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# Floristic diversity of Pathra and its adjoining areas, Paschim Medinipur District, West Bengal

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ABSTRACT

Pathra is a village in Paschim Medinipur district. Recently this village has come to limelight due to its archaeological recognition having many temples and related structures of historical importance. Many people come to this for sightseeing and as such biodiversity of this area might be affected due to anthropogenic activities. In this background, with a view to understand the floristic diversity in this region the present study has been undertaken.Present botanical exploration at Pathraand its adjoining areas reportsthe 97 species (angiosperms 94 & pteridophytes 3). 94 angiosperms (dicots 81& monocots 13) belong to89 genera (dicots77& monocots 12) and 45 families (dicots 38 & monocots 7). The ratio of monocots and dicots is 1: 6.23. Only 3 species of petidophytes i.e. *Adiantum lunatum* Burm.f., *Dryopteris filix-mas* (L.) Schottand *Pteris longifolia* L. under 2 familieshave been recorded from this area.In present study 18 alien species have been recorded, of whichfour are fast growing invasive (e.g. *Argemone mexicana*L., *Lantana camara* L., *Mikania micrantha* Kunth and *Parthenium hysterophorus* L.).In most of the cases they create hazards over the normal growth of the vegetation.Biodiversity of a particular vegetation pocket acts as the sources of medicine, ethno-medicine, keystone species as well as controlling nutrient cycle, check pollution etc, so from the conservation and as well as floristic point of view such pocketsshould be maintained scientifically from their gradual disappearance through anthropogenic activities, natural calamities etc.giving topmost priority.Botanical name of the plants, families, habit, flowering-fruiting times and mode of propagations has also been discussed in this paper.

## INTRODUCTION

Formulation of data bank in the form of flora, monograph of a particular area or a region through survey of vegetation, sacred grove etc are the ultimate measurement of bio-diversity index. The biodiversity of particular vegetation pocket is the treasure trove of the raw material resources for the preparation of ethno-medicines, modern medicines, wooden materials, building materials, etc.In a broad sense these materials can be utilised initially for strengthening socio-economic status of local areas and subsequently the sustainable development of a country. Rapid urbanisation, industrialisation, clear felling of trees, ecological fragmentation, climate change, etc

are the prime causes for gradual lossof bio-resources (genetic resources) day by day from our mother earth. So through pocket to pocket vegetation survey one can assess the quantum of diversified flora as well as fauna of a particular area. Ultimately from such works, data bank / information bank, etc in near future will be the prerequisite for evolving the state, regional as well as national level flora.

Earlier to understand the status of vegetation a galaxy of investigators, researchers have explored the flora from different parts of undivided Midnapore district (1-5). Later floristic works were also done by (6-9) mainly from Paschim Medinipur district. Besides them, a group of investigators (10-21) also reported their works on medicinal plants from this region. Until now no comprehensive floristic works have been done from this area. So in the present paper attempthas been made to investigate thoroughly the different floristic compositions at Pathra and its adjoining areas.

## MATERIALS AND METHODS

### *The study area:*

For the survey of floristic diversity, the study area Pathra and its adjoiningareas was selected. Pathra is a village of temples under Gram Panchayat and has a latitude $22.4116^0$N and longitude $87.4183^0$E and covering total geographical area is 341.15 hectares. From $8^{th}$ Century to $12^{th}$ Century, it was an important hub for Hindus, Jains and Buddhists. It is situated about 15 km from the district town head quarters, 10 km from Birendra Sasmal Setu (Locally at Amtala / National Highway, No.60) and 13 km from National Highway (No.6). The River Kansabati flows besides the Pathra. At rainy season this river remains in the spate, resulting inundation of the low-lying areas. The soil of Pathra is basically of alluvial type and to some extend mixed mural type. The floristic assemblage / vegetationare of tropical mixed types. The temperature varies from $34^0$C-$44^0$C (during summer) and goes down to around $9^0$C (during winter). Though the climatic set up of Pathra is not varied enough, there grows different type of habit groups.

### *Collection data/ specimens:*

Field surveys were done in different seasons (at least three times in a year) at Pathra and its adjoining areas. The specimens were collected in flowering and fruiting stage. The collected specimens were identified with the help of literatures (22-24). Field and herbarium methods were followed according to (25). Finally the voucher specimens were deposited at Ramnagar College Herbarium. For updating species names the websites (26) were consulted.The list of accepted plant nameswere arranged alphabetically (Table-1) along with their families, local name (s), habit, flowering and fruiting periods, mode of propagation and their major uses were presented in tabulated form.

The present communication is concerned with the enumeration of floristic diversity (floral compositions)at Pathra and its adjoining areasalong with their conservations.

**Table-1:** Showing the floristic compositions collected from Pathra and its adjoining areasof Paschim Medinipur district.*Symbols used:* Fl.=Flowering & Frt.=Fruiting; "NR'=Not Recorded; Months: 1=January to 12=December.

| Name of the plant | Family | Local name | Habit | Fl. &Frt. periods | Mode of propagation | Major uses of plants, weeds, etc |
|---|---|---|---|---|---|---|
| **DICOTYLEDONS [MAGNOLIOPSIDA]** | | | | | | |
| ***Abrus precatorius*** L. | Fabaceae | Lal kunch | Climber | 9-12 | Seeds | Jewellery system |
| ***Acalypha indica*** L. | Euphorbiaceae | Muktajhuri | Herb | 1-12 | Seeds | Weed |
| ***Achyranthes aspera*** L. | Amaranthaceae | Apang | Herb | 10-2 | Seeds | Medicinal |
| ***Alstonia scholaris*** ( L.) R.Br. | Apocynaceae | Chattim | Tree | 8-3 | Seeds | Medicinal |
| ***Alternanthera sessilis*** (L.) R. Br. *ex* DC. | Amaranthaceae | Chaanchi | Herb | 1-12 | Seeds | Weed |
| ***Amaranthus spinosa*** L. | Amaranthaceae | Kanta-note | Herb | 1-12 | Seeds | Weed |
| ***Amaranthus viridis*** L. | Amarantahceae | Ban-notey | Herb | 1-12 | Seeds | Weed |
| ***Anisomelis indica*** (L.) Kuntze | Lamiaceae | Gobura | Shrub | 9-12 | Seeds | Weed |
| ****Argemone mexicana3***L. [Fig.-4] | Papavaraceae | Sialkanta | Herb | 1-8 | Seeds | Weed |
| ***Aristolochia indica*** L. | Aristolochiaceae | Iswarmul | Climber | 7-2 | Seeds and rootstocks | Medicinal |
| ***Artabotrys hexapetalous***(L.f.) Bhandari. | Annonaceae | Kantali champa | Climber | 4-1 | Seeds | Timber yielding |
| ***Azadirachta indica*** A. Juss. | Meliaceae | Neem | Tree | 3-7 | Seeds | Medicinal |
| ***Barleria cristata*** L. | Acanthaceae | Swethjhanti | Herb | 9-2 | Seeds and stem cuttings | Medicinal |
| ***Blumea lacera*** (Burm.f.) DC. | Asteraceae | Barokuksima | Herb | 12-5 | Seeds | Medicinal |
| ***Boerhavia diffusa***L. | Nyctanginaceae | Punarnova | Herb | 6-12 | Seeds and rootstocks | Weed |
| ***Bougainvillea spectaabilis*** Willd. | Nyctaginaceae | Kagajphul | Climber | NR | NR | Ornamental |
| ***Bryophyllum pinnatum*** (Lam.) Kurz | Crassulaceae | Patharkuchi | Herb | 10-3 | Stem and leaf cuttings | Medicinal |
| ***Canscora diffusa***(Vahl) R.Br. *ex* Roem.& Schult. | Gentianaceae | - | Herb | 1-12 | Seeds | Weed |
| ***Capparis zeylanica*** L. | Capparaceae | Kalokera | Climber | 3-10 | Seeds | Medicinal |
| ***Cardiospermum halicacabum***L. | Sapindaceae | Sibjhul | Climber | 4-1 | Seeds | Ornamental |
| ***Cascabela thevetia*** (L.) Lippold | Apocynaceae | Gulancha | Tree | 8-3 | Seeds | Ornamental |
| ***Cayaponia laciniosa*** (L.) Jeffery | Cucurbitaceae | Mala | Climber | 4-12 | Seeds | Medicinal |
| ***Cayratia pedata*** (Lam.) Gagnep. | Vitaceae | Goalilata | Climber | 8-12 | Seeds | Weed |
| ***Cayratia trifolia*** (L.) Domin | Vitaceae | Amal-lata | Climber | 4-12 | Seeds | Weed |
| ***Cissus quadrangularis*** L. | Vitaceae | Harbhanga, Harjora | Climber | 5-11 | Stem cuttings | Medicinal |
| ***Cleome 3viscosa***L. | Capparidaceae | - | Herb | 7-10 | Seeds | Weed |
| ***Clerodendrum infortunatum*** L. | Verbanaceae | Ghetu | Shrub | 2-7 | Seeds | Weed |
| ***Clitoria ternatea*** L. | Fabaceae | Aparajita | Climber | 3-12 | Seeds | Ornamental |
| ***Coccinia grandis*** (L.) Voigt. | Cucurbitaceae | Telakucha | Climber | 5-12 | Seeds, stem cuttings | Medicinal |
| ***Cocculus hirsutus***(L.) Diels | Menispermaceae | Daipata | Climber | 11-5 | Seeds, stem cuttings | Medicinal |
| ***Crataeva nurvala*** Buch.-Ham. | Capparidaceae | Barun | Tree | 2-7 | Seeds | Timber yielding |
| ***Croton bonplandianu*s** Baill. | Euphorbiaceae | Churchuri | Herb | 1-12 | Seeds | Weed |
| ***Cuscuta reflexa*** Roxb. | Cuscutaceae | Swarnalata | Climber | 8-4 | By stem | Medicinal |

| Name of the plant | Family | Local name | Habit | Fl. &Frt. periods | Mode of propagation | Major uses of plants, weeds, etc |
|---|---|---|---|---|---|---|
| ***Cocculus hirsutus***(L.) Diels | Menispermaceae | Daipata | Climber | 11-5 | Seeds, stem cuttings | Medicinal |
| ***Crataeva nurvala*** Buch.-Ham. | Capparidaceae | Barun | Tree | 2-7 | Seeds | Timber yielding |
| ***Croton bonplandianus*** Baill. | Euphorbiaceae | Churchuri | Herb | 1-12 | Seeds | Weed |
| ***Cuscuta reflexa*** Roxb. | Cuscutaceae | Swarnalata | Climber | 8-4 | By stem | Medicinal |
| ***Cyclea barbata***Miers | Menispermaceae | - | Climber | 7-3 | Seeds and stem cuttings | Weed |
| ***Datura stramonium***L. | Solanaceae | Dhutra | Shrub | 8-3 | Seeds | Medicinal |
| ***Desmodium gangeticum*** (L.) DC. | Fabaceae | Salpani | Tree | 2-6 | Seeds | Weed |
| ***Dregea volubilis*** (L.f.) Benth.*ex* Hook.f. | Asclepiadaceae | Titakunja | Climber | 4-12 | Seeds | Medicinal |
| ***Eclipta 4prostrata44***(L.) L. | Asteraceae | Kesut | Herb | 1-12 | Seeds | Dye yielding |
| ***Eupatorium odoratum*** L. | Asteraceae | - | Shrub | 8-1 | Seeds | Weed |
| ***Ficus hispida***L.f. | Moraceae | Domur | Tree | 4-8 | Seeds | Medicinal |
| ***Ficus infectoria*** Willd. | Moraceae | Jagya Domur | Tree | 2-12 | Seeds | Religious |
| ***Ficus religiosa***L. | Moraceae | Ashatha | Tree | 6-8 | Seeds | Religious |
| ***Flacourtia jamgomas*** (Lour.) Raeusch. | Flacourtiaceae | Paniala | Tree | 3-10 | Seeds | Timber yielding |
| ***Gnaphalium indicum***Thwaites. | Asteraceae | Kalpahi bon | Herb | 1-5 | Seeds | Medicinal |
| ***Gouania leptostachya*** DC. | Rhamnaceae | - | Climber | 7-12 | Seeds | Weed |
| ***Grangea maderaspatana*** (L.) Poir. | Asteraceae | Namuti | Herb | 12-4 | Seeds | Weed |
| ***Gymnema sylvestre***(Retz.) R. Br. *ex* Sm. | Asclepiadaceae | Gurmar/ Meshshringa | Climber | 8-3 | Seeds and stem cuttings | Medicinal |
| ***Hemigraphis hirta*** (Vahl) T. Anderson | Acanthaceae | Mushakani | Herb | 7-1 | Seeds and rootstocks | Weed |
| ***Hiptage bengalensis* (L.)** Kurz | Malpighiaceae | - | Climber | 3-7 | Seeds | Ornamental |
| ***Hybanthus linearifolius*** (Vahl) Urb. | Violaceae | Nunbora | Herb | 1-12 | Seeds | Weed |
| ***Hydrocotyle asiatiaca*** L. | Apiaceae | Thankuni | Herb | 7-1 | Stem cuttings | Medicinal |
| ***Ipomoea quamoclit*** L. | Convolvulaceae | Tarulata | Climber | 8-12 | Seeds | Ornamental |
| ***Jatropha gossypiifolia***L. | Euphorbiaceae | Lal veranda | Shrub | 4-8 | Seeds and stem cuttings | Medicinal |
| ****Lantana camara*** L. [Fig.-2] | Verbenaceae | Bhutbhairabi | Shrub | 1-12 | Seeds & stem cuttings | Weed |
| ***Leucas aspera*** (Willd.) Link | Lamiaceae | - | Herb | 8-10 | Seeds | Weed |
| ***Lindenbergia indica*** Vatke | Scrophulariaceae | Haludbasanta | Herb | 3-12 | Seeds | Weed |
| ***Luffa cylindrica***(L.) M. Roem. | Cucurbitaceae | Parul/ Dhundul | Climber | 6-12 | Seeds | Medicinal |
| ***Lysiloma latisiliquum***(L.) Benth. | Fabaceae | Subabul | Tree | 11-3 | Seeds | Fodder |
| ***Mangifera indica***L. | Anacardiaceae | Aam | Tree | 2-7 | Seeds | Fruit yielding |
| | | | | | | |
| ****Mikania micrantha***Kunth | Asteraceae | Taralata | Climber | 1-12 | Seeds | Medicinal |
| ***Ocimum basilicum*** L. | Lamiaceae | Dula tulsi | Herb | 8-1 | Seeds | Medicinal |
| ***Oldenlandia diffusa*** (Willd.) Roxb. | Rubiaceae | Khetpapra | Herb | 3-6 | Seeds | Weed |
| ****Parthenium hysterophorus*** L. | Asteraceae | Jayadrath | Herb | 1-12 | Seeds | Weed |
| ***Passiflora foetida***L. [Fig.-1] | Passifloraceae | Begambahar (wild) | Climber | 4-1 | Seeds | Ornamental |
| ***Pergularia deamia*** (Forssk.) Chivo. | Asclepiadaceae | Dudhlata | Climber | 9-1 | Seeds | Weed |
| ***Phyllanthus 44reticulatus***Poir. | Euphorbiaceae | Panjuli | Herb | 2-10 | Seeds and rootstocks | Weed |
| ***Pisum sativum*** L. | Fabaceae | Matar | Climber | 4-12 | Seeds | Fruit yielding |
| ***Quirivelia frutescens* (L.)** M.R. Almeida & S. M. Almeida | Apocynaceae | Shyamlata /Siamalata | Climber | 10-3 | Seeds | Medicinal |
| ***Ruellia 4prostrata*** Poir | Acanthaceae | Chotpoty | Herb | 4-1 | Seeds | Weed |
| ***Rungia pectinata*** (L.) Nees | Acanthaceae | - | Herb | 5-12 | Seeds | Weed |
| ***Senna sophera*** (L.) Roxb. | Fabaceae | Sena | Shrub | 7-12 | Seeds | Weed |
| ***S.tora*** (L.) Roxb. | Fabaceae | Chakunda | Shrub | 7-12 | Seeds | Weed |
| ***Sida cordifolia***L. | Malvaceae | Swetberela | Herb | 8-1 | Seeds | Weed |
| ***Solanum sisymbriifolium*** Lam. | Solanaceae | Swetrangani | Shrub | 8-12 | Seeds | Medicinal |
| ***Streblus asper*** Lour. | Moraceae | Seorah | Tree | 6-11 | Seeds | Timber yielding |
| ***Tinospora sinensis*** (Lour.) Merr. | Menispermaceae | Padmagulancha | Climber | 6-2 | Seeds and stem cuttings | Medicinal |

| Name of the plant | Family | Local name | Habit | Fl. &Frt. periods | Mode of propagation | Major uses of plants, weeds, etc |
|---|---|---|---|---|---|---|
| ***Tragia 55involucrata*** L. | Euphorbiaceae | Bichuti | Climber | 10-1 | Seeds and rootstocks | Poisonous |
| ***Tridax procumbens*** (L.) L. | Asteraceae | Targanda | Herb | 1-12 | Seeds | Weed |
| ***Vernonia coerulea***J. Kost. | Asteraceae | Chhoto kuksima | Herb | 1-12 | Seeds | Weed |
| ***Vincetoxicum indicum*** (Burm.f.) Mabb. | Asclepiadaceae | Antamul | Climber | 10-2 | Seeds and stem cuttings | Medicinal |
| ***Ziziphus oenoplia*** Mill. | Rhamnaceae | Shiakul | Climber | 4-12 | Seeds | Fruit yielding |
| **MONOCOTYLODONS [LILIOPSIDA]** | | | | | | |
| ***Borassus flabellifer*** L. | Arecaceae | Taal | Tree | 2-8 | Seeds | Fruit yielding |
| ***Chloris barbata*** Sw. | Poaceae | - | Herb | 8-10 | Seeds and rootstocks | Weed |
| ***Cynodon dactylon*** (L.) Pers. | Poaceae | Durva | Herb | 1-12 | Seeds and rooted slips | Medicinal |
| ***Cyperus rotundus*** L. | Cyperaceae | Mutha | Herb | 6-10 | Seeds and rhizomes | Medicinal |
| ***Digitaria ciliaris***(Retz.) Koeler | Poaceae | - | Herb | 8-10 | Seeds | Weed |
| ***Dioscorea alata*** L. | Dioscoreaceae | Ban alu | Climber | 8-12 | Root stocks | Medicinal |
| ***Dioscorea bulbifera*** L. | Dioscoreaceae | Khamalu/ Chuprialu | Climber | 9-12 | Root stocks | Medicinal |
| ***Eleusine indica*** (L.) Gaertn | Poaceae | - | Herb | 8-11 | Seeds and rootstocks | Weed |
| ***Gloriosa superba*** L. [Fig.-3] | Liliaceae | Ulatchandal | Climber | 8-12 | Seeds and root stocks | Ornamental |
| ***Musa paradisiaca*** L. | Musaceae | Pakakala | Herb | 12-6 | Rhizome | Fruit yielding |
| ***Phoenix sylvestris***(L.) Roxb. | Arecaceae | Khejur | Tree | 12-6 | Seeds | Fruit yielding |
| ***Scindapsus officinalis*** (Roxb.) Schott | Araceae | - | Climber | 7-9 | Seeds | Ornamental |
| ***Typhonium trilobatum***(L.) Schott | Araceae | Ghet kachu | Herb | 6-10 | Rhizome | Ornamental |
| **PTERIDOPHYTES** | | | | | | |
| ***Adiantum lunatum*** Burm.f. | Petridaceae | - | NR | NR | NR | Weed |
| ***Dryopteris filix-mas*** (L.) Schott | Dryopteridaceae | - | NR | NR | NR | Weed |
| ***Pteris longifolia*** L. | Petridaceae | - | NR | NR | NR | Weed |

** Fast growing invasive alien species*

**Table-2**: Numerical break up of taxa occurring at Pathra and its vicinity,Paschim Medinipur, West Bengal.

| Type | Family | Genus | Species |
|---|---|---|---|
| Dicots | 35 | 76 | 81 |
| Monocots | 7 | 10 | 13 |
| Pteridophyta | 2 | 3 | 3 |
| **TOTAL** | **44** | **89** | **97** |

**Table- 3:** Dominant families with number of species

| Sl. No. | Families | Total No. of genus | Total No. of species |
|---|---|---|---|
| 1. | Asteraceae | 9 | 9 |
| 2. | Fabaceae | 6 | 7 |
| 3. | Euphorbiaceae | 5 | 5 |
| 4. | Amaranthaceae | 3 | 4 |
| 5. | Acanthaceae, Asclepiadaceae & Poaceae | 4 | 4 |

**Table-4**: Habit groups along with their numbers

| Sl. No. | Type | Total numbers |
|---|---|---|
| 1 | Herbs | 37 |
| 2 | Climbers | 34 |
| 3 | Shrubs | 9 |
| 4 | Trees | 14 |
| 5 | Pteridophytes | 3 |
| | **Total** | **97** |

## OBSERVATIONS AND DISCUSSION

Recent floristic survey at Pathra and its adjoining areas revealed the record of 97 species under 89 genera and 44 families. Attempts have also been taken to record the habit groups, flowering fruiting periods, mode of their propagations, dominant families along with grouping of plants for their common uses (Table-1).

With proper enumeration of the recorded 97 species it was found that herbs acquired the highest position in the list i.e. 37 species then followed by climbers (34 species), trees (14 species), shrubs (9 species) and pteridophytes (3 species).

In general the survival of the species is carried out by the process of reproduction. They can reproduce following different methods. From the above survey report it was shown that out of 97 species, only 66 species reproduces by the agent of seed; 9 species through seeds and root-stocks; 9 species by seeds and stem cuttings; 2 species each by the process of stem cuttings, rhizomes, root-stocks; stem and other 1 species each through stems and leaf-cuttings; seeds and rhizome. The reproductive process of 3 pteridophytes is yet to be ascertained.

In view of the selection of 5 families, out of recoded 44 families in respect of their highest number of genus and species, it was found that in the family Asteraceae scoredthe highest (9/9) followed by Fabaceae (6/7); Euphorbiaceae (5/5); Amaranthaceae (3/4) and Acanthaceae, Asclepiadaceae and Poaceae (4/4).

Regarding the flowering and fruiting period it was found that 46 species showed it in

January; 42 species in February; 44 species March; 47 species in April; 49 species in May; 52 species in June; 57 species in July; 69 species both in August, September; 73 species in October; 67 species in November and 68 species in December. The extended flowering and fruiting periods i.e. throughout the years was exhibited bythe species like *Acalypha indica* L., *Alternanthera sessilis* (L.) R. Br. *ex*DC., *Amaranthus spinosa* L., *Amaranthus viridis* L., *Canscora diffusa* (Vahl) R.Br. *ex* Roem.& Schult., *Croton bonplandianus* Baill., *Cynodon dactylon* (L.) Pers., *Eclipta prostrata*(L.) L., *Hybanthus linearifolius* (Vahl) Urb., *Lantana camara* L., *Mikania micrantha* Kunth , *Parthenium hysterophorus* L., *Tridax procumbens* (L.) L., *Vernonia coerulea* J. Kost..From the above observation it was clear that most of the species showed flowering and fruiting activity among the months of August to December and lowest activity among the months of January to March.

Regarding the types of plants and their common uses it was revealed that 40 species are weeds; 31 species are medicinal; 10 species are ornamentals; 6 species are fruit yielding; 4 species are timber yielding; 2 species are religious and rests 4 species, 1 species each can be utilised as fodder, poisonous, dye yielding and in the jewellery system.

Out of 97 species listed species, only 18 species arealien, they are *Argemonemexicana*L., *Cardiospermum halicacabum* L.,*Cascabela thevetia* (L.) Lippold , *Chloris barbata* Sw., *Clitoria ternatea* L., *Croton bonplandianus* Baill., *Digitaria ciliaris* (Retz.) Koeler, *Eupatorium odoratum* L., *Ipomoea quamoclit* L., *Jatropha gossypiifolia* L., *Lantana camara* L., *Lysiloma latisiliquum* (L.) Benth., *Mikania micrantha* Kunth, *Parthenium hysterophorus* L.,*Passiflora foetida* L., *Senna tora* (L.) Roxb., *Solanum sisymbriifolium* Lam., *Tridax procumbens* (L.) L..

Fig.-1: *Passiflora foetida* L. Fig.-2: *Lantana camara* L.


Fig.-3: *Gloriosa superba* L. Fig.-4: *Argemone mexicana* L.

## CONCLUSION

Pathra is a village temple under Gram Panchayat and historically it was an important hub for Hindus, Jains and Buddhists from 8th Century to 12th Century. From the vegetation point of view Pathra is a treasure trove, there are growing as many as 97 floristic components ultimately constitute a flora of its own. The denizensof Pathra and its adjoining areas have religious beliefs, taboos, socio-cultural peace over this place from time immemorial.This floral diversity can be considered as "treasure house" due to its bio-resource, bio-prospection and ultimate source of information for the conservators, academicians and researchers. The floristic elements (plants) are also the source of medicines, food, fodder, fuel, pollinators, keystone species, water conservation, nutrient cycle monitoring, soil conservation and ultimately conservation of germplasm of wild relatives. At present the minds of the young people are changing towardssuch type of biodiversity pockets.Most of these vegetations pockets are now in threatened condition. So the first and foremost point is the massive involvement of the local people to conserve our local vegetation structure (local plant biodiversity) to ensure the sustainable development through extensive local area exploration, documentation of floral components (recommendable database) and their utilisation to fulfil our needs as well as for the interests of future generation. It appears from the study that Pathra and its adjoining areas are rich in floral diversity and human activities as a visiting place has not affected its floristic components to a great extent. Above all floristic as well as conservation point of view such pockets should be maintained scientifically from their gradual disappearance through grazing, natural calamities etc giving topmost priority.

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# Major Pestiferus Snails and Slugs of Paschim Medinipur: an Account on Diagnosis, Damage and Control

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ABSTRACT

Land snails and slugs form an important component in the forest ecosystem. In terms of number of species composition, the phylum Mollusca, to which land snails and slugs belong, is the largest phylum after Arthropoda. Mollusca serves unique ecosystem services including recycling of nutrients and they also serves as a prey for small mammals, birds, snakes and other reptiles or even carnivorous molluscs. However, land snails have the largest number of species extinctions, compared to any other taxa. Till date 1,129 species of land snails are recorded from IndianTerritory. Present study is a regular survey work performed during January, 2019 to December, 2019 on pestiferous, terrestrial mollusc of Paschim Medinipur district. Result depicts that two land snails namely *Achatina fulica* and *Macrochlamys indica* and one slug,*Laevicaulis alte* are major pest on crops, orchards and nurseries of flower and ornamental plant of Paschim Medinipur district. Beside record, their nature of damage and adopted control measures are being reported here.

## INTRODUCTION

Molluscs are the second largest group of animals in the world and mostly distributed in the tropical region. Molluscs exploit all most all habitat of the world except icecaps as because they are poikilothermic animal. They are commercially viable, nutritionally enriched and ecologically sound group of valuable organism, play important role in ecosystem service. Land snails and slugs include several distinct terrestrial gastropods and belong to the second largest phylum after arthropods in terms of number of species composition with more than one lakh named species (Lydeard et al. 2004). Terrestrial gastropods constitute about six per cent of the total species on Earth (Clark & May 2002). A large part of molluscan fauna in many tropicalregionsoftheworldisstillpoorly explored.They are an important component of the forest ecosystem by recycling nutrients (Graveland et al. 1994; Dunk et al. 2004) and are prey for a number of small mammals, birds, reptiles, amphibians and other invertebrates, including carnivorous mollusc (Deepak et al. 2010). Terrestrial mollusc population is largely depends upon the soil calcium content, moisture

contain and $P^H$ of the soil. In calcium poor habitats land snails can form an important source of calcium for other animals. Terrestrial molluscs also serve as an indicator of ecological conditions, and are very sensitive to climatic change (Shimek 1930; Simone 1999; Èejka & Hamerlík 2009). Thus, they are useful for reconstructing past environments (Bar-Yosef Mayer 2002; Gümü° 2009).

Literature survey shows that a few workers like, Blanford (1863; 1870 and 1880) gave contribution to Indian Malacology by giving description of new genera and species of terrestrial mollusc from various parts of India. Preston (1915) gave description of freshwater gastropods, Annandale (1919) and Annandale and Prashad (1919) gave description of some freshwater molluscs from Bombay Presidency in Records of Indian Museum, Hora (1925) gave information of molluscs from Western Ghats, in Bombay Natural History Society. Tonapi and Mulherkar (1963) and Tonapi (1971) gave contribution to studies on freshwater molluscs of Poona district of Maharashtra in Bombay Natural History Society. Subba Rao andMitra (1979), Subba Rao (1989) and Surya Rao et al. (2002) gave description of freshwater snails from Poona district of Maharashtra and other part of India in Zoological Survey of India, Kolkata. Indian Malacology was pioneered by William Henry Benson (1803-1870), who contributed significantly to our knowledge on Indian land snails in the mid-19th century (Naggs, 1997). From India 1488 species of snails and slugs belonging to 26 families and 140 genera have been recorded (Ramakrishna and Mitra, 2002 and Madhyastha, et al., 2004). The molluscan fauna of West Bengal has not been studied thoroughly and the available information on the terrestrial molluscs is scattered. Molluscs being epidemic remain undiscovered or under described, partly because of insufficient exploration and partly because of their often minute size (Madhyastha, et al., 2004). Land snail research, in India has truly been at snail's pace (Aravind, et al., 2005; 2008; 2010 and Aravind and Naggs, 2012). More recent studies in India, have mainly concentrated on inventorying regional snail faunas (like state or protected areas), checklist of district fauna (Chanda, 2017) and less on species description, ecology and conservation. Little information is available on species limits, distribution ranges and patterns of diversity. Recent analysis of Indian land and freshwater molluscan literature has confirmed that there are hardly some studies found on the ecology and conservation of Indian land snails compared to the wide range of historical literature available on taxonomy (Aravind, et al., 2010). In the period of intensive study, there was a drastic decline in studies on Indian land snails. There are no studies on the population status (except, Rout, 1986), phylogeny and taxonomic revision of different families or genera of Indian land snails (Sen, et al., 2012). Present report of pestiferous snail and slugs of Paschim Medinipur is certainly being the addition of regional knowledge of macro-faunal diversity of the area under study.

## MATERIALS AND METHODS

Specimens were collected from selected three Blocks Garbeta-1, forest best ecosystem; Midnapur sadar, municipal ecosystem and Debra, agriculture based ecosystem are tabulated with latitude and longitude in table-1.

**Table-1.** Study Sites in Paschim Medinipur District

| Sl. No. | Name | Longitude & Latitude |
|---|---|---|
| 1 | Garbeta-1 | 22̊ 51′36″N&87̊ 21′36″E |
| 2 | Midnapur Sadar | 22̊ 25′53.6″N&87̊ 19′5.48″E |
| 3 | Debra | 22̊ 22′8.6″N&87̊ 33′15.9″E |

Specimens were collected by hand picking in the early morning and preserved in 70% alcohol in the department of Zoology, Raja N. L. Khan Women's College (Autonomous), Midnapur, Paschim Medinipur, West Bengal. Identification of species is done following existing literature (Towie, A. 1989; Aravind, et al., 2005; 2008; 2010 and Aravind and Naggs, 2012).

## RESULT AND DISCUSSION

During the present study author identified three major and common molluscan pests in the study area. Two snail species namely *Achatina fulica* (Ferussac, 1821) and *Macrochlamys indica* Benson, 1832 and one slug, *Laevicaulis alte* (Ferussac, 1822) are the major molluscan pest in the study area. Their systematic position, diagnosis, damage and control measure are given bellow.

### *Achatina fulica* (Ferussac, 1821)

*Achatina fulica* is a giant land snail, native to East Africa, fast growing, polyphagous plant pest. It has been introduced from its native place to many part of the world as a commercial food source for human consumption, pisciculture and fodder for livestock management.

**Systematic Position**

Phylum: Mollusca

Class: Gastropoda

Superfamily: Achatinoidea

Family: Achatinidae

Genus: Achatina

Species: *A. Fulica* (Ferussac, 1821)

**Diagnosis**

*Achatina fulica* has a narrow, conical shell, which is twice as long as it is wide and contains 7 to 9 whorls when fully grown (Fig. 1). The shell is generally reddish-brown in colour with weak yellowish vertical markings but colouration varies with environmental conditions and diet. A light coffee colour is common. Adults of the species may exceed

20cm in shell length but generally average about 5 to 10cm. The average weight of the snail is approximately 32 grams.

**Fig. 1:** A. Fulica from RNLKW College garden, Midnapur.

**Damage**

*Achatina fulica* is one of the most destructive pests affecting subtropical and tropical areas, causing large damages to farms, commercial plantations and domestic gardens. It can also be found on trees, decaying material in decomposition and next to garbage deposits. Furthermore, *A. fulica* could be an intermediate host of *Angiostrongylus costaricencis*, the etiological agent of abdominal angiostrongylosis and its dispersion could imply a possible risk of transmission of this disease (Mead, 1995). The Giant African Snail (*Achatina fulica*Bowdich, 1822) promotes substantial ecological and economic impacts in areas where it has been introduced (Raut and Barker, 2002). This herbivorous mollusc isdestroying significant amount of vegetable loss in field of Garbeta-I and Midnapur Sadar blocks of Paschim Medinipur District. It mainly feed on the foliages of divers cultivable vegetables of the study area.

***Macrochlamys indica* Benson, 1832**

The first complete description of this species was given by Godwin- Austen and the name *M. indica* is accepted (Blandford and Godwin-Austen, 1908). *Macrochlamys indica* is considered to represent a potentially serious threat as a pest, an invasive species which could negatively affect agriculture, natural ecosystems, human health or commerce.

**Systematic Position**

*Macrochlamys indica*

Phylum: Mollusca

Class: Gastropoda

Superfamily: Helicarionoidea

Family: Ariophantidae

Genus: Macrochalmys

Species: *M. Indica* Benson, 1832

**A**

**B**

**Fig. 2:** *Macrochlamys indica*; A, from Garbeta-I and B, from Midnapur town.

**Diagnosis**

The shell is perforate, depressed, smooth, polished throughout, translucent, pale brownish tawny, not distinctly striated, but with microscopic longitudinal impressed lines, slightly flexuous and not close together (Fig. 2). The spire is low, conoid. The structure is slightly impressed. The shell has 5.5 whorls that are slightly convex above. The last whorl is not descending. The last whorl is rounded at the periphery and moderately convex beneath. The aperture is slightly oblique and broadly lunette. The peristome is thin in one plane, with columellar margin is curved, oblique, and never quite vertical, carried forward and briefly reflected above. The width of the shell is 16-18.5 mm. The height of the shell is 8.5 mm.

**Damage**

Machrochlamys indica (Benson, 1832) is considered to represent a potentially serious threat as a pest, which could negatively affect agriculture, natural ecosystems, human health or commerce. It is polyphagous in nature and feed on several vegetables in home garden and nurseries. It eats whole leaf and stem of tender shoots and make hole on mature leaf of vegetables (fig. 2). Macrochlamysindica is common in wild areas where they thrive well on the leaves and flowers of the wild plants also. During the present study this species has been recorded from Garbeta-I and Midnapur Sadar Blocks.

***Laevicaulis alte* (Ferussac, 1822)**

*Laevicaulis alte* is considered a serious agricultural pest in India where it is invasive. It was first described by Férussac (1821) as *Vaginulus alte* from Central Africa. Smiroth (1914) changed it to *Laevicaulis alte*. Common name of the species is tropical leatherback slug or black slug in India.

**Systematic Position**

Phylum: Mollusca

Class: Gastropoda

Superfamily: Veronicelloidea

Family: Veronicellidae

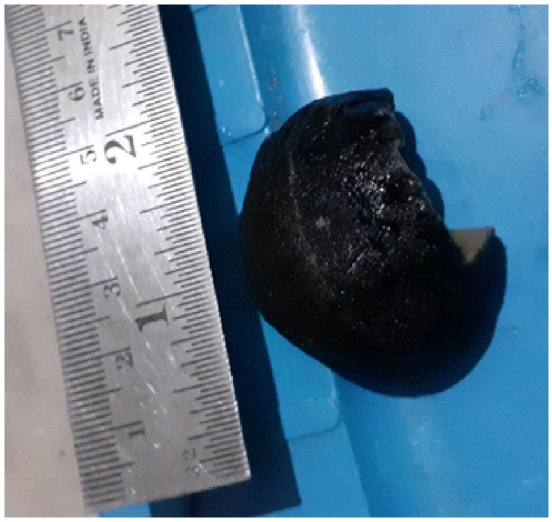
Genus: Laevicaulis

Species: *L. alte* (Ferussac, 1822)

**Diagnosis**

*Laevicaulis alte* is a round, dark-coloured slug with no shell, 7 or 8 cm long (Fig.3), when fully stretched. Its skin is slightly tuberculated. The central keel is beige in brownish colour. This

slug has a unique, very narrow foot; juvenile specimens have a foot 1 mm wide and adult specimens have a foot that is only 4 or 5 mm wide. The tentacles are small, 2 or 3 mm long, and they are only rarely extended beyond the edge of the mantle.



**A**

**B**

**Fig. 3:** *L. alte* ; A, Dorsal and B, ventral view, collected from Debra

**Damage**

Most slugs **feed at night,** and the **slime trails**, if present, can alert you to the level of activity. Damage is usually most severe during warm humid periods. Slugs can use their rasping tongues to make holes in leaves, stems, buds, flowers, roots, corns, bulbs and tubers of many plants. There are many control options available for slugs and snail but despite this they remain a persistent pest. Slugs can make a meal of a wide range of vegetables and ornamental plants, especially seedlings and other soft growth. Hosts, delphiniums, dahlias, sweet peas and tulips are regularly attacked by slugs in the study area. In the vegetable garden peas, beans, lettuce and potato tubers are often damaged. It is a most serious pest in the luminous soil of the Kansaboty River bank of Debra Block of Paschim Medinipur District.

**General control measures of snails and slugs in the study area**

Farmers of the study area follows mainly two type of control measures namely physical and chemical measures and their preference is to the second one.

**Physical control**

Hand collection with subsequent squashing of the slugs and snails is the oldest mechanical methods (Mahrous et al., 2002). Farmers of the study site simply collect and chopped the animal to reduce its population. Hand-picking during night, when the slugs and snails have left their hiding places was found effective (Hamir, 2010). Some of the farmers use sodium chloride (common salt), an effective dehydrating agent

as barrier on snail infested area. Practice of collection of the snails daily andkilling them in strong solution of common salt or in boiling water is a common practice to destroy the molluscan pest population. Some farmers also use cattle salt, caustic soda and dry quick lime as protective barriers against snail and slug infestation.

**Chemical control**

Majority of the farmers of the study area uses metaldehyde, methiocarb (Mesurol), common salt or combinations of these chemicals as effective molluscicides. Metaldehyde stimulate the mucous gland which cause excessive sliming and leading to death due to dehydration (Henderson, 1970; Henderson and Triebskorn, 2002; Abd El-Wakeil, 2005). Methiocarb was found more poisonous than metaldehyde against slugs (Getzin and Cole, 1964; Abd El-Wakeil, 2005). Some of farmers also use urea as toxic manure for snail and slug control of the study site.

## CONCLUSION

Consequently, molluscs represent some of the most thoroughly studied pest species, with a substantive body of literature relating to population and behavioural ecology and control. Yet molluscs are also among the most intractable of pests (Barker and Watts, 2002). The pest gastropods not only directly damage the agricultural crops in the field but also lower the quality by soiling with slime and faeces. The snail affected portions of agricultural product are contaminated by rotting agents such as bacteria and fungi, which lead to further damage of fruits and vegetables in storage.Such commercially important snail and slugs are gradually increasing and infesting newer fields in the study area need effective control measure for safe guarding the crops and vegetables. Slugs grow registrant to molluscicides very rapidly and difficult to control as reported by local people. To conclude, it can be said that land mollusc density and richness were associated with abiotic factors such as rainfall and humidity as well as characteristic of soil leaf litter distribution on ground and biotic factors such as vegetation cover and various anthropogenic pressure such as land use, cattle grazing etc. So from control point of view proper management of vegetation and land use pattern should be considered to maintain a steady state of the population. Therefore, further research is needed to develop effective control measure for terrestrial molluscan pests in the area under study.

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# Enumeration, Isolation and Identification of Crustacean Surface Attachment Bacteria

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ABSTRACT

The presence of microbial epibionts on marine and freshwater zooplankton (crustaceans) and other invertebrates have been documented frequently. The ecological context and impact of these intricate relationships are not well understood so far. Recent studies have examined the interrelationship of bacterial epibionts on freshwater crustaceans.Presently,eighty-three bacteria were isolated from three different aquatic environmental study areas and seven of them were primarily screened on account of their colony characters, morphological and biochemical character. Among the seven isolates, SA-5 and SA-7 strains showed maximum different morphology with respect to others isolates.Molecular characterization of two isolates indicates that SA-5 and SA-7 are *Aeromonas sp.*.The present study provided the baseline information regarding the pathogenic and non-pathogenic bacteria in association with crustacean zooplankton. Present study also tries to find out the differences and commonalities across epibionts in the realm of epibiont-host nutrients cycling which in turn to generate relevant hypothesis in the context of host microbial interaction.

## INTRODUCTION

Several human pathogens and fecal-pollution indicators may persist as viable organisms in natural environments, owing to their ability to activate different types of survival strategies. These strategies include adhesion on both abiotic and biotic surfaces and the entrance to the so-called viable but non-culturable (VBNC) state (Coveney et al., 1977; Hickman et al., 1977). In an 18-month survey for the detection of enterococci in both lake water and seawater(Signoretto et al., 2004) have shown that *Enterococcus faecalis* was detected mostly bound to plankton and in the VBNC state.Bacterial function in aquatic systems encompasses two major processes: (1) the degradation of organic matter; and (2) the regeneration of soluble nutrients to the biological system (McCoy et al., 1969). Organic matter emanating from the zooplankton and phytoplankton of lakes constitutes a sizable fraction of the organic materials pool which bacteria utilize (Ormerod et al., 1978). Detailed examinations of the relationships between plankton and bacteria are necessary to determine the various pathways of organic matter flux. Bacteria-phytoplankton interactions have been the focus of many studies (Henrici

*et al*., 1938;Niewolak *et al.*, 1971; Nalewajko *et al.*, 1972; Rieper *et al*., 1976). Phytoplankton provides organic substances for the heterotrophic activities of bacteria by excreting carbonaceous materials during growth and by releasing them through lytic processes when the population undergoes senescence. Studies concerning zooplankton and bacteria have been concerned primarily with the utilization of bacteria as food for (Coveney et al., 1977). Investigators interested in the distribution of bacteria in river pond and cannels have demonstrated the importance of surfaces in the ecology of lake bacteria; in fact, some of the apparently high concentrationsof bacteria encountered may be surface-induced (McCoy et al., 1969&Hillbricht-Ilkowska *et al*., 1966). Microorganisms, both sessile and free-swimming, provide surfaces that can support microbial films (Sieburth *et al*., 1976). The objective of the present experimental research effort was to study the interrelationship ofbacterial community of zooplankton (copepods and cladocera), including the distribution and relative abundance of these bacteria from investigation site of Midnapore, West Bengal, India.

## MATERIALS AND METHODS

### Study site:

The present investigation was carried out from aquatic ecosystems of freshwater lotic zone, and around certain wetlands of Midnapore, PaschimMedinipur, West Bengal, India. The samples were collected from six study sites, two river sites located outside of the town which are used for domestic and agricultural purpose, two ponds located at heart of the town which are heavily used by colonies people of the town and two cannels(present within town), used mainly municipality sewage drainage system.

### Collection of the environmental samples:

Water and plankton sample were collected from the six-study site in the month, of june, 2018. Zooplankton samples were collected from subsurface zone of the five sub- sampling sites in the water body of sampling site, using Nylobolt plankton net (25 µm mesh size). A total hundred liters of water were filter from each sampling site (Midya *et al*., 2018).

### Isolation and screening:

The zooplankton were identified to the lowest taxonomic level following standard references, Emir (1994), Ruttner-Kolisko (1974), Segers (1995), Nogradyand Pourriot (1995), and DeSmet (1998) for Rotifera and Kiefer (1978), Reddy Ranga (1994), and Dussart and Defaye (2001). Quantitative study of zooplankton was done under a phase contrast microscope with the help of Sedgewick rafter counting cell and the values was expressed in number per litter.

Fresh plankton sample were further concentrated and homogenized. These concentrated samples were incubated in alkaline peptone water (APW) and incubated at 37°C for 18-24 hrs. Pellicle growth from the surface of APW was subsurface on to selective chitin agar media and incubated at 37°C for 48-72hrs (Midya *et. al*., 2019)

**Effect of pH, temperature and salt concentration on native isolates:**

The effects of pH on newly isolated bacterial growth were determined by adjusting the pH of the nutrient agar media at different level of pH (4.0 -12.0). The effect of temperature on bacterial growth was determined by incubating inoculated medium at different temperatures (15, 18, 22, 28, 35, 37, 40, 50 and 55$^{o}$C) in nutrient broth through separate incubation. In order to determine the effect of salt concentration on bacterial growth the selected bacterial strain was grown in presence of varied level of NaCl (1-10%) with nutrient agar media (Kuddus M.S., *et al*., 2013)

**Tests for arginine, lysine, and ornithine decarboxylases:**

Inoculate tube of each of 3 decarboxylase broth media (HiMedia, India: having composition (g/ l):Peptic digest of animal tissue- 5.0, Beef extract- 5.0, Dextrose- 0.5, Bromocresol purple- 0.01, Cresol red- 0.005, Pyridoxal- 0.005, Final pH- 6.0 ±0.2, at 25°C] with loopful of TSA culture. After inoculation, add 10 mm thick layer of sterile mineral oil to each tube; include basal medium control. Replace caps loosely and incubate 24 hr at 35°C. Examine every 24 hr for 4 days.

**Effect of substrate supplementation on bacterial growth:**

The isolated bacteria were grown in phenol red broth(HiMedia), India: having composition (g/ l):Proteose peptone- 10.0, Beef extract- 1.0, Sodium chloride- 5.0, Phenol red- 0.018, Final pH- 7.4±0.2, at 25°C], supplemented with four different substrates sugar disc such as fructose, maltose and lactose (disaccharide) respectively at 37°C for 96 hrs (Gerhardt, 1994 & Kuddus M.S., *et al.*, 2013).

**Isolation of genomic DNA for 16S rRNA and PCR amplificationwith specific primer for molecular identification of the isolates:**

Genomic DNA was extracted from the bacterial cells by using the standard phenol–chloroform method (Ruzzante, D.E. *et al.*, 1996).Extracted DNA was checked for its quality by agarose gel electrophoresis on UV transilluminator and concentration by nanodrop. The extracted DNA was amplified with 16S rDNA specific primer pair, Forward primer (27F) 5¹-AGAGTTTGATCMTGGCTCAG-3¹, and Reverse primer (1492R) 5¹-TACGGYTACCTTGTTACG-ACT-3¹ (Lane et al. 1991).Each 25 µ1 reaction mixture contained: 1.5 µ1 of 10 X Taq polymerase buffer contains (100 mMTris (pH 9), 500 mMKCl , 15 mM MgCl2 , 0.1% Gelatin)(Genei) , 1.5 µ1 of dNTP mixture ((dATP, dCTP, dGTP, dTTP10 mMconectration) (Genei),Enzyme: Taq polymerase-0.5 µ1 (3 U/ìl) (Genei), 1 ìl each primers (5pM/ ìl) (Eurofin), Template DNA-2µ1 (100 ng) and 17.5 µ1sterile molecular biology Grademilli-Q water (Hi-media). The reaction conditions were 94°C for 5 min, 94°C for 45 s, 56°C for 1 min, 72°C for 1 min (for 35 cycles), and then 72°C for 10 min and final holding at 4°C. The PCR-amplified product

was analyzed on 1.5% agarose gel containing ethidium bromide (0.5 mg/mL) and 1 kb DNA molecular weight marker and documented using a gel documentation system. The PCR amplicon for the partial 16S rDNA gene was further processed for sequencing. Sequencing was carried out using the same set of primers in both the directions to check the validity of the sequence. Sequencing of the 16S rDNA gene amplicons were done by outsourcing (Scigenom, Kochi). After that the homology of the partial 16S rRNA gene sequence of these two isolates was analyzed using the BLAST algorithm in Gen-Bank (http:// blast.ncbi.nlm.nih.gov/Blast.cgi). Only the highest-scored BLAST result was consideredfor identification.

## RESULTS & DISCUSSION

### Planktons biodiversity and microbial load in study area:

In this present study the zooplanktons number in different study area were reveals that the number of copepoda was higher than the cladosera. In case of cannel water both average number and standard deviation of copepoda and cladocera were 69.40 & 1.13; 50.30 & 0.93, respective Biodiversity of copepoda and cladocera were to much higher in cannel water with respect to pond and river water aquatic systems. But in case of river water average numbers of copepoda decrease, where the average number of cladocera is lowest in pond water aquatic systems (Fig-1). Length of copepod and cladocera were 906.96 µm 1848.84,respectively (Fig-2). Dynamics of culturable microbiological parameter was interestingly varied in different auatic system. Microbial abundance i.e.the culturable total bacterial Colony Forming Unitper ml (CFU/ ml) was higher in cannels aquatic($5.61 \times 10^5$) sysytem than pond ($5.34 \times 10^5$) and river ($5.52 \times 10^5$) aquatic systems (Fig-1). So, the present study reveals that the microbial and planktons abundance both are higher in cannel aquatic system. A number of eighty-three bacteria were isolated from three different aquatic environmental study area and seven of them were primarily screened on account of their colony characters (Table-1). Among the seven isolates, SA-5 and SA-7 strains showed maximum different morphology with respect to others isolates. All the isolates were pure cultured and kept in refrigerator for further use.

### Bacterial growth depends on pH, temperature and salt concentration*:*

Comparison of pH of these two strains showed that pH-7 was optimum for strain SA5 and SA7, but it's favor for growth between pH 6–8 and pH 5–8, respectively. At higher the pH values, we may presuppose that the pooled enzymes and proteins were not in same structural configuration that's why further enhance the growing environment, for that one which has diminished the bacterial growth.Generally, an increase in temperature will increase enzyme activity.But if temperatures get too much high then enzyme activity will diminish and the protein (also enzyme) will denature. On the other hand, lowering temperature will decrease enzyme

activity.Every bacterial species has a specific growth temperature requirement which is largely determined by the temperature requirements of its enzymes. In this study, both isolates were grown in mesophiles (i.e. 15 to 45ÚC) condition.Water passes out of a bacterium so as to balance salt concentrations on each side of its cell membrane. Without water, bacterial proteins such as enzymes cannot function and eventually the cell collapses in on itself. Some bacteria can tolerate salt; they are called as halotolerant bacteria. Certain strains of *Staphylococcus*, *Vibrio* and *Aeromonas* were responsible for infections, blood poisoning, and even death, these are also halotolerant. These pathogens have a salt alert system that uses sponge-like molecules to prevent water loss. Here both strains were growing simultaneously up to 6% NaCl, after that the growth rate was diminish with increasing the salt concentration, these strains are also called as salt tolerant bacteria (Fig-3).

**Biochemical efficacy of native isolates:**

Both the native isolates strains (mentioned above) were examined for evaluating their various types of biochemical efficacy. It was found that both the strains were Gram negative, non-spore forming, motile, catalasepositive, indole and methyl red test positive, also capable to hydrolyze starch agar medium.But both the isolates were Voges-Proskaure test negative and can't utilize Urease, also do not produce $H_2S$ gas. On the other contrary varied results were observed incase of citrate utilization. Strain SA-5 can utilize citrate but strain SA-7 can't utilize citrate.(Table-2).Utilization of different sugar and oxidation of mannitol results showed that both isolates are utilizing all sugar and also oxidize mannitol.

**Molecular identification of bacteria:**

The isolates SA-5 and SA-7 were identified as *Aeromonas sp.* through biochemical test. The strains were rod shaped, Gram negative, motile and non-spore forming (Table 2). The identity of the strains was further confirmed by 16S rDNA analysis. The sequences of the 16S rDNA region of twelve isolates have been submitted to the NCBI database. The DNA sequences of 16S rDNA region were searchedfor homology with Basic Local Alignment Search Tool (BLAST) against the nucleotidedata base maintained by National Centre for Biotechnological Information (NCBI), NIH,USA. Isolates are used in the study exhibited 97 to 100% sequence similarity to the *Aeromonas sp.* vailable in NCBI database with lowest E-value and maximum query coverage andmaximum identity (Fig-4).

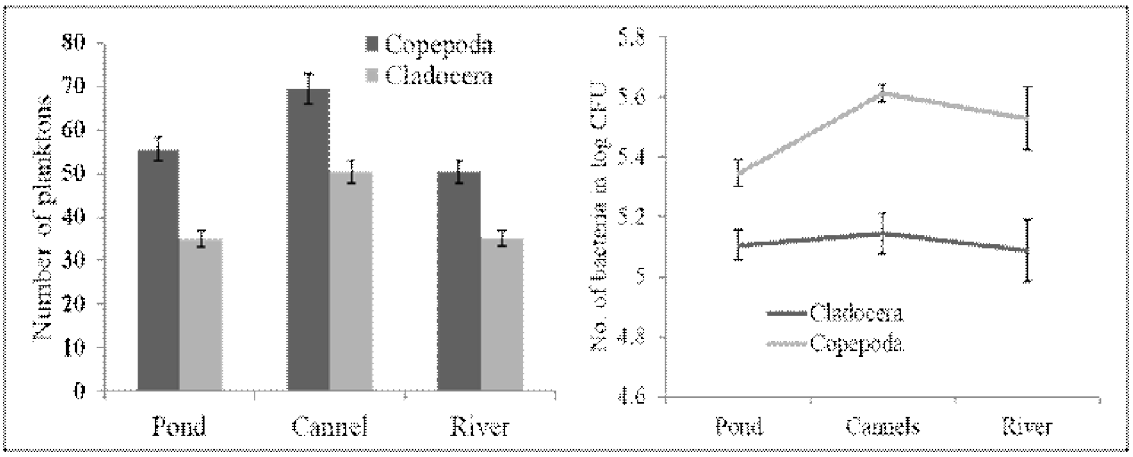


**Fig-1:** Temporal variation in zooplankton composition in different wetlands of study sites.

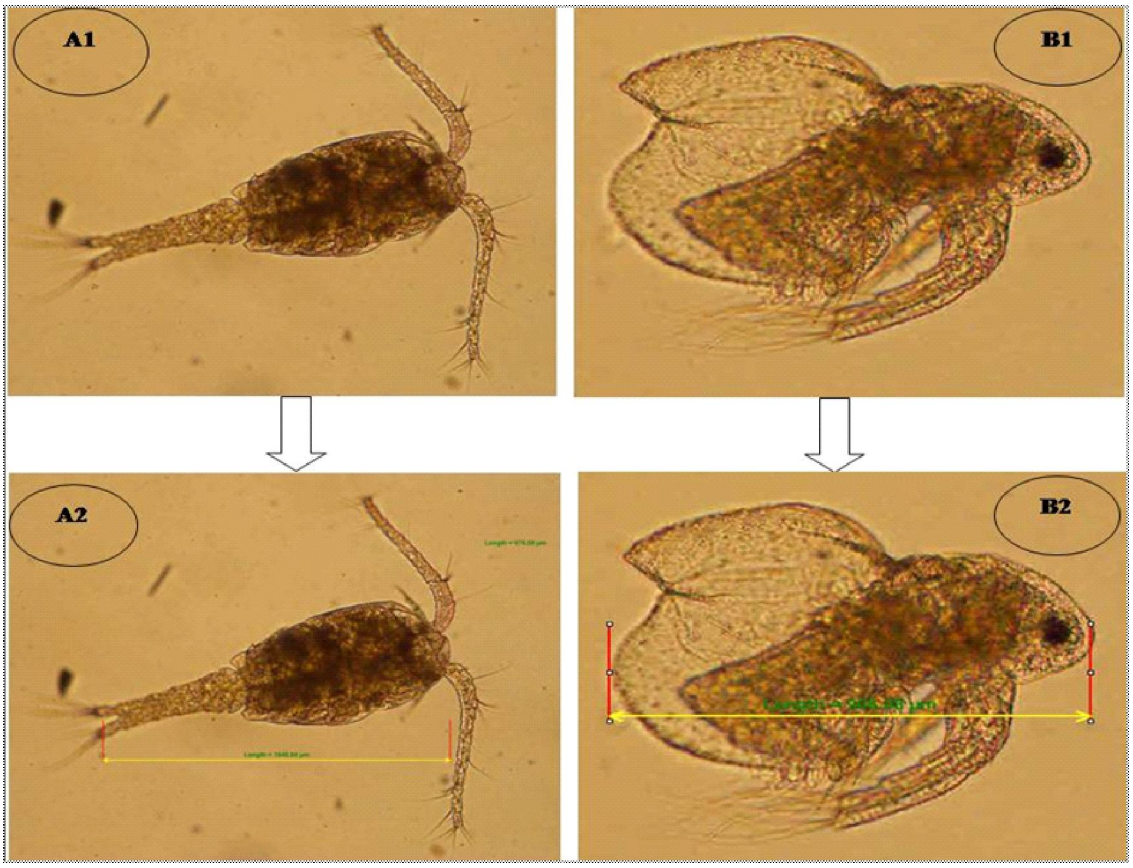


**Fig-2:** Microscopic view and body length of zooplankton (A) & (B)

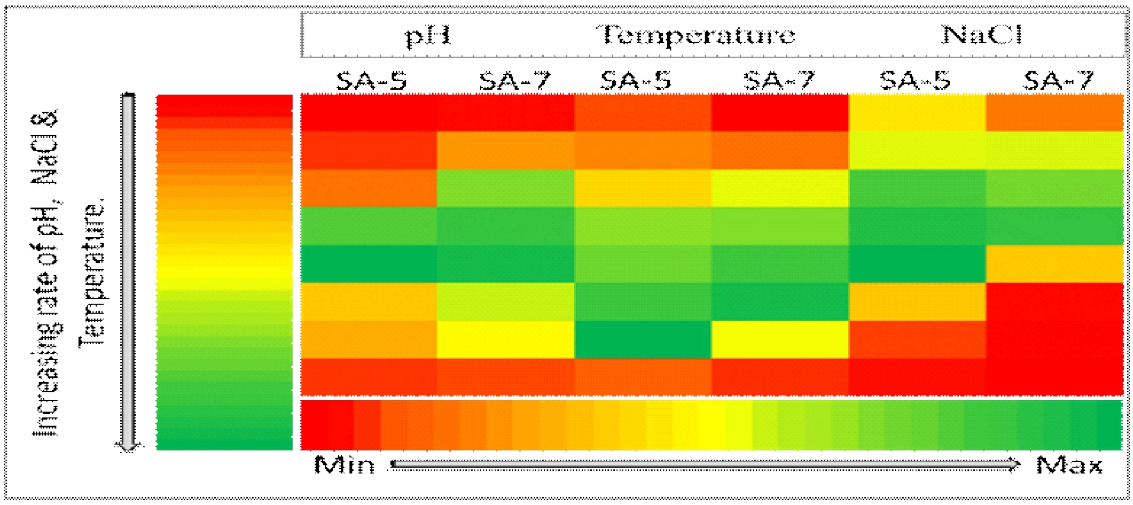


**Fig-3:** Favorable growth conditions of two isolates were obtained in different pH, temperature and NaCl concentration. Heat map generated data reflecting values in several conditions.

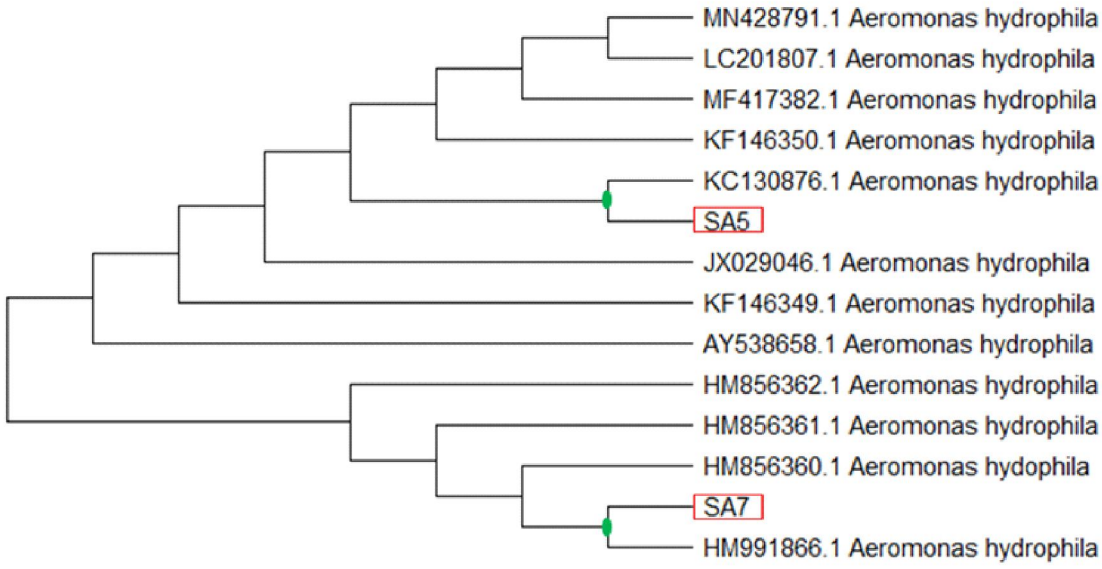


**Fig-4:** The evolutionary history was inferred using the Neighbor-Joining method.Phylogenetic tree of 14 taxa generated by comparing 16S rDNA homology in MEGA-X showing the location of isolates SA5 and SA7. The GenBank accession numbers are shown in parentheses.

**Table-1:** Colony characterization of native isolates.

| Colony properties | SA-5 | SA-7 |
|---|---|---|
| Colony shape and size | Irregular, 2–3 mm | Circular, 2 mm |
| Elevation | Convex | Convex |
| Margin | Undulate, | Entire edge, |
| Gram reaction | Negative | Negative |
| Cellular morphology | Rod | Rod |
| Color | Creamish white | Creamish |
| Opacity | Opaque | Opaque |
| Texture | wrinkled surface | smooth surface |

**Table-2:** Biochemical assay of native isolates, isolated from plankton surface area.

| Biochemical assay | SA-5 | SA-7 |
|---|---|---|
| Utilization of lactose, | | |
| Maltose and cellobiose | +ve | +ve |
| Oxidation of mannitol | +ve | +ve |
| Spore | -ve | -ve |
| Citrate utilization | +ve | -ve |
| Motility | +ve | +ve |
| Urease production | -ve | -ve |
| Catalase test | +ve | +ve |
| Starch hydrolysis | +ve | +ve |
| Indole | +ve | +ve |
| $H_2S$ | -ve | -ve |
| Methyl red | +ve | +ve |
| Voges–Proskauer reaction | -ve | -ve |
| Decarboxylases of arginine, lysine, and ornithine | +ve | +ve |
| Growth at pH | 6–8 | 5–8 |
| Growth at temperature (ÚC) | 15-40 | 18–37 |
| Growth in NaCl | 2–8% | 2–6% |

## CONCLUSION

The result concluded that these two strains SA-5 and SA-7 are novel mesophilic andslightly salt tolerant bacterial strains that have the ability to producedifferent types of enzyme in short time. Both the isolated strains of *Aeromonassp*. have the ability to hydrolysisstarch in optimize temperature and pH.Infection of *Aeromonas* remains among those infectious diseases of potentially severe risk to public health. *Aeromonas* infectious disease outbreaks have to create a hurting awareness of the personal including economic, societal, and also public health expenses associated with the impact of contaminated drinking water in the aquatic environment. The evidences from many scientific reports suggest that the prevalence of diseases of *Aeromonas* infections may be noticeably underestimated in some developing nations and that schedule endemic exposure to waterborne and food-borne pathogens have been occurring more frequently than originally perceived. A variety of factors likely demographical, societal, environmental, and physiological emergence have to play significant roles in enhancing the occurrence of transmission of pathogens to hosts and the rising trend in antibiotics resistance property in some bacteria makes extensive studies on the particular group of bacteria.Previous study suggests that, the *Aeromonas* sp. have fungicidal efficacy. So, these strains may use in agriculture and different industrial purpose in different way.


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# Overview of fish vaccines with focus on nanovaccines

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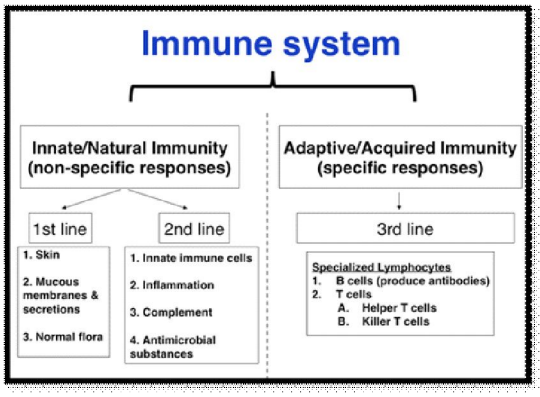
ABSTRACT

The development of a strong aquaculture industry depends, in part, on advances in the diagnosis and treatment of fish diseases. Use of antibiotics has attracted lot of criticism due to the issues like antibiotic residues, bacterial drug resistance and toxicity. In this present scenario, vaccination would be the best alternative to combat bacterial and viral diseasesforlong term protection. The first report on fish vaccination was by David *C. B. Duff and he is regarded as "Father of fish vaccination". In 1942 he reported prolonged use of* chloroform-inactivatedbacteria through feed could protect trout fish from a serious bacterial disease.Vaccination believes in saying "*prevention is better than cure*"& it is a sustainable means of preventing diseases.The major causative agents of infectious diseases in finfish aquaculture include bacteria (54.9 %), viruses (22.6 %), parasites (19.4 %) and fungi (3.1 %)[1].

Traditional vaccines for fish are generally safe but these are often less immunogenic.However, genetic immunization or DNA immunization mediated by plasmid DNA in transfected cellsactsas a bioreactor to produce the vaccine.Some of the advantages of polynucleotide immunization is that it is extremely safe, induces a broad range of immune responses (cellular and humoral responses), long-lived immunity.There is a great need to develop better delivery systems to improve the transfection efficiency in vivo.Recently, biocompatible nanoparticles (NPs) have gained enormous attention as delivery vehicles for vaccines.NPs can be surface-engineered with peptides, proteins, polymers, cell-penetrating peptides, and other targeting ligands, which make them a versatile delivery vehicle for vaccine formulations.




**Basis of fish vaccination (the immune response)**

In multicellular organisms immunity is necessary for *survivability*to resist harmful microorganisms from entering into body. Immunity involves both specific (acquired or adaptive)and nonspecific (innate or inborn)components. The nonspecific components act as eliminators of a wide range of disease causing organisms or pathogens irrespective of their antigenic make-up.Specific or acquired components, involves special class of cells (T & B-cells) responsible for adaptive immunity& these are targeted to particular pathogens.

Innate mechanisms require no previous exposure to the particular antigen- this includes: physical barriers such as skin and mucus layers, specialized cells such as macrophages and natural killer cells and particular soluble molecules such as complement ( a part of the immune system that enhances the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism ) and interferon (group of signaling proteins made and released by host cells in response to the presence of several viruses). The cell-mediated response in fish is similar to that in mammals and relies on the presence of antigen results in a cascade of events that includes cytokine production that regulates or enhances the cellular response.Fish are the most primitive vertebrates to possess adaptive immune system. Adaptive immunity arose early in vertebrate evolution.The innate system is the earliest immune mechanism.Fish have cellular (T mediated) and humoral (B lymphocytes & antibodies mediated) immune responses. Fish and mammals show some similarities and some differences regarding immune function. The two head kidneys (anterior part of trunk kidney) arethe site of formation of blood cellular components( haemopoietic ) and also it is the central organ for immune-endocrine interactions.The thymus is situated near the opercular cavity in teleosts which produces T lymphocytes and B cells.They have complement system & cytokines (are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems) also as a backup of immune system.Immunomodulatory products, including nucleotides, glucans and probiotics when given as dietary supplementation confer increased resistance to fish against viral, bacterial and parasitic diseases and improves the effectiveness of vaccination and the better ability to osmoregulate. The use of these products reduces the need for therapeutic

treatments, enhances the effects of vaccines.

**Three generations of fish vaccines**

Attenuated and inactivated vaccines are identified as the FIRST GENERATION, which use a primary method in their production. Attenuated pathogens, full organisms or inactivated bacterial toxin, which are effectively immunogenic, are used in making these vaccines. This type of vaccine is known as a traditional vaccine. Example of this type iskilled vaccines against *Streptococcus* spp. or/ and *Lactococcus* spp. infections in rainbow trout (*Oncorhynchus sp.*).

SECOND-GENERATION vaccines have subunit elements, recombinant or synthetic proteins, non-protein antigens, and epitopes of different species and strains of pathogens.Subunit vaccines are produced by using the part of the DNA which encodes for the production of the specific antigens to trigger an adequate immune response into another type of organism.Subunit vaccines take advantage of using only antigenic components for vaccination and since subunit vaccines cannot replicate in the host, there is no risk of pathogenicity to the host. One commercial subunit vaccine (peptide; VP2) is currently used in Norway (against IPNV).

THIRD GENERATION vaccines have the principles of immunogenic potential administration of a plasmid containing a gene encoding the antigen, known as genetic vaccines& used since the beginning of 1990s. Different names have been given for this kind of vaccine, such as DNA vaccines, RNA vaccines, and plasmid vaccines.In 2005, APEX-IHN (Novartis/Elanco) for protecting Atlantic salmon against Infectious Hematopoietic Necrosis Virus (IHNV) in British Colombia became the first DNA vaccine licensed for commercial use in aquaculture.

*Flow diagram (in simplified way)*

*of immune mechanisms in the vaccinated fish by expression of the VHSV G* protein in transfected cell's nucleus harboring the DNA vaccine in plasmid*

Uptake of the vaccine plasmid in the nucleus—-àplasmid promoter will drive transcription of the G gene —-àmRNA transcripts —-à exported to the cytoplasm—-àribosomes binding to the mRNA will mediate translation of the polypeptide into the endoplasmatic reticulum (ER)—-à transmembrane G protein will be folded and glycosylated and associate into non-covalently associated trimers while on its way from ER to the Golgi cisternae—-à reaches the cell membrane in its mature form—-àprotruding as spikes into the extracellular space and appear like a virus infected cell—-àendosomal TLR activates &initiate an IFN type I response—-àactivate intracellular antimicrobial programmes and influence the development of innate and adaptive immune responses—à

*Viral hemorrhagic septicemia virus (VHSV) is the causal agent of a serious disease in many marine and freshwater fish species worldwide.the virus has a negative-sense, single-stranded RNA genome.The VHSV G-protein gene DNA (using reverse genetics) vaccine had a high protective efficiency, giving

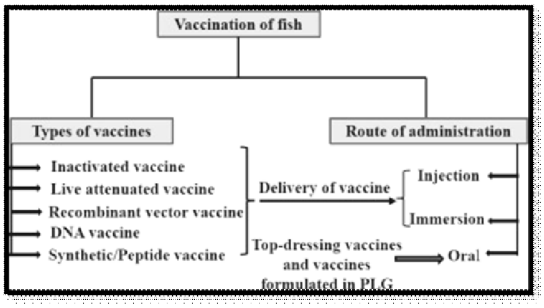
relative percentage survival (RPS) values of at least 93%.VHSV is attenuated using deletion of whole virulent NV gene.Among the components, the glycoprotein (G) is located on the surface of the virus and can induce immune response because of the presence of epitopes.

**Concept & use of vaccines in aquaculture**

The major goal of vaccination is to induce a specific long-term protection against a certain disease. Among various diseases viral disease outbreak is the most serious issue as it may cause severe losses to the economy in the aquaculture industry worldwide.A typical fish vaccine either contains or produces a substance that serves as an antigen. This component then stimulates the immune system within the fish against a particular pathogen.

TRADITIONAL VACCINES

Such vaccines typically come in one of two different types– inactivated vaccines and attenuated vaccines. Both involve giving the fish a dose of the pathogen we want to protect the fish against, but inactive vaccines use dead pathogens whilst attenuated vaccines use live pathogens in a weakened form so they don't actually cause illness.




Attenuated vaccines can provide better immunity than inactivated vaccines, and can also be used to treat young fish (by immersion).However since they use a live pathogen, there is a small risk that some residual virulence may remain and the treated animal – or those that come in contact with the animal, could become infected.Most successful use of inactivated vaccine has been against furunculosis ( *Aeromonas salmonicida* ) in salmon. Two live attenuated vaccines have been developed and are commercially available in the U.S. for enteric septicemia of catfish (caused by the bacterium *Edwardsiella ictaluri*) and columnaris disease (caused by the bacterium *Flavobacterium columnare*).

MOVING FORWARD

Thanks to advances in fields such as genetics, immunology, and biotechnology, new methods for fish vaccines have emerged in recent years that can be developed and produced over much faster time frames.

Replicating recombinant vector vaccines consist of a fully competent viral vector backbone engineered to express an antigen from a foreign transgene. Live vaccines replicate within the host. Most live virus vaccines in use today are attenuated, their reduced virulence typically achieved by adapting the wild-type virus to a new environment.

DNA vaccines against other types of fish pathogens have so far in limited use and vaccination strategy is more complicated. The

primary function of DNA vaccines is a bacterial plasmid DNA containing a construct for a given protective antigen, is to establish specific and long-lasting protective immunity against diseases where conventional vaccines fail to induce protection.Intramuscular injection of DNA vaccines has been successfully used against viruses such as infectious haematopoietic necrosis virus (IHNV) or viral haemorrhagic septicaemia virus (VHSV)diseases in fish.

*Subunit vaccines* are prepared from proteins or sugars derived from the disease-causing organism. Synthetic peptide vaccines are produced from short sequences of amino acids prepared synthetically to act as antigens.Against infectious pancreatic necrosis virus (*IPNV*) in farmed salmonid fish disease subunit vaccine are prepared fromVP2 and VP3 capsid proteins and used as oralvaccine inCanada, USA.

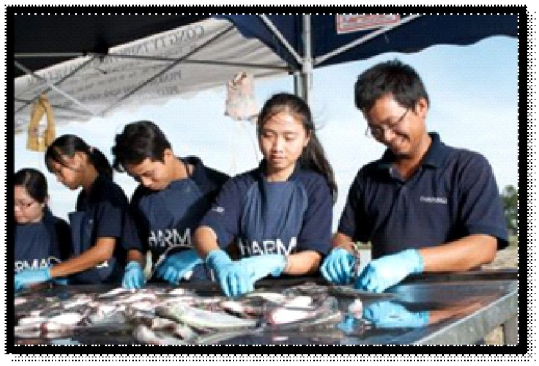
**Different routes of delivery of vaccines in fish**

| Parameter | Oral delivery | Immersion delivery | Injection delivery |
|---|---|---|---|
| Efficacy | Low | Low/moderate | High |
| Labor input | Low labor input | Low labor input | Intensive labor input |
| Size of fish | Unlimited | Unlimited | Limited |
| Individual handling of fish | No | No | Yes |
| Open cages in seawater | Highly applicable | Not easily applicable | Not easily applicable |
| Closed systemvessels in freshwater | Highly applicable | Highly applicable | Applicable |
| Stress | Low | Low | High |
| Route of exposure | Gut/intestine | Natural portals of entry | Parenteral |

The route of administration of vaccine is a vital factor influencing the efficacy and feasibility of vaccination. Injection, bath, and oral are the major administration routes used in aquaculture. Immersion vaccination is currently the most suitable method for mass vaccination of fish. As the fish is immersed in the vaccine solution, all mucosal surfaces including skin, gills, nostrils, eyes, vent and intestinal surfaces are exposed to antigens in the vaccine solution.Oral and bath immunizations (mucosal routes) are the ideal way for fish at all life stages, especially the larval stage when fish are often most susceptible to viral disease.But poor performance of oral vaccines is due to result of antigen degradation during passage through the hostile "stomach" like environment prior to reaching the second segment of the intestine where absorption takes place. It is to be noted that some *teleost fish have no anatomical stomach* at all & it produces both HCl and enzyme(s) from a single kind of cell. Injection is by far the most effective route, providing the highest protection.However, it is labour-intensive, causes handling stress to fish, and it is not feasible to vaccinate large numbers of juvenile fish via injection administration.Recently, automated vaccinating machines have been introduced that can lower labour costs and reduce stress to the fish.However, progress has been made regarding encapsulated technology of vaccine antigens which can be mixed with food for oral administration to increase it's stability.Nanotechnology has helped to formulate efficient vaccine delivery system that protects encapsulated antigen until it reaches to target area through gut & maintains sustained release.Other levels of commercial application

include intramuscular injection of DNA vaccines, nasal vaccination and hyperosmotic pretreatment before immersion vaccination.

**ALPHA JECT® Panga 2 injection vaccine is given to *Pangasius* fish in Vietnam.The fish are netted out in small groups, drained for a few seconds from sea water and immersed in the dilution of vaccine.**



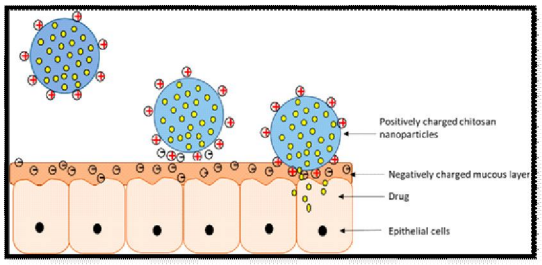
**Why thinking of nanovaccines**

When oral vaccination is most preferred but it suffers its degradation in the gastrointestinal tract.In contrast,encapsulating antigenic materials using several polymeric and lipid-based nanoparticle carriers could be an effective approach.Nanoparticle systems possess adjuvant properties that can enhance the efficacy of the antigens. An ideal vaccine carrier protects the structural integrity of the antigen and having effective delivery systemto reach mucosal surface in order to produce sufficient mucosal, humoral and cellular responses. Due to their very small size, nanoparticles enter living cells through cellular endocytosis.DNA vaccine incorporated into nanoparticles may be a more promising approach as the size of particle influences the delivery to a particular type of immune cells ensuring optimal antigen presentation, for example nanoparticles tends to be phagocytized preferentially by dendritic cells. This in turn may result in presentation via MHC class I and activation of the specific CTL(cytotoxic T lymphocyte)response typical of viral infections. Microparticles are more likely to be phagocytosed by macrophages and the antigens presented via MHC class II, generating a humoral response.

## Nanovaccines

Nanotechnology is the study & application of extremely small things ( about 1-100 nanometer or nm or 0.000000001 m) and this term is used across all other science fields. Nanoparticles are known to exhibit interesting properties different from their parent material. Due to their nano size, nanoparticles can be taken up by cellular endocytosis mechanism which facilitate the cellular uptake of antigens and increase the ability of antigen presentation.

Studies have demonstrated that application of nanotechnology increases solubility, stability, targeting, biocompatibility and permeability of vaccines. Nanovaccines, thus developed are made of nanoparticles formulated with antigens either encapsulated within or adsorbed on to the surface against which an immune response is desired. Numerous vaccine nanocarriers have been designed and investigated for their utility in the delivery of antigens and adjuvants to immune cells.The most explored nanoparticles in fish vaccine studies are synthetically derived polymeric PLGA and chitosan (deacetylation of chitin,which is the *structural* element in the exoskeleton of crustaceans like crabs, shrimp etc.) for administration of viral as well as bacterial antigens.Chitosan can be earmarked as a "green nanoparticle". It is highly abundant, biodegradable and biocompatible, making it an attractive candidate for vaccine delivery.Nanoconjugation of bicistronic DNA vaccine against *Edwardsiella tarda* using chitosan nanoparticles have shown protective efficacy and immune modulatory effects in *Labeo rohita* vaccinated by different delivery routes. Nanoparticles can boost the immune response in multiple ways. They can induce inflammatory reaction at the injection site, which recruits immune cells to the proximity of antigens, a phenomenon termed "reverse targeting".Phagocytic cells recruited to the injection site can phagocytize and subsequently digest the nanoparticles if they are biodegradable.




*Schematicrepresentation of chitosan loaded nanoparticles (NP)structure and interaction with the mucus layer.NP upon reaching the mucosal layer bind to the negatively charged mucus by virtue of electrostatic attraction and release the drug over time.*

## Types of nanoparticles & their merits & demerits.

| Type of nanoparticles | Merits | Demerits |
|---|---|---|
| Polymeric nanoparticles | Better immunogenicity can be obtained by easy modification of surface properties, biodegradable and targeted antigen delivery | Low aqueous solubility and synthesis require use of organic solvents, low antigen loading, premature release of antigens, insufficient antigen protection |
| Inorganic nanoparticles | Easy to modify, less chances of premature release and better protection of adsorbed antigens | Low aqueous solubility and low biodegradability |
| Nanoliposomes | Possess intrinsic adjuvant properties, accommodates both hydrophilic and lipophilic antigens and relatively stable in gastrointestinal fluids when modified | Low mucus penetration, limited antigen loading and poor gastrointestinal stability of naked liposomes. |
| ISCOMS | Easy to encapsulate and built in adjuvant property of Quil A | Do not form depot and difficult to incorporate hydrophilic antigens |
| Virus like particles | Possess self-adjuvant properties, mimics original virus and high gastrointestinal stability | Lack of reproducibility |
| Nanoemulsions | Possess self-adjuvant properties, encapsulates both hydrophilic and lipophilic antigens | Premature release of antigens and poor gastrointestinal stability |

Hyaluronic acid (HA) is another natural polymer composed of D-glucuronic acid and N-Acetyl-

Dglucosamine and is a component of cartilaginous tissue.It is biocompatible, biodegradable, hydrophilic and due to high abundance in nature and makes it as one of the attractive candidate nanoparticles for vaccine delivery.Alginate is an extract of naturally available brown algae and also it can be found as a polysaccharide in some bacteria. It is also biodegradable, biocompatible, non-toxic, acid resistant, mucoadhesive and most suited for oral vaccine delivery.Further, the nanoparticles can be classified as biodegradable or non-biodegradable based on their properties to get decomposed in biological system. In general, the other forms of nanoparticles used in vaccine studies include virus-like particles (VPL's),nanoliposomes, immunostimulating complexes (ISCOMs), nanoemulsions and metal nanoparticles.

There are several inorganic nanoparticles based on carbon, calcium phosphate, gold, silver, silicate, aluminium, titanium etc., among which carbon nanotubes (CNTs) and calcium phosphate are evaluated as vaccine delivery systems in fish vaccines.CNT can be listed as emerging nanoparticles in the biomedical research and are investigated as antigen delivery systems. The inorganic nanoparticles have good adjuvant properties and stabilities but they have certain limitations in their chemistry and physical properties.

Plasmid (pGPD + IFN) **constructed ——— àchitosan NPs———-àincorporated into feed—-à DNA plasmid enters cells( myocytes, APC) ——à target cells uptake DNA by endocytosis —-à translocates to the nucleus —-à expression of the DNA-encoded protein antigen—à antigen is produced by the host cellular machinery—-à antigen protein is degraded and presented by major histocompatibility complex (MHC)-I in immune cells———-à directly stimulate naïve $CD8^+$ T cells———-àvaccinated groups evade the bacterial infection elucidating the protective efficacy

**Bicistronic DNA vaccine (designated as pGPD + IFN as adjuvant) containing a regular antigenic gene (glyceraldehyde-3-phosphate dehydrogenase gene of *Edwardsiella tarda*) along with an additional immune adjuvant gene (Interferon gamma gene of *Labeo rohita*)DNA vaccine can be delivered effectively by oral or immersion route.

**Global fish vaccine manufacturers**

Aquaculture vaccines is the fastest growing input in the global aquaculture market. The major commercial markets for these vaccine manufacturing companies are currently, the salmon and trout industries in Northern Europe, Chile, Canada and the USA. Till now, no known vaccines are marketed or used in India despite the fact that market attractiveness is very high[2].Recently Vietnam aquaculture industry got the government approval for use of ALPHA JECT® Panga 2 injection vaccine that provides protection against the main diseases in the *Pangasius* fish.

Commercial vaccines are available for the catfish industry in the USA and, on a smaller scale, for European seabream, seabass and tilapia. Some limited-use, locally developed

vaccines are also available in countries such as China, Russia, Spain and Germany. Key players in the market include: Pharmaq AS (Zoetis, LLC) (Norway), Merck Animal Health (Merck & Co., Inc.) (USA),KoVax Ltd. (Part of Phibro Animal Health) (Israel),Hipra (Spain),Tecnovax SA (Argentina),Veterquimica S.A. (Chile), Nisseiken Co. Ltd. (Japan),Virbac S.A. (France), Elanco (USA),Kyoritsuseiyaku Corporation (Japan)& few others.

MERCK Animal Health is reputed for marketing following vaccines-

NORVAX® Minova 6: Inactivated, multivalent vaccine against furunculosis, classical vibriosis, coldwater vibriosis, wound disease and infectious pancreatic necrosis (IPN) for intraperitoneal injection in Atlantic salmon.

AQUAVAC® Strep Sa 2: Vaccine for the active immunization of susceptible fish species to reduce mortality and disease due to Streptococcosis caused by *Streptococcus agalactiae*.

AQUAVAC® ERM (ORAL): Inactivated vaccine against Enteric Redmouth Disease caused by *Yersinia ruckeri* (Hagerman strain) in rainbow trout (*Oncorhynchus mykiss*). Available as an immersion vaccine as well as an oral administration vaccine.

AQUAVAC® VIBRIO: Inactivated vaccine against vibriosis caused by *Vibrio anguillarum* serotype OI and O2"" (*V. ordalii*) in rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*).

**Conclusion**

Nanotechnology-based gene delivery vectors have shown tremendous potential at overcoming physiological and biochemical barriers towards efficient gene delivery. While the nanoparticles have certain unique features & shown the undisputable potential for applications fish vaccine delivery, but the very nature of these particles have negative effects as well [3]. Nanoparticles can cross the blood brain barrier (BBB), the applications have to be made carefully as it may cause serious troubles. Current research focuses on elucidating the toxicity of nanoparticleswhich are postulated to range from inûammatorycell inûltration &cellular necrosis to ROS(reactive oxygen species )-induced apoptosis.More research is still needed regarding nanoparticles-based vaccine delivery platforms.

Advances in genome sequencing of pathogens have accelerated the opening of opportunities to investigate new approach of vaccine development. Recently, the genome of salmon and several other fish species have been fully sequenced. Development of polyvalent vaccines and standardization of a vaccination calendar with molecular biology and modern technologies can develop new dimension of vaccination. Plant-based edible fish vaccines can also contribute a lot in the field of fish vaccination.

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# Study on the physico-chemical and Coliform load of waste water collected from Haldia Industrial site

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ABSTRACTS

Spatial variations of some physico-chemical and microbial profile of Haldia Industrial waste water werestudiedat seven sampling stations along the Green belt canal of Haldia Industrial site during the month of January and February, 2020.The site receives domestic, agricultural and high industrial wastes. The waste water is being influxes into the Haldi River through Green belt canal and finally Bay of Bengal. Considering the ecosystem of HaldiRiver for the sustainability of aquatic animals, water quality monitoring was carried out. The pH, TSSandTDS vary inthe range of 7.30 – 7.75, 100 mg/L -525 mg/L and 225 mg/L -625 mg/L respectively. Dissolved oxygen, biochemical oxygen dem and (BOD) valueat different sites varies within a narrow range 0.92 mg/L–13.2mg/L and 4.0 mg/L – 48mg/L,respectively. The coliform count at the sampling site varied from 70-2400 MPN/100ml.Present result indicates that all the studied parameters are deviate from standard WBPCB (Cornwell, 1985) report.So, this result informed that proper effluent treatment plant or bioremediation is required before influx into the green belt canal otherwise may also trigger outbreaks of waterborne disease and alter the aquatic system of Haldia River.

## INTRODUCTION

Haldia is one of the most rapidly growing towns in West Bengal and in on the deltaic tidal range of the Ganga basin. It is located at distance of 125 km South-West of Kolkata and 50 km from the Bay of Bengal at the confluence of three rivers Hooghly, Haldi&Rupnarayan in Purba Midnapore district. Haldia is also one of the biggest ports in the Eastern region and focal point for industrial development in West Bengal. The Haldia Planning Area (HPA) is bounded by the rivers Hooghly, Haldi&Hajli canal and covers a total area of around 326.85 sq. km. spread over 258 mouzas. The HPA is divided

in four police stations namely, Haldia, Mahisadal, Sutahata&Durgachak. Haldia is a port based industrialtown in West Bengal, India. Ministry of Urban Development, Govt. ofIndia has announced in 2016 that this town will be converted as one of the four smart cities in West Bengal. Haldia is also one of the biggest ports in the Eastern region and the focal point for industrial development in West Bengal. The population of Haldia as a Town has increased from 9968 in 1971 to around 200827 in 2011.

It is an industrial hub having a base of chemical and petrochemical industry. Many large industries like M/s. Indian Oil Corporation Refinery, M/s. Haldia Petrochemicals Ltd., M/s. MCC-PTA, M/s. South-Asian Petrochemicals, oil/gas terminals for HPCL, BPCL and Reliance have been set up in this region. Besides, other large industries operating in this area are detergent manufacturing unit, chemical unit, pesticide manufacturing unit, lead-acid battery manufacturing unit, vegetable oil producing unit, textile unit, tank firms either storing edible oil or petroleum products etc. A number of non-recovery type coke oven plants are also coming up in this area due to the locational benefits of obtaining imported coking coal directly through Haldia Port. In future, the industrial base is likely to be diversified, and many new industries are proposed as per the perspective plan for Haldia Planning Area.

Throughout the year, these units are generating tonnes of solid, liquid and gaseous waste materials that are exposed and released to the environment nearby. As per rule, majority of them are following the green belt mandate and treatment of wastes prior to disposal, many of them are offenders also.


The Green Belt Canal (GBC) which was originally built for fire-water supply to the port area is presently carrying most of the trade

effluent of the industries located at Haldia Municipal Area. The GBC has a stretch from the Oil Jetty-1 in the Haldia Dock Area to the Patikhali gate end (Fig.1)Boundary (in Red line) of critically polluted area in Haldia demarcated by CPCB (Green line shows the area where industries are located within the identified area and have major impact).The GBC and the Hooghly River is regularly monitored at specific locations. The GBC is guarded by metallic gates at both ends and does not seem to have a definite flow profile. The Patikhali gates are opened to discharge the effluent. The green belt canal receives liquid effluent that is mostly treated, from different industries through a no. of outfalls. Except 3 nos. of units, viz. IOC and United Phosphorous Ltd., all other units discharge their effluent to the canal indirectly i.e. to any other canal/drain/channel which is linked to GBC. IOC discharges only the overflow of its catch pit no. 6 to the canal. The industries are mostly located along the bank of river Hooghly and on the both sides of Haldia Petrochemical Link Road. Most of these industrial units discharge their effluent into the Green Belt canal leading to the river Hooghly. The water quality of Green Belt canal is regularly monitored by the State Board in eleven sampling stations.

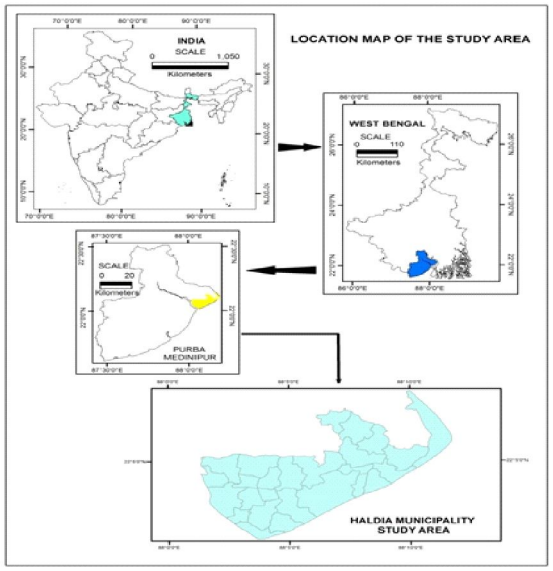
In 2017, Central Pollution Control Board has ordered Tata Chemicals to shut its Haldia Plant which produced fertilizers like diammonium phosphate and single superphosphate for non-compliance of norms on liquid effluent discharge. Chemicals released from pesticide industry are persistent in nature. They can affect soil ecology, enter into the food chain through plant food materials and affect human health. Oil refinery and petroleum industries are most potent in their ability to generate chemicals which are persistent, toxic, bioaccumulable/ biomagnifiable, carcinogenic/ mutagenic and dispersible. The hazardous oily waste is composed of total petroleum hydrocarbons (TPH), water, and sediments which is more polluted (Dibble et al., 1979). The TPH constitutes is more toxic because this complex mixture is alkane; aromatic; nitrogen, sulfur, and oxygen containing compounds (NSO); and asphaltene fractions (Bhattacharya et al., 2003).The effect of oil contamination has severe impacts in the plant and animal ecosystem including human health (Mandal et al., 2007). Crude oil exposure is more injurious and damage to lungs, liver, kidneys, intestines and other internal organs. Polycyclic aromatic hydrocarbons (PAH) may lead to cancer, Inhalation leads to headache, nausea, dizziness, respiratory irritation, BTEX (Benzene, Toluene, Eethyl benzene & Xylene) cause mutations, cancers, birth defects, nervous disorders, and liver disease, depression, irregular heartbeats etc. ( Lee et al., 2006; Chen et al., 2008; Lewis et al.,2008 and Rice et al., 2007). Oil contaminated soil loose its fertility for crops and have impact on seed germination. (Yoshida et al., 2006 and Gong et al., 2001). Hence disposal of the oily waste in an improper manner may cause a serious environmental problem (Yustle et al., 2000). Polluted water and soil have detrimental impact on soil fertility, microbial population in the soil and crop quality. They

cumulatively can affect sustainable agriculture. Keeping the anthropogenic pressure comes from Haldia Industry, present projecthighlight the level ofphysico-chemical and microbial profile of waste water from Haldia Industrial site.

## MATERIALS AND METHODS

### 1. Location of the study area

Haldia is one of the most rapidly growingtowns in West Bengal on the deltaic tidal range of the Ganga basin. It is located at distance of 125 km south-west of Kolkata and 50 km from the Bay of Bengal at the confluence of three rivers Hooghly, Haldi and Rupnarayan in Purba Midnapore district. The extension of Haldia (township) is 22º01´262 2 N to 22º04´182 2 N latitude and 88º01´562 2 E to 88º08´40 2 2 E longitude. Haldia municipality has 26 wards but industries are concentrated mainly in ward No.8, 9, 11 and 12.So sample has been collected both from more industrial wards (No.11 &12)and residential wards (No.19 & 23).




### 2. Collection of Water sample:

Water samples are collected from the waste laden area of the each industrial unit at 7 points (effluent release point near IOC second Gate, IOC Main Gate, Hoogly Met Coke Gate, Exide Gate, TATA Chemicals, Petrochemicals Unit, UPL gate etc.) in sterile amber colored bottles and transported to the laboratory for analysis.

Figure:1 GBC near Haldia petrochemicals

### 3. Determination of Physico-chemical parameters of water

3.1 Determination of pH

The pH of the surface water was measuredat a time with a potable digital pH meter (Model BST-BT-BT65; sensitivity =±0.01).

3.2 Determination of Total Dissolved Solid (TDS) and Total Suspended Solid (TSS)

The total suspended solid, total dissolved solids were analyzed the laboratory as per the standard procedure (APHA, 1998).

3.3. Determination of DO and BOD of water sample For chemical variables of water like Dissolved Oxygen (DO) and Biochemical oxygen Demand (BOD) was analyzed by the standard method (APHA, 2005;Trivedy and Goel, 1986).

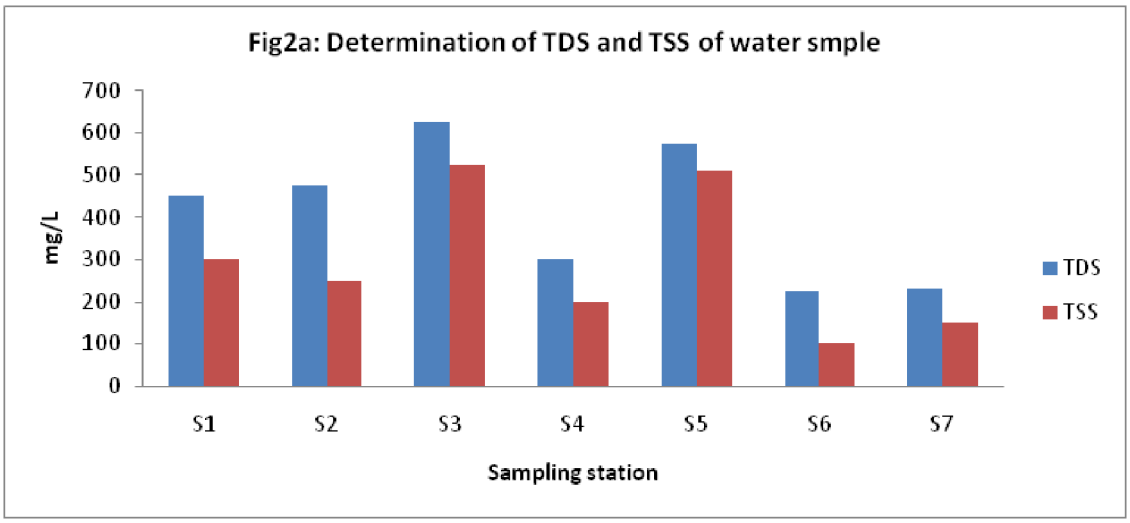
3.4 Determination of total coliform in water

The total coliform and fecal coliform of water sample was determined Multiple fermentation (5test tube) Technique by (APHA, 1998).

## RESULTS

**Table .1 Determination of pH, TDS, and TSS of the collected water sample**

| Sampling station | Water sample | pH | Total Dissolved Solids (TDS, mg/L) | Total Suspended Solids (TSS, mg/L) |
|---|---|---|---|---|
| S1 | Green belt canal, IOC 2nd Gate | 7.36 | 450 | 300 |
| S2 | Green belt canal, IOC main Gate | 7.55 | 475 | 250 |
| S3 | Hoogly Met coke | 7.75 | 625 | 525 |
| S4 | Behind Exide | 7.68 | 300 | 200 |
| S5 | Tata Chemicals | 7.30 | 575 | 510 |
| S6 | Haldia Petrochemicals | 7.47 | 225 | 100 |
| S7 | Near UPL Gate | 7.65 | 230 | 150 |



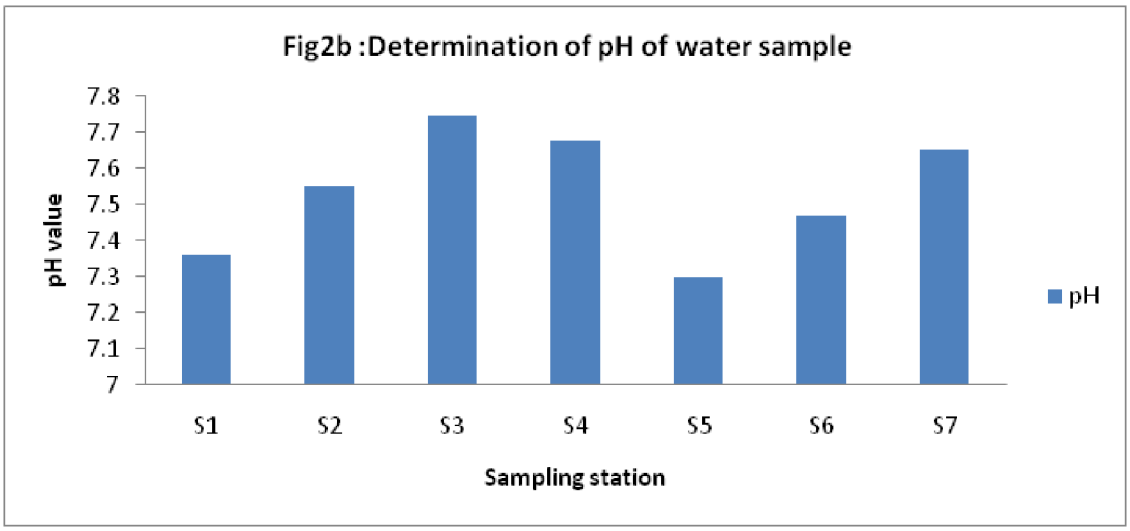



**Table.2Standard value of treated Industrial wastewater by WBPCB**

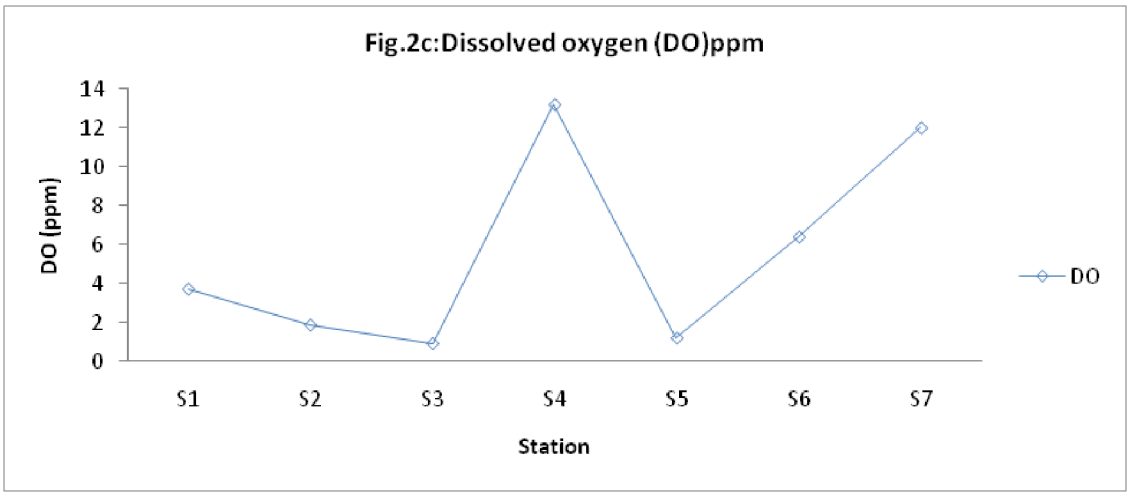
| Sl. No | Parameters | Unit | WBPCB Standard |
|---|---|---|---|
| 1 | $p^H$ | - | 6.5-8.5 |
| 2 | TSS | mg/L | 100 |
| 3 | TDS | mg/L | 200 |
| 4 | DO | mg/L(ppm) | 6 |
| 5 | BOD | mg/L(ppm) | 30 |
| 6 | COD | Mg/L | 250 |
| 7 | Coli form | MPN/100ml | <200 |

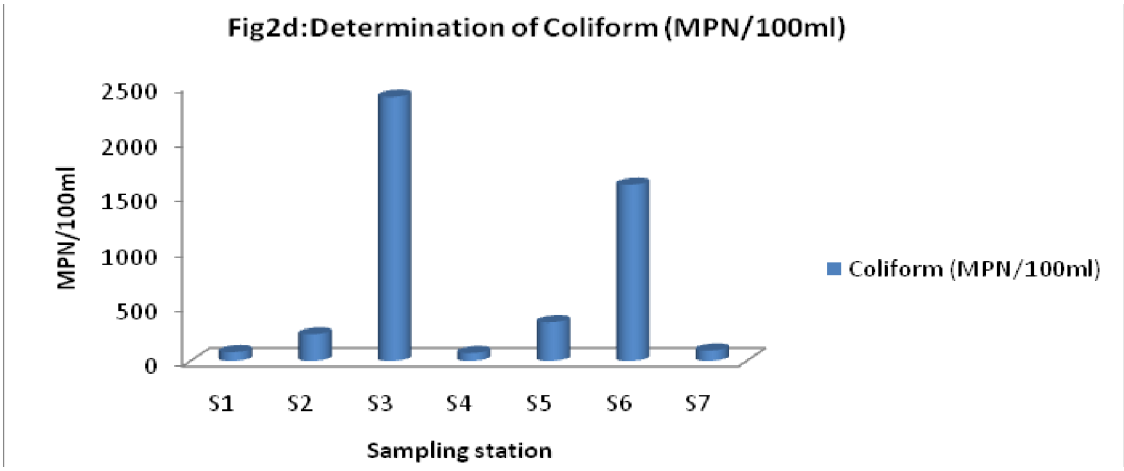
**Table.3Typical range of composition of untreated Industrial wastewater (Davis and Cornwell, 1985)**

| Sl. No | Parameters | Unit | WBPCB Standard |
|---|---|---|---|
| 1 | $p^H$ | - | 6.5-8.5 |
| 2 | TSS | mg/L | 100-350 |
| 3 | TDS | mg/L | 200-1000 |
| 4 | DO | mg/L(ppm) | 6-8 |
| 5 | BOD | mg/L(ppm) | 100-300 |
| 6 | COD | Mg/L | 250-1000 |
| 7 | Coliform | MPN/100ml | <230(91/492/EEC)<br><200(ONRW) |

**Table: 4 Determinations ofDO, BOD, and Coliform**

| Sampling Station | Water sample | Dissolved Oxygen (DO ) ppm | Biological Oxygen Demand (BOD) ppm | Coliform (MPN/100ml) |
|---|---|---|---|---|
| S1 | Green belt canal, IOC 2nd Gate | 3.72 | 21 | 79 |
| S2 | Green belt canal, IOC main Gate | 1.88 | 6.8 | 240 |
| S3 | Hoogly Met coke | 0.92 | 42 | 2400 |
| S4 | Behind Exide | 13.2 | 13.6 | 70 |
| S5 | Tata Chemicals | 1.2 | 48 | 350 |
| S6 | Haldia Petrochemicals | 6.4 | 36 | 1600 |
| S7 | Near UPL Gate | 12 | 4.0 | 94 |






## DISCUSSION

### Water pH

The water pH of allthe sampling stations showed slightly alkaline (Fig.2b) and remainedalmost constant (pH7.30 – 7.75) which is within the standard value (Table-1,2&3). Alkaline pH of each water bodies was due to discharge of alkaline chemicals from theHaldia industrial area or due to rainwater-runoff fromembankments rich in soluble alkaline matters. Comparatively high pH of the water atHoogly Met coke may be attributed to discharge of huge raw coke waste in to the Green Belt canel. According to Boyd (1990), the high pH can also affect fishhealth. For most freshwater species, a pH range between6.5 - 9.0 isideal, the observed pH is usually between pH 7.30 and 7.75. Therefore,the observed pH of water in different stations indicates within the normal range for aquaculture.

### Total Dissolved Solid (TDS) and Total Suspended Solid (TSS)

The population of Haldia as a Town has increased from 9968 in 1971 to around 200827 in 2011. It is an industrial hub having a base of chemical and petrochemical industry. Many large industries like M/s. Indian Oil Corporation Refinery, M/s. Haldia Petrochemicals Ltd., M/s. MCC-PTA, M/s. South-Asian Petrochemicals, oil/gas terminals for HPCL, BPCL and Reliance have been set up in this region. Besides, other large industries operating in this area are detergent manufacturing unit, chemical unit, pesticide manufacturing unit, lead-acid battery manufacturing unit, vegetable oil producing unit, textile unit, tank firms either storing edible oil or petroleum products etc. Such industrial waste consist different types of dissolved solid materials likecarbonates, bicarbonates, chlorides, sulfates, phosphates, nitrates, calcium, magnesium,sodium, potassium, iron, manganese, and a few others.. TheTDS valueof seven water sampling station varies from225 mg/L -625 mg/L (Table- 1) which deviate from standard permissible level (Table2&3). The TDScorresponding to each location isshown in Fig.2a. The maximum value

of TDS is 625mg/L at Hoogly Met coke. It is due to discharge of unused high coke in to the water body. This water alsocontains huge quantity of organic and inorganic matterswhich come from this industry. The minimum valueof TDS is 225 mg/L at site Haldia Petrochemicals. This Low value indicates the presence of insoluble hydrocarbon in the water which is influxed fromHaldia Petrochemicals. The concentration of TSS for the water samples ranged from 100 mg/L -525 mg/L which were also exceeded the desirable limit(Table-2&3).

**Dissolved oxygen and Biochemical oxygen demand of water sample**

The dissolved oxygen (DO) is one of the most important parameters ofwater quality assessment. It plays an important role on the biotic life ofan aquatic system and this can be used as an index of water quality forpollution studies (Thirumala et al, 2011).The range of DO value of the studied water sample is 0.92 mg/ L–13.2mg/L which indicate that the DO value of four stations are below the permissible level and other three stations are just above the permissible level.Therefore,present investigation indicates that Green belt canal water is considered as unhealthy for aquatic animals. Maximum values of DOobserved were 13.2 mg/lit at Behind Exideand minimum valuesobserved were 0.92 mg/ lit at Hoogly met cock station during the studyperiod (Fig.2c and table-4). High values of DO at Behind Exide could be due to high expose with tidal water than other stations. In general, a saturation level of at least5 mg/lit is required (Lioyd, 1992) for aquatic animal. Values lower thanthis can put undue stress on the fish, and levels reaching less than 2mg/L may result to death (but 3 mg/L to some species). Biochemical Oxygen Demand (BOD) detects the presence of organic load as well as microbial population. With increase the BOD value decrease the water quality. Drinking water usually has a BOD ofless than 1 mg/l. But, when BOD value reaches 5 mg/l, the water isdoubtful in purity (WHO, 2011). The BOD of water sample variedfrom 4.0 mg/L – 48mg/L throughout the sampling stations(Fig.2d) which is within the permissible level and some stations exceed the standard but average result indicate the presence of high level of anthropogenic stress. Many scientist also reported that an increase in BOD level as indicative of increasing pollution and hazards for aquatic animals (Kudesia and Verma, 1986; Mahadevan and Krishnaswamy, 1984, Sinha, 1988). With high anthropogenic pressure in water causes ecological unbalanced(Chandrashekar et al, 2003).Therefore, proper wastewater management required to minimize the anthropogenic load in Green belt canal.

**Coliform in water**

Coliform bacteria consist of several genera belonging to Family *Enterobacteriaceae*.Fecal coliform which belongs to this group isfound mostly in feces and intestinal tracts of humans and other warmblooded animals. It is not pathogenic; however, it is a goodindicator of the presence of pathogenic bacteria. High levels of fecalcoliform in the water may cause typhoid fever, hepatitis, gastroenteritis,dysentery and eat

infection. In recent times increased attention isgiven to the possibility of cultured fish as vector of human pathogenicbacteria (Islam et al, 2000). Fish living in natural environment areknown to harbor pathogenic Enterobacteriaceae (pillay, 1990). Invasionof fish muscles due to the breakage of immunological barrier offish by pathogen is likely to occur, when the fish are raised in pondwith coliform of greater than $10^4$per 100 ml, in pond water (Guzmanet al, 2004).The maximum density of total coliform (TC) in the water was recordedinHoogly Met cokeand Haldia Petrochemicals station, which may be due to high exposure with fecal material of local people compare to other stations and low amount of TC was enumerated in Behind Exidestation(Table- 4&Fig.2d) but the average coliform load in all the stations are not suitable for sustainability aquatic animals.Therefore, one of the important recommendation outputs of the present study is that the local authority in Haldia Municipality should take this serious issue of water quality degradation in Haldi River water. Moreover, there should be a regular orconstantly monitoring for the quality of the stream, because this could increase the risk of direct threats to humanhealth and environment, because more pollution could increase the concentrations of unhealthy water pollutants forall organisms

## CONCLUSION

Present study highlight the pollution level of Green belt channel which information will be more important for Waste water quality management of Haldia Municipality as well west Bengal Pollution control board.Therefore, one of the important recommendation outputs of the present study is that the local authority in Haldia Municipality should take this serious issue of water quality degradation in Haldi River water. Moreover, there should be a regular orconstantly monitoring for the quality of the stream, because this could increase the risk of direct threats to humanhealth and environment, because more pollution could increase the concentrations of unhealthy water pollutants forall organisms.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# Pengba, *Osteobrama Belangeri* (Valenciennes,1844) A Prospective Species For Diversification Of Carp Polyculture System And Suggestions Towards The Adoption Of Scientific Fish Farming In Haldia, West Bengal.

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ABSTRACT

With emphasis on diversification of Indian carp culture, attempts have been made to incorporate other potential candidate species into mainstream carp culture in Haldia. As for field trial of Osteobrama belangeri fish, farmers of Haldia stocked in their composite fish culture ponds. Osteobrama belangeri, locally known as Pengba in Manipur, India and nga-hpeh-oung and nga-net-hua in Myanmar, is a medium-size cyprinid endemic to the eastern part of Manipur, Myanmar and Yunnan Province in China. Although the species is listed as near threatened by the IUCN, there is a sizable population in Myanmar but is extinct in the wild in Manipur. A few progressive fish farmers in Haldia have tried to culture Pengba. Grow-out of Pengba has been confined mainly to earthen ponds. The culture practice is normally adopted in combination with other carps. An annual production levels of 14-15 t/ha can be achieved with the adoption of scientific carp culture. Pengba is suitable for pond culture because it is herbivorous and thus can be included in composite fish culture in place of grass carp. It has also been possible to produce most of its preferred foods through fertilization of culture ponds and provision of supplementary artificial feeds. Seed production technology and its supply is a major constraint. Hence, intensification of induced breeding and attempts to culture this fish has been given high priority. This article summarizes culture aspects, extra income generation of farmers and future prospects for Pengba in West Bengal.

## INTRODUCTION

O*steobrama belangeri* a very popular medium-size carp fish species, belonging to *Family-Cyprinidae* , locally known as pengba in Manipur, India and nga-hpeh-oung and nga-net-hua in Myanmar. Present species is available in the eastern part of Manipur, Myanmar and Yunnan Province in China. Although the species islisted as near threatened by the IUCN, there is a sizable population in Myanmar but is extinct in the wild in Manipur.However, availability of the fish decline from the natural habitat due to prohibition of breeding migration of the fish after the construction of Ithai barrage for supply of water to the Loktak Hydro-Electric Project (Singh and Devi, 2012).It has great demand in the state Manipur due to its association with the cultural heritage of the state and its unique taste.Successful induced breedingwas achieved after several efforts made by the Indian Council of Agricultural Research (Behera *et al.* 2009). ICAR-CIFA, it hassuccessfully commercialised its captive breeding technology of Pengba. With emphasis on diversification of Indian carp culture, attempts have been made to incorporate other potential candidate species into mainstream carp culture in Haldia. During field trial of pengba fish , farmers of Haldia stocked in their composite fish culture ponds. The constraints to carp seed production in India have been described by Basavaraja (1994) and reported that, success of aquaculture enterprise is largely depends on the availability of the adequate quantity of quality fish seed to maximize the productivity and increasing production level in the country.Despite its culture potential, no systematic attempt has been made to culture and propagate this species in our region. Although it is a high-value species, especially in the north-eastern states, monoculture may not be a suitable proposition, considering its lower growth potential compared to Indian major carps. Pengba is suitable for pond culture because it is herbivorous and thus can be included in composite fish culture in place of grass carp.The production of quality seed depends on various external and internal factors which regulates the growth and survival of fish larvae (Faruque *et al.*, 2010). It has also been possible to produce most of its preferred foods through fertilization of culture ponds and provision of supplementary artificial feeds. Seed production technology and its supply is a major constraint. Hence, intensification of induced breeding and attempts to culture this fish has been given high priority. This article summarizes culture aspectsand future prospects for pengba in West Bengal.

### Materials and Methods:

Six fish farmers of Haldia blocks of Purba Medinipur are experimentally cultured Pengba fish in their ponds are given details in table below.

| Sl No. | Name & address of farmers | Mouza | Area of pond in acre |
|---|---|---|---|
| 1 | Arup Mantri, Vill+PO- Dwaribetia, Dist- Purba Medinipur | Dwariberia | 7 |
| 2 | Panchanan Mantri, Vill+PO- Dwaribetia, Dist- Purba Medinipur | Dwariberia | 7 |
| 3 | Sarat Chandra Bhaumik, Vill+PO- Basanchak, Dist-Purba Medinipur | Basanchak | 10 |
| 4 | Sofi Ahamed, Vill- Chaklalpur,PO- Barbasudevpur | Chaklalpur | 5 |
| 5 | Krishna Prasad Samanta, Vill+PO- Barghasipur | Barghasipur | 5 |
| 6 | Mrinmoy Samanta, Vill+PO-Dwariberia | Dwariberia | 5 |

The traditional scientific process of composite fish farming is followed during the culture of Pengba in combination with Indian major carps.

**Result and Discussion**

Six progressive fish farmers of Haldia have brought Pengba fish fry from CIFA, Bhubaneswar and stockedin their composite fish culture ponds. So far not much technical information on its culture is available; however, Fish farmers stocked Pengbe fish of 2gm size ( approx.) along with Rohu, Catla, Silver Carp & Mrigel was stocked@20 no per decimal. All other fishes are stocked simultaneously. Catla Fish fingerlings of 300-350 gm size (approx) was stocked @ 4 nos. per decimal. Rohu Fish fingerlings of 100-150 gm size (approx) was stocked @ 20 nos. per decimal. Silver carp Fish fingerlings of 150-200 gm size (approx) and Mrigel fingerlings of 150-200 gm size (approx) was stocked respectively @ 8 nos. and 4 nos per decimal. After fourmonths, there was 600 grams of Rohu and 1 kg of silver carp. Rohu & Silver were harvested and sold to market. Again, replenishment of harvested species i.e.Rohu Fish fingerlings of 100-150 gm size (approx) and Silver Carp fingerlings of 150-200 gm size (approx) was restocked respectively @ 20 nos. and 8 nos per decimal.

After 8month the first release of the fishes, pengba (400 gms of average body weight of each pengba ) fish was harvested and gradually the rest of the fish was harvested and sold according to the market and get a good profit. 1.5 kgs of catla, 600gms of Rohu, 1kgs of Silver Carp and 1kgs of Mrigel fish was harvested. Total 6kg of catla, 24 kg of Rohu, 16kg Silver Carp, 4 kg of Mrigel and 8kg of pengba fish as per decimal area was sold. As the Pengba fish eats aquatic vegetation including algae of ponds / water bodies, having vegetation - algae is preferred for stocking with this fish species. It feeds on artificial feed readily and grows well in well manured (organic) composite culture pond. There is no need for special care and / or

husbandry, excepting good management practices, recommended for Composite Fish culture. A few progressive fish farmers in Haldia have tried to culture p

Pengba. Grow-out of Pengba has been confined mainly to earthen ponds. The culture practice is normally adopted in combination with other carps depending on the compatibility and type of feeding habits of the fishes. Result depicts that the annual production of 14-15 t/ha can be achieved with the adoption of scientific carp culture procedure. The general practice of Pengba culture includes, control of predatory and weed fishes in pond, stocking of fingerlings at a combined density of 7000-8000 no. /ha, pond manuring and fertilization with organic manures like cattle dung or poultry droppings and inorganic fertilizers, the provision of a mixture of rice bran and mustard seed press cake as supplementary feed, fish health monitoring and water management. They were using "joibo Juice" which play a vital role to maintain water quality as well produced natural food for fishes. Joibo juice is nothing but a fermented product i.e. combination of 300gm Ground nut oil cake, 250gm rice bran, 25 gm Yeast, 300 gm molasses & 10gm wheat powder per decimal water area achieve good result.

## CONCLUSION

The present study revealed that ithas already drawn up a strategic plan for doubling freshwater aquaculture production through an increase in productivity and area. Because Pengba forms an important component of carp culture as extra income fish, it can be expected that there will be a major increase in its production in fish farm. Commercial-scale seed production of Pengba can be achieved in captivity through induced breeding by various hatcheries. Because the breeding protocol for seed production is simple and cost-effective, it can be taken up by small and marginal farmers. In this context, Haldia has been making a unique example in diversified aquaculture, fish farming in the successful cultivation of various fish through fish farmers, explaining the importance of fish biodiversity in the fancy initiative of Haldia Block Fisheries Department. The integrated fishery management practices related to proper resource utilization, species diversification with new fish species introduction i.e. Amur Common Carp, Pengba, Milk fish, GIFT Tilapia, Pearl spot and Conservation of indigenous endangered fishes i.e. Pabda, Magur, Singi, Koi, Tengra rearing successfully done by this fish farmers. Fish farmers of Haldia are trends to farming by using "Organic Juice". As per scientific recommendations has helped in long-term rural livelihood improvement for the fish farmers. Fish farmers also recognized in state as well as National level.

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# Metabolomics approaches in oral submucous fibrosis : Areview

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ABSTRACT

The present reviewexploresthe applications of "OMICS" approaches in the diagnosis and treatment of an oral pre-malignant disease (OPMD) named Oral Submucous Fibrosis (OSF). The study is mainly concentrated on "omics" techniques leading to its identification of novel biomarkers and molecular signatures that are capable of diagnosing OSF and thus are applied in clinical practice for the proper treatment.Various "Omics" techniques were applied to differentiate OSF from control cases in many research works. Several online databases (PubMed, Google Scholar, and Springer) were explored for a detailed study of the papers of these research works that are being published in the last 20 years. The searches were made as "omics + OC diagnosis", "metabolomics + OPMD", "OSF + biomarkers", "metabolic profiling of OSF + control group", "Modern OC therapies", "Molecular approaches + OSF" etc.From the various reported literature on OSF detection and treatment, the obtained result is that metabolic signatures are found inthe saliva, serum, and tumor tissue samples of oral cancer patients that are capable to differentiate among oral cancer, oral-precancerous lesions, and healthy control individuals.Though there are various advancements of Oral Cancer (OC) therapies in the past few decades, the traditional methods of diagnosis that are still in practice can detect oral pre-cancerous conditions like OSF only at their advanced stage,whendisease becomes incurable. Thus, a need for early detection of these diseases before their progression towards OCis essential,at least inpre-malignant conditionswhere it can only be diagnosed by metabolic profiling of the biomarkers. Modern OMICS strategies hold the potential to serve this need by giving a major contribution to metabolomic profiling.

## 1.1. Introduction

The most common type of cancer worldwide is Oral Cancer (OC) which shows a higher prevalence in males than in females [Chaturvedi, P., et al., 2013]. Almost 1.7 million new cancer cases were diagnosed worldwide in 2019. Among these, OC ranks third in India and the sixth most abundant type of cancer in the world [Lu, M. and X. Zhan, 2018].OSF is an irreversible, chronic premalignant lesion that is characterised by scarring and hardening of the oral mucosa. Though its pathogenesis is unknown to date it is suspected to be induced by the use of tobacco, the habit of chewing betel quid, smoking, consumption of alcohol, and chronic inflammation [Rajalalitha, P. and S. Vali, 2005].

Over the past few decades, the prevalence of

OSF in India increased from 0.03-6.42% [Rajalalitha, P. and S. Vali, 2005]. The disease that was previously constricted only in South East Asia and India has now gained its popularity throughout the United Kingdom, the USA, and other developed countries, thus, is a serious problem to global health [Hazarey, V., et al., 2007.]. The most threatening part of OSF is that it has a high malignant transformation rate that has increased by 7.6% within a span of 17 years [Rai, V., et al., 2018]. The transformation of OSF to OC involves altered thickness of the epithelium and keratin proteins, the presence of micronuclei during dysplasia along with maturation, and interaction of collagen fibers with mast cells and myofibrils [Arakeri, G., et al., 2017]. The sites that are most commonly affected due to OSF are as follows- labial and buccal mucosa, soft palate, retromolar pads, the floor of the mouth, pharynx, esophagus as well as para tubal muscles in eustachian tube of the ear [Rajalalitha, P. and S. Vali, 2005].Alist of histopathological changes observed in oral mucosa during different stages of progression in OSFis presented in Table 1. These are the signs and symptoms observed by clinical practitioners or dentists to diagnose OSF.

Table 1. Depicts histopathological Grading of OSF [7]

| Very early (stage I ) | Early (stage II) | Moderately advanced (stage III) | Advanced (stage IV) |
|---|---|---|---|
| Dispersion of collagen fibers accompanied by edema | Juxta-epithelial hyalinization | Moderately Hyalinised collagen present | Collagen completely Hyalinised |
| Blood vessels are often dilated and congested | Blood vessels are dilated and congested | Blood vessels are normal and constricted | Blood vessels are destroyed and narrowed |
| Strong fibroblastic responses | Presence of young fibroblast cells | Less fibroblastic response; adult fibrocytes seen | Hyalinised areas are devoid of collagen |
| Normal and non-keratinized epithelium | Shortening of epithelial rete-pegs followed by keratinization | Atrophic epithelium with loss of rete-pegs and muscular degeneration | Epithelial dysplasia with degeneration of muscle cells (malignant transformation) |

## 1.2. Materials and Methods

Extensive studies of related literature are exploredmainly based on the keywords Omics, Metabolomics, OC, OSF on the search engines like Google Scholar, Springer, and PubMed from 2000 till aug 2020. Relevant papers containing specific information about OSF diagnosis are selected, summarized and presented in this review article.

## 1.3.Molecular signatures of OSF based on metabolomics

Prerequisite of biomarkers for early dectection of OSF is need of the hour. The progression of

OSF towards malignancy is a very subtle process that does not exhibit any early signs or symptoms. India has seen a rise of OSF cases from 25000 to 2 million from 1980 to 1993 [Rai, V., et al., 2018]. Image analysis is used by physicians for diagnosing OSF. Since, ischemia or hypoxia does not occur in this disease, so it becomes very difficult to assess it accurately unless significant stromal changes appear [Rai, V., et al., 2018, Bari, S., et al., 2017]. Presently, doctors rely on incisional biopsy to confirm OSF[Guta, M. and S. Mhaske, 2008]. But the process being invasive and painful is mostly not preferred by patients. Moreover, biopsy can only be done once the symptoms or lesions arise. By that time, the disease becomes incurable [Rajendran, R., et al., 2005]. Under such circumstances, development of non-invasive diagnostic procedure over surgical procedure is significantly important [Lee, C.-K., et al., 2009].The statisticsof the published papers on various samples are embodied from 2000-2020 in (Fig.1).

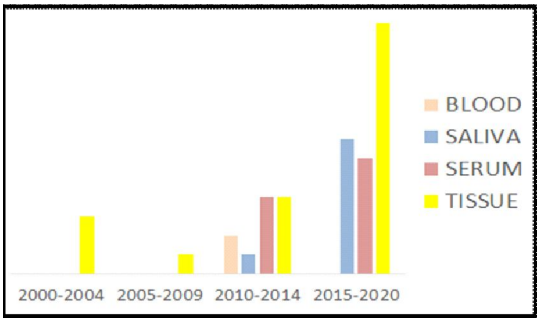


Figure 1. Graphical representation of number of studies conducted on OSF on the biological specimens from 2000-2020

### 1.4.Molecular Approaches for OSF

Juxta-epithelial inflammatory reactions in OSF are often succeeded by the fibro-elastic changes that occur in lamina propria which is also accompanied by epithelial atrophy [Lee, C.-K., et al., 2009].miRNAs regulate both the transcriptional and posttranscriptional regulatory mechanisms of epithelial-mesenchymal transition (EMT) and thus may serve as biomarkers and therapeutic targets for EMT-based pathological conditions, including OSF[Pandya, S., et al., 2009]. There is a notable rise in p63 level and CD105 expressions in OSF that is accompanied by loss of E-cadherins in the oral mucosal membrane [Zhou, S., et al., 2017]. These above mentioned molecular markers of OSF can easily be assessed by computer-aided quantitative assessment framework based on the altered status of cellular lesions, neo-angiogenesis and hypoxia in malignant potential diseases like OSF [Anura, A., et al., 2016].

### 1.5. “Omics” Science in OSF Detection

Among various developments in modern biological era, one of the most notable works are applications of “omics” approaches that include genomics, transcriptomics, proteomics and metabolomics in detecting abnormalities at the level of DNA, RNA, protein, metabolite and development in the field of medical imaging [Anura, A., et al., 2016]. OSF during its progression towards malignancy cause copy number changes in chromosome along with loss of heterozygosity (LOH) [Lu, M. and X. Zhan, 2018]. Genetic sequencing have shown p53 expression in precancerous cells have positive correlation with its increase in malignant

transformation [Bathi, R.J., 2003]. Various changes are observed at protein expression level along with their structural modification and deferred activity during conversion from healthy cell into neoplastic cells. Altered localization of these proteins affects cellular functions and can easily be diagnosed by cancer proteomics [Srinivas, P.R., et al., 2001]. RT-PCR, Western Blotting and other immunohisochemical analysis are applied to observe CYPA (Cyclophilin A) expressions, a potential biomarker of OSF. Results revealed that cell proliferation and apoptotic processes are stimulated by RNA interferences due to inhibition of CYPA expressions [Hou, X., et al., 2017].Quantitative proteomics using isobaric tags for relative and absolute quantification (iTRAQ) for tissues showed that ANXA4 and FLNA are proteins found to be up regulated in OSF [Liu, W., et al., 2016]. Other studies using proteomic analysis with two dimensional electrophoresis (2DE) and MALDI TOF mass spectrometry showed that Hsp 70 1B, Calreticulin, and Lumican variant levels in OSF tissues were significantly increased whereas Enolase 1 was decreased[Das, T., et al., 2018]. Various technologies examined for different biomarkers in the last 20 years are listed in Table 2.

### 1.6.Metabolomics in OSF

Metabolism is defined as a collection of processes that generate energy and various cellular level building blocks by the cell utilizing the molecules, substances, and nutrients accumulated from the surroundings. These products formed along with the various cellular intermediates formed during these processes constitute the metabolites [Adeola, H.A., et al., 2017]. Metabolomics is the detailed

**Table 2. Summarises modern techniques along with different biomarkers identified and applied for detection of OSF.**

| Technique | Observed parameter | Samples examined | References |
|---|---|---|---|
| Two-dimensional gel electrophoresis and Mass Spectroscopy | Protein levels | Tissue and Serum | Srinivasetal.,2001[17] |
| Staining | P53 expression | Tissue | Bathi etal.,2003[16] |
| Next-generation sequencing (NGS) | DNA, RNA and Protein metabolites | Tissue | Luetal.,2016 [2] |
| Optical Coherence Tomography (OCT) | Epithelium Thickness | Tissue | Leeet al.,2009[12] |
| Computer based quantification of chromogenic immune-histochemical (IHC) images | Cytoplasmic, nuclear and stromal expression | Tissue | Anura et al,2016 [15] |
| NanoLC MALDI MS/MS | Lipid metabotypes | Tissue | Bagetal.,2016[21] |
| Itraq | Protein expression | Tissue | Liuetal.,2016[19] |
| 2-DE MALDI TOF MS | Protein expression | Tissue | Dasetal.,2018[20] |
| FTIR | Metabolic Profiling | Serum | Rai et al., 2019[22] |
| $^{1}$H Nuclear Magnetic Resonance Spectroscopy | Glucose metabolytes | Serum | Raietal.,2019[23] |

analysis and technical study of these substances with low molecular weights called metabolites present in cellular level, tissue level or of whole organisms and are dependent upon can be manipulated by various factors. It enables the analysis of a wide range of exogenous and endogenous metabolites which include substances like lipids, peptides, amino acids, nucleic acids, vitamins, organic acids, carbohydrates and thiols[Kapila, Y.L., 2015].

Upregulation of miRNA in serum [Singh, P., et al., 2018], over-expressed alpha-enolase [Bag, S., et al., 2018] are some of the indicators of OSF observed in metabolic profiling.

### 1.7."Omics" Application in OSF diagnosis

5-aminolevulinic acid (ALA) induced cellular accumulation of protoporphyrin IX (PpIX, the photosensitizer) issuccessfully used todistinguish between normal and neoplastic cells andtodiagnose tumour tissues. Though Auto fluorescence spectroscopy can also diagnose malignant cells but cannot differentiate pre-malignant conditions like OSF from normal cells.Due to atrophic epithelium in pre-malignancy, the conversions of ALA into PplX in epithelial cells are very specific and thus results in accurate diagnosis [Wang, C.-Y., et al., 2009]. Optical coherence tomography (OCT) is a non-invasive, real-time, three-dimensional imaging technique that can replace incisional biopsy for diagnosis of OSF. The scanning images from Swept-source OCT (SS-OCT) shows that the boundary between epithelium (EP) and lamina propria (LP) becomes smoother due to thinning of EP layer in collagen-rich OSF tissues while EP-LP boundary is reported to be irregular in control cases [Bag, S., et al., 2016].

Combination of *invivo* fluorescence spectroscopy with principal component analysis (PCA) and partial least square discriminate analysis (PLS-DA) provide a rapid, acute, cheap, non-invasive and technically advanced procedure for screening of OSF [Musharraf, S.G., et al., 2016]. Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS) can indicate the alterations in lipid biogenesis during OSF and cancer by significant changes in expression of crucial lipid metabolites. Gas chromatography-Mass spectroscopy (GC-MS) along with chromo-metric analysis reveals the up-regulated fatty acid (FA) synthesis and reduced amount of histidine, threonine, arginine, tyrosine, isoleucine, leucine and glucose in OSF tissues along with increased levels of alanine and methionine [Goel, R., et al., 2014, Misra, B.B., et al., 2019].$^{1}$-H nuclear magnetic resonance (NMR) spectroscopy revealed up-regulated glucose metabolism, increase in lipid metabolites and considerably altered FA metabolic pathway as a potential biomarker for OSF detection [Rai, V., et al., 2019].

### 1.8. Discussion

The present review extensively discussed the "Omics" studies that is analysis of high-throughput data like protein-protein interaction, protein-DNA interaction or allosteric regulation of biological sample with decreasing cost and time to reveal critical biological network and help us to promoteour knowledge in abnormality detection as well as treatment.

Elaborate biochemical and genetic changes were observed in oral mucosa when it progresses towards hyperplasia and then advances up to metastasis. Variety of transcription factors that gets altered during malignancy are now considered as potential biomarkers and are studied through Western Blotting, RT-PCR and other image analysing techniques. There is also considerablechanges in the metabolic products in OSF patients that are studied and comes under the field of metabolomics. Spectroscopy, optical coherence tomography (OCT), chromatography and nuclear magnetic resonance (NMR) are some of the techniques thatprovide us with information regarding the metabolic profile of an individual. The altered levels of glucose, lipid and amino acid regulation detected via these techniques benefitus to distinguish betweennormal individualsandthat of OSF or OSCC patients.

### 1.9. Conclusion

From the above discussion it may be resolved that in modern times, metabolic profiling of biomarkers present in blood, saliva and serum have proved their significant need in detection and treatment of OSF. A number of studies performed in the last decadeidentified the deregulations of those biomarkers taking place in OSF that are capable to differentiate them from normal individuals. Detection of OPMD cases at such an early stage makes OSF curable and the non-invasive techniques used in their treatment and detection makes it more acceptable to the patients since there is no risk of infection, excessive blood loss and pain. The use of advanced techniques like HPLC, GS-MS, OCT, NMR and FTIR has also made the diagnosis cost-effective thus, can be used foreconomically backward people towardstreatment of OSF. Higher specificity and accuracyof these advanced technologies on the way to detecting novel metabolomic markers and molecular signatures hold enough potential to be applied in clinical medicine.

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# On the use of red ant *Oecophylla smaragdina* by the indigenous people of Binpur, Jhargram, West Bengal.

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ABSTRACT

The red ants (locally known as *Kurkut*) *Oecophylla smaragdina* in the Purnapani and Jorum forests of Binpur-I and Binpur-II block respectively of Jhargram are collected by indigenous people during September to February period to use them as food, medicine and marketing commodities. They prepare delicious food by using the adult and larva of the ants. Also they are habituated to use these ants to get relief from the trouble of cold and cough. The live and dry ants as well as 'chutney'prepared by using these ants are sold in the market and the festival fairs.

## INTRODUCTION

Ants are found almost everywhere in possible niches on the earth except the Antarctica and islands like Greenland, Iceland, parts of Polynesia and the Hawaiian Island (*Thomas, 2007; Jones, 2008).* They play an important role to regulate the ecosystems to ensure pollination and dispersal of seeds in many plant species and also serve as food and medicinal resources for human (Long, 1901; Mahawar and Jaroli, 2008; Lengyel et al., 2010; Rastogi, 2011; Van Huis et al. 2013). In India tribal people are habituated to use certain ant species as their food, medicine as well as a source of income on way of selling the same in the market (Oudhia, 2002; Narzari and Sharmah, 2015; Jena et al., 2020) in Chhattisgarh, Assam and Odisha.However, no information on the said aspect is on record from Jhargram-tribal dominated areas of West Bengal. But, the indigenous people of the Binpur area are accustomed to use the red ants (local name *Kurkut) Oecophylla smaragdina* as food, medicine and commercial itemand information on the said aspect is presented in this article.

## METHOD

We visited the tribal people-residing areas of Purnapani and Jorum forests of Jhargram District (formerly Paschim Medinipur) of Jangalmahal under Binpur-I and Binpur-II blocks ($22^0$36'0 North, $86^0$55'0 East) respectively several times since 1994 to collect data on the use of ants by the local people. Attempts were made to note the ant species, their nesting sites, collection techniques of the adult and larvae of the ants by the local people from the trees and the mode of use of ants and the collection period during January to December.

## RESULTS

It is revealed that the tribal people collect only the red ants *Oecophylla smaragdina* (Fig. 1) from the nests constructed in the trees collectively by two or more people (Fig. 2). They are habituated to collect these ants during

September to February period when large numbers of ants' nests are found in different trees.

F

Fig. 1. Red ants on leaf

The collected *Oecophylla smaragdina* are used as food and medicine by the local people. Also, they were seen to use these ants as commercial item. Most of the rich families are accustomed to prepare an item known as 'Chutney' which is considered a palatable food item to all people. This said chutney is prepared by adding required amount chili, mint, onion, ginger, garlic, cumin, mustard oil and salt. As this type of preparation of ants is costly, the poor families are habituated to use these ants by different means. They have developed the art of pasting these ants by the help of a mortar. The paste thereafter is mixed with pieces of onion, drops of mustard oil,chili and salt. These preparations are used for 2 to 3 days with rice at their lunch or dinner. Sometimes, these ants are boiled and consumed by the people as such.The chutney, because of it's good taste is also sold in the market and festival-fair (Fig.3) at high price.

Fig. 2. Collection of kurkut at Purnapani of Jhargram by the local people

Also the people are used to keep the sun-dried ants in containers for selling the same in high price during other months of the year when these ants are not found in forest.The people are accustomed to sell the live, dried and readily consumable ants in the market. Thus the local people earn money to a great extent.

Fig. 3. Red ants' chutney

*Oecophyllas maragdina* are also used rather eaten by the tribal people to get rid of the suffering from cold and cough. Many non-tribal people were seen to purchase the live ants, both adults and larvae to feed their pets especially the birds and fishes.

## DISCUSSION

From the results it is evident that the red ant *Oecophylla smaragdina* are used by the tribal and other indigenous people of Binpur, Jhargram, West Bengal as food, medicine and commercial item. Though, Long (1901) reported the use of red ants as food by the different ethnic groups of India in 1901 the second report was came into the sight while Roy and Rao (1957) stated in their article that the Muria tribal people of Bastar district of Chhattisgarh are accustomed to consume 'chutney' prepared using these ants.

Subsequently,Veeresh (1999) in his article stated that the people residing at Kanara region of South India and Nagaland consumed red ants either in raw form or as chutney or following cooking. Consumption of red ants by the tribals of Madhya Pradesh did not escape the sight of Srivastava et al. (2009). Narzari and Sharmah (2015) reported that the Bodos of Assam are habituated to swallow the roasted, smoked and fried *Oecophylla smaragdina*. Also, in Arunachal Pradesh certain section of people used red ants in different forms in their regular diet (Chakravorty et al. 2016). After few years, information on the consumption of fried pupal and adult morphs of the said ant species by the Koch Rajbongshi of North Salmara subdivision of Bongaigaon district of Assam was forwarded by Das and co-workers (2019). Moreover, recently Jena et al. (2020) reported on the habit of consumption of chutney prepared by using red ants in the tribal families of Mayurbhanj district of Odisha.

The use of *Oecophylla smaragdina* as medicine by various tribes and ethnic groups of India is also well documented from the studies of Oudhia (2002), Mahawar and Juroli (2008), Padmanabha and Sujana (2008), Kumari and Kumar (2009) and Rastogi (2011). According to Oudhia (2002) *Oecophylla smaragdina* are used extensively as medicine in Chhattisgarh. Though Padmanabha and Sujana (2008) stated that the paste made of red ants *Oecophylla smaragdina* is eaten as a remedy for myopia, Mahawar and Juroli (2008) opined that the said ant is used as medicine to cure various diseases. Kumari and Kumar (2009) reported the use of larval, pupal and adult morphs of red ants to cure gout and joint pain as well as to recover from weakness following suffering from typhoid, gastritis and bronchitis by the local people of Panch Pargana area of Jharkhand. Rastogi (2011), more specially stated the use of red ants as a remedy for cold and flu, headache and to stimulate the secretion of gastric juices.

Besides these uses of red ants, many of the above-mentioned workers have reported the collection of *Oecophylla smaragdina* in large quantities from the forests with a view to earn money on way of selling the same in the market. But, from the present studies it is well evident that the concerned people of Binpur area are accustomed to sell not only the live red ants but also the prepared food item viz. the chutney in the market and/or festival fair in Binpur, West Bengal.

It is to be mentioned here that the red ants *Oecophylla smaragdina* are used as food and medicine in Africa (Nkouka, 1987; Bani, 1995) as well as in Australia (DeFoliart, 1989). The food values of these ants have been discussed

at length by Chakraborty et al. (2016) while specific report on the components of *Oecophylla smaragdina* acting as medicine to cure various ailments is still wanting.

Thus it is concluded that the red ants *Oecophylla smaragdina* are used as food, medicine and commercial commodities in many parts of the globe and the present article confirming the use of ants in West Bengal, India for the first time. Certainly these red ants are very much involved with the socio-economy of the ethnic groups and the said aspect will be highlighted through subsequent publications.

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# Diversity of indigenous ornamental fishes of Purba Medinipur, West Bengal, India

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ABSTRACT

Present study has been conducted on indigenous ornamental fish diversity of Purba Medinipur district, West Bengal, India. During the 21 months of study period we have identified 44 indigenous ornamental fish species under 17 families of 7 orders from area under study. On the basis of overall survey the order Cypriniformes represented the largest diversity including 15 species under 2 families. Cyprinidae is the most dominant family, contributing 29.45% species among total species. According to IUCN (2020.1) status of the fishes are included 86.37% Least Concern, 4.54% Vulnerable, 4.45% Near Threatened, 2.27% Not Evaluated& 2.27% are Data Deficient. Locally a good number of species have been detected very low in density from the study area is an alarming condition, needed immediate conservation strategies to safeguard these valuable natural resource.

## INTRODUCTION

Ornamental fishes can be defined as attractive colourful fishes of peaceful nature, that are kept as pets in confined spaces of an aquarium or a garden pool with the purpose of enjoying their beauty for fun and fancy (Dey, 1996). Since ornamental fishes are usually kept in glass aquarium, these are also popularly known as aquarium fishes. Ornamental fishes are the most popular pets in the world (Singh, 2005). Aquarium keeping has emerged as the second most popular hobby in recent years next to photography (Chapman, 1997). It offers a feast to our eyes and relaxation to the mind especially when we feel tired or depressed. Ornamental fishes are also called 'living jewels' for their beautiful colours and playful behaviour. Ornamental fishes are typically small sized, colourful and most often bizarre shaped in appearance (Dey, 1996). However, these fishes need not necessarily be always colourful. In fact, certain fish species loved by aquarist are quite ugly, in such cases the peculiar appearance is a source of attraction for the aquarium lovers and naturalists (Dey, 1996). With the inspiring popularity of aquarium keeping in households in many parts of the world ornamental fish has become an important part in international trade and has become a global industry (Tlusty et al., 2013). About 288 exotic varieties of ornamental fishes are popular in West Bengal (Bhaskar et al., 1989). Not only do exotic fish have ornamental values, many of our inland fish also have ornamental values such as their various attractive colours, spots, shapes, transparent bodies, behaviours such as their shaking, sudden rise and fall, crawl down, wagging the tail

excessively change colour at different time and seasons etc., which will attract the eyes of aquarist.

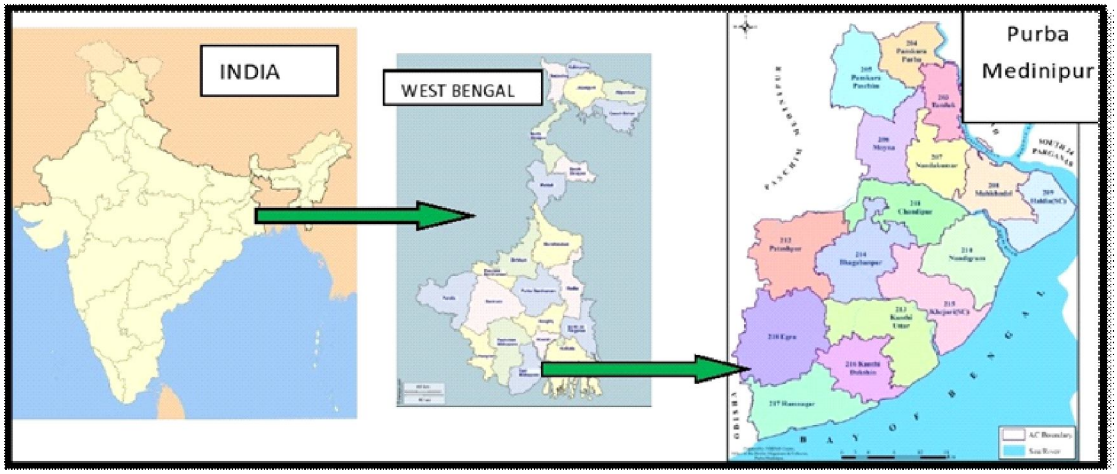
Basu et al (2012) enlisted 70 indigenous ornamental fishes from West Bengal. Purba Medinipur district is an important district of West Bengal, India. Purba Medinipur district was formed on 1st January, 2002 from undivided historical Midnapore. Another part is designated as Paschim Medinipur. It is part of the lower Indo-Gangetic Plain and Eastern Coastal plain. The major rivers are Haldi, Rupnarayan, Rasulpur, Bagui and Keleghai, flowing in north to south or south-east direction of Purba Medinipur district. Various indigenous ornamental fish found in the rivers of Purba Medinipur districts. Paul & Chanda (2014) enlisted 48 numbers of indigenous ornamental fishes from Paschim Medinipur district. Sit et al. (2020) enlisted total 9 *Puntius* species as an ornamental fish from Purba Medinipur, Paschim Medxinipur & Jhargram district. Dutta *et al.*, (2013) reported 21 species of ornamental fishes from coastal region Digha to Talpati as well as Purba Medinipur, West Bengal. During present study a thorough survey has been conducted in all 25 blocks of Purba Medinipur district.The objective of the present study is to report the diversity of indigenous ornamental fish, their distribution & conservation status in Purba Medinipur district. Present study will be a base line for conservation planning of the aquatic environments of these river as well as Purba Medinipur district. Present study follows the work of Mishra *et al.* (2003), R. P. Barman (2007), Goswami *et al.* (2012), Patra *et al.*(2017), Moglekar *et al.* (2017) & Benerjee *et al.* (2019).

## MATERIAL & METHODS

### Study Site:

The geographical position of the study site (21° 38´N- 22° 31´N; 87° 17´E-88° 12´E) is shown in figure 1.Climatic conditions of Purba Medinipur are under the influence of South-West and North-East monsoon.

**Fig 1: Geographical position of Purba Medinipur District.**



**Collection& preservation of specimens:**

The study has been conducted over a period 21 months (June 2018 to February 2020). Specimens have been collected from all the blocks of Purba Medinipur districts. Specimens were immediately preserved in 4% formaldehyde and brought to laboratory of PG Dept. of Zoology, Raja N. L. Khan Women's College (Autonomous). Then the fish specimens have been washed, identified and finally preserved in 5-6% formaldehyde in separate container for each species.

**IDENTIFICATION**

The specimens have been identified morphologically & meristimatrically such as body length, depth, colour, colour band, shape, size, fin number, fin shape, fin rays number, lateral line scale etc. on the basis of existing literature such as Talwar and Jhingran, 1991; Jayaram, K. C,1999 & 2010, www.fishbase.org.ver 2020.

**DATA ANALYSIS**

The identified fish species have been categorized as different levels of threatened condition such as Least concern (LC), Vulnerable (VU), Near threatened (NT), Not evaluated (NE), Data deficient(DD) on the basis of IUCN Red list (IUCN, 2020.1). All the statistical graphs have been done with the help of Microsoft Excel 2007.

**RESULT & DISCUSSION**

During the study period we enlisted 44 species of indigenous ornamental fish under 26 genera, 17 families and 7 orders from Purba Medinipur district of West Bengal that has been given in the table no-1. Among the collected species order Cypriniformes is the most dominant group contributing 34.09%, followed by Perciformes 29.60%, Siluriformes 20.45%, Synbranchiformes 9.09%, Cyprinidontiformes, Osteoglossiformes, Beloniformes, each with 2.27% of the total species (Fig.3&4). Order Perciformes contributed 6 families to the total families, followed by Siluriformes 4, Synbranchiformes & Cypriniformes each with 2, Cyprinidontiformes, Osteoglossiformes & Beloniformes each with 1 family (Fig.2). Cyprinidae is the most dominant family contribute 29.45% species followed by Bagridae 11.36%, Ambassidae 9.09%, Mastacembelidae 6.81%, Channidae 6.81%, Gobioidei 4.54%, Siluridae 4.54%, Cobitidae 4.54%, Osphronemidae 4.54% and Anabantidae, Notopteridae, Claridae, Heteropneustidae, Belonidae, Aplocheilidae, Branchidae, Badidae each with 2.27% among total species (Fig.5). A comparison between observation of Dutta *et al.* (2013) and present study reveals that there is a probability to found 53 species of ornamental fish from Purba Medinipur district as because 12 species are common with the record of Dutta *et al.* and there is a gap of 41 species (32 in record of Dutta *et al.* and 9 in present study) between two reports.

According to IUCN (2020.1) status of the fishes has been included 86.37% Least Concern, 4.54% Vulnerable, 4.54% Near Threatened, Not Evaluated and Data Deficient each with 2.27% (Fig.6). On the basis ofblock wise availability (High e" 70% Blocks; Medium= 40%-69% Blocks; Low=20%-39% Blocks; Very Low d" 20% Blocks) local status of fish species have

been determined for their conservation.The local status of fish species contributes 36.36% very low, 25% low, 13.63% medium & 25% high amount in Purba Medinipur district (Table-1). It is found that maximum number of fish species have been low amount into the study area. Several anthropogenic factors damaged the fish population mainly culture of Indian major carp, effluence of different industrial waste, surface runoff containing various pesticides from nearby agriculture fields and also over fishing are somefactors for fish diversity loss in the Purba Medinipur district. From the above factors, we can conclude the necessary of the conservation of indigenous ornamental fishes of Purba Medinipur district is essential.

**Table 1: Indigenous Ornamental fishes of Purba Medinipur with their block wise distribution**

| Name of the order | Name of the family | Name of the species | Local name | IUCN Status | Local Status | Distribution (blockwise) |
|---|---|---|---|---|---|---|
| 1.Osteoglossiformes | 1.Notopteridae | **1.***Notopterus notopterus*(Pallas,1769) | pholui | LC | M | Egra-I & II, Contai-I & III, Mahisadal, Moyna, Panskura, Potaspur-I & II, Bhawanpur-I & II, Tamluk,Nandigram-I&II,Chandipur |
| 2.Cypriniformes | 2.Cyprinidae | **2.***Amblypharyngodon mola*(Hamilton,1822) | mourla | LC | H | Egra-I&II, Contai-I&II, Mahisadal, Moyna, Nandigram-I , Panskura, Potaspur-I&II,BhawanpurI&II,Tamluk, SahidMatangini, Nandigram-II,Kolaghat,Chandipur.Deshopran ,Nandakumar, Haldia, Khejuri-I & II, Ramnagar-I & II, Sutahata |
| | | **3.***Danio rerio*(Hamilton,1822) | nirali | LC | L | Mahisadal, Moyna, Potaspur-I, Potaspur-II, Bhawanpur-I,Nandigram-I&II |
| | | **4.***Esomus danricus*(Hamilton,1822) | darke | LC | M | Egra-I&II, Contai-I&II, Mahisadal, Moyna, Nandigram-I&II, Panskura, Potaspur-I&II,BhawanpurI&II,Tamluk, Chandipur, Deshopran |
| | | **5.***Puntius chola*(Hamilton,1822) | punti | VU | H | Egra-I&II, Bhawanpur-I & II, Contai-I&III, Mahisadal, Moyna, Nandigram-I&II, Panskura, Potaspur-I, Potaspur-II, , Tamluk, SahidMatangini, Chandipur.Deshopran,Nandakumar, Khejuri-I & II, Ramnagar-I & II, Sutahata |

| Name of the order | Name of the family | Name of the species | Local name | IUCN Status | Local Status | Distribution (blockwise) |
|---|---|---|---|---|---|---|
| | | **6.***Puntius conchonius* (Hamilton, 1822) | punti | LC | VL | Bhawanpur-I, Mahisadal, Moyna, Nandigram-I&II |
| | | **7.***Puntiusticto*(Hamilton,1822) | punti | LC | VL | Mahisadal, Moyna |
| | | **8.***Puntius sophore*(Hamilton,1822) | punti | LC | H | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I &II, Panskura, Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk, Haldia,SahidMatangini,Kolaghat,Chandipur,Deshopran, Nandakumar |
| | | **9.***Puntius phutunio*(Hamilton,1822) | punti | LC | L | Mahisadal, Nandigram-I&II, Bhawanpur-I &II,Tamluk |
| | | **10.***Puntius gelius(*Hamilton-Buchanan,1822) | punti | LC | VL | Nandigram-I &II |
| | | **11.***Puntius sarana* (Hamilton-Buchanan,1822) | punti | VU | VL | Tamluk, SahidMatangini, Nandigram-II,Kolaghat, Deshopran |
| | | **12.***Salmophasia bacaila*(Hamilton,1822) | chela | LC | L | Mahisadal,Moyna, Bhawanpur-I & II, Potaspur-I, Nandigram-I,Potaspur-II |
| | | **13.***Salmophasia phulo*(Hamilton.1822) | chela | LC | VL | Bhawanpur-I , Mahisadal, Moyna, Potaspur-I, Potaspur-II |
| | | **14.***Osteobrama cotiocotio*( Hamilton, 1822) | gobinda | LC | VL | Mahisadal, Nandigram-I, Bhawanpur- II |
| | 3.Cobitidae | **15.***Lepidocephalichthys guntea*(Hamilton,1822) | gunte | LC | L | Mahisadal, Moyna, Nandigram-II, Potaspur-I, Potaspur-II, Bhawanpur-I &II |

| Name of the order | Name of the family | Name of the species | Local name | IUCN Status | Local Status | Distribution (blockwise) |
|---|---|---|---|---|---|---|
| | 3.Cobitidae | **15.***Lepidocephalichthys guntea*(Hamilton,1822) | gunte | LC | L | Mahisadal, Moyna, Nandigram-II, Potaspur-I, Potaspur-II, Bhawanpur-I &II |
| | | **16.***Lepidocephalichthys thermalis*(Valenciennes,1846) | gunte | LC | VL | Mahisadal, Moyna, Potaspur-I, Bhawanpur-I |
| 3.Siluriformes | 4.Bagridae | **17.***Mystus cavasius*(Hamilton,1822) | tengra | LC | H | Egra-I & II, Bhawanpur-I & II, Contai-I & III, Mahisadal, Moyna, Nandigram-I&II, Panskura, Potaspur-I, Potaspur-II, Haldia,SahidMatangini,Kolaghat,Chandipur,Deshopran, Nandakumar, Khejuri-I & II, Ramnagar-I & II, Sutahata |
| | | **18.***Mystus vittatus*(Bloch,1794) | tengra | LC | H | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I, Panskura, Bhawanpur-I & II, Potaspur-I, Potaspur-II, Tamluk, SahidMatangini,Chandipur,Deshopran,Nandakumar |
| | | **19.***Mystus tengara*(Hamilton, 1822) | tengra | LC | VL | Potaspur-I&II, Tamluk, Chandipur, Nandakumar |
| | | **20.***Mystus bleekeri (Day, 1877)* | tengra | LC | VL | Potaspur-I&II, Tamluk, Deshopran |
| | | **21.***Mystus gulio (Hamilton-Buchanan, 1822)* | tengra | LC | M | Egra-I&II, Contai-I&II, Mahisadal,Tamluk, Haldia,SahidMatangini,NandigramII,Kolaghat,Chandipur, Deshopran, Nandakumar |
| | 5.Claridae | **22.***Clarias batrachus*(Linnaeus, 1758) | magur | LC | L | Moyna, Nandigram-I, Potaspur-I, Potaspur-II,Bhawanpur-I , Nandakumar, SahidMatangini, Chandipur |
| | 6.Siluridae | **23.***Ompok pabo*(Hamilton,1822) | pabda | NT | L | Bhawanpur-I & II, Panskura, Potaspur-I, Potaspur-II,Tamluk |

| Name of the order | Name of the family | Name of the species | Local name | IUCN Status | Local Status | Distribution (blockwise) |
|---|---|---|---|---|---|---|
| | | **24.***Wallago attu* (Bloch & Schneider, 1801) | boal | NT | VL | Mahisadal, Potaspur-I |
| | 7.Heteropneustidae | **25.***Heteropneustes fossils* (Bloch,1794) | singhi | LC | H | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I, Panskura, Potaspur-I, Potaspur-II, BhawanpurI&II,Tamluk,Chandipur,Deshopran,Nandakumar, Haldia, Deshopran, Khejuri-I & II, Ramnagar-I & II, Sutahata |
| 4.Beloniformes | 8.Belonidae | **26.***Xenentodon cancila*(Hamilton,1822) | gangtur | LC | VL | Moyna, Potaspur-I, Potaspur-II, Bhawanpur-I |
| 5.Cyprinodontiformes | 9.Aplocheilidae | **27.***Aplocheilus panchax*(Hamilton,1822) | techokha | LC | L | Bhawanpur-I & II, Mahisadal, Moyna, Nandigram-I, Potaspur-I, Potaspur-II, Bhawanpur-I &II, Nandakumar |
| 6.Synbranchiformes | 10.Mastacembelidae | **28.***Macrognathus aral*(Bloch &Schneider,1801) | pankal | LC | L | Panskura, Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk |
| | | **29.***Macrognathus pancalus*(Hamilton,1822) | pankal | LC | H | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I&II, Panskura, Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk, SahidMatangini,Chandipur,Deshopran,Nandakumar |
| | | **30.***Mastacembelus armatus*(Lacepède, 1800) | pankal | LC | L | Mahisadal, Nandigram-I,Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk |
| | 11.Branchidae | **31.***Monopterus cuchia*(Hamilton,1822) | cuchia | LC | VL | Moyna, Potaspur-I, Potaspur-II,Bhawanpur-I |
| 7.Perciformes | 12.Ambassidae | **32.***Chanda nama*(Hamilton,1822) | chanda | LC | M | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I, Panskura, Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk |

| Name of the order | Name of the family | Name of the species | Local name | IUCN Status | Local Status | Distribution (blockwise) |
|---|---|---|---|---|---|---|
| | | **33.***Parambassis baculis*(Hamilton,1822) | chanda | LC | VL | Mahisadal, Moyna, Potaspur-I, Potaspur-II, Bhawanpur-I |
| | | **34.***Parambassis lala*(Hamilton,1822) | chanda | LC | L | Mahisadal, Moyna, Nandigram-I&II, Potaspur-I, Potaspur-II, Bhawanpur-I |
| | | **35.***Parambassis* ranga (Hamilton, 1822) | chanda | LC | L | Contai-I&III, Moyna, Nandigram-I, Potaspur-I, Potaspur-II |
| | | | | | | |
| | | **33.***Parambassis baculis*(Hamilton,1822) | chanda | LC | VL | Mahisadal, Moyna, Potaspur-I, Potaspur-II, Bhawanpur-I |
| | | **34.***Parambassis lala*(Hamilton,1822) | chanda | LC | L | Mahisadal, Moyna, Nandigram-I&II, Potaspur-I, Potaspur-II, Bhawanpur-I |
| | | **35.***Parambassis* ranga (Hamilton, 1822) | chanda | LC | L | Contai-I&III, Moyna, Nandigram-I, Potaspur-I, Potaspur-II |
| | 13.Badidae | **36.***Badis badis*(Hamilton,1822) | dhobachi | LC | VL | Moyna, Nandigram-I,Potaspur-I, Bhawanpur-I,Tamluk |
| | 14.Anabantidae | **37.***Anabas testudineus* (Bloch,1792) | koi | DD | H | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I&II, Panskura, Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk, Chandipur, Deshopran, Nandakumar, Khejuri-I & II, Ramnagar-I & II, Sutahata |
| | 15.Osphronemidae | **38.***Trichogaster fasciata*(Bloch & Schneider,1801) | kholse | LC | H | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I, Panskura, Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk, Haldia, Chandipur, Deshopran, Khejuri-I & II, Ramnagar-I & II, Sutahata |
| | | **39.***Trichogaster lalius*(Hamilton,1822) | kholse | LC | M | Egra-I&II, Contai-I&III, Mahisadal, Moyna, Nandigram-I, Panskura, Potaspur-I, Potaspur-II, Bhawanpur-I &II,Tamluk, Khejuri-I & II, Ramnagar-I & II |

| Name of the order | Name of the family | Name of the species | Local name | IUCN Status | Local Status | Distribution (blockwise) |
|---|---|---|---|---|---|---|
| | 16.Channidae | **40.***Channa punctata*(Bloch, 1793) | latha | LC | H | Egra-I&II, Contai-I&II, Mahisadal, Moyna, Nandigram-I, Panskura, Potaspur-I&II,BhawanpurI&II,Tamluk, Haldia, Kolghat, Chandipur, Khejuri-I & II, Ramnagar-I & II, Sutahata |
| | | **41.***Channa orientalis*( Bloch & Schneider,1801) | chang | NE | VL | Moyna, Potaspur-I |
| | | **42.***Channa striata*(Bloch, 1793) | shol | LC | VL | Panskura, Potaspur-I, Potaspur-II, Moyna |
| | 17.Gobioidei | **43.***Glossogobius giuris*(Hamilton, 1822) | bele | LC | H | Mahisadal, Moyna, Nandigram-I, Potaspur-I, Potaspur-II, Bhawanpur-I &II, Haldia, Khejuri-I & II, Ramnagar-I & II, Sutahata, Egra-I&II, Contai-I&II,Tamluk, Panskura,Chandipur, Deshopran |
| | | **44.***Stigmatogobius sadanundio (*Hamilton-Buchnan,1822) | Vacha | LC | M | Nandigram-I, Tamluk,Haldia, Khejuri-I & II, Ramnagar-I & II, Sutahata, Egra-I&II, Contai-I&II,Tamluk |

Abbreviations: IUCN = International Union for Conservation of Nature; VU = Vulnerable; NE= Not Evaluated; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; VL= very Low; L = Low; M = Medium; H = High

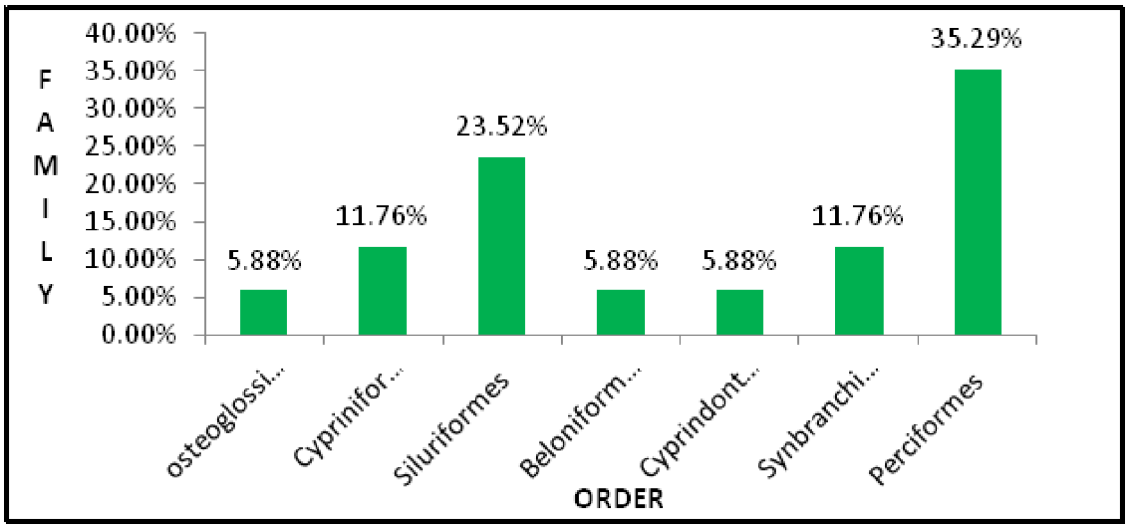



**Fig 2: Orderwise family distribution of indigenous ornamental fishes in Purba Medinipur district**

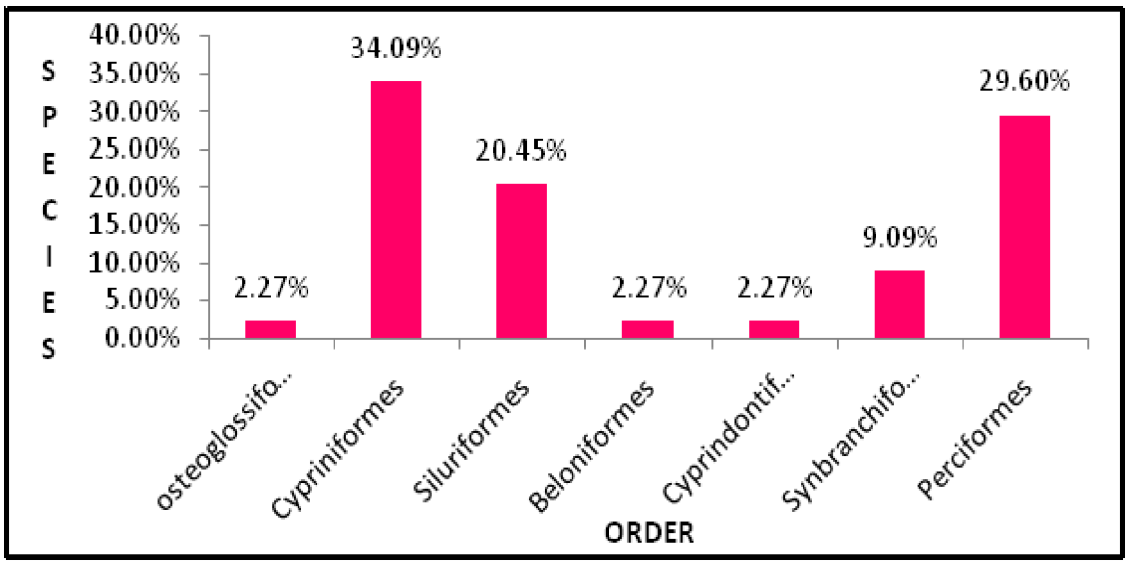



**Fig 3: Orderwise species distribution of indigenous ornamental fishes in Purba Medinipur district**

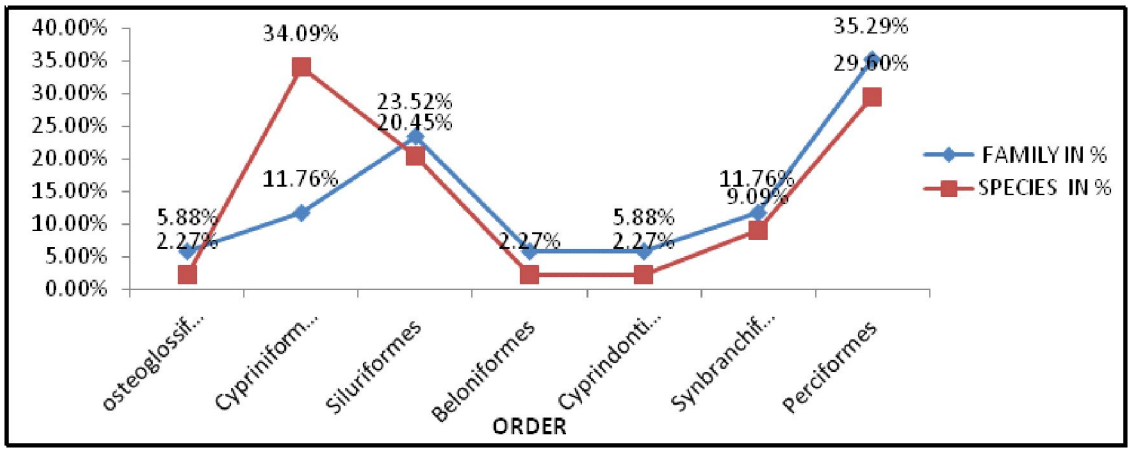



**Fig 4: Relationship among family & species of indigenous ornamental fishes in various orders**

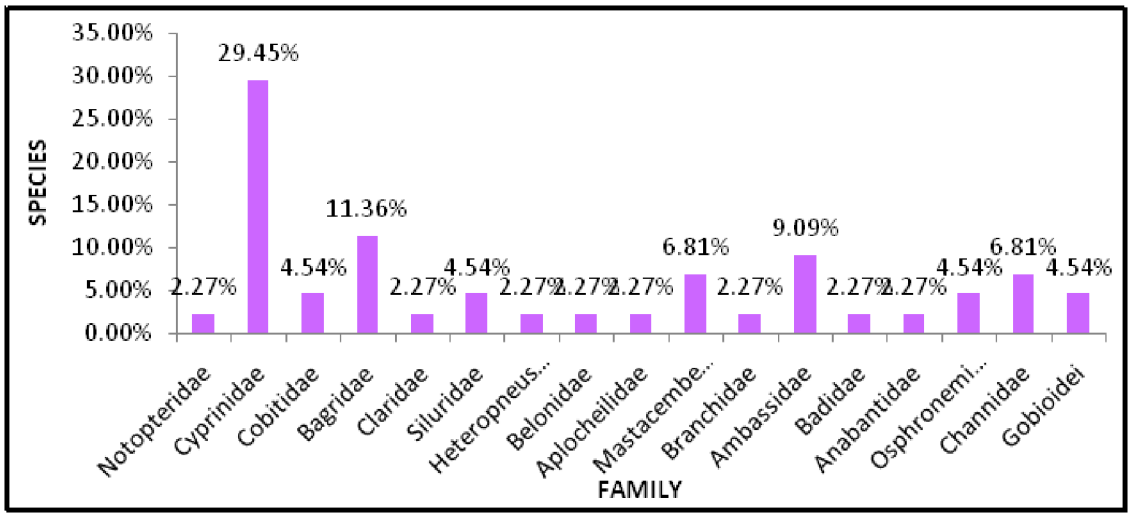



**Fig 5: Family wise species distribution of indigenous ornamental fishes Purba Medinipur district**

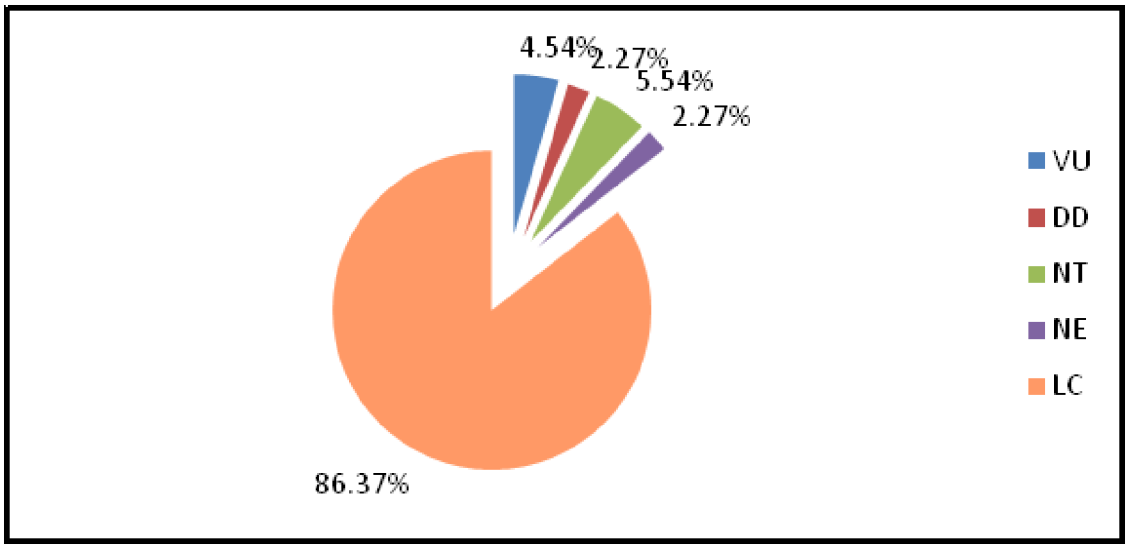



**Fig 6: Percentage contribution of species under IUCN (2020.1) categories**

## Conclusions

A total number of 44 indigenous ornamental fish species has been found in Purba Medinipur district. During present study it has been observed that maximum number of species is available in very low amount in the study area indicates the urgency of conservation to the sustainability of these valuable aquatic resources. Captive breeding fish ranching is being suggested to save this indigenous ornamental fish in its natural water bodies.

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# A report on the study of zooplanktons of Mirik Lake in Eastern Himalayan biodiversity hotspot, Darjeeling India

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ABSTRACT

Mirik Lake is situated in Darjeeling Himalaya which is a hill station and incidentally one of the biodiversity hotspot in India. Studies on the occurrence of different zooplankton species has been done in different seasons for two years period during April, 2002 to March, 2004. A total of five zooplankton genera viz. *Bosmina*, *Chydorus*, *Alona*, *Macrothrix* and *Mesocyclops* have been recorded. Hydrobiological parameters viz. temperature, pH, hardness, alkalinity, dissolved oxygen and free carbon-dioxide have been measured. Level of ten different heavy metals has been estimated while the level of Lead and Selenium were above the maximum permissible limit. Being a tourist spot this hill lake is susceptible to be polluted by hotel wastes and other activities related to tourism. Existence of zooplankton belonging to such a few number of above mentioned genera is assumed to be related to the pollution of lake water. Possibility of considering *Bosmina longirostris* and *Macrothrix* sp. as bioindicator species could not be easily overruled. Since, no previous report is available on zooplankton composition of this lake; the present study is very interesting in the context of zooplankton biodiversity of Darjeeling Himalaya lakes.

## Introduction

Hill lakes are interesting for study of aquatic biodiversity of high altitudes. Zooplanktons in general constitute an important part of biodiversity of the aquatic bodies found in hill areas (Dulmaa 1965; Buyantuyev et al. 1996; Bondarenko et al. 2002). Zooplanktons are the smallest herbivores in aquatic bodies, which consume phytoplankton directly and thus related to the productivity of the habitat concerned. Zooplankton community may act as good agents for giving an indirect estimate of productivity of the lakes and other aquatic bodies (Krebs 2001).

Mirik Lake is one of the interesting lakes situated in Darjeeling hills of West Bengal at an altitude of 1767 meters above the sea level (Jha and Barat 2003). The natural beauty of the lake attracts all tourists visiting Darjeeling hills and thus the lake became a victim of pollution caused by the activities of the tourists. Consequently, the natural occurrence of zooplankton is expected to be affected by tourism. Though the occurrence of different species of zooplankton in different hill lakes of the world is available (Watson 1974; Sharma 2001; Bondarenko et al. 2002; Sharma and Bhattarai 2005); yet there is no record till date available except on rotifers (Mondal et al 2012) on the zooplankton of Mirik lake of Darjeeling hills even when it is situated in one of the biodiversity hotspots in Indian subcontinent. In the present study the investigator tried to identify and enlist different members of zooplankton community occurring in Mirik

Lake, side by side keeping records on some of the hydrobiological parameters and the results have been communicated.

**Materials and Methods**

The study was conducted for 2 years period during April, 2002 to March, 2004. Samples were collected in different seasons of the year during the study period. Plankton samples of subsurface water were collected randomly from different spots of Mirik Lake using No. 25 Plankton Net following conventional method (Shegal 1983). Zooplanktons are fixed in 4% formalin and stained in alcoholic eosin after proper dehydration andmounted in entellan to make permanent slides for observation, identification and preservation of the same.

Water samples have also been collected at the time of zooplankton collection and the water samples were analysed following standard methods (Trivedy and Goel 1986; APHA 1995) for estimation of the hydrobiological parameters viz. dissolved oxygen, free carbon dioxide, alkalinity, salinity, chloride, hardness (for both calcium and magnesium). The temperature and pH of the water samples were also noted time to time. Part of the water samples were brought to Bose Institute, Kolkata, for testing the presence and quantification of heavy metals, if any. The zooplankton specimens were identified by the help of Scientists of Zoological Survey of India, Kolkata.

**Results**

A total of five zooplankton genera viz. *Bosmina*, *Chydorus*, *Alona*, *Macrothrix*, and *Mesocyclops* have been seen during different seasons of the two years observation period. All zooplanktons were under the class Crustacea of which four genera were under order Cladocera and one was under the Order Copepoda. The names and systematic position of the zooplankton species as identified by the Scientists of Zoological Survey of India, Kolkata is represented below.

Phylum – Arthropoda

Sub-Phylum – Mandibulata

Class – Crustacea

Sub Class – Branchiopoda

Order – Cladocera

I. Family : Bosminidae

Genus : Bosmina

Species : *longirostris and* others

II. Family : Chidoridae

i) Sub Family : Chydorinae

Genus : *Chydorus*

Species : *sphaericus* (O.F. Muller) and others

ii) Sub Family : Aloninae

Genus : Alona

III. Family : Macrothricidae

Genus : *Macrothrix*

Order : Copepoda

I. Family : Cyclopidae

Genus : *Mesocyclops*6

Results regarding some of the important physicochemical parameters of the water sample in different months of the study years are shown in Table 1 Level of a few heavy metals were higher than the maximum

permissible limit as prescribed by WHO and ICMR (Mukherjee 1997) while most of the detected heavy metals were present below the permissible limit. Level of some detected heavy metals present in the water has been presented in Table 2.

**Discussion**

No previous study is in hand regarding zooplankton composition except on rotifers (Mondal et al 2012) of Mirik lake. In the present study five genera of zooplankton has been identified. But the number of genera obtained in the present study is less than the number expected in freshwater lakes of hills in general (Rylov 1937; Shulga 1953; Dulmaa, 1965; Watson 1974; Klisko 1998; Bondarenko et al. 2002). Presence of zooplankton species belonging to such a less number of genera may be assumed to be an outcome or the impact of pollution.

Level of the important physicochemical parameters in different seasons of the year as appeared in Table 1 reveals the following facts. The dissolved oxygen varied from 3.43 to 8.72 mg/L. During the months of May and August it is well below the normal range perhaps due to increase in organic load during this period which happens to be a peak season for the tourists; ensuring high inflow of tourists and crowding in hotels producing a large amount of organic pollutants. Alkalinity ranges from 37.5 to 100.0mg/L, however the levels during different seasons are within the normal range (Kendeigh 1974). Lowest level was recorded during the late monsoon period might be due to increase in water volume and the highest level was recorded during the winter presumably due to the decrease in water volume as well as temperature. Hardness, Ca++ and Mg++ level gradually decreased from summer to monsoon to winter. Salinity (Chloride) varied from 21.30 to 44.02 mg/L showing decreasing trend during monsoon due to increase in water volume. The pH of water varied from 2.61 to 7.02, the water becomes highly acidic during the winter and again become neutral to alkaline during the rainy season. The average temperature of the water body ranges from 20.1 to 23.1ÚC in the present study but earlier reported maximum temperature was 29?C and minimum was13?C during summer and winter respectively(Jha and Barat, 2003). Present study reveals that highest temperature is prevalent during summer as well as monsoon and during winter it was lowest and around 20ÚC.

Lower level of dissolved oxygen, free carbon dioxide, hardness, temperature and pH and higher level of alkalinity (Table 1) favours *Bosmina longirostris* and other species of genera *Bosmina* and *Macrothrix*. During summer and monsoon seasons relatively higher level oxygen, free carbon dioxide, hardness, temperature and pH and lower level of alkalinity is somehow favourable for *Bosmina longirostris*, *Chydorus sphaericus*, and other species of genera *Chydorus* (species not identified), *Alona* and *Mesocyclops*.

The levels of different heavy metals recorded during the entire study period have been represented in Table 2. Out of the 10 metals

concentration of Cadmium, Mercury, Chromium and Nickel is below the detective level. The concentration of Copper, Arsenic, Zinc and Manganese are well below the maximum permissible limit. However the concentration of Lead and Selenium are very high compared to the permissible limit. The maximum permissible limits are 0.05 and 0.01 mg/L (ICMR 1975; WHO 1986) while observed levels are 0.95 and 0.58 mg/L for Lead and Selenium respectively. The source of Lead and Selenium might be the rocks of the hill. The physicochemical factors and level of heavy metals may have some role on the zooplankton community but the nature and confirmation of the exact impact needs further detailed study.

Two years study revealed that pollution caused by organic and inorganic matters as well as heavy metals must have some impact on biology of zooplanktons which may lead to extermination of some of the zooplankton species at least from the water body of Mirik Lake. The species occurring in different seasons are not similar, some of the species occurring in all seasons while some others restricted to some seasons only. Seasonal variation in zooplankton diversity in perennial lakes of Tamilnadu is reported by Manickam et al, 2017. In the present study several species under five genera of crustacean zooplankton have been identified. *Bosmina longirostris* has been seen throughout the year whereas other species of genus *Bosmina* is seen during winter. During summer and monsoon seasons the species seen are *Bosmina longirostris*, *Chydorus sphaericus*, and other species of genera *Chydorus* (species not identified), *Alona* and *Mesocyclops*. During post monsoon and winter season the species seen are *Bosmina longirostris* and other species of genera *Bosmina* and *Macrothrix*. Since *Bosmina longirostris* is seen throughout the year it may be presumed that they are tolerant to all kinds of pollution occurred during different seasons. Due to increase in water level/volume during rainy season more number of species appeared as mentioned above. This can be explained in this way that level of pollution fell down due to dilution in the concentration of pollutants with the increase in water volume thus inviting more species by providing more healthy condition. It is also to be noted that during rainy season chance of loading of pollution materials to the lake water becomes very less since tourists do not prefer to visit during rainy season. The occurrence of less number of species during winter condition may be due to the rise in level of pollutants with the fall of water level as well as temperature. Thus some species of genus *Macrothrix* seems to act as good bioindicator specifically for winter season exhibiting fair pollution tolerance ability in adverse situations while other species could not survive except some species of the genus *Bosmina*. However confirmation of the bioindicator property of any species requires details study about its ecology. So confirmation of the fact – whether the above mentioned species are acting as bioindicators or not - demands further extensive study on the bioecology of the species especially in the particular habitat concerned.

**Table 1.** Physicochemical parameters of Mirik Lake water during different months of the study period.

| MONTH | | | | PHYSICOCHEMICAL PARAMETERS | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dissolved O2 (mg/L) | Free CO2 (mg/L) | Alkalinity (mg/L) | Hardness (mg/L) | Ca++ (mg/L) | | Mg++ (mg/L) | Chloride (mg/L) | pH | Average Temperature (°C) |
| March | 8.72 | 19.98 | 47.5 | 31.5 | 6.81 | 3.53 | 29.11 | 6.82 | 21.5 |
| May | 3.43 | 8.69 | 65.0 | 24.0 | 4.41 | 3.17 | 44.02 | 7.02 | 20.1 |
| June | 7.56 | 10.99 | 55.0 | 24.0 | 5.61 | 2.44 | 22.72 | 7.01 | 23.1 |
| August | 4.94 | 11.23 | 37.5 | 16.0 | 2.81 | 2.19 | 21.30 | 4.60 | 23.0 |
| September | 6.15 | 2.30 | 100.0 | 14.0 | 1.60 | 2.44 | 28.40 | 2.61 | 20.5 |

**Table 2.** Level of some heavy metals (mg/L) in the water of Mirik Lake, Darjeeling, W.B., India.

| Heavy metal | Mirik Lake | Maximum permissible limit (WHO, 1984) |
|---|---|---|
| Copper | 0.008 | 0.500 |
| Lead | 0.950a | 0.050 |
| Arsenic | 0.030 | 0.050 |
| Zinc | 0.101 | 1.500 |
| Manganese | 0.050 | 5.000 |
| Selenium | 0.580a | 0.010 |
| Cadmium | BDL | 0.010 |
| Mercury | BDL | 0.001 |
| Chromium | BDL | 0.500 |
| Nickel | BDL | - |


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# Allelopathic influence of *Eupatorium odoratum* L. on germination and seedling growth of somepulses

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ABSTRACT

A case study on the allelopathic potential of *Eupatorium odoratum* on three common pulse seedsviz., *Pisum sativum*,*Phaseolus mungo* and *Lens esculenta* revealed significant inhibition of seed germination and seedling growth.The shade dried leaf powder (1, 2, 5 and 10 g) was soaked separately in 100 ml distilled water for 12 and 24 hours. The aqueous extracts showed inhibitory effects on seed germination, root and shoot length of 12 days old seedlings. The inhibitory effects were proportional to the concentrations of leaf extracts, and the two higher concentrations (5 and 10%) had more inhibitory effects. Among the test cropseeds, *Pisum sativum* showed least sensitive to the application of various concentrations of leaf extracts while *Phaseolus mungo* and *Lens esculenta* seeds were more susceptible to the allelopathic effects of *Eupatorium odoratum*. The results suggest that leaf extracts of *Eupatorium odoratum*had potent allelopathic activity although the magnitude of activity differed depending on concentration.The present study could be important in planning the field under different crops in view of the prevalent agro-ecosystem for higher yield. It is also suggested that these pulses should not be planted close to *Eupatorium odoratum*due to its adverse effects on their growth and development or the crop field should be kept free from this obnoxious weed.

## Introduction

The inhibitory effect of one plant by another through releasing allelochemicals is commonly called “Allelopathy”. Itinfluences one plant upon another growing in its surrounding area by the release of certain metabolic toxic products. Allelopathy can be regarded as a component of biological control in which plants are used to decrease the vigour and development of other plants. Allelopathy is a natural occurrence whereas plant produces one or more biochemical substances that influenceseed germination, growth, survival and reproduction of other plants. Allelopathy involves the release of chemicals into the ecosystem. The biochemical substances are released by plant leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Kruse *et al*., 2000). These chemicals have harmful effects on the crop in the ecosystem resulting in the reduction and delaying in germination, mortality of seedlings and reduction in growth and yield.It has been shown that where *Eucalyptus* stand is replaced by the agricultural crop, that crop will not grow well, at least for a number of years (Fikreyesus *et al*., 2011). Several studies revealed that large areas of theground surface beneath the *Eucalyptus* remains completelybare and ground vegetation

is very limited in extent. The allelopathic effects of *Eucalyptus* species have greatly been investigated on different plant species (Willis, 2010; Yamagushi *et al.*, 2011).Different plant parts, including flowers, leaves, leaf litter and leaf mulch, stems, bark, roots, soil and soil leachates and their derived compounds, can have allelopathy activity that varies over a growing season (Madane and Bhimrao, 2015; Sikolia and Ayuma, 2018). Allelopathic chemicals can also persist in soil, affecting both neighbouring plants as well as those foliar and leaf litter leachates of *Eucalyptus* species, for example, are more toxic than bark leachates to some food crops (Sasikumar *et al.*, 2002). These parts possess allelochemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, amino acids and have an inhibitory or stimulatory effect on the seed germination of crop plants (Mali and Kanade, 2004; Ghodake*et al.*, 2012). The leaf extract has much allelopathic property studied by Kumbhar and Patel (2012).*Eupatorium odoratum*leaf extracts caused inhibitory effect on seedling development of some legume crops (Rafiqul *et al.*, 2003).Hence, the present study is undertaken to assess allelopathic effects of *Eupatorium odoratum* which is a common bushy herb and to analyse how it exerts influence on common pulses.

## Materials and Methods

### Preparation of *Eupatorium* leaf extracts:

One hundred grams (100 g) of fresh mature leaf of *Eupatorium odoratum*, after shade duringfor 10 days, were powdered with the help of grinder and stored in polyethene bags.Theshade dried leaf powder (1, 2, 5 and 10 g) was soaked separately in 100 ml distilled water for 24 hours at room temperature.The collected extracts were filtered through fine cloth to remove debris and finally filtered using Whatman No. 1 filter paper. The filtrate was a stock solution and then prepared 1, 2, 5, and 10% concentration with distilled water. The four extract levels, besides the control (distilled water), were undertaken to perform the experiment under both laboratory and field conditions.The vigorousidentical seeds of *Pisum sativum,Phaseolus mungo* and *Lens esculenta*were surface sterilized with 1% sodium hypo-chloride for 10 minutes, then rinsed with distilled water for several times to eliminate excess adhering water on seeds. Then surface sterilized seeds were soaked for treatment with different concentrations of plant extracts along with distilled water (control) for 12 and 24 hours.All the treated seed samples were placed on 90 × 15 mm sterilized Petri dishes containing wet blotting paper and covered with a lid. The Petri dishes were then kept in a germinating Biochemical Oxygen Demand (B.O.D) type chamber, regulated at 25ÚC constant temperature with 12 hours photoperiod for five days. For each treatment, four replicates were used and each replicate containing 20 seeds.The percentage of seed germination was calculated after five days. The average growth of shoot and root was measured and compared with the corresponding controls and data were statistically analyzed. The data were recorded on percent seed germination and shoot and root length of 12 days old seedlings

during the course of experiment.

**Results**

**Effects on seed germination:**

As shown in Table-1, among various leaf extract concentrations, maximum germination percentwas observed in 1% aqueous extract while the minimum was found in 10% extract in all tested seeds. In *P. sativum* 98%, 88%, 76%, 70% and 60% of seed germination takes place in control, 1%, 2%, 5%, and 10% extract solution respectively when the seeds were soaked for 12 hours. On the other hand, about 98%, 71%, 57%, 55% and 39% of seed germination takes place in control, 1%, 2%, 5%, and 10% extract solution respectively when the seeds were soaked for 24 hours in the same species. Different concentrations of leaf extract of *E.odoratum*significantly inhibited the germination and seedling growth of *Pisum sativum,Phaseolus mungo* and *Lens esculenta*as compared to the control. It is clear from the results that application of aqueous extracts reduced germinationpercent of all the experimentalpulses. Seed germinabilityas shown in Table-1, among various concentrations of leaf extracts and time period on seed treatment, maximum germination percent was observed in control while the minimum was found in 10% leaf extract on both 12 and 24 hours duration of treatment. The study revealed that the inhibitory effect of leaf extracts increased with increasing extract concentration and time (Table-1). Seed germination of *P. sativum* wasstrictly inhibited and only 60 and 39 percent seed germinationwas observed in 10% extract when the seeds soaked for 12 and 24 hours respectively. On the other hand, only 40 percent and 38 percent seed germination was observed on *P. mungo* and *L. esculenta* when soaked in 10%extractfor 12 hours treatment respectively (Table-1).

**Shoot Length:**

Comparison of treatments revealed that shoot length of *Pisum sativum, Phaseolus mungo* and *Lens esculenta* was reduced with the application of leaf extracts. Shoot length decreased significantly with increasing concentrations of leaf extract of *E. odoratum* in all the treatments (Table-2). The highest inhibitory effects on shoot length were found in 10% concentration of leaf extract while the lowest was found in 1%. Shoot length of *P. sativum*was inhibited and only **2.9±0.2 cm** and **2.2±0.4**cm lengthwas observed in 10% extract when the seeds were soaked for 12 and 24 hours treatment respectively. On the other hand, only **3.0±0.1 cm** and **2.2±0.5 cm shoot length** was observed on *P. mungo* and *L. esculenta* when soaked in 10% extract for 12 and 24 hours treatment respectively (Table-2).

**Root length:**

Root length was found to decrease significantly with increasing concentration of leaf extract of *E. odoratum* in all the treatments (Table-3). The highest inhibitory effects on root length were found in 10% concentration of the leaf extract. The highest root length wasobserved in control where the length of roots was recorded **3.2±0.3**

**cm, 3.2±0.4 cm and 3.0±0.1 cm** in *P. sativum*, *P. mungo* and *L. esculenta* respectively. Root length of *P. sativum* was drastically inhibited and only **2.2±0.5 cm** and **2.1±0.2** cm length was observed in 10% extract when the seeds experienced soaking for 12 and 24 hours treatment respectively. On the other hand, only **2.0±0.4 cm** and **1.7±0.1 cm root length** was observed on *P. mungo* and *L. esculenta* when seeds underwent soaking in 10% extract for 12 hours treatment respectively (Table-3).

**Discussion**

The allelopathic effect of leaf extract iscaused due to the various phytotoxic compounds present in the extracts which may independently or conjointly impair to plant growth and inhibit seed germination. The results of this study showed that all the leaf extracts had allelopathic effects on germination and seedling growth, and inhibition was amplified with increasing concentrations used. These results were correlated with the findings of Kil and Lovett (1999),who reported inhibition of seed germination and seedling growth of some herbaceous plants such as chick pea, maizeand pea by aqueous leaf extracts of *Eucalyptus camandulensis* Dehnh. Plants may favourably or adversely affect other plants through allelochemicals, which may be released directly or indirectly from leaf, produced by dead plants or organic residues. This study examined the inhibitory nature of interference of aqueous leaf extract of *E. odoratum* on *Pisum sativum*, *Phaseolus mungo* and *Lens esculenta*. Some workers have reported on the allelopathic potential of common weeds on seed germination, seedling growth and yield of several crop species (Kong *et al*., 2007; Ilory *et al*., 2011). The results of present study were found similar to those of Malik (2004)and Yamagushi *et al*.,(2011)who studied allelopathic effects of *E. globulus* leaf extract on seed germination and seedling growth of some crop plants.

In our study, comparison of treatments revealed that shoot length of *Pisum sativum*, *Phaseolus mungo* and *Lens esculenta* was reduced with the application of *Eupatorium* leafextract irrespective of concentration. The lengths of shoot and root were highly reduced in all leaf extracts of *E. odoratum* and the magnitude of inhibition increased with increasing concentrations. Zhang and Shenglei (2010) reported that the length of radicles and plumules of radish, cucumber and chinese cabbage treated with leaf litter, root exudates of three *Eucalyptus* species were shorter than control and higher concentration induced greater phytotoxicity. In addition, leaf extracts of *E. camaldulensis* decreased root and shoot lengths of tomato (Fikreyesus *et al*., 2011).

From the present study, it can be concluded that aqueous extracts of leaves of *E. odoratum*renderedallelopathic effects on seed germination and seedling growth of *Pisum sativum*, *Phaseolus mungo* and *Lens esculenta*. The extracts reduced germination and growth of seedlings and this inhibitory effect increased with increasing extract concentrations.

## Conclusion

The present study thus concludes that *Eupatorium odoratum* has strong allelopathic property as its leaf extracts succeeded in suppressing seed germinability and seedling growth of three pulses, namely *Pisum sativum*, *Phaseolus mungo* and *Lens esculenta*. Moreover, allelopathy is a concentration dependent phenomenon as its effect increases as the concentration of the extracts increases. Compared with the control (0%), higher concentrations remarkably reduced the seed germination percentage, shoot and root length. Therefore, it is suggested that the weed *Eupatorium odoratum* should be removed from pulse crop fields before the allelochemicals wash down in soil with rain water.

**Table-1**: Allelopathic effects of *Eupatorium odoratum* on seed germination (%)of different pulses. Results are the mean of 6 replicates.

| | **Seed germination (%) on 12 hrs of treatment** | | | | | **Seed germination (%) on 24 hrs of treatment** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Crops** | **Control** | **1%** | **2%** | **5%** | **10%** | **Control** | **1%** | **2%** | **5%** | **10%** |
| *Pisum sativum* | 98 | 88 | 76 | 70 | 60 | 98 | 71 | 57 | 55 | 39 |
| *Phaseolus mungo* | 98 | 77 | 60 | 58 | 40 | 98 | 70 | 54 | 50 | 29 |
| *Lens esculenta* | 96 | 61 | 55 | 51 | 38 | 96 | 55 | 49 | 44 | 30 |

**Table-2**: Allelopathic effects of *Eupatorium odoratum* on shoot length (cm) of different pulses. Results are the mean of 6 replicates (±SE).

| | **Shoot length(cm) after 12 hrs of leaf extract treatment on seeds** | | | | | **Shoot length(cm) after 24 hrs of leaf extract treatment on seeds** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Crops** | **Control** | **1%** | **2%** | **5%** | **10%** | **Control** | **1%** | **2%** | **5%** | **10%** |
| *Pisum sativum* | **4.8±0.6** | **4.4±0.2** | **4.0±0.2** | **3.2±0.3** | **2.9±0.2** | **4.5±0.3** | **3.5±0.4** | **3.0±0.2** | **3.0±0.1** | **2.2±0.4** |
| *Phaseolus mungo* | **4.8±0.5** | **4.2±0.1** | **3.9±0.4** | **3.3±0.2** | **3.0±0.1** | **4.6±0.3** | **3.5±0.1** | **3.1±0.2** | **3.0±0.1** | **2.2±0.5** |
| *Lens esculenta* | **4.1±0.5** | **4.0±0.3** | **3.2±0.2** | **3.0±0.4** | **2.2±0.5** | **4.0±0.2** | **3.2±0.4** | **3.2±0.2** | **3.0±0.3** | **2.2±0.2** |

**Table-3**: Allelopathic effects of *Eupatorium odoratum* on root length (cm) of different pulses. Results are the mean of 6 replicates (±SE).

| | Root length(cm) after 12 hrs of leaf extract treatment on seeds | | | | | Root length(cm) for 24 hrs of leaf extract treatment on seeds | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Crops** | **Control** | **1%** | **2%** | **5%** | **10%** | **Control** | **1%** | **2%** | **5%** | **10%** |
| *Pisum sativum* | **3.2±0.3** | **3.0±0.4** | **2.6±0.3** | **2.2±0.5** | **2.2±0.5** | **3.0±0.3** | **3.0±0.1** | **2.2±0.8** | **2.2±0.2** | **2.1±0.2** |
| *Phaseolus mungo* | **3.2±0.4** | **3.1±0.4** | **2.8±0.1** | **2.5±0.4** | **2.0±0.4** | **2.8±0.4** | **2.8±0.4** | **2.2±0.6** | **2.1±0.2** | **2.0±0.3** |
| *Lens esculenta* | **3.0±0.1** | **2.6±0.2** | **2.1±0.5** | **2.1±0.1** | **1.7±0.1** | **2.8±0.1** | **2.5±0.6** | **2.1±0.1** | **1.9±0.5** | **1.5±0.1** |

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