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About The Conference

International Conference on "Recent Trends in Life Sciences" organized by the Department of Zoology, Modern College of Arts, Science and commerce, Ganeshkhind, Pune–16 and sponsored by SPPU Pune under QIP on 2nd and 3rd February2018. The theme of the conference included Biodiversity, Biotechnology, Biophysics and Bioinformatics, Nanotechnology, Agro bio science, Botanical science, Wetland Conservation and management, Environmental science Earth and Geological science, Food and Nutrition, Microbial Medical sciences, Animal behavior sciences, E-learning, Networking in life science Molecular taxonomy. The aim of this conference was to bring on board students, teachers, stake holders &scientists on a common platform who are avid readers, do research and active conservation specialists. This international conference allowed exchanging thoughts, provoking debates, which would lead to constructive & novel research and conservation principles applicable to Indian scenario. Besides, this, the conference provided exposure to the academician, researchers, consultants and policy makers on high quality research.

The Inauguration of the conference was carried out by the auspicious hands of Guest of Honour Prof. W.N.Gade, former Vice Chancellor, S.P.P.U. The Chief Guests were Dr. Nanda Bahadur Singh, Professor, Tribhuvan University, Nepal and Dr. R.S.Pandit, Head, Department of Zoology, S.P.P.U. The Dignitaries present were Prof. P.G.Dixit, Deputy Secretary and Visitor, Modern College Ganeshkhind; and Dr.S.G.Deshpande, Scientist from NCl, Pune. The welcome speech was given by our respected Principal, Dr. S.S.Kharat. The felicitation of the guest was followed by Release of Proceeding.

OBJECTIVE OF THE CONFERERNCE

The aim of the conference is to bring on board students, teachers, stake holders & scientists on a common platform who are avid readers, do research and active conservation specialists. This international conference will allow exchanging thoughts, provoking debates, which would lead to constructive & novel research and conservation principles applicable to Indian scenario. This conference will allow discussing inextricable issue that represents urgent priorities for scientist, conservationists and policy maker. Besides, this conference will also give exposure to the academician, researchers, consultants and policy makers on high quality research will lead to high impact output.

THEME

- Biodiversity
- Biotechnology
- Biophysics and Bioinformatics
- Nanotechnology
- AgroBioscience and Botanical Science
- Wetland Conservation and Management
- Environmental Sciences
- Earth and Geological Science Food & Nutrition
- Microbial and Medical Science
- Animal Behavior science
- Information technology,
- E-learning,networking in Life science
- Molecular Taxonomy.

ABOUT SAVITRIBAI PHULE PUNE UNIVERSITY

Savitribai Phule Pune University established in 1948. It is spread over 411 acres, which is home to 46 academic departments. The university is named after Savitribai Phule, a 19th-century Indian social reformer who is known for her contribution towards empowerment and emancipation of women through education. The university has affiliated colleges, departments, and research institutes, which are primarily in Pune. It has been given "A^+" ranking by NAAC for its overall performance and was ranked 14 in India overall by the National Institutional Ranking Framework in 2017 and 10th among universities. As of 2017 the university had 43 departments, 433 affiliated colleges and 232 recognized research institutions, with an enrolment of 496,531 students for undergraduate and graduate courses. More than 70 research institutions have been recognized by the

Savitribai Phule Pune University for research, including institutions of national importance like the Inter-University Centre for Astronomy and Astrophysics (IUCAA), National Chemical Laboratory (NCL), National Centre For Cell Science (NCCS), National Institute of Virology (NIV), among others.

ABOUT COLLEGE

Progressive education Society's Modern College of Arts, Science and Commerce, Ganeshkhind, Pune (Estd. 1992) offer 21 graduate, 10 postgraduate and some Ph.D programs of Savitribai Phule Pune University, in various classical and interdisciplinary courses in social sciences, commerce, life sciences, natural sciences, to name a few, with students from 18 countries and 24 Indian states. The college is also running UGC funded B. Voc. (Food Processing and Technology). The college has been awarded with highly prestigious Star status from the Department of Biotechnology (DBT), Government of India in 2017, being the first college under S.P. Pune University to get this coveted status. The college has been re-accredited with 'A' Grade y NAAC, Bangalore, and is supported under DST-FIST program. The college has received different awards instituted by S.P.Pune University viz. Best college Award, Best Principal Award, Best college Award for Student Welfare Board and Program Officer, Best NSS Unit and Program Officer Award, Best College Magazine. Maharashtra Energy Development Agency (MEDA) has honored us with a State level award for excellence in Energy Conservation and Management recognizing us as a Green Educational Institute State-of –the art facilities including ITC-enabled classrooms, well equipped Laboratories, hi-tech Computer Labs, and well- stocked Library along with a Digital Library, and computerized Administrative Office enables college to provide learner-centric environment. The college has excellent research facilities and vibrant academic and research environment.

ABOUT LIFE SCIENCE DEPARTMENT

Lifescience departments include Department of Zoology, Botany, Biotechnology and Microbiology and were established from 1993. These departments have emerged as excellent centers for providing quality education at under-graduate and post-graduate levels. All the departments regularly organize activities for students and faculty members for updating and meeting the demands of industry and academia. Well equipped laboratories and qualified staffs with rich experience in teaching, research and industry are key strengths which have enabled to excel in research based teaching learning processes. Faculty members are active in research and received research support from different funding agencies like University Grants Commission (UGC), Board of College and University development (BCUD-SPPU), Science and Engineering Research Board (SERB-DST), Indian Space Research Organization (ISRO). Currently ten minor and major projects funded by these agencies are in progress. Department of zoology is a recognized research center and have recognized guides for M.Phil. and Ph.D and is affiliated to S.P.Pune. University.

CONTENTS

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Themed Section:Science and Technology

Biosystematic study of Glyptapanteles malshri sp. Nov (Hymenoptera: Braconidae)

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ABSTRACT

The order Hymenoptera is extremely important from the view of Biological control of insect pests. Biology forms the basic information for the workers involved in biological control programmes for formulating mass rearing. The genus glyptapanteles is rcognised bu Ashmead in 1905. It is one of the larger segregates of the old "Apanteles". 5-10% of the species in temperate regions and about 25% in tropic , probably 1000 species in Wilkinsons group A or Nixons species group. 198 females and 111 males, wings, antenna, legs were mounted and studied. 150 early second instars of Plutella xylostella (Binn) on cabbage were exposed to five mated females of Glyptapanteles malshri sp. Nov. Parasitoid eggs and larvae were collected after 12h interval by dissecting parasitized host larvae in saline.

Keywords : Hymenoptera, Biological control, Glyptapanteles

I. INTRODUCTION

The parasitic hymenoptera is an important component in biological control programme. Biological control and taxonomy are interrelated and interdependent. Taxonomists need for the identification of biological control agents, understanding their evolutionary history, compilation and to guide explorations for native and exotic parasitoids. The detailed taxonomical works on Indian species were those of Wilkinson (1928, 1929), Bhatnagar (1948), Rao (1961), Nixon (1967), Rao and Chalikwar (1970), and Sathe and Inamdar (1988, 1989). In assessments of parasitic hymenoptera a reliable approach would be to study their lifecycle stages. Biometrical data is helpful in separation of different instars of the species. Fulton (1940), Cardona and Oatman (1971), Rojas – Rouse and Benoit (1977), and Sathe and Nikam (1985) have attempted such type of studies. It is estimated that there are about 250,000 species of parasitic Hymenoptera in the world, of which only about 50,000 have been described (Gupta 1988).

The family Braconidae having almost 40,000 species is divided into 21 subfamilies, some important among them are Euphorinae, Microgastrinae, Braconiae, etc. The subfamily Microgastrinae is of economic importance because they breed from the lepidopterous hosts. It includes the three genera into which Foerster Microgaster Lateriella, Microgaster, Microplitisand Apanteles. Apanteles genus was given by Foerster in 1862. Nixon (1965) divided this genus into 44 species groups. Some of these groups are very large like ater, ultor, etc: some groups, on other hand, have less than half a dozen species. Rao (1961) compared critically this genus with the help of all available literature and type specimens and divided Apanteles into two subgenera viz. Areolatus and Carinatusby presence or absence of propodeal areola as the main, valid and important character for the division. The catalogue of Apanteles Shenefelt (1972) lists 1118 valid species and nearly 200 more have been described since then for a total of about 1300 species. 2000 species have been included under this genus by Mason (1981) from different parts of the world. Organized

The genus Glyptapanteles is recognized bu Ashmead in 1905. It is one of the larger segregates of the old "Apanteles". 5-10% of the species in temperate regions and about 25% in tropic , probably 1000 species in Wilkinson's group A or Nixon species group virtripennis, octonarius, pallipes, siderion ,demerter, fraternus, triangulator are included under Glyptapanteles. The vitripennis being especially well developed in cool and humid temperate climate while the octonarius in humid warm temperate and tropical climate. The genus Glypatapanteles is less well represented from dry climate.

Mason kept the following neartic species to Glyptapanteles(new combination) : (octonarius group). Apanteles affray Muesebeck A.cassianus Riley A.floridanum Mues., A.herbertii Ashmead.

Glyptapanteles malshri sp. Nov.:

Length 4.08 mm excluding ovipositor , forewing 4.00mm long , antenna 3.56 mm long , weakly trapered to apex.

Head:

0.80 mm long, it is circular and convex smooth ; interorbital space is 0.80mm which Is width of head , ocelli in triangle , ocellar space equal to the interocellar Space , front ocellar is 0.16mm frons smooth dark brown , shiny . Antenna 16 segmented 3.56mm, smaller than length of the body , first 7 segments having transverse band , first segment smaller than other 15 segments penultimate segment 0.25mm .

Flagellar formula:

2 L/W = 2.5 ; 14 L/W =2.4 ; L 2/14= 1.1 , W 2/14 = 1.0 Eye pubescent, 0.37mm long , 0.1mm wide ; molar space rugose.

Thorax:

1.68mm long ; mesonotum lacking setae on the sublateral lobes, punctuate ; width of tegulae is slightly broader , brown , 0.12mm long . Propodeum 0.48mm broad and 0.40mm long , smooth, only middle region is coarsely punctuate , no trace of areola, prepctal carina absent. Fore wing length 4.00mm ; stigma is dark black in colour and hairy ; radius and intercubitus slightly equal ; radius is strong ; basal vein strongly angulated Hind wing 3.5mm long , vennal lobe convex with fringe of hair , areolet open . Hind leg 4.67mm long , yellow in colour ; hind tibia with strong spines on outer side ; length of femur is 1.04mm tibia is dark brown colour , 1.08 mm long , spurs equal , 0.24 mm long , sharply pointed ; 0.60mm length of hind basitarus ; tarsal segment are 1.08mm long claws 0.12mm long , curved inside , black in colour , pointed .

Abdomen:

Spindle shaped , 1.60mm long ; tergite I never wider at apex , 0.28 mm long , the sides gradually converging apically and strongly rounded to apex ; tergite II 0.23mm long tergite III ; basal two tergites completely smooth and polished , ovipositor 0.28mm long and ovipositor , few hairs concentrated near the apex .

Male:

Similar to female , length 4.0mm .

Cocoon:

White , 3.4mm long .

Host:

Plutella xylostella (Binn) , on cabbage .

Holotype:

Female , India , Maharashtra , Kolhapur , on cabbage , Brassica apitata L , collection , January to June 1988 - 1989 ; antenna legs , wings , on slides , labelled as above .

Paratype:

23 females, 52 males , sex -ratio , Male : Female , 1 : 0.44 . Same data as in holotype , reared from larvae of the above mentioned host in India , Maharashtra , Kolhapur , collection in January to June 1988 - 1989 .

Discussion:

Glytapanteles malshri species run close to Glyptanteles militaris (weed) in Mason's key in its characters.

1. Ovipositor sheath is shorter than ovipositor and with few hairs concentrated near the apex.

2. Areolet open (2 r-m absent) .
3. Tergites I always tapering apically, tergite II sub triangular and wider posteriorly.
4. In propodeum, areola absent but trace of longitudinal median carina present .

It differs with
1. Propodeum is with two lateral carinae.
2. Antenna smaller than its body .
3. The first 7 segments having transverse band .
4. Vannal lobe of hind wing convex and fringed with hairs .
5. Hind leg 4.67mm long , faint brownish - yellow in colour .
6. Tergite Irugose and punctuate.

Glyptapanteles malshri sp. Nov. (Fig I):

Egg (Fig I-2):

At the time of oviposition the egg of G.malshri is translucent, white , smooth surface and is cylindrical , slightly acute . Usually only one egg is deposited per host. The ends of the egg are somewhat rounded and there is no visible stalk or pedicel. The chorion is transparent and lacks surface sculpturing but somewhat smaller than the newly laid eggs .Eggs is randomly deposited in the hemocoele of the larvae. At deposition, the eggs contents are homogeneous. However, as development proceeds, the embryo was distinctly visible with nine narrow segments in the middle portion of the body . Free embryonic cells have been found in the host blood, it appears that may constitute part of the food of the parasitoid. The ripe ovarian 25 eggs averaged 0.52 mm in length (range 0.49- 0.54) and 0.187 mm in width (range 0.175 - 0.196 mm). Egg hatching period is 1-2 days.

Larvae:

G.malshri has 3 larval instars.

First instar (Fig I-3):

It is noted that the first instar found floating freely in the body cavity of the host, usually at about 5th or 6th abdominal segments. The head of the parasitoid larva directed towards the head of its host. Eclosion is protracted process which may require up to four hours. The larva forces its head through the egg, splits from anterior side. The body consists of a broad quadrate Head, 3 thoracic and 7 abdominal segments. There are two raised oral papillae situated anterior to the mouth which are capable of contraction and retraction. This instar is manipulate type . The mandibles are long and sharply pointed when at the rest their edges cross each other. These are not densely sclerotised at this stage and are capable of free and quick movement. The tracheal system was not seen in this stage. The mean body length and width of 25 individuals averaged 1.31 mm (range 1.28 - 1.38 mm) and 0.24 mm (range 0.21- 0.26 mm) respectively . The mean length and width of head capsule in 25 individuals were 0.101 mm (range 0.098 - 0.11 mm) 0.085 mm (range 0.079 - 0.095 mm respectively. The averaged length of 25 mandibles was 0.05 mm (range 0.032 - 0.061mm) and width was 0.015 mm (range 0.012 - 0.017 mm) while vesicle averaged in its length and width 0.22 mm (range 0.21 - 0.24 mm) and 0.24 mm (0.19 - 0.29 mm) respectively. Mature first instar is almost pale yellowish in colour. The head become less prominent and narrower than the rest of the body. The vesicles is minute in young host instar, but it appers to be well developed, bladder like by 2nd day after eclosion. The first instar lasts for 3 days.

Second instar(Fig I-4):

Second instar was first found on the 5th day after oviposition. It was hymenopteri form and somewhat oval in shape. The opaque body is creamy white and consists of a narrow head, 13 well defined segments and a prominent vesicle. The cuticle is smooth and appears to lack setae. The cephalic structure is very weakly sclerotized, so that the mandibles are easily discernible even in cleared specimens. The head is smaller and more sclerotized. Evagination of the last segment has prominently developed into a vesicle with clearly seems to consist of a single layer intestine. The paired salivary glands were very conspicuous forming series of loops. The tracheal system is well developed with two longitudinal trunks. Into the head , some

short branches are extended and posteriorly then run almost the entire length of the larva. These longitudinal trunks are connected just behind the head by a dorsal commissural. Still no spiracles have seen. Spines or setae were not apparent on the body. The mean body length and width of 25 individuals were averaged 1.72 mm (range 1.53 - 1.91 mm) and 0.378 mm (range 0.355 - 0.389 mm) respectively. In 25 individuals, head capsule measured 0.189 mm in length (range 0.172 - 0.183 mm). The averaged length and width of 25 mandibles were 0.63mm (range 0.048 - 0.79 mm) and 0.23 mm (range 0.016 - 0.027 mm) respectively . Measurement of vesicles in 25 individuals averaged 0.53 mm in length (range 0.15 - 0.58 mm) and 0.64 mm in width (range 0.61 - 0.67 mm) . The second instar lasts for only one day.

Third instar(Fig I-5):

The third instar appeared 7th day after oviposition. The body of larva is creamy white and opaque , consists of the head and 13 well defined segments . It tapers slightly toward both the ends. Early last instar have an anal vesicle, the structure gradually decrease in size and lastly disappears in matured larvae.

The cephalic structure is well developed and is described according to the terminology of short (1952 - 1953). The head is well developed with two prominent mandibles and sclerotized facial structure. The head is divided into a dorsal epicranial part and ventral buccal region. The epicranial part consists of a frons with two lateral rudimentary antennal stockets and a clypeus. The buccal area consists of a supra oral labrum the mouth and two dark brown sclerotized mandibles with saw like teeth on the dorsally directed cutting edge. Each mandible is with a broad proximal base tapering distally to a sharp point. The broad base articulates dorsally with the anterior pleurostomal process and ventrally with the posterior pleurostomal process. A strongly curved hypostoma with a ventrally directed sclerotized hypostomal spur lies behind each maxilla. The labial sclerite is supported by lateral stipital sclerites on each side. The labium has two oval labial palpi a silk rest of the body and is apparently telescopic.

Digestive system is well developed; which consists of the mouth, a slender oesophagaus, a large mid intestine closed at its posterior end and the anus. The silk glands found surrounding the digestive tract. In 3rd instar larva 8 pairs Of spiracles are very prominent. One pair is situated in 2nd thoracic segment and one pair in each of the 7 abdominal segments. While rest of the tracheal system is similar to 2nd instar. The average diameter of thoracic spiracular opening was 0.009 mm. The average body length and width Of 25 third instar were 2.75mm (range 2.52 -2.85mm) and 0.679 mm (range0.45 -0.832 mm) respectively . The measurement of head capsule in 25 individuals averaged 0.32mm in length (range0.31 - 0.34mm) and 0.301mm in width (range0.292 - 0.304mm) . The average length and width of mandible in 25 cases were 0.102mm (ranges 0.090mm - 0.104mm) and 0.040 mm (range 0.038 - 0.042 mm) respectively. The average length and width of vesicles were 0.25mm (ranges 0.20 - 0.31mm) and 0.30mm (range 0.26 0.33 mm) respectively, vesicles were smaller than second instar. The third instar lasted 1 -2 days. The parasitoid larvae were found floating in the posterior half of host larva. The mature parasitoid larvae exist from the host larvae, with the help of their mandibles by cutting the lateral line and thus killing their host .

Biometry:

Biometry studies of different instars of G.malshri showed that there is an increase in the length and width of larval form as well as in head capsule, mandible with respect to age (Table-1). The result obtained clearly indicated that there exists (length -$P < 0.50$, width -$P < 0.30$) correlationship between the age of the larval instar and the size which was tested with regression analysis ($r = 1.0$) for length and ($r = 0.974$) for width . The statistical result is tabulated in the (Table-2).

Cocoon (Fig I-6):

After emergence, the last instar larva of parasitoid form a silvery white, densely spun, cylindrical cocoon

which is round at both ends. The cocoon formed is attached with host food plant parts. The mean length and width of 25 cocoon were 3.4 mm (range 3.35 - 3.50mm) and 1.3 mm (range 1.21 - 1.35 mm) respectively.

Prepupa :

The prepupa appeared on the 9th day after oviposition and last for one day . It is differentiated from late 3rd instar by the appearance of the constriction in the middle portion of the body and by the pupal structures, such as segmentation of the abdomen , can be seen through the integuement. The length of 20 individuals were 3.2 mm (ranges 3.1 -3.4mm) and width 0.92 mm (range 3.1 - 3.4 mm) and width 0.92 mm (range 0.88 - 0.95mm).

Pupa(Fig I-7):

The pupa of G.malshri is of the exarate or free type , it is creamy white . The eyes were blackish and ocelli brown. As development proceeds, the entire pupa gradually darkens. The pupal appendages found loosely oppressed to the body. With the help of developing ovipositor the female pupa can be readily distinguished. The average length and width of 25 individuals were 2.90 mm (range 2.88 - 3.00 mm) and 1.05 mm (range 1.00 - 1.08mm) respectively. Under laboratory conditions 26 +- 1° c , the pupal period lasts for 6 - 7 days .

The average duration of the life cycle of G.malshri from egg to adult emergence was 15 - 16 days.

Emergence:

Emergence of the adult G.malshri as found at day time. The adult emerged from cocoon by cutting off at side a circular cap, which was pushed aside and usually remain attached. After emergence the adult spent a brief time for cleaning their bodies. If food available, feeding could occur immediately. Usually make emerged before female.

Adult(Fig I-1,8):

The male differentiated from the female by its sexual characters and dark abdomen. Antenna was 16 segmented and 4.08 mm long , shorter than body , propodeum contain longitudinal median carina and also both of lateral carina , vannal lobe of hind wing convex and fringe of hair , legs are faint yellow colour , Tergite rugoes and punctuate at apex . Length of female was 4.5mm, ovipositor 0.28mm long.

Mating:

Mating amongst the adult parasitoid was observed within 12 hr after emergence and it lasted for about 1 minute. Both sexes attracted towards each other when caged in plastic container (size 4 x 4 cm). The male recognized the female. After several attempts the male catch-up the female. By catching, the male suddenly mounted the female, and if there was no resistance, copulation took place. During mating, both sexes were remained, stationary. The males found perusing other females after separation and copulated with several. However the female apparently mate only once.

Preoviposition:

Immediately after emergence, both sexes were placed in small glass tube. The adults were supplied honey as food. At the time of emergency females already have a number of mature ovarian eggs but not deposited as soon as host material was encountered. It takes 20 hr before oviposition the preened and newly emerged

Females do not respond the host larva. The substrate is examined with the antennae by extending forward.

Oviposition:

After landing on the cabbage boll the female found searching for its host by moving around and tapping the cabbage surface with her antennae. If damage part come across , she become excited and start searching vigorously , and later , stabbing intention movements are made . The female examine continuously untill she located the probable position of the host larva on

the cabbage. She then quickly inserted the ovipositor in the host larva, the parasitoid deposited an egg in tge larva , requiring less than 2 - 3 sec . If the host was not contacted, the female withdrew her ovipositor and inserted it in a new place . The probing operation was persistently repeated until the host larva was parasited.

Host age selection(Table -3,4):

In this experiment optimum age for maximum was find out, Result are recorded in Table 12 & 13. The number of parasitoid emerged from host of age 2,3,4,5,6,7,8,9 and 10 days old larvae were 8,28,44,72,60,46,28,18 and 7 respectively , while parasitoids have not emerged from the hosts which were one day old . Maximum 48% parasitism was recorded on 5 days old hos000t0s a00nd meam number of parasitoids emerged per replicate under this age was 72 . Host larvae , older than 5 days that have been progressively less suitable . The regression analysis indicated that there was a significant correlation between host age parasitism (r=0.067, P<0.10)

Longevity:

Neither sex survived for more than two days without food and water. The result is shown in table 14. The mean survival of males fed with 10% and 20% honey was 5.3 and 7.0 days and in females 5.52 and 7.5 days respectively. Maximum survival of females was 13 days while make survived for 12 days when fed with 50% honey. In general, females live longer than males.

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Table no. 1: Biometrical Measurement of Larval instars *G. Malshri*

Sr.No.	Body structure	Larval instars		
		First	Second	Third
I	Larval Body			
	Length	1.31	1.725	2.75
	Width	0.24	0.378	0.678
II	Head			
	Length	0.1.1	0.189	0.323
	Width	0.085	0.180	0.301
III	Mandibles			
	Length	0.05	0.063	0.323
	Width	0.015	0.023	0.040
IV	Vesicle			
	Length	0.22	0.53	0.25
	Width	0.24	0.64	0.30

Table no. 2: **Statistical Linear Regression relationship between larval age and Length of body of** *G. malashri*

Instars No.	Age in days	X2	Larval leg.	Y2	xy	Expected value y
1	2	4	1.31	1.7161	2.62	1.82
2	4	16	1.723	2.9687	6.892	1.927
3	6	36	2.75	7.5625	16.5	2.675
	12	56	5.783	12.2098	26.012	
Mean x= 4,Mean :y= 1.927,a=0.437,b=0.3725,r= 1.0 t=0.615,p < .50						

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A Taxonomical Shortnote On Microgomphus Torquatus (Anisoptera: Odonata) From Pavana River (Pune District, Ms: India)

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ABSTRACT

Microgomphus torquatus (Anisoptera) species belonging to Family: Gomphidae, which is also called as Clubtails. This type of the species commonly identified by their unique eyes, which are well separated and black, brown to yellow colouration. This family derived its name gomphides due to its last abdominal segment which are bulbous, club or dilated downward in shape. As compared to other dragonflies it shows variability in body size. Usually this species shows abdomen about 22-25 mm in length in case of male and 26-27 mm in case of female, Hind wings about 20-22 mm in case of male while 23-24mm in case of the Female. Wings are tinted in colouration with brownish shade and abdomen generally 29- 30 mm in length. They show greenish brown coloration, legs are whitish and also possessing black spines on it. They occur in the habitat from River surroundings with shady vegetations.

Keywords: Microgomphus torquatus, Gomphidae, Clubtails, Pavana River etc.

I. INTRODUCTION

Pseudagrion species are belonging from the family Coenagrionidae, one of the major families which is distributed all over the Planet in which there are 1000 known species of damselflies found worldwide, Coenagrionidae are small and generally held their wings close above the abdomen. The genus Pseudagrion is particularly well developed in Africa with more than 40 species exhibiting much disparity in habitat requirements, appearance and behavior. This genus is also diverse and widespread in Asia with 28 species including, Ceriogrion coromandelianum Fabricius 1798 is common and widespread in most parts of tropical Asia. Depending upon the habitat specificity regarding temperature, atmosphere, changing climate they act as a very good indicator of environment. As they show carnivorous mode of feeding habitat they ultimately act as a bio indicator (Tiple et. al, 2008). Sonawane in (2014) reported the distribution of the Ceriogrion coromandelianum Fabricius, 1798. In which the genus Ceriogrion is particularly well developed in Africa with more than 40 species. Ceriogrion coromandelianum was reported as a common damselfly over Lakes, Ponds and Rivers. As in Ceriogrion coromandelianum species their habitat and also feeding ability noticed similarity in Microgomphus torquatus in this research work. Sonawane and Khandagle (2014) reported a field note on odonates of Ganesh Lake, Akurdi-44 (Pune District, MS: India).

The main objective of the study was measuring the population, diversity and abundance of damselflies throughout year 2013. During this survey water quality also matter for the occurrence of the species specificity. According to Nair (2011) some of the species of the Family: Gomphidae showed mosquitovorous ability in which Microgomphus

torquatus, Dysphaea ethela, Caconeura sp. Onycothemis testacea, Zygonyx eiris had been noticed. Microgomphus torquatus also showed remarkable thing that this species occurs within undisturbed habitats with good water and forest ecosystem (Shady Area). The reported species showed some differences in anal appendages which help in separating genera level classification.

Mostly Gomphides inhabited majoritily aquatic habitat with flowing water like rivers for their breeding purposes, Nair (2011). Out of the 90 species found in peninsular India, (Subramanian, 2005) 9 species reported from the Orissa by Nair in 2011. Sonawane et al., (2013) reported seasonal variation and abundance of damselflies (Zygoptera) at Gupteshwar Lake 18.2509 Latitude (Nº); 74.3345 Longitude (Eº) habitat specifying several species of the odonates preferably. The cannibalism property also reported in this work and the cannibalism by Microgomphus torquatus over Disparoneura quadrimaculata. Andrew et. al., 2008 reported the impact of changing in landscape going on since from last fifty years or so in the peninsular India over odonates.

DISTRIBUTION:

Microgomphus torquatus showed ancient finding from the Western Ghats including the Deccan, Pune and Satara region. This species which showed their occurrence very rare mentioned localities. While Fraser (1934) reported that distribution of this species is very common during rainy season.

PAVANA RIVER

The Pavana River is located at 18.383'' Latitude (Nº) from north side and 73.4510" Longitude (Eº) East-side. Originates in the Western Ghats and is a tributary of Bhima River from opening to the Lonawala city east side. It is located at a distance of 1800 feet ASL. The river Pavana merges with the river Mula in Pune city. The Pavana River is slightly polluted, than the river Mula. It is more polluted than the river Bhima.

SCIENTIFIC CLASSIFICATION

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta.
Order: Odonata
Suborder: Anisoptera
Family: Gomphidae
Genus: Microgomphus
Species: torquatus

II. METHODS AND MATERIAL

Specimens were collected with the help of Insect collecting Net during morning time; total

32 species were collected in total 3 month survey reports. Specimen were collected individually and kept in butter paper envelop so as to make them to allow for void their excreta. After that they were placed into the vial with 100% Ethanol for permanent preservation it get dehydrated. Morphometrical analysis was done by using thread because the collected specimen got stip and also certain post mortem changes. Wet preservation method used because dry preservation will give more brittleness to the specimen.

DETAILS OF SIGHT

While describing taxonomical shortnote on Microgomphus torquatus from Pavana River at Rawet Bridge, Pune Maharashtra on 25 September 2014 to 10 November 2014, a tandem pair of yellowish -colored dragonfly was spotted at 09:51 a.m. The pair was initially thought to be the common Gomphides species after field guide, morphological characters this species were identified the Clubtail which was commonly, found species. However the yellow colored with black striped marking on head of the male suggested otherwise and on closer investigation, it was recognized to be Microgomphus torquatus. The pair remained in tandem for two minute at submerged aquatic vegetation of water lancet.

III. RESULTS AND DISCUSSION

SPECIMEN DETAILS

The following descriptions are based on the pair captured on 17 August 2014. The male Microgomphus torquatus is generally of a moderate yellow color from the head to the thorax with black strips over it. The eyes are yellowish, being darker above and lighter below with light dark black dot in eyes as they shows compound eyes. The abdomen has continuous yellow rings at every interval throughout the abdomen.

Taxonomical features: The morphological characters observed are –

1. Remarkable black 'Y' marking on thorax region.
2. Wings with tinted brownish in colouration at the base.

A. On Thorax of the Microgomphus torquatus shows yellow and black colour alternating strips which are separated by blackish brown midline of the thorax.

B. Male:

Head: Labium yellow in colour, Labrum is greenish yellow in colour, the base and the front border narrowly black, the basal marking produced as a triangular mark in the middle line; frons bases of mandibles ante and post- clypeus greenish yellow in colouration. Prothorax: yellow and black strip, Thorax: black, marked with yellow. laterally greenish yellow, with black strips which tapers apically. Wings are transparent Hyaline. Pterostigma pale yellow in colouration. Abdomen black, marked with greenish yellow as follows Segment – 1. Segment 2 with a mid dorsal blobbed spot not quite reaching the apical border the sides broadly yellow , including the large , abdomen segment no 3 showed black strip.

Male (♂) anal appendages:

- Superior anal appendages yellowish in colour, with small yellow spot over it and black border beneath.
- Superior anal appendages have a minute distinct spines at apex region.
- Genitalia black, yellow coloured at base & very prominent

C. **Female (♀)**

Females are generally looking like male but dull in colouration but larger with blunt abdomen. There are 3-4 spines on the thorax of the abdomen. Lateral lining of the abdomen showed black demarcation. Abdominal segment no 8th with yellow coloured mark.

Female (♀) anal appendages:

- Anal appendages in male are relatively very small but expandable and yellow in colouraton.
- Conical, pointed

Microgomphus torquatus inhabits slow running water in open, degraded habitats with rich aquatic vegetation as well as rivulets on the lateral side of the river. The habitat is shaded with trees and high grassy vegetation along Pavana River Region. The location was near submerged vegetation and edge of the river side is trapped under the tall trees, which had created a small- undisturbed area around the river region. This habitat was very different from the other shady areas because marshy place also including around one side of the river. Nodal Index is nothing but square from costa to median (Nodus) and nodus to wings spot respectively for every wing individually.

Fig.: 1 Microgomphus torquatus (♂)

Fig: 2 Head of Microgomphus torquatus

Fig: 3 Thorax of Microgomphus torquatus

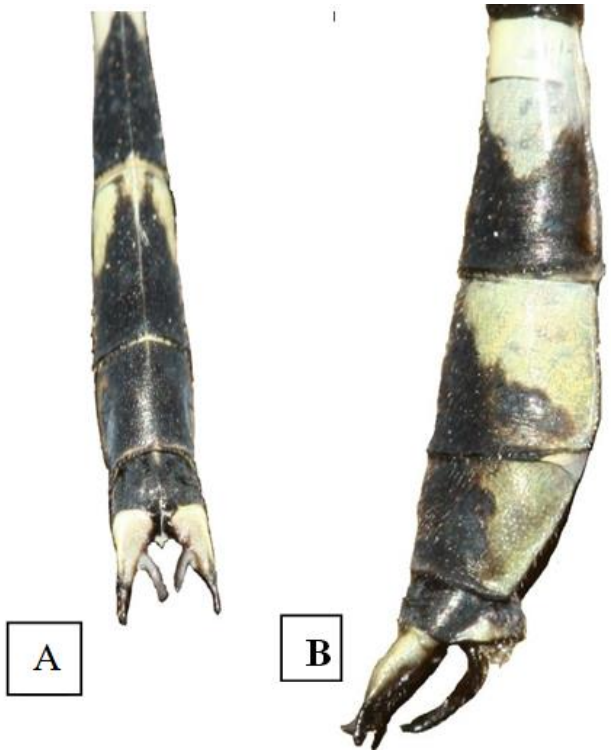

Fig: 3, A: Anal appendages of Microgomphus torquatus (♂) dorsal view & B: anal appendages of Microgomphus torquatus (♂) Lateral side view.

Fig 4: Cannibalism Microgomphus torquatus over Disparoneura quadrimaculata (♀).

No.	Authors	Left Fore wing (mm)	Right Fore wing (mm)	Left hind wing (mm)	Right hind wing (mm)
1	Fraser F.C. 1933	9-12	12-8	9-10	9-8
2	Sonawane A.R. 2014	12-7	12-7	9-11	10-8

(♂)/(♀)	Abdomen Length (mm)	Hindwing (mm)	Wings spot	Eyes
Male(♂)	22-25	20-22	Pale brown	Above: brown above golden combination below.
Female(♀)	26-27	23-24	Pale brown	Light brown

Table: 2, Morphometry of Microgomphus torquatus {(Male (♂) & Female (♀)}

Microgomphus torquatus were reported to be absent around the lakes and ponds. Therefore the distribution of this species is as not usual as seen throughout the state. There are several open ponds in Maharashtra included parks and other forested areas in India but Microgomphus torquatus have been recorded in few part of Maharashtra (Satara, Nagpur). Thus yet unknown inhibiting factors could be restricting the species distribution in India. One reason could be due to ecological niche segregation from the more common and dominant Gomphidae family.

IV. ACKNOWLEDGEMENT

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Amputation induced Reactive Oxygen Species regulate tissue regeneration in Clinotarsus curtipes tadpoles

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ABSTRACT

Regeneration is the ability of an organism to repair a wound which is partially destroyed tissue of animal's body or the damage involving the loss of an organ or the larger part of the body. Understanding the molecular mechanisms that promote tissue regeneration is necessary for advancements in regenerative medicine. Amphibian tadpoles' residents of aquatic systems, species Clinotarsus curtipes have the ability to regenerate their tails after amputation within 2 weeks effectively. Tail amputation induces a massive recruitment of inflammatory cells to the site of injury and produce high levels of ROS. Traditionally, ROS have been thought to have a negative impact on cells. But in this case they seemed to be having a positive impact on tail re-growth. Lowering levels of ROS impairs tail regeneration while sustained increased ROS are essential for downstream signaling pathway for proper tail regeneration. The findings demonstrate that injury-induced ROS production is an important regulator of tissue regeneration.

Keywords: Downstream signaling, Reactive Oxygen Species, Regeneration

I. INTRODUCTION

A large number of organisms are able to regenerate body parts. However tetrapods have the highest ability of regeneration. Amphibianshave long been used in the study of tail regeneration (Goss 1969). Urodeles regenerate their lost limbs and tails throughout their lives, but anurans do so only in a limited period in the tadpole stage.

Tadpoles have remarkable abilities to regenerate their tailsfollowing amputation via the coordinated activity of numerous growth factor signaling pathways, including the Wnt, Fgf, BMP, notch, and TGFβ pathways. Tail amputation induces a sustained production of reactive oxygen species (ROS) during tail regeneration. Sustained increased ROS levels are required for Wnt/β-catenin signaling and the activation of one of its major downstream targets, fgf20 7, which, in turn, is essential for proper tail regeneration (Love et al; 2013).

Oxygen derived free radicals are also very important mediators of cell injury and death (Joseph and Knight; 1995). Cells contain antioxidant defenses that respond to variations in cellular oxidant production. The imbalance between oxidants and antioxidants is termed oxidative stress (Sies; 1997). Evidence is present that implicates oxidants and antioxidants as a factor that can stimulate alteration in gene expression (Allen, 1991; Schulze-Osthoff and Baeurle, 1998) and the appearance of new tissue is preceded by the transcription of tissue specific genes and the concomitant suppression of transcription of genes that are specific to pluripotent stem cells(Allen, 1991). The aim of the present investigation was to study the

oxidative stress during amputation induced tail regeneration in C. curtipes.

C. curtipes, is endemic to the Western Ghats of peninsular India inhabiting the forest floors and sheltersunder the leaf litter (Dutta, 1997).An interesting feature of C. curtipes tadpoles is their size. Larval C. curtipes are the largest known anuran tadpoles inhabiting the Western Ghats. These tadpoles with long larval life are bulky, sluggish, andmove slowly in shoals making them vulnerable to diverse predators. Thus,evolution of large body size could be a morphological adaptation for survival especially since the tadpoleshave long larval period (Narahari P. et al, 2014). Oxidative stress was assessed by investigating lipid peroxidation (LPO). Furthermore, the antioxidant enzymes analyzed included superoxide dismutase (SOD) and catalase that are directly involved in processing of ROS. Reduced glutathione (GSH) a non-enzymatic antioxidant expressed during cell division and regeneration has been analyzed.

II. METHODS AND MATERIAL

A. Chemicals

Chemicals used in this study were of analytical grade. Thiobarbituric acid (TBA), bovine serum albumin (BSA), 5, 5'- dithiobis-2-nitrobenzois acid (DTNB), mercaptoethanol,nicotinamide adenine disodium salt (NADH), reduced glutathione (GSH), Folin-Ciocalteus reagent were obtained from Sigma Chemicals, India. All other chemicals were of the highest purified grade available.

B. Tadpoles collection and rearing

Tadpoles of *C. curtipes* were collected from a stream in the Western Ghats near Anamod, (15° 4′N and 74° 33′E) Karnataka, India during November–March, 2011 and transported quickly to the laboratory where they were maintained in plastic tanks with aged tapwater. The tadpoles were fed with boiled spinach leaves and the water was changed every alternate day.

C. Tail amputation

Limb bud stage tadpoles were taken for the experiment. Tadpoles were anaesthetized with MS222 prior to the tail amputation. Following amputation the experimental tadpoles were transferred to aged tap water. The control (original) tadpoles were transferred to aged water. 7 days, 15 days and 25 days post amputed tadpoles were re-amputed to study oxidative stress. Tail of non-amputated tadpoles were also taken after 7 days, 15 days and 25 days of the experiment. For each assay a pool comprising of 25 tadpoles was taken.

D. Estimation of oxidative stress

The original tail tips of limb – bud stage tadpoles were analyzed as the original group. Regenerated tail of 7 days, 15 days and 25 days post amputation were taken for analysis.

1) Lipid peroxidation: The lipid peroxidation was assayed as thiobarbituric acid reacting substance (TBARS) by the thiobarbituric acid (TBA) assay of Ohkawa et al., (1979). A 10% homogenate was prepared with 1.5% KCl. The homogenate was centrifuged at 4 ◦ C for 5 min at 1000 x g. The supernatant was used for the assay. The assay mixture contained 1 ml of homogenate mixed with 2ml of TBA-TCA-HCL complex. Solution was heated for 15 mins in boiling water bath. After cooling the absorbance was measured at 532nm. The protein was estimated by the Lowry et al (1951). The concentration of TBARS was calculated from the extinction co efficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Wills, 1969).

2) Superoxide Dismutase (SOD): SOD activity was measured using the method of Misra and Fridovich (1972). 10% homogenate was prepared in 0.25M sucrose. The homogenate was centrifuged at 12000

rpm for 20min. supernatant was used for the assay. The assay mixture contained 0.2 ml EDTA, 0.1ml NBT in 0.1ml of sample. Tubes were incubated for 5-8mins in light. Reaction was stopped by adding 0.05ml riboflavin after time interval of 1 min. Absorbance was measured at 560nm.

3) Catalase: Catalase activity was measured according to the method of Aebi (1974). A 10% homogenate was prepared in 0.25M sucrose and centrifuged at 12000 rpm for 20 min. Supernatant was used for the assay. The assay mixture contained 0.5ml of sample mixed with 2.5 ml of hydrogen peroxide. Tubes were incubated for 2mins. Reaction was stopped by adding 0.5ml of 1M HCl after time interval of 1min.Absorbance was measured at 240nm.

4) Reduced glutathione (GSH): The assay of GSH was done according to the method of Ellman et al (1959). A 10% homogenate was prepared in 5 % metaphosphoric acid. The homogenate was kept at room temperature for 15 min. Centrifuged for 30 min at 1000 x g at room temperature. 0.5ml supernatant was used. 2.5 ml of DTNB (5,5' dithiobis- 2 – nitrobenzoic acid) was added to the supernatant and centrifuged at 4500 x g for 5 min. Absorbance was measured at 412nm.

5) Statistical analysis: Statistical analysis was followed by the method of Gomez and Gomez (1984). The ANNOVA test to find out the significant difference between means was calculated by Duncan's multiple range test.

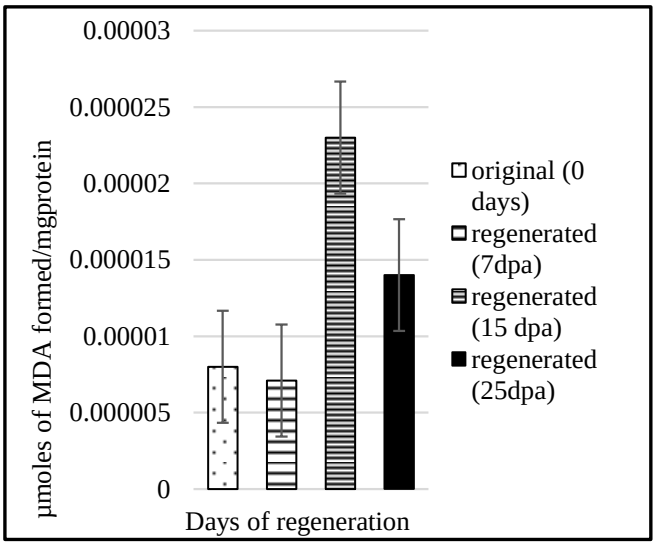
III. RESULTS AND DISCUSSION

The regenerated tail of 7 days post amputation were compared with the original tail. In the regenerated tail of 7 days amputated tadpoles LPO was lower than that of the original tadpoles (Fig. 1). The level of LPO had decreased to 0.7147 x 10^{-5}umoles MDA/mg protein in the regenerated tadpole tail from 0.8076 x 10^{-5}umoles MDA/mgprotein. An elevation was observed in the regenerated tail of 15 dpa and thereafter a reduction was observed in its level in the 25dpa tadpoles.

Table 1: Changes in oxidative stress parameters of the original and regenerated tails of tadpoles of *Clinotarsus curtipes*.

Oxidative parameter	Original (0 days)	Regenerated (7 days post amputation)	Regenerated (15 days post amputation)	Regenerated (25 days post amputation)
LPO[a]	0.8 x 10^{-5}	0.71 x 10^{-5}	2.3 x 10^{-5}	1.4 x 10^{-5}
SOD[b]	0.53	14	37.21	22.14
CAT[c]	1.89	7.80	21.3	13.3
GSH[d]	9.3 x 10^{-3}	10.71 x 10^{-3}	25.86 x 10^{-3}	16.6 x 10^{-3}

[a]level of lipid peroxidation (umoles MDA formed/mg protein).



[b] Activity of SOD (units of SOD/mgprotein)
[c]Activity of Catalase (umoles/ mg protein/ min)
[d] Level of Reduced glutathione (umoles/mgprotein)

Fig. 1. Lipid peroxidation (umoles of MDA formed/mgprotein)in the original and regenerated tailof tadpoles following 7, 15 and 25 days of post amputation in the tadpoles of *C. curtipes.*

In comparison to the original tail highest level in the activity of SOD was observed in the regenerated tails of 7 days post amputation. (Fig. 2). SOD activity showed significant increase in 7 and 15dpa tails of tadpoles, thereafter a decrease was seen in the 25dpa tails.

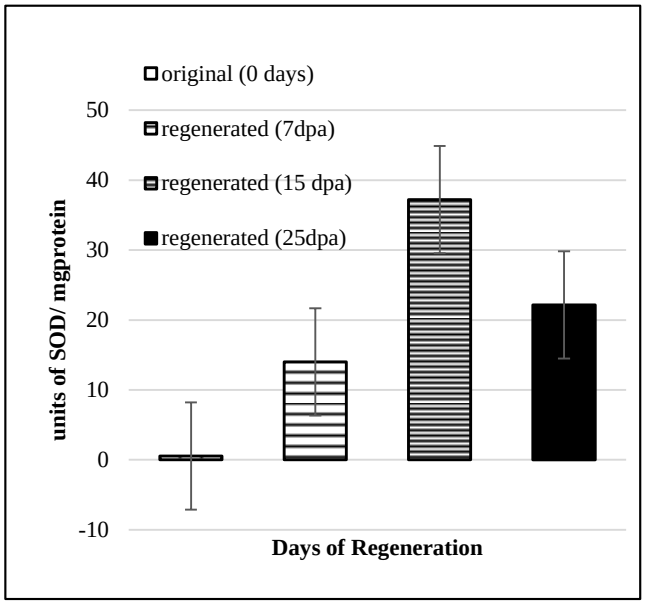


Fig. 2 Superoxide dismutase (units of SOD/mgprotein) in the original and regenerated tail oftadpoles following 7, 15 and 25 days of post amputation in the tadpoles of *C. curtipes.*

An elevation in the catalase activity was observed in the regenerated tail of 7 and 15 dpa tadpoles of *C. curtipes* in comparison to the original tail. (Fig. 3). The activity of catalase remained higher throughout and always higher than original tail. The Level of Catalase evaluated per milligram of protein was 1.89 umoles/mg protein in original and 7.8 umoles/mgprotein in the regenerated tadpoles of 7 dpa and 21.3 µmoles/mgprotein of 15dpa tadpoles of *Clinotarsuscurtipes* species. One time decrease was observed in the 25dpa tadpoles in comparison to 15dpa tadpoles.

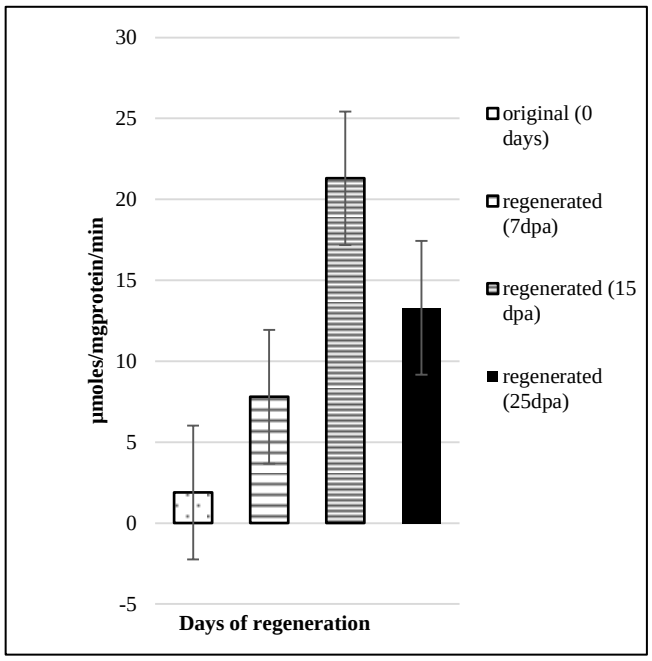
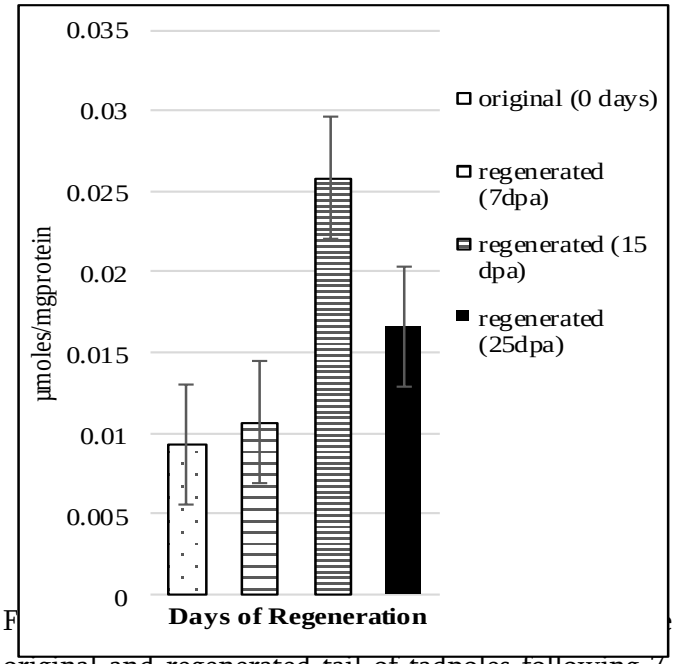


Fig. 3 Catalase activity (umoles/mgprotein/min) in the original and regenerated tail of tadpoles following 7, 15 and 25 days of post amputation in the tadpoles of *C. curtipes.*

The level of reduced glutathione (GSH) also showed elevation in the regenerated tail of 7 and 15 dpa in comparison to original tail of the *C. curtipes* tadpoles. While regenerated tail of 25dpa showed decrease in comparison to the 15 dpa tadpole tail.



F original and regenerated tail of tadpoles following 7,

15 and 25 days of post amputation in the tadpoles of *C. curtipes.*

A comparative account of the changes in oxidative stress parameters in the regenerated tail and original tail has been summarized in Table 1.

Different kinds of physiological factors can result in oxidative stress. For example, the antioxidant status of animals has been investigated in fish under anoxia and reoxygenation (Lushchak et al., 2001), in frogs under anoxia and recovery (Hermes-Lima and Storey, 1996,T.V. Bagnyukova, K.B. Storey, V.I. Lushchak, may 2002) and with respect to temperature changes (Perez-Campo et al., 1990; Joanisse and Storey, 1996).

We have observed a higher level of SOD and Catalase in the regenerated tails of 7 and 15 dpatadpoles (Fig. 2 and Fig. 3). Catalase enzyme is a scavenger of H_2O_2. H_2O_2acts as a secondary messenger molecule, affecting the proliferation and differentiation of cells. (Sehulze –Osthoff and Fiers, 1991).It has been observed in frog muscle that increased catalase activity minimizes cellular oxidative stress by lowering the concentration of H_2O_2. (Hermis-Lima and Story, 1998).In the present study the elevation in catalase activity is suggested to be due to increase in level of H_2O_2 in earlier stages i.e. 7 and 15dpa tadpoles.

A comparatively high activity of SOD (Fig. 2) was observed in the regenerated tails of 7 and 15 dpa tadpoles. SOD is widely distributed in aerobic organisms and plays an important role in the control of radical superoxide levels in the cellular compartments (Sasaki et al., 1997, Idowu Emmanuel Taiwo, AmaezeNnamdi Henry, Adie Peter Imbufe and OtubanjoOlubunmiAdetoro 2013.) An increase in the level of SOD has been observed during development in several amphibians (Bajra De Quiroga and Gutierrez, 1984). It has been explained that increase in SOD rather than the antioxidant properties of the enzyme seem to play a role in stimulating gene expression. (Allen and Balin, 1980). The level of LPO comparatively showed decrease at the same time in 7dpa whereas showed sudden increase in the 15dpa tadpoles. Our findings support the view of Allen and Balin. LPO disintegrates the biomembrane rich in polyunsaturated fatty acids (PUFA) which are susceptible to oxidation (Rady, 1993).

It is evident in amphibians that GSH concentration increases during mitotic phase of regeneration and decreases during redifferentiation of regenerating tissue. (Fig 4) This rise is due to the high level of cell division during this period. Decrease in GSH is seen in cells exhibiting a decline in mitotic acvity. In our observation there gradual increase in 7 dpa tadpole whereas a sharp increase in the 15dpa tadpole tail due to the high level of cell division during this period. The decline in the level in 25dpa tadpoles is due to the slowing of rate of cell division.

III. CONCLUSION

The remarkable observations of the present study includes higher level of SOD and Catalase and a lower level of LPO in the 7 dparegenerated tail of tadpoles. The above findings demonstrate that injury-induced ROS production is an important regulator of tissue regeneration and establishes a hyper oxidative stress condition in the regenerated tadpole tail in comparison to the original tail.

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Study of Butterfly Diversity In Mulshi Tahsil, Pune District, Maharashtra, India

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ABSTRACT

The present study was carried out at Mulshi Tahsil of Pune District, Maharashtra, India, during August 2007 to August 2009. A total of 71 species of butterflies belonging to 48 genera under five families were recorded from Mulshi Tahsil. Out of 71 species, 10 species of butterflies are scheduled species. Family Nymphalidae is dominating in study area, followed by Lycaenidae, Hesperiidae, Pieridae, and Papilionidae. The seasonal pattern in the abundance of butterflies, their biotopes and nectar food plants were also studied. Mud- puddling is also observed among some butterflies. Forest biotope is found to be rich for butterfly species. Thirty two Nectar food plants were identified belonging to 15 plant families. Plants of Asteraceae family were found to be preferred by Butterflies as nectar food plants. Visits of Butterflies were more frequent to flowers with tubular corollas than to non-tubular ones, to flowers coloured yellow and to flowers with a bloom for longer period in the year. Highest species abundance was observed in the months during August to November. A decline in species abundance was observed from the months of December to January and continued up to the end of May. These findings are important with respect to monitor butterfly and plant diversity and to define conservation strategies in the Mulshi Tahsil.

Keywords: Butterfly diversity, food plants, seasonal abundance, biotopes, Mulshi Tahsil.

I. INTRODUCTION

The increased industrialization and urbanization of Pune has affected the ecology of this important industrial District in the Maharashtra State, to a great extent. It has fourteen Tahsils, out of which, Mulshi Tahsil was selected for the study of Butterfly Diversity with respect to seasonal abundance, biotopes and nectar food plants in Mulshi. Butterflies are scaled wing insects belonging to order Lepidoptera of class Insecta. Butterflies are an integral part of the forest ecosystem. They show distinct patterns of habitat utilization. Being highly sensitive to changes in the environment, they are easily affected by even relatively minor perturbations in the habitat, so much so that, they have been considered as indicators of environment quality and health of an ecosystem (Rosenberg et al., 1986).Feeding is a significant activity and food may be often the most decisive factor affecting distribution, abundance and movements of animals. In Butterflies, this has a special relevance because food and mode of feeding are

different in the larval and adult stages (Kunte, 2000).Butterflies and their caterpillars are dependent on specific host plants for foliage, nectar and pollen as their food. Thus Butterfly diversity reflects overall plant diversity, especially, that of the herbs and shrubs, in the given area. The herbs and shrubs start their life cycle in the beginning of the Monsoon and complete it by the end of post- monsoon season.While some shrub like Lantana camara shows flowering throughout the year.

Chandra et al., (2007) gave a checklist of butterflies of Madhya Pradesh and Chhattisgarh States, reporting 174 species / subspecies of 100 genera under the eight families. Chandrakar et al., (2007) studied the butterflies' fauna of Melghat reporting 51 species of butterflies from the seven families. Chowdhury and Das (2007) enumerated 64 species of Butterflies belonging to 49 genera spread over the five families, from Indian Botanic Garden, Howrah, West Bengal. Singh and Kosta (2007) studied the butterfly fauna of Madhya Pradesh, including Chhattisgarh, India. Sharma and Borkar (2008) updated an account of butterflies from the Lonar Wildlife Sanctuary and reported 53 species of 36 genera under the seven families. Sindhu and Mehta (2008) recorded 60 species of Lycaenid butterflies from Himachal Pradesh. Trigunayat et al., (2008) reported and updated list 69 species of butterflies belonging to 48 genera and the five families from

Keoladeo National Park, Bharatpur, Rajasthan. Gaikwad et al., (2009) studied Butterfly diversity in Amba Reserved Forest Kolhapur and reported 106 species and subspecies, distributed over 82 genera belonging to eight families of Butterflies. Ranganekar and Dharwadkar (2009) documented three additions to the known Butterfly Fauna of Goa, India.Singh (2009) recorded 147 species of Butterflies from Kedarnath Musk Deer Reserve, in Chamoli and Rudraprayag, districts of Uttarakhand State in India, along with their seasonality, altitudinal distribution and relative abundance. Tiple et al., (2006) have analyzed the factors influencing nectar plant resource visits by Butterflies and its implications for conservation at Amravati University campus. Further, Tiple et al., (2009) investigated Butterfly-Flower morphological interrelationships for 108 Butterfly species and 20 plants at Nagpur. Triigunayat and Saxena (2009) documented Butterfly diversity from Dholpur Distt., Rajasthan and their study reflected 48 species from 34 genera spread over five families.

Four tropical habitats in North-Western Ghats. These four sites were close to Pune city within a radius of 20km. Rane and Ranade (2004) studied Butterflies of Tamhini- Dongarwadi area, Mulshi, Maharashtra. Padhye et al., (2006) studied season and landscape wise distribution of Butterflies in Tamhini, Northern Western Ghats of India. Sharma (2009) studied the fauna of Bhimashankar Wildlife Sanctuary, Maharashtra. Chandekar et al., (2014) studied the seasonal patterns in the abundance of butterflies, their biotopes and nectar food plants from Maval Tahsil, Pune District, Maharashtra, India. The diversity studies are important for environmental protection. The present study was undertaken for study of diversity of butterflies from Mulshi Tahsil.

II. METHODS AND MATERIAL

The Study Area:

Tahsil Mulshi with head quarter Paud is located 42 Km from Pune towards west direction. It is situated at 18°.32' North latitude & 73°.37' East longitude, altitude is about 576 meters. This Tahsil area is also irregular in shape, having an area of 1039 sq. km., bordered by Tahsil Haveli in the east, Tahsil Velhe in south, Tahsil Maval towards the north and district Raigd in west.

This area receives heavy rainfall. This place is one of the attractive picnic spots developed in recent time.

The small village Mulshi in Mulshi Tahsil has its natural beauty and attracts a lot of tourists. The village falls in the Sahyadri ranges and has beautiful deep forests, and the place is famous for its mountains and plateaus named Lavmai. Most of the area is covered with forest. Sag, Teak, Oak, Mango, are the trees found in the forest. Rice is grown in this region. Mula River flows through this area. There is a lake Valanewadi named just 5 kms away from Mulshi. Also located close by are two marvelous forts, namely Dhangad and Korigadh. The hills Hattihant and Pagota, reaching a height of almost 1000 meters, are amazing sights to behold. Famous 'Mulshi' dam is located here which also generates power. The study area was fully explored during August 2007 to August 2009 and then probable areas were decided. To study the seasonal patterns/diversity in Butterfly abundance in relation to nectar food plants, the entire year was divided into three seasons. The three seasons of the year are Pre-Monsoon i.e. from February to May, Monsoon i.e. from June to September and Post-Monsoon i.e. from October to January. The study area was visited twice in each season during the two years i.e. 2007-2008 & 2008- 2009. In the said investigation the selected sites were surveyed mainly between 7.30 am to 12.30 pm. Butterfly species were identified directly in the field visually with the help of field guides followed by photography, in difficult cases, rarely by capture. Collection was restricted to those specimens that could not be identified directly. All scientific names and common English names were designated as per Varshney (1983) Wynter Blyth (1957) respectively. Classification of Butterflies is after Gaonkar (1996). Benthum & Hooker (1862-1863) system of classification is followed for plants.

III. RESULTS AND DISCUSSION

During the study, seventy one species of Butterflies belonging to five families were recorded in Maval Tahsil (Table2). Out of seventy one species, eight belong to Papilionidae, ten to Pieridae, twenty-eight to Nymphalidae, thirteen to Lycaenidae and twelve to Hesperiidae. Species belonging to family Nymphalidae, were the most dominant (40%) followed by Lycaenidae (18%), Hesperiidae (17%), Pieridae (14%), and Papilionidae (11%). The status recording was as follows: VC- very common (75-100 sightings), C- common (50-75 sightings), NR- not rare (25-50 sightings), R- rare (5-25 sightings) and VR- very rare (1-5 sightings). Among the species fifteen were found very common, thirty-one species common, eighteen species not rare, six species were found rare and one species was observed in very rare category from study area. Ten species (Pachliopta hector, Pareronia valeria, Appais indra, Cepora nerissa, Euploea core, Neptis jumbah, Hypolimnas misippus, Lampides boeticus, Jamides alecto and Acytolepis puspa) come under the protection of the Indian Wildlife (Protection) Act 1972. Out of the seventy-one species twenty-seven species were recorded from botanical and nursery garden, sixty-four from forest area, thirty-five from grassland, sixty on plantation and fifty- two from scrub biotope.

Suborder : Grypocera		
V. Family: HESPERIIDAE		
1. Subfamily: Coeliadinae		
23	Common Banded Awl	*Hasora Chromus* Cramer
2. Subfamily: Pyrginae		
24	Malabar Spotted Flat	*Celaenorrhinus ambareesa* Moore
25	Common Spotted Flat	*Celaenorrhinus leucocera* Kollar
26	Common Small Flat	*Sarangesa dasahara* Moore
27	Fulvous Pied Flat	*Pseudocoladenia dan* Fabricius
28	Tricoloured Pied Flat	*Coladenia Indrani* Moore

3. Subfamily: Hesperiinae		
29	Dark Palm Dart	*Telicota ancilla* Herrich-Schaffer
30	Pale Palm Dart	*Telicota colon* Fabricius
31	Rice Swift	*Borbo cinnara* Wallace
32	Small Branded Swift	*Pelopidas mathias* Fabricius
33	Indian Palm Bob	*Suastus gremius* Fabricius
34	Chestnut Bob	*Iambrix salsala* Moore

TABLE - 2(Continued)

List of Butterfly Species Observed from Study Area Mulashi

S.#	Common Name	Scientific Name
Suborder : Rhopalocera		
I. Family: PAPILIONIDAE		
1. Subfamily: Papilioninae		
35	Common Bluebottle	*Graphium sarpedon* Linnaeus
36	Tailed Jay	*Graphium agamemnon* Linnaeus
37	Common Mormon	*Papilio polytes* Linnaeus
38	Red Helen	*Papilio helenus* Linnaeus
39	Blue Mormon	*Papilio polymnestor* Cramer
40	Lime Butterfly	*Papilio demoleus* Linnaeus
41	Common Rose	*Pachliopta aristolochiae* Fabricius
42	Crimson Rose	*Pachliopta hector* Linnaeus
II. Family: PIERIDAE		
1. Subfamily: Coliadinae		
43	Small Grass Yellow	*Eurema brigitta* Cramer
44	Common Grass Yellow	*Eurema hecabe* Linnaeus
45	Spotless Grass Yellow	*Eurema laeta* Boisduval
46	Common Emigrant	*Catopsilia pomona* Fabricius
47	Lemon Emigrant	*Catopsilia crocale* Cramer
48	Mottled Emigrant	*Catopsilia pyranthe* Linnaeus
2. Subfamily: Pierinae		
49	Common Wanderer	*Pareronia valeria* Carmer
50	PlainPuffin	*Appias indra* Moore
51	Common Gull	*Cepora nerissa* Fabricius
52	Pioneer	*Belenois aurota* Fabricius
III. Family: NYMPHALIDAE		
1. Subfamily: Danainae		
53	Blue Tiger	*Tirumala limniace* Cramer
54	Dark Blue Tiger	*Tirumala septentrionis* Butler
55	Striped Tiger	*Danaus genutia* Cramer
56	Plain Tiger	*Danaus chrysippus*Linnaeus
57	Glassy Tiger	*Parantica aglea* Stoll
58	Common Indian Crow	*Euploea core* Cramer
2. Subfamily: Charaxinae		
59	Common Nawab	*Polyura athamas* Drury
60	Black Rajah	*Charaxes Solon* Fabricius
3. Subfamily: Satyrinae		
61	Common Evening Brown	*Melanitis leda* Linnaeus
62	Common Bush Brown	*Mycalesis perseus* Fabricius
63	Common Five Ring	*Ypthima baldus* Fabricius
64	Common Four Ring	*Ypthima huebneri* Kirby
4. Subfamily: Heliconiinae		
65	Tawny Coster	*Acraea violae* Fabricius
66	Common Leopard	*Phalanta phalantha* Drury
5. Subfamily: Limenitinae		
67	Chestnut Streaked Sailer	*Neptis jumbah* Moore
68	Commom Sailer	*Neptis hylas* Linnaeus
6. Subfamily: Cyrestinae		
69	Common Map	*Cyrestis thyodamas* Boisduval
7. Subfamily: Biblidinae		
70	Angled Castor	*Ariadne ariadne* Linnaeus
8. Subfamily: Apaturinae		
71	Black Prince	*Rohana parisatis* Westwood

IV. DISCUSSION & CONCLUSIONS

The species abundance increased from the beginning of monsoon, from the months June to July and reached a peak in the months from August to November. A decrease in species abundance was observed from the months December to January and continued up to the end of May, indicating the abundances of diverse species was positively affected by approaching warmer days, high relative humidity and more rainfall. These factors help to flourish diverse vegetations, which are vital food sources for many Butterfly species. Gutierrez & Mendez (1995) reported that the abundance of Butterflies is not affected by altitudes but it is more related to the availability of food plants. Plants play vital role in increasing the Butterfly diversity and their abundance in the area. In study area, maximum species of Butterflies were recorded in forest biotope than followed by plantation, scrub, grassland and boticanal garden biotope. However, grassland and botanical garden are not observed as rich biotopes; heavy grazing pressure on grassland and use of pesticides in gardens has adversely affected diversity of Butterflies in these biotopes. Maval Tahsil, forest biotope is rich in butterfly diversity as observed in present study. The nectar flowering plants visited by Butterflies, as observed in our findings, namely Alstonia scholaris, Ageratum conyzoides, Nothapodytes nimmoniana, Carissa congesta, Asclepias curassavica, Calotropis gigantea, Senecio bombayensis, Zinnia eleganas, Cassia auriculata, Urena lobata, Pentas karmesiana, Gnidia glauca and Vitex negundo . Mulshi Tahsil is rich in floral diversity as compared to earlier reports from Amravati University Campus, Nagpur and Bhor Tahsil. The herbs from study area namely Celosia argentea and Tridax procumbens are more used by the Butterflies, probably due to long flowering period. The shrub Lantana camara is having flowering period throughout the year, so it is more used by Butterflies as their food plant. Number of Butterfly species also feed on other sources of food like tree sap, rotting fruits, rotting animals, animals dropping, minerals from wet soil and varying combinations of all these. Details of habitat used by Indian Butterflies are not known. Fresh information on the habitat and microhabitats of Butterflies will be very useful in all the regions of India (Kunte, 2000). Wildlife habitats are getting destroyed at an alarming rate with disastrous effects on biodiversity. While a large number of species have become extinct in the recent past, the survival of many others is threatened. Thus, habitat loss is considered as major threat to biodiversity of Butterflies (World Resources, 1994-95).These findings will prove to have their own importance.

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Effect of Organophosphate Pesticide, Chlorpyriphos on Biochemical Constituents In Snakehead Fish, Channa Gachua (F. Hamilton)

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ABSTRACT

Farmers still use the pesticides as the most effective weapon to protect the crop. Chlorpyriphos is most widely used organophosphate pesticide and being broad spectrum of harmful effects. Therefore present investigation aimed to study the effect of chlorpyriphos on biochemical constituents of fish, Channa gachua. In acute toxicity (96 hrs.) experiment the fish C. gachua exposed to LC0 and LC50 concentrations of pesticide. Significant depletion in glycogen content was observed in muscle, gill, liver, kidney and brain when compared to the control. The total protein content was decreased in all above tissues whereas fluctuations was also observed in lipid content. From the results it was concluded that the harmful pesticide caused significant depletion in biochemical composition of fish, this may be due to the utilization of these constituents for high energy demand, to cope up with pesticidal stress. Hence the harmful pesticide intoxication has made defective consequences in the normal metabolic pathways which led increasing the rate of mortality in fish population.

Keywords: Chlorpyriphos, Biochemical Constituents, Pesticides, Acute Toxicity

I. INTRODUCTION

People attempted to increase the world's food production to solve the problem of malnutrition, and overcome the basic need of population, this is achieved by the use of fertilizers to nourish the plants and use of pesticides to protect them from pests (Ganeshwade, 2011). Now a days large quantity of pesticides are used to control insect pest but these harmful chemicals entered into water bodies and pose a great threat to aquatic organisms. Chlorpyriphos is an organophosphate pesticide widely used in agricultural and domestic field to control of insect pests and vectors of different diseases. It acts on the nervous system of insects by inhibiting acetylcholinesterase. Its main break down products in the environment is chlorpyriphos oxon which is more toxic. Fishes are good indicator of aquatic pollution. Nutritional value of fish depends on their biochemical composition which is affected by water pollution.

Study of biochemical parameters like glycogen, protein and lipid helps to access the effect of harmful toxicant on vital tissues of fishes. Alterations in biochemical contents in different tissues of fish due to different pesticides have been reported by many workers e.g. Naveed et al. (2006) in fish, Channa punctatus exposed to lihocin, Sing et al. (2010) in fish, C. punctatus exposed to phorate, Ganeshwade (2011) in fish, Puntius ticto exposed to dimethoate, Stalin and Das (2012) in Cirrhinus mrigala exposed to fenthion, Magar and Dube (2013) in C. punctatus exposed to malathion, Chamarthi et al. (2014) in C. carpio exposed to quinalphos, Justin and Josef (2015) in Oreochromis mossambicus exposed to acetamipride.

Though the numerous workers have been reported the alterations in biochemical components, the level of depletion in biochemical constituents and the nature of toxicity is different according to species of fishes and type of pesticide. Therefore, the present investigation was designed to understand the effect of acute exposure of chlorpyriphos on glycogen, protein and lipid content in fish, C. gachua.

II. METHODS AND MATERIAL

A. Material:

1) Experimental fish - Live specimen of C. gachua of size15 ± 1 cm and weight 50 ± 5gm were obtained from Krishna river around karad city with the help of fisher man. The collected fish were kept in 1% solution of KMnO4 for some time to protect from dermal infection. Finally they were kept in glass aquarium and fed on commercial fish food. They were acclimatized in laboratory for 10 days at room temperature.

2) Pesticide - Commercially available organophosphorus pesticide chlorpyriphos was used for present research work. The pesticide brought from Local agro chemist shop. **B. Methods:**

1) Experimental set up – Under the experiment healthy fishes were divided in to three groups each group contained ten fishes. Group 1st was considered as control and group 2nd and 3rd as experimental groups. Fishes in the experimental groups were exposed to 35 ppm (LC0) and 50 ppm (LC50) concentration chlorpyriphos for 96 hrs. At the end of each exposure period the fishes were sacrificed and desired organs were quickly excised to study biochemical parameter by employing following methods.

2) Biochemical methods – i) Anthrone method was used to determine total glycogen content in tissues, (Siefter et al., 1950). ii) Total protein content was determined with Folin Ciocalteau reagent according to Lowry Method (Lowry, et. al., 1951). iii) Lipid were extracted by Floch, et al.(1957), and estimated by sulphophosphovaniline method (Barnes and Blackstock, 1973).

The results obtained in the present study were expressed as mean ± SD (standard deviation). The statistical differences between various groups were analyzed by the One Way ANOVA and the significance was observed at the $p>0.05$, $p<0.05$, $P<0.01$ and $p<0.001$ level.

III. RESULTS

In the present study the amount of glycogen, protein and lipid in some important organs like muscle, gill, liver, kidney and brain was estimated in control fish and in fishes exposed to lethal concentration of chlorpyriphos for 96 hrs. (acute). The calculated values of total glycogen, protein and lipid along with percent change in the different organs in control fish and fishes exposed acutely to different concentration of pesticides with standard deviation are shown in table 1-3.

A. Glycogen

The estimated values of the glycogen in control fish were 1.8512 mg in liver, 1.4318 mg in kidney, 1.2622 mg in brain, 0.8514 mg in muscle and 0.7588 mg in gill per gram wet weight of each tissue. Changes in glycogen content in different organs of the test fish exposed acutely to 35 ppm concentration of chlorpyriphos are shown in Table 1. Highly significant decrease ($P<0.001$) was observed in the glycogen content in the kidney (22.77) and liver (22.44) of fishes exposed to this concentration of chlorpyriphos. Moderately significant ($P<0.01$) decrease was observed in gill (23.54) followed by brain (18.53) and in muscle (22.46) the decrease was less significant ($P<0.05$) in comparison with other tissue. Whereas highly significant ($P<0.001$) depletion in the glycogen content was noticed in all the organs

of test fish exposed to 50 ppm concentration. The percent depletion was higher (70.53) in liver which was followed by gill (64.11), muscle (60), brain (59.27) and kidney (49.02) (Table 1).

Table 1. Showing changes in the glycogen (mg/gm. wet weight tissue) in different organs of control fish and in fishes exposed to different concentrations of chlorpyriphos at 96 hrs. exposure period (acute).

Organ	Muscle	Gill	Liver	Kidney	Brain
Control	0.8514 ±0.09	0.7588± 0.08	1.8512± 0.08	1.4318 ±0.08	1.2622 ± 0.07
35 ppm (LC0)	0.6967 ±0.08 (-18.17) *	0.5802 ±0.07 (-23.54) **	1.4411 ±0.08 (22.14) ***	1.1057 ±0.05 (-22.77) ***	1.0283 ±0.12 (18.53) **
50 ppm (LC50)	0.3405 ±0.05 (-60) ***	0.2723 ±0.05 (-64.11) ***	0.5454 ±0.06 (-70.53) ***	0.7298 ±0.06 (-49.02) ***	0.5518 ±0.06 (-59.27) ***

Table 2. Showing changes in the protein (mg/gm. wet weight tissue) in different organs of control fish and in fishes exposed to different concentrations of chlorpyriphos at 96 hrs. exposure period (acute).

Organ	Muscle	Gill	Liver	Kidney	Brain
Control	19.824± 0.69	24.228± 0.65	51.940 ± 0.91	37.130 ± 1.26	12.908 ± 0.50
35 ppm (LC0)	17.626± 0.24 (-11.08) ***	19.194± 1.35 (-20.77) ***	44.916± 0.96 (-13.62) ***	31.656± 1.32 (-15.19) ***	10.096± 1.13 (-23.33) ***
50 ppm (LC50)	09.244± 0.73 (-53.36) ***	11.536± 0.69 (-52.38) ***	32.504± 0.81 (-36.54) ***	19.952± 0.84 (-46.26) ***	6.676± 0.72 (-48.28) ***

Table 3. Showing changes in the lipid (mg/gm. wet weight tissue) in different organs of control fish and in fishes exposed to different concentrations of chlorpyriphos at 96 hrs. exposure period (acute).

Organ	Muscle	Gill	Liver	Kidney	Brain
Control	3.908 ± 0.05	57.68 ± 0.12	3.499 ± 0.18	4.620 ± 0.09	5.895 ± 0.15
35 ppm (LC0)	3.292 ± 0.17 (-18.70) ***	5.349 ± 0.28 (-7.82) **	3.561 ± 0.12 (1.75) NS	4.228 ± 0.22 (-9.26) **	5.493 ± 0.18 (-7.30) **
50 ppm (LC50)	2.601 ± 0.63 (-33.43) ***	4.382 ± 0.10 (-24.02) ***	3.896 ± 0.10 (11.36) **	3.587 ± 0.16 (-22.35) ***	4.824 ± 0.16 (-18.16) ***

Values are mean ± SD, n=5, figures in parenthesis are percentage Decrease over control.

Values significant at NS = $P > 0.05$ (Non-significant), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Protein

The estimated values of the protein in control fish were 51.940 mg in liver 37.130 mg in kidney, 24.228 mg in gill, 19.824 mg in muscle and 12.908 mg in brain per gram wet weight of each tissue. Highly significant depletion in protein ($P<0.001$) in all tissues was observed in fishes exposed to 35 ppm concentration of this pesticide. In brain (24.18) percent depletion more pronounced which was followed by gill (20.77) and kidney (15.19). The percent depletion of protein in liver (13.62) and muscle (11.28) was less than other tissues. But, the fishes exposed to 50 ppm concentration of chlorpyriphos for 96 hrs. showed drastic depletion in protein. The decrease in protein was also highly significant ($P< 0.001$) in all tissues in muscle the percent depletion (53.36) was higher, followed by gill (52.18), brain (48.28), and kidney (46.26). In liver (36.54) depletion in protein content was less than other tissues (Table 2).

Lipid

The estimated values of the lipid was 5.895 mg in brain, 5.768 mg in gill, 5.260 mg in intestine, 4.658 mg in stomach, 4.620 mg in kidney, 3.908 mg in muscle and 3.499 mg in

liver per gram wet weight of each tissue. The lipid content in all tissues was decreased after exposure of fishes to 35 ppm concentration of this pesticide except liver. In muscle lipid content was decreased more significantly ($P<0.001$) and the decrease was 18.70. The percent depletion in kidney, gill and brain was moderately significant which was 9.26, 7.82 and 7.30 respectively. But in liver non-significant increase in lipid content (1.75) was observed at this concentration. In fishes exposed to 50 ppm (LC 50) concentration of this pesticide highly significant ($P<0.001$) decrease in lipid content was observed in all tissues except liver. The percent depletion in muscle was higher (33.43) than other tissues. In gill the percent depletion was 24.02 followed by kidney (22.35) and brain (18.16). However, moderately significant ($P< 0.01$) increase was observed in liver (11.36) as compared to control (Table 3).

In all the organs the glycogen content was found decreased significantly as compared to the control fish, but percent depletion was more in fishes exposed to LC 50 concentration than the LC0.

III. DISCUSSION

The contamination of water by widely utilized organophosphate pesticide such as chlorpyriphos is a potential problem for fishes and aquatic organism. In present study attempt has been made to study acute effect of chlorpyriphos on biochemical composition of tissues like muscle, gill, liver, kidney and brain of fresh water fish C. gachua. The biochemical constituents are source of energy in animal body is present in the form of stored energy which is used in starved and stressed condition. Glycogen is the prime and important biochemical constituents in tissues of animal. It acts as building blocks and reservoir of chemical energy in the cell which can increased or decreased according to organismal need (Kumar and Ali, 2013). Glycogen provides the energy for the animal which is essential for performing different process (Lehninger, 2004). Carbohydrates are mainly stored in liver of animal in the form of glycogen and it is exported in the form of hexose units for maintenance of blood glucose and readily available for glycolysis (Herper, 2003). Protein serves as an alternative source of energy for animal when the insufficient energy is available from the other sources like carbohydrates and lipids. Protein is the building blocks of animal body. It is the most important constituents for growth, development and maintenance of life (Waghmare and Wani, 2014). Protein plays an important role in cellular metabolism and regulates intra and extra cellular interactions media as a part of cell membrane and enzyme (Anita and Venkata Rathnamma, 2016). All cells contain lipid in the form of globules scattered in the cytoplasm (Tamizhazhagan et al., 2016). Lipid is an essential biochemical constituent in tissues of all animals, and plays vital role in the energy metabolism. (Kumar and Ali, 2013). Lipids are also the strong form of energy like glycogen. Lipid form an essential component of protoplasm and during extreme starvation considerable amount can be extracted from tissues.

Due to pesticidal stress, such prime and important energy source is affected significantly and alters various processes in the fish. The biochemical constituents like glycogen, proteins and lipids are important to indicate the susceptibility of organs system to pollutants (Verma and Tonk, 1983). The results obtained in the present work showed fluctuations in the glycogen, protein and lipid content in muscle, gill, liver, kidney, and brain of fish, C. gachua exposed to lethal concentrations of

chlorpyriphos at acute exposure period. The alterations were time of exposure and concentration of pesticides dependent. Similar results have also been observed by Venkataramana et al. (2006) in cardiac muscle of Glossogobius giuris exposed to malathion, Thenmozhi et al. (2011) in liver , muscle and gill of L. rohita exposed to malathion, Veeraiah et al. (2013) in gill, muscle, brain, liver and kidney of fish, Labeo rohita after sublethal exposure of indoxacarb, by Tripathi and Yadav (2015) in gill, muscle, brain, liver, kidney and gut of L. rohita exposed to phenthoate, by Pechiammal and Kiruthika (2016) in gill liver kidney and muscle of Cirrhinus mrigala exposed to rogor, by Tamizhazhagan et al. (2016) in muscle, liver and kidney of L. rohita exposed to monochrotophos, by Verma and Rawat (2017) in ovary of Heteropneustes fossilis exposed to chlorpyriphos, by Padmavathi et al. (2017) in muscle and liver of Mystus vittatus exposed to cypermethrin. The biochemical changes occurring in the body of the organisms give first indication of stress. Here, in present investigation depletion in glycogen content was might be due to the rapid utilization of stored glycogen for energy production in pesticide stressed condition at acute exposure. Required Energy in the form of ATP might be produced due to anaerobic breakdown of glycogen which may leads to breakdown of more amount of glycogen to cope up the energy need under stress condition. A fall in glycogen levels indicates its rapid utilization to meet the enhanced energy demands in pesticide treated animals through glycolysis or hexose monophosphate pathway (Cappon and Nicholas, 1975). Pesticides are known to act on endocrine system (Edwards, 1973). Hence, it contributes to the decreased glycogen synthesis. Decreased glycogen synthesis is also attributed to the inhibition of the enzyme glycogen synthatase which mediates glycogen synthesis.

The decreasing trend in the protein content as observed in the present study in all tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; or due to the directing of free amino acids for the synthesis of necessary proteins, or for the maintenance of osmotic and ionic regulation (Schmidt Nielson, 1975). According to Kamble (1999) these toxic compounds inhibit the incorporation of amino acids into proteins and increased degradation of protein into amino acids. This phenomenon supports by Pugazhedy et al. (2012), according to them reduction in protein might be due to the blocking of protein synthesis, protein denaturation or interruption in the amino acid synthesis under pesticidal stress. Protein might be used in cell repair, tissue organization and formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in the cytoplasm.

The depletion in lipid level might be due to the lipolysis in different tissues to overcome the high energy demand by the fish under pesticidal stress, rate of lipolysis might be accelerated for the production of energy and subsequently used for glucose synthesis. On the other hand it is suggested that the lipid synthesis may be inhibited and mobilization of stored lipids through β-oxidation and unsaturation of lipid molecules (Jha, 1991). Pesticide may affects on cellular structure of the tissue and lipid might be used in cell repair and tissue organization due to that depletion in lipid content may occur. The increased lipid content in liver may be due to cellular degeneration in liver results into collapsed metabolic activity which inhibit emulsification of lipids. As the liver is commonest site for accumulation of lipids it plays central role in lipid metabolism. The accumulation of lipid might be due to mobilization of lipids to the liver for metabolism and energy production but in liver the excess lipids are saturated (i.e. hyperlipidemia) exceeding capacity of the liver to metabolize it (Harsh Mohan, 2010). Pesticides and their metabolites interferes with the enzymes involved in the metabolic pathway, they exerts their effect on synthesis of biochemical

compound and stored biochemical compounds in the form of energy utilized to cope with pesticidal stress on metabolism and physiology of fish.

IV. CONCLUSION

Present study revealed that the pesticide chlorpyriphos is potent to cause toxic responses, and biochemical alterations in fish. The natural physiological functioning of an organisms gets distributed on exposure to toxicants, it induces its effect first at cellular or even at molecular level, but ultimately leads into physiological, pathological and biochemical alteration. Pesticides even in minute extent, causing a stress to aquatic organisms which reflected by the behavioral, biochemical and pathological changes and at the end death of the animal was evident. But an agricultural efforts reducing the use of pesticides and implementing natural remedies for pest control can become one solution for pesticidal pollution.

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Study of Diversity of Millipedes (Arthropod : Diplopod) At In Around the Northern & Western Ghats of Rajgurunagar, (M.S.) India

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ABSTRACT

The millipedes are belong to Arthropod: Diplopods. The Diplopods are commonly known as millipedes which mean "Thousand legers", though it is a gross exaggeration. The millipedes are sluggish, timid and secretive creatures avoiding enemies and hiding in dark and damp places such as under stones, logs and barks, among decaying leaves or in moss or rotten wood. Current knowledge on the effects of climate, food quality and land cover on millipedes is reviewed. The different period was used to study of diversity of millipedes species. In present research concludes the diversity of millipede rich in part of Northern Western Ghats of Rajgurunagar (M.S.) India. A total 5 species of millipedes belonging to order polydesmida and spirobolida, were recorded from tropical or agricultural landscape of Rajgurunagar. At the time of diversity study, Trigoniulus corallines and Orthomorphase were observed more than other millipede species, which supports the environmental determinism condition.

Keywords: Diplopod, Northern Western Ghats, Millipede Diversity Extracts.

I. INTRODUCTION

In India there are about seven zone of biodiversity are recommended as hot spot. The Northern Western Ghats is one of the regions in this hot spot zone of biodiversity. (Myers N.,Mittermeier A,2000). In the Northern Western Ghat, many exotic species are need to research for its conservation. The Northern Western Ghats are deciduous and tropical moist forest, which environment is suitable to millipede, that's the reason to study the millipede in given environment.

The millipedes mean thousand legers. The millipedes are sluggish limed and secretive creature. It move slowly because of its short legs, these help to push soil. The millipede body is divided in to three parts head, thorax and abdomen. In which there are first three at thoracic regionand one pair at abdomen. The protection from enemies millipedes mostly leave at dark and damp places. Such as under the stone logs and bark of tree also rotting leaves mosses and rotten wood.

The millipedes are belongs to phylum Arthropoda and class Diplopods. Millipedes are classified into 16 orders and 145 families (Shelley R.M.2007, Sierwald p.2001). The eighty thousands species are estimated in that twenty thousand species are distributed in world. The millipedes are directly or indirectly economically important to human being.

The Diplopods are generally known as millipede. The millipedes are widely distributed in all over world. Theenvironmental changes commonly affect on the millipedes mainly high temperature. It also biological indicator, it shows variation in environment and climatic condition(Brunner H 2001). The millipedes

are seasonal arthropods, it commonly found in rainy season and rarely found in summer season, because fluctuation in temperature are affect on millipede. (Ashwini KM ,Shridhar KR,2006).

II. METHODS AND MATERIAL

The study was carried out during the all seasons in the year 2015 to 2017. In monsoon seasons green forest and grassland regeneration is very low. We are selected three locations from given area of different altitude. Done the survey of Northern Western Ghats and observe occurrence of different species of millipedes. Then millipedes are collected for photography by hand picking method and identify the species using field guider and standard literature and Wikipedia site. At every sampling time noted the air, soil temperature at 10 cm above depth of land using thermometer. Standard methods were used for the richness and evenness of millipede species at different altitude.

III. RESULTS AND DISCUSSION

In present study total 5numbers of species belongs 5 genera were recorded from Rajgurunagar. At the Northern western Ghats of Rajgurunagar in which order polydesmida

belongs to four species and order spirobolida belongs to one species were recorded.

Miliped e species	Order	Family	Genus
Trigoniul us Corallines	S pi rob olid a	T r i g o n i ulidae	T r i g o n i u l a s
Aphelori a virginiesi s	P o l y d e s m i d a	X ys t ode s m i da e	A p h e l o r i a
Orthomor pha	P o l y d e s m i d a	Paradoxoso matidae	O r t h o r n o r p h a
coarctata			
Harpaphe haydenia na	Polydes m ida	X ys t ode s m i da e	H a r p a p h e
Oxidus gracilis	P o l y d e s m i d a	Paradoxoso matidae	Oxidus

At Northern Western Ghats of Rajgurunagar millipede fauna was not well known. The following species under two orders of millipede were identified from the study area.

1] Order –Polydesmida.

It is the largest order of millipede. The order polydesmida are the flat backed millipedes, with fused sclerites. These millipedes generally have 20 segments as about 2700 species recorded in there order.
Under these order we are recorded 4 species.

a) **Apheloria virginiesis** – It is common millipede species. It is also called the black and gold flat millipede. There millipede is identified by its flattened look and black body with orange and yellow spots or highlights, with medium sized antenna. It is commonly found at leaf litter.

Fig.1- Apheloria virginiesis

Fig.2-Harpaphe haydeniana

Fig.3-Trigoniulus Corallines

Fig.4-Orthomorpha coarctata

Fig.5-Oxidus gracilis

b) Orthomorpha coarctata (Saussure,1860)- It is also called Asiomorpha coarctata. The male are 14.5 to 20.5mm in length and female are 16.5 to 27.5 mm in size. Middle body is segmented, with longer gonopods.

c) Harpaphe haydeniana (Wood,1964) –There species also in order polydesmida and family xystodesmidae. There millipede is black colored, with yellow spots at both sides of the body.The length of the millipede is 5 cm, with 15-20 body segments.

d) Oxidus gracilis (Koch,1847) –It is in order polydesmida belongs to family paradoxosomatidae. There millipede is brown in color with faint yellow patches on body, the length of these animal is 4-5 cm in long with 15-20 segments.

2] Order-Spirobolida.

It is also included in class Diplopoda (millipede). The spirobolids are generally tropical species. Both pairs of legs on the seventh segment of the male are modified into gonopods. The spirobolids are generally tropical species, some of which are very brightly coloured (Lewis, 1984). From given order we are recorded only one species.

a) Trigoniulus corallines (gervais,1847)-It is also called rusty millipede. These millipede are medium to large in size,brick red in color, mostly it is found in botanical gardens found in bunch.

From the given study area the Harpaphe haydeniana and Orthomorpha coarctatathese two species are abundance in given ecosystem .The millipede species reported from the Northern Western Ghats, total 5 species which were present in selected area . In that the 4 species are already introduced in 2013 (C.R.Choudhri and S.V.Theurkar,2013) and one species is not introduced.

The millipede species are active at morning in rainy season. It is mostly present at wet landscape. The millipedes are good decomposers especially in forest ecosystem in montane areas. (Aldgasam and Ramanathan, 2013). There is an urgent need for

intensive inventorying and monitoring of millipede in different habitats especially in the Northern Ghats (M.S.) India in order to promote the conservation of diversity of millipede.

IV. CONCLUSIONS

Most of the species are abundant at rainy season than summer and winter season, it is depends on arability of food. These investigated that the effect of seasonal fluctuation on diversity of millipede. Millipede need to conserve from the given area of hot spot and increase their species richness.

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Studies on Larval Digenetic Trematodes of Godavari River, Gangapur Project : Xiphidiocercariae

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ABSTRACT

The earlier work on the snails and cercariae of this region was carried out by Karyakarte and Yadav(1974 to 1979 under PL-480 Project on "Control of Molluscan Agents of Helminth Parasites of Agricultural and Veterinary Importance" (Project No. A 7-ADP-39). The account of their finding was published in the year 1981. They examined 8 species of snails viz , Viviparous bengalensis, Melanoids tuberculatus, Melanus scabra, Lymnea acumulata , Lymnea luteola, Lymnea auricularia, Indoplanorbis exustus, Anisus (Gyrulus) convexiusculus. Out of these 8 species, they reported cercarial infection in 6 species of snails and 2 species, M. scabra and V. bengalensis were free from larval infection. Their work included description of 11 species of fresh water cercariae belonging to Monostome, Amphistome and Distome groups. The work on larval trematodes was further continued in this region in the Trematology Laboratory. Present paper deals with two species of freshwater cercariae which belong to Xiphidio cercariae. The group xiphidio cercariae having a piercing spine or stylet in the oral region is represented here with two different types of cercariae. The cercariae are abundant during the period between December and May in the hepatopancreas and gonads of the snails occurring in Nashik region. The collection is mainly from the snails Lymnea Luteola and Melania tuberculata The snails were collected at Gangapur project (Water reservoir) Godavari river, Darna river, Waldevi river, Girnare, ponds and ditches around Godavari river. The cellulosa group is represented hereby two cercariae a known Cercariae indicae LVII sewell, 1922 and a new Cercaria disciforma n.sp.

Keywords: Monostome, Distome, Amphistome, Trematology, Cellulosa group.

I. INTRODUCTION

Luhe (1990) made the first attempt to classify the cercariae in a comprehensive manner. He classifies various cercariae into five different groups. The groups were Monostome cercariae, Distome cercariae, Amphistome cercariae. Lophocercous cercariae, Gastrostome cercariae. Labour (1911) made a survey of British marine cercariae and divided into two main groups Gastrostomata and Prosostomata. Cort (1914) made a survey of larval trematodes from Norh American freshwatrer snails Faust (1919b,1921,1924,1926) studies larval trematodes from South Africa and China. Miller (1936) made a comparative account of Furcocercus cercariae and in 1936 he studied the North American cercariae. Sewell (1922) studied the freshwater cercariae from India and he modified the Luhe's classification and divided major groups into number of smaller groups. Porter (1938) studied the freshwater Larval trematodes found in certain South American Mollusca. While classifying the cercariae he followed the ideas of Luhe (1909) and Sewell (1922). Soparkar (1921) gave a note on some Furcocercuos cercariae from Bombay. Chandler (1953) gave a key to the Furcocercuos cercariae. Khan (1960 to 1961) studied larval trematodes infecting

freshwater snails in London and some adjoining area. Nasir (1964) gave a key to the cercariae from British freshwater Molluscs. In 1972 he gave some aspect of Xiphidocercarial classification and in 1973 he reported twenty new species of Venezuelan cercariae, Ito et al(1977) study on the freshwater cercarial in Leyte Island, Philippines. After Sewell, few workers have described some cercariae from India (Singh 1952, P, Srivastava 1958, Malaki and Singh 1962, Gupta and Taneja 1970, and 1970a, Mohandas 1977 and 1979, Karyakarte and Yadav 1981, A Farahank 2006, 2007, Nkwengulila 1998, Gulam M.A. 2011, Eric 2005, Shimura 1980, Oleg Ditrich 1997, Sami Bdir 2011, Sey 2003, Todd 2004, Thapana 2011, Uthpala 2010,),Karkaykarte & Yadav
1981) Present paper deals with two species of freshwater cercariae which belong to Xiphidiocercariae. The classification followed in the paper is of Luhe (1909), Sewell (1922) and Porter (1938).

II. METHODS AND MATERIAL

(1) Collection and maintenance of snails:

Studies on cercariae commenced with collection of first intermediate host (snails). They were collected either hand picked or dragging a net through water and were transported to the laboratory. The snails were then transferred to glass water bowls and well aerated acquaria already provided with a rich water plants such as Vallisneria, Hydrilla, Chara, Spiirgyra and fimbria etc. After a short period of acclimatization the snails were transferred to individual test tubes kept on wooden rocks in order to detect the cercariae. In the laboratory most preferably the same pond water was used for the snails from which they were collected as the purified tap water supplied to the laboratory proved unsuitable perhaps due to chemical purification

(2) Observations:

The snails collected were kept under observation for some time. The snails which are fully grown showed larval infection while the young ones were normally free from larval infection. Due to the infection, it was observed that the snails grow in size and show a phenomenon of gigantism. Many a time the shell grow enormously and ballooning was observed.

For the study of cercariae heavily infected snails were selected. Two methods were followed for the morphological observations.

1) Natural emerging method
2) Crushing method.

1) In natural emerging method the snails (2to3 at a time) were kept in separate test tubes. This was a constant source of living cercariae naturally emerging from the snails. The sunlight and artificial light play an important positive role in stimulating th emergence of cercariae . It was observed that some cercaraie emerge only in darkness.

2) Crushing method

This method of investigation of cercariae found suitable for morphological observation on various developmental stages such as sporocysts and rediae. This quick method was useful for studying the seasonal percentage of infection of cercariae.

The cercariae collected were subjected to various artificial methods for the study of various internal structures.

3) Movement relaxation :

Sometimes cercariae were found to be so active that observation under power was impossible without some method interfering with or controlling their

movement. Hence dilute solutions of gum, starch, gelatin were used to slow down their movements.

4) Vital stains :

For the study of structural details in live condition vital stains were used such as Neutral red, Methyl green, Nile blue, Azur II and Nile blue sulphate.
For the study of flame cells Indian ink and Amphibian ringer solution were found to be suitable.
For the preparation of permanent mounts the cercariae were fixed in 1% hot formalin, stained in Delafieid's haematoxylin, cleared in clove oil and mounted in D.P.X.

(5) Measurements :

Most of the specimens were measured in live state. In the preset work the measurements given for two species of cercariae and their parthenitae represent averages of twenty specimens of each species. The diagrams have been made with the aid of a camera lucida. Sketches were drawn at different magnification using oil immersion objective if necessary. This method gave the most uniform results.

All the measurements are in millimeters.

The most suitable time making the diagrams for morphological study of living cercariae was immediately after they emerged from the snails without vital staining otherwise became opaque after remaining in water for half an hour.

Responses :
The responses of cercariae to various stimuli were studied in the laboratory conditions at temperature 28®C
(A) For the study of phototaxis a glass apparatus was fabricated and used. The cercariae allowed to move into four limbs of the apparatus. Three limbs were subjected to various light intensities and fourth the dark one.
(B) For geotaxis U tube was used.
(C) Emergence of cercariae was concluded after series of such observations.

I) XI PHIDIOCERCARIAE

CER CAR IAE MICROCOTYLAE CELLULOSA CROUP
I) CERCAR IAE INDICAE LVII SEWELL , 1922

In the present work xiphidiocercariae are represented by two species. These cercariae were collected from the snail host Lymnea luteola and Melania scabra. The snails collected at Gangapur Project, Godavari river, Darna River, Waldevi river and nearby places. As indicated in collection data of snails and cercariae it is evident that the larval forms of Cercariae indicae LVII Sewell, 1922 are abundant during the month from December to May for both years 2015 and 2016. Two cercariae belonging to Cercariae Microcotyle group of Cellulosa in Xiphidiocercariae are described here. The Cellulosa group is represented here by two cercariae, a known Cercariae indicae LVII Sewell, 1922 and a new Cercaria disciforma n.sp.

COLLECTION DATA

1) Cercariae indicae LVII Sewell, 1922

Percentage of infection during the years 2014 and 2015

Percentage of infection (Mean) = 4.16

The cercaria is greenish in colour with spinose cuticle. The eye spots are absent. It is an active swimmer and has a tendency to swim at the bottom. The main body of cercaria is oval. The tail is two times longer th. The cercaria measures 0.34 (0.30 to 0.38) in total length (main body 0.11 X (0.09 to 0.13) and Tall

is 0.23 (0.20 to 0.26). The breadth of main body Is 0.08 (0.05 to 0.11) and that of tail is 0.03 (0.02 to 0.04). The oral sucker Is bigger than acetabulum, subterminally located and measures 0.03 (0.03 to 0.04) in length and 0.04 (0.03 to 0.05) in breadth. The stylet is pointed and measures 0.03 (0.02 to 0.04) in length. The ventral sucker is located in the posterior half of the body. Its anterior margin just touches the equator. It is 0.02 (0.01 to 0.03) long and 0.03 (0.02 to 0.04) wide. The mouth leads into a very short prepharynx which is turn opens into a pharynx which is muscular and measures 0.010 (0.007 to 0.013) in length. Oesophagus and intestinal caeca are totally wanting. The cercaria is typical in haying two pairs of salivary glands. The glands are dimorphic and differ in their location. One pair (smaller) is located in between oral and ventral suckers. The other pair (larger) is spindle shaped end extends in the postacetabular region in between ventral sucker and excretory pore. Both the ducts open slightly anterior to mouth.

The excretory bladder is spindle shaped and the excretory tubules branch marginally in the acetabular region into anterior and posterior sub-branches. The flame cells could not be traced. The caudal excretory duct opens at the tip of the tail.

Sr. NO.	Month	Locality	No.of snails examined	No,of snails infected	% of infec-tion
1	January 2015	Gangapur Project Girnare Godavari River Waldevi River	513	45	8.96
2	February 2015	-do-	215	21	9.76
3	March 2015	-do-	232	27	11.63
4	April 2015	-do-	292	38	13.01
5	May 2015	-do-	345	51	14.78
6	June 2015	-do-	-	-	-
7	July 2015	-do-	-	-	-
8	August 2015	-do-	-	-	-
9	September 2015	-do-	-	-	-
10	October 2015	-do-	-	-	-

SPOROCYST :

The sporocyst is oval, containing 3.0 - 11 mature cercariae at a time. It is greenish in colour and measures 0.38 (o.31 to 0.45) in length and 0.17 (0.14 to 0.20) in width. The wall of the sporocyst is dotted over with a number of irregularly distributed orange granules.

RESPONSES :

(1) Phototaxis Negative
(2) Geotaxis Positive

11	November 2015	-do-	-	-	-
12	December 2015	-do-	12	6	2.83
13	January 2016	-do-	425	17	4.00
14	February 2016	-do-	293	15	5.11
15	March 2016	-do-	410	29	7.07
16	April 2016	-do-	340	31	9.11
17	May 2016	-do-	312	34	10.89
18	June 2016	-do-	-	-	-
	July 2016	-do-	-	-	-
20	August 2016	-do-	-	-	-
21	September 2016	-do-	-	-	-
22	October 2016	-do-	-	-	-
23	November 2016	-do-	-	-	-
24	December 2016	-do-	255	7	2.74
	Annual Percentage of infection 2016	Total	2035	133	3.24

III. DISCUSSION

As the oral sucker Is provided with a stylet, the present form belongs to Xiphldlocercaria In Leptoceroous group.

In possessing undivided tail, main body less than 0.20 acetabulum smaller than the oral sucker and located in the posterior region of the body, salivary glands two pairs confined in the pre-acetabular region the present form belongs to Microcotyle group - Luhe 1909 of Xlphldiocercariae. Amongst Mlcrocotyle group it falls in with the members of sub- group Cellulosa as It possess two pairs of salivary glands.

In Cellulose group the present form resembles Cercaraie Indicae LVII Sewell, 1922. As the present form Is reported from Lymnea luteola a new host record and also from a different local It y# it was thought advisable to report it here.

Host : Lymnea luteola
Habit : Hepatopencreas and gonads
Locality : Gangapur Project , Girnare Godavari River, Waldevi River, Nashik District, Maharashtra State India.

2) Cerearia disciforma n.sp

This cercaria, belonging to XiphIdlocercaria group

Sr No.	Months	Locality	No.of snails examined	No, of snails infected	% of infec-tion
1	January 2015	Gangapur Project Girnare Godavari River Waldevi River	400	48	12.00
2	February 2015	-do-	325	26	8.00
3	March 2015	-do-	370	25	6.75
4	April 2015	-do-	380	22	5.78
5	May 2015	-do-	420	21	5.00
6	June 2015	-do-	420	16	3.80
7	July 2015	-do-	325	-	-
8	August 2015	-do-	312	-	-
9	September 2015	-do-	285	-	-

10	October 2015	-do-	415	-	-
11	November 2015	-do-	315	-	-
12	December 2015	-do-	295	-	-
	Annual Percentage of infection 2015	Total	4259	201	4.67
13	January 2016	-do-	292	33	11.30
14	February 2016	-do-	515	29	6.98
15	March 2016	-do-	400	24	6.00
16	April 2016	-do-	440	22	5.00
17	May 2016	-do-	310	12	3.87
18	June 2016	-do-	390	11	2.82
19	July 2016	-do-	200		
20	August 2016	-do-	472		-
21	September 2016	-do-	390	-	-
22	October 2016	-do-	360	-	-
23	November 2016	-do-	320	-	-
24	December 2016	-do-	272	38	13.97

was collected from the snail Melanla tuberculata The collection was observed in the hepatopancreas and also in gonads. The snails collected at Gangapur Project (Water Reservoir) Godavari river, Darna river, Waldevi river and near by places. The snails occur in Nashik region 2012 and 2013 but the infection was found from Decemeber to June during both the years and 2012 and 2013.

3) Collection data
4) Percentage of infection during the years 2015 &

in having a length of 0.05 (0.04 to 0.06) and width of 0.011 (0.010 to 0.012) with a pointed anterior end. It shows small thickening about one third the length from the anterior end. The ventral sucker is in the posterior part of the body, smaller than the oral and measures 0.03 (0.02 to 0.04) in diameter. The ratio between width of ventral to oral sucker is 1: 1.6. The mouth is ventral and leads into a pharynx which measures 0.02 (0.01 to 0.03) in length and 0.03 (0.02 to 0.04) in width. There is no trace of oesophagus and intestinal caeca. There are two pairs of salivary glands. The outer pair is larger than the inner one. The glands are confined in the preacetabular region and open near the mouth with the help of narrow ducts.

The excretory bladder is Y-shaped. The protonephridial tubules are coiled and at the tip of them fairly prominent flame cells are observed. The caudal excretory canal is present in the tail but flame cells are not observed.

SPOROCYST

The sporocyst is yellowsih in colour and oval in shape with limited number of mature cercarie (Four at a time). It measures 0.41 (o.39 to 0.43) in length and 0.25 (0.23 tfc 0.27) in width. The wall of the sporocyst is dotted over with a number of small round retractile granules

DISCUSSION

The present Xiphidiooercaria reported from Melania tuberculata belongs to Cercariae microcotylae group, the characters being long and undivided tail, body length less than 0.20 and acetabulum smaller than the oral sucker lying behind the middle of the body.

As there are only two pairs of salivary glands, it is included in the cellulose group From India only two species are reported in Cellulosa group namely Cercariae indicae

LVII Sewell, 1922 and Cercaria naukuchlensis Malaki and Singh, 1962.

Amongst these two forms the present form shows affinities with C. indicae; LVII Sewell, 1922.

C. indicae; LVII and the present form have same type of stylet, oral sucker larger than the acetabulum and same type of excretory bladder. Close examination, however, shows that the present form is quite different from the known one.

The body is oval in C. indicae LVII and rounded in the present form.

The acetabulum is located very near to the equator in the known form and away from it by a considerable distance in the new form. Both the pairs of salivary glands are preacetabular in the form under discussion whereas one pair is preacetabular and other paracetabular in the form described by Sewell.

The excretory system differs into two. In the known form there are eight flame cells whereas, in the present form the excretory tubule terminates into single flame cell. Thus there are only two flame cells in the present form.

The known form is reported from Melanoides tuberculatus from a tank, Indian Museum, Calcutta whereas, the new form from MeIania tuberculata at Gangapur Project, Godavari river, Nashik. Maharashtra State, India.

A new species is established and named as Cerceria disciforman.sp.

Host : Melania tuberculata

Habitat : Hepatopancreas and gonads

Locality : Gangapur Project (Water Reservoir) Girnare,Godavari River Waldevi River

Dharna river , Girnare District Nashik Maharashtra State, India.

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Hypoglycemic Activity Of Ethanolic Extract of Aloe Vera In Control And Diabetic Mice

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ABSTRACT

Herbal medicines are frequently considered to be less toxic and have fewer side effects than synthetic ones. Hence, the present study was designed to evaluate hypoglycemic activity of Aloe vera. Diabetes mellitus was induced by single intraperitoneal injection of alloxan 120 mg/kg body weight. Ethanolic extract of Aloe vera was administered intraperitoneally 300mg/kg body weight in diabetic mice. The treatment effects were compared with standard antidiabetic drugs insulin 1IU/kg body weight. A significant decrease in blood glucose level and improvement in the body weight was observed. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract. Therefore, the result suggests that Aloe vera leaf extract is potent hypoglycemic agent.

Keywords: Aloe Vera, Hypoglycemia, Intraperitoneal Diabetes Mellitus, Alloxan

I. INTRODUCTION

Diabetes mellitus is one of the most challenging metabolic disorder of 21st century which affects essential pathways in the body such as carbohydrate, protein and lipid metabolism (Karau et al., 2012). It leads to hyperglycemia resulting from a defect in insulin secretion, or insulin resistance in the peripheral tissues or both (Ansarullah et al.,2011). The classical symptoms of hyperglycemia viz poluuria, polydipsia, polyphagia and weight loss (Chan et al., 2009).It is assumed that in 2030 the number of diabetic patient will increase to 439 million which was 285 million in 2010 (Shaw et al.,2010).India has been declared as "Diabetic Capital of the World" by the International diabetes federation because 20% of the total diabetic patients in the world found in India. It is evident that this disease leads to hyperglycemia and to many other complications such as hyperlipidemia, hypertension, atherosclerosis, retinopathy and neuropathy (Anfenan, 2014). The oral hypoglycemic drugs glyburide, metformin, glimepiride and poglitazone are basic drugs for diabetes mellitus (Bandawane et al., 2011).These hypoglycemic drugs putforth serious side effects especially gastrointestinal discomfort, nausea and metallic taste (Vishakarma et al., 2010). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Pushparaj et al.,2000). Only few have received scientific scrutiny. Aloe vera can be mentioned as a plant of considera ble interest.

Aloe vera belongs to the Xanthorrhoeaceae family. It is a cactus like plant, grows in hot dry climates and currently, because of demand it is cultivated in large quantities (Grieve, 1975). This herb used internally to fight again poor appetite, colitis constipation, digestive problem.

Thus the present study is designed to study hypoglycemic activity of Aloe vera in alloxan induced diabetic female mice.

II. METHODS AND MATERIAL

Preparation of Aloe vera leaf extract The fresh A.vera leaves (Voucher specimen: KJS-1) were washed thoroughly with water, peel was removed and only pulp was collected. The pulp was lyophilized. Extraction of lyophilized material was carried out by soxhlet method (Aswar et al.,2011). The extract obtained was dried at 370 C in oven. The obtained yield was stored in refrigerator at 80C until further use. The residual extract was resuspended in distilled water and used in study as per desired concentration when needed.

Animals

Healthy adult female mice (Mus musculus Linn.) were used for present investigation. Mice were obtained from (Rajarambapu College of Pharmacy, Kasegao, Sangali). Adult female mice (4 month age) 28 ±2 were selected. All the animals were kept under a 12:12 hr L:D cycle. The animals were housed in aluminium cages and allowed to live in groups of 3-4 animals per cage. They were fed with Amrut mice feed, marketed from Pranav Agro Industries, Pvt. Ltd. Sangli and water ad libitum. Animals were divided into four groups.

Experimental design

Female mice were divided into four groups containing 6 animals per group.

Control group: Female mice were given intraperitoneal injection of 0.15 M Acetate buffer PH 5.4 for 15 days.

Diabetic group: Female mice were given single intraperitoneal injection of alloxan 120 mg/kg body weight (Fayed et al.,1988; Helal,2000 and Syiem et al.,2002).

Recovery group: Female mice from diabetic group were given intraperitoneal injection of A. vera leaf extract at dose of 300 mg/kg body weight once a day for 15 days (Rjasekaran et al.,2004).

Diabetic + Insulin group: Female mice from diabetic group were given intraperitoneal injection of Insulin 1IU/kg body weight (Rajesh Mandade, 2012).

Determination of blood glucose level

Fasting blood glucose was measured by collecting a drop of blood from the tail after incision with a sharp blade. The blood glucose was determined by using a rapid glucose analyzer with a glucose strip inserted in sugar scan digital blood glucose monitoring glucometer. The result was expressed in terms of milligram per deciliter of blood (Kumar et al., 2006).

Determination of body weight

Animals from each group weighed before starting experiment, animals from control, diabetic and recovery group and insulin treated group. The record of these observations was maintained.

Statistical analysis

The data was statistically analyzed by One way ANOVA followed by Tukey HSD test. All the values were expressed as mean ± S.E. The difference was considered significant when $p<0.001$

III. RESULTS

Table 1: Showing blood glucose level (mg/dl) in control and experimental adult female mice.

Values are mean ± S.E. Number in parenthesis denote number of animals. P< 0.01 = significant, P< 0.001 = highly significant.

Sr. No.	Animal group (n=6)	Adult female mice	
		Blood glucose level (mg/dl)	Statistical significance
I.	Control group	85 ± 2.91	1:2, P<0.01
II.	Diabetic group	220.2 ± 1.92	2:3, P<0.01
III.	Recovery group	124.6 ± 13.39	2:4, P<0.01
IV.	Diabetic + Insulin group	121.4 ± 7.4	3:4, P<0.01

Table 2: Showing body weight (gm) in control and experimental adult female mice.

Values are mean ± S.E. Number in parenthesis denote number of animals. P< 0.01 = significant, P< 0.001 = highly significant.

Sr. No.	Animal group (n=6)	Adult female mice	
		Body weight (gm)	Statistical significance
I.	Control group	28.6 ± 1.14	1:2, P<0.01
II.	Diabetic group	23 ± 1.58	2:3, P<0.01
III.	Recovery group	26.6 ± 1.1	2:4, P<0.01
IV.	Diabetic + Insulin group	27.8 ± 1.3	3:4, P<0.01

The initial body weight in the adult female control group was evaluated to be 28.6 ± 1.14 gm (1:2, p < 0.01) whereas it as 23 ± 1.58 gm in diabetic group. After the treatment of diabetic mice with A. vera (300 mg/kg body eight) the body weight it was found significantly increase to 26.6 ± 1.1 gm (2:3, p < 0.01) while highest weight gain was observed in insulin treated group 27.8 ± 1.3 gm (2:4, p< 0.01).These data shown that the effect of A.vera on weight gain was intermediate to insulin.

The mean level of blood glucose in the adult female control group was observed to be 85 ± 2.91 mg/dl (1:2, p < 0.01) whereas it was 220.2 ± 1.92 mg/dl in diabetic group. After the treatment of diabetic mice with A.vera (300 mg/kg body weight) the blood glucose level found significantly decrease to 124.6 ± 13.39 mg/dl (2:3, p < 0.01) while highly decreasing blood glucose level was observed in insulin treated group 121.4 ± 7.4 mg/dl (2:4, p < 0.01). These data shown that the effect of A. vera on blood glucose level was intermediate to insulin.

IV. DISCUSSION

Diabetic mellitus is a complex metabolic disease caused by defect of insulin signaling pathways which show the defect from pancreatic β-cell deficiency (Kahn, 1994). Scientists are in search of easily available, inexpensive therapeutics, having minimum side effects for better treatment (Manna et al., 2010).
In the present study alloxan was used for induction of diabetes. Our results are similar to previous findings (Jain et al., 2011). Alloxan selectively destroy pancreatic cell, after being taken up by the pancreatic cells via GLUT-2 glucose transporters, alloxan produces reactive oxygen species in a cyclic redox reaction with its reduction product, dialuric acid, superoxide radicals hydrogen peroxide and hydroxyl radicals which are responsible for the death of the β-cells (Lensen, 2008; Muhtadi et al.,2015). However, the exact mechanism after treatment of plant not yet

known. According to literature hypoglycemic action of plant extract might be enhanced insulin secretion, increase peripheral glucose uptake or decrease counter regulatory hormone like cortsol, glucagon and growth hormone (Abunasef et al.,2014).

Body weight is an indicator of good health and efficient metabolic homeostasis, body weight before and after commence of experiment, showed that alloxan –induced diabetes resulted in a significant decrease in body weight with respect to control group, which was in accordance with previous reports (Komolafe et al., 2009; Sharma et al., 2013; Singh et al., 2016). Loss of weight has been one of the symptom of diabetes mellitus (Sellamuthu et al., 2009). Weight loss associated with diabetes may be due to increased muscle wasting and loss of tissue proteins (Kato et al.,2008; Nagwa et al, 2017). Deficiency of insulin in the diabetic mice lead to decreased amino acids at the level of protein synthesis (Mohamed et al., 2013). Glycosuria is known to cause a significant loss of calories which result weight loss inspite of increased appetite.These event related to insulin deficiency. In the present investigation treatment of A. vera groups tend to gain weight. This weight gain in diabetic groups showed that weight loss prevented by interaction of several bioactive compounds. Treatment allowed to access the glucose both to supply energy and spared some to build tissue require for growth by decreasing metabolic rate and glycosuria.

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Use Selected of Fungicides for the Control Of Fusarium oxysporum f. sp. Ciceris Causing Wilt of Chickpea (Cicer arietinum L.)

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ABSTRACT

The aim of this study was to investigate the effect of fungicides on the management of Fusarium oxysporium f. sp. ciceris causing wilt of chickpea. In chemical control, the different fungicides were used to test the sensitivity against Fusarium oxysporum f. sp. ciceris by food poisoning technique. The linear growth of mycelium was measured daily and the results were expressed in terms of percent control efficacy (PCE) up to 8 days for Fusarium oxysporum f. sp. ciceris. It was noted that as the concentration increases linear growth decreases up to certain concentration. Above that concentration, there was total inhibition of the growth of fungal pathogen. This is considered as minimum inhibitory concentration (MIC) of respective fungicides. The different fungicides used were Thiram,Carbendazim, calcium polysulfide, captafol, captan, carbamate, carbamorph, carbanilate, carbendazim, carboxin Mancozeb, Copper oxychloride

Keywords: Fungicides, Fusarium oxysporium f. sp. ciceris & food poisoning technique.

I. INTRODUCTION

Mungbean (Vigna radiata (L.) Wiczek] is one of the important pulse crops in India. It is an important source of protein. A seedling disease that usually appears within a month of sowing, when patches of dead seedlings at the primary leaf stage are seen, scattered over the field. Worldwide it is grown on an area of 13.5 million hectares with a production of more than 13 million tons. It is an important crop of Indian sub-continent that usually contributes more than 66% in terms of global production, (Anon., 2013). The confirmatory symptom is rotting in the collar region that is covered with white mycelial growth; this differentiates collar rot from other seedling diseases caused by Fusarium. Yaqub and Shahzad. 2006. studied effect of fungicides on in vitro growth of Sclerotium rolfsii. Fungicides are biocidal chemical compounds or biological organisms used to kill fungi or fungal spores. Ilyas et al (1996) evaluated of some fungicides against Fusarium oxysporum f.sp. lini. Crop production is adversely affected by plant diseases which destroy in agriculture as well as drastically affect modern agriculture system. Iqbal SM, Bashir M, Rauf CA, Malik BA (1996).studied of fungicides against soil-borne pathogens of chickpea These diseases are mainly caused by seed borne pathogens like, Fusarium oxysporum f. udum etc. These are responsible for most of the losses as they spread very quickly and affect chickpea plant plant. Qayoom et al. (2006) studied of different fungicides against Fusarium wilt of cotton caused by Fusarium oxysporum. A fungistatic inhibits their growth. Fungi can cause serious damage in agriculture, resulting in critical

losses of yield, quality, and profit. Haidukowski, et al. (2005).studied the effect of fungicides on the development of Fusarium head blight, yield and deoxynivalenol accumulation in wheat inoculated. Fungicides are used both in agriculture and to fight fungal infections in plant. Cromey et al.(2001). Control of Fusarium head blight of wheat with fungicides.

With the individual fungicide treatment minimum inhibitory concentration (MIC) was determined. The minimum inhibitory concentration (MIC) of Thiram,Carbendazim, calcium polysulfide, captafol, captan, carbamate, carbamorph, carbanilate, carbendazim, carboxin Mancozeb, Copper oxychloride, Captan and Captafol. to Fusarium oxysporium f. sp. Ciceris

II. METHODS AND MATERIAL

The effect and sensitivity of different fungicides was tested by food poisoning technique as described by (Borum & Sinclair, 1968 Nene and Thapliyal, 1982). In this technique, the different concentrations of fungicides ranging from 100 to 1200 µg/mL were prepared on the basis of active ingradient. After sterilization 10 mL of prescribed fungicide concentration was added into 10 mL of PDA medium (Potato Dextrose Agar medium) in sterilized beaker. After mixing well the solution was poured in sterile petriplate and allowed to solidify. After solidification, a 5mm disc of 8 days old culture of test fungus was inoculated in the center of PDA plate.

These plates were incubated at 28□1□C. All treatments along with control i.e. by adding 10mL of sterilized distilled water in 10mL of media was prepared. Such all treated plates were prepared in triplicates of each treatment. The observations were recorded in the form of linear growth of fungal pathogen in millimeter (mm) daily for 8 days. The linear growth was measured up to the growth in control plate when filled completely. The minimum inhibitory concentration (MIC) of fungicide was recorded. The percent control efficacy (PCE) of fungicide was calculated by using following formula.

$$PCE = 100 \times [\, 1 - (X/Y) \,]$$

Where, X= Diameter of the colony treated with fungicide. Y= Maximum growth of fungus on control.

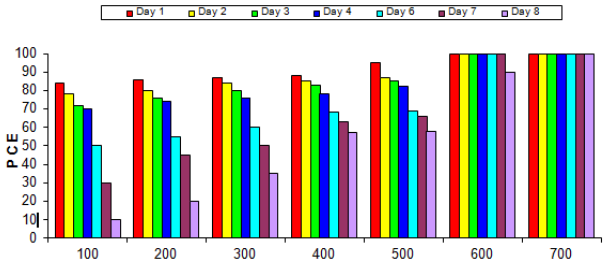
III. RESULTS

The captafol was used against Fusarium oxysporium f. sp. ciceris and observed PCE as noted in Table 1 and Fig. 1. The different concentrations were used from 100□g/mL to 1000□g/mL. The least PCE was at 100□g/mL and highest was at 1000□g/mL. The MIC was found to be 900□g/mL.

Table 1 : Effect of Captafol on percent control efficacy (PCE) of Fusarium oxysporium f. sp. Ciceris

Conc. □g/mL	Percent Control Efficacy (PCE)							
	Incubation period (Days)							
	1	2	3	4	5	6	7	8
100	78	76	70	50	35	23	20	6
200	80	79	73	53	40	35	24	10
300	82	80	72	65	59	45	35	30
400	84	81	75	68	63	60	55	43
500	85	82	77	73	70	63	57	50
600	90	84	81	78	72	68	63	53
700	93	85	82	79	77	75	65	60
800	100	100	100	85	83	77	67	63
900	100	100	100	100	100	100	100	75
1000	100	100	100	100	100	100	100	100

S.E ±	2.59	2.85	3.69	5.17	6.56	7.62	8.26	8.56
C.D. at 5%	5.85	6.44	8.33	11.68	14.82	17.22	18.66	19.41



Concentration (µg/mL)

Fig.1. Effect of Carbendazim on percent control efficacy (PCE) of Fusarium oxysporum f. sp. Ciceris

IV. DISCUSSION

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Effect of Heavy Metals on Antioxidant Enzyme of Fresh Water Major Carp Labeo Rohita from Mula Dam Ahmednagar

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ABSTRACT

Accidental industrial spills may lead to a high concentration of toxic metals in the aquatic environment which may lead to acute and chronic toxicity. Of all aquatic species, fish are most sensitive to water borne contamination and are recognized as bioindicators for water quality monitoring. The present study was planned to enumerate the accumulation of heavy metals like Fe, Cu, Ni and Zn in various tissues of fish like muscles, gills, liver, and kidneys). Level of these metals in different organs of fish was determined by Atomic Absorption Spectrophotometer (AAS). Total proteins and levels of antioxidant enzymes, Thiobarbituric acid reactive substance (TBARS), Superoxide dismutase (SOD) and Catalase (CAT) in the tissues of the Indian major carps were measured.

Keywords: Oxidative stress, Pollutants, heavy metal, antioxidant enzymes, Labeo rohita Mula dam

I. INTRODUCTION

Heavy metals are the metals or metalloid of the environmental concern. The term heavy metal originates with reference to the harmful effects of various elements like iron, copper, nickel, and zinc. Heavy metals have been reported to have a significant influence on all life forms mostly aquatic fauna. Heavy metals have been reported to affect aquatic animals like fish and indirectly pose a threat to human life. Heavy metals arrive in to the aquatic ecosystem through natural as well as anthropogenic sources, including industrial and domestic sewage, storm, run-off, leaching from landfills/dumpsites and atmospheric deposits (Forstner et al, 1983). The increasing concentration of heavy metals in the tissues of fish leads to biomagnifications in the successive tropic levels of food chain. Several studies have been carried out to examine the contamination of fish by various heavy metals.

Due to their high protein contents and less saturated fat value fish makes up a major part of the human diet (Sivaperumal et al.,2007; Raychaudary et al., 2008; Raouf et al ., 2009 ; Yilmaz et al., 2009, Bhattacharya et al .,2010). Fish makes up major forms of the aquatic fauna and are considered as the best bio-indicator of heavy metal pollution in aquatic systems (Alinnor et al., 2010). Fresh water bodies are being continuously contaminated with heavy metals released from various sources (Adnano 1986). When compared with other toxicant, heavy metals are considered to be unrelenting components of the aquatic habitats. Heavy metals are omnipresent, soluble in water, easily transported by water and are usually easily consumed by aquatic biota (Mendel et al., 2005). The study of bio- accumulation of heavy metal in the living tissues

of aquatic animals is a significant method to monitor the pollution level of water bodies and at the same time can prove to be helpful method to study the biological role of heavy metals present at an increased level in fish and other aquatic organisms (Ahmad et al .,2010).

II. METHODS AND MATERIAL

Study area: Mula Dam is located 19º20'to 19º35' N latitude & 74º25' to 74º25' to 74º36 E latitude. The dam was artificially built across the Mula river in 1971 and contains natural water and capacity of dam is 26 TMC. It experiences an average rain fall 58 cm. Maximum depth being 67.97 m. The physiography of basin is semi agricultural & semi-arid with cultivated top soil bank (A J Dhembare, 2011).

Experimental Animal: The fresh water major carp Labeo rohita was collected from Mula Dam by local fishermen using multifilament, nylon gill net of mesh sizes ranging from 30 mm. After collection, samples were kept in ice pack and brought to the laboratory on the same day and then frozen at -20ºC until dissection, according to standard FAO methods.

Heavy Metal Analysis: One gram of muscle, liver, kidney and gill racers from each sample was dissected for analysis. The dissected samples were transferred to a Teflon beaker and digested in an acid solution to prepare the sample for heavy metal analysis (Kenstar closed vessel microwave digestion) using the microwave digestion program. The samples were digested with 5 ml of nitric acid (65%). After complete digestion the samples were cooled down to room temperature and diluted to 25 ml with double distilled water. All the digested samples were analyzed for metals like Fe, Cu, Ni and Zn using Atomic Absorption Spectrophotometer (Perkin-Elmer AA 700). The instrument was calibrated with standard solutions prepared from commercially available chemicals procured from Merck, Germany (Kingston, Jassie et al., 1988).

III. RESULTS

The bioaccumulation rate of Cu, Iron, Zinc and Mn in different tissues of Labeo rohita follows the pattern

Copper (Cu) : Liver > Kidney >Muscles >Gills
Iron (Fe) : Kidney> Liver > Gills >Muscles
Zinc (Zn) : Liver > Kidney >Muscles >Gills Manganese (Mn) : Liver > Muscles > Gills > Kidney.

The concentration of heavy metals and enzymatic biomarkers determined from different tissues of Labeo rohita is tabulated as follows.

Table 1. : Concentration of metals like Cu, Cr, Mn and Zn in the tissues of Labeo rohita caught from Mula dam.

Organs	µg/g dry weight			
	Cu	Fe	Zn	Mn
Liver	172.05 ±81.87	175.52±58.83	132.04±45.79	80.24±52.71
Muscle	10.70±3.47	50.67 ±5.3	31.12±9.82	52.28±31.03
Gill	6.83±0.67	117.56±18.18	25.37±3.36	20.17±4.34
Kidney	31.75±6.72	187.99±74.36	83.56±27.05	10.54±4.03
FAO/WHO guidelines	30	100	100	1.2

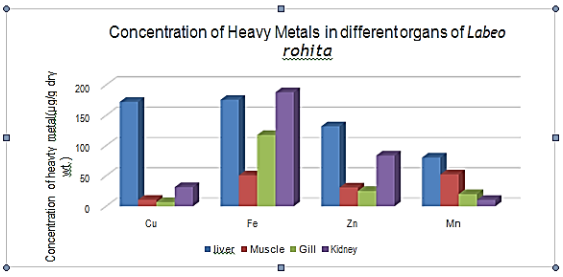
Values are expressed as Mean ± S.E

Table 2: Enzymatic Biomarkers of Labeo rohita caught from Mula dam.

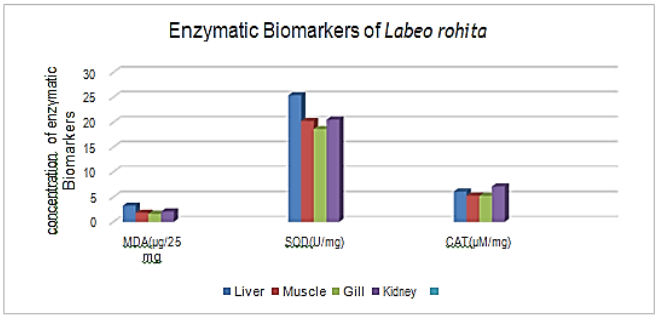
Organs	MDA (µg/25mg)	SOD (U/mg)	CAT (µM/ mg)
Liver	3.26±0.0666	25.37±1.4830	6.10±0.1907
Muscle	1.82±0.0777	20.24±0.9909	5.27±0.3354
Gill	1.62±0.0664	18.65±0.8130	0.13±0.1475
Kidney	2.07±0.0683	20.53±0.4916	7.06±0.1982

Values are expressed as Mean ± S.E

Graphic representation of heavy metal concentration in various tissues *of Labeo rohita*



Graphic representation of enzymatic biomarkers in various tissues *of Labeo rohita*



IV. CONCLUSION

The toxic effects of heavy metals in fish have been demonstrated in the present study. It is richly clear that metals induce an early response in the fish as proved by alterations both at structural and functional levels of different tissues include enzymatic and genetic effects, thereby affecting the innate immune system of exposed fish or increasing susceptibility to multiple types of diseases.

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FTIR and GCMS Studies in Phytochemical Characterization of Physalis Angulata and Solanum Virgianum

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ABSTRACT

The Present study focuses on analysis of phytochemicals present in various parts of Solanum virgianum and Physalis angulata(Solanaceae family)using GCMS and IR spectroscopy. The work also highlights the different phytochemicals present in various parts like fruits and leaves of the plants from Solanaceae family. The GCMS has identified 14 components each from Physalis angulata fruit and leaf extracts, and 13 from Solanum virgianum leaf extracts. However, 11components were identified from Solanum virgianum fruit extracts. The FTIR spectrum confirmed the presence of functional groups like alcohol,phenol,alkene, alkanes flavones , Azides, Arenes in the fruit and leaf extracts .

Keywords: Solanum virgianum, Physalis angulata, Solanaceae , GCMS , FTIR spectroscopy.

I. INTRODUCTION

Knowledge of the chemical constituents of plant is essential, for the discovery of therapeutic drugs as well as for finding new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances 3,12 . A great variety of phytochemicals are synthesized and accumulated by woody plants in their cells, which include alkaloids, flavonoides, tannins, cyanogenic glycosides ,phenolic compounds ,saponins and lignin 17 . Phytochemical which possess many economical and physiological roles are widely distributed as plant constituents

Physalis angulata and Solanum virgianum Dunal from the Solanaceae family are rich in secondary metabolites with specific pharmacological properties.

The metabolic constituents, particularly secondary metabolites differ with the variety of plant, tissue type and at times with the growth conditions24 .There is enormous difficulty in standardizing any formulation if the exact composition of chemical constituents is not known 4,18 . So there is a need to study the various chemicals present in different parts of plant to evaluate their potential as medicinal plants. With the advancement in analytical technology, details about metabolites present in plants can be identified with help of instruments like GCMS and IR spectroscopy 10,14,21 . Present study deals with chemical screening of Solanum virgianum and Physalis angulata using Gas Chromatography-Mass Spectrometry(GCMS) and Infrared (IR) spectroscopy.

II. METHODS AND MATERIAL

2.1 Plant materials :

Solanum virgianum and Physalis angulata were collected from Ahmednagar district, Maharashtra state and identified 2.The leaves and fruits were isolated, dried and powdered. The samples were extracted with ethanol using soxhlet apparatus. Analytical techniques GCMS and Fourier Transform Infrared (FT-IR)

spectroscopy were used for further phytochemical analysis5,16 .

III. RESULT AND DISCUSSION:

1. GCMS analysis: Identification of components detected by GCMS was based on direct comparison of the retention times and mass spectral data with those for standard compounds from National Institute of Standards and Technology (NIST) library. The GCMS identified 15 phytochemicals each from Physalis angulata fruit extract , Physalis angulata leaf extracts and Solanum virgianum leaf extracts, Solanum virgianum fruit extracts, however, showed the presence of 12 phytochemicals. The compounds , identified in the analysis and which are unique to particular extract are reported below with their retention times and area percentage.

1a. GCMS analysis of Physalis angulata fruit extract (PAF): The phytochemicals present in Physalis angulata fruit extract are shown in table no.1and figure no.1 displays the GCMS chromatogram. Heptadecane, Octadecane ,Dibutyl phthalate were present.

1b. GCMS analysis of Physalis angulata leaf extract (PAL): The phytochemicals present in Physalis angulata leaf extract are shown in table no 2and figure no 2 displays the GCMS chromatogram. In the fruit and leaf extracts,octasiloxane and heptasiloxane were common.

1c. GCMS analysis of Solanum virgianum fruit extract (SVF): The phytochemicals present in Solanum virgianum fruit extract are shown in table no. 3 and figure no. 3 displays the GCMS chromatogram.

1d. GCMS analysis of Solanum virgianum leaf extract (SVL): The phytochemicals present in Physalis angulata leaf extract are shown in table no. 4 and figure no. 4 displays the GCMS chromatogram.

The phytochemical constituents obtained after GCMS of Physalis angulata fruit(PAF) and leaf (PAL)are shown in Table No.1and2.respectively. It shows presence of heptasiloxane monolinoleoyl glycerol trimethylsilyl ether and octasiloxane in both leaf and fruit extract. Present study also showed presence of compounds which are unique to fruit and leaf part of the plant. Solanum virgianum fruit extract contains 9,12- Octadecadienoicacid (Z,Z) methyl ester, tocopherol and carotene. Solanum virgianum leaf extract contains levoglucosenone, benzenetriol,heptadecane. The details about the retention time and area percentage of the compound indicated in the Table No.3 and4.

2 FTIR analysis: The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation

2a. FTIR analysis of PAF extract:The IR spectrum is shown in figure no.5.The PAF extract yielded maximum peak level 3600 cm-1 and minimum peak 783 cm-1 FT-IR studies confirms the presence of functional groups in PAF extract and is listed in table no

5. Many of the functional present in Physalis angulata fruit and leaf extract are similar like aliphatic and carboxylic group but the fruit extract contains Arenes and Physalis angulata leaf contains flavones .

2b. FTIR analysis of PAL extract:The IR spectrum is shown in figure6 and FT-IR studies confirm the presence of functional groups in PAF extract and is listed in table no

6. The PAL extract yielded maximum peak level 3600 cm-1 and minimum peak 783cm-1 .

2c. FTIR analysis of SVF extract:The IR spectrum is shown in figure7and FT-IR studies confirm the presence of functional groups in SVF extract and is listed in table no 7.The SVF extract yielded maximum peak level 3448 cm-1 and minimum peak 723 cm-1 Many of the functional present in Solanum virgianum fruit and leaf extract are similar like aliphatic group ,alcohol group but the fruit extract contains azides ,arenes and Solanum virgianum leaf contains nitrile isocyanates and conjugated carbons.

2d. FTIR analysis of SVL extract:The IR spectrum is shown in figure 8 and FT-IR studies confirm the presence of functional groups in SVL extract and is listed in table no 8.TheSVL extract yielded maximum peak level 3600 cm-1 and minimum peak 715 cm-1 .

IV. CONCLUSION

Phytochemical screening of plant extract is vital, as it helps us to verify the exact composition of metabolites in various plants .The research is helpful, in establishing relationship between the chemical and their biological, physiological roles. The study also emphasizes use of sophisticated instruments like GCMS and IR Spectroscopy in phytochemical research.

V. ACKNOWLEDEGMENT

The authors are thankful to SAIF Chandigarh for providing GCMS facility.

Table No.1:Retention time, Area%, Molecular formula and Major peaks of chemicals detected by GCMS of Physalis angulata fruit extract (PAF).

Peak no.	Rt	Area %	Molecular formula	Compound
1	8.22	1.55	C10H30O5Si5	Cyclopentasiloxane
2	10.72	2.92	C14H44O6Si7	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13tetradecamethyl
3	13.95	3.85	C16H50O7Si8	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethy
4	14.95	4.52	C16H48O8Si8	Cyclooctasiloxane, hexadecamethyl
5	16.67	1.02	C16H48O6Si7	Heptasiloxane, hexadecamethyl
6	18.17	9.15	C16H22O4	Dibutyl phthalate
7	19.37	16.17	C19H34O2	9,12Octadecadienoicacid (Z,Z), methyl ester
8	19.97	5.85	C21H38O2	nPropyl 9,12octadecadienoate
9	23.21	3.66	C24H38O4	Diisooctyl phthalate
10	24.25	0.53	C20H60O10Si10	Cyclodecasiloxane, eicosamethyl
11	26.66	1.70	C28H39ClO9	9Desoxy9xchloroingol 3,7,8,12tetraacetate
12	28.50	1.88	C23H48	Heptadecane, 9hexyl28
13	30.17	1.67	C26H54	Octadecane, 3ethyl5(2ethylbutyl)
14	33.07	2.56	C30H50O6	Olean12ene3,15,16,21,22,28hexol

Figure 1 : GCMS chromatogram of Physalis angulata fruit (PAF)

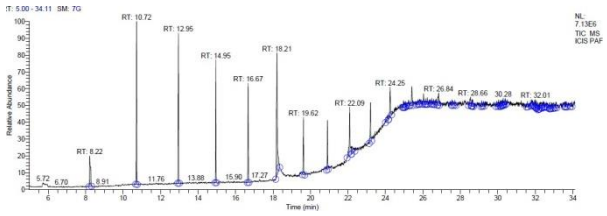


Figure 2: GCMS chromatogram of Physalis angulata leaf (PAL)

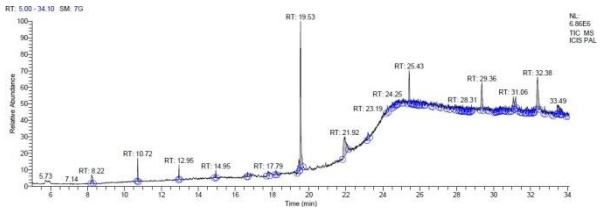


Table no 2: Retention time, Area%, Molecular formula and Major peaks of chemicals detected by GCMS of Physalis angulata leaf extract (PAL).

Peak no.	Rt	Area %	Molecular formula	Compound
1	8.22	1.55	C10H30O5Si5	Cyclopentasiloxane
2	10.72	2.92	C14H44O6Si7	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13tetradecamethyl
3	12.95	3.85	C16H50O7Si8	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethy
4	14.95	1.63	C16H48O8Si8	Cyclooctasiloxane, hexadecamethyl
5	16.67	1.02	C16H48O6Si7	Heptasiloxane, hexadecamethyl
6	18.21	10.43	C20H30O4	1,2Benzenedicarboxylic acid, butyl 2ethylhexyl ester
7	19.62	3.78	C20H60O10Si10	BenzoicCyclodecasiloxane, eicosamethyl
8	22.09	3.68	C24H72O12Si12	Tetracosamethylcyclododecasiloxane
9	22.88	0.65	C27H54O4Si2	Monolinoleoylglycerol trimethylsilyl ether
10	23.32	1.06	C27H54O4Si2	1Monolinoleoylglycerol trimethylsilyl ether
11	24.25	0.53	C20H60O10Si10	Cyclodecasiloxane, eicosamethyl
12	27.14	1.52	C12H38O5Si6	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11dodecamethyl
13	28.92	0.70	C28H38O10	3Desoxo3,16dihydroxy12desoxyphorbol 3,13,16,20tetraacetate
14	29.06	2.11	C14H44O6Si7	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13tetradecamethyl

Peak no.	Rt	Area %	Molecular formula	Compound
1	5.89	1.09	C8H24O4Si4	Cyclotetrasiloxane, octamethyl
2	10.72	4.08	C14H44O6Si7	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13tetradecamethyl
3	12.95	2.41	C16H50O7Si8	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethyl
4	16.67	0.84	C18H54O9Si9	Cyclononasiloxane, octadecamethyl
5	19.42	10.06.	C19H34O2	9,12Octadecadienoicacid (Z,Z), methyl ester
6	23.21	0.75	C27H54O4Si2	Monolinoleoylglyceroltrimethylsilyl ether
8	26.84	0.94	C28H53NO5Si2	Pregnan20one,3,11dihydroxy17,21bis[(trimethylsilyl)oxy],Omethyloxim,
9	29.39	4.41	C29H50O2	dlàTocopherol
10	31.87	1.33	C42H64O2	psi.,.psi.Carotene,,1',2,2'tetrahydro1,1'dimethoxy
11	33.55	3.31	C30H42O11	9Desoxo9xihydroxy3,7,8,9,12pentaacetate Ingol

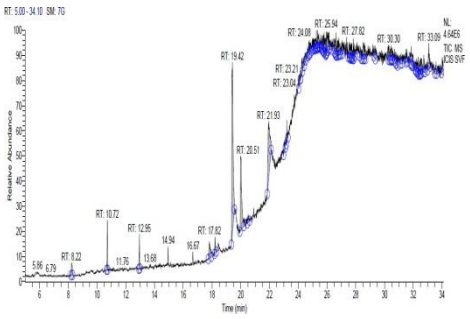


Figure no 3: GCMS chromatogram of Solanum virgianum fruit (SVF)

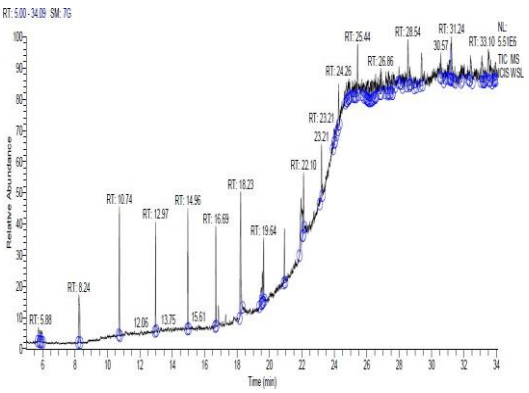


Figure no 4: GCMS chromatogram of Solanum virgianum (SVL)

Table no 4 : Retention time, Area%, Molecular formula and Major peaks of chemicals detected by GCMS of Solanum virgianum leaf extract (SVL).

Peak no.	Rt	Area%	Molecular formula	Compound
1	8.24	3.55	C10H30O5Si5	Cyclopentasiloxane decamethyl
2	10.74	3.90	C6H6O3	Levoglucosenone
3	12.97	2.85	C16H50O7Si8	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl
4	14.95	4.52	C6H6O3	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
5	16.67	1.02	C16H48O6Si7	Heptasiloxane, hexadecamethyl
6	18.23	7.40	C17H20O2	2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl)propane
7	19.64	2.22	C6H6O3	1,2,3-Benzenetriol
8	22.10	2.30	C24H72O12Si12	Tetracosamethylcyclododecasiloxane
9	23.21	2.06	C6H10O5	1,6-Anhydro-à-d-galactofuranose
10	24.26	1.84	C20H60O10Si10	Cyclodecasiloxane, eicosamethyl
11	25.44	2.58	C27H54O4Si2	1Monolinoleoylglyceroltrimethylsilyl ether
12	28.54	3.35	C26H54	Heptadecane, 9hexyl28
13	31.24	2.44	C34H52O4	Lanosta7,9(11),20(22)triene3á, 18diol, diacetate

Table no 5: FTIR Result for Physalis angulata Fruit extract (PAF)

Wavelength in cm-1	Functional groups	Name of the Functional groups
3600-3000	O-H	Alcohol
2926 , 2854	C-H	Aliphatic
1745	C=O	Carboxylic acid
1643	C=C	Arenes
1159 ,1097	C-O	Alcohols/ Phenols
715	=C-H bending	(out-of-plane bending) cis –RCH=CHR

Figure no 5: FTIR spectrum of Physalis angulata Fruit extract (SNF)

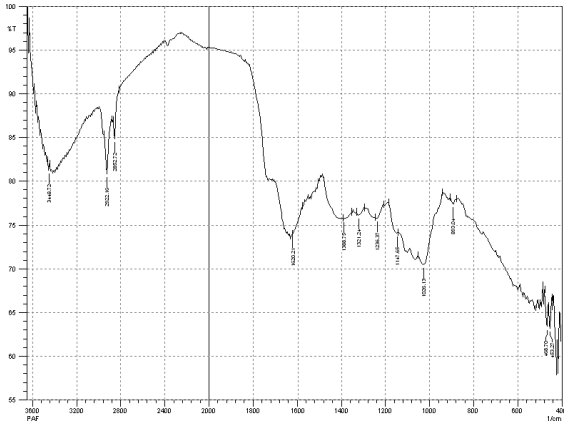


Table no 6: FTIR Result for Physalis angulata Leaf extract (PAL)

Wavelength in cm-1	Functional groups	Name of the Functional groups
3600-3000	O-H	Alcohol
2931	C-H	Aliphatic
1745	C=O	Carboxylic acid
1651	C=C	Conjugated carbonyl (may be flavone)
1159 ,1097	C-O	Alcohols/ Phenols
783	=C-H bending	(out-of-plane bending) cis – RCH=CHR

Figure no 7: FTIR spectrum of Withania somnifera Fruit extract (SVF)

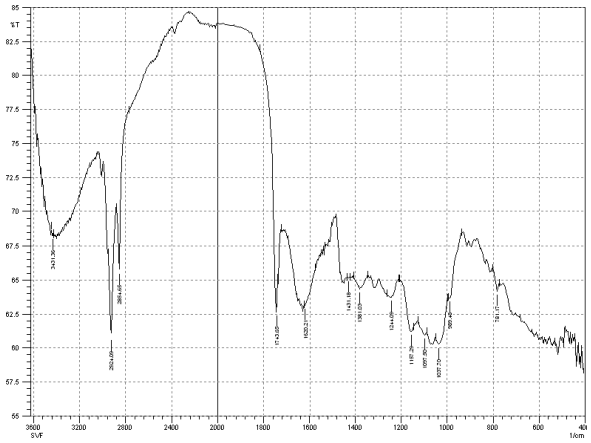


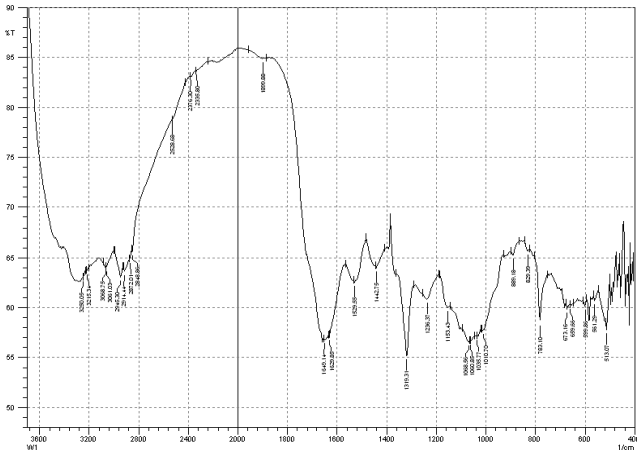
Table no7 :FTIR Result for Solanum virgianum Fruit extract (SVF)

Wavelength in cm-1	Functional groups	Name of the Functional groups
3600-3000	O-H	Alcohol
2924 , 2854	C-H	Aliphatic
2100-2270	-N=C=N,-N3,-N=C=O	Azides and ketones
1629	C=C	Arenes
1163,1103,1031	C-O	Alcohols/ Phenols
898,723	=C-H bending	(out-of-plane bending) cis – RCH=CHR

Table no8 :FTIR Result of Solanum virgianum Leaf extract(SVL)

Wavelength in cm-1	Functional groups	Name of the Functional groups
3600-3000	O-H	Alcohol
2945, 2914	C-H	Aliphatic
2240-2260	-N=C=O	Nitrile isocyanates
1629	C=C	Conjugated
1068,1060	C-O	Alcohols/ Phenols
783	=C-H bending	(out-of-plane bending) cis – RCH=CHR

Figure no 8: FTIR spectrum of Solanum virgianum Leaf extract(SVL)



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Study of Micrometry of Dog Hair of Different Breeds

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ABSTRACT

Dogs which belongs to different climatic conditions, different breeds or genetic makeup they have different structure of hair which is related to its thickness and length and other morphological characteristics. Following data is useful for the easy identification of dog breeds form the single hair follicle. Every breed has different characters with respected to its hair these different characters are related to its thickness of hair, size of medulla, size of cortex. These study is helpful for forensic investigations, in some situations of crime scenes forensic team get animal hair through these hair follicle they can detect the dog breed which is provide good clue for crime cases. In both micrometric & histological study of hair of different dog breeds it results particular identities for every dog breed and these identities will be greatly helpful for identification of different dog breed through the single hair follicle of dog.

Keywords : Micrometry, Morphology, hair follicle.

I. INTRODUCTION

When we think of dogs, we tend to think of animals that were selected for behaviour performed in a service of people. Dogs pull sledge, guard property, protects herds of sheep, guides the blind, track and retrieve game and so on. We also think of dogs in terms of breeds often try to identify to breeds. Some think of breeds as if they were ancient species separately derived from different strains of wolves, jackals or even coyotes. But breeds of dogs are most part of modern invention. Like other domesticated animals dogs may have originated as scavengers and been domesticated for use of food and fibre or use for daily routine work like protection of domesticated goats from predators, hunting of wild animals like pigs, deer ,rabbits and many more.

The dog Canis familiaris is a direct descendent of wolf, coyotes and jackals they also belongs to canine family. In other words 'dogs as we know them are domesticated wolves not only their behaviour changed domestic dogs are different in form from wolves mainly smaller and with shorter muzzles and smaller teeth.

Darwin was wrong about dogs. He thought their remarkable diversity must be reflecting interbreeding with several types of wild dogs. But DNA finding say differently. All modern dogs are descendents of wolves, thought this domestication happened twice, producing groups of dogs descended from two unique common ancestors.

Classification of Dog:-

Kingdom: -	Animalia
Phylum: -	Chordata
Sub-Phylum:-	Vertebrata
Class: -	Mammalia
Order:-	Carnivora
Family:-	Canidae
Genus :-	Canis
Species:-	familiaries

Dogs are classified as Canis familiaries under Linnaeus published in systema nature a characterization of species which include Canis species. Canis is a Latin word meaning dog (Harper) and the list include the dog like carnivores: domestic dogs, wolves, foxes and jackals. The dog was classified as Canis familiaris which means dog family or family dog .In 1982 the first edition of Mammal species of the world listed Canis familiaries under Canis lupus (Honaki.at.el)

The hair bulb is a structure of actively growing cells which eventually produce hair. Cells continually divide in the lower part of the bulb and push upwards, gradually hardening. When they reach the upper part of the bulb they arrange themselves into six cylindrical layers.

The three inner layers become the hair, made up of the cuticle, the cortex and the medulla
– although the medulla isn't always present, especially in hairs with a thinner diameter. The outer three layers become the lining of the follicle and form the inner root sheath and basement membrane, around which lie undifferentiated cells. Specific cells in the hair bulb, called melanocytes, make thepigment called melanin that gives your hair its colour.(Anka Lungu at. el.(2003)). Hair is made of the protein keratin and dead epidermal or skin cells and it grows from follicles in the dermis or inner layer of the skin. Some hair is densely packed into stiff, fibrous outgrowths that, depending on species and location on the body, become horns, fingernails, and toenails. Dogs have three types of hair: soft downy undercoat that is especially abundant in northern breeds but exists in most breeds that developed in cool or cold climates; stiffer and often longer guard hair that form a protective layer to protect undercoat and skin from harsh weather and cold water; and whiskers, those specialized hair that grow in clumps on the face. Medulla of animals is found in many shapes and this is also one of the major difference between two different animal spesies or organism which is from same species but divided into different breeds.following types of medulla's found in animal hair which is catagorised with its different shape

1. Discon= oval shape medulla is present.
2. Globular= medulla is not continuous and present in bunches.
3. Continuous= medulla looks like continuous thread like in structure.
4. Fragmental= medulla is present in fragments of irregular shapes.
5. Lattice= clump of medulla is present
6. ladder= square or ladder shape of medulla is occur.
7. Branched= medulla get divided like branching of trees.
8. Aeroform=small portions of medulla is get stick to cortex wall. medulla is not contiuous.

On the basis of medullary index and corticular index and other important micrometric characteristics we can differentiate and identify individual dog breed which can help in forensic point of view and other scientific study

II. METHODS AND MATERIAL

The present study was carried out in the department of Zoology, Modern College of Arts, Science and Commerce, Ganeshkhind, Pune-411016. The study material includes hair samples of different Dog Breeds, collecting hair samples by the method of plucking, combing, rubbing & cutting. Dog Hair including 10 different types of Dog Breeds, Which are 1) Saint Bernard 2) French Mastiff 3) English Cocker Spaniel 4) Golden Retriever 5)

Rottweiler 6) Caucasian Shepherd 7) German Shepherd 8) Pitbull 9) Chow Chow 10) Siberian Husky. The entire region of Hair follicle containing tip and root portion and shaft region was selected for Micrometric examination or study. All the samples

were stored in serially marked collecting small zip plastic bags.

Preparation:-Each Hair sample was cleaned before Micrometric examination 2-3 times by water because of that all dust and chemicals get removed from the hair. Then hair sample is deep into 30% Hydrogen peroxide (H2O2) solution for 3 hours or as per requirement of bleaching to differentiate between cortex and medulla lucid; then such hair samples are washed by water again and afterwards hair sample are deep into the 100% Ethanol or Absolute Alcohol for preservation of sample. Then hair samples are ready for micrometric and histological study by removing ethanol by water or other removing agents like Xyline, Toluene, chloroform, benzene petrol.

Mounting of Hair Strand:-Each dried hair samples were cleaned in water and mounted on microscopic slide by placing Hair samples on slide in drop of Water. A cover slip placed on hair allowing the water or medium to spread under cover slip-encasing hair. Each slide labelled by marker and examined appropriately.

III. OBSERVATION

The mounted slides were examined for morphological characteristics and micrometry. The characters of hair examined are 1) Hair Length 2) Colour 3) Tip 4) Scale Type 5) Hair Scale Height 6) Root length 7) Medulla Type 8) Hair Position and Structure 9) Shaft Diameter 10) Medulla Diameter 11) Cortex Diameter 12) Medullary index 13) Corticular index. These observations are done for 2 to 3 times. Medullary index= Maximum Diameter of Medulla /Maximum Diameter of Shaft Corticular Index=Maximum Diameter of Cortex/ Maximum Diameter of Shaft.

Morphological characteristics of Hair of Dog Breeds

No.	Dog Breed	Medullary index	Corticular index
1	Saint Bernard	3	3
2	French Mastiff		5
3	English Cocker Spaniel	3	9
4	Golden Retriever		5
5	Rottweiler		5
6	Caucasian Shepherd	3	3
7	German Shepherd		
8	Pitbull		
9	Chow Chow		5
10	Siberian Husky	3	3

Table-Scale Height of dog hair

Sr. No.	Name of Dog Breed	Scale height in (μm)
1	Saint Bernard	1.5
2	Rottweiler	2.5
3	Caucasian Shepherd	3.0
4	German Shepherd	2.0
5	Golden Retriever	2.5

Medullary index and corticular index of hair of different Dog Breeds.

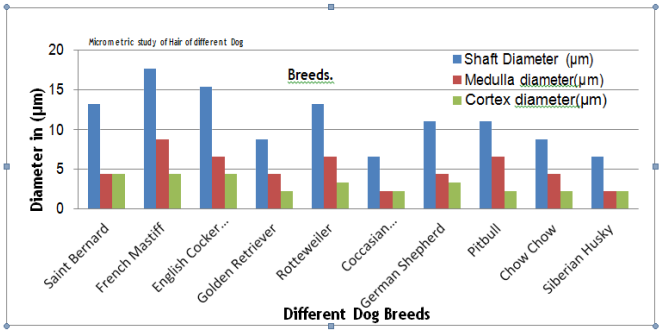
Table-Scale Height of dog hair

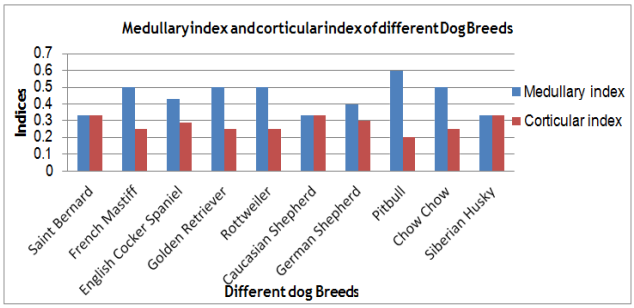
Sr. No.	Name of Dog Breed	Scale height in (μm)
1	Saint Bernard	1.5
2	Rottweiler	2.5
3	Caucasian Shepherd	3.0
4	German Shepherd	2.0
5	Golden Retriever	2.5

Sr. No.	Name of Dog Breed	Length In cm	Colour	Tip	Scale type	Root Length (µm)	Medulla type	Position & structure	Shaft Diameter (µm) at 10X	Medulla Diameter (µm) at 10X	Cortex Diameter (µm) at 10X
1	Saint Bernard	4.5	White & Brown	Thin Tapering	Irregular Wave	79.2	Amorphous less Dense Stacked	Less cure, Wavy With Slope	13.2	4.4	4.4
2	French Mastiff	1.7	Brown & White Patch	Thin Tapering	Smooth Regular Wave	132.0	Continuous Dense Stacked	Straigh	17.6	8.8	4.4
3	English Cocker Spaniel	4.5	Red with Brown	Tapering or Narrow	Smooth	121.0	Light Dense Stacked Continuous	Shiny with Slope	15.4	6.6	4.4
4	Golden Retriever	6.0	Faint Brown	Wavy, Thin, Transparent	Regular wave	Not done	Amorphous Packed like Vacuoles	Silky Straight & Curve	8.8	4.4	2.2
5	Rottweiler	4.0	Black	Wavy, Dense, Thin	Regular petal	Not done	Highly dense Stacked	Straight & Slight Curve	13.2	6.6	3.3
6	Caucasian Shepherd	6.5	Brown	Wavy transparent	Irregular Wave Mosaic	77.0	Stacked	Wavy with Slope	6.6	2.2	2.2
7	German Shepherd	3.5	Brown & Black	Thin Wavy and Transparent	Irregular Wave	132.0	Discon	Wavy With Slope	11.0	4.4	3.3
8	Pitbull	1.5	Black & White patch	Tapering	Regular Wave	99.0	Continuous	Straight & Plane	11.0	6.6	2.2
9	Chow Chow	11.0	Brownish White	Tapering Thin	Single Chevron	55.0	Fragmental & Interrupted	Shiny with Curve	8.8	4.4	2.2
10	Siberian Husky	5.0	White With Gray	Tapering	Regular Petal	77.0	Stacked & Less Dense Pigmentation	Wavy With Slope	6.6	2.2	2.2

Medullary index and corticular index of hair of different Dog Breeds.

No.	Dog Breed	Medullary index	Corticular index
1	Saint Bernard	3	3
2	French Mastiff		5
3	English Cocker Spaniel	3	9
4	Golden Retriever		5
5	Rottweiler		5
6	Caucasian Shepherd	3	3
7	German Shepherd		
8	Pitbull		
9	Chow Chow		5
10	Siberian Husky	3	3





IV. RESULT

As shown in following table

1) Saint Bernard

In the present micrometric study, Saint Bernard having 4.5 cm long hair having White and Brown colour. Hair tip is thin and tapering. Scale type is irregular wave. Root length of hair is 79.2 µm. Medulla type is amorphous, less dense stacked. Position and structure of hair is wavy with slope. Shaft diameter of hair is 13.2 µm. Medulla diameter of hair is 4.4 µm. Cortex diameter of hair is 4.4 µm. Medullary index and corticular index of hair is 0.33 and 0.33 respectively. Scale height of hair is 1.5 µm.

2) French Mastiff

Micrometric study of hair shows, French Mastiff having 1.7 cm long hair having Brown and White colour. Hair tip is thin tapering. Scale type is smooth regular wave. Root length of hair is 132.0 µm. Medulla type is continuous, dense stacked. Position and structure of hair is straight. Shaft diameter of hair is 17.6 µm. Medulla diameter of hair is 8.8 µm. Cortex diameter of hair is 4.4 µm. Medullary index and corticular index of hair is 0.5 and 0.25 respectively.

3) English Cocker Spaniel

In micrometric study of hair, English Cocker Spanial having 4.5 cm long hair having Brown or Red colour. Hair tip is tapering. Scale type is smooth. Root length of hair is 121.0 µm. Medulla type is continuous, light dense stacked. Position and structure of hair is shiny with slope. Shaft diameter, medulla diameter & cortex diameter of hair is 15.4 µm, 6.6 µm & 4.4 µm respectively. Medullary index and Corticular index of hair is 0.43 and 0.29 respectively.

4) Golden Retriever

Micrometric study of hair shows Golden Retriever having 6.0 cm long hair having faint Brown colour. Hair tip is wavy, thin, and transparent. Scale type is regular wave. Medulla type is amorphous, packed like vacuoles. Position and structure of hair is straight, silky and curve. Shaft diameter, medulla diameter and cortex diameter of hair is 8.8 µm, 4.4 µm and 2.2 µm respectively. Medullary index and corticular index of hair is 0.5 and 0.25.

5) Rottweiler

Micrometric study of hair shows Rottweiler having 4.0 cm long thick hair having Black colour. Hair tip is dense pigmented, wavy and thin. Scale type is regular petal. Medulla type is highly dense stacked. Position and structure of hair is straight and slight curve. Shaft diameter, medulla diameter and cortex diameter of hair is 13.2 μm, 6.6 μm and 3.3 μm respectively. Medullary index and corticular index of hair is 0.5 and 0.25 respectively. Scale height of hair is 2.5 μm.

6) Caucasian Shepherd

Micrometric study of hair shows Caucasian Shepherd having 6.5 cm long hair having Brown colour. Hair tip is wavy and transparent. Scale type of hair is irregular wave mosaic. Root length of hair is 77.0 μm. Medulla type is stacked. Position and structure of hair is wavy with slope. Shaft diameter, medulla diameter and cortex diameter of hair is 6.6 μm, 2.2 μm and 2.2 μm respectively. Medullary index and corticular index of hair is 0.33 and 0.33 respectively. Scale height of hair is 3.0 μm.

7) German Shepherd

Micrometric study of hair shows German Shepherd having 3.5 cm long hair having Brown and Black colour. Hair tip is thin, wavy and transparent. Scale type is irregular wave. Root length of hair is 132.0 μm. Medulla type of hair is discon. Position and structure of hair is wavy with slop. Shaft diameter, medulla diameter and cortex diameter of hair is 11.0 μm, 4.4 μm and 3.3 μm respectively. Medullary index and corticular index of hair is 0.4 and 0.3 respectively. Scale height of hair is 2.0 μm.

8) Pitbull

Micrometric study of hair shows that Pitbull having 1.5 cm long hair having Black colour and White patch on chest. Hair tip is tapering. Scale type is regular wave. Root length of hair is 99.0 μm. Medulla type is continuous. Position and structure of hair is straight and plane. Shaft diameter, medulla diameter and cortex diameter of hair is 11.0 μm, 6.6 μm and 2.2 μm respectively. Medullary index and corticular index of hair is 0.6 and 0.2 respectively.

9) Chow Chow

Micrometric study of hair shows that Chow Chow having 11.0 cm having faint Brownish colour. Hair tip is thin tapering. Scale type is single Chevron. Root length of hair is 55.0 μm. Medulla type is fragmented and interrupted. Position and structure of hair is shiny with curve. Shaft diameter, medulla diameter and cortex diameter of hair is 8.8 μm, 4.4 μm and 2.2 μm respectively. Medullary index and corticular index of hair is 0.5 and 0.25 respectively.

10) Siberian Husky

Micrometric study of hair shows that Siberian Husky having 5.0 cm long hair having White and Gray colour. Hair tip is tapering. Scale type is Regular petal. Root length of hair is 77.0 μm. Medulla type is stacked with less dense pigmentation. Position and structure of hair is wavy with slope. Shaft diameter, medulla diameter and cortex diameter of hair is 6.6 μm, 2.2 μm and 2.2 μm respectively. Medullary index and corticular index of hair is 0.33 and 0.33 respectively.

V. DISCUSSION OR CONCLUSION

The present study observe that the medullary index of Dog Breed ranges between 0.5 to 0.43 and the corticular index of Dog Breed ranges between 0.2 to 0.33.Study was conducted by the microscopic observation including Colour, Tip pattern, Scale type, Medulla type, Root length of hair, position and structure of hair, Scale height of hair, and also shaft diameter, medulla diameter, & cortex diameter and other micrometric characteristics. In colours of Dog Breed hair is Brown, White, Black and Mixed. According to Dog Breeds specification-hair position and structure of hair also differs among the breeds i.e. the diameter of Medulla and cortex and the other important microscopic characteristics are useful parameters to differentiate between Breeds. So this studies helpful in Investigation of Dog Breeds.

Following data is useful for the easy identification of dog breeds form the single hair follicle. Every breed has different characters with respected to its hair these different characters are related to its thickness of hair, size of medulla, size of cortex.

This study is helpful for forensic investigations, in some situations of crime scenes forensic team get animal hair through these hair follicle they can detect the dog breed which is provide good clue for crime cases.

This study is helpful for restriction to animal poaching from wild as well as domestic level, in the case of dogs many breeds are banned or illegal e.g. Fighter dog.
If these type of studies get occur widely and add as data in the software format then it will be the good key for identification and benefits of these key is we need only single hair follicle of organism, it's much better than capturing and observing to organism.

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Phytochemical and Taxonomical Studies In Celosia Argentia L. (Amaranthaceae)

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ABSTRACT

In recent times, focus on plant research has increased all over the world and a large number of evidence has collected to show immense potential of medicinal plants used in various traditional systems.Over the last few years, researchers have aimed at identifying and validating plant derived substances for the treatment of various diseases. Similarly it has been already proved that the correct identification and authentication of taxa is most important in plants science. The Celosia argentia L. has enormous traditional uses against various diseases. The present review aims to Phytochemical, Morphological and anatomical review of Celosia argentia L.In the present work phytochemistry and taxonomical enumeration of Celosia argentia L. is carried out.

Keywords: Phytochemistry, Morphology, Anatomy, Celosia argentia L., Amaranthaceae.

I. INTRODUCTION

Celosia argentia L. (Family: Amaranthaceae) is a common weed plant in India, profoundly used as Ayurvedic medicine, and used as medicine on Musculoskeletal disorder, calculii, burning and painful urination, dysuria etc.

Celosia argentia L. is an annual plant commonly known as plumed cockscomb or M fungu, also known as “Sitivara, Vitunnaka, Suni shannaka, Indivara” in Sanskrit and Survali, Safed murga in Hindi language. The plant is especially famous for its attractive bicolor flowers which are used in the treatment of skin disorders and body odaur.In recent trend the re-emerging connection between plants and human health especially depends on their antioxidant activities that may delay or reduce the hazardous effects of free radicals. The major causative for the generation of free radicals in food, drug and living systems is the oxidation process. (Pourmorad et. al.2006)

II. MATERIALS AND METHODS

A. Collection and Identification

Celosia argentia L. (Family: Amaranthaceae) was collected from Aurangabad region of the Maharashtra. The survey of the study area was conducted during 2016-2017. Identification of the collected specimens was made with the help of standard Floras (Hooker, 1872-1897; Naik, 1998).Herbarium specimens are deposited in the Department of Botany, Shri Chhatrapati Shivaji College, Omerga. Library and Herbarium of Botanical Survey of India, Pune was consulted for review of literature and also for identification of the specimen.

B. Histochemical screening

Histochemical screening was performed as per standard methods given in by (Gangulee et. al. 1959), Evans (1996), Gibbs (1974), Harborne (1973), Peach & Tracey (1955), Rastogi & Mehrotra (1999) and Johansen (1940).

C. Anatomy with illustration

The T. S. of Root, Stem and leaf were taken by fine blade and the sections were stained by the method of double staining, and the illustration of all sections and habit of plant were made by 0.2, 0.4 and 0.6 tip drawing pen on A4 sized drawing paper.

D. Qualitative analysis

Test for qualitative analysis of starch, protein, fat, tannin, saponin, glycosides and alkaloids was taken and confirm the presence or absence of compounds in plant parts i e. Root, Stem and Leaves. (See : Histochemical screening)

E. Quantitative analysis

Total Ash values, Moisture contents, Sugar in root, alkaloids, Nitrogen, Potassium, Calcium, Phosphorus, Crude protein, free amino acid were calculated in percentage. (See : Histochemical screening.

III. RESULT AND DISCUSSION

Celosia argentea L. (Family- Amaranthaceae) is commonly known as plumed cockscomb or silver cocks comb in this region, flowering and fruits - September to March.

Non recorded uses: seeds used in kidney stone and fever and Recorded uses: The seeds used for treatment of jaundice, gonorrhea, wound and fever in this region.

A. Morphology

Celosia argentea is an erect herb with 0.4 – 02 m hight bearing many ascending branches, leaves lanceolate with the excurrent, glabrous lamina and slender petiole. The primary steam having width 2 - 0.3 ± 0.1cm. Inflorences is dense spike.

Many flowers in spike 2.5-20 ± 1.5-2.2cm., periandh segement 6 -10mm narrowly elliptic oblong acute to rather baunt, shorately mucronate with the excurreent midrib with 2-4 lateral nerves ascending more than half way up each segement margin highline filaments very delicate free part subquallinj the staminal sheath sinuses rounded with number with very minute ,intermediate teeth, anther and filament creamy to magenta stigma 2-3 very short and filiform style 5-7mm long ovary 4-8 ovulate capsule 3-4mm ,avoid to almost globular c.1.25-1.5mm lenticular black shin.

B. Micromorphology

1) T. S. of Root

T.S. of Root shows uppermost layer is cork which is thick protective covering to the root cells with having diameter about 3-5µm. The cortical zone of T.S. of root composed of irregular cells which having measured about 5-6 x 6-8 µm. After cortex there is presence of stele the, stele is composed of vascular strand. The vascular strand is composed of phloem & xylem elements. The xylem elements shows diameter about 4-5 x 4.5-5µm. was as phloem parenchyma shows various phloem elements with ranging about 1.5-2 x 2.5-3µm. in diameter.The pith is present at the center of stele, pith is composed of 5-6 layers of parenchymatous cells & that cells were measured about 4-5 x 4.5-5.5µm.

2) T. S. of Stem

Stem shows uppermost layer is of epidermis, which is composed of thick walled compactly arranged barrel shaped cells. The epidermal cells ranging about 3.4 x 2.5-3µm in diameter. The epidermis is followed by cortex. The Cortes is composed of 4-7 layers of cells the critical cells measured about 2.5-3 x 3-3.5µm. The cortex is followed by stele. The stele were delimiting by endodermis which is single layered compactly arranged cells, the endodermis is followed by 2 to3 layers of pericycle. The pericycle is composed of phloem parenchymatous like cells. Below to pericycle there are patches of xylem elements surrounded by phloem parenchyma. The phloem parenchyma measured about 1-1.5 x 1.5-2 µm and the xylem elements were measured about 3-5 x 5-6 µm. The pith is present at the center of stele which is composed of 4-5 layers of pith parenchyma. The pith parenchyma measured about 4-5x5.5-6µm.

3) T. S. of Leaf

The T.S of leaf shows bilayer of epidermis i.e. - upper epidermis & lower epidermis. The upper and lower epidermis is composed of compactly arranged thick walled cells & both of epidermis is covered with cuticle. The upper epidermal cells were measured about 4-5x1.5-5µm. Palisade cells were measured about 2-3 x 8-12µm.

The palisade cells were rich in chlorophyll. The spongy parenchyma is present is between two palisade layers which is also rich in chlorophyll. The vascular strand presents at the center of T.S which delimiting by bundles sheath cells. The bundle sheath cells were surrounded to phloem xylem elements.

The xylem elements are of two types' protoxylem & metaxylem. The metaxylem measured about 4-5 x 5-6µm & protoxylem measured about 1.5-1.7 x 1.8-2µm. The xylem elements surrounded by phloem parenchyma which is measuring about 2- 3 x 3-3.5µm.

4) Trichome and Stomata

Celosia argentia shows the uniserate multicellular type of trichome present rarely on leaf margin, the Tetracytic type of stomata found in Celosia which is measured about 5-6 x3-4 µm.

C. Qualitative analysis

Root gave the positive test for the starch protein, fat, glycosides of alkaloid while negative test for tannin.

Stem reveals presence of the starch, protein, fat, tannin and saponin in cortical as well as in pith parenchyma.

Alkaloids in hypodermal collenchymatous while fats in pith parenchyma. The fresh leaf of T.S. sections shows the starch, protein, fat, tannin, alkaloids and saponin in mesophyll

Celosia argentea				
Sr. No.	**Test**	**Root**	**Stem**	**Leaf**
1	Starch	+	+	+
2	Protein	+	+	+
3	Fat	+	+	+
4	Tannin	-	+	+
5	Saponin	+	+	+
6	Glycoside	+	+	+
7	Alkaloids	+	+	+

Quantitative analysis

Phytochemical investigation

Total sugars

Total sugar content in root 2.1%, reducing sugar is found in 1.92% and non reducing sugar is 0.18%, stem 1.8%, reducing sugar is found in 0.88% and non reducing sugar is 0.92%, in leaf 2.9%, reducing sugar is found in 1.6% and non reducing sugar is 1.3%

Total alkaloids

Total alkaloids in root is found in 11.12%, in stem 8.2% and leaf 9.8% is found

Nitrogen

Amount of nitrogen in root 2.1%, stem 3.2% and in leaf 5.3% is found

Potassium

Amount of potassium in root is 0.191%, stem 6.431% and in leaf 0.319% is found

Calcium

Amount of calcium in root 0.30%, stem 0.21% and in leaf 3.1% is found

Phosphorus

Amount of Phosphorus in root 2.1%, stem 7.8% and in leaf 9.1% is found

Crude protein

Amount of Crude protein in root 19.2%, stem 18.7% and in leaf 22.3% is found

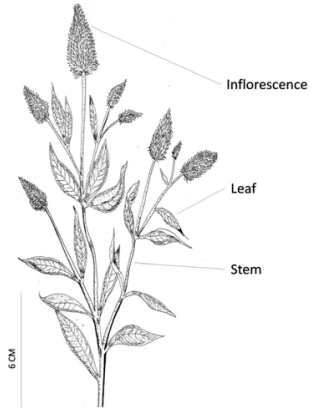
Total free amino acid

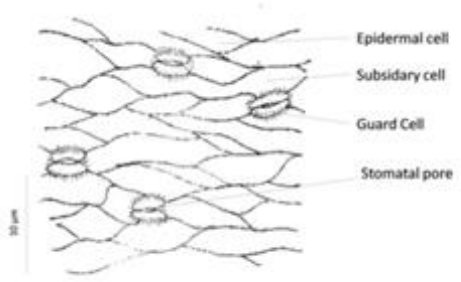
Amount of Total free amino acid in root 0.2%, stem 0.3% and in leaf 2.1% is found

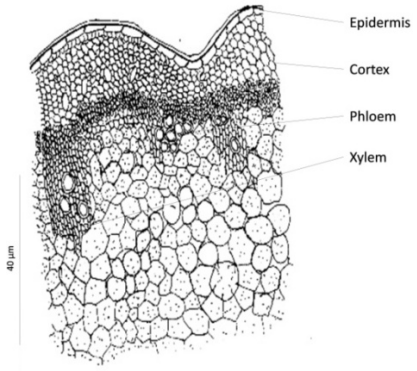
Physiochemical investigation

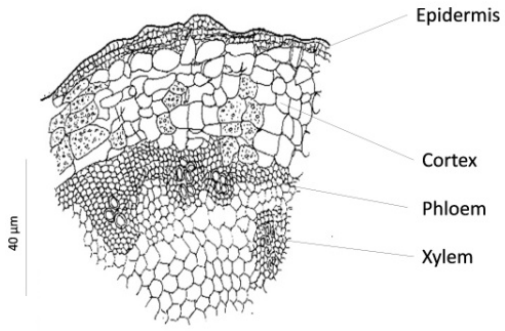
Table.02: Physiochemical investigation

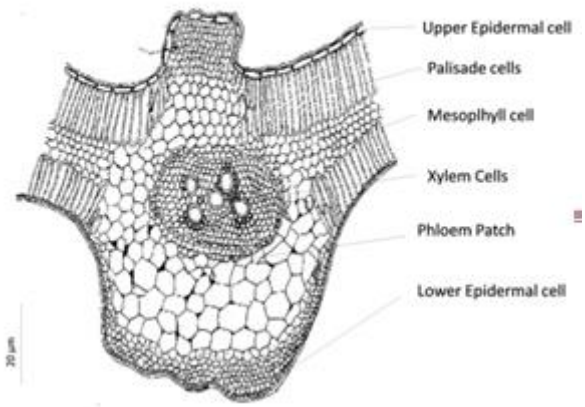
Sr. No.	Parameter	Percentage of content in Plant Part		
		Root	Stem	Leaf
1.	Total ash	18.1%	20.1%	11.0%
2.	Water insoluble ash	15.0%	13.2%	10.1%
3.	Water soluble ash	03.1%	06.9%	00.9%
4.	Acid soluble ash	16.0%	17.0%	08.0%
5.	Acid in soluble ash	02.1%	03.1%	03.0%
6.	Moisture content	05.7%	06.1%	06.8%
7	Total sugar	02.1%	01.8%	02.9%
8	Reducing sugar	1.92%	0.88%	01.6%
9	Non reducing sugar	0.18%	0.92%	01.3%
10	Total alkaloids	11.12 %	08.2%	09.8%
11	Nitrogen	02.1%	03.2%	05.3%
12	Potassium	0.191 %	0.431 %	0.319%
13	Calcium	0.30%	00.21 %	03.1%
14	Phosphorous	02.1%	07.8%	09.1%
15	Crude protein	19.2%	18.7%	22.3%
16	Total free Amino acid	00.2%	00.3%	02.1%











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Micro-Morphological Studies In Some Species of Dichanthium Willemet From Maharashtra, India

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ABSTRACT

The present study was carried out to determine the micro-morphology of some species of Dichanthium Willemet (Poaceae) from Maharashtra. Species of Dichanthium were collected from the different localities of Maharashtra for detailed micro-morphological study. Epidermal studies pertains to epidermal cell size, epidermal frequency, Stomatal frequency and index, presence and absence of micro-hairs, papillae, its frequency and types of silica bodies of species of Dichanthium. All the 7 studied members show the variation in the types and arrangement of the epidermal cells of leaf.

Keywords : Micro-morphology, Stomatal frequency and index, micro-hairs, papillae, prickles, silica bodies, Dichanthium, Maharashtra.

I. INTRODUCTION

Grasses belong to the family Poaceae consist of approximately 10,000 species and 785 genera (Yanis et al., 2010; The plant list, 2010; Hilu, 2006). The grass family is one of the largest of all plant families. Poaceae is the fifth most species rich flowering plant family (Tim, 1999). It ranged among the top five families of flowering plants in terms of the number of species, but they are clearly the most abundant and important family of the earth's flora (Campbell, 2014). Grasses are distributed worldwide and exhibited great variations in size and shape. Before the later part of the 19th century, taxonomists were confined to the use of the features of reproductive organs as floral characters were considered to provide the most valuable characters to taxonomic affinities (Nwokeocha, 1996). Leaf epidermal studies have proved to be very important in providing information of taxonomic importance. Leaf epidermis study provides valuable data regarding the identification of grasses and is recognized as a source of useful taxonomic characters, because of variations in leaf characters that are taxonomically useful (Barkworth, 1981). The leaf is the most widely used in plant taxonomy (Stace, 1984). Strivastava (1978) described the leaf epidermis as the second most important character after cytology for solving taxonomic problems. Metcalfe (1960) has described comprehensive general account of anatomy and micro- morphology for Poaceae members; also leaf anatomy for tribe Eragrosteae was studied in particular by Renvoize (1983). Earlier, Significance of microhairs as known from the work of Amarshinghe and Watson (1990). The present paper on micro–morphological studies of leaf epidermis is useful in evaluation of the patterns of anatomical variations in epidermis for species identification, classification and also in establishing the taxonomic relationships between the seven species of Dichanthium.

II. MATERIALS AND METHODS

Species of Dichanthium were collected from the different localities of Maharashtra for detailed micro-

morphological study. Herbarium specimens are deposited in YCCSK herbarium (Department of Botany, Department of Botany, Yashwantrao Chavan College of Science, Karad, Dist. Satara, Maharashtra, India). For micro-morphological study, the fresh and dried leaves were used throughout preparation. The leaves were boiled in 1:5 conc. H2SO4 with few crystal of Potassium Dichromate in test tube. After washing with Distilled Water the peels were made by scraping pieces of treated leaves with the help of safety razor blade; the samples were stained with saffranin andmounted in glycerine. Individual cells were identified and measured by micrometer. 20-25; peels were made from each species from several dozen of leaves. All peels were examined and the representative areas were photographed using Olympus research microscope, with ×40 objective andmeasurements of different cells are taken.

III. RESULT

The species wise details of the epidermal complex and epidermal ornamentation are as under:

1. Dichanthium annulatum (Forssk.) Stapf

The amphistomatic type of leaf is present in D. annulatum. Stomata of upper and lower epidermis usually in a single row interrupted by the inter-stomatal or epidermal cells, unequal in length to the inter-stomatal cells, subsidiary cells usually with elongated concave ends towards stomata.

A. Adaxial Surface :

Narrowly oblong shaped long cells were observed in upper surface of D. annulatum with approx width 140 × 20 µm, the long cells with regularly sinuous walled, sparsely papillate, contiguous with single papillae on each cell. The exodermic small papillae have 15 × 20 µm in diameter. The short cells were absent from inter-coastal zone and it's observed in Coastal zone and measured about 5 × 20 µm. The Prickle hairs were measured approx 10 × 20 µm. Silica bodies Dumbbell Long Shank shaped with 23 × 15 µm. Micro-hairs usually bicellular type, slender with the apical cell 35 µm which is shorter than the basal cell 40 µm in length. Stomata from upper surface were measured about 35 × 20 µm. The inter- stomatal cell is irregularly present and having 80 × 25 µm. The epidermal cells were measured about 50 × 30 µm diameter.

B. Abaxial Surface:

Narrowly oblong shaped long cells were observed in lower surface of D. annulatum with approx width 220 × 12 µm, the long cells with regularly sinuous walled, non papillate. The short cells were absent from coastal zone while observed in inter-coastal zone and measured about 5 × 7 µm. The Prickle hairs were measured approx 41 µm in length. Silica bodies Dumbbell Short Shank shaped with 26 × 12 µm. Micro-hairs usually bicellular type with the slender apical cell having 30 µm in length which is shorter than the basal cell. The basal cell was measured about 50 µm in length. Stomata were measured about 30 × 20 µm. The inter-stomatal cell is regularly present between two adjacent stomata with 40 × 20 µm. The epidermal cells were measured about 50 × 30 µm in diameter.

2. Dichanthium armatum (Hook.f.) Blatt. & McCann.

Amphistomatic type of leaf is present in D. armatum. Stomata of upper and lower epi- dermis usually in a single row interrupted by the inter-stomatal or epidermal cells, unequal in length to the inter-stomatal cells, subsidiary cells usually with elongated concave ends towards stomata.

A. Adaxial Surface :

Narrowly oblong shaped long cells were observed in upper surface of D. armatum with approx width 70 × 10 µm in length, the long cells with regularly sinuous walled, sparsely papillate. Long cells were interrupted by Short cells. The small exodermic papillae present on long cell, having diameters 15 × 10 µm. Long cell

having more than 5 papillae on each long cell. The short cells were present in coastal zone and it's also observed in inter-coastal and measured about 7.5 × 7.5 µm. The Prickle hairs were measured approx ×µm length. Silica bodies with Dumbbell Nodular shaped measured 15 × 7.5 µm. The Micro-hairs usually bicellular type having slender apical cell and broad basal cell with 20 µm in length. Stomata were measures about 20 × 15 µm from upper surface. The inter-stomatal cell is irregularly present with 50 × 20 µm. The epidermal cells were measured about 85 × 30 µm diameter.

B. Abaxial Surface:

Narrowly oblong shaped long cells were observed in lower surface of D. armatum with approx 120 × 10 µm in measurement. The long cells with regularly sinuous walled and sparsely papillate. More than 5 exodermic papillae with 5 × 5 µm in diameter present on each Long cell. The long cells were interrupted by Short cells. The short cells were present in intercostals zone and it's also observed in Coastal zone which is measured about 5 × 10 µm. Prickle hairs were measured approx 55 µm in length. Silica bodies with Regular complex Dumbbell shaped having 15 × 10 µm in measurement. Micro-hairs usually bicellular with slender apical cell 30 µm and globular basal cell 30 µm in length. Stomata were measures about 27 × 22 µm. The inter-stomatal cell is irregularly present with 50 × 25 µm. The epidermal cells were measured about 120 × 20 µm diameter.

3. Dichanthium caricosum (L.) A Camus

The amphistomatic type of leaf is present in D. caricosum. Stomata of upper and lower epidermis usually in a single row interrupted by the inter-stomatal or epidermal cells, unequal in length to the inter-stomatal cells, subsidiary cells usually with elongated concave ends towards stomata.

A. Adaxial Surface :

Broadly oblong shaped long cells was observed in upper surface of D. caricosum with approx length 100 × 10 µm, the long cells with regularly sinuous walled, non papillate. Long cells were interrupted by Short cells. The short cells were present in coastal zone and it's also observed in inter-coastal zone which measured about 5 × 12 µm. The Prickle hair was measured approx 30 µm. Silica bodies Dumbbell short shank shaped with 21 × 15 µm. Micro-hairs usually bicellular type having slender apical cell measured about 30 µm and basal cell with 20 µm in length. Stomata were measures about 27.5 × 15 µm. The inter-stomatal cell is regularly present between two adjacent Stomata with 60 × 23 µm. The epidermal cells were measured about 55 × 30 µm diameter.

B. Abaxial Surface:

Narrowly oblong shaped long cells were observed in lower surface of D. caricosum with measured about 70 × 12.5 µm. The long cells with regularly sinuous walled, non papillate, which is interrupted by Short cells. The short cells were present in coastal zone and it's also observed in inter-coastal zone and measured about 5 × 10 µm. Prickle hairs were measured approx 45 µm. Silica bodies with Dumbbell short shank shaped with 27 × 14 µm. The Micro-hairs usually bicellular type having apical slender cell having 15 µm in length and basal cell with 20 µm in length. Stomata were measures about 27 × 22 µm. The inter-stomatal cell is regularly present between two adjacent Stomata with 59 × 24 µm. The epidermal cells were measured about 55 × 15 µm diameter.

4. Dichanthium oliganthum (Hochst. ex Steud.) Cope

The hypostomatic type of leaf is present in D. oliganthum. Stomata of lower epidermis usually in a single row interrupted by the inter-stomatal cells, unequal in length to the inter-stomatal cells, subsidiary cells usually with elongated concave ends towards stomata.

A. Adaxial Surface :

Broadly oblong shaped long cells was observed in upper surface of D. oliganthum with 110 × 20 µm in measurement, the long cells with regularly sinuous walled, non papillate and interrupted by Short cells. The short cells were present in coastal zone and it's also observed in inter-coastal zone and measured about 10 × 13 µm. The Prickle hairs were measured approx 130 µm. Silica bodies Dumbbell short shank shaped with 20 × 15 µm. The Micro-hairs usually unicellular type, slender with 90 µm in length. Stomata and inter-stomatal cells were absent from upper surface of D. oliganthum. The epidermal cells were measured about 70 x 40 µm diameter.

B. Abaxial Surface:

Narrowly oblong shaped long cells were observed on lower surface of D. oliganthum with 90 × 10 µm in measurement, the long cells with regularly sinuous walled, sparsely papillate and interrupted by Short cells. The exodermic small papillae having diameter 7 × 7 µm. Each long cell with more than 5 papillae. The short cells were present in coastal zone and it's also observed in inter-coastal zone and measured about 5 × 16 µm. Prickle hairs were measured approx 180 µm. The Silica bodies with Dumbbell short shank shaped structure in with 16 × 10 µm. The Micro-hairs usually slender and bicellular type cells which having 25 µm in length. Stomata were measures about 30 × 20 µm. The inter- stomatal cell is regularly present between two adjacent Stomata with 40 × 25 µm. The epidermal cells were measured about 50 × 20 µm diameter.

5. Dichanthium panchganiense Blatt. & McCann.

The amphistomatic type of leaf is present in D. panchganiense. Stomata of lower and upper epidermis usually in a single row with irregularly interrupted by the inter-stomatal cells which is unequal in length to the stomatal cell. Subsidiary cells usually having elongated concave ends towards stomata.

A. Adaxial Surface :

Broadly oblong shaped long cells was observed in upper surface of D. panchganiense with approx 70 × 20 µm, the long cells with regularly sinuous walled, sparsely papillate, interrupted by Short cells. The exodermic papillae 2.5 × 3 µm, with more than 4 papillae on each long cell. The short cells were present in coastal zone and it's also observed in inter-coastal zone and measured about 5 × 6 µm. Prickle hairs were measured approx 27.5 µm. Silica bodies Dumbbell nodular shaped with 13 × 10 µm. Micro-hair usually bicellular type, broad apical cell having 5 µm in length and basal cell with 15 µm in length. Stomata were measures about 25 × 15 µm. The inter-stomatal cell is regularly present between each adjacent Stomata with 22 × 15 µm. The epidermal cells were measured about 50 × 15 µm diameter

B. Abaxial Surface:

Narrowly oblong shaped long cells were observed on lower surface of D. panchganiense with approx width 125 × 20 µm and interrupted by Short cells. The long cells with regularly sinuous walled, sparsely papillate. The exodermic papillae having 7 × 10 µm in diameter, more than 5 papillae is present on each long cell. The short cells were present in coastal zone and it's also observed in inter-coastal zone and measured about 5 × 10 µm. Prickle hairs were measured approx 25 µm. Silica bodies with Dumbbell nodular shaped measured about 22 × 7.5 µm. Micro-hairs usually bicellular type with slender cells having 15 µm in length. Stomata were measures about 22.5 × 16 µm. The inter-stomatal cell is regularly present between two adjacent Stomata with 45 × 15.2 µm. The epidermal cells were measured about 50 × 15 µm diameter.

6. Dichanthium pertusum (L.) Clayton

The amphistomatic type of leaf is present in D. pertusum. Stomata of lower and upper epi- dermis usually in a single row with regularly interrupted by

the inter-stomatal cells which is unequal in length to the stomatal cell, Subsidiary cells usually having elongated concave ends towards stomata.

A. Adaxial Surface :

Broadly oblong shaped long cells was observed in upper surface of D. pertusum with approximately measured about 120 × 10 µm, the long cells with regularly sinuous walled, non papillate and irregularly interrupted by Short cells. The short cells were present in coastal zone and it's also observed in inter-coastal zone and measured about 5 × 10 µm. Prickle hairs were measured approximately 70 µm. Silica bodies Dumbbell short shank
shaped measured about 20 × 12 µm. Micro-hair usually bicellular type, apical cell having 5 µm in length and basal cell with 20 µm in length. Stomata were measures about 25 × 20 µm. The inter-stomatal cell is regularly present between two adjacent Stomata with 40 × 20 µm. The epidermal cells were measured about 40 × 30 µm diameter

B. Abaxial Surface:

Narrowly oblong shaped long cells was observed in lower surface of D. pertusum with approximately measured about 85 × 10 µm, the long cells with regularly sinuous walled, non papillate and irregularly interrupted by Short cells. The short cells were present in coastal zone of lower surface, measured about 5 × 15µm. Prickle hair was measured approximately 35 µm. Silica bodies cross thik shank shaped and measured about 25 × 15 µm. Micro-hair usually bicellular type, broad apical cell having 17 µm in length and basal cell with 23 µm in length. Stomata were measures about 30 × 20 µm. The inter-stomatal cell is irregularly present between two adjacent Stomata with 70 × 25 µm. The epidermal cells were measured about 40 × 15 µm diameter.

7. Dichanthium tuberculatum (Hack.) Cope

The amphistomatic type of leaf is present in D. tuberculatum. Stomata of lower and upper epidermis usually in a single row with regularly interrupted by the inter-stomatal cells which is unequal in length to the stomatal cell, Subsidiary cells usually having elongated concave ends towards stomata.

A. Adaxial Surface :

Broadly oblong shaped long cells was observed in upper surface of D. tuberculatum with approximately measured about 90 × 15 µm, the long cells with regularly sinuous walled, non papillate and regularly interrupted by Short cells. The short cells were present in coastal zone and measured about 5 × 15 µm. Prickle hairs were measured approximately 51 µm. Silica bodies cross thick shank shaped which is measured about 20 × 15 µm. Micro-hair usually bicellular type, apical cell having 15 µm in length and basal cell with 35 µm in length. Stomata were measures about 30 × 20 µm. The inter-stomatal cell is regularly present between two adjacent Stomata with 50 × 25 µm. The epidermal cells were measured about 40 × 30 µm diameter.

B. Abaxial Surface:

Narrowly oblong shaped long cells was observed in lower surface with approximately measured about 120 × 10 µm, the long cells with regularly sinuous walled, non papillate and regularly interrupted by Short cells. The short cells were present in coastal as well as in inter-coastal zone of lower surface, measured about 11 × 10 µm. Prickle hair was measured approximately 48 µm. Silica bodies Dumbbell short shank shaped and measured about 20 × 10 µm. Micro-hair usually bicellular type, broad apical cell having 25 µm in length and basal cell with 35 µm in length. Stomata were measures about 30 × 23 µm. The inter-stomatal cell is irregularly present between two adjacent Stomata with 49 × 20 µm. The epidermal cells were measured about 75 × 15 µm diameter.

1) **Table 1: Comparative micro-morphology of five members of *Dichanthium* species**

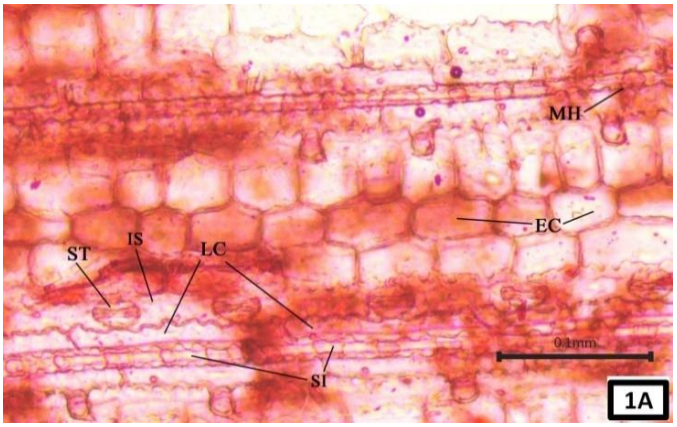
SPECIES NAME	Leaf Surface	Long cell (µm)	Short cell (µm)	Stomata (µm)	Micro hair (µm)	Prickle hairs (µm)	Silica Bodies (µm)	Inter-Stomatal Cell (µm)	Epidermal cell (µm)	Papillae (µm)
Dichanthium annulatum	US	140 × 20 B1	5 × 20	35 × 20	70	20	23 × 15 A2	80 × 25 B1	50 × 30	15 × 20
	LS	220 × 12 B2	5 × 7	30 × 20	80	41	26 × 12 A1	30 × 20 B1	40 × 20	Absent
Dichanthium armatum	US	70 × 10 B2	7.5 × 7.5	20 × 15	20	59	15 × 7.5 A3	50 × 20 B1	85 × 30	15 × 20
	LS	120 × 10 B2	5 × 10	27 × 22	60	55	15 × 10 A4	50 × 25 B1	120 × 20	5 × 5
Dichanthium caricosum	US	100 × 10 B2	5 × 12	27.5 × 15	50	30	21 × 15 A1	60 × 23 B1	55 × 25	Absent
	LS	70 × 12.5	5 × 10	30 × 20	35	45	27 × 14 A1	59 × 24 B1	55 × 15	Absent
		B2								
Dichanthium oliganthum	US	110 × 20 B2	10 × 13	Absent	90	130	20 × 15 A1	Absent	70 × 40	Absent
	LS	90 × 10 B2	5 × 16	30 × 20	25	180	16 × 10 A1	40 × 25 B1	50 × 20	7 × 7
Dichanthium panchganiense	US	70 × 20 B1	5 × 6	25 × 15	20	27.5	13 × 10 A3	22 × 15 B1	60 × 15	2.5 × 2.5
	LS	125 × 20 B2	5 × 10	22.5 × 16	15	25	22 × 7.5 A3	45 × 15.2 B1	50 × 15	7 × 10
Dichanthium pertusum	US	85 × 10 B2	5 × 10	25 × 20	25	70	20 × 12 A1	40 × 20 B1	40 × 30	Absent
	LS	120 × 10 B2	5 × 10	30 × 20	40	35	25 × 15 A5	70 × 25 B1	40 × 15	Absent
Dichanthium tuberculatum	US	90 × 15 B2	5 × 15	30 × 25	50	51	20 × 15 A5	50 × 25 B1	40 × 30	Absent
	LS	120 × 10 B2	11 × 10	30 × 23	60	48	20 × 10 A1	49 × 20 B1	75 × 15	Absent

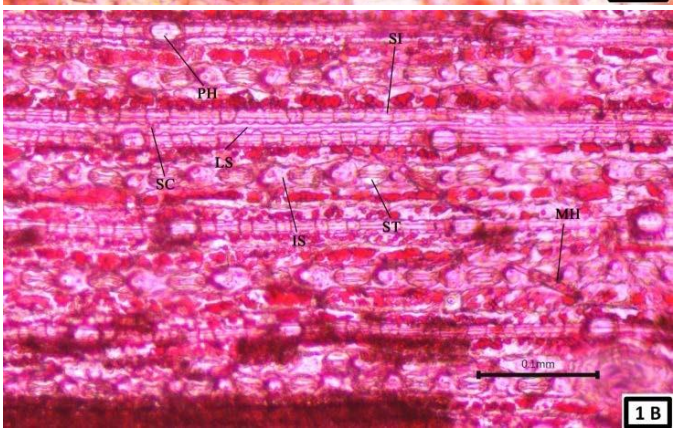
US: Upper Surface, LS: Lower Surface,
A1: Dumbbell Short Shank, A2: Dumbbell Long Shank, A3: Dumbbell Nodular, A4: Regular Complex Dumbbell, A5: Cross thick shank

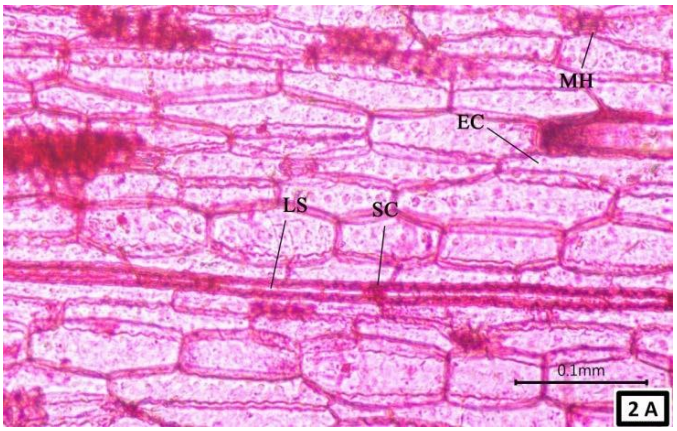
B1: Elongated concave ends, B2: Elongated Sinuous

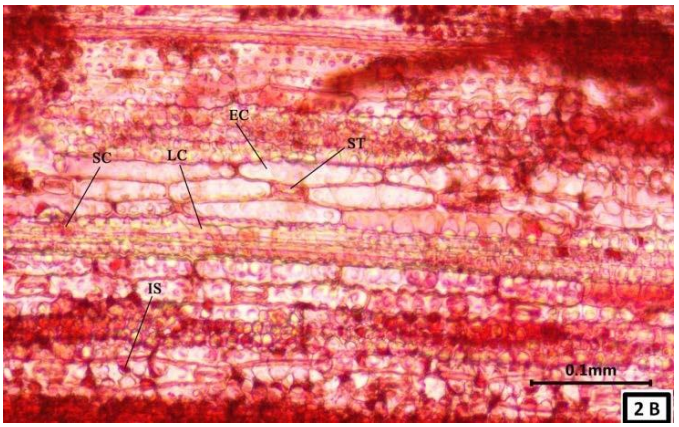
IV. DISCUSSIONS

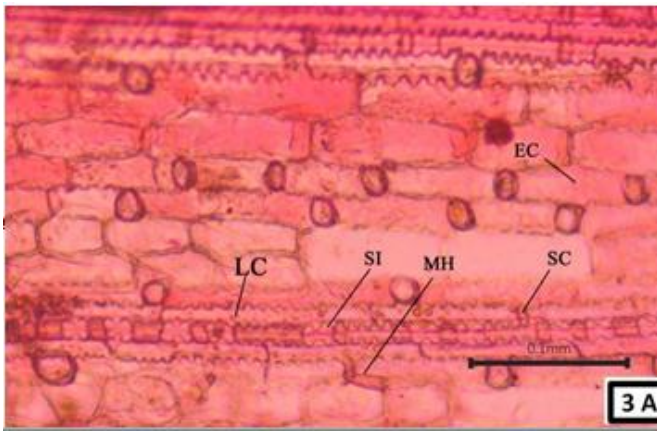
Interrelationships of the family Poaceae have been described by Watson et al. (1985) and Clayton (1981). The genus Dichanthium is quite distinct in having a high ratio of basal/distal cell length ratio of bicellular microhairs, and 4-5 long short cells in a row (Faruqi, 1961). Micromorphological features of the leaf surfaces of seven Dichanthium species have a significant value in identification. The long cells were of rectangular and sinuous type in all the members. The short cells mostly alternating with long cells are found in all members. The costal zone shows presence of short cells alternating with silica cells in all species. The measured size of interstomatal cells was variable and were absent in upper surface of D. oliganthum. Stomatal nature and stomatal index has been calculated and presented in Tab. 1. Out of these 7 species only D. oliganthum showed absence of stomata on upper surface. The size of prickles were larger in D. oliganthum than other species. In D. annulatum and D. panchganiense papillae were recorded only on upper surface. Papillae were absent in D. caricosum, D. pertusum and D. tuberculatum. Cork cells showed the silica deposition of Dumbbell shapes in all seven Dichanthium species.







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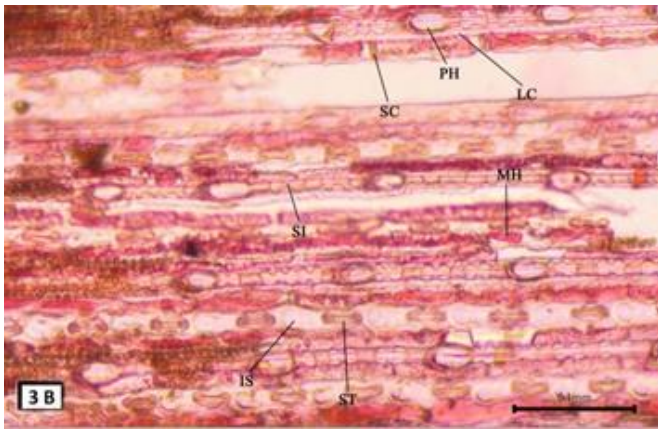
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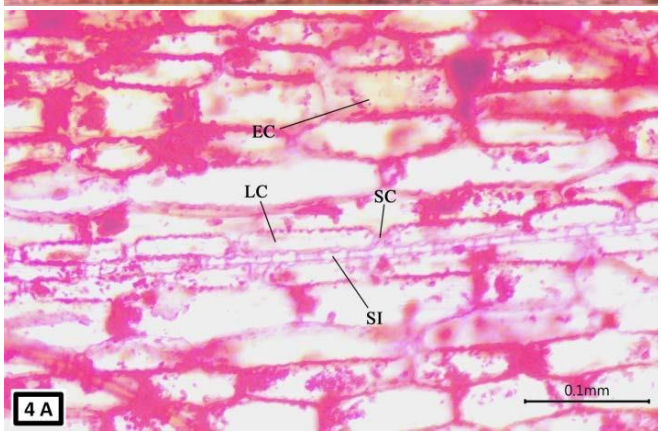
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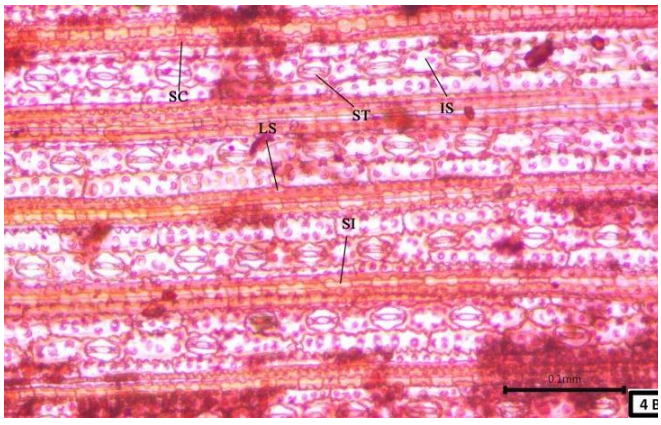
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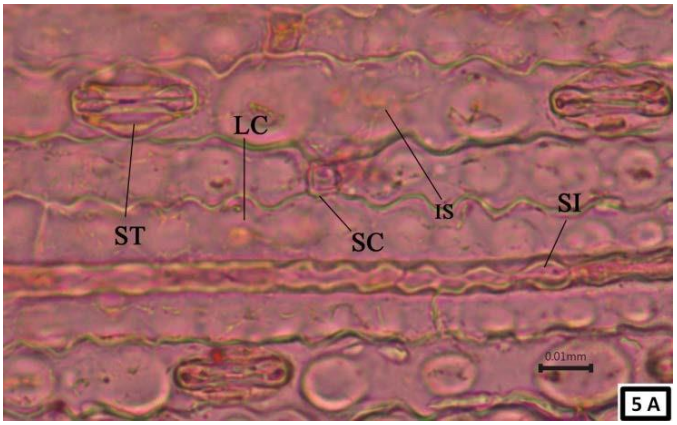
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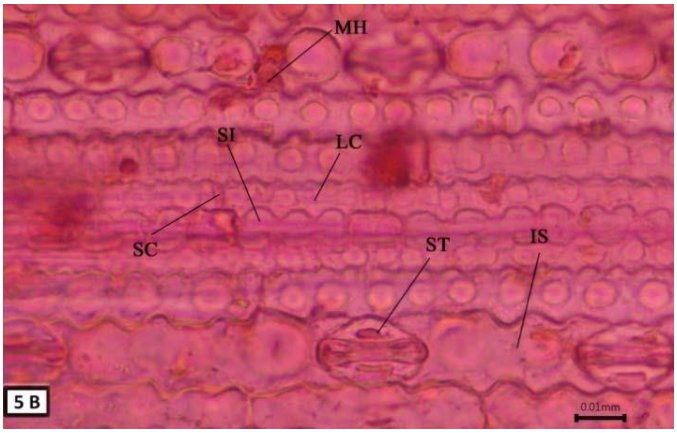
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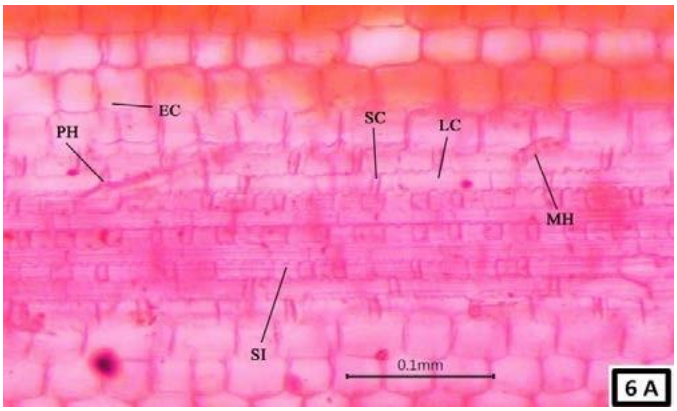
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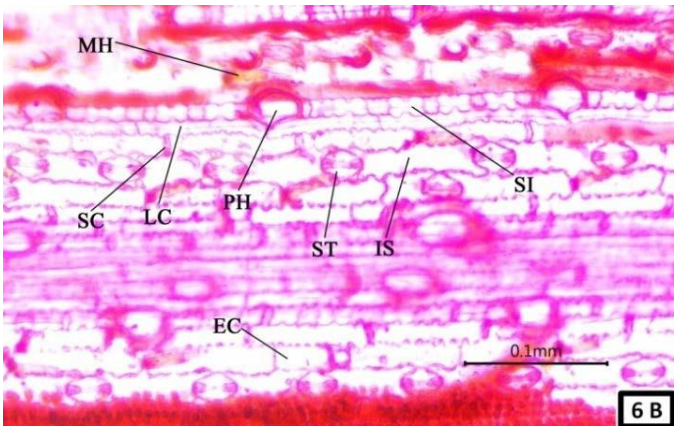
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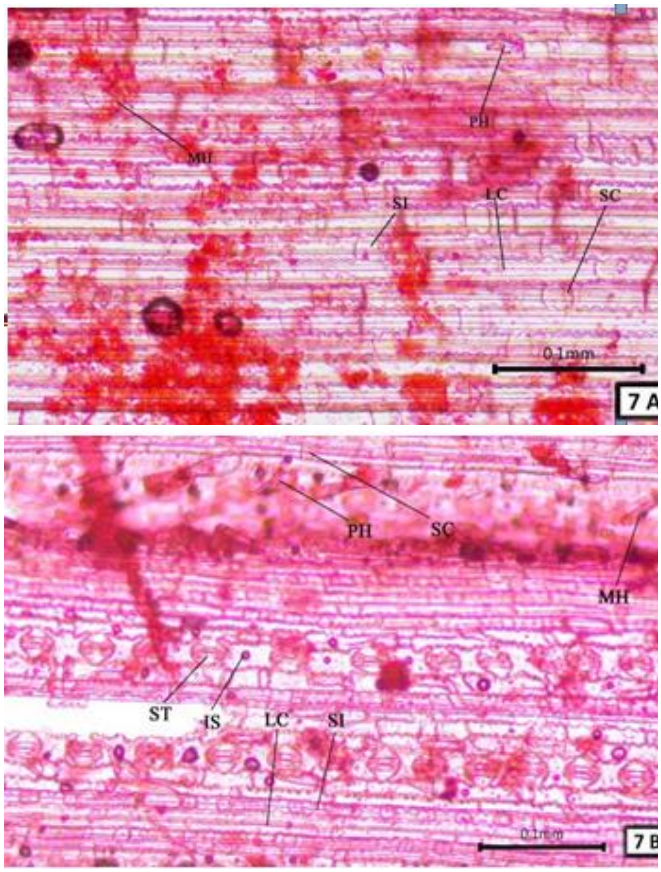

Fig. 1: Photomicrographs of leaf blade

1A. Abaxial surface of D. annulatum showing Microhair Epidermal cell, Stomata, Silica cells, Long cell and Inter stomatal cell.

1B. Adaxial surface of D. annulatum showing Microhair,

Epidermal cell, Stomata, Silica cells and Long cell.

2A. Abaxial surface of D. armatum showing Epidermal cell, Stomata, Silica cells, Long cell and Inter stomatal cell.

2B. Adaxial surface of D. armatum showing Epidermal cell, Stomata, Silica cells, Long cell, Prickle and Inter stomatal cell. 3A. Abaxial surface of D. caricosum showing Epidermal cell, Stomata, Silica cells, Long cell and Inter stomatal cell.

3B. Adaxial surface of D. caricosum showing Microhair, Epidermal cell, Stomata, Silica cells, Long cell and Inter stomatal cell. 4A. Abaxial surface of D. oliganthum showing Epidermal cell, Silica cells, Long cell and Inter stomatal cell.

4B. Adaxial surface of D. Oliganthum howing Stomata, Silica cells, Long cell and Inter stomatal cell.

5A. Abaxial surface of D. panchganiense showing Stomata, Silica cells, Long cell and Inter stomatal cell.

5B. Adaxial surface of D. panchganiense showing Microhair, Stomata, Silica cells, Long cell and Inter stomatal cell. 6A. Abaxial surface of D. pertusum showing Microhair, Epidermal cell, Stomata, Silica cells and Long cell.

6B. Adaxial surface of D. pertusum showing Microhair, Epidermal cell, Stomata, Silica cells, Long cell and Inter stomatal cell. 7A. Abaxial surface of D. tuberculatum showing Microhair, Silica cells, Prickle hair, Long cell and Inter stomatal cell.

7B. Adaxial surface of D. tuberculatum showing Microhair, Stomata, Silica cells, Long cell and Inter stomatal cell.

(Legends. LC: Long cell, SC: Short cell, ST: Stomata, MH: Microhair, PH: Prickle Hair, SI: Silica cell, EC: Epidermal cell, P: Papillae).

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Exploring Anti-Inflammatory Potential in Leaves of Jamun (Syzygium Cumini)

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ABSTRACT

Inflammation is initiated as healing process by the tissue in response to injury by pathogens, irritants or cell damage. Anti-inflammatory drugs used today to cure the disease have serious side-effects. The focus of research is to study the bioactive compounds from medicinal plants with anti-inflammatory property. The work is carried out with Syzygium cumini leaves (family-Myrtaceae). Leaves are reported to posses anti-inflammatory activity in crude extract of leaves and bark. In our study, ethanolic extract of leaves was screened for the anti-inflamatory activity and bioactive compound was partially purified by Thin Layer Chromatography (TLC) technique. Partially purified bioactive compounds were further analysed using various in vitro models such as inhibition of albumin denaturation assay and HRBC membrane stabilization assay. Aspirin and diclofenac sodium were used as standard drug. It is cleared from the result that the bioactive compound tannins at concentration of 100µg/ml posses 99.50% inhibition of heat induced protein denaturation as compared with the standard drug Aspirin i.e. 89.26%. In HRBC membrane stabilization activity tannins at concentration of 1mg/ml showed 82.94% protection of HRBC membrane while the standard diclofenac sodium showed 70.41% protection. Overall study claims that ethanol extract of S. cumini leaves posses potential anti-inflamatory activity due. Further study is required to purify and identify the specific bioactive compound.

Keywords: Herbal preparation, Bioactive compounds, S. Cumini, TLC, Aspirin, Diclofenac sodium

I. INTRODUCTION

Inflammation is the healthy and an essential immune response shown by the body that may enable body to survive during infection or injury which is also termed as host defense mechanism. Inflammation process also maintains tissue homeostasis in noxious conditions [1]. Its key features are being characterized by redness, warmth, swelling, pain in joints with loss of joint function [2]. Inflammation is mainly classified into Acute and Chronic inflammation. Acute inflammation is an initial response given by the body to harmful stimuli and is achieved by increase in the movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A various biochemical pathways such as the local vascular system, the immune system and various cells within the injured tissue show the inflammatory response [3]. Chronic inflammation is chiefly marked by new tissue formation and is prolonged. Currently inflammation is treated with most commonly worldwide used drug NSAIDs [4]. In inflammation, injured tissue cells release kinins, prostroglandins and histamine that are collectively involves increase in vasodilation (widening of blood capillaries) and permeability of the capillaries which lead to increase blood flow to the injured site. Management of inflammation related disease is the real issue in the

rural community; the population in this area uses many alternative drugs such as various different substances produced from medicinal plants, [5] Most of the anti-inflammatory drugs available in market have problems with efficacy and side effects and the need of safe novel effective anti-inflammatory compounds can be fulfilled by herbal medicine as they have no side effects [6]. Syzygium cumini (Family Myrtaceae), also called as Eugenia cimini and Syzygium jambolanum. Other common names used for this are jambul, Black Plum, java Plum, jamblang, jamun, Indian Blackberry etc. Tree is found in Asian subcontinent, Eastern Africa, South America etc. The tree fruit is annually and is sour to taste. Different parts of jamun are reported to have medicinal properties including anti-diabetic, antioxidant, anti-inflammatory, macological, anti-bacterial, anti-fungal, anti-HIV, anti-diarrheal, anti-ulcerogenic, antifertility, anti-leshmanial, nitric oxide scavenging, free radical scavenging and radioprotective [7, 8].Ethanol S. cumini seeds is reported to have potential antidiabetic activity[9] . Insufficient data is present which can show the effective anti-inflammatory activity on s.cumini .Very few literatures are available for the activity of seed, leaf and bark. We did not find any literature on isolated compounds on S.cumini leaf and bark extract.

II. METHODS AND MATERIAL

Collection of Plant material and Extract preparation

Syzygium cumini leaves were collected from Nakshatra Udyan, Vidya pratishthan, Baramati, Dist:Pune, (MS), India. The leaves were shade dried for 5-7 days and powdered with morter pestle. The Powder was then filtered by using 85mm mesh and extracted by soaking 30g of powder in 50ml of ethanol for 72 hrs at 37ºc in dark. The extract was filtered and concentrated by evaporation [10].

Determination of mineral content

The 5gm crude leaf powder was converted into ash by keeping in muffle furnace for 2 hours, mixed with 100ml nitric acid and filtered through filter paper . Standard working solution was prepared to determine mineral content in atomic absorption spectrophotometer [11].

Qualitative analysis of phytochemicals

The leaf extract of S.cumini were analysed for the presence of phytochemicals according to standard methods reported [11, 12].

Test for alkaloids: 2ml of extract was added to 2N HCL. and treated with few drops of Mayer's reagent were added. Inferenced by Cream colour precipitate.

Test for flavonoids: 3-5 drops of 1N NaOH were added to 2ml extract. Inferenced by formation of yellow orange color.

Test for phenolic compounds: 3-5 drops of 5% Fecl3 solution were added to 2ml extract. Inferenced by deep blue color formation.

Test for saponins: 2ml of extract was mixed with 6 ml of water. Persistent foam was observed.

Test for tannins: 2ml of the aqueous extract was mixed with 2ml of D/W and few drops of fecl3 solution were added. Formation of green precipitate confirmed the presence of tannis.

Test for phlobatanins: 2ml of extract was mixed with 2ml of 1%HCL and the mixture was boiled. Inferenced by deposition of red precipitate.

Test for coumarins: 3ml of 10% NaOH was added to 2ml of aqueous extract. Formation of yellow color was observed

Test for anthocyanins: 2ml of aqueous extract was mixed with 2ml of 2N HCL and ammonia. Turning of Pink-red to blue violet was observed

Test for leucoanthocyanin: 5ml of aqueous extract was added to 5ml of isoamyl alcohol. Inferenced by formation of upper layer red in colour

Test for terpenoids: 2ml of extract was mixed with 2ml of acetic anhydride and con of H2So4. Formation of blue green ring confirmed the presence of terpenoids.

Test for steroids: 1ml of extract was taken and mixed with 10ml of chloroform & equal volume of concentrated sulphuric acid was added slowly from the

side. Upper layer turned red & sulphuric acid layer showed yellow with green fluorescence.

Test for fatty acid: 0.5 ml of extract was mixed with 5ml of ether, extract was allowed to evaporate on filter paper & filter paper was dried. The transparency on paper was observed.

Thin layer chromatography

The leaf extracts were analysed with the help of Thin Layer Chromatography (TLC) to separate specific bioactive compound from the extracts. TLC was done using different solvent ratio described in [9,13]. The slide were then dried and kept in iodine saturation tank for spot visualization. The movement of the analyte was expressed by its retention factor (Rf). Values were calculated for different sample by formula [14].

Rf (Retention factor) = Distance travel by solute / Distance travel by solvent

The active compounds were retrieved in 1ml of ethanol and filtrate was concentrated by evaporation. Further the concentrated filtrates of different bioactive compounds were stored for further analysis [15].

In-vitro

Inhibition of albumin denaturation assay

The assay was determined by methods described by [5] and [16] with slight modification. The 2ml reaction mixture contained test extract at different concentrations (100µg/ml-1000 µg/ml) and aqueous solution of bovine serum albumin fraction (1%). pH of reaction mixture was adjusted to 6.3 by 1N HCl. The samples were incubated at 37 ℃ for 20 min and then heated at 57 ℃ for 30 min. After cooling the samples, 1ml of Phosphate buffer saline was added to each sample tubes. The turbidity was measured spectrophotometrically at 660 nm against blank. The experiment was performed in triplicate. Standard drug used was aspirin at concentration of 1mg/ml [17]. Percent inhibition of protein denaturation was calculated as follows

Percentage inhibition (%) = (O.D control – O.D sample) X 100/ O.D control

HRBC membrane stabilization assay

HRBC membrane stabilization method has been used to study the cyto-protective activity. The assay was performed according to simple modification of [18,19]. Healthy human blood was collected and mixed with equal volume of sterilized alsever solution (2% dextrose, 0.8% sodium citrate, 0.5 citric acid, 0.42% sodium chloride in distilled water).The blood was centrifuged at 3000rpm & packed cells were washed with iso-saline (0.85%, pH 7.2) and suspension was made with iso-saline. Assay mixture contained 1ml phosphate buffer (0.15M pH 7.2), 2ml of hypo saline (0.36%), 0.5ml of RBC suspension & 1ml of various concentration of test samples i.e. (200ug/ml, 400ug/ml, 600ugml, 1000uglml). Diclofenac sodium was used as standard drug. In control 2ml of Distilled water was used instead of hypo saline. All reaction mixtures were incubated at 37º c for 30min and centrifuged at 3000rpm for 20min.Absorbance was taken at 560nm. The percent haemolysis was calculated by comparing with the control produced in 100% Distilled water. The % HRBC membrane stabilization was calculated by using the standard formula.

% Protection = 100 – (Absorbance of control - Absorbance of sample/Absorbance of control) × 100

III. RESULT AND DISCUSSION

Mineral analysis

Mineralization of S.cumini leaf and bark extract by atomic absorption spectrophotometry analysis has revealed the presence 6 important elements including 2 macro nutrients like Ca++ & Mg++ and 4 micro nutrients like Fe++, Zn++, Mn++ and Cu++. Major elements are found higher as compared to trace element. Each nutrient yielded as Ca++ 48.80ppm, Mg++-5.37ppm, Fe++-3.8ppm and Zn++-1.7ppm in leaf extract and Ca++ 64.44ppm, Mg++- 5.3ppm, Fe++-2.5ppm and Zn++-1.3ppm in bark extract which were far lesser then the reported values Ca++-156ppm, Mg++-11.2ppm, Fe++-12,7ppm and Zn++-2.38ppm[20].

The presence of macro and micro elements reflect their function as essential nutrient elements and often as cofactor activators in metal- ligand complexes [21].

Table 1- Mineralization of leaf and bark extract by atomic absorption spectrophotometry

	Mineral content (mg/L)					
Elements / P Part	Ca++	Cu++	Fe++	Mg++	Zn++	Mn++
Leaves extract	48.80	1.43	3.88	5.31	1.70	2.54
Bark extract	64.4	1.53	2.52	5.37	1.30	1.61

In plant leaf and bark extract the minerals found to be dominant were calcium followed by magnesium, iron and manganese, whereas zinc and copper were found to be in trace amounts. Ca++ showed highest concentration in both plant extract than the other elements.

Table 2 Phytochemical analysis of aqueous leaf and bark extract. (+): Positive (-): Negative

Secondary metabolites	Bark extract	Leaf extract
Alkaloids	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Phlobatannins	+	+
Coumarins	_	_
Anthocyanins	_	_
eucoanthocyanins	_	_
Emodins	_	_
Steroids	+	+
Fatty acids	+	- --

As per the evaluation Out of 12 phytochemicals 8 bioactive compounds were present in bark extract, while 7 were present in leaf extract. One of which might be the responsible factor for the anti-inflammatory activity for the plant extracts.

Thin layer chromatography.

Profiling of plant leaf extract in different solvent system confirmed the presence of diverse group of phytochemicals like alkaloids, flavonoids, tannins and phenols. The separation was confirmed by comparing RF values with reported values in literature as shown in Table3. TLC is simple cost-effective technique which has been used routinely from several decades to separate chemical and biochemical compounds. Study revealed the application of qualitative estimation of bioactive compounds from medicinal plants. The presence of bioactive compounds like alkaloids, tannins, phenol and flavonoids was determined and confirmed by comparing obtained Rf values 0.71, 0.74, 0.97 and 0.41 with reported Rf values 0.72, 0.74, 0.97 and 0.42 in literature [13, 14, 22, 23]. The separation of alkaloids was done by using benzene: methanol (80:20) which gave the best results. Mobile phase used for flavonoids - ethyl acetate: methanol: water (100:20:12), phenols- chloroform: methanol (27:0.3) and tannins- methanol: water (6:4) were used as reported in the reference [14] which resulted into the proper separation. Rf values of all the compounds was cross checked with the reported values in the references, results are tabulated in Table 3. The total yield of retrieved compound as for flavonoids- 3.12g/ml, alkaloid- 980mg/ml, phenols- 90mg/ml, tannins- 100mg/ml which were used as stock concentrations for further analysis. That were separated by using specific solvent ratio and Rf value obtained was confirmed by crosschecking with the reported values in the literature [13,22,23]

Sr. No	Purified bioactive compound	RF value	Reported value	Solvent used	Total yield

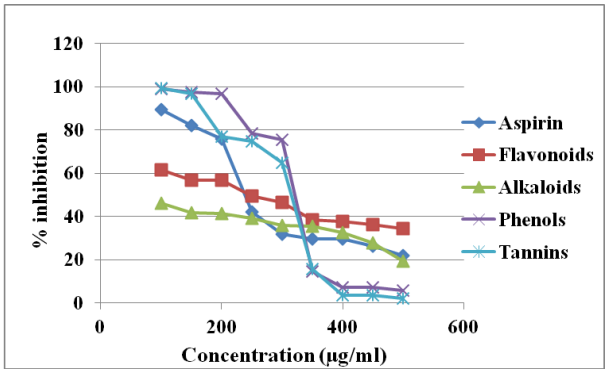
1	lkaloid s	0.41, 0.90, 0.97, 0.72, 0.18	0.41, 18, 0.55, 0.64, 82, ,0.92 [14]	Benzene: Methanol (80:20)	980mg/ ml
2	lavonoi ds	0.89, 0.74, 0.62, 0.33	85, 0.73, 0.62, .57,0.33[14]	thylacetate: Methanol: Water (100:20:12)	.12g/ml
3	Tannins	0.96, 0.42, 0.74, .93 ,0.3 9	0.83, 94, 0.46, 0.74 [22]	lethanol :W ater (6:4)	100mg/ ml.
4	Phenols	.98,0.52 ,0.81, .46,0.76 ,0.70	.52,0.65, .87,0.83. 0.46 [23]	Chloroform: Methnol (27:0.3)	0mg/ml

Inhibition albumin denaturation assay

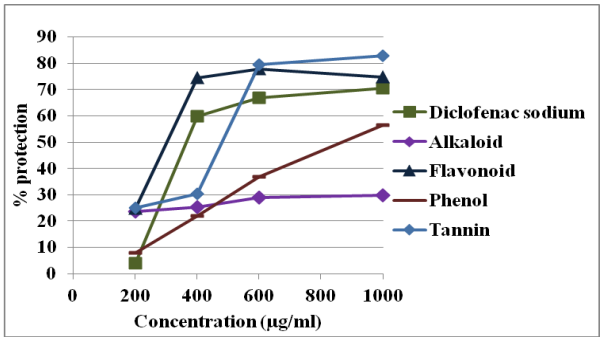
Inflammation is nothing but the normal protective response to tissue injury caused by physical, chemical or microbial agents. Denaturation of protein is well documented cause of inflammation. Protein denaturation is the process in which protein lose their secondary and tertiary structure. Most of the biological protein loses their biological function when denatured [5]. Our study revealed the mechanism of anti-inflammatory activity and the ability of bioactive compounds to inhibit the protein denaturation. BSA denatured by heat treatment. The denatured BSA expresses antigens associated to type 2 hypersensitive reaction that are related to various diseases. Heat denatured proteins are as effective as native protein in provoking delayed hypersensitivity [24]. Among the four bioactive compounds tannins showed maximum 99.50 % inhibition at the concentration of 100µg/ml as compared to standard anti-inflammation drug aspirin 89.26% as shown in Graph 1.

HRBC Membrane stabilization assay

The lysosomal enzyme released during inflammation produces various disorders. The extracellular activity of these enzymes is related to acute or chronic inflammation [25]. Since, HRBC membrane is similar to lysosomal membrane. Exposure of RBC's to injurious substances such as hypotonic medium shows haemolytic effect that result in lysis of the membrane and oxidation of haemoglobin. Membrane stabilization brings about prevention of leakage of serum proteins and fluids into the tissue during the period of increased permeability caused by inflammatory mediators [18]. Thus the phytochemicals from the leaf extract possess the potent anti-inflammatory property. The anti-inflammatory activities are probably due to their inhibitory effect on enzymes that are involved in production of chemical mediators of inflammation and metabolism of arachidonic acid [26]. A bioactive component of leaf extract at concentration 200-1000µg/ml protects the human RBC membrane against lysis induced by hypotonic solution. At concentration of 1000µg/ml the active component tannins shows maximum 82.94% protection as compared with standard diclofinac sodium 70.41% at same concentration as shown in Graph 2.



Graph 1- Effect of bioactive compounds on heat induced percent denaturation



Graph 2- Effect of bioactive compounds on hypotonicity induced haemolysis of HRBCs

IV. CONCLUSIONS

Overall study concluded that S.cumini leaves and bark contains total 7 phytochemicals out of which tannins showed highest activity, hence proved to be potent anti-inflammatory agent. Ca is present in higher concentration in both the extracts.

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Cultivation of Banana Plant From Agriculture Waste, Goat Manure & Organic Fertilizer

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ABSTRACT

The experiment was conducted in farm of Nagansur, Taluka- Akkalkot, District Solapur during 25 July 2015 to 25 May 2016 to study effect of Agriculture Waste, Goat Manure & Organic Fertilizer on Banana plant growth, fruit yield. The experiment was laid out by pit formation in the farm. The observation was recorded periodically on growth of plant in all three pits named A, B, and C. The height of plant A after 120 day it measure 88 cm. The height of plant B after 120 day measure 143 cm. The height of plant C after 120 day measure 107 cm. From this it was conclude that the organic fertilizer is more effective than the chemical fertilizer. The Banana plants receiving the recommended dose of fertilizers along with bio-fertilizers recorded plants were superior in growth; fruit was more than other plants. The treatments with bio-fertilizers recorded higher values compare to without bio-fertilizers and the organic fertilizer gives more amount of product yield. And also increase the soil fertility for long time the chemical fertilizer casus various disease

Keywords : Agriculture waste, Goat manure, Bio-fertilizer, Nagansur, Banana

I. INTRODUCTION

The banana plant is the largest herbaceous flowering plant. All the above-ground parts of a banana plant grow from a structure usually called a corm. Plants are normally tall and fairly sturdy, and are often mistaken for trees, but what appears to be a trunk is actually a false stem or pseudo stem. Bananas grow in a wide variety of soils, as long as the soil is at least 60 cm deep, has good drainage and is not compacted. The leaves of banana plants are composed of a stalk (petiole) and a blade (lamina). The base of the petiole widens to form a sheath the tightly packed sheaths make up the pseudo stem, which is all that supports the plant. The edges of the sheath meet when it is first produced, making it tubular. As new growth occurs in the centre of the pseudostem the edges are forced apart. Cultivated banana plants vary in height depending on the variety and growing conditions. Most are around 5 m (16 ft) tall, with a range from 'Dwarf Cavendish' plants at around 3 m (10 ft) to 'Gross Michel' at 7 m (23 ft) or more. Leaves are spirally arranged and may grow 2.7 meters (8.9 ft) long and 60 cm (2.0 ft) wide. They are easily torn by the wind, resulting in the familiar frond look.

Organic fertilizers are derived from animal matter, animal excreta (manure), human excreta, and vegetable matter (e.g. compost and crop residues). Naturally occurring organic fertilizers include animal wastes from meat processing, peat, manure, slurry, and guano. In contrast, the majority of fertilizers used in commercial farming is extracted from minerals (e.g., phosphate rock) or produced industrially (e.g., ammonia). Organic agriculture, a system of farming, allows for certain fertilizers and amendments and disallows others; that is also distinct from this topic.

A Bio fertilizer is a substance which contains living microorganisms which, if applied to seeds, plant

surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, soluble phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria" (PGPR). Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, bio-fertilizers do not contain any chemicals which are harmful to the living soil.

Present study was done to improve method of cultivation of banana crop by using agriculture waste, Goat manure and organic fertilizer. In India, about 80% of the farmers are using chemical fertilizers for production of any crop. Chemical fertilizers affect human health. The organic fertilizers are cheap in rate, easily available and these fertilizers improve soil fertility for long time and also do not affect human health. India is the agriculture country. We produce more quantities product yield with using chemical fertilizer but it is not healthy to human health. Chemical fertilizer is affects on our health and also causes many disease including hereditary disorders. This work can promote our Indian farmers to use bio fertilizer and organic fertilizer.

Study area:

The study was done in the Nagansur, Taluka-Akkalkot, District-Solapur. Nagansur is a village at south of Akkalkot Taluka with population of 15000. The main source of economy of the village is agriculture, that to grapes farming is the main occupation, it had 500 acres of grape farm. The geographical coordinates i.e. latitude and longitude of Nagansur is 17.392994 and 76.165217 respectively

II. METHODS AND MATERIAL

Formation of Pits:

This work is on field work, it was done from date 25 July 2015 to 25 May 2016 for whole study. In this first three months were for collection of a soil sample and analysis of soil parameters as like N, P, K, pH of soli sample. After that prepared 3 pits which were named as pit A, pit B, pit C. The width and depth of pit's are 1.5 feet by 1.5 feet respectively. Then each pit was filled with about 2 kg black soil and Pit B filled with addition of agriculture waste & goat manure. The pit C has contains only black soil. Observation was taken for minimum and maximum temperature. After the observation of temperature each Pit contains following matter. Pit A contains: only black soil

Pit B contains: agriculture waste + goat manure + organic fertilizer

Pit C contains: only black soil

After that the decomposing culture is mix in the pit B for decomposition of agriculture waste & organic matter. In 4-5 day gap continuous supply the water for well mix of decomposing Culture. After 30 day complete agriculture waste is decomposed now it is ready to plantation.

How to prepare decomposing culture solution:

Take 200 gram of decomposing bacteria and dissolve 20 liter of water this solution is kept 20 hour for totally dissolve of decomposing bacteria After 20 hour totally dissolve and it is ready to use. In this way prepare of decomposing solution in pit B of plant were kept approx 5-7 kg of goat manure & caw dung in Pit C of plant were kept about half kg of chemical fertilizer it include DAP, Urea & some micro nutrient in pit A nothing of fertilizer are use either chemical fertilizer or organic fertilizer.

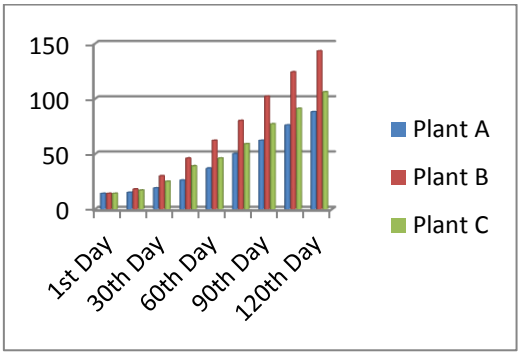
Plantation:

The plantation was done on 29 August 2015, with each plants are equal in height 14 cm and each plant having 4 green leaves. The height and width of each plant was equal. As the plantation day10 liter of water supplied to each plants, after the plantation the continue observation of plants were done periodically and regularly after 2 day interval 15 liter water is supply of each plant. For plant B after the 60 day re-again supplied of bio-fertilizer. And also taken care of plants for any fungal or bacterial infection and any other injury.

Observation of Growth of the Plant:

Day	Plant A (cm)	Plant B (cm)	Plant C (cm)
1st Day	14	14	14
15th Day	15	18	17
30th Day	19	30	25
45th Day	26	46	39
60th Day	37	62	46
75th Day	50	80	59
90th Day	62	102	77
105th Day	76	124	91
120th Day	88	143	107

Graphical Representations Growth of Plant A,B,C.



III. RESULT & DISCUSSION

The height of plant A after 120 day it measure 88 cm. The height of plant B after 120 day measure 143 cm. The height of plant C after 120 day measure 107 cm .So I observed that the variation of height of plant. The height of plant B is measured longer than plant C than plant A. And the plant B having more green leaf. For the plant B I supply only organic fertilizer & plant growth is very fast.

IV. CONCLUSION

On the basis of plant growth in the present investigation, it was conclude that the organic fertilizer is more effective than the chemical fertilizer. We used more amount of bio fertilizer, organic fertilizer for plant growth. The Banana plants receiving the recommended dose of fertilizers along with bio-fertilizes recorded plants were superior in growth, fruit was more than other plants. The treatments with bio-fertilizers recorded higher values compare to without bio-fertilizers and the organic fertilizer gives more amount of product yield. And also increase the soil fertility for long time the chemical fertilizer casus various disease.

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Antagonistic and Phosphate Solubilization Potential of Trichoderma SP From Rhizosphere of Red Gram Cultivated in Marathwada Region

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ABSTRACT

The region where soil and roots makes contact is called rhizosphere. The microbial growth is enhanced by nutritional substances such as amino acids and vitamins released from plant root tissues. It represents a tremendously complex biological system. The growth of plant is also influenced by the products of microbial metabolism. The inorganic phosphorus which is unavailable to plant is solubilized by fungi & bacteria. Biocontrol of plant disease is an alternative to chemical pesticides which causes environmental pollution and development of resistant strain. In the present work screening of phosphate solubilizing Trichoderma sp. from rhizosphere of red gram cultivated in Marathwada region was done by using PVK medium. Soil samples were collected from different rhizosphere region of red gram cultivated in Marathwada region. From this isolates of Trichoderma sp. were isolated & discussed in this paper. Isolates of Trichoderma sp. were identified depending upon their morphological and cultural characters. Efficient isolates of PSTS were further tested for antimicrobial activity against Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281.It was observed that one isolate showed high PSE & antimicrobial activity; hence it could be exploited as biofertilizer and biocontrol agent in agriculture.

Keywords: Antagonism, Biocontrol, Phosphate Solubilization, Rhizosphere, Trichoderma SP

I. INTRODUCTION

Cajanus cajan L. (Red gram) is also known as pigeon pea (Arhar or tur in local language).It is one of the most extensively used pulses in India. Pigeon pea probably evolved in South Asia and appeared about 2000 BC in West Africa, which is considered a second major center of origin. It is a leguminous shrub that can attain height of 5 M. It is a major pulse crop of India. Among total pulses, the red gram accounts for 14.5% in area and 15.5% in productivity. Maharashtra is the largest producer with approximately 10.51 lakh hector area with average productivity of 6.03 Q/ha. Being important nitrogen fixing crop, it is widely grown for enriching the soil. Its deep penetrating roots helps in bringing nutrients from deeper layers of soil.

The term rhizosphere was introduced by German scientist Hiltner. It is the region where soil and roots makes contact. It is a unique subterranean habitat for microorganisms [1]. The microbial growth is enhanced by nutritional substances like amino acids, vitamins and other nutrients released from plant tissues. The growth of plant is also influenced by the products of microbial metabolism that are released into the soil [2]. It is a hotspot of microbial interaction. There are several beneficial microorganisms in the rhizosphere, which can improve soil quality, enhance

crop production, protection, conserve natural resources and ultimately create more sustainable agricultural production and safe environment[1], [2] . Phosphorus is a major growth limiting nutrient for plants. Like the case for nitrogen there is no large atmospheric source for it [3]. The inorganic phosphorus which is unavailable to plant is solubilized by fungi and bacteria. Trichoderma is free-living fungi that are highly interactive in root, soil and foliar environments. Trichoderma was found to be the most effective organic phosphorus mobilizers as compared to other fungi [4].

Biocontrol of plant pathogen is an alternative to chemical pesticides, which cause environmental pollution and development of resistant strain. The use of beneficial microorganisms (biopesticides) is considered as one of the most promising method for disease control [5]. The nature and practice strategies for detection and characterization systems for biological control of plant and soil born pathogen have been elucidated earlier [6]. Trichoderma sp have been shown to act, and are commercially applied as biological control agents against fungal pathogens [7, 8]. Biological control agents colonize the rhizosphere, the site requiring protection & leave no toxic residues, as opposed to chemicals. In the present work screening of Trichoderma species from rhizosphere soil of red gram cultivated in Marathwada region was done. Then its phosphate solubilizing efficiency & antagonistic activity was studied.

II. METHODS AND MATERIAL

Collection of rhizosphere soil sample

Soil samples were collected from rhizosphere of red gram cultivated in different locations of Aurangabad, Jalna, Latur, Osmanabad, Nanded, Parbhani, Hingoli and Beed district area in sterile plastic bags & stored at low temperature.

Isolation & identification of Trichoderma sp

The soil sample was serially diluted by serial dilution method. The last dilutions were spread on Czapek Dox agar and PDA medium .Plate was incubated at 25 ºC for 3-5 days. Morphologically distinct colonies were picked on the basis of their morphology [9]. The isolates Trichoderma sp. were identified by morphological character, cultural characters and reproductive structure [8], [10], [11], [12].

Phosphate solubilization

The isolates of Trichoderma sp were spot inoculated on PVK agar (M520 Hi-media) plates containing: Glucose 10.0 gram, Tri calcium phosphate – 5.0 gm, (NH4)2SO4-0.50 gm, KCl-0.20 gm, MgSO4.7H2O-0.10 gm, MnSO4- Trace, FeSO4- Trace, Yeast extract-0.50 gm, Agar-15gm in 1000 ml of distilled water. The plates were incubated at 28 ºC for 4-6 days. After incubation phosphate solubilizing fungi were detected by the appearance of transferent halo zone around its growth [11]. The zone diameter around the colony is measured and phosphate solubilizing efficiency was calculated by using following formula [13].

PSE = Solubilization diameter ÷ Growth diameter ×100 (1)

Detection of antimicrobial activity

The antimicrobial activity of isolates of Trichoderma sp. was determined by using agar diffusion (well) method. The water extract of isolates of PSTS was loaded into the well

bored and test organism seeded agar plates. For bacteria nutrient agar & for fungi Czapek Dox agar was used. The test organisms used were Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281.The plates were kept in freeze for 20-30 minutes and incubated at 30 ºC for 24 hours for bacteria & 25 ºC for 2-3 days for fungi. The diameter of zone of inhibition was measured & noted in the table.

III. RESULT & DISCUSSION

Soil samples were collected from different rhizosphere region of red gram cultivated in Marathwada region & from this isolates of Trichoderma sp. were isolated and identified. Trichoderma sp. was confirmed on the basis of morphological and cultural characters. On the PDA and Czapek Dox agar plate after 3-5 days at 25 ºC, the colonies were initially white in color which turns yellowish green in color after further incubation. Colony characters are: size- large, shape- irregular, elevation- flat, margin- lobate and surface texture- concentric. After staining with lactophenol cotton blue, microscopic observation showed septate hyphae which form firm tufts, conidiophores erect developing from side branching, branching usually opposite, conidiophores bear terminal conidial heads and conidia were oval to elliptical and smooth. Isolates of Trichoderma sp. were further subjected for detection of phosphate solubilization activity. Phosphate solubilizing efficiency was determined by using formula (Eq.1) & noted (Table 1).

Table 1: Phosphate solubilizzation efficiency of isolates of Trichoderma sp. at temp 28 ºC on PVK medium.

Sr. No.	Isolates of *Trichoderma* sp.	PSE
1.	PSTS01	113
2.	PSTS02	181
3.	PSTS03	175
4.	PSTS 04	134
5.	PSTS 05	147
6.	PSTS 06	180
7.	PSTS 07	207
8.	PSTS 08	132
9.	PSTS 09	187
10.	PSTS 10	158

Identification of Trichoderma sp was done according to morphological character, cultural characters and reproductive structure. Phosphate solubilization was confirmed by observation of clear zone around the colony on PVK agar plate. The zone is formed due to microorganism which cleaves phosphate molecules present in the medium [14]. The isolates of PSTS were subjected for study of antimicrobial activity against Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281. Zone diameter was measured & noted (Table 2)

Table 2: Antimicrobial activity of isolates of phosphate solubilizing Trichoderma p.

Sr. No.	Isolates of *Trichoderma* sp	*B. subtilis* NCIM 2010	*E. coli* NCIM2064	*Pr. vulgaris* NCIM2027	*X. compestris* NCIM2956	*F. oxysporum* NCIM 1281
1.	PSTS01	+	+	-	-	+
2.	PSTS02	++	++	+	+	-
3.	PSTS03	+++	-	++	-	++
4.	PSTS 04	-	+	+	+	-
5.	PSTS 05	+	+	-	+	++
6.	PSTS 06	++	-	++	+	+
7.	PSTS 07	+++	++	++	+	++
8.	PSTS 08	+	+	-	-	-
9.	PSTS 09	+++	-	+	+	+
10.	PSTS 10	++	+	-	+	+

(--: no antimicrobial activity, +: antimicrobial activity) It was observed that isolate PSTS07 showed high phosphate solubilization and antimicrobial activity against Bacillus subtilis NCIM2010; E. coli NCIM2064; Proteus vulgaris NCIM2027; Xanthomonas compestris NCIM2956 & Fusarium oxysporum NCIM1281, hence it could be exploited as biofertilizer and biocontrol agent. Phosphate solubilization takes place through different microbial processes. Phosphate solubilizing microorganism is being used as biofertilizer since 1950 [15, 16]. Microorganisms release organic acids which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms [14, 17]. The Trichoderma sp. are known to produce a number of secondary metabolites like trichodermin, trichodermol, harzianum A, harzianolide etc., apart from lytic enzymes that play significant role in the antagonistic activity [18]. Trichoderma sp. produces different antibiotics against fungal phytopathogens. Among the antibiotics, the production of gliovirin, gliotoxin, viridin, pyrones, peptaibols and others have been described [19].Continuous and indiscriminate use of chemicals leads to development of resistant strain towards pesticides. Chemical fertilizers also adversely affect the useful microorganisms in the soil. Biofertilizer is an attractive alternative to chemical fertilizers which causes environmental pollution.

IV. CONCLUSION

On the basis of plant growth in the present investigation, it was conclude that the organic fertilizer is more effective than the chemical fertilizer. We used more amount of bio fertilizer, organic fertilizer for plant growth. The Banana plants receiving the recommended dose of fertilizers along with bio-fertilizes recorded plants were superior in growth, fruit was more than other plants. The treatments with bio-fertilizers recorded higher values compare to without bio-fertilizers and the organic fertilizer gives more amount of product yield. And also increase the soil fertility for long time the chemical fertilizer casus various disease.

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Studies on Identification of Potential Bivoltine Hybrid of Silkworm Bombyx Mori L. for Quality Cocoon Productivity In Western Region of, Maharashtra State

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ABSTRACT

Maharashtra is largest silk producer out of which Nontraditional states of India and western zone is contributed more than 40 % out of which silk production of 252 MT and bivoltine silk is excel of industry where climate fluctuations during the rearing lead to adverse effects on bombyx mori L and .badly suffer. aims to identify potential hybrids for tolerance to adverse temperature and relative humidity. fourth day of fifth stage silkworm larvae were subjected to high temperature of 37 ± 1° C with RH of 52 ± 5 % for 8 hours until spinning .two hybrids. Significant varied were found for potential in adverse climate .out of which five hybrids were evaluated base of index value 50 (CSR6XCSR26) X (CSR2XCSR27) double hybrid (70.6) and (CSR17XCSR19) (62.6) and new double hybrid highly potential over control CSR2xCSR4 in respect of cocoon yield (11.23 %) single cocoon weight(5.4 %) cocoon shell weight (7.9 %) and silk percentage (2.8 %) and filament length (6.9 %) and over all parameters are indicate to significantly and to be recommended in field.

Keywords: Bivoltine Hybrid, Bombyx Mori L Performance

I. INTRODUCTION

The mulberry silkworm, Bombyx mori L. (Lepidoptera: Bombycidae), is an economical insect and domesticate by provide as fed of mulberry leaves which is a sole food plant for the purpose of silk production in the world . At present bivoltine sericulture is an excel of the silk industry of the india and other country and need to be compete for silk market of international level the india is an second largest producer of silk in world and is contributing nearly 5% in productivity of bivoltine silk. Bivoltine silkworm is highly susceptible .for adverse climate and need to careful domestication over centuries has apparently deprived this commercial insect of the opportunity to acquire adverse climate. . lots of factors responsible for poor results of the bivoltine silkworm rearing in laboratory and field level under tropical conditions, the major one is lack of adverse climate. Many quantitative and qualitative characters decline sharply at higher temperatures. Therefore, one of the key considerations in developing bivoltine breeds for tropics could be the need for adverse temperature tolerateThe recent silkworm breeding and those in stress-induced protein synthesis have opened up new avenues to evolve to robust productive silkworm hybrids (; Vasudha et al. 2006; Srivastava et al. 2007;Moghaddam et al. 2008).

BivoltineSericulture in India is practiced predominantly in tropical environmental regions such as Karnataka, Tamil Nadu, Andhra Pradesh, and West Bengal and to a limited extent in the temperate environment of Jammu and Kashmir. This situation provides scope for bivoltine hybrids as a commercial venture as hybrids are hardy and have the ability to

survive and reproduce under fluctuating climatic conditions. of nontraditional sericulture zone of Maharashtra state.

However, hybrid quality is low when compared to the existing international standards. For example, cross breeds of polyvoltine female x bivoltine male are generally reared in these regions during the summer, but the quality of cocoon production is not as high as it is for bivoltine silkworm hybrids (Ramesha et al. 2009). Bivoltine silkworm breeds are known for their qualitative and quantitative traits in the sericulture industry. During the last decade, a number of silkworm hybrids have been developed (Basavaraja et al. 1995; Data et al. 1997) and selected for exploitation at the field level during favorable season in coarse of silkworm rearing.

II. OBJECTIVES

The purpose of this study is to obtain new data about identification for t in silkworm larvae, not only to augment current knowledge on gene expression under stress conditions, but also to provide valuable information that will allow identification of thermotolerant bivoltine silkworm breeds based on the silkworm rearing performances relative to nine important economical genetic traits.

III. METHODS AND MATERIAL

The five silkworm breeds used were, CSR2xCSR4 CSR17xCSR19 ,(CSR6XCSR26)x (CSR2xCSR27) CSR50 xCSR51 PM XCSR2. These hybrids with varied qualitative and quantitative parameter, are observed and maintained during the silkworm rearing silkworm eggs from each hybrids were reared and cocoons were harvested and maintained until emergence of moths. Disease free female moths emerging on the peak day of pupal stage were allowed to mate for 3 to 4 hours and held until fertilization and depaired the moths. Mother moth will kept for oviposition and laid eggs more than 500-550 per female which are need to be acid-treated within 20 hours after eggs layings of silkworm before initiate the hibernation process with the method developed by Yokoyama (1962) to prevent hibernation. The eggs were incubated at 25 ± 1° C temperature and 70 to 80% RH after surface treatment with 2% formalin solution. 20 to 30 eggs were chosen from each hybrids and pasted onto egg sheets. Three such egg sheets for each breed were prepared, wrapped in white tissue paper and boxed with black paper to synchronize the embryonic development. On the day of hatching, the eggs were exposed to light in order to obtain uniform hatching and freshly chopped mulberry leaves were fed to the young larvae. The whole process, from silkworm egg incubation to completion of rearing activities, was carried out under hygienic conditions in a silkworm rearing under laboratory conditions and disinfected with bleaching powder and astra/sanitech.
Silkworm rearing was conducted for each breed in plastic rearing tray by provided as feeds to larvae of the V1 variety of mulberry leaves from the well-maintained irrigated mulberry garden at research institute A standard rearing procedure was adopted as recommended by Datta (1992). The young larvae (1st-3rdinstars) were reared at 26-28° C with 80-90% RH and late age larvae. (4thand 5thinstars) were maintained at 24-26° C with 70-80% RH until the 3rd day of fifth instar until formation of silky cocoon.

The study was conducted out between april and may.2016 Silkworm rearing was taken with standard method under the recommended temperature and relative humidity until the 3rd day of the fifth instar. On the 4th day of the fifth instar, 150larvae per breed in three replications of 50 larvae were selected for the high temperature treatment. High temperature treatment was obtainedan environmental growth chamber with precise and automatic control facilities for uniform maintenance of temperature and humidity. The temperature used was 37 ± 2oC and RH 51 ± 6%. Fresh mulberry leaves were given twice a day

to larvae A control group was maintained at ambient temperature of standard rearing conditions at 25 ± 1oC and RH 65 ± 5%. Thermal exposure was given every day for six hours until spinning (10:00-16:00) since continuous exposure to high temperature conditions reduces quantitative breeds improve to tolerate adverse temperature and relative humidity under laboratory experiment for identification of potential bivoltine hybrids in non traditional sericulture region western part of Maharashtra . Observations were carried out daily and mortality due to raise the temperature and reduce the relative humidity in atmosphere in each of the breeds of silkworm was observed . After arrangement by artificial treatment, for optimum climate the practices silkworm larvae were shifted to the mountage for spinning at normal temperature of 25 ± 2° C and RH 65 ± 5%. For cocoon formation . the Cocoons were harvested 5th days and accessed for survival to . pupation rate was significant

assesment of silkworm rearing, perfomence on larvae and cocoons for the eight parameter (larval weight, cocoon yield for 10,000 larvae by number and weight, pupation rate, cocoon and shell weight, shell ratio, filament length) were collected and calculated

IV. RESULT

Differences were marked amongst the bivoltine breeds at high temperature. Data were obtained for larva weight, yield by 10,000 larvae by number and weight, pupation, cocoon weight, shell weight, shell ratio, filament length and for 5bivoltine breeds under normal and high temperature treatments. There was evidence of clear declines in all economic parameter in all of the high temperature Among control and high temperature treated groups, maximum larval weight in (CSR6XCSR26)X(CSR2XCSR27)-W(41.8 g) CSR50XCSR51(39.8 g), CSR17XCSR19 (38.5 g) and minimum in PMXCSR2 (33.1 g) with an average of 36.8 g was estimated. Highest yield /10,000 larvae by number was observed in double hybrids (9245) and lowest in PMXCSR2 (9112) with an average of 8745 under lab conditions the double bivoltine hybrid is potential in adverse climatic as high temperature and low humidity in summer season.

PERFORMANCE OF SILKWORM REARING

Name of breeds	Larval wt (gm)	Cocoon yield/ 10000 (By no)	Pupation rate(%)	Cocoon wt (gm)	Shell wt (gm)	Silk (%)	Filament length (m)
(CSR6 XCSR26)X(CSR2XCSR27) (Control)	41.8	9245	93.6	1.76	.39	22.1	1098
(Treated)	38.2	7247	70.1	1.54	.31	20.1	955
CSR50 XCSR51 (Control)	39.8	9148	92.2	1.71	.37	21.6	1083
(Treated)	35.2	7025	69.4	1.51	.30	19.8	944
CSR17 XCSR19 (control)	38.5	9123	91.5	1.72	.36	20.9	1084
(Treated)	34.9	7019	69.3	1.50	..29	19.3	935
CSR2X CSR4((control)	38.3	9121	91.2	1.69	..34	20.1	1065
(treated)	34.4	7012	68.5	1.49	.28	18.7	926
PMXCSR2 (control)	37.2	9112	90.9	1.48	.27	18.2	989
(treated)	33.1	6989	68.3	1.41	.23	16.3	905

V. Discussion

On the summer season and due to high temperature the silkworms showed a decline with the increase of temperature above standard level. A similar result was observed Temperature stress causes a number of abnormalities at the cellular level as the normal pattern of protein synthesis halts. Another important effect of temperature (or stress of any kind) is the unfolding of cellular proteins. Cellular proteins are typically folded in their native conformations while functioning in cells. This process can result in aggregates of unfolded protein that at best diminish the pool of functional proteins a The isolated hemocytes of polyvoltine breeds exhibited the induction (Joy and Gopinathan 1995). For instance, polyvoltine breeds reared in tropical countries are known to tolerate slightly higher temperature, as are cross breeds that have evolved for a tropical climate (Ramesha et al. 2009).

The success of the sericulture industry depends upon several variables, but environmental conditions such as biotic and abiotic factors are of particular importance. Among the abiotic factors, temperature plays a major role on growth and productivity of silkworms (Benchamin and Jolly 1986). and that fluctuation of temperature during different stages of larval development was found to be more favorable for growth and development of larvae than constant temperature. There is ample literature stating that good quality cocoons are produced within a temperature range of 22-27° C and that cocoon quality is poorer above these levels (Krishnaswami et al. 1973; Datta 1992; Datta et al. 1996, 1997). However, polyvoltine breeds reared in tropical countries are known to tolerate slightly higher temperature (Hsieh et al. 1995), as are cross breeds that have been developed for tropical climates. In order to use bivoltine races in tropical and area of pune , it is necessary to have a stable cocoon crop in a high temperature environment. High temperature affects nearly all biological processes including the rates of biochemical and physiological reactions (Hsieh et al. 1995; Willmer et al. 2004), and can eventually affect the quality or quantity of cocoon crops in the silkworm. Several reports (Ueda and Lizuka 1962; Shirota 1992; Tazima and Ohuma 1995; Hsieh et al. 1995) demonstrated that silkworms were more sensitive to high temperature during the fourth and fifth stages, which are recommended for the recognition and selection of adverse temperature tolerant silkworm breeds, under high temperature conditions.

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