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STUDIES ON THE EFFECT OF CHLORPYRIFOS (ORGANOPHOSPHATE) AND CYPERMETHRIN (SYNTHETIC PYRETHROID) ON THE GROWTH OF *Paramecium*

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ABSTRACT
Interest in the toxicity of pesticides has increased as they enter waterways from agricultural and urban runoffs and may end up in aquatic environment and bioaccumulate in the food chain. The aim of this study was to determine the effect of two insecticides; Chlorpyrifos and Cypermethrin on aquatic protozoa (Paramecium). The test was carried out in laboratory, where Monoxenic culture of Paramecium was used to test the effect of Chlorpyrifos and Cypermethrin at 50, 25, 12.5, 6.25, 3.125, 1 respectively, at time interval of 24, 48, and 72 hours. The effect of Chlorpyrifos on Paramecium growth showed significant different with P value 0.0228, while for Cypermethrin, there was no significant difference with P value of 0.1333. The damage was more at 24hrs interval, as the number of population growth increased with time. Hence, the effect of the insecticides reduced. Conclusively, both insecticides showed harmful effect to Paramecium and may disturb the aquatic ecosystem.
Keywords: Chlorpyrifos, Cypermethrin, *Paramecium*, Monoxenic culture, Toxicity.

INTRODUCTION

The use of pesticides has increased rapidly since World War II, and recently studies have shown that pesticide residues frequently occur in surface water in agricultural areas (Ulen *et al., 2002)*.Hence, nowadays agricultural practice uses considerable pesticides to control weeds, insects and fungal pathogens. These pesticides often enter surrounding water through run offs, soil erosion and aerial deposition (Downing *et al.*, 2004).

Interest in the toxicity of pesticides such as organophosphate compounds has increase in the recent time as they enter waterways from agriculture and urban runoffs and may end up in aquatic environments and bioaccumulation in the food chain (Amanchi and Hussain, 2010). Thus pesticides contribute greatly to freshwater environmental pollution, potentially causing harm to a large variety of non-target organism (Amanchi and Hussain, 2007). Also, many aquatic species are taxonomically related to the target organism of pesticides, thus, aquatic ecosystems in agricultural areas are at risk of being negatively affected by these chemicals (Wendt-Rasch, 2003), though when introduced to aquatic system, cause significant toxicological risk, such as genotoxicity, neurotoxicity and mammalian gonadal toxicity (Shakoori *et al.*, 2008), to the resident organism (Scott *et al.*,1990).

Microorganisms are of vital importance as their assemblages (bacteria, protozoa, algae and fungi) serve critical roles in aquatic environment, like nutrient cycling, primary productivity and decomposition. If pesticides or other agents change this microbial interaction they may ultimately affect ecosystem functioning (Downing *et al.*, 2004).

According to Larsen, (1997) experiment with ciliates demonstrate a clear inhibitory effect for different pesticides, because they are unicellular, sensitive, and can be in very close contact to environment, thus respond rapidly to any unfavourable stress (Amanchi, 2010). Hence, they react quickly to limnological change of aquatic environment therefore can be used as pollution indicators (Srivastava, 2013). They have many advantages such as high reproductive rate, easy to culture and accessibility of experimental manipulation which render them as test organism for laboratory tests (Weisse, 2006). As a result *Paramecium spp.* was used in this experiment to test the effect of two insecticides; Chlopyrifos and Cypermethrin.

The aim of this research was to study the effect of two insecticides; chlorpyrifus and cypermethrin on aquatic organisms, and the objectives were; to observe the harmful effects of the insecticides; Chlorpyrifos and Cypermethrin on aquatic protozoa, to observe the dosage dependent effect of these two insecticides that is capable of killing aquatic protozoa and to find the time taken for these insecticides to kill the organisms.

MATERIALS AND METHODS

Monoxenic Culture of *Paramecium*:

Paramecium was selected as test specie for present the study because of its ease to culture and maintainance in the laboratory. Monoxenic culture of *Paramecium* was prepared according to Fraga(2001). It was prepared by boiling 5 grams of wheat grass in 1L of distilled water, cooled and filtered. Stigmasterol solution was prepared by adding 500mg of stigmasterol into 100ml of 100% ethanol while wheat grass buffer was prepared by mixing 7.8g TrisBase, 5.6g Na2HPO4, 2.1g $NaHPO_4$ and 0.7g Na_2EDTA into distilled water and autoclaved.1ml of stigmasterol and5ml of wheat buffer was added to make the wheat media. *Enterobacter* was cultured on EMB agar media and a single colony was inoculated in the wheat grass media and incubated at 37°C overnight. Next day, 10 ml of pond water was added into the wheat grassmedia and maintained at 28 ± 1^0C for 5 days. Subsequently, subculture was prepared after every 5 days to ensure maintenance of *Paramecium*. *Paramecium* growth was observed by placing 10µl of *Paramecium* culture on a slide and viewed under compound microscope.

Dosage Preparation:

Chlorpyrifos 20% EC (Organophosphate Insecticide) while Cypermethrin 25%EC (Synthetic Pyrethroid) insecticides were used, manufactured by SWASTIK Pesticide Limited. Recommended dose for Chlorpyrifos is 2L/hec (Prasad, 2010) and for Cypermethrin, it is80ml/200L per hectare (India Mart, 2014) on wheat grass. The Stock Solution and working concentration were calculated according to the area of the trough used, which is 531.14 cm^2. Working concentrations were prepared as 50, 25,12.5, 6.2, 3.125, 1% respectively (Palma *et al.*, 2008). Of each working concentration; three troughs were used as triplicate, and one control was used for each series of insecticide dose.

Bioassay

In each trough 10ml of *Paramecium* culture was added, the working concentrations were then added in triplicate accordingly, while the control trough contain the *Paramecium* culture only. *Paramecium* growth was observed and then calculated at 24, 48 and 72 hours interval, using compound microscope. And counting was done as per the guide of www.hope.edu/academic/biology/meciums/paranumb.htm.

RESULTS AND DISCUSSION

Table 1 shows the effect of Chlorpyrifos insecticide on *Paramecium* growth, the result indicate that the test organism survived at 1% and 3.125%, while in the rest of the concentrations no survival were recorded after 24hrs of application. The result of Chlorpyrifos is in agreement with the research conducted by Lin *et al*. (2012) where they studied the acute and chronic toxicity to *Paramecium* which revealed remarkable effect on growth of *Paramecium* and the higher the concentration, the greater the effect, similarly, in this test the growth of *Paramecium* reduced distinctively with increase in the dose of Chlorpyrifos. Consequently, work done by DeLorenzo *et al*. (1999) also indicates its effect. But at lower doses, the population recovers with time and start to increase slowly, this may be due to its ability to hydrolyse and volatilize in water (U.S.EPA, 2006).

However, with Cypermethrin, the effect registered showed that all organisms (*Paramecium*) survived all the concentrations used against them as shown in Table 2. Hence it indicates little or no effect at all. Likewise, the result of Cypermethrin shows little effect on growth of paramecium which is similar to the work of Larsen (1997), where he concluded that, Cypermethrin has no effect on growth of protozoan compared with the control cultures. Work done by Hill (2006) also indicate minor effect on some aquatic species and no effect on microorganisms, thus, he concluded that, the effects are mostly transient and are unlikely to cause any adverse changes in the population or productivity. However certain researches done by scientists like, Saha and Kaviraj (2008) showed high toxic effect of pyrethroids on aquatic species irrespective of taxa.

Figure 1 indicates the comparison between the insecticides Chlorpyrifos and Cypermethrin on the test organism *Paramecium*. And it clearly shows that Chlorpyrifos have more effect on growth of *Paramecium,* as the number of growth is higher in Cypermethrin.

From the comparative study above, it was found that Cypermethrin was much more safe than Chlorpyrifos, wherein the number of organisms were more with Cypermethrin than Chlorpyrifos at all-time interval. The damage was more at 24 hour time interval, later it reduced its effect which was shown by increased in the number of organisms. However for Chlorpyrifos at all-time interval, doses up to 6.25% the number of paramecia were nearly zero, stating its harmful effect.

Table 1: Effect of different concentrations (%) Chlorpyrifos (Organophosphate) on the growth of *Paramecium*.

		Time (Hours)/ No. of Paramecium				
Concentration (%)	Initial no. of Paramecium	24	48	72	Mean	Std. Error
Control	11000	160000	280000	400000	280000	69282
RD	9000	0	0	0	0	0
50	10000	0	0	0	0	0
25	12000	0	0	0	0	0
12.5	10000	0	0	0	0	0
6.25	10000	0	0	0	0	0
3.125	11000	800000	120000	240000	386667	209550
1	9000	120000	160000	360000	213333	74237

P = 0.0228(p < 0.05 i.e Significant difference between means) using repeated measures ANOVA

Table 2: Effect of different concentrations (%) of Cypermethrin (Synthetic Pyrethroid) on the growth of*Paramecium*.

		Time (Hours)/ No. of Paramecium				
Concentration (%)	Initial no. of Paramecium	**24**	**48**	**72**	Mean	Std. Error
Control	12000	200000	200000	280000	226667	26667
RD	10000	80000	200000	240000	173333	48074
50	10000	40000	160000	160000	120000	40000
25	11000	40000	200000	280000	173333	70553
12.5	9000	120000	200000	200000	173333	26667
6.25	11000	160000	200000	280000	213333	35277
3.125	10000	120000	280000	200000	200000	46188
1	10000	80000	120000	20000	73333	29059

P = 0.0435 (p < 0.05 i.e Significant difference between means) using repeated measures ANOVA

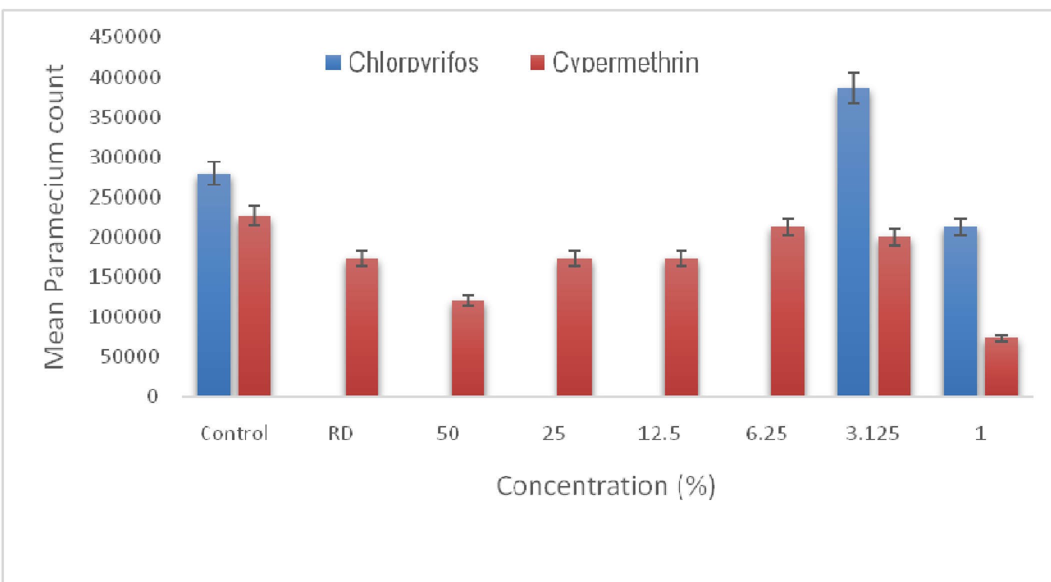

Fig. 1: Relationship between Chlorpyrifos and Cypermethrin.

CONCLUSION

Paramecium is a responsive bioindicator of environmental stress, thus, serve as experimental model to eukaryotes for biomonitoring studies and assessment of pesticides toxicity. Chlorpyrifos was found highly toxic to *Paramecium* than Cypermethrin in this study. And Chlorpyrifos may have a real ecological impact on aquatic ecosystem. Thus, in conclusion, the study proves that both Cypermethrin and Chlorpyrifos are harmful to aquatic organisms thereby may disturbs the ecological system; however Chlorpyrifos is much more potent in decreasing food web. This indicates a need for performing chronic studies

Recommendations

Government should pass policies that will stop the use of toxic pesticides and outline guidelines to land management that are safe for the environment and public health.

Further studies are needed to better understand the processes that influence the susceptibility of aquatic organisms to insecticides.

Pesticides bioassays require standardization

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