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Subtoxic Effect of Organophosphate, Chloropyriphos on Earthworm *Lampito mauritii*

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Abstract

Earthworms play a very important role in the soil fertility as it significantly influences the physical, chemical and biological properties of soil. Chloropyriphos, an organophosphate is most widely used by farmers of western odisha in crop fields as an insecticide on grain, cotton, fruit, nut, and vegetable crops. The subtoxic effect of this pesticide was studied on the anecic earthworm, *Lampitomauritii*. Chloropyriphos has significant detrimental effect on the growth, feeding, excretion and regeneration of *Lampitomauritii*, but increased the respiration when it was exposed to subtoxic level of the pesticide. These effects on the earthworm can also be used as the bioindicator for assessment of the sub-lethal effects of pesticides on soil fauna.

Keywords

Organophosphate, chloropyriphos, subtoxic effect, bioindicator, growth, feeding, respiration, regeneration.

Introduction

The soil is a habitat for various organisms like bacteria, symbiotic and non-symbiotic nitrogen fixing bacteria,actinomycetis viruses, fungus, algae, protozoa, nematodes, enchytraeids and earthworms, arthropods, molluscs etc. which play an important role as the biological ploughs and suppliers of nutrients for growth of plants.The soil scientists opine that soil biodiversity and their interaction among themselves and with soil restores the soil fertility of degraded soil and also increases the crop yield without heavy input of fertilizers and pesticides.

The soil biota can be grouped in various groups according to their size and they influence the soil in different ways as depicted in the Table1 (Dash, 2012).

Table 1: Role of different groups of soil biota

Micro flora	Mineralization and immobilization of nutrients in aggregates in soil	Catabolism of complex organic matter into nutrients that bind to aggregates The hyphe entangles particles into aggregates
Microfauna (≤ 100μm)	Regulation of microbial population after nutrient turnover	Affect the aggregates through interactions with microflora
Mesofauna (≤ 2mm)	Regulation of microbial population after nutrient turnover and fragment plant residues	Production of fecal pellets, creation of biopores and promotion of humificational pellets
Megafauna (> 2mm)	Fragmentation of plant residues, stimulation of microbial activity and distribution of of organic matter and microbes	Mixing of organic and mineral particles, creation of biopores and production of fecal pellets and promotion of humification

The Earthworms

Earthworms, the "Goldenplough of our agricultural history" arethe terrestrial oligocheates. Which are also called as "intestines of the earth". They help in breakdown of dead plants and animal material in soil and plant litter, and thus in recycling of nutrients. The horizontal and vertical tunnels made by them helps in oxygen and water circulation in soil. Earthworms make significant contribution to improve soil fertility and air porosity. Their castings are rich in nitrogen, phosphorus, potassium and other nutrients; this helps soil to make it more fertile and healthy (Lakhani, 2013).

They are natural invertebrates of agroecosystem belonging to the family lumbricidae and dominant in the temperate and tropical soils. They are hermaphrodites, both male and female reproductive organs are present in every single earthworm but self-fertilization does not generally occur. At the time of laying eggs, the sexually mature worms have a distinctive epidermal ring shaped area called, the clitellum, which has gland cells that secrete material to form a viscid, girdle like structure known as cocoon.(Sharma et al., 2005).

Earthworms also have the power to regenerate segments, which are lost (Sharma et al.,2005).They represents a significant proportion of the soil biomass and hence makes an important contribution to the decomposition of organic matter, cycling of nutrients and pedogenesis (Sizmuret al.,2011).

The earthworms in play important role in paedogenesis is through mixing of the particles during its movement through its gut, depositing their casts throughout the soil column, and also improves aeration and drainage of the agricultural soils (Kavithaet al.,2011). Earthworms are also important contributors to the recycling of carbon and nitrogen in the ecosystem, so, they are used as bioindicators (Callahan,1988).

They are involved in decomposition of litter, intermixing of inorganic and organic matter, enhancement of soil microbial activity, increased nutrient availability, formation of macroporosity and formation and stabilization of soil aggregates (Edwards and Bohlen, 1996).

Due to their feeding and burrowing behavior they directly or indirectly help in improving physical, chemical and biological process of the soil. Earthworms participate in the mixing of organic and inorganic fractions of soil, formation of stable clusters, dynamic and recycling of nutrients from the decomposition of organic matter, their burrows help to the aeration, infiltration drainage of soil (Ouelletet al.,2008).

Classification of earthworms

Earthworms can be classified into three ecological groups according to their life history strategies, epigeics, anecics and endogeics (Dash, 2012). Epigeics are found in the litter layer, dorsolaterally and ventrally pigmented and feed on litter and coarse particulate matter. Anecics are found in soil, dorsally pigmented, feed on litter, humus and soil and creates vertical burrows in soil profiles. Endogeics are found in soil, unpigmented and feed on soil containing organic matter and burrow both horizontal and vertical tunnels. Contribution of epigeics to soil fertility includes fragmentation of litter, ingestion of soil biota and their dispersal, mucus production. Anecics help in fragmentation of litter, transportation, mucus production, macrostructure formation, ingestion and dispersal of soil biota, population control of some soil biota, and mineralization. Endogeics help in mucus production, macrostructure formation and immobilization of nutrients and moisture.

Lampitomauritii (**Kinberg)**

Fig 1: ***Lampitomauritii (***Kinberg)

Lampitomauritti (Kinberg) is an anecic earthworm with as grassland, crop field, forest litter and soil, garden soil, domestic garbage, compost heap/pit, and sewage as its habitat. The species was first reported from Mauritius. Its origin was in India (Palani/Cardamom Hills in Western Ghats), widely distributed in India.It feeds on decaying plant material and soil micro-fungi. It deposits casts on soil surface in the formof stable aggregates.

It can tolerate temperature upto 32°C in field but its favourable temperature is about 25^{-}26°C with soil moisture 10-20 g% for favourable growth and reproduction.Its number doubles in 35-40 days, rate of coccon production is 12-45 and hatching success is about 60% depending on soil temperature and moisture content laying coccons for 12-20 months(Julka and Senapati, 1987).

It has greyish to brownish body colour; many setae are present per segment; first dorsal pore in 10/11-11/12 intersegmental furrow; clitellum covers 14-17th segment; male pores on slightly raised areas of 18th segment; 3 pairs of spermathecal pores in intersegmental furrows of 6/7/8/9 segments; paired female pores on 14th segment; length 95-155 mm; body live weight usually 1 g.

Pesticides

The term pesticide encompasses a variety of chemicals including insecticides, herbicides, and rodenticides (Frazer 2000).Agrochemicals such as pesticides alter soilbiological and chemical properties, and these could beused as markers of soil healthwith the earthworms being acommon and popular organismal model (Xiao et al., 2006).

Pesticdes are classified depending upon target species to their mode of action. According to application the main classes of pesticides includes herbicides (that kill weeds), fungicides (that kills fungi) and insecticides (that kills insects) and depending upon chemical nature the classes includes organochlorines, organophosphosphates, thiocarbamates, pyrethroids and neonicotinoids.

An organochlorine, chlorinated hydrocarbon or chlorinated solventis an organic compound containing at leastone covalently bonded chlorine atom. Some examples include alpha-BHC, beta-BHC, gamma-BHC (lindane), heptachlor, endosulfan and dichlorodiphenyltrichloroethane (DDT).

Organophosphosphates bind to acetylcholinesterase and other cholinesterases resulting in disruption of nerve impulses killing the insect or interfering with its ability to carry on normal functions. Examples include acephate, chloropyriphos, diazonion, fenthion, monochrotophos and malathion.

Carbamates are closely related to the organophosphorus compounds in mode of action and resistance development. Bendiocarb, carbofuran, carbaryl, dioxacarb, fenobucarb, fenoxycarb, isoprocarb and methomyl are some of the examples of carbamate insecticides.

The chemical compound, pyrethroid pesticides, has been developed to mimic the insecticidal activity of the natural compound pyrethrum. These are nonpersistent, which is a sodium channel modulators, and are much less acutely toxic than organophosphates andcarbamates. Cypermethrin, cyfluthrin, deltamethrin, etofenprox, fenvalerate, permethrin, phenothrin, prallethrin, resmethrin and tetramethrin are some of the pyrethroids.

Neonicotinoids are synthetic analogues of the natural insecticide nicotine (with a much lower acute mammalian toxicity and greater field persistence). These chemicals are nicotinic acetylcholine receptor agonists. These are broad spectrum systemic insecticides often used as substitutes for organophosphates and carbamates. Acetamiprid, clothianidin, imidaclopridthiacloprid and thiamethoxam are some of neonicotiniods.

Chloropyriphos

Chloropyriphosbelonging to organophosphate is one of the most widely used pesticides in the Western Odisha. Chloropyriphos is acutely toxic to bees, birds, mammals, aquatic life, and certain species of algae, andis registered for the control of cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, mosquitoes, and lice. It is used as an insecticide on grain, cotton, fruit, nut, and vegetable crops. Its IUPAC name is *O*,*O*-diethyl *O*-3,5,6-trichloro-2pyridinyl- phosphorothioate.

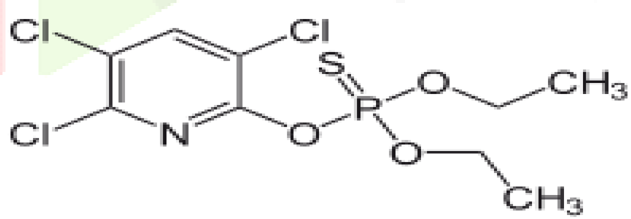

Fig 2: **Structure of Chloropyriphos**

Generally farmers use pesticidesin agricultural field to increase the crop productivity but it also have a negative impact on soil fauna especially to the earthworms. So the present work aims to assess the effects of organophosphate on anecic earthworm (*Lampitomauritii*) as organophosphates like chloropyriphos are most widely used in Western Odisha.

Materials and methods

Chloropyriphos as pesticide

Chloropyriphos is a crystalline organophosphate insecticide acts on the central nervous system of insects by inhibiting acetyl cholinesterase activity.

The certified dose of organophosphate (Chloropyriphos) application is 2ml diluted in 1 litre per acre. Active ingredient (AI) is about 20% and trade name as Cheminova Classic 20 % EC.

The lethalitytests were conducted from concentrations starting from 400 ppm, 200 ppm, 100 ppm, 50 ppm, 25 ppm, 20 ppm. As the worms survived for more than a day in the pesticide concentration of 20 ppm, the microcosm experiments have been conducted with chloropyriphos solution containing 0,5,10 and 20 ppm as active ingredient (AI).Experiments beyond 20 ppm were not conducted as instant lethality was observed when worms were exposed to it.

Earthworms Used

Systematic position of *Lampitomauritii*(Kinberg) is as follows:

Phylum	Annelida
Class	Clitellata
Order	Oligochaeta
Family	Megascolecidae
Genus	*Lampito*, Kinberg
Species	*mauritii*, Kinberg

For soil metabolism and feeding adult earthworms were used. For rest of the study, immature *Lampitomauritii* has been utilized.

Growth

The biomass of earthworms reflects the physiological requirements of tissues. The population changes of earthworms in field do not represent the true effect of the pesticides (Thompson, 1970).

In the present study the change in the biomass of the earthworms i.e., change in weight of earthworms at different concentrations of chloropyriphos i.e., 0 ppm, 5ppm, 10 ppm and 20 ppm has been studied.

500g (300 g soil and 200 g cow dung) 2mm sieved, air dried soil was taken in polythene packets with moisture maintained at 20 ± 2 g% by addition of distilled water in control sets i.e. '0' ppm and respective chloropyriphos solution in experimental sets i.e. 5, 10 and 20 ppm. 10 replicates of each concentration were taken. To each packet worms about 1.5 g were inoculated after 5 days of moisture addition during which microbial activation of the soil was done with temperature maintained at 25 ± 2˚c. Similar preparation of soil packets were done for all the experiments.

The change in the weight over initial weight was observed after 10, 20 and 30 days and percentage change in weight of earthworms over zero day culture was estimated. Statistical analysis, ANOVA was performed to infer the results.

Feeding (Stable Aggregate Formation)

Anecic and Endogeic earthworms conserve nutrients by formation of stable aggregates. Formation of stable aggregates is directly proportional to feeding. So estimation of the stable aggregate formation also indicates feeding by the earthworms.

Formation of stable aggregates is governed by percentage of clay, amount of organic matter in soil, bacterial mucilage, fungal mycelia and colloid contribution of macrofauna (Lavelle, 1984).

The earthworms were inoculated in zero day and estimation of stable aggregates was done at an interval of 10 days. After 10 days culture sets were sieved with water. The stable aggregates formed were collected, air dried and weighed. Carbon content of aggregate formed was estimated by Walkley and Black (1934) titration method. From the amount of carbon energy conversion has been calculated.(According to available standard values energy conversion for carbon is 41.44 kJ,g^{-1},dry wt. (Remmert, 1980).

Regeneration

The guts of the immature worms were cleaned and about 50% of post clitellar region was cut with sharp blade. 10 worms were inoculated into each culture packet. 10 replicates ofeach culture packet for each concentration of chloropyriphos were taken. One of the ten replicates was sampled on 10^{th}, 20^{th} and 30^{th} day for the estimation of number of regenerated segments. The worms sampled were preserved in 5% formalin.

Soil Metabolism

Respiratory metabolism was quantified by Alkali absorption method (Witkamp, 1966). Carbon dioxide evolution was measured at $25 \pm 2^{\circ}$c and expressed as mg of CO_2, g^{-1} live worm tissue, hr^{-1}, kg^{-1} soil.

The respiratory metabolism was maintained at 0, 10, 20 and 30 days. Statistical analysis for all the parameters were done by ANOVA test (Gupta, 1980).

Results

Growth

Fiig.3 indicates growth, change in weight of *Lampitomauritii* earthworm and Fig.4 represents percentage change in weight of earthworms under the impact of chloropyriphos in laboratory culture.

After 10 days there was a positive increase of about 5.97% in weight at ‘0’ ppm exposure to chloropyriphos. But on exposure to 5, 10 and 20 ppm chloropyriphosdecrease of about 1.17, 2.37 and 2.94% over zero day was observed, respectively. By 30^{th} day there was 29.12% increase in weight over zero day in control but decrease of 45.25% in sets with 20ppm chloropyriphos. Two way ANOVA indicated significant decrease in weight on exposure to chloropyriphos (0.05 level of significance).

Feeding (Stable Aggregate Formation)

After 10 days of exposure to chloropyriphos at 0, 5, 10 and 20ppm the weight of stable aggregates formed was 12.82, 8.42, 6.40 and 6.19 g dry wt. of stable aggregates, g^{-1} live tissue.

There was decrease of about 34, 50 and52% in stable aggregate formation at 5, 10 and 20ppm respectively as compared to 0ppm chloropyriphos (Fig. 5). By 30 days there was decrease of about 34.36, 60.28 and 66.05% over 0ppm chloropyriphos on exposure to 5, 10 and 20ppm respectively. Analysis of varianceshowedsignificant impact of chloropyriphos on the stable aggregate formation by the worms at 0.05level of significance.Significant change in energy content of stable aggregates (Fig.6) was also found at 0.05 level of significance.

Regeneration

After 10 days of exposure to 0, 5, 10 and 20 ppm chloropyriphos, the increase in regenerated segments was 8.6, 6.6, 4.2 and 2.6 respectively (Fig.7). There was a significant (at 0.05 level of significance)decrease of about 23.25, 51.16 and 69.76% at 5, 10 and 20 ppm chloropyriphos exposure as compared to 0ppm chloropyriphos. After 30 days of culture there was a decrease of about 15.06, 37.95 and 55.4 % (Fig.8) on exposure to 5, 10 and 20 ppm chloropyriphos as compared to 0 ppm exposure.

Respiration

The rate of respiration increased with duration and treatment (Fig.9). After 10 days of exposure the rate of respiration was found to be 3.69, 5.32, 6.86 and 7.83 mg of CO_2 evolved, g^{-1} live worm tissue, hr^{-1}, kg^{-1} soil at 0, 5, 10 and 20 ppm of chloropyriphos respectively. At 30 days of exposure there was increase of about 14%, 42% and 55% in rate of respiration on exposure to 5, 10 and 20 ppm chloropyriphos over 0 ppm chloropyriphosThe mg of CO_2 i.e. evolved, g^{-1} live tissue, hr^{-1}, kg^{-1} soil increased from lower concentrations to higher concentrations of chloropyriphos as the days increased. Analysis of variance showed significant impact of chloropyriphos treatment on respiratory metabolism of earthworm at 0.05 level of significance.

Discussion

Growth

The biomass is a good indicator of the physiological requirements of tissues and material cycling (Thompson, 1970). In the present study significant reduction in biomass and length of the earthworm was found on exposure to sub-lethal dose of chloropyriphos.

Zhou *et al*., (2007) assessed and found chloropyriphos had adverse effect on growth in earthworm exposed to 5 mg/kg chloropyriphos for eight weeks. Weight of earthworms was a more sensitive index compared to the mortality in indicating toxic effects of acetochlor and methamidophos. Reduction in weight of earthworms was also observed on exposure to endosulfan and femaniphos (Choo and Baker, 1998), diazinon (Booth et al., 2000), aldicarb, cypermethrin, profenofos (Moslehet al., 2003) dichlorovos (Farukh and Ali, 2011), carbendazim, glyphosate,dimethoate (Yasmin and D'Souza, 2007) and cartap hydrochloride (Panda and Patnaik, 2021).

Decrease and retardation in Growth rate might be decreased due to enhancement in respiration as under stress most of the energy is channelizedtowards maintenance cost i.e. respiration (Senapati et al., 1992). Biomass reduction is correlated to decrease in feeding habits of the earthworms under the influence of pesticides (Khalil, 2013). Weight loss of affected worms might be due to the channelization of energy for detoxification and repairement of the body to avoid toxicity aalso for their survivability (Farrukh and Ali, 2011).

Feeding (Stable Aggregates Formation)

Earthworms feed on pre-decomposed or undecomposed organic matter according to their ecological category.Bossuyt*et. al*., (2004)found earthworms to have a dramatic effect on aggregate size distribution,greatly increasing the macroaggregate fraction and thus overall C incorporation into macroaggregates

The feeding habit results in physical aggregation of the soil. Earthworm activity creates structures, casts and galleries which modify the circulation and accumulation of water (Tisdall and Oedes, 1982). The feeding and behaviour of the worms depends on the stability of the casts structure formed by themselves (Guild, 1955). They eject a significant amount of nutrients in their casts which is due to increase microbial activity in their gut and from their own metabolic activity.

Soil aggregation influences root respiration and soil gas exchange. Reduction in surface casting, abundance of earthworms, biomass and increased litter accumulation when benomyl and related fungicides are sprayed (Wright, 1977).

The pollutants including the pesticides affect their ingestion by earthworms. Wang *etal.*, (2022) found that earthworms when grown in soil containing microplastics lead to weight loss (Zhou *et al.*, 2020; Ding *et al.*, 2021; Cui *et al.*, 2022).

Decline in stable aggregate formation by *Lampitomauritii* earthworm on application of different doses of chloropyriphos and thereby interfere in positive contribution of earthworms.

Regeneration

Regeneration of lost parts is proportional to growth (Stephenson, 1930). Kulkarni and Wakale (2012) studied thatendosulfan dose showed regeneration after 30 days and suggested as a response to the toxic effects of endosulfan.Regeneration is hampered on removal or damage of nerve cord (Zhinkin, 1936)

In the present study regeneration rate has been significantly reduced by application of chloropyriphos at sub-lethal level.

Respiration

Energy loss of an organism is represented by respiratory metabolism. Most of the organism's energy is utilized in respiration. Soil respiration is being used for estimation of biological activity (Lundergarh, 1927). Earthworms on exposure to carbaryl and endosulfan showed an increased value of CO_2 evolution.Temperature stress results in 3 fold increase in the oxygen consumption in summer as compared to winter have been reported by Senapati and Dash (1983). Increase in muscular activity leads to increase in respiratory metabolism. Higher muscular activities and energy utilization are also to required to overcome oxidative impairment as found in fish, *Labeorohita* under the impact of cypermethrin (Marigoudar et al. 2009).

The results of present study also coincides with study of Dasgupta et al. (2012) who found that CO_2 evolution increased in *P. excavates* under the influence of pesticides carbaryl, chlorpyrifos and endosulfan and it might be due to metabolic stress and nickel (Patnaik and Behera, 2013). In order avoid stress energy is utilised in muscular activity and result of which CO_2 is released (Blagodatsky and Smith, 2012; Lubbers et al., 2015).

It is used as an important parameter for the organism. In the present study enhancement in the respiratory metabolismhas been observed which results in the loss of more energy utilised by the earthworms

Conclusion

Chloropyriphos being an organophosphate is most widely used by farmers of westernOdisha in crop fields with certified dose of 2 ml l^{-1}, having active ingredient about 20%. When the worms are exposed to lethal dose the metabolic functions of the body ceases but at sub-lethal dose the earthworms remains in the ecosystem but it does not contribute its positive role towards the system as a result of exposure to toxic substances. The soil metabolism increases as most of the energy is directed towards respiration while all other parameters like growth, feeding and regeneration decreases.

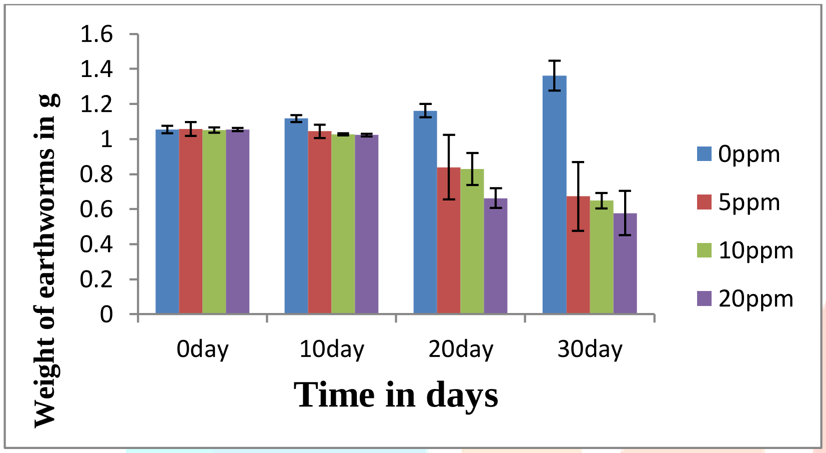

Fig 3: Change in weight of earthworm *lampitomauritii* under the impact of Chloropyriphos in laboratory culture.

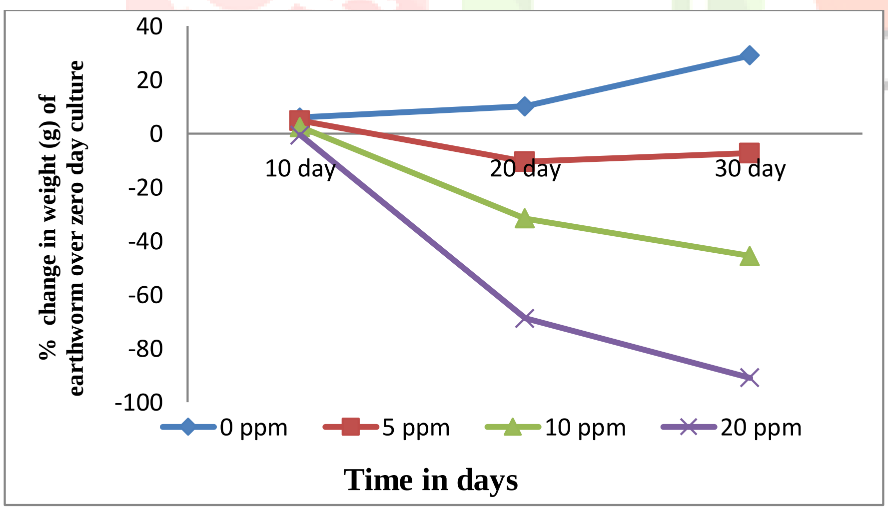

Fig 4: Percentage change in weight of earthworm *lampitomauritii* under the impact of Chloropyriphos in laboratory culture

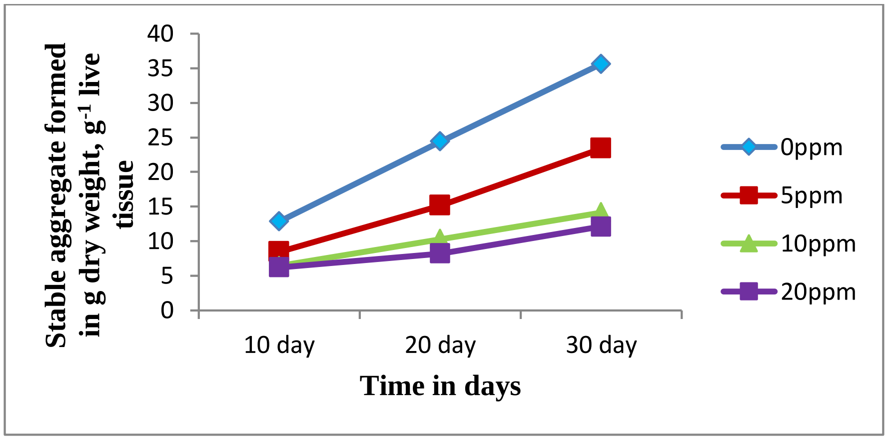

Fig 5: Stable aggregate formed by earthworm *Lampitomauritii* earthworm under the impact of Chloropyriphos in laboratory culture

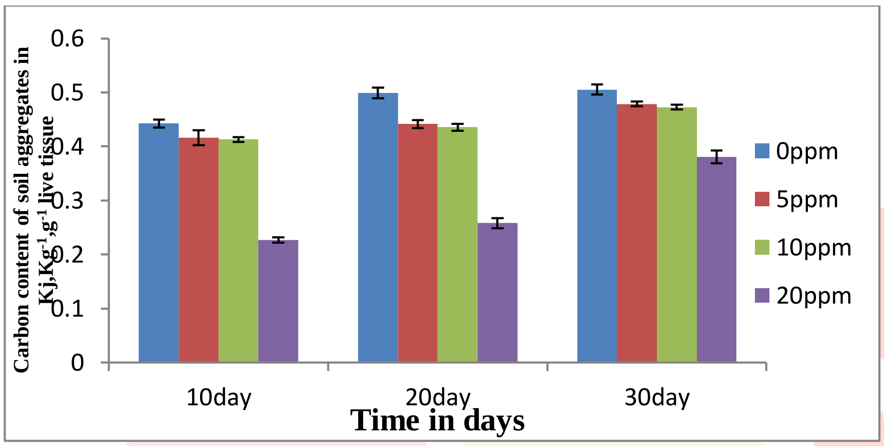

Fig 6: Carbon content of soil aggregates formed by earthworm *lampitomauritii* under the impact of Chloropyriphos in laboratory culture

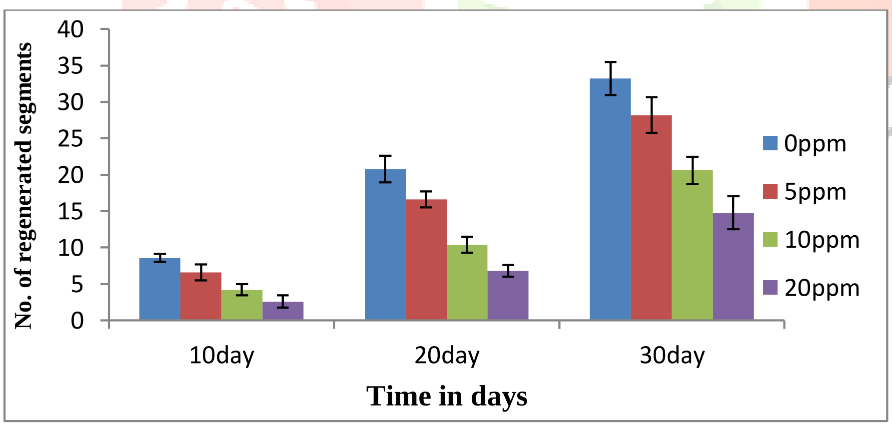

Fig 7: Regeneration of *Lampitomauritii* earthworm under the impact ofChloropyriphos in laboratory culture.

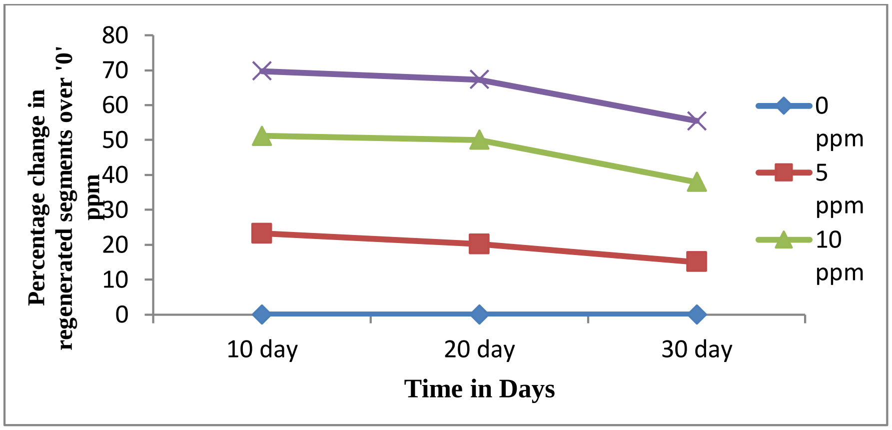

Fig 8: Percentage change in Regeneration of *Lampitomauritii* earthworm under the impact of Chloropyriphos over '0' ppm in laboratory culture

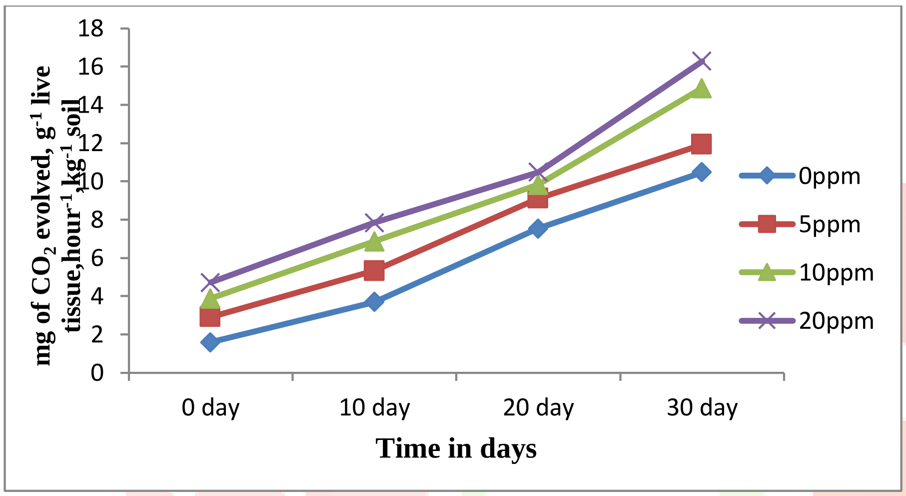

Fig 9: Respiration of *Lampitomauritii* earthworm under the impact of Chloropyriphosin laboratory culture

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