon and Breach Science Publishers S.A.

Voss OL and Williamson QR (1971) *Cephalopods of Hong Kong*. Government Press, Hongkong:138p.

RESEARCH REPORT

G-6-PD deficiency and Sickle cell anaemia in Badhiys Muslims of Purnia District (Bihar)

Kumar Sanjeeva[1], Md. Jahangeer and Pandey BN*

[1]Principal, Purnea College, Purnia (Bihar), India
P. G. Department of Zoology, Purnea College, Purnia - 854301 (Bihar) India.
*Corresponding author email – b.n.pandey@hotmail.com

Manuscript details:

Received: 03.10.2015
Revised: 02.11.2015
Accepted: 10.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Sanjeeva Kumar, Md. Jahangeer and Pandey BN (2015) G-6 PD deficiency and Sickle cell anaemia in Badhiys Muslims of Purnia District (Bihar). *International J. of Life Sciences*, 3(4): 395-398.

ABSTRACT

G-6 PD deficiency and sickle cell anaemia were studied in Badhia Muslims of Purnia distrct (Bihar) which are migrated from West Bengal. In a sample size of 509, no case of G-6 PD deficiency and sickle cell anaemia were observed. Once upon a time, Purnia district was popularly known as Kala Pani due to its bad climate and malaria was endemic in this area. However, now a day the climate has improved and the area is not endemic to malaria. It needs further investigation to know if there is any correlation between decreasing trends of malaria with G-6-PD deficiency, and sickle cell anaemia

Keywords:G-6-PD deficiency, sickle cell anaemia, Badhiya Muslims, malarial endemicity and Purnia district

INTRODUCTION

Hereditary hemolytic disorders like hemoglobinopathies, thalassemia syndrome and glucose-6-phosphate dehydrogenase (G-6-PD) enzyme deficiency are important genetic and public health problems in India. The sickle cell anemia especially affects 60-70 million people all over the world. The victims include the growing children, adolescent girls, pregnant women and a large chunk of ignorant people. Inherited disorders of hemoglobin cause high degree of hemolytic anemia, clinical jaundice, painful crisis, frequent infections, splenomegaly, growth retardation, etc. and are responsible for high infant morbidity and mortality, maternal mortality and fetal wastage in India. Although both hemoglobinopathies and G-6-PD deficiency are prevalent in malaria endemic areas but to the best of our knowledge, no study has ever reported combined conditions in a single individual from India. The present study highlights G-6-PD deficiency and sickle cell anaemia in a randomly conducted study in Badhiya Muslims of Purnia district (Bihar), India.

MATERIALS AND METHODS

Blood samples (509) were collected in test-tubes containing an anticoagulant, Acid Citrate Dextrose (ACD) solution from individuals from different villages of Purnia district.

The samples include unrelated individuals. The G-6-PD deficiency was detected with brilliant crystal blue dye test of Motulsky & Campbell-Kranel (1961). Testing of sickling was done on the spot, by using freshly prepared solution of sodium-meta-bisulphate ($Na_2S_2O_3$) in the manner described by Daland and Castle (1948). A two percent solution of salt was prepared in sterile distilled water immediately before the tests. A small droplet of fresh blood from the finger tip was mixed with a drop of solution on a clear microscope slide and the mixture immediately covered with a sealed cover glass. The first observation was made after fifteen minutes in which sickling could be seen in most of the positive cases, but the final reading was taken after half-an-hour under the dry objective of the microscope. Longer time was avoided to avoid false sickling. All the positive cases were re-tested in order to ensure the reliability of the results.

RESULTS AND DISCUSSION

G-6-PD deficiency was mainly found in populations originating from tropical areas of the world. In India, G6PD deficiency was first reported in 1963 by Baxi *et al*. and the prevalence rate varies. The frequency of G-6-PD deficiency is 4.5% (varies from complete absence to 27.1%) among Indian population and it is quite high among the Scheduled tribes as compared to the other ethnic groups (Bhasin *et al*., 1994). The frequency is higher among the tribals than the caste populations. The frequencies of G-6PD deficiency among Indian population as a whole ranges from complete absence to 27% (Bhasin and Walter 2001). It is higher among the scheduled tribes as compared to other ethnic groups. G6PD-deficient allele frequency is comparatively higher in North and West Indian zones, whereas in South India it is uniformly low except in Andhra Pradesh and Tamil Nadu. Prevalence of G6PD deficiency is generally 0–10%, although some communities may have higher prevalence: 27.5% for the Vataliya Prajapati community in Western India (Gupte *et al*., 2005) and 27.1% for the Angami Nagas, a tribal group in Northeastern India Seth and Seth, 1971). However, in the present study no case of G-6PD deficiency was found. It has been found that the distribution of G-6PD deficiency is not closely related to that of malaria (Bhasin *et al*., 1994). Saha *et al*., (1990) observed a very low frequency of G6PD among Naga, Hamar, Lepcha and Adi populations in the region of high malarial endemicity.

Anaemia is a condition in which the red corpuscles are reduced or the amount of haemoglobin is lessened. Sickle cell disease (SCD) is an autosomal recessive genetically transmitted hemo-globinopathy responsible for considerable morbidity and mortality. The sickle gene is an example of balanced polymorphism. Heterozygotes have a selective advantage and are protected against *Plasmodium falciparum* malaria while there is an increased premature death rate of homozygotes (Stuart and Nagel, 2004). It is prevalent in many parts of India including Central India, where the prevalence in different communities has ranged from 9.4-22.2% (Shukla and Solanki, 1985). Dunlop and Mazumder reported the first case of sickle cell hemoglobin in India.

Haemoglobinopathies are concerned with the abnormality in the protein molecule of red blood cells. This abnormality is due to defective synthesis of globin chains or its structure. A genetic defect that results in abnormal structure of one of the globin of the haemoglobin molecule is termed as haemoglobinopathy. These inherited genetic diseases of haemoglobin are controlled by a single gene and are transmitted from generation to the next. In India the presence of Sickle cell gene (HbS) was first detected in Nilgiri Hills of southern part (Lehman and Cutbush, 1952). Sickle cell is most common pathological

haemoglobin variant worldwide (Weatherall *et al*., 2006). The Indian subcontinent is a rich reservoir of sickle cell anaemia (SCA), thalassaemia (β- thal) and various abnormal haemoglobins. In India, HbS gene ('HbS' for HbAS carrier) is mostly confined to tribes in central and south India and the frequency ranges from 5 to 35 per cent (Bhatia and Rao, 1987) and in most of the Indian populations, castes and tribes the high incidence of various abnormal haemoglobins has been reported . In Central India study on sickle cell anaemia has been carried out mostly on tribal groups and very few on castes and other populations (Agarwal, 2005). WHO (2006) has reported an estimate of about 20-25 million homozygous individuals for sickle cell disease worldwide of which 5-10 million are in India (Serjeant,2006).

In India, the trait occurs most commonly among the tribal peoples in central India (southeastern Gujarat, Maharastra, Madhya Pradesh, Chhattisgarh, western Odisha) with a smaller focus in the south of the country (northern Tamil Nadu and Kerala), and trait frequencies as high as 40% have been described in some groups (Serjent, 2013). Among the Indian populations the frequency of sickle cell trait is 3.1% (Varies from complete absence to 41.0%). It is present in high frequency among the Scheduled tribes (5.4%) as compared to other ethnic groups (Bhasin *et al*, 1992, 1994). The frequency of HbS in Brahmins is 4.17%, in Kalar 5.41%, in Rajput 2.04%, in Muslims 3.73% in Maratha 2.08% in Bania 9.09% while in Teli it is 3.65% of Central India Urade (2012). Shah *et al*., (2012) have reported the allele frequency of sickle cell gene, i.e., 16.96% and 8.6% respectively in Moghul and Naga populations of Manipur. The earlier researchers have shown a complete absence of gene HbS in Muslims (Hakim *et al*., 1972; Saha *et al*., 1976; VijayKumar *et al*., 1987; Gorakshakar *et al*., 1987). But Urade has reported presence of HbS gene in Muslims (3.73%). In the present study of Badhiya Muslims no case of sickle cell was found. However, this finding needs verification by enlarging study area as well as sample size as once upon a time, malaria was prevalent in this area and there is abundance of swampy area in this region. This trait is reported to be present in scheduled castes and communities, who are living in close proximity with tribal populations. The trait has been transmitted among these groups due to admixture with tribal groups (Bhasin *et al*., 1994). The sickle cell trait is either absent (Bihari group of Indo-european family) or present in very low frequency (0.010) among Munda group of austro-asiatic speakers.

Haemoglobin polymorphism with G-6PD deficiency is advantageous to the communities against lethal effect of malaria especially against *Plasmodium falciparum* at population level but their combination is harmful at the individual level because of low level of blood cells indices to cope with the routine human physiology, where malaria is endemic. There is unexpected association between haemoglobinopathies and G-6PD deficiency (Jacques *et al*., 2007; Balgir, 2010). However, in the present study no case of G-6PD deficiency and sickle cell trait was found. Though, once upon a time malaria was prevalent in this area. Thus there is a decrease tendency of G-6PD deficiency and sickle cell anaemia in the study area which is according to observation made by Pandey and Ranjana, 20013 and Pandey *et al*., 2014. However, sickle cell has not been observed in Muslim populations in India (Balgir, 2007)

REFERENCES

Agarwal MB (2005) The Burden of Haemoglobinopaties in India – Time to Wake Up? *JAPI*. 2005; 53:1017–1018.

Balgir RS (2007) Genetic burden of red cell enzyme glucose-6-phosphate dehydrogenase deficiency in two major Scheduled Tribes of Sundargarh district, Northwestern Orissa, India, *Current Science*, 92 (6): 768–774.

Balgir RS (2010) Birth control necessary to limit family size in tribal couples with aberrant heterosis of G-6PD deficiency and sickle cell disorders in India: An urgency of creating

awarness and imparing ge-netic counselling. JAPI, 58, 357 – 362.

Baxi AJ, Balakrishnan V, Undevia JV and Sanghvi LD (1963) Glucose-6-phosphate dehydrogenase deficiency in the Parsee community, Bombay.," *Indian journal of medical sciences*, 17: 493–500

Bhatia HM and Rao VR (1987) Genetic atlas of the Indian tribes. Bombay: Institute of Immunohaematology, ICMR.77.

Bhasin MK, Walter H and Dankar- Hopfe H (1994) The distribution of Genetical Morphological And Behavioural Traits among the peoples of Indian Region, (Bangladesh, Bhutan, Maldives, Nepal, Pakistan and Sri Lanka). Kamala – RaJ Enterprises, New Delhi.

Daland GA and Castle WB (1948) A simple and rapid method of demonstrating sickling of red blood cells. The use of reducing agent. *J. Lab. Clin. Med*., 33: 1082.

Dunlop KJ, Mazumder UK (1952) The occurrence of sickle cell anemia among group of tea garden labourers of Upper Assam. Indian Med Gaz., 87:387-391.

Gorakshakar AC, Sathe MS, SR Shirsat SR and Bhatia HM (1987) Genetic Studies in Ratnagiri and Sindhudurg Districts of Maharashtra—Incidence of ABO, Rh (D), InaAntigens, G-6PD Deficiency and Abnormal Haemoglo- bins," Journal of the Indian Anthropological Society, 22:38-46.

Gupte SC, Shaw AN and Shah KC (2005) Hematological findings and severity of G6PD deficiency in Vataliya Prajapati subjects. Journal of Association of Physicians of India, 53: 1027–1030.

Hakim SMA, Baxi AJ, Balkrishnan VK, Kulkarni V, Rao SS and Jhala HT (1972) Haptoglobin, Transferrin and Abnormal Haemoglobin in Indian Muslims," *Indian Journal of Medical Research*, 60 (5): 699-703.

Jacques S, Denise Ilboudo D, Damintoti K, Sawadogo L, Maria E, Binet S, Nitiema H, Ouedrago P, Pignatelli and Nikiema JB (2007) Glucose- 6 phosphate dehydrogenase deficiency and sickle cell disease in Burkina Faso. *Pakistan J. Biol. Sci*., 10, 409 – 414.

Lehman H, Cutbush M (1952) Sickle cell trait in southern India. *Brit. Med. J*. 1(4755):404–405.

Motulsky AG and Campbell- Kranel JM (1961) Glucose-6- phosphate dehydrogenase deficiency of the red cell. In Proceedings of the conference on Genetic Polymorphisms and Genetic Variations in Diseaase. Grune and Stration, New York.

Pandey BN and Ranjana K (2013) Incidence of malaria in relation to serogenetic factors in population of Purnia district (Bihar). *J. Exp. Zool. India*, 16 (2):467 – 469.

Pandey BN, Mishra, AK, Kumar D and Vatsa S (2014) Sero-genetical study among rural women of Terai belt of Bihar, India. *FBLS*, 2(1): 1- 4.

Saha NR. Kirk L, Shanbhag S, Joshi SR and Bhatia HM (1976) Population Genetic Studies in Kerala and the Nilgiris (South West Asia)," *Human Heredity*, 26:175-197.

Serjeant GR (2006) Geograhpy and clinical picture of sickle cell disease. 2006 DOI: 10.1111/ J.1749-6632.1989 tb 24157.

Serjeant GR (2013) The Natural History of Sickle Cell Disease. Cold Spring

Seth PK and Seth S (1971) Biogenetical studies of Nagas: glucose-6-phosphate dehydrogenase deficiency in Angami Nagas, *Human Biology*, 43 (4): 557–561.

Shah A, Hussain R, Fareed M and Afzal M (2012) Gene frequency of sickle cell trait among Muslim populations in a malarial belt of India, i. e. Manipur. *Egypt. J. Med. Hum.Genet*., 13(3): 323 – 330.

Shukla RM, Solanki BR (1985) Sickle cell trait in Central India. *Lancet*, 1: 297 – 298.

Stuart MJ, Nagel RL(2004) Sickle cell disease. *Lancet* 2004; 364:1343–60

Vijaykumar M, Malhotra KC, Walter H, Gilbert K, Landenberg P, Dannewitz A, Sorensen A, R Chakraborty R, Reddy AP and Mukherjee BN (1987) Genetic Studies among the Siddis of Karnataka, India. A Migrant Population from Africa," *Zeitschrift fur Morphologie und Anthropologie*, 77 (2): 97-121.

Urade BP (2012) Incidence of sickle cell anaemia and thalassaemia in central India. *Op. J. Blood Dis*. :71–80.

Weatherall D, Akinyanju O, Fucharoen S, Oliveri N *et al*. (2006) 2nd ed. New York: Oxford University Press; Inherited disorders of hemoglobin, in disease control priorities in developing countries; pp. 663–680.

Bhasin MK, Walter H and Danker-Hopfe H (1992) The Distribution of Genetical, Morphological and Behavioural traits among the People of Indian Region. (Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan,Sri Lanka).Kamla -Raj Enterprises, Delhi

RESEARCH REPORT

Potential of Some Botanicals Against *Curvularia & Fusarium* Species

Bhajbhuje MN

Seed Pathology Lab., Department of Botany, Jawaharlal Nehru Mahavidyalaya, Wadi, Nagpur - 440 023 (M.S.) India.
E-mail : dr_mnbhajbhuje@rediffmail.com

Manuscript details:

Received: 01.10.2015
Revised: 02.11.2015
Accepted: 11.11.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Bhajbhuje MN (2015) Potential of Some Botanicals Against *Curvularia & Fusarium* Species, *International J. of Life Sciences*, 3(4): 399-402.

Acknowledgement:
The author indebted the facilitation of this work by Prof. R.P. Thakre, Mycologist and Prof. & Head, P.G. Dept. of Botany, RTM, Nagpur University, Nagpur.

ABSTRACT

Impatiens balsamina L. is one of the most potent medicinal plants extensively grown worldwide including India. The seeds of this plant are reported to be heavily infested with diverse group of fungi. The fungal organisms associated with seeds are known to deteriorate the nutrient content of seeds. As the plants serve as ecofriendly and economic bio control agents, the potential of aqueous extract of three plants each of family Acanthaceae and Euphorbiaceae was determined against three species each of seed borne isolates of *Curvularia* and *Fusarium* from stored seeds of *Impatiens balsamina* L employing poisoned food technique. Majority of the plant extracts were reported to be toxic, leading to inhibition for the growth of the test fungi. The aqueous leaf extract *Adathoda vasica* was found to be most potent reducing significant level of mycelial growth of test fungi.

Key Words: *Impatiens balsamina* L., *Curvularia*, *Fusarium*, fungicides, seed borne fungi, inhibition, antifungal activity.

INTRODUCTION

An annual herb, *Impatiens balsamina* L. (Gulmendi) of family Balsaminaceae, is native to southern Asia in India, Myanmar and Burma and extensively grown as ornamental plant worldwide in response to its use in traditional Ayurvedic medicine. Application of the extracts had a long lasting skin moisturizing effect and prevent dryness, rough skin chap, dandruff and splitting hair ends, hence it is used to prepare lotions, creams, hair tonics, cosmetics, bath preparations and detergents (Toki *et al*., 2000). Alcoholic extract of the flowers has been reported to be useful for pains in the joints. Roots are used to treat jaundice and digestive disorders. Juice from balsams leaves treats warts and snakebite; while the flower can be applied to burns to cool the skin. The plant has been used for the

treatment of thorn or glass -puncture wounds, abscesses, in grown nails and chronic ulcers cause by allergic reaction of detergents (Rajasekaran *et al*., 2009).

Impatiens balsamina L. is infested with several fungal organisms and most of them are seed-borne. Rajendran et. al., (2014) has isolated some microbial population including fungal and bacterial species from various parts of this plant. Moreover, the seeds are reported to be heavily infested with variety of fungal species (Madavi & Bhajbhuje, 2015). Fungal organisms associated with seeds bring about several undesirable changes making these hazardous for Ayurvedic preparation (Gangwar and Ghosh, 2014). An application of synthetic fungicides in minimizing spread of pathogenic fungal diseases is traditional practice. The foliar spraying of these fungicides is hazardous to the environment and also responsible to disturb the food chain. Moreover, the indiscriminate use of pesticides may result into development of resistance in the pathogens. To overcome these problems, treatment of extract of various parts of some plants may serve as alternative control remedy because the plants serve as ecofriendly and economic bio control agents.. Keeping this in view, the present report aims to study the potential of some plant extracts against seed-borne species each of *Curvularia* and *Fusarium* encountered on seeds of *Impatiens balsamina* L.

MATERIALS AND METHODS

Locally available plants each belonging to family Acanthaceae and Euphorbiaceae were collected. After oven drying, powder of various plant parts was made and stored at room temperature. The aqueous extracts of various plant parts were screened to study their antifungal potential on the mycelial growth of seed-borne fungal isolates of *Impatiens balsamina* L by poisoned food technique (Swami and Alane, 2013). After grinding, ten grams of the plant part with 100 ml sterile water; it was filtered through two layers of muslin cloth. The extract was heated to 55^0C in water bath for 15 minutes then poured to Potato Dextrose Agar (PDA) medium to obtain 1:1 final concentration. After autoclaving the medium at 15 lbs pressure for 20 minutes, it was allowed to cool at room temperature and then poured into sterile Petri plates. A small disc (0.7 cm diameter) of the actively growing fungus culture grown on Potato Dextrose Agar for seven days was cut with a sterile cork borer and transferred aseptically in the centre of the Petri plate containing agar medium along with plant extract. Suitable controls were kept where the culture discs were grown under the same condition on Potato Dextrose Agar medium without plant extract. The diameter of fungus colony was compared with the control, and considered as a measure of the fungitoxicity. Per cent inhibition was computed (Vimal and Das, 2015)

RESULTS AND DISCUSSION

The plants can provide a wealth of antimicrobial agents, and hundreds have been investigated for biological control. Scientific proof for antifungal activities of plants usually stagnates with the studies of respective plant parts against diverse group of fungal organisms (Gayatri and Ramesh, 2013). The literature survey concerns to antimicrobial activities of plants indicated that an aqueous as well as organic solvent extract of various parts of the plants have been used against the plant-pathogenic fungi to inhibit their activities involving degradation and deterioration of substrates (Swami and Alane, 2013; Nanthakumar *et al*., 2014; Vimal and Das, 2015). Majority of plants in the families Euphorbiaceae and Acanthaceae possess anti-microbial activity (Somchit *et al*, 2010; Sharma *et al*., 2013; Gangwar and Ghosh, 2014). The phenols and tannins content in extract may contribute to the antimicrobial effect (Nanthkumar *et al*., 2014).

The data presented in the table 1 indicated that all the plant extracts inhibited the mycelial growth of the fungal organisms on culture

Table 1: Effect of extract of plant parts against seed-borne fungal organisms

S. No	Plant	Diameter of fungal growth (mm)					
		Curvularia lunata (Wakker) Boedijn	*Curvularia ovoidea* (Hirosa & Watan) Munt	*Curvularia tetramera* (Mck.) Boe. ex Gilman	*Fusarium miniliformae* Sheldom	*Fusarium oxysporum* Schlecht	*Fusarium solani* (Mert.) APP. & Wollenw
	Control	75	72	78	62	71	78
1	*Acalypha indica*	36 (52.0)	41 (43.1)	44 (43.6)	32 (48.4)	33 (53.5)	36 (53.8)
2	*Adathoda vasica*	32 (57.3)	34 (52.8)	31 (60.3)	28 (54.8)	30 (57.7)	32 (59.0)
3	*Rungia repens*	39 (48.0)	37 (48.6)	40 (48.7)	34 (45.2)	34 (52.1)	38 (51.3)
4	*Euphorbia antiquorum*	34 (54.7)	39 (45.8)	34 (56.4)	37 (40.3)	38 (46.5)	40 (48.7)
5	*Euphorbia hira*	41 (45.3)	41 (43.1)	37 (52.6)	40 (35.5)	40 (43.7)	43 (44.9)
6	*Jatropha gossypifolia*	43 (42.7)	40 (44.4)	45 (42.3)	38 (38.7)	31 (56.3)	33 (57.7)

*Figures in parenthesis indicate percent inhibition of mycelial growth of fungus over control.

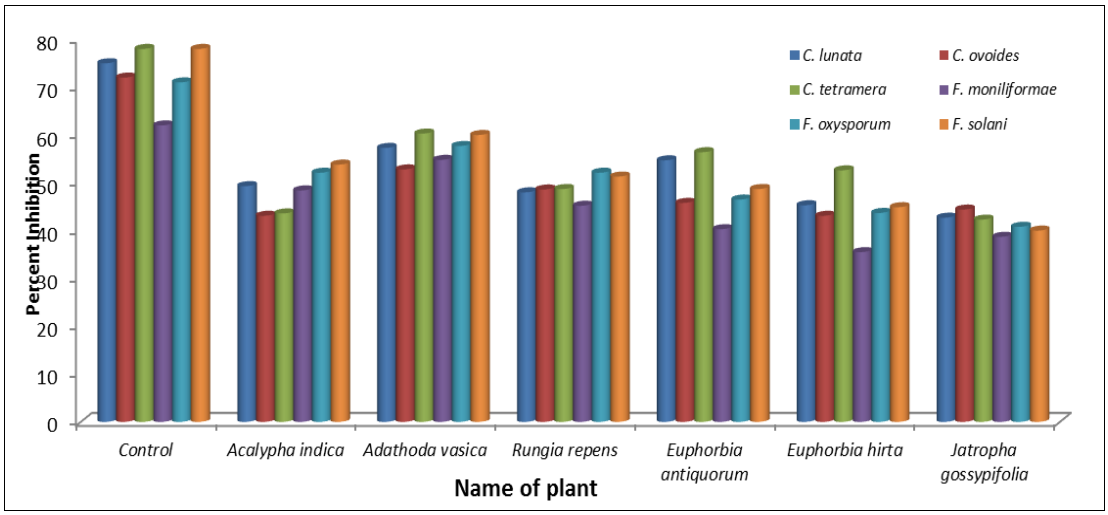


Fig.1: Effect of plant extract on mycelia growth of fungal organism

medium. The leaf extract of *Acalypha indica caused* maximum inhibition of mycelial growth of *Fusarium solani* (53.8%), *F. oxysporum* (53.5%) and *Curvularia lunata* (52.0%). *Adathoda vasica* effectively caused maximum inhibition of mycelial growth of test fungal isolates including *Curvularia tetramera* (60.3%), *Fusarium solani* (59.0%), *F. oxysporum* (57.7%) and *Curvularia lunata* (57.3%) *Fusarium moniliformae* (54.8%) and *Curvularia ovoides* (52.8%). *Rungia repens* was found to be inhibitory to *Fusarium oxysporum* (52.1%) and *F. solani* (51.3%), and it was less significant for other test fungi. *Euphorbia antiquorum* caused 56.7% and 54.4% inhibition of growth of *Curvularia lunata* and *C. tetramera* respectively while 40 – 48% inhibition was recorded for all three species of *Fusarium* and *Curvularia ovoides*. *Euphorbia hirta* proved highly inhibitory to *Curvularia tetramera* (52.6%) while *Jatropha gossypiflora* inhibited the growth of *Fusarium solani* (57.7%) and *F. oxysporum* (56.3%) (Fig. 1).

Much work has been done on the use of plant extracts against the plant-pathogenic fungi. Extract of *Acalypha indica* was effective against *Candida albicans, C. tropicalis, Microsporum canis, Aspergillus fumigatus* (Somchit *et al*., 2010). Leaf,

flower and stem extracts of *Adhatoda vasica* caused inhibition of mycelial growth of *Alternaria alternate, Phytophthora* sp., *Fusarium oxysporum, Aspergillus niger*, *Rhizoctonia solani, Curvularia lunata*, *Cladosporium* sp. *Curvularia penneseti* (Swami and Alane, 2013). *Rungia repens* was reported anti-pyretic and analgesic (Swain *et al*., 2011). The extract of *Euphorbia antiquorum* was effective against *Candida albicans, C. cruzi, Candida tropicalis, C. parapolisis and Aspergillis sp due to presence of many biologically active molecules such as alkaloids, cynogenic glycocides, phenols,flavonoids and terpenoids* (Vimal and Das, 2015). The extract of inflorescence of *Euphorbia hirta* exhibited antifungal activity against *Aspergillus flavus* targeting the cell membrane (Gayathri and Ramesh, 2013). The extract of *Jatropha gossypifolia* are active against human microbial pathogens thus emerging as potential sources of new antimicrobial compounds The plant has great promising potential as a source of antimicrobial compounds against microorganisms (Swain *et al*., 2011). These reports confirmed antimicrobial activity of the plant extract against the fungal organisms. *Euphorbia pulcherrima* was effective against *Colletorichum gloeosporioides, C. dematium, Aspergillus flavus and Fusarium oxysporum* (Swami and Alane, 2013).

CONCLUSION

The present report reveals that the aqueous extracts had antifungal activity in wide range of magnitude and can used as alternative control to the chemical fungicides. Application of extract of these plant parts to seeds, may control mycelial growth of seed borne fungal organisms associated with seeds of *Impatiens balsamina* L.

REFERENCES

Gangwar AK, Ghosh AK (2014) Medicinal uses and Pharmacological activity of *Adhatoda vasica International Journal of Herbal Medicine,*2(1) : 88-91

Gayathri A, Ramesh V (2013) Antifungal activity of *Euphorbia hirta* L. inflorescence extract against *Aspergillus flavus* A mode of action study. *Int. J. Curr. Microbiol. App. Sci.,* 2(4):31-37

Madavi Shivkumar, Bhajbhuje MN (2014). Mycodiversity of seed borne flora of Rose balsam (*Impatiens balsamina* L.) *In proceeding of UGC-Sponsored Nat. Seminar on Life Sciences Revolution: PCR & its applications* organized by Sindhu MV. NGP In *Int. J. Biosci., Agric. & Technol*., Special issue Dec. 2014 : 268-275.

Nanthakumar R, Udhayasankar MR, Ashadevi V, Arumugasamy K, Shalimol A (2014) *In vitro* antimicrobial activity of aqueous and extracts of *Rhinacanthus nasutus*- a medicinal plant. *Int. J. of Pharma, Chemical& Bio.Sci.*, 4(1), 164-166

Rajasekaran AJ and De Lucca JW Cary (2009) Aflatoxin control through approaches. *Toxin Reviews*, 28(2): 89 -101

Rajendran R, Manikandan A, Hemalatha K, Prabhavathi P. Karthik SS (2014). Efficancy of *Impatiens balsamina* extracts for antimicrobial activity. *PSGCAS Search: A Journal of Science & Technology*, 2 (1) : 60-64.

Sharma V, Kumawat TK, Seth R, Sharma A. (2013) Bioefficacy of Crude Extracts from *Jatropha gossypifolia* against Human Pathogens. *Int. J. of Biotech. and Bioengg. Res.*, 4 (4) : 401-406

Somchit1 MN, R. Abdul Rashid R, Abdullah A, Zuraini A, Zakaria ZA, Sulaiman MR, Arifah, AK, Mutalib AR (2010)*In vitro* antimicrobial activity of leaves of *Acalyphaindica* Linn. (Euphorbiaceae) *African Journal of Microbiology Research*, 4(20) : 2133-2136.

Swain SR, Sinha BN, Murthy PN (2011) Comparative evaluation of antipyreticand analgesic activities of *Rungia repens* nees and *Rungia pectinata* linn *Asian Journal of Pharmaceutical and Clinical Research*, 4(2) : 103-106

Swami CS, Alane SK (2013) Efficacy of some Botanicals against Seed - borne fungi Of Green Gram (*Phaseolus Aureus* Roxb.) *Bioscience Discovery*, 4(1):107-110

Toki M, Kondo M and Maeda M (2000) A move from traditional to more patient-oriented management. Kokai Tokkyo Koho. 143: 488.

Vimal JB, Das SM (2015) Antifungal activity of *Euphorbia antiquorum* L. late an *in vitro* study. *International Journal of Applied Research*, 1(3): 25-28.

REVIEW ARTICLE

Adverse Impacts of Changing Climatic Conditions on the Environment due to increasing Pollution

Piyush Gupta

Assistant Professor, Department of Chemistry, SRM University, NCR Campus, Modinagar, India
Email: piyushg.1977@gmail.com

Manuscript details:

Received: 27.07.2015
Revised: 20.10.2015
Accepted: 26.11.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Gupta Piyush (2015) Adverse Impacts of Changing Climatic Conditions on the Environment due to increasing Pollution. *Int. J. of Life Sciences*, 3(4): 403-408

ABSTRACT

This paper deals with the different perceptions about global environmental crisis due to the over burden of pollution. Environmental problems are not as bad as they are made out to be. The earth is a self-evolving and self-regulating living system and it will survive (Gaia Theory). The common issues associated with the earth's environment are Environmental pollution, Water scarcity, Contamination of food, Waste management, Global Climate Change, Land degradation, Desertification (80% of forests are destroyed), Acid Rain, Ozone Layer Depletion, Soil Erosion, Loss of Biodiversity and Mass extinction (Most species are endangered and 50% may go extinct by 2025) etc.

Keywords: Environment, Climatic change, Pollution, Global Warming, Pollutants, Waste, Health

INTRODUCTION

Weather and climate play a significant role in people's health. Changes in climate affect the average weather conditions that we are accustomed to. Warmer average temperatures will likely lead to hotter days and more frequent and longer heat waves. This could increase the number of heat-related illnesses and deaths. Increases in the frequency or severity of extreme weather events such as storms could increase the risk of dangerous flooding, high winds, and other direct threats to people and property. Warmer temperatures could increase the concentrations of unhealthy air and water pollutants. Changes in temperature, precipitation patterns and extreme events could enhance the spread of some diseases (USGCRP, 2009). Deforestation, landslides, soil erosion, floods, droughts, illegal constructions, more exploitation, mountaineering, garbage, heavy movement, resource depletion, volcanic eruptions and melting of glaciers are leading to utmost

severe challenges that we can face while surviving on the earth (Mckibben, 2010). Most of the world cities lack adequate water and sanitation. 80 countries of the world suffer from serious water shortages, one fourth of the population have no access to safe drinking water, half of the population lacks sanitation facilities. In India there is no drinking water in more than 60,000 villages. The per capita availability of freshwater is declining globally. Contaminated water remains the greatest single environmental cause of human sickness and death. Diarrhoea kills one million children per year 45 million affected by bad water per year.

The decline of quantity and quality of surface and groundwater is impacting aquatic ecosystems and their functionality. By 2025, approx 1.8 billion people will be living in countries or regions with absolute water scarcity, and two-thirds of the people in the world may face water stress. Aquatic ecosystems continue to be heavily exploited, putting at risk sustainability of food supplies and biodiversity. Global marine and freshwater fish catches show large-scale declines, caused mostly by persistent overfishing. There will be no fish to catch, by 2050. The average population density in coastal areas is now twice as high as the global average. More than 100 million people live in areas no more than one metre above mean sea-level. 21 of the world's 33 mega-cities are located in coastal areas, with most of them in developing countries. the loss of key ecosystems such as wetlands, mangroves and coral reefs. Sea level rise due to climate change, are increasing the risk of flooding and reducing coastal protection from storms, tsunamis and erosion. Increase in urban population from 732 million in 1950 to 3.2 billion in 2006. Half the world population lives in cities (from 2008). Asia and Africa to double their urban populations to roughly 3.4 billion by 2030 (Hunter and Cohen, 2011).

Eight of the world's 10 most populous cities sit on earthquake faults. 6 out of 10 are vulnerable to storm surges. Urban air pollution kills an estimated 800,000 people each year, roughly half of them in China. More than 50 countries have lost between 90 and 100 % of their forests. Tropical forests are being cleared at the rate of one hectare every second. In whole world 24% mammals, 12% birds, 25% reptiles, 30% fish are threatened or endangered. 100-1000 times faster than natural process of extinction. In India more than 10% flora and fauna are threatened, many on verge of extinction.

IMPACTS FROM HEAT WAVES

Heat waves can lead to heat stroke and dehydration, and are the most common cause of weather-related deaths. Excessive heat is more likely to impact populations in northern latitudes where people are less prepared to cope with excessive temperatures. Young children, older adults, people with medical conditions, and the poor are more vulnerable than others to heat-related illness. The "urban heat island" refers to the fact that the local temperature in urban areas is a few degrees higher than the surrounding area. Climate change will likely lead to more frequent, more severe, and longer heat waves in the summer (see 100-degree-days figure), as well as less severe cold spells in the winter. A recent assessment of the science suggests that increases in heat-related deaths due to climate change would outweigh decreases in deaths from cold-snaps (USGCRP, 2009). Urban areas are typically warmer than their rural surroundings. This climate change would increase the demand for electricity in the summer to run air conditioning, which in turn would increase air pollution and greenhouse gas emissions from power plants. Heat waves are also often accompanied by periods of stagnant air, leading to increase in air pollution and the associated health effects.

IMPACTS FROM REDUCED AIR QUALITY

At least one billion people in the world breathe unhealthy air. More than 2 million people globally die prematurely every year due to outdoor and indoor air pollution. Although air pollution has

decreased in some countries across the world, but some unwanted emissions are increasing. Indoor air pollution due to the improper burning of solid biomass fuels imposes an enormous health burden. Indian cities are the most polluted in the world. Despite significant improvements in air quality since the 1970s, as of today millions of people lived in counties that did not meet national air quality standards (EPA, 2010).

Increases in Ozone

Ground-level ozone is formed when certain air pollutants, such as carbon monoxide, oxides of nitrogen (also called NO_X), and volatile organic compounds, are exposed to each other in sunlight. Ground-level ozone is one of the pollutants in smog. If emissions of air pollutants remain fixed at today's levels until 2050, warming from climate change alone could increase the number of Red Ozone Alert Days (when the air is unhealthy for everyone) by 60-70% (CCSP, 2008). Scientists project that warmer temperatures from climate change will increase the frequency of days with unhealthy levels of ground-level ozone, a harmful air pollutant, and a component in smog. Smog decreases visibility and can be harmful to human health. Ground-level ozone can damage lung tissue and can reduce lung function and inflame airways. This can increase respiratory symptoms and aggravate asthma or other lung diseases. It is especially harmful to children, older adults, outdoor workers, and those with asthma and other chronic lung diseases (NRC, 2010). Ozone exposure also has been associated with increased susceptibility to respiratory infections, medication use, doctor visits, and emergency department visits and hospital admissions for individuals with lung disease. Some studies suggest that ozone may increase the risk of premature mortality, and possibly even the development of asthma (EPA, 2006).

Changes in Fine Particulate Matter

Sources of fine particle pollution include power plants, gasoline and diesel engines, wood combustion, high-temperature industrial processes such as smelters and steel mills, and forest fires. Due to the variety of sources and components of fine particulate matter, scientists do not yet know whether climate change will increase or decrease particulate matter concentrations across the world (EPA, 2009). A lot of particulate matter is cleaned from the air by rainfall, so increases in precipitation could have a beneficial effect. At the same time, other climate-related changes in stagnant air episodes, wind patterns, emissions from vegetation and the chemistry of atmospheric pollutants will likely affect particulate matter levels. Climate change will also affect particulates through changes in wildfires, which are expected to become more frequent and intense in a warmer climate (NRC, 2010).

Particulate matter is the term for a category of extremely small particles and liquid droplets suspended in the atmosphere. Fine particles include particles smaller than 2.5 micrometers (about one ten-thousandth of an inch). These particles may be emitted directly or formed in the atmosphere from chemical reactions of gases such as sulfur dioxide, nitrogen dioxide, and volatile organic compounds. Inhaling fine particles can lead to a broad range of adverse health effects, including premature mortality, aggravation of cardiovascular and respiratory disease, development of chronic lung disease, exacerbation of asthma, and decreased lung function growth in children (EPA, 2009).

Changes in Allergens

Climate change may affect allergies and respiratory health. The spring pollen season is already occurring earlier in the world due to climate change. The length of the season may also have increased. In addition, climate change may facilitate the spread of ragweed, an invasive plant with very allergenic pollen. Tests on ragweed show that increasing carbon dioxide concentrations and temperatures would increase the amount and timing of ragweed pollen production (Confalonieri et al., 2007). A recent interim assessment finds that:

- Climate change may increase surface-level ozone concentrations in areas where pollution levels are already high.
- Management of air quality would be more difficult.
- Policy makers should consider the potential impacts of climate change on air quality when making air quality management decisions.

Ozone Hole

The "hole" in the stratospheric ozone layer over the Antarctic is now the largest ever. Due to decreased emissions of ozone depleting substances and assuming full Montreal Protocol compliance, the ozone layer is expected to recover, but not until 2060 (or even later).

Global Warming

Earth's surface is continuously warming up due to Global warming. The Himalayan glaciers are receding in India. The Arctic is melting. Antarctic ice shelves are breaking off. Clouds are bursting. The weather is becoming unpredictable. This global warming is leading to floods, earthquakes, land sliding volcanic eruptions, etc. The frequency of these natural disasters and the number of people affected are increasing. Sea level rise is threatening the existence of small islands (Lawson, 2008; Weart, 2003 and Bell and Strieber, 1999).

Interconnected Things

Increasing population, urbanisation, unscientific developmental activities, food prices, increasing disparities, marginalisation of the poor, farmers in distress, increasing militarisation, terrorism, large scale corruption, frauds, financial crisis, economic ups and downs are another factors which should be controlled (Miller Jr. , 2008).

Natural Disasters

Nepal earthquake also known as the Gorkha earthquake killed more than 8,800 people and injured more than 23,000 in April 2015. It was the worst natural disaster to strike Nepal since the 1934 Nepal-Bihar earthquake. In June 2013, a multi-day cloudburst centered on the North Indian state of Uttarakhand caused devastating floods and landslides becoming the country's worst natural disaster since the 2004 tsunami. From 14 to 17 June 2013, the Indian state of Uttarakhand and adjoining areas received heavy rainfall, which was about 375% more than the benchmark rainfall during a normal monsoon. This caused the melting of Chorabari Glacier at the height of 3800 metres, and eruption of the Mandakini River which led to heavy floods near Gobindghat, Kedar Dome, Rudraprayag district, Uttarakhand, Himachal Pradesh and Western Nepal, and acute rainfall in other nearby regions of Delhi, Haryana, Uttar Pradesh and some parts of Tibet. In Cyclone Sidr, B'desh, 3000 were dead and 500,000 homes were gone. In Cyclone Nargis, Myanmar, 8000 were dead, Worst drought in China, drying rivers, Quake in China, 70,000 were dead and 4.8 million became homeless. California wildfires, 2000 sq. km, 500,000 evacuated. Arctic ice lowest since 1970, Northwest Passage open, Greenland ice melt maximum since 50 years, global glacier melt doubled. Amazon deforestation is increasing. An Ocean dead zone doubles every decade. SE Asia Ocean running out of fish, threat to livelihood of people. China emits 14% more than US. Russian tanker spill 2000 tons near Black Sea. China birth defects 40% up since 2001, due to pollution.

IMPACTS FROM EXTREME WEATHER EVENTS

The frequency and intensity of extreme precipitation events is projected to increase in some locations, as is the severity (wind speeds and rain) of tropical storms. These extreme weather events could cause injuries and, in some cases, death. As with heat waves, the people most at risk include young children, older adults, people with medical conditions, and the poor. Extreme events can also indirectly threaten human health in a following number of ways:

- Reduce the availability of fresh food and water.
- Interrupt communication, utility, and health care services.
- Contribute to carbon monoxide poisoning from portable electric generators used during and after storms.

- Increase stomach and intestinal illness among evacuees.
- Contribute to mental health impacts such as depression and post-traumatic stress disorder (PTSD) (USGCRP, 2009).

IMPACTS FROM CLIMATE-SENSITIVE DISEASES

Changes in climate may enhance the spread of some diseases. Disease-causing agents, called pathogens, can be transmitted through food, water, and animals such as deer, birds, mice, and insects (USGCRP, 2009). Climate change could affect all of these transmitters.

Food-borne Diseases

Higher air temperatures can increase cases of salmonella and other bacteria-related food poisoning because bacteria grow more rapidly in warm environments. These diseases can cause gastrointestinal distress and, in severe cases, death. Flooding and heavy rainfall can cause overflows from sewage treatment plants into fresh water sources. Overflows could contaminate certain food crops with pathogen-containing faeces.

Water-borne Diseases

Heavy rainfall or flooding can increase water-borne parasites such as *Cryptosporidium* and *Giardia* that are sometimes found in drinking water. These parasites can cause gastrointestinal distress and in severe cases, death. Heavy rainfall events cause stormwater runoff that may contaminate water bodies used for recreation (such as lakes and beaches) with other bacteria. The most common illness contracted from contamination at beaches is gastroenteritis, an inflammation of the stomach and the intestines that can cause symptoms such as vomiting, headaches, and fever. Other minor illnesses include ear, eye, nose, and throat infections.

Animal-borne Diseases

Mosquitoes favor warm, wet climates and can spread diseases such as West Nile virus. The geographic range of ticks that carry Lyme disease is limited by temperature. As air temperatures rise, the range of these ticks is likely to continue to expand northward. Typical symptoms of Lyme disease include fever, headache, fatigue, and a characteristic skin rash. The spread of climate-sensitive diseases will depend on both climatic and non-climatic factors. Many developed countries have public health infrastructure and programs to monitor, manage, and prevent the spread of many diseases. The risks for climate-sensitive diseases can be much higher in poorer countries that have less capacity to prevent and treat illness than developed countries.

OTHER HEALTH LINKAGES

Other linkages exist between climate change and human health. For example, changes in temperature and precipitation, as well as droughts and floods, will likely affect agricultural yields and production. In some regions of the world, these impacts may compromise food security and threaten human health through malnutrition, the spread of infectious diseases, and food poisoning. The worst of these effects are projected to occur in developing countries, among vulnerable populations. Although the impacts of climate change have the potential to affect human around the world, there is a lot we can do to prepare for and adapt to these changes (Confalonieri et al., 2007).

CONCLUSION

The impacts of climate change on health will depend on many factors. These factors include the effectiveness of a community's public health and safety systems to address or prepare for the risk and the behavior, age, gender, and economic status of individuals affected. Impacts will likely vary by region, the sensitivity of populations, the extent and length of exposure to climate change impacts, and society's ability to adapt to change. In addition, the impacts of climate change on public health around the globe could have important consequences (Joseph, 2006; UNEP 2005).

Many of the expected health effects are likely to fall mostly on the poor, the very old, the very young, the disabled, and the uninsured. Climate change will likely result in regional differences in impacts, due not only to a regional pattern of changes in climate but also to regional variations in the distribution of sensitive populations and the ability of communities to adapt to climate changes. Adaptation should begin now, starting with public health infrastructure. Individuals, communities, and government agencies can take steps to moderate the impacts of climate change on human health.

Organizations and governments around the globe are taking up the issue in arms. The simplest global phenomenon is called the Environmental Impact Assessment (EIA). Environmental impact assessment is a planning tool and a formal process that is now generally regarded as an integral component of sound assessment and decision making which will determine the potential environmental, social and health effects of a proposed development. The EIA is thus one of the forerunning tools to study, identify and improve on past, present and future environment hazards.

REFERENCES

Bell Art and Strieber Whitley (1999) The Coming Global Superstorm.

CCSP (2008) *Analyses of the effects of global change on human health and welfare and human systems* . A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. Gamble JL, Ebi KL, Sussman FG and Wilbanks TJ. U.S. Environmental Protection Agency, Washington, DC, USA.

Confalonieri U, Menne B, Akhtar R, Ebi KL, Hauengue M, Kovats RS, Revich B and Woodward A *(*2007) Human health. In: *Climate Change: Impacts, Adaptation and Vulnerability*. *Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Parry M, Canziani OF, Palutikof JP, van der Linden PJ and Hanson CE (eds.), Cambridge University Press, Cambridge, United Kingdom.

EPA (2006) *Air Quality Criteria for Ozone and Related Photochemical Oxidants*. U.S. Environmental Protection Agency, Washington, DC, USA.

EPA (2009) *Assessment of the Impacts of Global Change on Regional U.S. Air Quality: A Synthesis of Climate Change Impacts on Ground-Level Ozone (An Interim Report of the U.S. EPA Global Change Research Program).* U.S. Environmental Protection Agency, Washington, DC, USA.

EPA (2009) *Integrated Science Assessment for Particulate Matter: Final Report*. U.S. Environmental Protection Agency, Washington, DC, USA.

EPA (2010) *Our Nation's Air: Status and Trends Through 2008*. U.S. Environmental Protection Agency. EPA -454/R -09-002.

Hunter Lovins L and Cohen Boyd (2011) Climate Capitalism: Capitalism in the Age of Climate Change.

Joseph Benny (2006) Environmental Studies, Tata McGraw-Hill Publishing Company Limited.

Lawson Nigel (2008) An Appeal to Reason: A Cool Look at Global Warming.

Mckibben Bill (2010) Eaarth: Making a Life on a Tough New Planet.

Meadows Donella, Meadows Dennis and Randers Jorgen. Beyond the Limits, 1992.

Miller Jr. GT (2008) Environmental Science, 11th Edition, Cenage Learning India Pvt. Ltd.

NRC (2010) *Adapting to the Impacts of Climate Change*. National Research Council. The National Academies Press, Washington, DC, USA.

NRC (2010) *Advancing the Science of Climate Change.* National Research Council. The National Academies Press, Washington, DC, USA, 2010.

United Nations Environment Programme UNEP (2005) Atlas of Our Changing Environment.

USGCRP (2009) *Global Climate Change Impacts in the United States*. Karl TR, Melillo JM and Peterson TC (eds.). United States Global Change Research Program. Cambridge University Press, New York, NY, USA, 2009.

Weart Spencer R (2003) The Discovery of Global Warming.

AUTHOR INDEX

IJLSCI EVENTS

ज्ञान-विज्ञान विमुक्तये

UGC SPONSORED NATIONAL CONFERENCE ON
INNOVATIONS IN AGRI-BIOSCIENCES
26th & 27th FEBRUARY 2016

Organized by

Department of Botany, Biochemistry & Biotechnology

Dr. AMBEDKAR COLLEGE

NAAC RE-ACCREDITED WITH "A" GRADE

Deekshabhoomi, Nagpur-440010 (M.S.) India

In Association with

ICAR - CENTRAL INSTITUTE FOR COTTON RESEARCH (CICR),NAGPUR.

INDIAN SCIENCE CONGRESS ASSOCIATION, NAGPUR CHAPTER

Important Dates

Abstract / Full length Paper Submission
Deadline - 20th Jan. 2016
Early Bird Registration - 15th Jan. 2016

CATEGORY	EARLY-BIRD	AFTER 15th Jan. 16
Students	Rs. 500/-	Rs. 600/-
Research Scholar	Rs. 800/-	Rs. 1000/-
Delegate (Academic)	Rs. 1500/-	Rs. 1800/-
Delegate (Industry)	Rs. 2500/-	Rs. 3000/-

Accommodation: On request through e-mail, guest house(s) will be provided with applicable charges on first come first basis. Requests reaching after **15th Feb 2016** cannot be entertained.

Cheque/Draft in Favour of:
Principal,
Dr. Ambedkar College,
Deekshabhoomi, Nagpur

Bank Details:
Name of Bank: Bank of India
Branch: Dharampeth, Nagpur
Acc. No. : 870210100017422
IFSC Code: BKID0008702

Six Best Presentation Awards For STUDENTS & RESEARCHERS

Contact for Enquiry/Queries

Mrs. B. A. Mehere
Mob: 9860431885
Subhash Somkuwar
Mob: 9527157556
Dr. D. N. Begde
Mob: 9823162400

Email ID: nciabs.dacn2016@gmail.com

INSTRUCTION FOR PREPARING THE MANUSCRIPT:

International Journal of Life Sciences publish research papers, case studies and short communications containing original research work of good standard and review papers of contemporary relevance from all over the world. Following types of contributions are considered for publication:
RESEARCH PAPERS (Full Length - Maximum of 12 pages-A/4 size).
CASE STUDIES
SHORT COMMUNICATIONS
REVIEW PAPERS including Mini Reviews

Author can send manuscript as an attachment on email to editorilsci@gmail.com OR editor@ijlsci.in.
Manuscript format should be in MS word only. Charts, Tables and Diagrams should be in MS Excel or MS Word and images in JPG format using maximum possible 150 dpi resolution. All the matter should be typed in Times New Roman Font size - 12 point with single spacing.

AUTHORISATION AND DECLARATION

Authors must accept full responsibility for the content of their articles. The Members of the Editorial Board and the Publisher of the journal are not responsible for the statements and opinions expressed by the authors in their articles/write-up published in the journal. To use the copyrighted material (for example table, figure etc.), the author/s must seek the permission from the Author/s and Publisher of the material and send the copy of the permission letters via e-mail to the Administrative Editor for records. In case, if it is not possible than do not use that material.
While submitting the paper the author (s) must give a declaration that "the article has not been published or sent for publication elsewhere".

INSTRUCTIONS FOR MANUSCRIPT-FORMAT

a) Page Layout: A4 (21cm × 28 cm) leaving 2 cm margin on all sides of the text. All the text Should be single spaced and the pages should be numbered consecutively.
b) Use MS word (2003-2007) for text and TIFF, JPEG or Paint for figures.
c) The first page should contain:
Title-bold, 16 point size
Name/s of author/s-bold, 10 point size (Surname or Family name first in bold) Affiliation/s-address of all the author/s followed by email of corresponding author in 10 point size,
Abstract: up to 200 words in 10 point size
Keywords: at least 5 keywords in 10 point size
d)Main Text- The paper should be divided into the following sections:
Introduction, Material and Methods, Results and Discussion, Conclusion, Acknowledgement and References.
Tables and Figures of good resolution (150 dpi) should be numbered consecutively and given alphabetically by surname or family name in the order of their appearance in the text.
e) Reference style
Text: All citations in the text should refer to:
1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication;
2. Two authors: both authors' names and the year of publication;
3. Three or more authors: first author's name followed by "et al." and the year of publication.
Citations may be made directly (or parenthetically).
Groups of references should be listed first alphabetically, then chronologically.
Examples: "as demonstrated (Baig, 2004a, 2004b, 2010; Chavhan and Pawar, 2011). Jadhao et al., (2000) have recently shown…

Reference to a journal publication:
Chavhan AB, Pawar SS, Jadhao RG and Patil KG (2013) Distribution of CC-chemokine receptor-5-Δ32 allele among the tribal and caste population of Vidarbha region of Maharashtra state, *Indian J Hum Genet.*, 19 (1) 65-70.

A Book:
Durbin R, Eddy SR, Krogh A, Mitchison G (1999) Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. Cambridge University Press.

A Chapter in a Book:
Leach J (1993) Impacts of the zebra mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra mussels: biology, impacts and control, Eds., Nalepa, T. and D. Schloesser. Ann Arbor, MI: Lewis Publishers. pp: 381-397.

Report:
Makarewicz JC, Lewis T, Bertram (1995) Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference Proceedings:
Chavhan AB (2012) Biomedical Waste Management: Awareness and Practices among Healthcare Providers in Amravati". Proceeding of national conference on Recent Trends and Innovative Development in the Frontiers of Life Science. pp- 148-153. (ISBN-978-93-81733-04-2).

A Thesis:
Chavhan AB (2012) Genetic polymorphism of Chemokine receptors in some tribes of Vidarbha, Maharashtra. Ph. D. Thesis, RTM University, Nagpur,India;.

Web references:
As a minimum, the full URL should be given and the date when the reference was last accessed.

PROOFS

Proofs will be sent to the corresponding author via e-mail as an Acrobat PDF (Portable Document Format) which should be returned to the Editorial office within 2 working days.

PROCESSING FEES

Please send the Membership subscription or processing fees. Processing fees (Rs. 2000/- for India & US$ 120/- for Overseas) has to be paid every time when one submits the manuscript. Life members need not pay processing fees. If one is Annual member, then one can submit two manuscripts during the annual membership. If one submits more than three manuscripts in a year, one has to pay processing fees from fourth manuscript onwards.

ISSN: 2320-7817 (Print) ISSN: 2320-964X (Online)
Journal home page : www.ijlsci.in

Contact : +91-997 055 9438
+91-942 077 5527

INTERNATIONAL JOURNAL OF LIFE SCIENCES

An International Peer Review Open Access Journal

G-3 Dattamandir, Vinayak nagar, Nagpur Road,
Amravati- 444603, India

MEMBERSHIP FORM

The Editor,

I wish to be a member of **International Journal of Life Sciences (IJLSCI)** and agree to abide by the rules setup by you. The filled in proforma is:

(1) Full Name (in block letters) :

(2) Gender :

(3) Nationality :

(4) Working status and Department :

(5) Field of Research :

(6) Mailing Address :

(7) City : State :

Country : Pin :

(8) Phone (O):..... (R): ..... (M): .....

(9) E-mail :

(10) Nature of Subscription: Annual Membership / Life Membership / Fellow Membership

MEMBERSHIP	ANNUAL		LIFE		FELLOW	
	Indian	Overseas	Indian	Overseas	Indian	Overseas
Individual :	INR. 2,500/-	US $ 150	INR. 10,000/-	US $ 550	INR. 15,000/-	US $ 850
Institutional :	INR. 3,500/-	US $ 250	INR. 15,000/-	US $ 850	INR. 25,000/-	US $ 1700

Mode of Payment

INRs./US$/Euro can be remitted by online payment/E-Money Transfer/Wester Union Money Transfer/Bank Demand Draft/Cheques (at par only)/Cash mentioning Receipt No. and date.
Detail of transfer process is as follows:

A/c No.	:	31033988127
MICR Code.	:	444002976
Branch Code	:	003866
IFS Code	:	SBIN0003866
Branch Address	:	State Bank of India, Old Biyani College Road Tapowan Road Distt Amravati Maharashtra 444602
Full Name of Receiver	:	**ARVIND BHIMRAO CHAVHAN**
Full Address (Including City, Street & Postal Code)	:	G-3 Dattamandir, Vinayak nagar, Nagpur Road, Amravati- 444603, India.

Date .....

Signature.....

Name .....

Email this Membership form along with Scan copy of Fees deposit receipt to editorijlsci@gmail.com / editor@ijlsci.in