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Reprotoxic Consequences of Bifethrin and Dichlorvos pesticides on fecundity of *Drosophila melanogaster*

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ABSTRACT

The present exploration deals with the repercussion of pyrethroid insecticide, Bifenthrin and organophosphate insecticide, Dichlorvos, on reproductive potential of *Drosophila melanogaster,* exposed to LC_{20}. Exposure of selected concentration was carried out by adding aliquots of selected concentration of insecticides, in culture medium and exposing second instar larvae to insecticide for 24 hrs. Subsequently, the second instar larvae of *Drosophila,* were then reared up to imago stage and thereafter, were cross-mated in different experimental sets , which includes, first set: insecticide treated male and normal female, whereas, second set: insecticide treated female and normal male, along with respective controls. The procured data, specifying fecundity of treated groups, for two different experimental sets, was compared with the natural population. It had been scrutinized that insecticides exposure, induced, a drastic reduction in fecundity of treated flies as compare to control groups. Furthermore, it had been analyzed that selected insecticide affected more predominantly, to male flies, as revealed from procure data. Subsequently, obtained results from different experimental tests were analysed by Z-test, which indicated statistically significant induction of reprotoxic consequences on fruit flies ($p<0.05$).

Key Words: Reprotoxicity evaluation, Bifenthrin, Dichlorvos, *Drosophila melanogaster*.

Introduction

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Pesticide applications are quite beneficial, in preventing various diseases like malaria, west nile virus, filariasis, tularaemia, yellow fever, dengue, dirofilariasis, zika fever, eastern equine encephalitis, chikungunya etc. and furthermore pesticide applications have escalated agricultural productivity by preventing food loss. But simultaneously excessive utilization of pesticides is associated with various health problems including skin, blindness, blisters ,nausea and death, various chronic consequences including disruption of endocrine system, suppression of the immune system, a high risk of leukemia , brain , kidney, breast, liver , lung and skin cancers enhanced occurrence of chromosomal aberrations , sister chromatid exchange, micronuclei formation and Comet cells cancer of pancreas, stomach, hepatic, bladder, and gall bladder non-Hodgkin's lymphoma multiple myeloma, soft tissue sarcoma, and lung sarcoma, Parkison's disease and reproductive influences [1-15]. Therefore the genotoxic and mutagenic evaluation of pesticides, has become crucial before their administration in the agricultural fields. For present research exploration two insecticides *viz*: including Bifenthrin and Dichlorvos, has been selected on the basic of excessive application in Jalandhar district. Bifenthrin, a pyrethroid insecticide, is obtained from *Chrysanthemum* flowers. Bifenthrin are used to control to aphids, worms, moths, beetles, mites, spiders, maggots, flies and fleas. Bifenthrin, is a neurotoxic pyrethroid that possess affinity to the voltage-gated sodium channels and causing the membrane to be permanently depolarized, ultimately resulting in paralysis. Dichlorvos, organophosphate insecticide, used for effective control against various caterpillars, thrips, aphids, spider mites and white flies in greenhouse, outdoor fruit and vegetable crops. Dichlorvos are known to be neurotoxic, basically inhibits the enzyme acetylcholinesterase resulting in various effects such as perspiration, nausea, vomiting, diarrhea, drowsiness and headache.

Present research exploration deals with assessment of toxic consequences of selected insecticides on fruit fly. Therefore, *Drosophila* larvae had been exposed to selected

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insecticides, and were reared upto adult stage, thereafter different experiment trial had been set , including first set of insecticide exposed male and normal female, whereas, second set comprise insecticide exposed female and normal male, along with respective controls. Subsequently, effects of selected synthetic agro-formulation were assessed by counting number of eggs laid down in different groups in comparison to natural population. The procured data, specifying fecundity of treated groups, for two different experimental sets, was compared with the natural population. Furthermore, procured data had been analysis by Z-test and it had been observed that specific chemical induced statistically significant consequences on reproduction.

Material And Methods

Insecticide: Bifenthrin is a pyrethroid insecticide, IUPAC name 2-Methyl-3-phenylphenyl) methyl (1*S*,3*S*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]- 2,2-dimethylcyclopropane-1-carboxylate, molecular formula $C_{23}H_{22}ClF_3O_2$. Dichlorvos is an organophosphate insecticide with IUPAC name 2, 2-dichlorovinyl dimethyl phosphate, molecular formula $C_4H_7Cl_2O_4P$. LC_{20} of selected insecticides was assessed on the basis of mortality of second instar larvae, exposed to serial dilution of selected insecticides for 24 hours, which corresponds to value 2.00 pl/ml for Bifenthrin and 1.99 pl/ml for Dichlorvos [Figure 1 and Figure 2].

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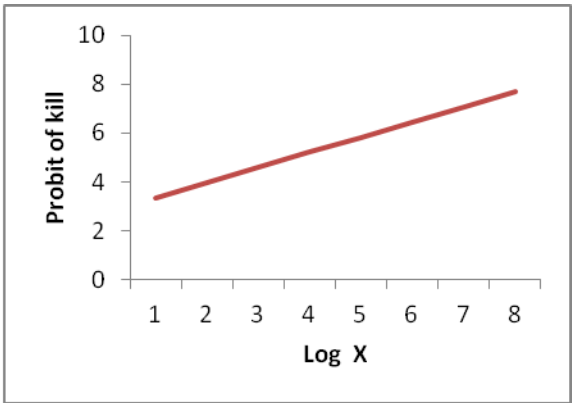

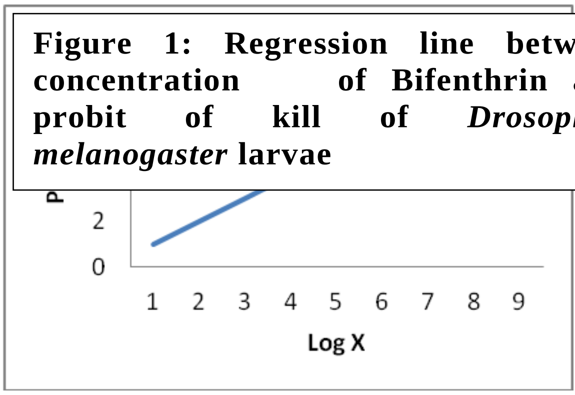

Figure 1: Regression line between concentration of Bifenthrin and probit of kill of *Drosophila melanogaster* larvae

Figure 2: Regression line between concentration of Dichlorvos and probit of kill of *Drosophila melanogaster* larvae

Test Organism: *Drosophila melanogaster* [2n=8] a holometabolous Dipteran insect, had been used as a test model to execute present research work, due to specific characteristics of the concerned test animals including more adaptability to laboratory conditions, short life span and high fecundity rate. For all experimental set up, primary stock of *Drosophila melanogaster*, Oregon strain was obtained from *Drosophil*a Laboratory, Department of Biotechnology, Punjab Agriculture University, Ludhiana, which had been incubated in BOD, set at 25+1 ^{0}C with relative humidity 40%-60% . The culture medium for rearing was prepared by mixing agar, yeast, maize powder and brown sugar in cultural vials.

LC_{20} standardization of selected insecticides and exposure to test organism: exact LC_{20} was considered from research exploration, carried out earlier in laboratory, which correspond to values which corresponds to value 2.00 pl/ml for Bifenthrin and 1.99

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pl/ml for Dichlorvos using *Drosophila melanogaster* and in earlier executed studies, for concentration standardization purpose, mortality of second instar larvae which had been exposed to serial dilution concentrations of 1% stock solution of Bifenthrin and Dichlorvos for 24 hours, was observed. Required concentration of serial dilution was prepared by adding aliquots of the stock solutions in culture medium. For each particular dose, three replicates of twenty larvae were kept simultaneously, with respective controls under controlled conditions of laboratory. In each experimental set, mortality of exposed larvae in treated group and in control groups was monitored after 24 hours. Exact value of LC20 was calculated, on the basis of observed mortality of larvae in each set by probit analysis.

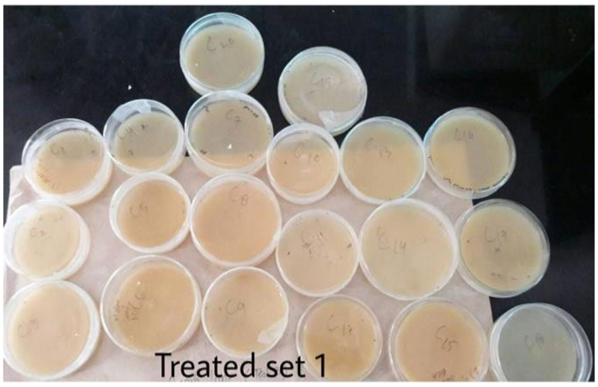

Figure 3: Experimental set of exposed flies

Figure 4: Egg laid by treated females

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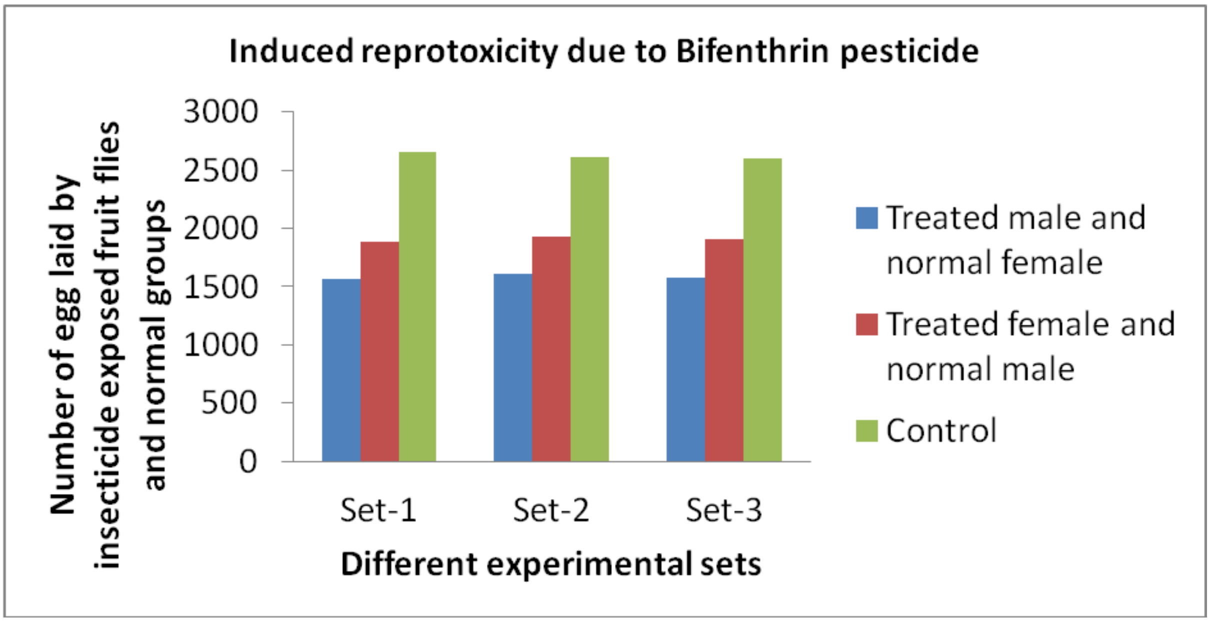

Figure 5: Statistical analysis of decreased fecundity in *Drosophila* exposed to Bifenthrin insecticide and control stocks.

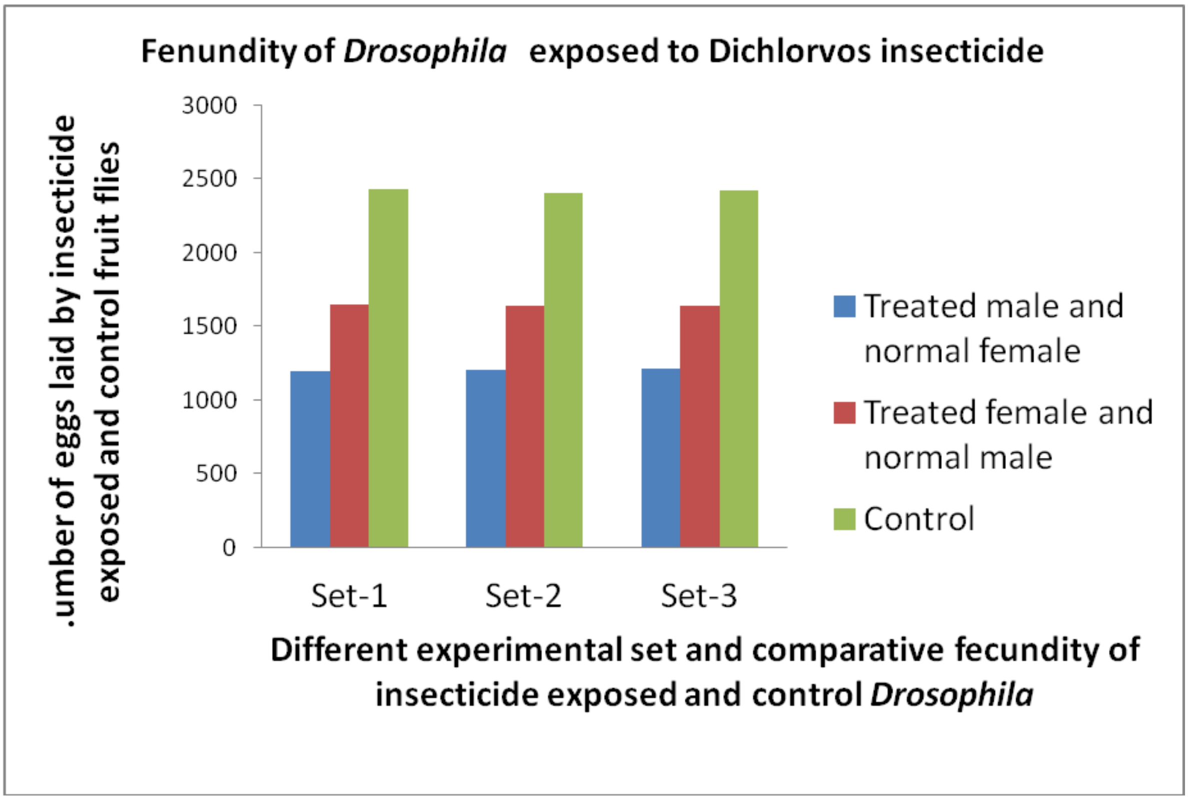

Figure 6: Statistical analysis of reduced fecundity of *Drosophila* exposed to Dichlorvos insecticide and control stocks.

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Insecticide exposure to fruit fly larvae and egg count: Exposure of selected insecticides to second larvae was given by mixing, standardized concentration of LC_{20} in culture medium. After 24 hours continuum, larvae had been shifted to culture medium. Adult flies had been cross-mated in different experimental groups: first experimental set include, cross mating of freshly emerged treated males with normal females, whereas second set comprise crossmating of treated females with normal males. All set of experiments were repeated in the form of triplicate and control groups were also maintained simultaneously. After 5 days the flies were removed and the laid eggs were counted under a dissection microscope for all experimental sets and obtained result were compared with that of natural population [Figure 3-6, Table 1,2] .

Table 1. Statistical analysis of consequences of Bifenthrin on reproduction of *Drosophila melanogaster*, in comparison to control stocks

Type	Set-1	Set-2	Set-3	Mean	Z value
Control (egg count)	**2650**	**2614**	**2603**	**2622**	**16.19**
Treated male and normal female (egg count)	**1561**	**1613**	**1579**	**1584**	
Treated female and normal male (egg Count)	**1890**	**1927**	**1904**	**1907**	**9.37**

Table 2: Statistical analysis of effects Dichlorvos pesticides on Fecundity of *Drosophila melanogaster*, which indicated significant reduced fecundity.

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Type	Set -1	Set-2	Set-3	Mean	Z value
Control (egg count)	**2433**	**2400**	**2420**	**2418**	**12.33**
Treated male and normal female (egg count)	**1191**	**1206**	**1214**	**1204**	
Treated female and normal male (egg Count)	**1647**	**1636**	**1642**	**1642**	**8.3**

Data Analysis*:* the obtained data was considered for analysis of mean, standard deviation and further statistical significance of data was analysed by carry out Z- test [Table 1,2].

Results

Present research exploration deals with adverse consequences of pyrethroid insecticide, Bifenthrin and organophosphate insecticide, Dichlorvos on fecundity of *Drosophila.* All experiments had been conducted in two different sets, simultaneously with controls. In first experimental group, freshly emerged treated male flies were cross mated with normal females whereas in second experimental sets, freshly molted treated females were cross mated with normal males. In the concerned research execution, it was observed that, Bifenthrin and , Dichlorvos significantly reduced the fecundity or egg production of *Drosophila*, in comparison to normal flies, which is clearly indicating its hazardous impact. Furthermore, it was reported that average fecundity decreases was more when normal female were crossed with treated males as compared to groups, in which treated females were crossed with normal

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males. Obtained results clearly indicate that selected insecticides exerted more detrimental effects on male fruit flies than female *Drosophila* as indicated by average decreased fecundity

Discussion

Few research explorations about cytotoxic, clastrogenic and reprotoxic instrict of pyrethroid insecticide, Bifenthrin and organophosphate insecticide, Dichlorvos, are procurable in scientific literature. Few research exploration , had been procurable in scientific literature that specify , harmful consequences of Bifenthrin including it had been found that the most prominent effect of Bifenthrin is blocking the sodium channels and depolarizing the pre-synaptic terminals and cause paralysis of organism by inhibiting nerve transmission [16] whereas, Lui *et al.,* (2011) revealed that Bifenthrin can mimic the action of estrogen and exerts an inhibitory effect on the luteinizing hormone gene in granulosa cells by inhibits luteinizing hormone induced PTGS2 gene expression in ovaries of rat [17]. It has been observed that bifenthrin increased the concentration of plasma glucose, ammonia, aspartate aminotransferase and creatine kinase in Cyprinus *carpio* [18]. Similarly, It has been observed that bifenthrin reduce fecundity, growth, and development of *Apis mellifera* [19]. Similarly, few research exploration are procurable for Dichlorvos including, Gupta, *et al.,* 2007 reported that Dichlorvos had significantly reduced reproductive potential of *Drosophila melanogaster* by adversely influencing proteins ACP70A and ACP36DE [20]. Similarly another exploration, on male albino rats, witnessed the reprotoxic consequences of concerned chemical, by decrease in sperm motility [21]. Furthermore, Krause and Homola, 1974 observed that Dichlorvos induced disturbance in spermatogenesis in rat [22]. In an exploration, it had been observed that Dichlorvos induced reproductive toxicity, by reducing weight of testis, decreasing the levels of FSH, LH and testosterone levels. Furthermore,

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cellular damage, necrosis and edema had also been reported in testicular tissue [23]. Similarly Dichlorvos was observed to reduce the fertility in human [24]. During present exploration, it had been concluded that exposure of Bifenthrin and Dichlorvos reduced fecundity in selected insecticides exposed *Drosophila* as compared to natural population.

Concluding remarks: During present research execution, it has been observed that Bifenthrin and Dichlorvos induced statically significant reprotoxicity in *Drosophila melanogaster* by decreasing egg laying capacity of test model at sublethal concentration LC_{20} for 24 hours. Reprotoxic consequences become conspicuous, in male flies as compared to female flies. Further intensive research explorations to assess reprotoxic instrict of selected insecticide are suggested, and alternative of pesticides should be implemented.

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