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Toxicological Impact of Non-Selective Herbicide Paraquat Dichloride on Lipid Content of Indian Major Carp *Cirrhinus mrigala* (Hamilton)

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ABSTRACT
Paraquat Dichloride is the most extensively used toxic weedicide across the globe. It is notorious as a 'suicide toxicant' and linked with major diseases like Parkinson's disease and Cancer. In agrarian countries like India, 83% of pesticides and herbicides manufactured, end up on farms and widespread use of them has caused adverse effects on the environment due to their toxic nature and unintentional intoxication of non-targeted species like insects, fish, birds, mammals, and other inhabitants of both aquatic and terrestrial ecosystems. When these pesticides leach into water bodies by floods, rains, drift, erosion, etc., they cause severe damage to the aquatic biota therein. The present study was designed to evaluate the toxic effects of paraquat on biochemical alteration in total lipid content, in the fingerlings of commercially important Indian Major Carp Cirrhinus mrigala (weight 3.37±0.01 gm. and length 4.07±0.69 cm) exposed to the acute lethal concentrations. The study was conducted for 96 hours in static renewal bioassay apparatus, in which water and toxicant were renewed every 24 hrs. After 96 hours of exposure to LC_0 and LC_{50} concentration of paraquat exposure to fish, live fishes were sacrificed. Gill, liver, muscle, and brain tissues were pooled out of fishes to estimate the total lipid content using Barnes and Black stock Phospovanillin method. Annotated observations specified that there was a significant ($p<0.05$) reduction in total lipid content, in the liver and muscle tissues of the LC_0 group, as compared to the control group, whereas, the total lipid content in gill, muscle, and brain tissues showed significant ($p<0.05$) decrease in the LC_{50} treatment group as compared to the control group. The liver tissue in the LC_{50} group showed a more moderately significant ($p<0.01$) reduction as compared to control. The present study concludes disorders in lipid metabolism and stress in fishes as a result of paraquat toxicity, affecting normal functionality and overall biochemical composition of tissues causing disturbances in overall homeostasis.

KEYWORDS
Paraquat Dichloride, Cirrhinus mrigala, Acute toxicity, Total lipid content.

1. INTRODUCTION

Paraquat Dichloride (1, 1'-Dimethyl-4, 4'-bipyridinium dichloride) (CAS No = 75-305-73-0) is a quick-acting, non-selective, quaternary ammonium herbicide used on broad spectrum of emerged weeds. It is extensively used herbicide in the world [1]. Paraquat is commercially produced as a brownish bright green colored, pungent-smelling, highly corrosive concentrated liquid of the dichloride salt in 20% strength under the product named 'Gramoxone' (Syngenta) [2]. Paraquat can penetrate human beings through various ways like mucosal absorption, respiratory system, digestive system, etc. and can cause effects of various magnitudes depending on the toxicity of their internal reactions [3]. Moderate Paraquat poisoning can eventually lead to death due to damage and progressive deterioration of affected organs, while severe poisoning can cause death in a short time due to acute multiple organ failure [4]. Soil residues of paraquat are increasing due to its continued widespread use and longer half-life [5]. Not just in the soils, but the ubiquity of all such pesticides in various components of the environment like surface waters is due to their cheap and extensive availability and high solubility in the aqueous medium [6,7]. Direct application, run-offs, leaching, sprays drift, and discharge from sewage and factories are various routes through which these pesticides reach the aquatic ecosystem [8]. The presence of Paraquat in various water bodies has been observed [9], which unfortunately exposes the non-targeted organisms, in the aquatic environments to the pesticides [10].

Lipids are esters of fatty acids and glycerol that are soluble in organic solvents but insoluble in water [11]. In fishes, lipids are most preferred source of metabolic energy for growth from egg to fingerlings [12]. Lipids are important source of metabolic energy for reproduction in fishes [13, 14] thus lipids play a vital role in the overall life processes of the fishes. Lipids in fishes undergo quick breakdown, resynthesize, and form various conversions internally as a result of various stimuli [15]. Lipid metabolism in fishes plays a very important role in vital life strategies and transitions related to metabolism, development, and regeneration [16]. Paraquat is a toxicant that causes repeated cycles of reduction and re-oxidation in presence of an adequate supply of reducing equivalents, to produce an enormous quantity of Reactive Oxygen Species (ROS). Degeneration of membrane lipids caused due to the peroxidation effect of ROS is most likely the suggested reason for paraquat toxicity [17]; still, its exact role is more ambiguous [18,19].

Present study was designed to investigate the toxic effects of paraquat dichloride on total lipid content in the commercially important Indian major carp, *Cirrhinus mrigala,* to determine the biochemical alterations in their lipid metabolism as baseline biomarker of damage to fish health and in evaluating environmental risks.

2. MATERIALS AND METHODS

Healthy fingerlings of *Cirrhinus mrigala* (weight-3.37±0.01 gm. and length 4.07±0.69 cm) were collected from Government Fish seed Production and Rearing center, Dhom (Wai), Satara District, Maharashtra State, India. They were brought to the laboratory in oxygenated plastic bags. At the laboratory, fishes were first disinfected by dip treatment in 0.1% $KmNO_4$ solution for 2 minutes. Then they were transferred to well-aerated glass aquaria filled with tap water, where they were acclimatized at room temperature to laboratory conditions for 15 days. During acclimatization and experimental procedure, the fishes were fed daily with 'Taiyo plus Discovery special fish food' at a rate of 2% of their average body weight. Natural photoperiod was maintained. During the acclimatization period, the aquarium water was changed every 24 hours to discard food remnants and fecal matter that can cause unnecessary stress in the enclosed water system. The water quality parameters were checked weekly to ensure normal conditions. Removal of any dead fish was done immediately to avoid possible water quality deterioration. After 15 days of laboratory acclimatization, the fishes to be used for the experiment were screened for any indication of physical damage, disease, stress, and mortality. Any suspected fishes were discarded immediately and only the healthy fishes were selected for the study. Before initiation of experimental protocols, the fishes were acclimatized to well-aerated 22-liter capacity plastic containers for 7 days, in which they were to be exposed to the toxicant. 24 hours before the test, feeding was discontinued to reduce the effect of vomiting and excreta due to toxicant.

2.1. Exposure to paraquat

The herbicide paraquat dichloride commercially marketed as brand name Gromoxone (24%w/w) by Syngenta was used as a toxicant in the present study. Before experiment, the LC_0 and LC_{50} concentrations for the toxicant used were determined by the static renewal bioassay method. The data so obtained was processed by Finney's Probit analysis to obtain both the lethal concentrations. Three transparent, openmouthed, cylindrical plastic containers with 22 liters capacity were set up. Each container was provided with continuous adequate aeration to maintain the desirable Dissolved Oxygen level in the water. All containers were filled with 20 liters of clean tap water and 10 fishes were released in each of them. The first container served as a control group, as the fishes in that group were not exposed to any toxicant. The fishes in the second and the third container were exposed to LC_0 (67.32 ppm) and LC_{50} (105 ppm) concentrations of paraquat respectively for 96 hours (acute toxicity). At every 24 hours, the water medium and the toxicant in all the containers were replaced with new fresh water and toxicant, to maintain the optimum concentrations throughout the experiment. Any dead fish were removed

immediately from the container. After 96 hours of exposure, live fish from all three containers were sacrificed and their organs gill, liver, muscle, and brain were pooled out separately to analyze the total protein content in them.

2.2. Analysis of the total lipid content

The total lipid content from all 4 tissues was estimated by Barnes and Black stock Phospovanillin Method (1973) [20]. Initially, a standard graph of lipid was derived using cholesterol ($C_{27}H_{46}O$) as a lipid concentration standard. Then tissue (100 mg) homogenates were prepared in 1 ml of chloroform-methanol (Folch's) mixture (2:1) by mixing with a glass homogenizer. After homogenization, a 0.2ml of 0.9% sodium chloride solution was added to it and shaken well to prevent the binding of acidic lipids to lipids. The entire solution was transferred to a separating funnel and allowed to stand overnight at 40^0C. Next, clear biphasic layer was formed with a lower phase containing lipids. This lower phase was extracted and the volume was made up to 10 ml by adding chloroform (99.5%). This 10 ml mixture act as an actual sample. 0.1 ml of this sample was taken into 3 separate test tubes. The sample in all these three test tubes was allowed to dry at 40^0C for 2 hours. After drying 0.5ml of concentrated sulphuric acid was added to all three test tubes and mixed well. All the test tubes were plugged well with non-absorbent cotton wool and placed into a boiling water bath for 10 minutes followed by cooling of test tubes to room temperature. This acid digest was added with 5 ml of phosphovanillin reagent, mixed well, and allowed to stand still for half an hour. Blank was prepared simultaneously along with sample using 0.1 ml chloroform + 0.5 ml concentration of sulphuric acid + 5 ml phosphovanillin reagent. After 30 minutes the absorbance values of the sample were measured at 530 nm against the blank by using a spectrophotometer. The concentration of lipid in each of the tissue samples was derived from their respective absorbance by referring to the standard graph. The final data from all the groups was expressed in arithmetic mean ± standard deviation format. The level of significance was derived using the student's T-Test with two-tailed distribution in the two-sample unequal variance (heteroscedastic) method.

3. RESULTS

The physicochemical parameters for water medium in the experiment were carried out using standard methods (APHA, 1989) [21] and the following values were derived: Temperature 27.9^0C, pH 7.2-7.8, Dissolved oxygen 5.86-6.51 mg/L, Dissolved Carbon dioxide 13.66 ± 0.31 mg/L, hardness 121.8 ± 3.32 mg/L, phosphates 0.5 ± 0.02 mg/L, nitrates 1.19 ±0.07 mg/L.

The results of the effects of paraquat toxicity on the total lipid content in various organs viz. gill, muscle, liver, and brain of the fish *Cirrhinus mrigala* in the control group, LC_0 concentration group, and LC_{50} concentration group after acute exposure (96 hours) are illustrated in Table 1 and Fig. 1.

In the control group fish *Cirrhinus mrigala*, the total lipid content was in the order of Brain > Liver > Muscle > Gills.

In the gills of the control group, the total lipids were 126.6±13.83 µg lipid/mg tissue. However, in the LC_0 concentration group fish gill exhibited 104.4±5.22 µg lipid/mg tissue and in the LC_{50} concentration group fish showed 90.12±3.74 µg lipid/mg tissue of gill.

The muscle tissue of the control group of fishes exhibited 176.41±1.04 µg lipid/mg tissue of the muscle, while in the LC_0 concentration group fishes exhibited 169.41±2.24 µg lipid/mg tissue of muscle and in the LC_{50} concentration group fishes; it was 156.5±5.26 µg lipid/mg tissue of muscle.

The total lipid in the liver tissue of the control group was 255.24±6.39 µg lipid/mg tissue. However, 241.92±4.36 µg lipid/mg tissue and 225.84±5.76 µg lipid/mg tissue of liver were observed in the LC_0 concentration group fishes and LC_{50} concentration fish group respectively.

The brain of the control group fish exhibited 659.4±7.28 µg lipid/mg tissue. While in the LC_0 concentration group fish showed 655.44±8.44 µg lipid/mg tissue of the brain and in the LC_{50} concentration group was 639.84±5.94 µg lipid/mg tissue of the brain.

Table 1: Effect of Paraquat Dichloride on the total lipid content in different tissues of the fish *Cirrhinus mrigala* after acute exposure:

Groups	Total lipid (µg protein/mg wet wt. of tissue)			
	Gill	**Muscle**	**Liver**	**Brain**
Control Group	126.6±13.83	176.41±1.04	255.24±6.39	659.4±7.28
LC_0	104.4±5.22	169.41±2.24*	241.92±4.36*	655.44±8.44
LC_{50}	90.12±3.74 *	156.5±5.26 *	225.84±5.76 **	639.84±5.94*

(Values expressed as Arithmetic Mean of (n=3); ±SD),

*= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)

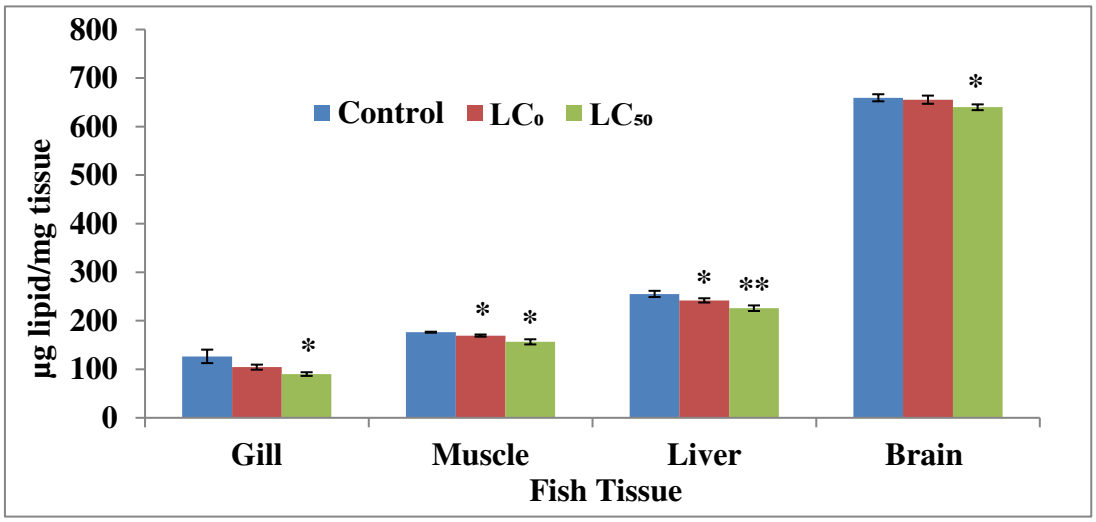

Fig. 1: Toxic Effect of the lethal concentration of Paraquat Dichloride on Total Lipid Content in Gill, Muscle, Liver and Brain tissues of the fish Cirrhinus mrigala after acute exposure (96 hours).

Data expressed in arithmetic mean ± Standard Deviation. Error bars represent SD of 3 individual observations. * indicates $p<0.05$(significant), ** indicates $p<0.01$(moderately significant), *** indicates $p<0.001$(highly significant).

The total lipid content in liver and muscle tissue showed a significant ($p<0.05$) decrease in the LC_0 treatment group. Liver tissue in the LC_{50} group showed a little more moderately significant ($p<0.01$) reduction as compared to control while gill, muscle, and brain tissues in the same LC_{50} treatment group also showed a significant ($p<0.05$) reduction than in the control group, on the contrary, there was no significant reduction in total lipid content, in the gill and brain tissue of LC_0 treatment groups as compared to the control group. The post-experimental total lipid content in the four tested tissues was in the order Brain > Liver > Muscle > Gills in the LC_0 group, similarly, in the LC_{50} group it was in the order, Brain > Liver > Muscle > Gills.

4. DISCUSSION

Lipids especially fatty acids have the major role of generating metabolic energy in the form of ATP by mitochondrial B-oxidation process, which is very well established in fishes [22]. Paraquat produces superoxide anions and other ROS during redox cycling and cellular NADPH oxidation [23] that induce severe oxidative stress at cellular and molecular levels causing severe damage. ROS produced can haphazardly attack and damage cellular macromolecules including lipids in living cells causing serious disruption in physiological cell processes [24,25] thus reducing the levels of total lipids in tissues. Fishes require high energy input and

antioxidants to neutralize such stress and to maintain homeostasis resulting due to the toxicant. Need for excess energy demand is further testified by the mobilization of lipid reserves from various tissues [26] that are converted into energy showing a reduction in total lipid content. Furthermore, severe damage to the liver may be the reason for declining levels of lipids due to inhibition of cholesterol biosynthesis in the liver or due to reduction of dietary cholesterol absorption as reported by Mishra *et al*., (2004) [27]. Besides starvation, renal disorders and malnutrition are also caused due to reduced nutrient absorption specifically fats in the intestine, due to toxicant toxicity, can probably be the main reasons for the reduction of total lipid content in the overall fish tissues. Similar results of paraquat exposure were reported by Bashini and Senthilkumar, (2018) [28] on total lipid contents in the fish *Labeo rohita.* Binukumari *et al*., (2016) [29] observed similar effects of pesticide monocrotophos on total lipid contents in various tissues of the fish *Labeo rohita.*

5. CONCLUSION

From the results of the present study, it can be concluded that paraquat toxicity cause metabolic dysfunction and stress in fishes leading to a decrease in total lipid content, affecting optimum functionality and biochemical composition of tissues and impairing overall homeostasis. Thus decrease in total lipid content can be good bio-indicators to determine the effect of paraquat dichloride and of other anthropogenic stressors in the aquatic ecosystems.

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Wound Healing Acceleration using Curcumin and Carbon Nanodot Incorporated Nanofibrous PVA Scaffold in Mice Model *(Mus musculus)*

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ABSTRACT

Wound healing is natural process which takes quite more time to heal naturally. Natural products such as curcumin is natural bioactive compound present in Curcuma longa, is well known for wound healing activity. It especially shows antibacterial, anti-inflammatory activity. During wound healing dressing material is important to reduce loss of body fluid, electrolytes, and nutrient and thus accelerates early wound healing. Here we have used carbon nanodots (CNDs) incorporated nanofibrous PVA (Polyvinyl alcohol) scaffold as dressing material. Mice (Mus musculus) were divided in three groups. Group I: Mice wound was not treated by any drug. Group II: Mice wound was treated with 5 mg curcumin1 time topically and kept for 5 days. Group III: Mice wound was treated with 5 mg curcumin1 time topically and 5mm X 6mm CNDs PVA scaffold and kept for 5 days. Wound area was studied for next 5 days from all above groups. Wound area recovery analysis was done by using photographs and morphometry of wound. Our CNDs incorporated nanofibrous PVA scaffold increased rate of wound recovery initially 10 times on 2ndday, 2 times on 3rd day and 1.2 times on 5th day as compared to only curcumin treated mice. Which indicates this scaffold is very much useful in immediate wound recovery within 24 hrs.

KEYWORDS

Wound healing, Curcumin, Carbon nanodot, PVA scaffold.

……

1. INTRODUCTION

Skin is barrier between body and environment. A wound is a type of injury which happens relatively quickly in which skin is cut or punctured. Wound healing is orderly progression of event that maintain the integrity of damaged tissue. Despite recent advances in wound care products, traditional therapies based on natural origin compounds, such as plant extract, natural products are interesting alternatives.

Natural compounds have been used in skin wound care for many years due to their therapeutic activities, including anti-inflammatory, anti-microbial and cell stimulating properties. Turmeric is used in Ayurveda in India it is used for skin treatment, in digestive disorders, upper respiratory tract disorders. The active compound is curcumin which having anti-inflammatory, anti-carcinogenic, anti-infectious, anti-oxidative property [1]. Curcumin plays key role in wound healing [2]. Antioxidative property of curcumin prevent oxidative damage and enhance healing of wound [3]. Curcumin is less soluble in water and has less bioavailability [4] Along with treatment dressing is practiced by many clinicians to avoid infection and proper healing process. The dressing material reduces healing time, side effect of drug and increases bioavailability [5,6]. Most of the time in wound healing process infection, loss of body fluids, electrolytes and nutrients occurs which severely create complication [7]. PVA gains widespread attention, since it is water soluble, nontoxic, noncarcinogenic, biodegradable, biocompatible, transparent, and with high charge storage ability, along with superior film-forming attributes [8,9]. Carbon nanodots gain attention in the field of *in vivo* and *in vitro* cell imaging because of its photoluminescence, biocamapatibility, and photo stability. Carbon nanodots enhance wound healing and give stratified epithelial tissue with mature dermal layer [10]. CNDs are preferred over metallic or semiconductive quantum dots due to its carbon origin, high stability, low cytotoxicity. CNDs from date molasses shows antioxidant property and cytocompatibility [11].

Our aim to find out whether CND incorporated nanofibrous PVA scaffold as dressing material with curcumin accelerates wound healing or not.

2. EXPERIMENTAL PROCEDURE

2.1 Animals

15 Male albino mice (*Mus musculus* Linn.) of age 6 months and weighing between 30-35 gm were used for present investigation. All the animals were maintained under controlled condition with 12 hr light and 12 hr dark cycles at temperature of 26^0C ± 2^0C in departmental animal house. Animals were divided into groups such as control and experimental and were caged separately. The animals were housed in plastic cages under proper condition of light, temperature and humidity. The animals were supplied with standard Nutrinix std- 1020 (Nutrivet life sciences, Pune, Maharashtra, India). Experimental study is conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2 **Methodology**

Curcumin from Sigma Chemical. Carbon nanodot incorporated PVA scaffold synthesized by chemistry department of our university was used for present study. Mice were first mildly anesthetized by isoflurane followed by shaving the dorsal

region. Shaved area was cleaned with 70% alcohol. About 30 mm^2 flap of skin was removed to the level of the subcutaneous layer to prepare wound [10]. Mice were divided into 3 groups

Group I: Mice wound were not treated by any drug.

Group II: Mice wound treated with 1 time 5 mg curcumin and kept for 5 days.

Group III: Mice wound treated with 1 time 5 mg curcumin and 5mm X 6mm CND PVA scaffold and kept for 5 days.

Wound area recovery analysis was done by using photographs of wound. Wound morphometry was done using Image J software developed by National Institute of health [12]. % Wound area recovery was calculated by formula

$$\% \text{ wound area recovery} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was the area of initial wound and A_t was area of at time [13].

2.3 Statistical analysis

All values were expressed in mean ±SD. Statistical analyses was carried out by one-way ANOVA followed by Tukey's HSD test. Values of $p < 0.05$ indicate significant difference.

3. RESULT

The wound healing potential of curcumin and carbon nanodot incorporated PVA scaffold evaluated by full thickness wound in mice. Figure 1 shows photographs of skin wound from day 1st to 5th day. Wound recovery calculated in all three groups. On 1st day,30 mm^2wound area was considered as 0% recovery in all three groups. On 2ndday there was significant decrease in group III wound area upto 13.401±0.197 as compared to group I and group II (Table No .1), that shows 55.33% wound recovery is more than 10 fold as compared to group I and II (Table No 2). While there was no significant difference observed in group I and II. On 3rdday significant difference was observed in group III as compared to group I and II. 62.13% recovery was observed in group III which is almost two fold more than group I and II. (Table No. 2). On 4th day there was significant decrease in wound area 7.35±0.155 in group III and 19.17±0.106 in group II as compared to group I (Table No. 1). On 5th day in group III decrease in wound area was upto 5.92±0.140 and in group II 8.70±0.257 was observed (Table No. 1), which shows 80.26% wound area recovery almost three fold in group III as compared to group I. 71% wound area recovery showed in group II that is two fold as compared to group I was observed (Table No.2).

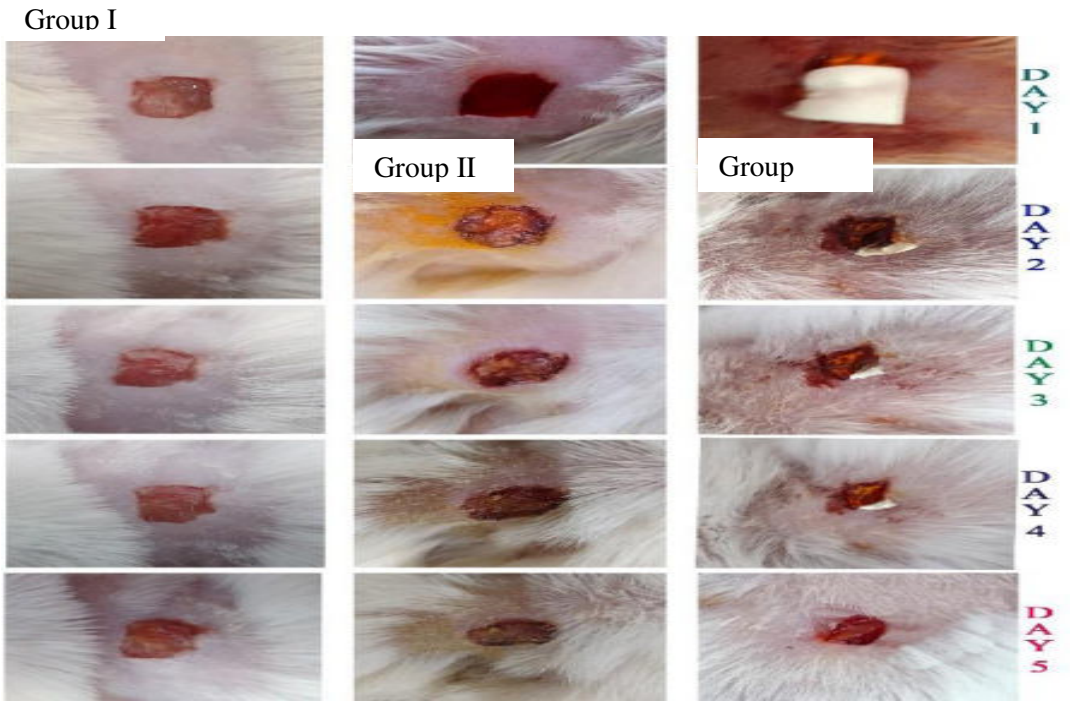

Figure 1Figure1: photographic representation of wound healing by curcumin and carbon nanodot incorporated PVA scaffold in mice model. Photographs showing healing progression in Group I (control), Group II (curcumin) Group III (curcumin + PVA scaffold) from 1st day to 5th day.

Table No. 1: Comparative analysis of wound area recovery (mm^2) in mice model. Values are mean ± S.D.

Days	**1**	**2**	**3**	**4**	**5**
Group I	30	28.23±0.147	21.47±0.512	21.812±0.054	20.64±0.273
Group II	30	28.00±0.09	21.12±0.574	19.17±0.106	8.70±0.257
Group III	30	13.401±0.197	11.36±0.440	7.35±0.155	5.92±0.140

Table No. 2: Comparative analysis of % wound area recovery in mice model.

Days	1	2	3	4	5
Group I	0	5.9	28.43	27.3	31.2
Group II	0	6.66	29.66	36.1	71
Group III	0	55.33	62.13	75.5	80.26

DISCUSSION

Wound healing is complex and dynamic process occurs relatively slow. Curcumin has been found to primarily act in the proliferative phase by increasing re-epithelialization, collagen deposition, fibronectin production and myofibroblast contraction [2]. Curcumin treatment decreases reactive oxygen species and increases collagen synthesis and cell proliferation in wound healing [14]. Curcumin treatment on the excision wounds of mice ex-posed to gamma radiation showed slight wound closure on 2^{nd} day after curcumin treatment [15]. Comparative analysis of %wound area recovery after treatment of curcumin on excisional wound showed slow recovery in mice on 2^{nd} and 3^{rd} day than control mice. But significant recovery was observed on 4^{th} and 5^{th} day as compared to control mice [16]. Electrospun PVA nanofibrous mats for wound dressings presented adequate mechanical properties and porosity to absorb aqueous fluids. They also presented antimicrobial properties against Gram-positive and Gram-negative bacteria [17]. The 2.5% PVA presented a high rate of healing in rats' wounds [18]. CNDs incorporated PCL gelatin scaffold fastening the wound healing process with re-epithelization and dermal regeneration was studied by Pal et al., 2017. Curcumin + CNDs incorporated PVA scaffold treated mice showed 10 times more recovery on 2^{nd} day, 2 times on 3^{rd} day and 1.2 times on 5^{th} day as compared to only curcumin treated mice. The nanofibrous architecture with bioactive carbon nanodots may provide cell adhesion and matrix and promote growth of epithelial cells aiding in faster wound healing. This indicate that the CND incorporated nanofibrous PVA scaffold is very much useful in early wound recovery within 24 hrs.

4. CONCLUSION

The present study suggests that the Curcumin + PVA scaffold composite possess better wound healing activity than alone curcumin and it accelerates wound closure rate in initial days and improve wound healing status. Moreover, based on the outcome of studies curcumin and carbon nanodot PVA scaffold supposed to be considered as a best wound healing device for future studies.

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Laboratory Studies on the Life-history of *Statilia maculata* (Thunberg, 1784) (Mantodea: Mantidae: Mantini)

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ABSTRACT

The observations were made on the life cycle, mating and cannibalism in Statilia maculata. Being a hemimetabolous insect, the life cycle consists of ootheca (egg), nymph, and adult stages. The incubation duration of ootheca is about 30 days while the nymphal duration ranges between 78-97 days with six moults. The adult female lived more (an average of 67 days) than the adult male (an average of 58 days). The entire life cycle period of males ranges between 124-167 days while in females it ranges between 118-192 days. Mating was allowed mostly when the female is engaged in feeding. The cannibalism was generally observed during the nymphal stage may be due to overcrowding as well as in females which devours the male. As S. maculata is a predator of many insects including crop pests, it can be used as a biocontrol agent as an Integrated Pest Management Tool.

KEYWORDS

Life cycle, Statilia maculata, Mating, Development, Cannibalism.

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1. INTRODUCTION

Praying mantis are the dynamic predators among all insects characterized by the triangular head, spiny raptorial legs and camouflaged body appearance. They are also known for predation, reproductive behaviour and laying eggs in the complex ootheca. The *Statilia maculata* [1], a well-known garden mantid is a friend of farmers as they are commonly found in agricultural fields. *S. maculata* is distributed in many states of India [2,3,4]. Mukherjee *et al.* [2] studied the biology and ecology of *S. maculata* very briefly, in which they focused on the oviposition, developmental stages, relative growth rate and oxygen consumption during different instars. Some behavioural studies on *S. maculata* were carried out by Watanabe and Yano [5]. In the present study, attempts have been made to study the life cycle of *S. maculata* along with observations on oviposition, food, mating, and cannibalism.

2. MATERIALS AND METHODS

The life cycle study of *S. maculata* was studied under laboratory conditions at 25°C to 35°C and 60% to 70% RH. The field-collected female of *S. maculata* was reared in aluminium meshed cages (15cm×15cm×22cm). The hatched nymphs were grouped into three groups of 20 each and transferred to different cages of the same size and observations were made.

Early instars i.e. 1st and 2nd instars were fed on a diet of *Drosophila* spp. on alternate days. Late instars were fed on the crickets, grasshoppers and plant hoppers etc. Excess feeding was avoided because some cannibalistic species of grasshoppers and crickets cause damage to the mantids. The measurements of each instar, adults and oothecae were recorded by vernier caliper and photography was done.

3. RESULTS AND DISCUSSION

In the present study, the life cycle of the *S. maculata* is studied along with various observations such as oviposition, hatching, nymphal development, food, mating, cannibalism and colour morphs. The developmental durations of each stage of the life cycle and measurements of each developmental stage are given in Table 1.

Table 1. Development of *Statilia maculata* (Thunberg, 1784)

Life Stage	Average duration (days)	Average Body Length (mm)	Average Pronotal Length (mm)
1st instar	8	7.4	1.9
2nd instar	8	12.8	3.42
3rd instar	9	18.3	4.9
4th instar	11.5	23.8	6.4
5th instar	12.5	29.1	7.8
6th instar	15	34	9.1
7th instar	23.5	42.3	11.25
Adult (male)	58	50	14.5
Adult (female)	67.5	52	14.8

3.1 Oviposition: The freshly laid ootheca (Image 1) is a soft, cream-coloured frothy mass containing eggs and after drying it becomes dirty brown coloured. After some days the colour changes to light brown with longitudinal invasive linings. It is dumbbell-shaped, pointed at both ends and laid on the leaflet of grass or a small twig, stick, bark or any suitable substratum. Oviposition takes place mostly in the early morning i. e. 6 am to 11 am. A mated female can lay 6 to 15 oothecae at the interval of 8 to 10 days in the entire life span.

3.2 Hatching: The hatching was observed after about 28 to 30 days of incubation in the morning. Small straw-coloured 66 nymphs hatched out from the first ootheca. Ten nymphs were reared in the laboratory while others were released in the field.

3.3 Nymphal development:

The nymphal stage of the life cycle consists of 7 instars with a nymphal period of 78 to 97 days.

Life Cycle of *Statilia maculata* (Thunberg, 1784)

1st instar (Image 2)**:** It measures about 7.4 mm long and the pronotum is about 1.9 mm. Freshly hatchlings are light brick coloured later on after sclerotisation it becomes dark brick or black coloured. Semitransparent mid and hind legs with alternate white and black patches on the junction of the tibia and tarsus. After hatching, till sclerotisation, they become steady and then active, feeding on *Drosophila* flies which were provided. The first instar lasts for 7 to 9 days.

2nd instar (Image 3)**:** It measures about 12.8 mm long and the pronotum is 3.42 mm. The second instar nymphs were very active, body colour changed to light brown with scattered black spots on the body; eyes showed faint longitudinal lines; a vertex has a horizontal band; in the mid and hind legs, tibia with white alternate bands, femur and coxa paler. It feeds on *Drosophila* sp. flies. It moults into the third instar within 7 - 9 days.

3rd instar: The third instar (Image 4) measures about 18.3 mm long with a pronotum of 4.9 mm. Nymphs are straw-coloured with grey-black abdominal margins; in fore legs, white marginal obtuse spines developed on coxa; in mid and hind legs femora and tibia with alternate dark and faint bands that are darker than the body. It feeds on *Drosophila* flies as well as small nymphs of grasshoppers and crickets. It moults in the 4th instar within 8 - 10 days.

4th instar (Image 5)**:** It measures about 23.8 mm long with a pronotum of 6.4 mm. Body with dirty straw body colouration with some small dark spots, the margins of abdomen somewhat lightened and become grey. The mid-dorsal line is not much prominent but surrounded by parallel light linings. The tergal plates show some dark spots; wing buds just appeared. It feeds on nymphs and adults of grasshoppers and crickets. It moults in the 5th instar within 10- 13 days.

5th instar (Image 6)**:** It measures about 29.1 mm long with a pronotum of 7.8 mm. It shows the initiation of wing pads and reaching up to the first segment of the abdomen. The body becomes dark straw-coloured with scattered dark even spots; a lateral faint line becomes prominent; in forelegs, coxa with a black rectangular spot at the base; femora with coloured spots, tarsus internally black; abdominal segments blackish at joints. It feeds on nymphs and adults of grasshoppers and crickets. It moults in the 5th instar within 11- 14 days.

6th instar (Image 7)**:** It measures about 34 mm long with a pronotum of 9.1 mm. The body colour becomes get faded, the dark band of the vertex gets fed, and the spots of the fore femora appeared dark and prominent, at the bases of the internal spines thin black line is present; wing pads appear much more reaching up to half of the second segment. Dark bands on the hind and mid-legs become fade. It feeds on nymphs and adults of grasshoppers and crickets. It moults in the 5th instar within 13-17 days.

7th instar (Image 8)**:** It measures about 42.3 mm long with a pronotum of 11.25 mm. The coxal spines appear clearly, milky at the tips, wing pads well developed and

extend towards laterally reaching up to the third segment of the abdomen, much darker. Legs are darker than the body in males, and paler in females. It fed on nymphs and adults of grasshoppers and crickets. It moults in the 5th instar within 22-25 days.

Adult (Image 9,10).**:** Morphological features of this species given in this study are following Mukherjee et al. (1995) and Vyjayandi (2007). Some additional characters are body length (vertex to the abdominal tip) 50 mm in male and 52 mm in female. Head- wide and triangular with globular compound eyes, vertex with transverse bands and frons light brown. Pronotum was elongated with the margins distinctly spinous. Base of prozona and anterior metazona with black patches. The fore coxa has a black elliptical spot with whitish spots. Femur red, yellow and black patches internally near the claw groove, the bases and tips of all tarsal segments are blackish ventrally

The adult male and female lived for 46-70 and 40-95 days respectively. The total life span of males ranges between 124 to 167 days and for females 118 to 192 days.

3.4 Mating: The mating behaviour is not much aggressive. The male approaches the female mostly when she is engaged in feeding. Several times a day mating was observed, during which the female rarely devours the male. Naturally, males are attracted to light much more quickly than females. When a male finds a female, then he approached the female and tries to mate.

3.5 Cannibalism: Cannibalism behaviour was observed mostly in the nymphal stage may be due to the scarcity of food. Even though food is available, sometimes cannibalism was observed, this may be due to overcrowding. The rest of the time nymphs are not much aggressive and are less cannibalistic than other species.

Earlier, Beeson [6] studied the life cycle of *Hierodula ventralis* and reported 8-9 moults and a 70-110 days nymphal period. Suckling [7] reported 6 moults and a minimum of 77 days of the nymphal developmental period in *Orthodera ministralis* in Palmerston North, New Zealand climate. The findings of the present study (6 moults and 78-97 days nymphal duration) are in agreement with the observations made by Suckling [7]. Mukherjee et al. [2] studied the life cycle of *Statilia nemoralis* and discussed the mechanism of oothecal formation and its characteristics, postembryonic development and adult body colouration. They found that there are seven instars and the nymphal duration of the first to seventh instar is 7, 9, 9, 8, 11, 13.5 and 25 days respectively which is more or less similar to the nymphal development of *S. maculata*. Battiston and Galliani [8] observed the life cycle of *Ameles spallanzania* and recorded the effects of climatic conditions on larval instars and oviposition strategy.

Raut and Gaikwad [9] studied the life cycle, *of Mantis religiosa religiosa* Linnaeus, 1758 and made observations on hatching, development of larval instars, mating behavior, sexual cannibalism, and larval cannibalism. The total average life span of males and females of *M. religiosa religiosa* is 165 and 196 days respectively. In the species under study, the total life span is found to be shorter i.e. it is 124 to 167 days and 118 to 192 days in males and females respectively. However, the incubation duration of ootheca is longer (28-30 days) in *S. maculata* than *M. religiosa religiosa* (18 days). Taking into consideration the deviations in the observations recorded in the life cycle of *M. religiosa religiosa* and *S. maculata* it is inferred that the duration of every life stage or the entire life cycle varies from species to species even if they belong to the same climatic conditions and habitats.

4. CONCLUSION

To know the insect world, along with the diversity studies observations on the life cycle, insect behaviour, etc. are also significant. As *S. maculata* is predatory insect feeding on several other insects including crop pests and also having high reproductive capacity, the information of the present study will be applied to the biological control of crop pests.

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First Record of Stink Bug *Tolumnia Basalis* Dallas, 1851 (Heteroptera: Pentatomidae) from Maharashtra

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ABSTRACT

Throughout the world, the genus Tolumnia Stal *is represented by 13 species. Out of these, only 6 species distributed over India, China, Philippines, Thailand, Java, Southeast Asia, Malaysia and western Indonesia have been provided with taxonomic keys. Among these 6 species, Tolumnia basalis is well spread in the Northeastern states like Himachal Pradesh, Uttarakhand and Meghalaya. During the present investigation, this species is the first time reported from the campus of Shivaji University, Kolhapur, Maharashtra. Owing to methodical monitoring of the Entomofauna of the Shivaji university campus resulted in the significant southward distribution of this species towards peninsular India.*

KEYWORDS

Stink bug, Tolumnia, First record, Kolhapur.

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1. INTRODUCTION

Stink bugs are the most diverse group of the family Pentatomidae of suborder Heteroptera represented by 896 genera and 4722 species in the world [1]. Members of the family Pentatomidae are known as "Stink bugs" or "Shield bugs" as they secrete pungent odour from stink glands situated near the bases of metacoxae in adults; while in nymphs these glands are situated beneath the dorsum of the abdomen [2]. Stink bugs are found nearly in all major zoogeographic regions with their phytophagous as well as predacious habits. Phytophagous stink bugs are capable to attack a large number of cultivated and uncultivated plants, causing damage to different crops. Taxonomically the genus *Tolumnia* is represented by 13 species from the world. Rider [1] carried out extensive taxonomic studies on pentatomids of the Oriental region and provided identification keys to only 6 species. Though phytophagous yet no pest status has been recorded for these species till now [3]. In the present communication, *Tolumnia basalis* [4] is the first time reported from

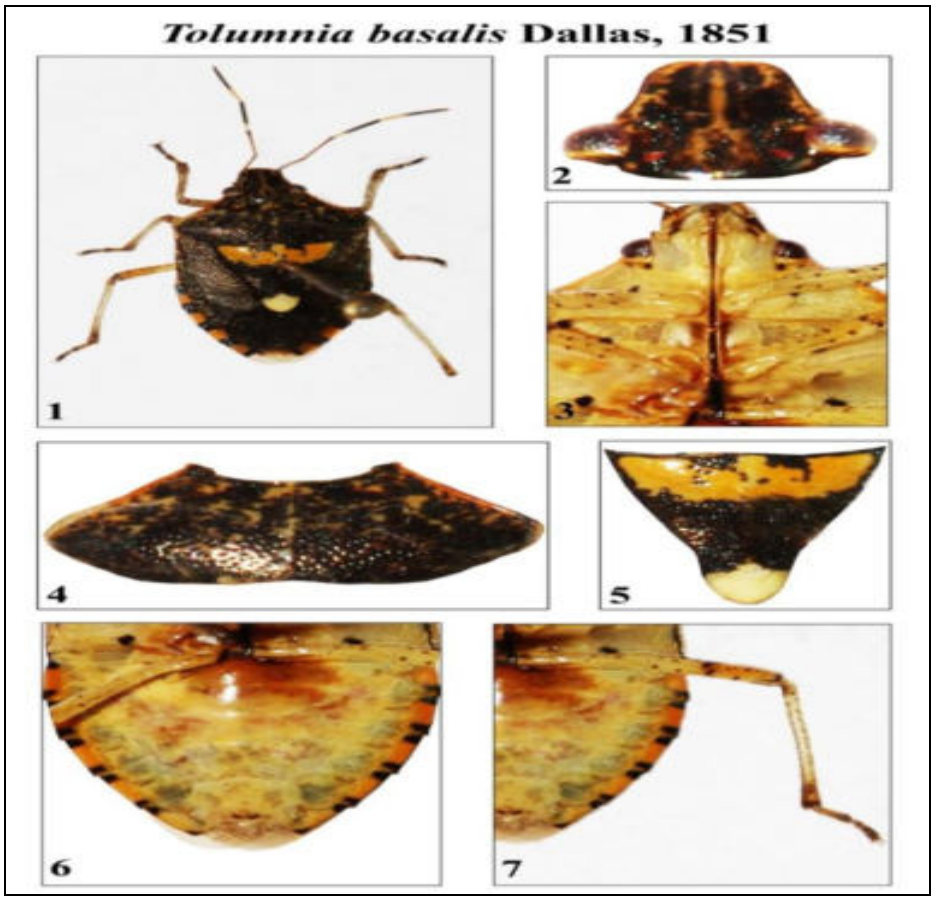
Maharashtra along with colour photographs depicting its morpho-taxonomic characters.

2. MATERIAL AND METHODS

The sampling was carried out in the Shivaji University Campus (16.6721^{o} N, 74.2545^{o} E), Karvir, Kolhapur, Maharashtra state on 6th October 2013. A single male specimen was caught in a light trap. The collected specimen was identified as *Tolumnia basalis* with the help of the description given by Kaur (2012) and deposited in NZC, Zoological Survey of India (ZSI), Western Regional Centre at Pune, labelled as Ent-3/792. Detailed taxonomic characters are described and photography was done.

3. RESULTS AND DISCUSSION

Tlumnia basalis (Dallas, 1851)



Images 1-7. Distinguishing characters of *Tolumnia basalis*. 1, *T. basalis* (Dorsal view); 2, Head (Dorsal view) ; 3, Rostrum passing through posterior coxae; 4

Pronotum (Dorsal view); 5, Scutellum showing scutellar base with irregular maculate pale yellow patch; 6, Abdomen (Ventral view); 7, Hind Leg (Ventral view)

Diagnostic Characters:

Male: Body length 9.09 mm (between head and tip of membrane) and breadth (between pronotal angle) 5.08 mm. Head longer than broad, moderately declivent subrounded at apex, brownish yellow, punctured with black, juga subequal tylus, eyes compound blackish red with their margin yellowish, ocelli pinkish (Image 2), rostrum elongate, just reaching at metacoxae (Image 3), antennae 5 segmented, 1st segment not reaching at the apex of head, 1st, 2n , and 3rd segment brownish yellow, apex of 4th and 5th segment black with their bases pale whitish yellow; pronotum brownish yellow, coarsely and densely punctured with black, anterior margin of pronotum concave and lateral margin brownish yellow, humeral angles subprominent, obtuse (Image 4); scutellum brownish densely punctured with dark black punctures, scuteller base with irregular maculate pale yellow patch, apex pale yellow and impunctate (Image 5); clavus concolorous to corium, costal margin thickly punctured with black; connexiva pale yellowish brown, anterior and posterior margins marked with black patch; membrane passing moderately beyond apex of abdomen suffused with black and brown, outer margin translucent (Image 1); abdomen medially convex, depressed laterally, finely punctured, admixture of pale brownish yellow with green tinge, lateral margins pale (Image 6); legs brownish yellow, finely spotted with black color (Image 7).

Rider et al. [5] reported this species from Fujian, Guangdong, Guangxi, Guizhou, Hainan, Jiangxi, Shaanxi, Yunnan, Zhejiang, Vietnam and Indonesia. In India, Kaur et al. [6] have reported *T. basalis* from Himachal Pradesh, Uttarakhand and Meghalaya. Herein this species shows considerable southward extension from Kolhapur, Maharashtra. More extensive and methodical monitoring may result in the enumeration of more species of the genus from the campus area since it is rich in diverse vegetation.

4. CONCLUSION

The Shivaji University Campus Kolhapur is a new locality for *T. basalis* extending its known geographical range south-westerly from its previous localities, Himachal Pradesh, Uttarakhand, and Meghalaya by about 2000 km.

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Paraquat Induced Alterations in Lipid Peroxidation Activity During Acute Exposure in Commercially Important Bottom Feeder *Cirrhinus mrigala* (Hamilton)

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ABSTRACT

The extravagant proliferation of blue-green algae and aquatic weeds in aquacultural habitats is a tenacious universal problem. Constraints in manual and mechanical de-weeding practices have given rise to the large-scale use of synthetic aquatic herbicides. Paraquat dichloride is one such exhaustively used aquatic herbicide. When sprayed in aquatic habitats, Paraquat pre-occupy in bottom hydrosoil and can persevere in them for longer durations. The economically important fish Cirrhinus mrigala is a bottom feeder Indian Major Carp and is an integral part of composite fish farming throughout south Asia. Its habitat in benthic niches makes it most susceptible to being exposed to Paraquat residues persisting in the benthic hydrosoil of aquacultural tanks and reservoirs. The present investigation was mapped-out to estimate the alterations in the Lipid Peroxidation (LPO) Activity in the Cirrhinus mrigala juveniles during acute exposure to lethal concentrations of Paraquat. Juveniles were exposed to the lowest-lethal concentration (LC_0) and the median lethal concentration (LC_{50}) along with the control group for 96 hrs. in a static renewal bioassay. Post-exposure live fish were euthanized and vital organs like gills, muscle, liver, and brain were pooled out to estimate LPO activity using Wills (1966) method. Annotated observations specified that the LPO levels showed a highly significant ($p<0.001$) increase in the LC_{50} group of gill tissue and a moderately significant ($p<0.01$) increase in the LC_0 group of the same tissue as compared to the control group, while the LC_O group of muscle tissue showed a significant ($p<0.05$) increase and LC_{50} group of the same tissue showed a moderately significant ($p<0.01$) increase in the LPO levels as compared to the control group. The LPO levels in the liver tissue showed a highly significant ($p<0.001$) increase in both the treatment groups compared to the control group while in the brain tissue the LC_0 group showed a moderately significant ($p<0.01$) increase and the LC_{50} group showed a highly significant ($p<0.001$) increase in the LPO levels as compared to the control group. From this study, it can be concluded that Paraquat toxicity induces significant lipid peroxidation in the vital tissues of fish that might cause disturbances in their internal homeostasis, stunted growth, and retardation in their development

causing economic losses to the stakeholders and damage to the non-targeted aquatic ecosystems.

KEYWORDS
Paraquat dichloride, Cirrhinus mrigala, Acute Toxicity, Lipid aeroxidation activity.

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1. INTRODUCTION

Aquatic ecosystems have been an ultimate sink for most of the anthropogenic, industrial, and agricultural chemicals globally [1]. Spray drift, leaching, direct application, run-offs, and factory/sewage discharge are the most prominent routes through which pesticides enter aquatic ecosystems [2]. Thus pesticides are regarded as serious aquatic pollutants with the potential to cause hazardous effects on non-targeted organisms like fish [3]. Water polluting agents including herbicides are causative factors of redox imbalance in fish cells and are responsible for the induction of oxidative stress in them [4]. Paraquat Dichloride (1,1'-Dimethyl-4,4'-bipyridinium dichloride) (CAS No = 75-305-73-0) is one such bipyridyl herbicide well-known for its redox cycle potential and generation of oxidative stress [5,6]. Paraquat is highly toxic to humans yet it is popular as a second-highest selling herbicide worldwide [7,8]. Its large-scale use, slow degradation process, and affinity toward bottom sediments have made its presence reportable in many water bodies across the globe that is responsible for its toxic effects on non-targeted aquatic organisms [9,10,11,12,13,14,15]. Such chemical contaminants in aquatic environments can adversely affect aquatic life, including fish [16,17,18,19]. *Cirrhinus mrigala* is one such commercially important fish species that is an integral part of composite fish culture throughout the South-Asian continent. Paraquat's persistence in benthic sediments makes benthic feeder *Cirrhinus mrigala* most susceptible to being exposed to it, hence was chosen as the most relevant test animal for this study. Besides, a paucity of data was noticed regarding the effect of Paraquat on bottom-feeder carp hence this study was designed to obtain that baseline data using alterations in Lipid Peroxidation (LPO) activity as a biomarker to detect non-targeted toxicity.

LPO is a prominent molecular mechanism in pesticide toxicity [20]. It is recognized as an attack of harmful Reactive Oxygen Species (ROS) causing oxidative stress/damage in tissues/organs [21,22,23]. Biota exposed to environmental pollutants can undergo increased ROS activity that can make the defensive antioxidant system incapable of their elimination causing oxidative stress and damage [24,25,26,27]. LPO is significantly important for aquatic animals as they contain comparatively more amount of Polyunsaturated Fatty Acids (PUFA) [28]. This study records the alterations in LPO activity in terms of Malondialdehyde (MDA) formed as an end product of PUFA peroxidation inside cells.

2. MATERIALS AND METHODS

2.1. Acquisition and acclimatization of Experimental Animals

Juvenile *Cirrhinus mrigala* fingerlings (weight 4.67±0.35 gm. and length 5.04±0.91 cm) were brought to the laboratory in jumbo-plastic bags supported with oxygen from the Government Fish seed Production center, Dhom (Wai), Satara District, Maharashtra State, India. At the laboratory, obtained fish were disinfected in 0.1% $KmNO_4$ solution and stored in well-aerated aquaria for 15 days. At room temperature, natural photoperiod was maintained for the well-acclimatization of fishes to laboratory conditions. During the entire experiment, juvenile fingerlings were fed with commercial fish food (Taiyo Discovery) at 2% of their total body weight, and aquarium water was changed every 24 hours to keep the enclosed-water system Optimum. Testing of standard water quality parameters was done every week to ensure normal conditioning. Weak, diseased, or dead fishes were discarded from the experiment and only healthy ones were chosen for the study. After 15 days, selective healthy fishes were introduced and acclimatized into well-aerated 22-liter capacity plastic containers filled with normal tap water for 7 days in which they were to be exposed to the toxicant. 24 hrs. before exposure, feeding was discontinued to minimize vomiting due to toxicant irritability and excess excretion.

2.2. Exposure to Paraquat

A commercially marketable product of Syngenta named ‘Gromoxone’ containing 24% w/w Paraquat Dichloride was used as a toxicant in this study. Prior to exposure protocols, the LC_0 and LC_{50} concentration values of Paraquat were derived using the 'static renewal bioassay method'. The data obtained from the bioassay was analyzed using ‘Finney’s probit analysis’ [29] to finalize both the lethal concentrations. Further, for the present protocol total of 3 polythene containers filled with 20 liters of tap water and adequate aeration support were set up. 10 fishes were released in each container. The first container functioned as a control group with no toxicant exposed to the fishes. The second and third containers served as treatment groups. In the second container, fishes were exposed to LC_0 (67.32 ppm) concentration of toxicant and in the third container, fishes were exposed to LC_{50} (105 ppm) concentration for 96 hrs. After every 24 hrs. the water and toxicant were renewed with fresh water and toxicant to maintain optimum toxicant concentration throughout the experiment. Dead fish were immediately discarded. Post 96 hrs. all live fish were euthanized and their vital organs like gill, muscle, liver, and brain were pooled out to derive the alterations in the lipid peroxidation activity, between similar tissues of different treatment groups.

2.3. Analysis of Lipid peroxidation activity

For the present experiment, E. D. Wills's method [30] was used to estimate the altered lipid peroxidation levels in all four tissues. Initially, the reaction mixture required to homogenize the tissues was freshly prepared. For its preparation 1 ml of Phosphate Buffer Saline (PBS, pH 7.4) + 1 ml of 75 mM Ascorbic Acid (AA) + 1 ml of 1 mM $Fecl_3$ + 0.01 ml of Chlorotetracycline was mixed together to obtain a 3.01 ml of reaction mixture. Then 100 mg of fresh tissue was homogenized in 10 ml of the reaction mixture. This mixture served as a stock solution. Then 0.1 ml of this stock solution was taken in 3 different test tubes (triplicates) and 1.9 ml of distilled water + 1 ml of 20% TCA + 2 ml of 0.67% Thio Butyric Acid (TBA) was added to all the test tubes. Simultaneously a blank was prepared by adding 2 ml distilled water + 1 ml of 20% TCA + 2 ml of 0.67% TBA in a separate test tube. All test tubes were placed in a boiling water bath for 15 minutes. After 15 minutes the test tubes were cooled and centrifuged at *1000 g* for 10 minutes. Finally, the absorbance's of supernatants thus obtained was recorded against the blank at 532 nm wavelength using a spectrophotometer. The lipid peroxidation levels were determined as nano-moles (nM) of MDA formed per milligram (mg) of tissue (nmol MDA/mg tissue) by using 1.56 x 105 M^{-1} cm^{-1} as its molar extension coefficient. The amount of Malondialdehyde (MDA) was calculated as follows:

2.4. Calculations

$$\text{MDA / mg tissue} = \frac{\text{O.D. of the sample}}{(0.156)\ (1)}$$

Where,
0.156 = Absorbance for 1 mM solution of malondialdehyde in a 1 cm thick cell at 532 nm.
1 = Amount of tissue taken in mg, present in 0.1 ml of a sample.
The final results were statistically represented in arithmetic mean ± standard deviation (SD) format in all the groups. The level of significance was derived using Student's t-test with two-tailed distribution in a two-sample unequal variance (heteroscedastic) method.

3. RESULTS

Physico-chemical parameters recorded for the water medium used for the experiment were measured according to the APHA (1998) [31] standard protocols. They were: Temperature 27.8ºC, pH 7.0-7.4, Dissolved oxygen 6.06-7.39 mg/L, Dissolved Carbon dioxide 14.62± 0.81 mg/L, hardness 156.2± 1.20 mg/L, phosphates 0.5 ± 0.27 mg/L, nitrates 1.89 ±0.73 mg/L.

The final results represent alterations in lipid peroxidation levels in all the selected vital organs viz. gill, muscle, liver, and brain of juvenile *Cirrhinus mrigala* fingerlings in all the three groups (control, LC_0, and LC_{50}) are illustrated in **Table 1 and Graph 1.**

In the control group of fish *Cirrhinus mrigala*, the lipid peroxidation activity was in the order of Brain > Liver > Muscle > Gills.In the gill tissue of the control group, the lipid peroxidation activity recorded was 1.36±0.04 nM of MDA/mg tissue. But in the LC_0 concentration group, fish gills exhibited 2.84±0.27 nM of MDA/mg tissue and in the LC_{50} concentration group, fish gills exhibited 4.82±0.25 nM of MDA/mg tissue.

In the muscle tissue of the control group, the lipid peroxidation activity recorded was 1.48±0.61 nM of MDA/mg tissue. But in the LC_0 concentration group, the muscle tissue exhibited 2.82±0.41 nM of MDA/mg tissue and in the LC_{50} concentration group, the muscle tissue exhibited 4.23±0.17 nM of MDA/mg tissue.In the liver tissue of the control group, the lipid peroxidation activity recorded was 4.15±0.14 nM of MDA/mg tissue. But in the LC_0 concentration group fish liver exhibited 5.01±0.12 nM of MDA/mg tissue and in the LC_{50} concentration group fish liver exhibited 6.72±0.17 nM of MDA/mg tissue. In the brain tissue of the control group, the lipid peroxidation activity recorded was 4.84±0.16 nM of MDA/mg tissue. But in the LC_0 concentration group, the brain tissue exhibited 5.88±0.05 nM of MDA/mg tissue and in the LC_{50} concentration group, it exhibited 7.84±0.08 nM of MDA/mg tissue.

Table 1: Effect of Paraquat Dichloride on the Lipid Peroxidation activity in different tissues of the fish *Cirrhinus mrigala* after acute exposure.

Groups	Lipid peroxidation activity (nM of MDA/mg wet wt. of tissue)			
	Gill	Muscle	Liver	Brain
Control Group	1.36±0.04	1.48±0.61	4.15±0.14	4.84±0.16
LC_0	2.84±0.27**	2.82±0.41*	5.01±0.12***	5.88±0.05**
LC_{50}	4.82±0.25***	4.23±0.17**	6.72±0.17***	7.84±0.08***

(Values expressed as Arithmetic Mean of (n=3); ±SD),

*= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)

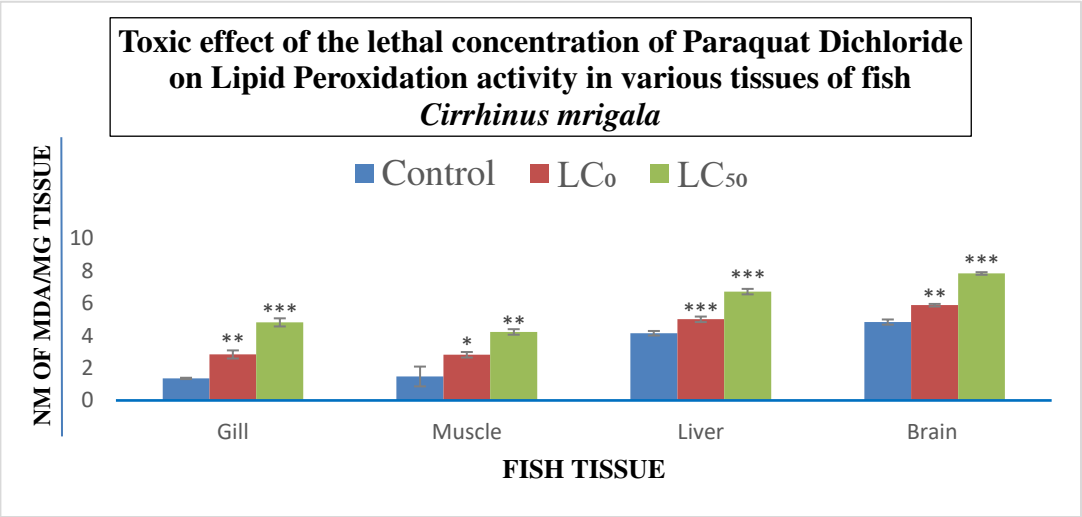

Fig 1: Toxic Effect of the lethal concentration of Paraquat Dichloride on Lipid peroxidation activity in Gill, Muscle, Liver, and Brain tissues of the fish *Cirrhinus mrigala* after acute exposure (96 hours). Data expressed in arithmetic mean±Standard Deviation. Error bars represent the SD of 3 individual observations. * indicates p<0.05(significant), ** indicates p<0.01(moderately significant), * indicates p<0.001(highly significant)**

The lipid peroxidation activity in gill tissue showed a moderately significant ($p<0.01$) increase in the LC_0 group (t.stat = -9.24) while it showed a highly significant ($p<0.001$) increase in the LC_{50} group (t.stat = -23.28). The lipid peroxidation activity in muscle tissue showed a significant ($p<0.05$) increase in the LC_0 group (t.stat = -3.13) while it showed a moderately significant ($p<0.01$) increase in the LC_{50} group (t.stat = -7.46). The lipid peroxidation activity in liver tissue showed a highly significant ($p<0.01$) increase in both LC_0 group (t.stat = -7.79) as well as in the LC_{50} group (t.stat = -19.8). The lipid peroxidation activity in brain tissue showed a moderately significant ($p<0.01$) increase in the LC_0 group (t.stat = -10.48) while it showed a highly significant ($p<0.001$) increase in the LC_{50} group (t.stat = -28.36). The post-experimental lipid peroxidation activity in the four tested tissues was in the order Brain > Liver > Gill > Muscle in the LC_0 group while in the LC_{50} group it was in the order, Brain > Liver > Gill > Muscle.

4. DISCUSSION

Lipid peroxidation is a result of oxidative damage due to ROS action causing interruptions in cell structure and function [32,33]. Fishes are effective biomarkers representing pollution that permit early diagnosis of aquatic problems concerned with environmental health [34,35]. When xenobiotics induce the abnormal

generation of ROS and it exceeds the internal protective mechanism of the fish, it causes damage to internal cellular components. This mechanism is known as oxidative stress [36]. Oxidative stress developed in multiple tissues due to pesticide effects has been popularly inspected as a possible mechanism representing their toxicity and degradation [37]. LPO produces Malondialdehyde (MDA) as an end product. The formation of MDA is an important indicator of induced oxidative stress resulting from free radicals that cause damage to components of cell membranes [38]. The results of the present experiment show significantly increased levels of MDA activity in the entire four tissues viz. gill, muscle, liver, and brain exposed to both LC_0 and LC_{50} concentrations of Paraquat Dichloride. These increased levels of MDA are an indicator of the peroxidation of lipids caused in vital fish tissue due to Paraquat toxicity. Toxic effects of Paraquat might have produced ROS during cellular NADPH oxidation and redox-cycling that induced oxidative stress causing damage to the vital tissues in the form of lipid peroxidation. Lipid peroxidation decreases the quality of consumable fish meat leading to a decrease in its nutritional quality, health problems, and financial losses to the stakeholders [39]. Tanilene Sotero *et al.,* (2017) [40] demonstrated a similar increase in lipid peroxidation activity in gill, liver, kidney, and muscle tissues of *Rhamdia quelen* juveniles exposed to various paddy herbicides used in integrated rice-fish culture.

5. CONCLUSION

From the results of the current study, it can be concluded that non-targeted organisms like fish can be greatly affected by Paraquat toxicity as it causes a significant increase in their lipid peroxidation levels. The increase in LPO activity may affect optimum functionality and internal homeostasis of the fish's vital organs leading to their mortality, stunted growth, and degradation in fish meat quality thus causing economic, health, and nutritional losses to the stakeholders and increase in the aquatic pollution. LPO activity is also a good bio-indicator of the effect of anthropogenic stressors on non-targeted organisms.

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Plant Essential Oils as Insecticidal Agents for Control of Pulse Beetle *Callosobruchus chinensis* L.

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ABSTRACT

The pulse beetle, Callosobruchus chinensis L. is a major insect pest of stored legumes and has developed resistance against regularly used synthetic insecticides. Thus, the experiments were designed to evaluate the contact and fumigant toxicity of Clerodendrum serratum and Clerodendrum viscosum essential oils against C. chinensis. Insecticidal responses varied with test oils and exposure period. Contact toxicity results of C. serratum and C. viscosum revealed the 70% and 53% mortality of beetles respectively at 12.8 µL/cm^2 oils concentration after 48 hrs exposure. In fumigant method assay, C. serratum and C. viscosum essential oils were effective against adult beetles which were produced 83% and 60% mortality respectively at 160 µL/ L air. However, in both contact and fumigant toxicity results, the fumigation method assays the C. serratum and C. viscosum found to be more effective for control the beetles. Among the two plant oils, the C. serratum oil to be more toxic compared to C. viscosum oil in both tests. From this study, we conclude that these oils have potential for the control of pulse beetle.

KEYWORDS

Bioinsecticides, C. Chinensis, C. Serratum, C. Viscosum, Essential oil.

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1. INTRODUCTION

The pulses are the second most important food grains worldwide and are an excellent source of proteins, as well as thiamine, niacin, calcium, and iron [1]. The pulse crops are important for the management of soil fertility through biological nitrogen fixation for sustainable agriculture [2]. The pulse grains are attacked by many insects, among them, the pulse beetle, *Callosobruchus chinensis* (L.) is the most destructive insect pest of stored grains. The infestation initiates from field conditions and continues in storehouses causing heavy losses [3]. Adult-laid eggs on seeds and hatched grubs are the infesting stage causing 34-65% of grain loss during storage conditions in the month of August to January in India [4]. Damaged grains are not suitable for human consumption, and they are incapable for making sprouts, thus they lose their market value.

For the control of stored-grain insects pest fumigation of phosphine and methyl bromide are still effective methods [5]. However, indiscriminate use and more dependence on these insecticides resulted in serious problems linked to food contamination, toxic hazards to human and non-target organisms [6]. These concerns have led to renewed search for new means to control insect pests without these negative impacts. In recent years, many research works emphasized on the use of plant products as low risk insect pest control agents due to their low mammalian toxicity and selectively varying sites of action [7]. Plant essential oils are among the most effective alternatives as insect pest control agents, particularly against stored-grain insect pests [8,9].

The genus *Clerodendrum* is one of the important genera of Lamiaceae family, it contains many plant species of herbs, shrubs, and trees. These plant parts are used as insect pests control agents as well as for pharmaceutical purposes [10]. Plant essential oils contain terpenes which show toxic effects against insects and phytochemicals are eco-friendly in nature, easy to degrade, and beneficial to human welfare [11]. Hence, in the present investigation, the *Clerodendrum serratum and Clerodendrum viscosum* essential oils were studied for their contact and fumigant toxicity effects on the adult pulse beetle, *C. chinensis.*

2. MATERIALS AND METHODS

2.1. Insect culture: The culture of pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) was established by collecting the adults from infested stored green gram (*Vigna radiata* L.) from grain traders, in Kolhapur, India. Beetles were maintained on insecticide-free newly harvested green grams under laboratory conditions (28 ± 2°C temperature, 70 ± 5% RH and natural photoperiod) [12]. Insect culture was repeated for 2 generations to get laboratory conditions reared adults and then were used for all experiments.

2.2. Plant collection and extraction of essential oils: The leaves of *Clerodendrum serratum* L. (syn. *Rotheca serrata*) and *Clerodendrum viscosum* L. (syn. *Clerodendrum infortunatum*) (family: Lamiaceae) plants were collected in the morning hours in and around Kolhapur vicinity. Plants were identified and confirmed by experts in the Department of Botany, Shivaji University, Kolhapur. The leaves were cleaned and then subjected to hydro-distillation for extraction of essential oils by using modified Clevenger apparatus [13]. The essential oils were extracted with n-hexane and then anhydrous sodium sulfate was used to remove water content. Oils were stored in airtight scintillation vials and then kept in a refrigerator at 4°C for further use.

2.3. Contact toxicity: The contact toxicity of *C. serratum* and *C. viscosum* plant essential oils were determined by direct contact method using the filter paper impregnated bioassay method [14] against the *C. chinensis*. Test solutions of each

essential oil were prepared by dissolving 2.5, 5, 10, 20, 40, 80, 160 µL in 1 mL of *n*-hexane. A 200 µL of each test concentration was applied uniformly to a 4 cm diameter Whatman No.1 filter paper disc using a micropipette to obtain gradient concentrations of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 µL/cm^2 and the control groups filter paper were treated with 200 µL *n*-hexane only. The solvent was allowed to evaporate at room temperature. Each filter paper was glued into the bottom of a 4 cm petri dish. In each petri dish, 10 beetles were released and covered with a lid. There were three replicates in each treatment. Mortality of beetles were recorded after 24 and 48 hrs of treatment and mortality results were expressed as µL/cm^2.

2.4. Fumigant toxicity: Fumigant toxicity of both plant essential oils were determined against *C. chinensis* [15]. A serial dilution of both plant essential oils was prepared in *n*-hexane to get seven concentrations of 2.5, 5, 10, 20, 40, 80, and 160 µL/ L air. A Whatman filter paper (2-cm diameter) was impregnated in test solutions and then placed on the underside of a 25 mL glass screw cap vial (2×7 cm) and in the control group, only *n*-hexane was used. The inside of the neck of the vial was painted with Vaseline to avoid direct contact of beetles with oil. Before releasing the 10 adults into a glass vial, the solvent was evaporated for a minute and the vial cap was screwed tightly. Three replicates were maintained in each treatment, and mortality was observed after 24 and 48 hrs, and results were expressed as µL/L air.

The mortality results were corrected by using formula [16]. A 48 hrs mortality results were analyzed through the one-way ANOVA by using SPSS software and means were separated by Least Significant Difference (LSD) admitting significance at $P< 0.05$ level. The LC_{50} values were determined [17].

3. RESULTS AND DISCUSSION

The essential oil obtained from *C. serratum,* and *C. viscosum* plant leaves were almost light-yellow in colour. The percent essential yield was calculated by comparing the weight of essential oil to the amount of leaves weight. The yield of essential oil obtained was 0.22% and 0.25% (v/w) from *C. serratum* and *C. viscosum,* respectively.

The contact toxicity of *C. serratum* and *C. viscosum* plant essential oils against *C. chinensis* by contact method mortality results increased with increasing concentration and exposure period (Table 1). The *C. serratum* essential oil showed greater efficacy compared with *C. viscosum* essential oil. The test insects initially after release, agitated movements were observed with a knockdown effect at higher concentrations of oil (12.8 µL/cm^2). Whereas at lower concentrations (0.6 µL/cm^2) insects recover after 20-25 minutes of exposure. During this period beetles were moved toward the lid area and prepared to stay away from the treated discs. Both plant oils showed a moderate insecticidal effect on the test insects in the contact method.

The fumigant toxic effect of *C. serratum* and *C. viscosum* essential oils were tested on adults of *C. chinensis* and observed the strong mortality of the insect. The *C. serratum* oil was significantly more toxic against beetles at 12.8 µL/L air compared to *C. viscosum* effect at the same concentration (Table 2). On the basis of LC_{50} values, both plant essential oils tested, the stored-grain insect pest of *C. chinensis* was the most susceptible to the *C. serratum* essential oil than the *C. viscosum* oil in both contact and fumigant toxicity (Table 3).

In the present study, contact and fumigant toxicity of *C. serratum* and *C. viscosum* essential oils against *C. chinensis* were studied. The results revealed that the insecticidal activity of the plant essential oils varied based. These biological activities of plant essentials depend on the plant species, age of the plant, soil, and phenology [10]. In the contact toxicity bioassay, tested oils showed the significant mortality of the beetles at higher concentrations whereas in fumigant toxicity method toxic effects were more rapid even in the lower concentrations. The toxicity of many plants essential oils might be attributed to the mono- and di-terpenes [11]. These terpenes are high volatile compounds thus they have fumigant activity that might be very important characteristics for controlling stored grain insect pests. Previously, monoterpenoids were confirmed as contact and fumigant toxicity agents for the control of stored insect pests [8,18,19].

The obtained results suggest that *C. serratum* and *C. viscosum* oils were potential for the use as contact and fumigant toxic agents to manage the *C. chinensis* adults. The oil concentrations were used in our work is only 2.5-60 µL/mL which is very insignificant when compared to the LC_{50}'s of oils that wide-ranging between 3000-5000 mg /kg rats body weight and it showed minor irritant effects after long time exposure [20]. Subsequently, it is direct evidence that essential oils are safer for mammals when compared to synthetic fumigants of methyl bromide and phosphine presently used around the world for control of stored grain insect pests. These chemicals cause environmental disturbances, resistance development in target pests, and depletion of the ozone layer [5].

The increased awareness of environmental and health hazards issues with relation to the use of chemical insecticides make use of bioinsecticides in which plant essential oils are more attractive for controlling the stored grain insect pests. Bioinsecticides have the advantage of proving the broad modes of action against insect pests. Based on our results, *C. serratum* and *C. viscosum* essential oils showed a significant insecticidal activity against *C. chinensis*. These plant oils will play a major role in the future for the control of stored product insects including *C. chinensis*.

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Table 1. Contact toxicity of plant essential oils against pulse beetle, *C. chinensis*

Concentration of Eos (µL/cm$^{2)}$	Percent mortality*	
	C. serratum	***C. viscosum***
0.2	07 ± 3.33^{ef}	03 ± 3.33^{e}
0.4	13 ± 3.33^{de}	10 ± 5.77^{de}
0.8	23 ± 3.33^{d}	20 ± 5.77^{cd}
1.6	37 ± 3.33^{c}	27 ± 3.33^{bc}
3.2	50 ± 5.77^{b}	33 ± 3.33^{b}
6.4	57 ± 3.33^{b}	47 ± 3.33^{a}
12.8	70 ± 5.77^{a}	53 ± 3.33^{a}
Control	00 ± 0.00^{f}	00 ± 0.00^{e}

*Mean ± SE of three replications. Means followed by the same letter in column are not significantly different P< 0.05 level.

Table 2. Fumigant toxicity of plant essential oils against pulse beetle, *C. chinensis*

Concentration of EOs (µL/ L air)	Percent mortality*	
	C. serratum	***C. viscosum***
0.2	07 ± 3.33^{ef}	03 ± 3.33^{e}
0.4	17 ± 3.33^{e}	10 ± 5.77^{de}
0.8	30 ± 5.77^{d}	20 ± 5.77^{d}
1.6	43 ± 3.33^{c}	33 ± 3.33^{c}
3.2	50 ± 5.77^{c}	43 ± 3.33^{bc}
6.4	67 ± 3.33^{b}	53 ± 3.33^{ab}
12.8	83 ± 3.33^{a}	60 ± 5.77^{a}
Control	00 ± 0.00^{f}	00 ± 0.00^{e}

*Mean ± SE of three replications. Means followed by the same letter in column are not significantly different at P< 0.05 level.

Table 3. LC_{50} and associated values of contact and fumigant toxicity of essential oils against pulse beetle, *C. chinensis*

Plants	LC_{50} (95% Fiducial Limit)	Regression Equation	χ^2	df
Contact toxicity				
C. serratum	3.86 (2.58-6.57)	Y= 4.3476 + 1.1105 X	4.67	19
C. viscosum	8.59 (4.99 -22.0)	Y= 4.0684+ 1.0299 X	6.92	19
Fumigant toxicity				
C. serratum	2.53 (1.78-3.76)	Y = 4.495 + 1.2565X	5.13	19
C. viscosum	5.47 (3.55-10.35)	Y = 4.1505 + 1.1592X	7.63	19

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Phase Contrast Microscopic Observations of Primary Culture of Non-Neuronal Cells from E15 Mouse Embryo

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ABSTRACT

The functioning of the brain is driven by non-neuronal and neuronal cells. The non-neuronal cells include astrocytes, oligodendrocytes, and microglia. These cells have distinct roles and morphological variability. To isolate these non-neuronal cells, most of the in vitro investigations employed adult or neonatal mouse pups (P0- P4). In the present investigation, the non-neuronal cells were isolated from the E15 mouse embryo (i.e.15th day of embryonic development). The dissociated cells were cultured on poly-L-Lysine coated 60mm culture plates. The cells were fed with Dulbecco's Modified Eagle Medium (DMEM), 10% Fetal Bovine Serum (FBS) supplemented with HEPES (4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid) (25mM), and gentamycin (40µg/ml). The cells were incubated in humidified CO_2 incubator at 37℃ and 5%CO_2. The morphology of non-neuronal cells was observed under a phase contrast microscope. These cells exhibited a variety of morphologies such as amoeboid, stellate, and ramified.

KEYWORDS

Astrocytes, Culture of Non-neuronal cells, Glial cells, Microglia.

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1. INTRODUCTION

The brain is a complex and integral structure that governs different activities of the body [23]. The mammalian brain contains two types of cell population; Neuronal cells and non-neuronal cells [5]. Neuronal cells comprise unipolar, bipolar, and multipolar neurons which had been extensively studied both in vivo and in vitro [7,9,10,26,31]. The non-neuronal cells in general are termed glial cells which comprise astrocytes, microglia, and oligodendrocytes [6]. In addition to neurons, these cells play an equally important role in brain functions. Microglia are the resident macrophages in the central nervous system [4]. Microglia are involved in the phagocytosis of foreign bodies and apoptotic cells [3]. Astrocytes play important role

in brain homeostasis and are involved in the formation of the Blood-Brain Barrier [1]. Astrocytes have a role in synaptogenesis and boost neuronal survival [45]. The Oligodendrocyte functions as a myelin-producing cell [8] and makes direct contact with a neuron at the synapse and node of Ranvier, and takes part in neuronal activities [11].

The glial cells exhibit different morphologies and extensions [32]. In vitro studies are very essential to observe cell morphology at the microscopic level [35]. The effects of Stress and aging-related alterations in the morphology of glial cells have been studied in vivo [13]. Most of the cell culture protocols for glial cell culture have used brains from neonatal rats or mice from postnatal days 1 to 4 [20,22,30]. Previous in vivo studies revealed that gliogenesis starts in the neopalium of the mouse at the embryonic day 12 [15]. Wang et al., [2011] demonstrated that radial glial-like progenitor cells were found in the embryonic neocortex of the mouse. Although the neurons remain the focus of many researchers, it is equally important to focus on non-neuronal cells as they are involved in neuroinflammation and neurodegenerative alteration in the aging brain [17,18]. The literature about the morphological observations of non-neuronal cells obtained from the embryonic brain is scanty. Therefore the present investigation is aimed to reveal the same.

2. MATERIALS AND METHODS:

In the present investigations E15 embryos of Swiss albino mice *Mus musculus* were used. All the experimental protocols were approved by the Institutional Animal Ethics Committee (1825/PO/EReBi/S/15/CPCSEA). All procedures described below were carried out strictly in aseptic conditions.

2.1 Preparation of Poly L Lysine coated culture plates:

2ml of 0.01% Poly L lysine in 0.1 M Borate buffer was added to a sterile 60mm culture plate to form a uniform layer of PLL and kept for 6 hrs. in the laminar airflow. Thereafter, the solution containing poly L lysine was discarded and the plates were washed three times with sterile distilled water and air-dried for 15 min. in the laminar airflow. The coated culture plates were soaked with calcium magnesium-free HBSS (Hanks' Balanced Salt Solution) for 6 hours. The soaked plates were used for the seeding of the cells.

2.2 Obtaining the E15 embryos:

The pregnant female albino mouse was euthanized by cervical dislocation on the fifteenth day of gestation in strictly aseptic condition. The embryos were removed from the uterus and transferred to chilled PBS containing 40 µg/ml gentamycin. Under the stereo zoom microscope, in the laminar airflow, the embryos were dissected for the isolation of cerebral cortices.

2.3 Isolation of cerebral cortices:

The skin of the skull was carefully removed using sharp forceps. Thereafter, the skull bone was gently cut open to expose the brain. The meninges were removed using fine forceps without damaging the internal tissues. The olfactory lobes were removed and the cerebral cortices of eight embryos were separated and transferred into a 15ml Falcon tube containing HBSS supplemented with HEPES (25mM) and Gentamycin (40µg/ml)

2.4 Dissociation of cells from cerebral cortices:

0.5 ml of trypsin (10X)was added to the above-mentioned falcon tube containing cerebral cortices and incubated at5% CO_2 and 37ºC for 10 min. in a CO_2 incubator. The HBSS containing trypsin was decanted and 0.5 ml of trypsin inhibitor was added to it. The trypsinized cortices were triturated by a sterile fire-polished Pasteur pipette to isolate cells.

2.5 Viable cell count:

100 µl of cell suspension and 100 µl of 0.25% Trypan blue were mixed in a sterile Eppendorf tube. 50µl of suspension was loaded into the Neubauer chamber. The cells were counted from four corner squares, the live cells appeared colorless and dead cells stained blue in colour.

2.6 Seeding of the cells:

2×10^5cells were seeded on the surface of the poly L lysine coated culture plate. The plates were incubated in CO_2 incubator at 37℃ and 5% CO_2 for 20 minutes to allow the adhesion of the cells. Thereafter, the cells were fed with 2ml of DMEM supplemented with 10% serum. After 24 hrs., the culture medium was replaced with a fresh culture medium to remove non-adhered cells. Every third day 2/3rd of the culture medium was replaced by a fresh medium.

3. RESULTS AND DISCUSSION

Immediately after seeding, the cells appeared oval and rounded [Fig.1 A]. On the 1st day of incubation, fine processes were developed [Fig.1B]. In DMEM supplemented with 10% serum, neurons cannot grow; rather, they require Neurobasal medium and B-27 Supplement [14,42]. Hence, neuronal cells were degenerated [Fig. 1D].The cell size of non-neuronal cells was increased, and nucleus and nucleoli were prominently seen [Fig. 1E].From the 7th day onwards some of the non-neuronal cells exhibited degenerative alterations, whereas, others continued to proliferate. The degenerating cells showed apoptotic bodies and fragmented nuclei [Fig. 1H].The extracellular matrix is important for cell adhesion which plays a vital role in cell survival, differentiation, proliferation and migration [2,19]. Saura, [2007] and De Vries et al.,

[2010] reviewed coating of the laminin favors astroglial growth but not microglia while poly L lysine is not prerequisite for astrocytes or microglia adhesion. Most of the evidence suggested that poly L lysine favors the adhesion of non-neuronal cells to the surface and also aids its differentiation [25,28]. In the present investigations, the cerebrocortical non-neuronal cells were grown well on a poly L lysine-coated culture plate.

Cells obtained from E15 mouse embryos revealed three non-neuronal cell morphologies: amoeboid [Fig. 2A], ramified [Fig. 2B], and stellate [Fig. 2C]. The microglial cell has two morphologies: amoeboid or fried egg morphology and ramified morphology [39]. The Amoeboid microglia are phagocytic and the ramified microglia are the next stage of amoeboid microglia which play an important role to stabilize the environment within the nervous system immunologically [12]. In the present investigation, there was no bacterial/mycoplasmal/fungal contamination, suggesting an immunologically inert condition. However, during seeding of the cells, there was presence of neuronal cells also, which could not survive in the absence of Neurobasal medium and B27 supplement. The dead neuronal cells from the Petri plate were phagocytosed by amoeboid microglia which eventually exhibited ramified morphology. Zhang et al., [2020] found that the ramification of amoeboid microglia is due to Transforming Growth Factor Beta (TGFβ) secreted by astrocytes. In the present investigations, astrocytes and microglia were growing together; therefore, TGFβ secreted by astrocytes might also be a trigger for ramified morphology of the microglial cells.

Astrocytes have a stellate or star-shaped and fibroblastic appearance [24]. They are classified into two types: fibrous astrocytes [fine processes and branching] [37] and protoplasmic astrocytes which possess highly branched bushy processes [33]. Vasile et al., [2017] reviewed that the astrocytes play an important role in the regulation of neural networks and cognitive functions. Astrocytes react to neuronal damage by forming a glial scar, which is known as astrogliosis. Astrogliosis is a pathological characteristic of neuronal damage [36].Any type of brain insults such as neuronal damage or infection causes the activation of microglia that induces astrogliosis [44]. Mason et al., [1988] studied the morphological alterations in the primary culture of astrocytes derived from the postnatal mouse cerebellum. Most of the studies on non-neuronal cells are based on cells obtained from adult mice or rats and newborn pups [21,29,41].The present study addressed the morphological observations of non-neuronal cells obtained from the embryonic brain.

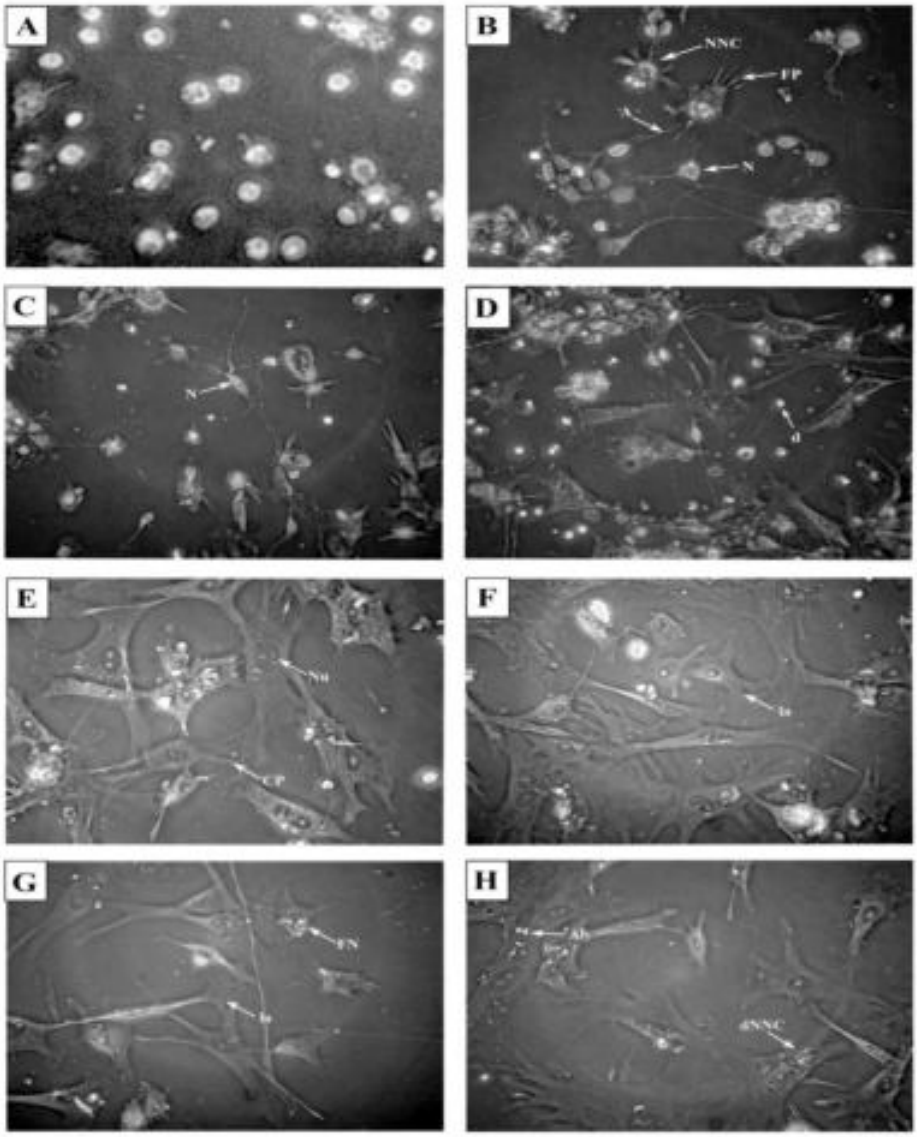

Fig. 1 Phase contrast microscopic images of mouse cerebrocortical non-neuronal cells in culture (400X)Fig. A: Non-neuronal cella on 0 day, **Fig. B:** Non-neuronal cells on 1st day, **Fig. C:** Non-neuronal cells on 2nd day, **Fig.D:** Non-neuronal cells on 3rd day **Fig. E:** Non-neuronal cells on 4th day, **Fig. F:** Non-neuronal cells on 5th day, **Fig. G:** Non-neuronal cells on 6th day **Fig. H:** Non-neuronal cells on 7th day, **Abbreviations:A:** Axon, **FP:** Fine processes, **N**: Neuron, **NNC:** Non-neuronal cell, **CP:** cell processes, **Nu:** Nucleus, **la:** lamellipodia, **dNNC**: degenerating Non-neuronal cell. **Ab:** Apoptotic bodies, **FN:** Fragmented Nucleus

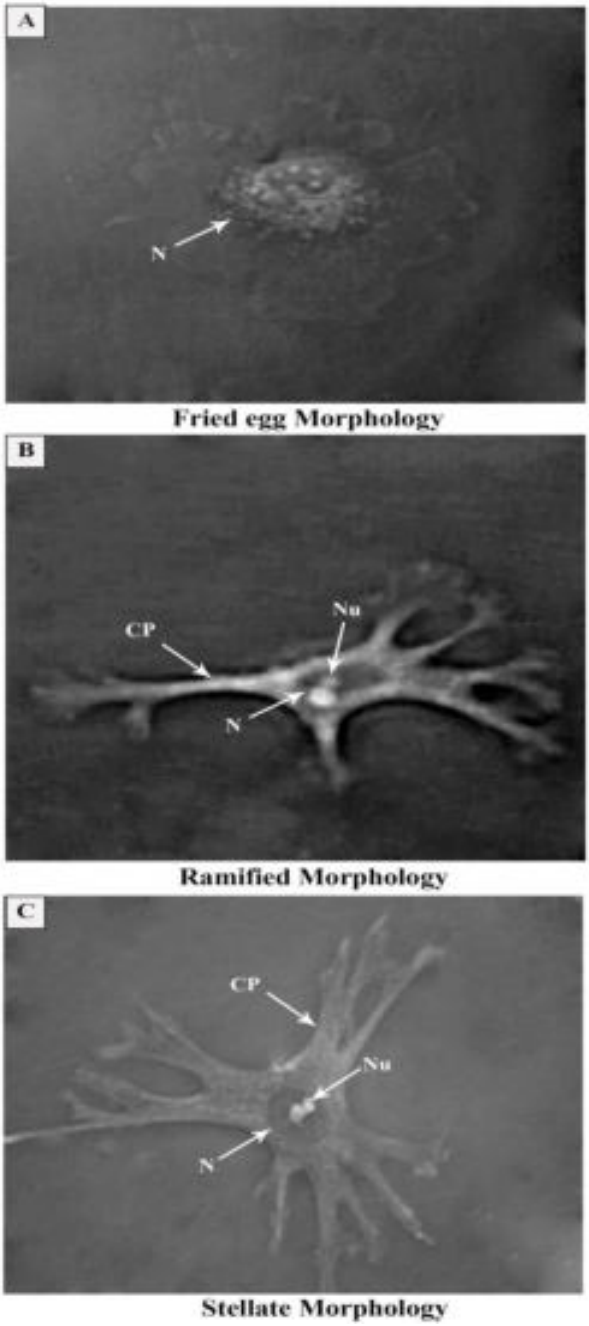

Fig. 2. Morphologies of cerebrocortical non-neuronal cells (400X)
Fig A: Amoeboid morphology, **Fig. B:** Ramified morphology, **Fig. C:** Stellate morphology.
Abbreviations: N- Nucleus, **CP:** Cell processes, **Nu:** Nucleolus

4. CONCLUSION

The present study on the phase-contrast microscopic observations of the primary culture of non-neuronal cells helps to focus on an individual cell level. This will help to study the effects of various growth regulators, paracrine factors, and neuromodulators on the non-neuronal cells in vitro.

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