PROTECTIVE EFFECT OF EMBLICA OFFICINALIS ON CHLORPYRIFOS (AN ORGANOPHOSPHATE INSECTICIDE) INDUCED MALE REPRODUCTIVE SYSTEM IN RATS.

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ABSTRACT

Chlorpyrifos is one of the mostly used organophosphates insecticides, has a major toxic effect in mammalian rat's reproductive system. The ancient Indian herbal formulation, Emblica officinalis plays a major role to subvert many diseases in human body. Through a preliminary study, we have tried to establish the ameliorative properties of Emblica officinalis with respect to reproductive rejuvenation in case of Rattus norvegicus, (Wistar Strain). Adult male albino rats were exposed in 7mg/kg/day chlorpyrifos, 20mg/kg/d amla, 7mg chlorpyrifos with 20mg amla/kg/d body weight for 30 days. The Present study indicates that chlorpyrifos provoked an alteration of body weight, male reproductive organs weight and sperm related parameters. These changes are potentially detrimental and lead to infertility in rats. However, when the subjects were treated with amla and chlorpyrifos, these parameters tend to be normalized, thus highlighting the debilitating effect of chlorpyrifos and remedial effect of Emblica officinalis.

KEY WORDS: Organophosphate compounds (OP), Chlorpyrfos (CPF), Emblica officinalis (EO), traditional use, ameliorative, fertility, male reproduction.

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INTRODUCTION

Pesticides enhanced the economic potentiality in agriculture to controlling the pest, on the debit side are the serious health implication for animals and as well as man. The population of non-agricultural countries also may be exposed to pesticides residues in food stuffs, especially fruits and vegetables, imported from other countries or as a result of bioaccumulation in the food chain, including marine products. Pesticides have a dual action- one is controlling the injurious pests and another is they also present a hazard to species not considered to be pest in the environment. Organophosphates pesticides show their biological effect mainly through the electrophilic attack of cellular constituents with simultaneous generation of reactive oxygen species (ROS). Chlorpyrifos (CPF) is an organophosphates insecticide with a broad range of activity against arthropods, pest of plants, animals including humans. CPF is also known as a residential pesticide for killing fire ants, cockroach and other household pests. Particularly this pesticide has an effect on behavioral and neurological aspect Emblica officinalis (E.O), commonly known as amla is one of the fruits which contain a bioactive component showing antioxidative property. It is a traditional medicine, widely used in India (Asia). Emblica officinalis (EO) enjoys a vital position in pharmacology. In Unani medicine, the dried E.O fruits are used to treat hemorrhage, diarrhea, dysentery vomiting, cough, jaundice, fever, scurvy, diabetes etc. It is a very rich source of ascorbic acid. Emblica is found to be a good herbal protector and at the same time non-toxic, reasonable and easily available botanical material. The aim of this study is to measure the male reproductive toxicity through the low dose chlorpyrifos administration. Administration of Emblica officinalis was found to have ameliorative properties on the adverse effect caused by consumption of chlorpyrifos on the reproductive system of male rats. In reproductive pharmacology, for our knowledge, there exists no previous scientific literature on Emblica as remedial representative for reproductive toxicity related disorder in male reproductive system.

MATERIALS AND METHODS

Animals

Healthy adult male albion rats (Rattus norvegicus, Wistar Strain) (weight approx. 170-220g) were used in the present study. The animals were housed individually in plastic cages, maintaining at a room temperature (21-24°C ± 3°C) in uniform light dark cycle (14:10:L:D). The animals were provided with diet (W.B.Dairy & Poultry Dev. Corp. Ltd.) and water ad libitum throughout the course of study. Animals were quarantined for 10 days before beginning of the experiments. The work related to rat experimentation was conducted with the permission from ethical committee (Vide ref no 892/ac/05/CPCSEA).

Chemicals

Chlorpyrifos was obtained from Nagarjuna Agrichem Limited (Hyderabad, Andhra Pradesh, India) for this experiment. All other chemicals were of analytical grade and were obtained from local commercial sources. EO was procured from local market. The fruits were washed, dried and crushed. The 20mg crushed material was extracted with 1 ml of water, and this extracted juice was given to the rat.

Animals’ treatment schedule

Rat were divided into two groups, control (n=5) and experimental groups (n=15). The experimental groups were divided into three groups. Group1 (G1) received 20mg EO/ kg bw/d (n=5), group 2(G2) received 7mg CPF/ kg bw/d (n=5) and group3 (G3) received 7mg CPF/kg bw/d with 20mg EO/kg bw /d (n=5), through
oral intubations. The control groups however received the same amount of water. After taking the body weight, both control and experimental rats were sacrificed after 30 days of treatment and samples were taken for organ weight measurement, Sperm motility analysis, sperm density, testicular sperm count and epididymal sperm morphology.

**Body and organ weights**
The body weight has been measured on the day of starting of experiment and also on the day of sacrifice (31\textsuperscript{st} day), both the control and experimental group, by automatic balance. The increment of body weight is showed in percentage. Similarly weight of different reproductive organs (Testis, Seminal vesicle, Epididymis) was also recorded.

**Testicular Sperm count**
Testicular sperm count was determined by the following Uzunhisarcikli et al., 2007 \textsuperscript{14}

**Sperm motility analysis**
Sperm motility was determined by the following Uzunhisarcikli et al., 2007 \textsuperscript{14}

**Epididymal sperm morphology**
Sperm morphology was assessed by the method of Filler, 1993 \textsuperscript{15}.

**Sperm density**
The reproductive organ epididymis was removed and fixed in Bouins fixative for 12-14 hrs. It was processed in a series of graded ethanol and embedded in paraffin. Section were cut at 5\textmu m thickness and stained with hematoxylin (Leblond & Clermont, 1952) and eosin for light microscopic examination. The qualitative changes were recorded. In the lumen of the epididymis sperm density was observed and was graded as normal (+++), moderately decreased (++) or severely decreased (+), depending on the concentration of spermatozoa in the tubular cross-sections through the microscope.

**Statistical analysis**
Data were statistically analyzed using \textit{t}-test. The maximum significant level chosen was \textit{P}<0.05.

**RESULTS**

**Evaluation of Body and organ weights**
Death was not observed in any of the experimental groups during experimental periods. It is observed from the table-1, that the body weight increased with the advancement of age, both in control and in the treated groups. The body weight decreased significantly, \textit{P}<0.001 and \textit{P}<0.05, after 30 days treatment for G2 group and G3 group respectively, However in case of G1 group it increased significantly (\textit{P}<0.001) than the control one. But the recovery group G3, Rats fed with Chlorpyrifos with Amla, gained body weight significantly (\textit{P}<0.001) than the treated group G2. The weight of testis, epididymis and seminal vesicle and relative weight of seminal vesicle of G2 group significantly decreased (\textit{P}<0.001) and (\textit{P}< 0.01) respectively, than the control value Table-2. When amla was singly fed, experimental organs showed no significant changes than the control groups. Due to recovery aspects, in group G3, the organ weight significantly increased than the G2 group, especially in testis (\textit{P}<0.001). However they showed no significance changes than the control group.
Table-1
Body weight

<table>
<thead>
<tr>
<th>Day &amp; Dose</th>
<th>No of animal exposed</th>
<th>Initial body weight (gm)</th>
<th>Final body weight (gm)</th>
<th>Body weight increased (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>180.4 ± 4.54</td>
<td>212.49 ± 5.40</td>
<td>17.78 ± 0.09</td>
</tr>
<tr>
<td>G1</td>
<td>5</td>
<td>188.42 ± 2.94</td>
<td>224 ± 2.91</td>
<td>19.51 ***</td>
</tr>
<tr>
<td>G2</td>
<td>5</td>
<td>190.8 ± 3.42</td>
<td>208.4 ± 3.90</td>
<td>9.16 ***</td>
</tr>
<tr>
<td>G3</td>
<td>5</td>
<td>188.70 ± 3.33</td>
<td>219.85 ± 3.71</td>
<td>16.56* aaa</td>
</tr>
</tbody>
</table>

Table-2
Organs weight.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>1.88 ± 0.06</td>
<td>0.89 ± 0.03</td>
<td>0.75 ± 0.04</td>
<td>0.35 ± 0.01</td>
<td>0.8 ± 0.05</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>G1</td>
<td>5</td>
<td>2.03 0.05</td>
<td>0.9 0.01</td>
<td>0.81 0.04</td>
<td>0.36 0.02</td>
<td>0.85 0.03</td>
<td>0.38 0.01</td>
</tr>
<tr>
<td>G2</td>
<td>5</td>
<td>1.23 ***</td>
<td>0.59 ***</td>
<td>0.47 ***</td>
<td>0.23 ***</td>
<td>0.55 ***</td>
<td>0.26 ***</td>
</tr>
<tr>
<td>G3</td>
<td>5</td>
<td>1.82 aaa</td>
<td>0.83 aaa</td>
<td>0.69 a</td>
<td>0.31 a</td>
<td>0.72 a</td>
<td>0.33 a</td>
</tr>
</tbody>
</table>

Evaluation of Testicular Sperm count
Figure 1 shows that the sperm count of G2 and G3 group significantly decreased (P<0.001) than the control group. Besides, the sperm count significantly increased (P<0.001) in G3 group than the G2 group. However, when amla was singly fed, there were no significant changes in testicular sperm count as compared to the control group.
A significant decline ($P<0.001$) was found in normal sperm morphology in G2 and G3 group than the control group. Simultaneously, a significant increment ($P<0.001$) was showed in abnormal sperm morphological structure in both the group G2 and G3 after 30 days of exposure (fig 2). Thick coil tail, tapered head and without head are the criteria of abnormal sperm morphology. In recovery aspect, the normal sperm morphology significantly increased ($P<0.001$) and abnormal sperm morphology significantly decreased than the group G2. No statistically significant changes were detected in normal and abnormal sperm morphology when only amla was singly administered.
Evaluation of Sperm motility analysis
A significant decline, \( P<0.001 \) and \( P<0.01 \), was observed in total epididymal sperm motility at the end of 30\(^{th}\) day in G2 and G3 group respectively than the control (fig 3). But the data shows a significant increment (\( P<0.001 \)) in group G3 than the treated group G2. Single amla fed group had no significant difference than the control group.

Figure 3
Sperm motility

![Sperm motility for 30 days](image)

*Fig.-1,2,3 and Table-1,2-All data are presented as mean ± S.E.M from five similar experiments. Data are significant at the level *** \( P<0.001 \), ** \( P<0.01 \), and * \( P<0.05 \) for G1 G2 and G3 than the control animals. G2, aaa \( P<0.001 \), aa \( P<0.01 \) and a \( P<0.05 \) for G3 than the G2*

Sperm density
In case of CPF treated group, (G2), sperm density is reduced in the lumen of epididymal tubule as compared to the control rats (Table3). In epididymis, the control animals showed normal sperm density (+++). However, the sperm density is decreased (++) in 7mg/ kg d CPF treated group. But when CPF was fed with amla, the sperms showed a normal density (+++).Single amla fed group showed normal sperm density (+++).

Table-3
Sperm density (microscopically observed) for 30 days treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+++</td>
</tr>
<tr>
<td>G1</td>
<td>++</td>
</tr>
<tr>
<td>G2</td>
<td>++</td>
</tr>
<tr>
<td>G3</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++ showing normal sperm density, ++ showing moderately decreased sperm density
DISCUSSION

The exponential increase in production, use and disposition of pesticides has a fundamental impact on the environment and creates unanticipated hazards to many organisms, including humans \(^{16-18}\). Organophosphates (OP) are the most widely used synthetic pesticides and it has a causable effect in fertility \(^{19}\). Herbal materials like amla, used in ancient medicines, are capable to subvert those toxic effects. The present study demonstrated that 30 day’s exposure of male rats to chlorpyrifos at the dose of 7mg/kg b.w/day resulted in decreased body weight, reproductive organ weight (testis, epididymis and seminal vesicle), testicular sperm count, normal sperm morphology, sperm motility and sperm density in tested tissues. Our results showed that the body weight and reproductive organ weight significantly decreased upon chlorpyrifos treatment \(^{20}\). According to Joshi et al., 2007; the reproductive organs weight decreased significantly at various dose levels (7.5, 12.5 and 17.5 mg/kg b.w./day) for 30 days chlorpyrifos treatment. Similar results were found in Chitra et al., 2004 \(^{21}\), in endosulfan treated rats, indicating injury at testicular, pituitary or hypothalamic level. The epididymis and seminal vesicles are androgen-dependent organs and a reduction in their weights may reflect a decline of androgen production. The decrease in testicular weight in treated rats may be due to reduction of tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of leydig cells \(^{22,23}\). In the present study, it is found that when amla was fed with CPF, there was a significant recovery in body and organ weight. Similar findings were observed when amla was fed singly, as compared to the control group. It may be due to recovery of organ or tissue injury or due to revitalized androgen secretion. According to Mode et al., 2009 \(^{24}\), broiler birds gain their body weight due to amla treatment for 28 to 42 days. This body weight gain might be due to the hepatoprotecting activity resulting in the improvement in the liver function \(^{25,27}\). The similar view was present in a master document of Natural Remedies Pvt.Ltd \(^{28}\).

The observed decline in testis weight could be an indicator of sperm toxicity due to CPF treatment \(^{29}\). Parallelly, sperm cell degeneration is an essential indicator of sperm toxicity. The sperm count is one of the most sensitive tests for spermatogenesis and it is highly correlated with fertility. The decrease in sperm motility and density in chlorpyrifos treated group may be due to inadequacy of androgen \(^{30}\) which caused anorgasmia in testicular functions by altering the activities of the enzymes which is contributing for spermatogenesis\(^{31,32}\). According to Zidan, 2009 \(^{20}\), percentage of sperm motility and sperm count significantly decreased in both the three pesticides (chlorpyrifos methyl, dizinon and profenofos,) and highest effect was noticed in case of diazinon exposure. The sperm morphology is considered as a better discriminator between fertility and infertility \(^{33}\). Sperm morphology and motility is a useful marker of toxic damage \(^{34}\). Similar result was showed by Abd El-Aziz et al, 1994 \(^{35}\), who revealed that diazinion treated rats exhibited a decrease in sperm motility, associated with an increment of dead sperm percentage. According to Aikten et al., 1984 \(^{36}\), sperm motility is an important functional measurement to anticipate their fertilizing capacity. Any negative impact on motility would gravely affect fertilizing ability of the organism \(^{37}\). Sperm motility is seriously affected by the low level of ATP content and it may be affected by alteration of the enzymatic activities of oxidative phosphorolytic process. Similarly oxidative phosphorolytic process is required for ATP production, a source of energy for the alleviated movement of spermatozoa \(^{19}\). Full ATP pool is crucial for normal spermatozoal movement and a slight deprivation of ATP leads to reduction in motility, which is one of the major causes of infertility \(^{38}\). According to
Chakraborty and Verma, 2009 39, oral administration of aqueous extract of *Emblica officinalis* along with ochratoxin for 45 days, significantly mitigated ochratoxin-induced alterations in reproductive parameters of mice. The recovery aspect of the herbal product, *Emblica officinalis*, find similar light in our study where there was a significant increase in sperm count, normal sperm morphology and sperm motility in case of amla treated group when compared to the CPF treated group. This shows the ameliorative effect of *Emblica officinalis*. Here amla works as an adjuvant. This might be due to the presence of bioactive compounds, namely: emblicanin A, emblicanin B, punigluconin and pedunculagin which are known to provide protection against oxygen radicals in various in vitro studies 8.

**CONCLUSION**

Under the light of this study, it is concluded that chlorpyrifos is responsible for alteration of body weight, male reproductive organs weight, sperm morphology, sperm count, sperm motility and sperm density. These changes are potentially detrimental and lead to infertility in rats. Our results reveal that due to chlorpyrifos exposure; male fertility diminishes, thus being harmful to any animal, mammals as well as human being. Based on our results obtained, it can be concluded that herbal formulation of *Emblica officinalis* boosts the male reproductive response. EO contains antioxidants which reduces oxidative stress and alleviates the toxicological changes. Amla fruit juice neutralizes the oxidizing potentials of reactive oxygen species generated and through these activities they maintain cell membrane integrity and viability. The present study highlights that amla plays an essential role to contain the chlorpyrifos toxicity in male reproductive system. The scavenging of oxygen radicals’ property possessed by amla can achieve a healthy protection against chlorpyrifos induced male reproductive toxicity; the recovery might hasten with increased amla intake.

**ACKNOWLEDGMENT**

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Emblica officinalis Garten fruits extract ameliorates reproductive injury and oxidative testicular toxicity induced by chlorpyrifos in male rats

Abir Lal Dutta* and Chitta Ranjan Sahu

Abstract

Organophosphate pesticides have destroying properties on male reproduction and chlorpyrifos adversely affects the male reproductive system. Emblica officinalis Garten plays a vital role to challenge many diseases in human body. We investigated the induction of oxidative stress in the male reproductive system of adult rats (Wistar Strain) exposed to widely used organophosphate pesticide, Chlorpyrifos, and tried to establish the ameliorative properties of Emblica officinalis Garten with respect to reproductive reconstruction in them. Rats were divided into 2 groups, control group and experimental group, and the experimental group was divided into 3 groups (G1-G3). All the groups had 5 rats each. Control group received water, experimental group, G1, received 20 mg/kg bw/day Emblica officinalis Garten, G2 received 12 mg/kg bw/day chlorpyrifos and G3 received 12 mg chlorpyrifos with 20 mg Emblica officinalis Garten /kg bw/day. Treatment was done orally from 30 days. Thereafter body weight, male reproductive organs weight, sperm count, sperm morphology, ACP, ALP, total protein, uric acid and testsis and serum testosterone level were determined using standard methods. The changes recorded are indicative of infertility in male rats because of chlorpyrifos exposure. When the subjects were treated with Emblica officinalis Garten in conjunction with chlorpyrifos, these parameters exhibited recovery and when treated with Emblica officinalis Garten alone, these parameters were more or less near to the control group. This highlights the debilitating effect of chlorpyrifos and scavenging property of Emblica officinalis Garten.

Keywords: Organophosphate pesticides; Chlorpyrifos; Emblica officinalis; Testicular toxicity; Reproductive injury; Oxidative stress; Natural medicine

Background

Pesticides, a unique group of compounds, are used to prevent, control or eliminate pests which are a major cause of crop losses in the field as well as in storage. The increase in population has resulted in a shift in cultivation of high yielding crops varieties to feed the teeming millions. In conjunction there has been widespread use of pesticides as insecticides, fungicides, herbicides and rodenticides etc. Application of pesticides amplified as the demand for control of pests and their resurgence increased (Giridhar and Indira 1997).

Occupational exposure to pesticides thus became a common and increasingly alarming phenomenon. Around 3 million acute poisonings and 220,000 deaths from pesticide exposure have been reported per annum (Marrs 1993; USDA 1994; Yasmashita et al. 1997). The health effects caused by this occupational exposure are massive and irreversible in some cases. The widespread use of organophosphorus compounds and the high rates of food contamination could leave humans, animals and birds being exposed to high levels of pesticidal toxicity (Suresh Babu et al. 2006). They rapidly spread in the environment, posing potential hazards to human health. These toxic chemicals, which are toxic to target as well as other non-target organisms, become an integral part of the ecosystem. The use of pesticides undoubtedly enhanced during the beginning of the 19th Century.

Chlorpyrifos (CPF) [O,O- diethyl-O (3,5,6 – trichloro-2-pyridyl)]- is one of the pesticides that exhibit a broad
spectrum of activity against arthropod and non-arthropod pests of plants, other animals including humans (Breslin et al. 1996). Chlorpyrifos is also known as a residential pesticide for killing fire ants, cockroaches and other household pests. Particularly this pesticide has an effect on behavioral, neurological and reproductive function too (Mueller-Beilschmid 1990). Varying concentrations of chlorpyrifos pollution in the environment has become a common phenomenon (Joshi et al. 2003), posing a potential hazard to human health. It has been reported that chlorpyrifos is linked to human genital deformities.

The chief mechanism of action of OP pesticides occurs by the inhibition of neuronal cholinesterase activity, a key enzyme that is concerned in neurotransmission (Richardson et al. 1993).

*Emblica officinalis* Garten, commonly known as amla (synonym Indian gooseberry), is one of the fruits which contain bioactive components that is thought to have antioxidative properties. As a traditional medicine, widely used in India (Ghosal et al. 1996; Bhattacharya et al. 1999), *Emblica officinalis* Garten enjoys a vital position in Ayurveda, an ancient Indian indigenous system of medicine. It belongs to the family Euphorbiaceae and is distributed in tropical Southeastern Asia, particularly in Central and Southern India (Warrier et al. 1995). For medicinal purpose, fresh or dried fruits are usually used. Unani medicinal system uses dried amla fruits to treat hemorrhage, diarrhea, and dysentery (Parroatta 2001). Apart from being a very rich source of ascorbic acid (Twarei et al. 1982), amla also bears fats, tannins and phyllemblin and minerals like phosphorus, iron, and calcium (Sidhu et al. 2011). *Emblica officinalis* Garten leaves and fruit have been used for fever and inflammatory treatments by rural population. The earlier study have demonstrated potent anti-microbial, antioxidant, adaptogenic, hepatoprotective, anti-tumor and anti-ulcerogenic activities in the fruits of *Emblica officinalis*, Leaf extracts have been shown to posses anti-inflammatory activity (Khan 2009).

This study was aimed at to (1) investigate the toxicity of chlorpyrifos on reproductive organs in rats. The body organ weight, sperm morphological abnormality, sperm motility, enzymatic assay, uric acid level and hormonal assay are the criteria used to evaluate the reproductive efficacy of treated rats. (2) see how the herbal product *Emblica officinalis* Garten mitigates the toxicological effects of chlorpyrifos as a bio compatible product.

A large number of compounds have been identified, by different researchers, having protective action against pesticidal toxicity, but those compounds were toxic at their effective dose level. *Emblica officinalis* Garten is found to be a good herbal protector and non-toxic as well, reasonable in cost-benefit aspect and easily available in nature (Chakrawarti et al. 2010).

**Methods**

**Animals**

Healthy adult male albino rats (*Rattus norvegicus*, Wistar Strain) (weight approx. 170-220 g) were used in the present study. The animals were housed individually in plastic cages, maintaining at a room temperature (21-24°C ± 3°C) in uniform light dark cycle (14:10:LD). The animals were provided with diet (W.B.Dairy & Poultry Dev. Corp. Ltd.) and water *ad libitum* through out the course of study. Animals were quarantined for 10 days before beginning of the experiments. The work related to rat experimentation was conducted with the permission from ethical committee (Vide ref no 892/ac/05/CPCSEA).

**Chemicals**

Chlorpyrifos was obtained from Nagarjuna Agrichem Limited (Hyderabad, Andhra Pradesh, India) for this experiment. All other chemicals were of analytical grade and were obtained from local commercial sources.

*Emblica officinalis* Garten was procured from local market. The fruits were washed, dried and crushed. 20 mg crushed material was extracted with 1 ml of water, and this extracted juice was given to the rat. The dose used as 20 mg *Emblica officinalis* /kg body weight for the experiment for removal of toxicity by *Emblica officinalis* is actually a dose below lethal compared to an oral LD 50 value of 1000 mg/kg body weight in rat. The dose used here is sufficient to carry on their ameliorative properties.

**Animals’ treatment schedule**

The oral LD 50 values of any pesticide are not equal and are dependent on the nature of pesticide along with the amount of pesticide exposed to the animals. Accordingly, the oral LD 50 of chlorpyrifos in particular for male rat is 135 mg/kg body weight. The reason for selecting a dose of 12 mg/kg body weight in the present experiment is due to its oral sub lethal dose that caused toxicity to the animals and simultaneously did not cause mortality of the animals. Rats were divided into two groups, control (n = 5) and experimental groups (n = 15). The experimental groups were divided into three groups. Group1 (G1) receive 20 mg *Emblica officinalis* Garten/kg bw/d (n = 5), group 2 (G2) received 12 mg Chlorpyrifos/kg bw/d (n = 5) and group 3 (G3) received 12 mg Chlorpyrifos with *Emblica officinalis* Garten 20 mg/kg bw/d (n = 5), through oral intubations. The control groups however received same amount of water. After taking the body weight, both control and experimental rats were sacrificed after 30 days of treatment and samples were taken for organ weight measurement, sperm motility analysis, sperm density, testicular sperm count, epididymal sperm morphology, quantitative study of protein, measurement.
of uric acid level, estimation of testosterone level in blood and serum, acid phosphatase and alkaline phosphatase activity.

Body and organ weights
The body weight has been recorded on the initial day of experiment and also on the day of sacrifice (31st day), both the control and experimental groups, by using automatic balance. The increment of body weight is presented in percentage. Similarly weight of different reproductive organs (Testis, Seminal vesicle and Epididymis) was also recorded.

Testicular sperm count
Immediately after dissection, one testis of each rat was placed in 1 ml phosphates buffer (pH 7.4). Tunica albuginea was cut by surgical blades, removed and the remaining seminiferous tubules were mechanically minced using surgical blades in 1 ml phosphate buffer. The testicular cell suspension was pipetted several times to make a homogenous cell suspension. One drop of the suspension was placed on the “Hemocytometer chamber” (Neubauer improved, Feinoptik Bad Blankenburg, Germany) and testicular sperm suspension was evaluated as million sperm cells per ml of suspension under 200X magnification using phase contrast microscope and the sperm were Counted manually. Testicular sperm count was measured by Uzunhisarcikli et al. (2007).

Sperm motility analysis
Sperm were collected as quickly as possible after a rat was dissected. The epididymis was cut by surgical blades into 1 mm3 pieces approximately in 1 ml phosphate buffer saline solution at 37°C. The solution was pipetted several times in order to homogenize the sperm suspension and one drop of the suspension was pipetted on a slide, covered by 24 × 24 mm cover slips and evaluated under 100X × 10X magnification using phase contrast microscope. Sperm motility was categorized in to “mobile” or “immotile”. Results were recorded as percentage of sperm motility (Uzunhisarcikli et al. 2007).

Epididymal sperm morphology
Sperm morphology was assessed by the method of Filler (1993). The epididymis was removed and placed in 2 ml of 0.9% saline. It was minced and allowed to incubate for 15 min at 37°C. The sperm were evaluated microscopically at 40X × 10X magnification for identifying the head, tail, mid piece and other sperm abnormalities. At least 300 sperm were evaluated from each slide.

Sperm density
The reproductive organ epididymis was removed and fixed in Bouins fixative for 12–14 hrs. It was processed in a series of graded ethanol and embedded in paraffin. Section were cut at 5 μm thickness and stained with hematoxylin and eosin for light microscopic examination (10X × 10X). The qualitative changes were recorded. In the lumen of the epididymis sperm density was observed and was graded as normal (++++), moderately decreased (+++), or severely decreased (+), depending on the concentration of spermatozoa in the tubular cross-sections through the microscope.

Quantitative study of protein (Testis, Seminal vesicle, Epididymis)
Total quantity of protein was estimated by the method of Lowry et al. (1951).

Estimation of uric acid
Uric acids level was quantified by Serum Uric acid Kit (1972).

Estimation of testosterone in testis and serum
Testosterone in testis and serum level was estimated by method of Mukherjee et al. (2006) and Orcyzk et al. (1979).

Estimation of acid phosphatase
Acid phosphatase enzyme activity was determined by the method of Walter and Schutt (1974). The amount of 4-Nitrophenol in the medium was estimated by measuring the yellow colour at 405 nm in a UV-visible Spectro photometer (Varian,Cary 50 Bio) against an analysis blank.

Estimation of alkaline phosphatase
Activity of alkaline phosphatase was estimated using the method of Ohmori (1937) with slight modification. The reaction product was measured at 405 nm in a UV-visible Spectro photometer (Varian,Cary 50 Bio) against the analysis blank. Activity was determined in the same way as done in estimation of acid phosphatase.

Statistical analysis
Data were statistically analyzed using t-test. The maximum significant level chosen was \( P < 0.05 \).

Results
Evaluation of body weights
Death was not observed in any of the experimental groups during experimental periods. But especially food intake in group 2 reduced during the experiment. It is observed from the Table 1, that the body weight has increased with the advancement of age, both in control
and in the treated groups. The body weight was noted to be decreased significantly ($P < 0.001$) for 30 days treatment of chlorpyrifos treated group (G2) and when *Emblica officinalis* Garten was fed singly for 30 days, the body weight increased significantly as compared to control. But when *Emblica officinalis* Garten was fed with chlorpyrifos (G3), the body weight regained significantly ($P < 0.001$) than the chlorpyrifos treated group (G2), suggesting some remedial role of amla.

**Evaluation of organ weight**

The absolute and relative weights of testis, seminal vesicle and epididymis were found to be decreased significantly ($P < 0.001$) in CPF treated group (G2) than the control group (Table 2). When *Emblica officinalis* was singly fed, the absolute and relative weights of experimental organs showed no significant changes than the control. Due to recovery effects, when *Emblica officinalis* was fed with chlorpyrifos in G3 group, the reproductive organ weight has significantly increased ($P < 0.001, P < 0.05, P < 0.01$, in testis, seminal vesicle and epididymis respectively, than the chlorpyrifos treated rat, though having a significant difference than the control one.

**Evaluation of testicular sperm count**

It is observed from Figure 1 that the sperm count of CPF treated group, G2, significantly decreased ($P < 0.001$) then the control group. However, when *Emblica officinalis* was fed with CPF for 30 days, the sperm count significantly increased ($P < 0.001$) than the CPF treated group G2, but still has a significant difference than the control rats. Singly fed *Emblica officinalis* have no significant change in testicular sperm count as compared to the control rats.

**Evaluation of epididymal sperm morphology**

A significant decline ($P < 0.001$) was found in normal sperm morphology in CPF treated group, G2, than the control group. Simultaneously, a significant increment ($P < 0.001$) was recorded in abnormal sperm morphological structures in CPF treated group for 30 days of exposure (Table 3). Thick coil tail, tapered head and without head sperm morphologies are the criteria of

<table>
<thead>
<tr>
<th>Table 1 Body weight after 30 days treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day &amp; dose</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>G1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>G2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>G3</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

All data are presented as mean ± S.E.M from five similar experiments. Data are significant at the level ***$P < 0.001$, for G1 G2 and G3 than the control animals. aaa $P < 0.001$, for G3 than the G2.

<table>
<thead>
<tr>
<th>Table 2 Organ weight after 30 days treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day &amp; dose</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>G1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>G2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>G3</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

All data are presented as mean ± S.E.M from five similar experiments. Data are significant at the level ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ for G1, G2 and G3 than the control animals. G2, aaa $P < 0.001$, aa $P < 0.01$ and a $P < 0.05$ for G3 than the G2.
abnormal sperm morphology. In recovery aspect, when CPF was administered with *Emblica officinalis* (G3), the sperm morphological deformities (thick coil tail, tapered head and without head) significantly decreased (*P* < 0.001) than the CPF treated group G3, still having a significant difference (*P* < 0.001) than the control group. No statistically significant changes were detected in normal sperm as well as abnormal sperm morphology in sole *Emblica officinalis* treatment in the group (G1) than the control group.

**Evaluation of sperm motility analysis**

A significant decline (*P* < 0.001) was observed in total epididymal sperm motility at the end of 30th day in CPF treated rat than the control group (Figure 1). But when *Emblica officinalis* was fed with CPF, the sperm motility increased significantly (*P* < 0.001) than the CPF treated group G2. However, it still has significant difference (*P* < 0.001) than the control rat’s sperm motility. Single *Emblica officinalis* fed group have no significant difference than the control group.

**Sperm density**

Due to CPF treatment, sperm density was reducing in the lumen of epididymal tubule in respected to control rats (Table 4). In epididymis, the control animals showed normal sperm density (+++). However, the sperm density severely decreased (+) in CPF treated group G2. However, when CPF was fed with *Emblica officinalis*, the sperm density shows a moderate decline (++). Single *Emblica officinalis* fed group showed normal sperm density (Figure 2).

**Quantitative estimation of protein**

According to Table 5, protein level of testis, epididymis and seminal vesicle significantly increased *P* < 0.001 in CPF treated group G2, than the control group. However, when animals were simultaneously treated with CPF and *Emblica officinalis*, the protein level significantly decreased than the G2 group, although these values have a significant difference than the control groups in testis, epididymis and seminal vesicle respectively. When *Emblica officinalis* was singly fed for 30 days, the

---

**Table 3 Sperm morphology (%) after 30 days treatment**

<table>
<thead>
<tr>
<th>Day &amp; Dose</th>
<th>Normal morphology (Mean ± S.E.M)</th>
<th>Abnormal morphology (Mean ± S.E.M)</th>
<th>Thick coil tail</th>
<th>Tapered head</th>
<th>Without head</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.968 ± 0.211</td>
<td>5.032 ± 0.211</td>
<td>3.772 ± 0.107</td>
<td>4.488 ± 0.105</td>
<td>3.89 ± 0.103</td>
</tr>
<tr>
<td>G1</td>
<td>94.526 ± 0.107</td>
<td>5.474 ± 0.107</td>
<td>3.92 ± 0.107</td>
<td>4.936 ± 0.116</td>
<td>4.394 ± 0.116</td>
</tr>
<tr>
<td>G2</td>
<td>66.856*** ± 0.237</td>
<td>33.144*** ± 0.237</td>
<td>11.838*** ± 0.124</td>
<td>13.728*** ± 0.136</td>
<td>16.850*** ± 0.158</td>
</tr>
<tr>
<td>G3</td>
<td>86.132*** ± 0.105***</td>
<td>13.868*** ± 0.105***</td>
<td>7.464*** ± 0.118***</td>
<td>7.332*** ± 0.066</td>
<td>8.978*** ± 0.114***</td>
</tr>
</tbody>
</table>

All data are presented as mean ± S.E.M from five similar experiments. Data are significant at the level ***P < 0.001 and *P < 0.05 for G1, G2 and G3 than the control animals. G2, aaa P < 0.001 for G3 than the G2.
reproductive organs except epididymis ($P < 0.001$) have no significant changes than the control value.

**Estimation of Uric acid**
A significant rise ($P < 0.001$) was found in uric acid level (Figure 3) of G2 group as compared to the control value. When CPF was fed with *Emblica officinalis*, the uric acid level significantly decreased ($P < 0.001$) than the CPF treated G2 group, but is still significantly higher than the control group. However, when *Emblica officinalis* was fed for 30 days singly, the uric acid level has no significant difference than the control value.

**Estimation of testosteron in testis and serum**
As shown in Figure 4, the testosterone level of testis and serum was significantly lowered ($P < 0.001$) in CPF treated G2 group as compared to the control value. In recovery aspect, when *Emblica officinalis* was fed with CPF for 30 days, the testosterone level of testis and serum were observed to be significantly higher ($P < 0.001$) than the CPF treated group G2, but still is significantly lower than the control value. When *Emblica officinalis* was singly fed for 30 days, the testosterone level of testis and serum showed a significant decline ($P < 0.05$ and $P < 0.001$ respectively) than the control value.

**Estimation of acid phosphatase (ACP)**
The acid phosphatase level of testis, epididymis and seminal vesicle were significantly decreasing $P < 0.001$ in CPF treated group G2 than the control value. However in G3, where *Emblica officinalis* was fed with CPF, the acid phosphatase level showed a significant rise $P < 0.001$ in respective tissues than the CPF treated group G2, but this value showed a significant difference $P < 0.01$ than the control value. Singly fed *Emblica officinalis* group showed no significant change in acid phosphatase level of testis, epididymis and seminal vesicle than the control value (Table 6).

**Estimation of alkaline phosphatase (ALP)**
The alkaline phosphatase level of testis and seminal vesicle were significantly lower and that of epididymis was significantly higher ($P < 0.001$) than the control value. However in *Emblica officinalis* treatment, G3 group shows a significant increment in alkaline phosphatase level $P < 0.001$ in testes and seminal vesicle and significant decline in epididymis than the G2 group; though these values still have a significant difference than the control group (Table 7).

**Discussion**
The exponential increase in the production and extensive use of pesticides has a profound impact on the environment and creates unforeseen hazards to any organism as well as man (Chia 2000; Karallieda et al. 2003; Karanthi et al. 2004). Organophosphates are among the most widely used synthetic pesticides. The wide spread use of organophosphate insecticides (OPIs) has a causable toxic effect on reproductive system (Joshi et al. 2007).

Reproductive organ weights are the criteria used for evaluation of reproductive toxicity (Zidan 2009). In general toxicity studies, it is well known that the alterations in body and organ weights are sensitive indicators of the detection of potentially toxic chemicals. In our study, during the exposure toxic symptoms were observed. The body and organ weight were decreased during chlorpyrifos exposure. It is agreed that the weight of reproductive organs decreases significantly at various dose levels (7.5, 12.5 and 17.5 mg/kg bw/day) of CPF for 30 days of treatment (Joshi et al. 2007). Decrease in testis weight could be a most sensitive parameter indicating the male gonadal toxicity. Similar results were found by Chitra et al. (1999), where body weight and testicular weight were reported to be decreased significantly in endosulfan treated rats, indicating impairment at testicular functions affecting androgenesis. Testicular steroidogenesis is regulated by hypothalamo-pitutary axis, which might be distressed by toxic inputs (Singh and Pandey 1989). The epididymis and seminal vesicles both are androgen-dependent organs. Testosterone is more essential for their growth and function and a reduction in their weights may reflect a decline in bioavailability and production of androgens. A similar type of decrease was found in body weight and reproductive organs weight of adult male rat for 90 days treatment of pirimiphos-methyl exposure (Ngoula et al. 2007). Weight of testis and epididymis were significantly lowered in the profenofos treated rats. The decrease in testicular weight in treated rats may be due to reduction of tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells (Sujatatha et al. 2001; Kaur and Mangat 1980). Similar results were recorded by Chadhuary and Joshi (2003), who reported a significant reduction in the rat testis weight after exposure of endosulfan for 15 and 30 days, at the dose levels of 5, 10 and 15 mg/kg bw/day. In addition, EL-Kashoury (2009), showed that the weight of testis was significantly lowered in male rats exposed

### Table 4 Sperm density (microscopically observed) for 30 days treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+++</td>
</tr>
<tr>
<td>G1</td>
<td>+++</td>
</tr>
<tr>
<td>G2</td>
<td>+</td>
</tr>
<tr>
<td>G3</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++ showing normal sperm density, ++ showing moderately decreased, + showing severely decreased sperm density.
to profenofos at the dose of 23.14 mg/kg bw for 60 days treatment. According to Zidan (2009), the reproductive organ weights (testis, epididymis and seminal vesicle) of male rats were significantly lowered at the dose level of 5 and 50 ppm of chlorpyrifos-methyl, diazinon and profenofos treatment for 65 days. The weight of testis and accessory sex organs are known to be dependable indicator of testicular androgen production (Price and Williams-Ashman 1961; Rind et al. 1963). Significant decrease in testicular weight may be a cause of decrease in the number of spermatogenic elements and spermatozoa (Sherins and Hawards 1978; Takihara et al. 1987). Abd El-Aziz et al. (1994) found that diazinon treatment decreased the weights of most genital organs when administered at two different doses of 1.5 and 3 mg/kg body weight in male rats for 65 consecutive days. The reduction of organ weights may be due to pesticides exposure which is affecting their hypothalamus, pituitary or both Okazaki et al. (2001). In the present study it is found that when amla was fed with CPF, there was a significant recovery seen in body and organ weight than the Chlorpyrifos treated group. It may be due to recovery of organ or tissue injury or due to revitalization of androgen secretion. Similarly, when amla singly fed, the body weight was increased, as compared to the control group. Its may be some adverse effects of different constituents of aqueous extract of Emblica officinalis. According to Mode et al. (2009), broiler birds gain their body weight due to amla treatment for 28 to 42 days. This body weight gain might be due to the hepatoprotecting activity resulting in the improvement in the liver function (Pande and Zeestress 2000; Babu et al. 2002; Ratankumar et al. 2004). According to Singh et al. (2006), Emblica officinalis

Table 5 Protein estimation (mg/g) of testis, epididymis and seminal vesicle after 30 days treatment

<table>
<thead>
<tr>
<th>Organ</th>
<th>Testis</th>
<th>Epididymis</th>
<th>Seminal vesicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.008</td>
<td>4.822</td>
<td>7.738</td>
</tr>
<tr>
<td></td>
<td>±0.019</td>
<td>±0.024</td>
<td>±0.047</td>
</tr>
<tr>
<td>G1</td>
<td>8.054</td>
<td>5.548***</td>
<td>7.842</td>
</tr>
<tr>
<td></td>
<td>±0.015</td>
<td>±0.030</td>
<td>±0.013</td>
</tr>
<tr>
<td>G2</td>
<td>12.772***</td>
<td>10.330***</td>
<td>11.644***</td>
</tr>
<tr>
<td></td>
<td>±0.039</td>
<td>±0.021</td>
<td>±0.013</td>
</tr>
<tr>
<td>G3</td>
<td>9.834***</td>
<td>7.030***</td>
<td>9.484***</td>
</tr>
<tr>
<td></td>
<td>±0.037 aaa</td>
<td>±0.027 aaa</td>
<td>±0.027 aaa</td>
</tr>
</tbody>
</table>

All data are presented as mean ± S.E.M from five similar experiments. Data are significant at the level ***P < 0.001 for G1, G2 and G3 than the control animals. G2, aaa P < 0.001 for G3 than the G2.
showed a recovery effect in body weight until 30 days of post-irradiation treatment.

A parallel essential indicator is sperm cell degeneration. The sperm count is one of the most sensitive tests for spermatogenesis and it is highly correlated with fertility. According to our study the sperm morphological abnormalities were increased, sperm count, sperm motility and density were decreased due to Chlorpyrifos treatment. Similar type of result was found in 5.4 and 12.8 mg/kg/d Chlorpyrifos treated rat group during 90 days treatment. The decrease of sperm motility and density after oral treatment of chlorpyrifos may be due to inadequacy of androgen (Chadhuary and Joshi 2003), which caused anorgasia in testicular functions by altering the activities of the enzymes which is causative for spermatogenesis (Siha et al. 1995; Reuber 1981). Similar type of results was found in 23.14 mg/kg body weight profenofos treated rats for 60 days (EL-Kashoury 2009). According to Zidan (2009), percentage of sperm motility and sperm count significantly decreased in both the three pesticides (chlorpyrifos methyl,dizinon and profenofos). Simultaneously total sperm abnormalities significantly increased for all the tested pesticides. According to our result thick coil tail, tapered head and without head were the selected parameters for sperm abnormalities studies, without head abnormalities showing maximum percentages, and these anomalies are considered as a better discriminator between fertile and infertile males (Guzik et al. 2001). Sperm morphology and motility are useful markers of toxic damage even in absence of any effect on male fertility. Two main regulatory processes, endocrine regulation via the gonadotropin hormones and local regulation via inter-cellular communication, control spermatogenesis. (Holdcraft and Braun 2004). The similar result was showed by Abd El-Aziz et al. (1994), who revealed that diazinon treated rats show decreased sperm motility associated with an increment of dead sperm percentage. Prior epidemiologic work on Chinese pesticide factory workers showed that OP exposure was associated with decreased...
sperm concentration and motility (Padungtod et al. 2000). It is established that sperm motility is an important functional measurement to anticipate sperm fertilizing capacity (Aikten et al. 1984). Any negative impact on motility would seriously affect fertilizing ability of the organism (Murugavel et al. 1989). Low level of ATP content seriously affects the sperm motility. Sperm motility may be affected by alteration of the enzymatic activities of oxidative phosphorolytic process (Tso and Lee 1981). Similarly oxidative phosphorolytic process is required for ATP production; it is a source of energy for the alleviated movement of spermatozoa (Joshi et al. 2007). Full ATP pool is crucial for normal spermatozoal movement and a slight deprivation of ATP leads to reduction in motility, which is one of the major causes of infertility (Poon et al. 2004). The decrease of sperm density in the epididymis is one of the indicators of reduction in spermatogenesis owing to the toxicity of any agent (Poon et al. 2004). Decline in sperm density may be due to direct spermicidal effects presence on Chlorpyrifos treated rats epididymis. The obtained results are in accordance with Narayana et al. (2006), who revealed that the sperm density of adult male rats was decreased due to various dose of methyl parathion exposure.

According to Chakraborty and Rm (2009), oral administration of aqueous extract of Emblica officinalis along with ochratoxin for 45 days significantly mitigates ochratoxin-induced alterations in reproductive parameters. The recovery aspect of the herbal product, Emblica officinalis, find similar light in our study, where there was a significant increase in sperm count, normal sperm morphology and sperm motility. This shows the ameliorative effect of Emblica officinalis, which might be due to the presence of bioactive compounds, namely: emblicanin A, emblicanin B, punigluconin and pedunculagin which are known to provide protection against free oxygen radicals in various in vitro studies (Bhattacharya et al. 1999).

Chlorpyrifos also induces biochemical changes in testis, epididymis and seminal vesicle. Our result reveals that the protein content was significantly elevated in male reproductive organs due to chlorpyrifos exposure. According to Joshi et al. (2007), the protein content of testis was significantly increased in chlorpyrifos treatment during 30 days exposure. Similar results showed the same trend in the protein content caused by several pesticides, at different exposure levels and or different concentrations, as reported by El-Kashoury and Tag El-Din (2010); EL-Kashoury (2009); Shivanandappa and Krishna Kumar (1981); Bulusu and Chakravarty (1992); Joshi et al. (2003) and Ngoula et al. (2007). Puga et al. (1974) demonstrated that the elevation of protein content may be due to the stimulation of growth proteins and RNA synthesis. Dikshith and Dutta (1972), Gupta et al. (1981) and Singh and Pandey (1989) showed that an elevation in the testicular protein may be due to the hepatic detoxification activities which resulted in the inhibitory effect on the activities of enzymes involved in the androgen biotransformation. In accordance with the findings of the present study, Rao and Chinoy (1983), suggested that the accumulation of protein occurred in testis epididymis due to androgen deprivation to target

### Table 6 Acid phosphatase activity (ACP) (μ p-nitrophenol/ mg tissue) of testis, epididymis and seminal vesicle after 30 days treatment

<table>
<thead>
<tr>
<th>Organ</th>
<th>Testis</th>
<th>Epididymis</th>
<th>Seminal vesicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>3.666 ±0.009</td>
<td>3.766 ±0.027</td>
<td>2.774 ±0.016</td>
</tr>
<tr>
<td>Amla 20 mg G1</td>
<td>3.626 ±0.018</td>
<td>3.728 ±0.018</td>
<td>2.738 ±0.007</td>
</tr>
<tr>
<td>12 mg CPF G2</td>
<td>1.974*** ±0.010</td>
<td>2.330*** ±0.021</td>
<td>0.878*** ±0.011</td>
</tr>
<tr>
<td>12 mg CPF + 20 mg Amla G3</td>
<td>3.562** ±0.022</td>
<td>3.638** ±0.012</td>
<td>2.680** ±0.015</td>
</tr>
</tbody>
</table>

All data are presented as mean ± S.E.M from five similar experiments. Data are significant at the level ***P < 0.001, **P < 0.01 for G1, G2 and G3 than the control animals. G2, aaa P < 0.001 for G3 than the G2.

### Table 7 Alkaline phosphatase (ALP) (μ p-nitrophenol/ mg tissue) activity of testis, epididymis and seminal vesicle after 30 days treatment

<table>
<thead>
<tr>
<th>Organ</th>
<th>Testis</th>
<th>Seminal vesicle</th>
<th>Epididymis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.186 ±0.010</td>
<td>7.838 ±0.012</td>
<td>1.400 ±0.024</td>
</tr>
<tr>
<td>G1</td>
<td>8.168 ±0.012</td>
<td>7.820 ±0.009</td>
<td>1.432 ±0.009</td>
</tr>
<tr>
<td>G2</td>
<td>4.802*** ±0.018</td>
<td>4.768*** ±0.022</td>
<td>6.386*** ±0.014</td>
</tr>
<tr>
<td>G3</td>
<td>7.052*** ±0.020</td>
<td>6.296*** ±0.017</td>
<td>1.688*** ±0.021</td>
</tr>
</tbody>
</table>

All data are presented as mean ± S.E.M from five similar experiments. Data are significant at the level ***P < 0.001 for G1, G2 and G3 than the control animals. G2, aaa P < 0.001 for G3 than the G2.
organs and this deprivation effect also led to a reduction in testicular and cauda epididymal sperm population, loss of motility in the latter and an increase in the number of abnormal spermatozoa. Chakrawarti et al. (2010) reported an earlier and faster recovery in Emblica officinalis treated groups. They reveal that the total protein content was adversely affected by cadmium with Emblica officinalis showing a protective action of the latter against cadmium treated group. A significant increase in the number of ribosome may be occurring due to their increased mobilization from ER and this leads to the augmented protein synthesis (Mukerjee and Goldfeder 1974).

Our result reveals that due to chlorpyrifos exposure uric acid level was significantly increased in serum. Similar result was found in various doses of methyl parathion treated rats (Prashanthi et al. 2006; Narayana et al. 2006). This may be due to stress induced toxicity leading to increased uric acid level in blood serum. The changes were less severe in Emblica officinalis treated group suggesting a protection against pesticides. Emblica officinalis is one of the richest sources of vitamin C and it mitigates the uric acid level in blood serum.

A significant reduction in ACP and ALP level was found in testes tissue of chlorpyrifos treated rats in the present study, reflecting suppression in testicular function (Johnson et al. 1970) and indicating a nonfunctional spermatogenesis. Our result is supported by the finding of Prashanthi et al. (2006) and Narayana et al. (2006). They revealed that the ACP level was significantly decreased in methyl parathion induced rat’s epididymis. According to EL-Kashoury (2009), the ACP and ALP level was significantly decreased in prophenofos treated testicular tissue of male rats. Chlorpyrifos induced cell damage results in the release of ACP and ALP into the blood stream, hence reducing its level in the reproductive tissue. This is similar to the findings of Abraham and Wilfred (2000). Decline in ALP activity indicated that chlorpyrifos treatment created a state of decreased steroidogenesis where the intra- and inter-cellular transports were reduced as the metabolic reactions channelize the required inputs for steroidogenesis (Yousef et al. 2001). Acid phosphatase is enzyme competent of hydrolyzing orthophosphoric acid esters in an acid medium. The testicular acid phosphatase gene is up regulated by androgens and is down-regulated by estrogens (Yousef et al. 2001), when the androgen production is inferior, may be the ACP activity is sermonized.

Due to chlorpyrifos exposure reduction in the serum testosterone level is demonstrated by Joshi et al. (2007). Our result is focusing the same light. Similar observation was noted by Zidan (2009), who revealed that there is significant alteration in chlorpyrifos methyl, diazinon.
and profenofos treated male rat testosterone. He also stated that testosterone is the principal male hormone produced by the interstitial Leydig cells of testes. Thus testes are responsible for the synthesis of the male sex hormones; so the decrease in testosterone level might be due to an extensive damage of Leydig cells. Besides, disorders of male genital function (hypogonadism) are manifested by a decrease in plasma testosterone level. Hypogonadism may occur with faulty seminiferous tubular function or defective Leydig cell function and this leads to aridity through decreased production of spermatozoa (Zidan 2009).

Biochemical and hormonal estimations of various parameters indicated that the values of Emblica officinalis treated groups were near the control values. Emblica officinalis extract has been shown to have antioxidant and antiperoxidant properties due to the presence of tannoids, mainly emblicanin-A, emblicanin-B, punigluconin, pedunculolinentic acid (Bhattacharya et al. 1999) and also steroid (Gupta et al. 2013). The in vitro antioxidant activity of tannoids was demonstrated by Ghosal et al. (1996). Some of the plants like Glycyrrhiza glabra (licorice), Rubia Cordifolia (Family-Rubiaceae) and Phyllanthus, Emblica have also been reported to possess antioxidant and free radical scavenging activities (Jose and Kuttan 1995; Tripathi et al. 1997; Korina and Afanasav 1997). The emblicanin are probable to the major antioxidant principles and it also reported that the antioxidant action present in vitro (Ghosal et al. 1996) and in vivo (Bhattacharya et al. 1999; Bhattacharya et al. 2000). Emblica officinalis is the rich sources of vitamin C, minerals and amino acids and also contains a wide variety of phenolic compounds (Rajkumar et al. 2011), those are the excellent scavengers of oxygen free radicals within the cells where reactive metabolites are produced (Uzunhisarcikli et al. 2007) by chlorpyrifos toxicity. Steroids are present in water soluble Emblica officinalis extract (Gupta et al. 2013), that may mimic the normal function of testosterone which plays an important role in reproductive development in mammals. But any of the other constituents of aqueous extract of Emblica officinalis may inhibit the secretion of testosterones when administrated singly amla.

The stress pathways caused by CPF and ameliorating pathways by which Emblica acts are presented schematically in (Figure 5 and 6).

**Conclusion**

Under the light of this study, it is concluded that chlorpyrifos is responsible for irreversible damage to male reproductive organs as well as decreased in body weight, sperm morphology, sperm count, sperm motility and sperm density, ACP, ALP and testosterone level, simultaneously significantly increase in abnormal sperm morphology, protein and uric acid level. These changes are potentially harmful and lead to reproductive infertility in rats. Our results reveal that chlorpyrifos induced oxidative stress diminishes the male fertility, thus being harmful to any animal, especially mammals like the human being.

Based on the results obtained it can be concluded that aqueous extract of Emblica officinalis Garten formulation, an herbal preparation ameliorate male reproductive

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**Figure 6** Diagram showing the probable mechanism of remedial effects of Emblica officinalis against the pesticides treated tissue.
tissue damages. Aqueous extract of *Emblica officinalis* 
Garten contains antioxidants, several flavonoids (Khan 2009) and steroids, these reduces the oxidative stress and recover the testicular tissue damage. *Emblica officinalis* fruit juice neutralizes the oxidizing potentials of reactive oxygen species induced by chlorpyrifos; through, these activities they maintain cell membrane integrity and viability. The present study mainly indicates that *Emblica officinalis* Garten play a core role to reduce the chlorpyrifos toxicity in male reproductive aspect. Any of the following ingredients may exert an inhibiting effect on testosterone secretion, that’s why testosterone level is decreased. Details study of different constituents of aqueous extract of *Emblica officinalis* separately feed is needed to find out the possible reasons of decreased testosterone level in serum and tests.

**Competing interests** 
Both authors declare that they have no competing interests.

**Authors’ contributions** 
ALD carried out this experiment; CRS supervised this work. All authors read and approved the final manuscript.

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Other Than Publication
Ornamental Fishes of Coastal West Bengal, India — Prospects of Conservation and Involvement of Local Fishermen

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ABSTRACT

This survey has been conducted to study the ornamental fishes of coastal Bay of Bengal, selecting the region from Digha to Talpati, a stretch of 64 Km coastal area. Twenty ornamental fish species were identified in the coastal water bodies in this stretch. Some of them are already established as aquarium fishes and rest demand ornamental piscine status for their unique features and qualities. Some of the ornamental fish species of this area like Brachygobious sua, Gobipterus chuno, Badis badis and Brachydanio rario are endangered due to excessive degradation of local ecosystems. Special care should be taken so that they continue to exist. There are ample justifications to establish ornamental fish industry to support the unemployed youth of the area and protect the piscine biodiversity.

Keywords: Ornamental Fish; Piscine Biodiversity; Unemployed Youth; Conservation; Indigenous Species; Coastal Bay of Bengal

1. Introduction

The demand of ornamental fishes is increasing remarkably due to their important role in the world trade for fish and fishery production [1,2]. About 288 exotic varieties of ornamental fishes are popular in West Bengal [3]. A few indigenous ornamental fish species are popular among the native people of coastal West Bengal as well as to the aquarium lovers of different countries. Singapore is considered to be a major supplier of ornamental fishes in the international market [4]. Singapore exports about US$80 million of ornamental fishes annually [5]. The export of ornamental fishes from India is worth only US$0.2 million, which is not sufficient with regard to the rising demand of these beautiful colored species in the world ornamental fish market. This may be attributed to the lack of involvement of technical people in ornamental fish farming [4]. A large number of very beautiful ornamental fish species are still neglected which are easily available in the natural waters of coastal Bay of Bengal. Due to congenial climatic conditions Kolkata and its surrounding districts have emerged as promising breeding centers for ornamental fish where a considerable number of small fish farmers and amateurs are engaged in this trade [6,7]. The indigenous fish fauna of this state includes a wide variety of small fish, which though are unsuitable for conventional fish farming but could be gainfully utilized as ornamental fish for their attractive coloration and other features [7]. These species are found in different qualities of water bodies, like deep perennial ponds, puddles, annual and seasonal ponds, paddy fields and bheries. Most of the fish species can tolerate variations in temperature, salinity, and pH.

2. Methods

The study was conducted during July 2011 to June 2012 from Digha to Talpati, a stretch of 64 km in length and few (variable) kilometers in width, along the coastal Bay of Bengal in India. This region is located at 21°37' North to 21°89' North and 87°29' East to 87°96' East of Purbo
Medinipur district (West Bengal, India). This zone was selected for the study as because it is a very remote and still is an ill developed area of the West Bengal, but is rich in fish diversity. This zone has potential to develop into an indigenous ornamental fish trade centre.

The fish species were collected from ponds, paddy fields, muddy water areas, wetlands and other natural water bodies like low-lying paddy fields by using fishing traps and fine nets. The collected specimen was preserved in 5% formalin and brought to the laboratory for further study. Wherever possible, potential ornamental species were collected alive and taken to the laboratory for culture in aquarium and earthen vats. The salinity (ranging from 0.5 to 9 ppm) and pH (5.8 to 8.2) of the water bodies were measured. Further studies were carried out to know about their food habit, feeding behavior, sexual behavior, parental care, and adaptation in aquarium condition. Reports from interrogation (verbal and questionnaire) were also collected as source of information.

3. Results

The fishes selected for our study were Colisa fasciata, Schneider 1801; Badis badis, Hamilton 1822; Oryzias malastigma, McClelland 1839; Brachygobius suad, Smith 1931; Lepidocephalichthys guntea, Hamilton 1822; Chanda ranga, Hamilton 1822; Chanda nama, Hamilton 1822; Brachydanio rerio, Hamilton 1822; Scatophagus argus, Linnaeus 1766; Ailia coila, Hamilton 1822; Gobioperus chun, Hamilton 1822; Puntius ticto, Hamilton 1822; Puntius stigma, Valenciennes 1844; Chela laubuca, Hamilton 1822; Notopterus notopterus, Pallas 1769; Nandus nandus, Hamilton 1822; Aplocheilus panchax, Hamilton 1822; Mastacembelus pancalus, Hamilton 1822; Puntius sarana, Hamilton 1822; Mestacembelus armatus, Scopoli 1777 and Macragnosthus aculeatus, Bloch 1786.

3.1. Colisa fasciata, Schneider 1801

Has attractive looks with rainbow and greenish colors. Oblique orange or bluish bars are present on the body. Vertical fins with alternating dark spots, anal fin with red margin are present. Sexual dimorphism, nice rhythmic up and down movement, laterally compressed body, with golden yellow oblong prismatic striations makes the fish more attractive. Filliform long extension of the pectoral fin rays makes it elegant. Handy fish, they maintain a wide range of supplemented food acceptability with ability to breed in aquarium, and show a very beautiful parental care. Their average size is about 8 - 12 cm [8]. They are often found in rice fields in Asia: Pakistan, India, Nepal, Bangladesh and upper Myanmar [9]. They are generally present in large rivers, estuaries, ditches, ponds [10] and lakes.

3.2. Badis badis, Hamilton, 1822

It has unique body shape with colourful transverse bands. They occasionally change their body colour. It can camouflage with the bowlike awaiting posture, and have an interesting pairing habit. Hardy fish, can tolerate wide range of water qualities (pH range 6 - 8), salinity and food acceptability. Able to breed in aquarium, average size varies from 6 - 8 cm. They are generally found in Asia: Bangladesh, Nepal and Ganges River drainage in India, the Mahanadi river drainage; Assam lowlands close to Brahmaputra (Kaziranga, Gauhati, and Dibrupur) [11] and also reported from Bhutan [12].

3.3. Oryzias malastigma, McClelland, 1839

They have a transparent body with glittering silver belly and silvery margin fins. Sexually dimorphic characters are also present. Male shows anal fin rays. Show elegant movement in Shoals, and jolly females breed several times a year and carry bunches of eggs from seven days up to one month. They are very hardy fish, can tolerate wide range of water qualities (temperature, pH and salinity). Can consume all sorts of natural and supplementary food of both animal and plant origin. They have excellent larvaevore food habit. They are generally found in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).

3.4. Brachygobius Sua, Smith, 1931

A tiny fish, 5/6 vertical black bands are present. An idle fish; always remains in resting condition, attached on the glass walls of aquarium. It seems to be the beauty spot of an aquarium. They always bumble, has comparatively large pectoral fin like honey bee. Although very idle, becomes swift and smart at the time of preying. Sometimes they swim gladly with jerking movement. They can camouflage for protection and predation.

3.5. Lepidocephalichthys guntea, Hamilton, 1822

In coastal Bengal, the fishermen popularly know L.guntea as loach. Black and brown spots with whitish long stripes along the laterals are present. Most of the time, they remain lazy and stay at the bottom of the aquarium. Its zigzag movement is very fast. The rapid rhythmic movement of operculum makes them attractive in aquarium. As it is detritus feeder, it plays a vital role to keep the aquarium clean. They have a maximum length of about 40 cm. They are very hardy fish which can withstand long starvation. It is distributed in Asia (Pakistan, Northern India, Bangladesh, Nepal, Myanmar and Tha-
ileand.) and also present in Salween basin [13].

3.6. Chanda ranga, Hamilton, 1822

Popularly known as Glassfish, they are transparent, with a pearly white spot in the belly. During breeding season it glitters and develops a deeper colour. Its average size is about 4 cm. Wide range of food and pH acceptability makes them more demandable. They are fresh and brackish water fish generally found in Burma, India, and Thailand.

3.7. Chanda nama, Hamilton, 1822

Chanda nama is an established aquarium fish. It is also known as Glassfish but is a little larger than Chanda ranga. With an average size of about 11 cm and may be male/unsexed [14]. During the breeding season, margin of all fins become glossy white and silvery like neon beam. They usually inhabit standing and running waters, clear streams, canals, beels, ponds, and inundated paddy fields, and are abundant during the rainy season [15]. Wide range of food and pH acceptability makes them more demandable. They are widely distributed in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).

3.8. Brachydanio rerio, Hamilton, 1822

Due to its longitudinal black and white striations, it is popularly know as Zebra fish. In rural West Bengal, it is known as “Hamani” in Birbhum and “Danria” in coastal Medinipur districts. They are very sporty and jolly and show a rhythmic zigzag movement. Brachydanio rerio requires large tank because they are 6 cm long and need a lot of free swimming space. They are found in shoal. They can tolerate a high degree of toxicity, salinity, turbidity and temperature (18˚ to 24˚) etc. Although it prefers zooplankton, it can also engulf mosquito larvae and tubifex eagerly. In case of food scarcity, it consumes phytoplankton and other aquatic soft weeds. They are easily bred in aquariums. Its colorful body and restless movement attract aquarium lovers. Zebra fish is generally found in the streams of the southeastern Himalayan region, including the countries India, Pakistan, Bangladesh, Nepal, and Myanmar (USGS NAS—Nonindigenous Aquatic Species). It commonly inhabits streams, canals, ditches, ponds, and slow-moving to stagnant water bodies, including rice fields [16].

3.9. Scatophagus argus, Linnaeus, 1766

Has a quadrangular strongly compressed body with steep dorsal head profile. Eye moderately large with the tail fins and all other fins are more or less transparent. Violet dark patches are found on the body. Its main food is zooplankton, micro-arthropod and tubifex. Maximum length is 38 cm [17] and common length is 20 cm (male/unsexed). Wide range of food and pH acceptability makes them more demandable. They are already popular as an aquarium fish. Though they comfortably and naturally live in brackish water yet they can easily adopt to freshwater aquarium. They are found in Indo-Pacific: Kuwait to Fiji, north to southern Japan, south to New Caledonia. Reported from Samoa [18], Tonga [19] and the Society Islands [20].

3.10. Ailia coila, Hamilton, 1822

It is a small transparent fish and is popularly known as “kajri” in costal Bengal. A prominent and bright black spot present its pectoral fin base, body is elongated and deeply compressed, mouth sub-inferior, upper jaw longer than lower jaw. Barbells are well developed and present in four pairs. Dorsal fin is absent and a small adipose fin present. Their pectoral fins are well developed and pelvic fins are small, Pectoral spine are slender and serrated, anal fin is large. Body color is silvery or whitish [21]. In aquarium they move in shoals with dancing tentacles. Found in India, Pakistan, Nepal and Bangladesh. Maximum length reported, 15.4 cm [22]. They are mainly plankton feeder and consume all sorts of aquatic micro arthropods and larvae.

3.11. Gobiopterus chun, Hamilton, 1822

Although it is an estuarine fish, they are found in fresh and brackish water in the lower courses of rivers. They are generally not found in fish markets. It can be a popular aquarium fish for its exclusively glass like transparent body. They breed throughout the year and are able to live in aquarium. Their average size is about 3.0 cm [23]. The clutches of eggs are visible in gravid females. Though it is an estuarine fish yet can easily live and breed in freshwater aquarium. They are highly carnivorous fish and prefer micro arthropods and zooplanktons. Its high demand as an aquarium fish is due to its miraculous appearance and sudden camouflaging character. Wide range of food and pH acceptability makes them more demandable. They are generally found in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).

3.12. Puntius ticto, Hamilton, 1822

Puntius ticto is an established aquarium ‘barb’. A body with silvery white glittering colour with a black spot on caudal peduncle is present. Maximum size is about 10.0 cm: [10]. Wide range of food habit and hardy character makes them popular to the aquarium lovers. The sexual dimorphism and seasonal colour change in breeding period makes them more demanding. Found in still, shallow, marginal waters and rivers, mostly with muddy bottoms. They can feed on crustaceans, insects and planktons. About 150 eggs are laid in a batch and about 20
batches are laid at a time. They are generally found in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).


*Puntius stigma* is also an established aquarium ‘barb’. They are commonly found in ponds, pools and ditches of Bengal. It usually grows to size of 2 to 3 inches but it attains 5 inches in length. They found in male and female characters. Generally male are well define, they have a deeper colour and extends to the end of the caudal fin, while in the female it is more or less diffuse and only extends up to the end of the caudal peduncle. Usually the female develop this coloration only when theses fishes are ready to lay eggs, while in the male, the colour band develops with the repining of germ cells. The body of mature female is proportionally deeper than male. Wide range of food habit and hardy character makes them popular to the aquarium lovers. Broad range of food and pH acceptability makes them more demandable. They are generally found in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).


*Chela labuca* have bilaterally compressed body with longitudinal light stripes. The upper part from the lateral line is olive-green while the lower part is glittering. Its attractive rainbow colour makes it a demanding aquarium fish. They are omnivorous in nature, and can be easily bred in aquarium and maximum size about 17.0 cm. A longitudinal stripe may be present on the body which extends up to operculum where it surrounds a deep black blotch. Wide range of food and pH acceptability makes them more demandable. They are generally found in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).

3.15. *Notopterus notopterus*, Pallas, 1769

*Notopterus notopterus* is a fresh water knife fish. It can withstand pH range from 6.0 - 6.5 [24]. Body is bilaterally flat. A large eye with long anal fin is attractive to the aquarium lovers. It has a silvery whitish body and is slow moving. They are highly carnivorous and prefer small fish, larvae and small aquatic arthropods, small crustaceans and zooplanktons. Wide range of food and pH acceptability makes them more demandable in the aquarium arena. The fingerlings body colour is dark violet which fades with age. Maximum length is about 60.0 cm male/unsexed [25]; but common length is 25.0 cm male/unsexed [10]. Found in India, Myanmar, Thailand and Malaysia.

3.16. *Nandus nandus*, Hamilton, 1822

It is a slightly large fish and is yet to be established as an aquarium fish. It has blackish brown colour sometimes with vertical blackish brown bands on the body. They can camouflage with the suitable substratum. They are highly carnivorous and have a protractible jaw with large mouth. They have Maximum size of about 20.0 cm [10] and can tolerate pH of 6.9 to 8. Frequently occur in ditches and inundated fields, they are generally seen in summer. Feeds on aquatic insects and fishes [23]. They are generally found in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).

3.17. *Aplocheilus panchax*, Hamilton, 1822

They are generally Surface feeder and larvaevore fish, present in pair or single. The average size is 9.0 cm [14]. They can breed several times a year. Sticky eggs are attached on aquatic weeds. The males are more attractive with silvery white or orange yellow bordered fins. They are generally present in lowland wetlands to estuaries area [26] and in ponds, ditches and canals [15, 27]. They are usually found in shallow lagoons and swamps among roots of mangroves along the waters margins [14]. They generally prefer, clear water areas with dense growth of rooted or floating macrophysics [28], sometimes they occurring in hyper saline water and feed mainly on insects [27] so they can be utilized for mosquito control. They are difficult to maintain in aquariums [29]. Wide range of food and pH acceptability makes them more demandable. They are generally found in Asia (Pakistan, India Nepal, Bangladesh and Myanmar).

3.18. *Mastacembelus panchal*, Hamilton, 1822

It has a bilaterally compressed body with pointed beak like upper jaw. The upper portion of the body is grayish blue or gray while the lower portion is sandy gray which in mature fish is yellowish. Three to five deep black spots are found at the base of the distal dorsal fins and one spot is present at the base of caudal fin. It is a mud dwelling bottom feeder and habitually stays at the bottom of the aquarium. They usually feed on entomostracans and insect larvae. Maximum length is 13.5 cm [22]. They generally breed in monsoon period or rainy season and spawn between May and August [30]. In aquaria, they spawn in the upper water-level where courtship between female and slender and smaller male generally take place [10].


It is a minor carp. Size is about 42.0 cm (male/unsexed); [15] and mostly remain in flock. Bilaterally compressed bodies with slivery white glitering scale are present. *P. sarana*’s movement is slow but royal in aquarium. Present in rivers, streams, lakes and backwaters region. Moving in shoals of four or five with each shoal having
several dozens, they exhibit a beautiful aquarium picture [17]. They feed on aquatic insects, fish, algae and shrimps. They are present in Asia: Afghanistan, Pakistan, India, Nepal, Bangladesh, and Bhutan [10], Sri Lanka [17], Myanmar [31] and Thailand [32].

### 3.20. Mestacembelus armatus, Scopoli, 1777

It looks like Pankal but smaller than Pankal (Mastambullas pancala) and generally a bottom dweller with detritus feeding habit. Body colour is sandy yellow and its shows wavy zigzag moment. Their movement is very catchy. Maximum length is about 90 cm. They are found both in highland streams and low wetlands [26]. Usually found in streams and rivers with sand, pebble, or boulder substrate. Seldom leaves the bottom except when disturbed. Also occurs in still waters, both in coastal marshes and dry zone tanks. Sometimes stays partially buried in fine substrate. They are reported to occur in areas with rocky bottoms in the Mekong mainstream during the dry season, but enter canals, lakes and other flood plain areas during the flood season [33]. They are marketed fresh and frequently seen in the aquarium trade [28]. They can tolerate high range of pH from 6.5 to 7.5 [34]. They are generally presented in Asia: Pakistan to Viet Nam and Indonesia.

### 3.21. Macrognathus aculeatus, Bloch, 1786

They show features similar to Pankal (Mastambullas pancala) except that they are smaller. In young stage the distal part of the body shows black mosaic, which fades away with the advancement of age. Maximum length is about 38 cm. They are generally freshwater and brackish water fish, and tolerate a high range of pH from 6.5 to 7.5 [34]. They generally feed crustaceans. Present in Asia: India, Nepal, Thailand, Malay Peninsula, Borneo, Indonesia Bangladesh [15].

### 4. Discussion

Data were obtained from interviewing nine hundred and thirty peoples about the present status of these twenty one colour fishes as well as their situation thirty years back, with regards to their availability. The information considered were those obtained from majority (>50%) of person interviewed Table 1. Perusal of the responses in the Table1 highlights the fact that most of the fish species show a declining trend with regards to their availability.

In another study two hundred and seventy nine water bodies were considered for the presence or absences of these ornamental fishes from July, 2011 to June 2012 with the help of skilled fishermen. The percentage availability of each species in each water body is given in Table 2. The study revels that most of the species are present at an alarmingly low percentage which calls for immediate restorative measures. The fishes which are lower in availability could be declared as threatened or endangered in this locality.

### Identifying the Underlying Causes for the Reduction in the Availability of Theses Ornamental Fishes in the Study Area

Nowadays the villagers mainly culture Indian major carps (IMC) for gaining more profit. Besides, the people are using enormous chemical pesticides to eradicate carnivorous and weed fishes for major carp cultivation. Sometimes it is a fascination to cultivate different exotic species like silver carp (Hypophthalmichthys molitrix), grass carp (Ctenopharyngodon idella) American rohu (Cynopterus carpio), Telapia (Tilapia mossambica) and (T. nilotica), African magur (Clarias garipinas), java punti (Puntius javanicus) and Pangas (Pangasius sp.) etc. for the purpose of destruction of unwanted weed fishes. It was found, however, that most of the time the ornamental fishes are also consumed by the local population. Thus, these ornamental fishes have to compete with the locally cultivated fishes which serve as food. Indiscriminate use of chemical pesticides and fertilizers in paddy fields, especially for high yielding crop cultivation and aquaculture, is one of the reasons for the decrease in the ornamental fish diversity.

Dewatering of ponds every year is found to be another cause for decrement of ornamental fishes as because they are considered as the natural breeding ground for the coloured fishes where they easily propagate during the rainy season. After dewatering of ponds mud dwelling fishes like Ban (Macrognathuous aculetum), Pankal (Mystambelus pankalal), Tur (Mas tacebellus), Guntea (Lepidocephalichthys guente) etc. are collected by the urea exposure over the bottom mud. Exposure to urea inevitably mars any chance for the survival of any ornamental fish remaining in the dewatered pond.

Another factor leading to the decline in the availability seems to be illiteracy and lack of awareness about the significance, both economical and ecological, of these ornamental fishes among the fishing folk. Rural collectors cannot discriminate between the desired coloured fish species and other trace fish species, which are not useful to the coloured fish market. Therefore, proper strategy for the conservation of these uncustomary biotic resources, especially the endangered and threatened species is needed [8]. Over population is one of the causes for decrease in the number of locally available coloured fishes in the coastal Bay of Bengal.

With the increasing potentiality of ornamental fish farming some fish have a good demand in global fish market for their beautiful coloration and life style [8]. Some of the indigenous ornamental fishes those were...
abundant even in the past decades [35-37] are becoming rare.

It is crucial time to protect the threatened biodiversity and the susceptible indigenous fishes especially the ornamental fish species. It is encouraging that the developing countries contribute to more than 60% of the total world trade fish market. Although India is still in a marginal position but its market is rising rapidly [8].

Indiscriminate harvesting of fish species from their natural habitat is regularly done by the rural people, which may lead to serious stock depletion. Through interviewing the local people, it is clear that the Lepidochelichthys guntea, Scatophagus argus, Gobiosperus chun, Oryzias malastigma, Brachygobius sua, Ailia coilia, Mestacemebus armatus, Macrogantthis aculeatus, Mastacembelus pancealus, present in very threatened condition (Table 1), but it is fact that in earlier occasion these fishes are present in plenty. It is evident especially in case of Puntius sarana which is not available now but was common thirty years back. To overcome this problem these fishes can be reared and breed in captivity and vocational training could be provided to the fisher folk in rural areas for rearing and breeding of those threatened fish [13].

A good number of stake holders are already involved in ornamental fish farming of coastal Bay of Bengal, but there is lack of proper marketing channel in this trade. In most of the cases the rural women and children collect the fishes from the natural habitats. The exporters buy these fishes from the farmers/collectors, against nominal price and export them to the global fish market [8]. Ornamental fish marketing is becoming popular as an easy and stress relieving hobby. About 7.2 million houses in the USA, 3.2 million in the European Union have an aquarium and this hobby is on an increase throughout the world. Presently USA, Europe and Japan have the largest ornamental fish market [8].

However, there is a nice scope to establish an ornamental fish industry to export the aquarium fishes. Ornamental fish farming that includes rearing, captive breeding, and culture have considerable economic importance [35]. Annually, India exports about of 250 million ornamental fishes, mainly to USA [38], with Tamilnadu and West Bengal contributing to about 150 millions and 30 million respectively. A rich fish diversity, favorable climate, cheap labor and easy distribution make India, specially West Bengal, suitable for ornamental fish culture. With kolkata (West Bengal, India) as a nodal distribution and export center, the neighboring districts can become the major ornamental fish-producing zones of India. In the state of West Bengal there are more than 2000 people involved in this trade including ornamental fish breeders, growers, seed and live food collectors. About 150 families are involved in ornamental fish farming to maintain their livelihood. More than 500 families use it as an additional income generating business [8].

There is an immense scope of ornamental aquaculture in coastal Bengal, only if the captive breeding of ornamental fish could be commercially done. It can be a good source of earning foreign exchange. Most of the unsold fish or excess of exports can be utilized as food and some fishes have a good domestic market [8].

Some unemployed educated youth of this backward estuarine area of coastal Bay of Bengal may earn considerable amount from this work. Most of these families work as a small home unit, through this cultivation they earn US$ 50 - 100 monthly. Generally the men are engaged in the seed collection and marketing. However it is the women and children who look after the activities like water exchange; feeding etc [8]. It will be easier, if they form self help group or co-operative, for ornamental fish marketing. Motivation and training can be imparted through the government agencies and NGOs, to take the ornamental fish farming as an alternative employment

<table>
<thead>
<tr>
<th>No</th>
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<th>± 30 years back</th>
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<td>1</td>
<td>C. fasciata</td>
<td>Plenty</td>
<td>Common</td>
</tr>
<tr>
<td>2</td>
<td>B. badis</td>
<td>Moderate</td>
<td>Very rare</td>
</tr>
<tr>
<td>3</td>
<td>O. malastigma</td>
<td>Plenty</td>
<td>Plenty</td>
</tr>
<tr>
<td>4</td>
<td>B. sua</td>
<td>Plenty</td>
<td>Very rare</td>
</tr>
<tr>
<td>5</td>
<td>L. guntea</td>
<td>Plenty</td>
<td>Rare</td>
</tr>
<tr>
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<td>C. ranga</td>
<td>Plenty</td>
<td>Plenty</td>
</tr>
<tr>
<td>7</td>
<td>C. nama</td>
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<td>Plenty</td>
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<tr>
<td>8</td>
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</tr>
<tr>
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<td>S. argus</td>
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<tr>
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<td>A. coila</td>
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<td>G. chuno</td>
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<tr>
<td>17</td>
<td>A. panchax</td>
<td>Plenty</td>
<td>Plenty</td>
</tr>
<tr>
<td>18</td>
<td>M. pancealus</td>
<td>Plenty</td>
<td>Rare</td>
</tr>
<tr>
<td>19</td>
<td>P. sarana</td>
<td>Common</td>
<td>Not available</td>
</tr>
<tr>
<td>20</td>
<td>M. armatus</td>
<td>Common</td>
<td>Vary rare</td>
</tr>
<tr>
<td>21</td>
<td>M. sculeatus</td>
<td>Common</td>
<td>Vary rare</td>
</tr>
</tbody>
</table>

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opportunity for this unemployed educated estuarine youth.

5. Conclusion

From this study we come to the conclusion that a considerable number of ornamental fish species which are available in the natural water bodies of the coastal Bay of Bengal are in a state of danger in near future. This calls for immediate intervention to prevent the undesired decline of the potentially marketable colourful variety of fishes. This can be done fruitfully with the active participation of the local populations in breeding, marketing, and conservation of these wonderful fishes. When we engage a good number of skilled educated people in this colour fish farming, it can solve two problems, one is unemployment, and the other is the ecological restorations of the water bodies. This can possibly happen when the local populace is provided with not only encouragement but also active support from the local governing bodies. Formation of self help groups and cooperative marketing strategies will ensure not only considerable income but also self viability. Besides, this will prevent emigration. With this coastal Bay of Bengal area taken as a model area, we can replicate the same in other states.

6. Acknowledgements

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Oryzias melastigma -- an effective substitute for exotic larvicidal fishes: enhancement of its reproductive potential by supplementary feeding


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Oryzias melastigma – an effective substitute for exotic larvicidal fishes: enhancement of its reproductive potential by supplementary feeding

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Abstract

A preliminary study was conducted on the efficacy of Oryzias melastigma in consuming mosquito larva so as to control mosquito and mosquito borne diseases, and enhancing its reproductive success using supplementary feed. Oryzias melastigma is a larvivore fish and widely distributed in the shallow water, wetlands of Gangetic plains and peninsular India. These studies indicate that O. melastigma is a prolific breeder and gregarious feeder of mosquito larvae. Increased reproduction by providing different supplementary feed, of which Ulothrix acted remarkably, may aid in wide spread use of this fish as a biological control measure against mosquitoes. One adult fish of any sex can consume 87.1% first instars mosquito larvae/day. So, early stages of mosquito larvae are effectively controlled, as compared to other successive stages. Ulothrix has considerable effect on egg production, successful hatching and regaining reproductive maturity of female in surprisingly quicker interval.
Keyword

Oryzias melastigma, Ulothrix, Larvicidal fish, Biological control, Indigenous, Reproductive potential

Background

Mosquito borne diseases are a major problem in all tropical and subtropical countries and are responsible for causing some of the most life threatening diseases in man, like malaria, dengue fever, filariasis, encephalitis and chikungunya. The harmful effects caused by chemicals, for instance DDT, in mosquito as well as on non target populations and the development of resistances in mosquitoes have prompted an alternative use of simple and sustainable methods of mosquito control (Milam et al. 2000).

Larvivorous fishes are being successfully exploited for mosquito control in various countries like Spain, Italy, Greece, Southern Europe, Northern Africa, India, Iran, Malaysia, Madagascar and many other countries (Bruce-Chwatt 1985). They are either employed for the destruction of larvae or to render the habitat unsuitable for mosquito to breed.

Larvivorous fishes are those that feed on immature stages of mosquitoes. They are mostly small, hardy and capable of maneuvering easily in shallow waters where mosquitoes breed. They must be drought resistant and capable of flourishing in both deep and shallow waters. They must have the ability to withstand rough handling and transportation over long distances. These fishes must be prolific breeders with a shorter span of life with having the ability to breed successfully in confined waters. They should be surface feeders, carnivores in habit and should have a predisposition to feed on mosquito larva even in the presence of other food materials. Among important criterion for all larvivorous species is that they should not be brightly coloured or attractive. Besides, they should be unpalatable with no food value so that they are discarded by fish-eating people (Job 1940).

O. melastigma (McClelland 1839) belonging to the Order Beloniformes, Family Adrianichthyidae and Subfamily Oryzinae (Jayaram 1981), is a tiny cyprinodontid weed fish. It is a carnivorous, surface feeder found in both lentic and lotic waters. It is a semitransparent and hardy fish which can tolerate a wide range of salinity (31ppm) (Manna 1989), temperature, and many other adverse water qualities. Popularly known as rice fish or minnow (Rosen and Parenti 1981) or Indian Medaka or Bechi, it is a sexually dimorphic species (Manna and Bannerjee 1984). It is found in limited areas of West Bengal, Tamil Nadu, Kerala, Orissa (Jayaram 1981; Manna and Bannerjee 1985) in India and also some riverine areas of Bangladesh. In West Bengal, they are mainly distributed in the lower Gangetic shallow water bodies of 24 pargans, Midnapore and Howrah district. They generally lay eggs two to four times in a year and show a notable parental care and their breeding rate is higher as compared to other minnows under certain conditions (Daniels and Ranjit 2002). This experiment has been designed to study the increase in reproductive success of O. melastigma by giving different supplementary feeds and to study its potentiality in mosquito control.
Methods

In the present experiment *O. melastigma* were collected from fresh water pond of Midnapore and maintained in a cement cistern (20 liters), where they could breed successfully. The eggs were collected time to time, hatched in laboratory condition and reared in separate glass aquaria (55.88cm x 30.48cm x 30.48cm), each filled with 20 liter of tap water (1/4th of aquarium). The hatchlings were fed with natural feed till maturity. Six months old, healthy, disease-free specimens of both sexes of first filial generation were used for the present study.

Five males and five females of above specifications were released in experimental aquaria. Five such replicas were used for each of the first to third instars larvae and for pupae. Two earthen vats were maintained for stocking of mosquito larvae collected from drains and ditches. Sieves of specific mesh sizes were used to separate the larval insters of mosquitoes. After one hour of fish release, 100 larvae of desired instars were introduced each time in each experimental aquarium at 2 hourly intervals (6 times a day) and were observed till the end of tenure of the schedule. Suction pipettes were calibrated to count different larval instars in specific numbers for introduction in each aquarium each time. The fishes were fed and observed for 12 hours daily, for 20 consecutive days and the numbers of larvae consumed were noted and the specimens left were counted and removed. During rest of the observation period (12 hours) no food was supplied to keep them for food appetite. Reasonably sufficient numbers of larvae were supplied each time to minimize their energy expenditure for food search and to increase energy budget for feeding of minnows. However, preference of different species of mosquito larvae by the juveniles and sub-adults of the *O. melastigma* was not tried.

In a separate experimental set up, five pairs of adult *O. melastigma* (6 month old) were kept in each of the six aquaria where they were provided with different kinds of food supplement such as rice, semolina (suji), *Ulothrix*, and combinations of rice and *Ulothrix*, semolina and *Ulothrix*, and, semolina, rice and *Ulothrix*. Another set of five pairs of adult fishes were fed with natural food and was used as control. Water parameters were maintained; temperature from 26°C to 31°C, pH from 6.34 to 6.61 and dissolved oxygen content from 5.28 to 6.47 mg/ml. The behavioral patterns up to three generations were critically studied.

After each successful fertilization, from each of the aquaria, egg clutches were removed carefully with the help of a blunt forceps. Clutch size was measured and transferred to separate aquaria. Viability of the eggs was calculated in terms of fertilization success and hatching success. Hatchlings were reared to maturity to calculate the sex ratio of the new born. The design of present work is summarised schematically in Figure 1.

Data was statistically analysed using t-test. The maximum significant level chosen was $p < 0.05$.

Results and discussion

Results based on multiple observations from 1st experimental set up demonstrate that adult *O. melastigma* feed successfully and vigorously on the early larval stages of mosquito. The first instars larvae were observed to be consumed at an average above 50 numbers/day/fish.
The consumption rate decreased successively towards the advanced stages of mosquito and reached below 10 numbers/day/fish for the pupae. The percent consumption of 1st, 2nd and 3rd insters and pupae by each fish/day are presented in Figure 2. Results obtained from the 2nd experimental set-up show different reproductive efficiencies in fishes provided with different supplementary feed. Fishes fed with *Ulothrix* show a marked increment in egg production, fertilization success and hatchling survival as compared to other groups, including control. While each control female produces 44.667 ± 1.196 eggs, female fed with rice and suji produces 45.25 ± 1.538 and 45.083 ± 1.554 respectively, the *Ulothrix*-fed female produces 67.083 ± 1.311 eggs per laying. When *Ulothrix* was fed in combinations with rice, suji and rice-suji mixture, the clutch size per female was observed to be 48.833 ± 1.036, 50.167 ± 1.120 and 50.083 ± 0.996 respectively. *Ulothrix*, whether fed singly or in combinations, shows reasonably significant improvement in clutch size produced per female.

**Figure 2** Percent consumption of different larval stages by *O. melastigma*.

When percentage of fertilized eggs from the clutches was calculated, it was observed that 60.583% of control eggs, 64.167% eggs of rice fed group, 62.917% eggs of suji fed group, 78.167% eggs of *Ulothrix* fed group, 67.333% eggs of rice + *Ulothrix* fed group, 67.583% eggs of suji + *Ulothrix* fed group and 71% eggs of suji + rice + *Ulothrix* fed group were fertilized. Hatching success, calculated as percentage of fertilized eggs hatched, shows highest viability of eggs of *Ulothrix* fed group. 95.833% of fertilized eggs hatched successfully in this group. 80.583%, 84.083% and 86.75% of fertilized eggs in control, rice fed and suji fed groups were observed to be hatched respectively. *Ulothrix* along with rice or suji or rice + suji resulted in 85.413%, 84.75% and 87.25% hatching success respectively. *Ulothrix* has shown significant increase in producing fertilized eggs, whether fed singly or in combinations. However, combinations have no significant effect in increasing hatching success (Table 1).

**Table 1** Results of supplemented feeding on reproductive efficiency of *O. melastigma*

<table>
<thead>
<tr>
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<th>C</th>
<th>R</th>
<th>S</th>
<th>U</th>
<th>R + U</th>
<th>S + U</th>
<th>R + S + U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clutch size</td>
<td>44.667 ± 1.196</td>
<td>45.25 ± 1.538</td>
<td>45.083 ± 1.554</td>
<td>67.083 ± 1.311**</td>
<td>48.833 ± 1.036*</td>
<td>50.167 ± 1.120**</td>
<td>50.083 ± 0.996**</td>
</tr>
<tr>
<td>Fertilized eggs (%)</td>
<td>60.583 ± 1.593</td>
<td>64.167 ± 1.604</td>
<td>62.917 ± 1.721</td>
<td>78.167 ± 2.584***</td>
<td>67.333 ± 2.426*</td>
<td>67.583 ± 2.454*</td>
<td>71 ± 1.871***</td>
</tr>
<tr>
<td>Hatching success (%)</td>
<td>80.583 ± 1.823</td>
<td>84.083 ± 1.721</td>
<td>86.75 ± 1.733*</td>
<td>95.833 ± 0.796***</td>
<td>85.413 ± 1.900</td>
<td>84.75 ± 1.883</td>
<td>87.25 ± 1.706*</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SEM from 12 similar experiments. Data are significant at the level ***p < 0.001, **p < 0.01, *p < 0.05.

Female: male ratio was observed above 1 in both the control and *Ulothrix* fed groups. The value was calculated as 1.07 in control which significantly increased to 1.33 in *Ulothrix* fed group. Removal of egg clutches from female enhances the reproductive maturity effectively. Adult females spawn biannually in the wild while the removal of eggs causes them to produce eggs 4 times a year in all the experimental groups except the *Ulothrix* fed group where it is found to increase phenomenally to 4 times a month i.e. 48 times a year. The higher ratio of female offspring and shortening of gestation period in *Ulothrix* fed group caused a dramatic rise in per annum production by a single pair of *O. melastigma*. It was calculated that a pair of control fish and their successive generations may cause a total production of about 4 Kg per year whereas an *Ulothrix* fed pair and their forerunners would add up to a quintal per year.

Mosquitoes, potent vector of various life threatening diseases, are and will be the major concerns for human health. To find an effective solution for their control in the wild calls for
introduction of efficient larvivorous fishes which prey on them in their natural habitat. A variety of larvivore fishes were tried and established as biological control measure against mosquitoes, of which most are exotic to Indian water bodies. For instance *Gambusia affinis* and *Poecilia (Lebistes) reticulate* have been extensively used in India for mosquito control. Though they have been found to be efficient in mosquito control, their adverse impact on local biodiversity must draw some attention of the ecologists. It has been reported that where *Gambusia* was introduced, often for mosquito control, has resulted in or has contributed to the exclusion of many native fishes which have similar ecological requirements (Page and Burr 1991). From a conservational view point, to protect the habitat of native species, World Health Organization has emphasized on research and introduction of indigenous fish species for mosquito control. The subject of present study, *Oryzias melastigma*, is a native species to Indian subcontinent distributed widely in many states of India and Bangladesh and is a hardy fish capable of propagating in shallow natural water bodies which happen to be the breeding ground of mosquitoes. From the present study it has been found that this fish has reasonably high feeding activity on mosquito larvae, especially on the early stages of mosquito life cycle. The efficiency of consumption gradually decreases as the life stages of mosquito progresses. This may be due to that the mouth of fish appears to be non-accommodative to engulf smoothly the larger larvae. Besides, as the larva develops, they sense the water movement more and learn to move away from the predator faster. It is also expected that the wriggling movement of the larvae is more attractive than the comparatively stationary late larvae and pupae.

The vigorous larvivore activity of this fish, as advocated in the present study, may suggest promote the rearing and breeding of *O. melastigma* for the control of mosquitoes. It also can be suggested that this fish is an efficient native alternative to the exotic vector control agents like *Gambusia affinis* and *Poecilia (Lebistes) reticulate*, as far as the larvae feeding ability is concerned (Chatterjee and Chandra 1997a, b).

Breeding of *O. melastigma* in captive condition has shown encouraging results with supplementary feed. It is found that the fish can be readily cultivated under laboratory conditions with different feed supplement exhibiting varying degrees of reproductive success. *Ulothrix* was observed to be most promising in enhancing the egg production, fertilization success and egg viability when supplemented singly or in combinations. *Ulothrix* must have some inducing effect on gonadal activity of both male and female fishes which need to be studied further. Results show that female to male ratio of produced offspring is on higher side in *Ulothrix*-fed pairs as compared to that of control pairs. Furthermore, removal of egg clutches from the female fed with *Ulothrix* resulted in an enhanced reproductive maturity leading to a remarkably high egg producing ability. All these reproduction enhancements aid in huge production of *O. melastigma* seeds that can be successfully released in the mosquito breeding grounds like drains/ canals, septic tanks, cement tanks, pools/ponds, pit latrines, marshy lands, wells, overhead tanks, water meter chambers and miscellaneous household in domestic containers, for controlling mosquito menace.

**Conclusion**

The present study strongly advocates the larvivore efficacy of *O. melastigma* and potency as biological control measure against mosquitoes. This fish is an Indigenous alternative to exotic larvivores. It can be recommended that breeding this fish in captive condition using *Ulothrix* as a feed supplement can meet the need of an effective larvivorous fish for mosquito control.
Competing interests

The authors declare that they have no competing interests.

Authors’ contribution

ALD carried out the experiment and drafted the manuscript; SKD drafted the manuscript and contributed to statistical inferences; DC contributed towards finalizing the manuscript; AKM supervised the execution of the experiments; PKM hypothesised the experiment. All authors read and approved the final manuscript.

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Figure 1

Cement Cistern

Male + Female

Eggs collected and hatched

Aquarium (5x4 numbers)

5 male + 5 female (6 months)

Aquarium (7 numbers)

5 male + 5 female (6 months)

100 larvae fed/2 hrs

Number and percentages of larvae consumed were counted

Aquarium

Control, Rice, Suii, Ulothrix, Rice + Ulothrix, Suii + Ulothrix, Rice + Suii + Ulothrix
Figure 2

Life stages of mosquito

% consumption/adult/day

1st inster
2nd inster
3rd inster
Pupa