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Reprotoxicity profiling of Bispyribac Sodium, Pinoxaden and Spinosad Pesticides on *Drosophila melanogaster*

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Abstract

Present research exploration is attributed to reproductive toxicity evaluation of three synthetic agro-chemical pesticidal formulations viz: Bispyribac Sodium, Pinoxaden and Spinosad, by implementing, test model, Drosophila melanogaster (2n=8). The target of present exploration is to evaluate reprotoxicity induced by selected pesticides. To accomplish the objectives of present research analysis, standardization of semilethal concentration LC_{20}, had been considered by exposing second instar larvae to serial dilution of selected pesticides, for continuation of 24 hrs. Thereafter, considering co-relation between exposed concentration and mortality rate, exact values of LC_{20}, had been calculated by probit analysis,which correspond to values 4.4 pl/ml for Sodium Bispyribac and 7.1pl/ml for Pinaxoden pl/ml for Bispyribac Sodium, and 2 pl/ml for Spinosad. Subsequently, second instar larvae of fruit fly were exposed to sub lethal concentration, LC_{20} of selected pesticides, for 24 hrs, by means of nutrition, adding to culture medium. Afterward, larvae molted into imago in normal culture medium, which were allowed to cross mate in three separate experimental sets, in triplicate with respective negative controls. In first set, both pesticide exposed male and female cross-mated. In second experimental set, treated male and normal female flies were allowed to crossmate, whereas in third experimental set, pesticide exposed females were allowed to mate with normal males. After comparison of fecundity rate in all experimental sets with natural population, it had been concluded that selected pesticides induced statistical significant decrease in fecundity, when scrutinized by Z-test ($p<0.05$). Additionally, it had been analysed that the male fruit flies were more susceptible than female fruit flies to selected pesticides.

Keywords: Reprotoxicity, Bispyribac, Pinoxaden Spinosad Drosophila melanogaster

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Introduction

Although tremendous implication of synthetic agro chemical pesticidal formulations has escalated agricultural productivity, and therefore enhanced significant economic benefits, but simultaneously concerned chemicals are also associated with acute and chronic health problems[1-9]. Exposure to a wide variety of pesticides, affect structural as well as functional integrity of genetic imprints and therefore, disturbs various biochemical processes occurring in living organisms, and normal process of reproduction. Hence, reprotoxicity evaluation of different agro-chemicals, by implementing various innovative procedures, with appropriate biomarkers of different test models, has become very crucial, before excessive application of such chemicals in agricultural fields. Considering particular cognition, present research work has been executed, that deals with reprotoxicity

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evaluation of three selected pesticides namely Sodium Bispyribac , Pinoxaden , and Spinosad, which had been registered in 2001, 2005 and in 1997 respectively, by EPA for commercial uses. Half life period of Sodium Bispyribac is 42 days, Pinoxaden is 16 days and Spinosad is 16 days. Sodium Bispyribac is a contact pesticides, used to control weeds including grasses, broadleaf plants, on wheat and barley. Sodium Bispyribac is a broad spectrum post- emergence pyrimidinyl carboxy pesticide, it gets absorbed by foliage which works by interfering with production of plant enzymes necessary for growth, Acetolactate Synthase (ALS)[10]. Pinoxaden is a highly selective systematic, post emergence pesticide, belongs to the phenylpyrazolin group of chemicals, used to control a broad range of grass species including *Alopecurus myosuroides, Apera spica-venti, Avena ludoviciana, Avena sterilis, Avena fatua, Lolium multiflorum, Lolium rigidum, Phalaris minor, Phalaris paradoxa and Poa trivialis* etc. It acts by inhibiting enzyme, Acetyl Co enzyme A Carboxylase (ACCase) that prevents fatty acid synthesis. Spinosad is a specific bioinsecticide which is produced by a soil bacterium *Saccropolyspora spinosa.* The concerned chemical belongs to Spinosyn family. Spinosad is extracted from *Saccharopolyspora spinosa* by fermentation which is toxic to thrips, spiders, mites, mosquitoes, ants, fruit flies etc. Spinosad is a neurotoxic bioinsecticide which target nicotinic acetylcholine receptors and Gamma-Aminobutyric acid (GABA).

Limited explorations are available, in scientific literature that deals with clastrogenic and reprotoxic impacts of selected pesticides, therefore the present research work has been executed, and that deals with reprotoxicity assessment of three pesticides namely Sodium Bispyribac, Pinoxaden and Spinosad, at sublethal concentration LC_{20}, using *Drosophila melanogaster.* Exposure of standardized concentration was provided to second instar larvae of *Drosophila melanogaster*, which were reared upto adult stage under controlled conditions of insectory. Detrimental consequences of selected pesticides, on fruit fly were assessed by crossmating flies in different sets along with control. Subsequently, effects of selected synthetic agro-formulations were assessed by counting number of eggs laid down in different groups in comparison to natural population. Thereafter, produced data was scrutinized for statistical significance of results by applying Z- test.

Material and Methods

Test organism: *Drosophila melanogaster* (fruit fly) is a holometabolous insect with peculiar characteristics including short life span, adaptability to laboratory conditions, small diploid number(2n=8) and presence of four polytene chromosomes due to homologous pairing and endoreduplication of chromosomes. Primary stock of *Drosophila melanogaster*, Oregon strain was received from *Drosophila* Breeding Laboratory, Department of Biotechnology, Punjab Agriculture University, Ludhiana. The *Drosophila melanogaster* culture for present experimentation was maintained on culture medium composed of agar powder, brewer's yeast, sucrose, dextrose and corn meal along with fungicides *i.e.* phosphoric and propionic acid in cultural vials at 25^0C and with relative humidity 40%-60% under dark conditions. The medium was kept hydrated for easy movement of larvae and kept away from dirt, direct sunlight and other heat sources.

Herbicidal Profile: Three pesticides had been selected for the present reprotoxicity evaluation including Sodium Bispyribac with IUPAC Name, Sodium2,6-bis[(4,6-dimethoxy-2-pyrimidinyl)oxy)benzoate, Molecular formula $C_{19}H_{17}N_4NaO_8$ and Pinoxaden with IUPAC name 8-(2,6-diethyl-4-methylphenyl)-1,2,3,4,tetrahydro-7-oxo-7-H-pyrazolo[1,2 d] [1,4,5] oxadiazepin-p-9-9-yl 2,2-dimethylpropanoate, Molecular formula C_{23} H_{32} N_2 O_4 and Spinosad with IUPAC Name 2-((6-deoxy-2,3,4-tri-O-methyl-a-L-mannopyranosyl)oxy)-13-(((5dimethylamino) tetrahydro-6-methyl-2H-pyran-2-yl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16btetradecahydro-14-methyl-1H-as-indaceno(3,2-d)oxacyclododecin-7,15-dione, Molecular formula C41H65NO16. Locally available packets of concerned pesticides *viz* Sodium Bispyribac, Pinoxaden, Spinosad, procurable under respective trade names Adora, Axial and Tracer, had been procured, and were used as such because basic motive of research execution is to assess the reprotoxicity and clastrogenic potential of concerned synthetic formulations, which are actually going to be used in the agricultural fields.

Standardization of Sublethal Exposure Limit: To achieve specific targets, of concentration standardization, mortality of second instar larvae, exposed to serial dilution concentrations of 1% stock solution of selected pesticides, for 24 hours, was

considered. Required concentration of serial dilution was prepared by adding aliquots of the stock solutions in distilled water and exposure of serial dilution of selected pesticides was provided to second instar larvae through culture medium. For each particular concentration, 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 and subsequently, larvae were shifted to fresh culture medium for further growth. Exact value of LC_{20}, for selected pesticides, was calculated, through probit analysis, by considering co-relation between observed mortality of larvae in each set and exposure limit. No mortality had been reported in the control groups. Exact LC_{20} exposure the value corresponds to 4.4 pl/ml for Sodium Bispyribac, 7.1pl/ml for Pinaxoden, 2 pl/ml Spinosad (Figure, 1,2,3).

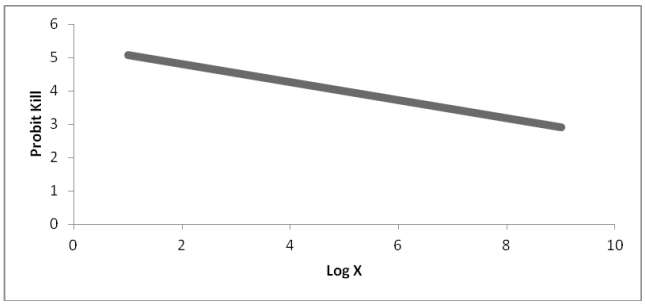


Figure 1: Regression line between Probit kill and concentration of Sodium bispyribac.

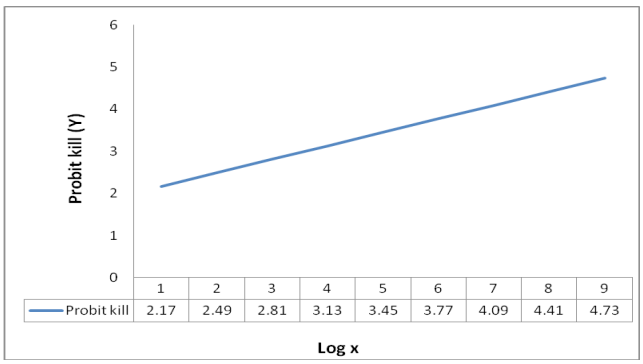


Figure 2: Regression line specifying co-relation between Probit kill and concentration of Pinoxaden .

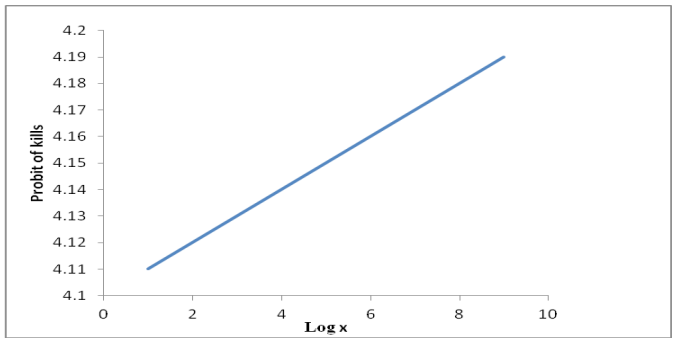


Figure 3: Regression line depicting co-relation between Probit kill and concentration of Spinosad .

Exposure of standardized LC_{20} to Larvae and Egg Counting: For the present research execution, pesticides treatment was given to the second instar larvae. After 24 hours the larvae which survived in treated as well as in untreated groups were transferred to new culture vials and were reared until upto adult stage. Adult flies were allowed to crossmate in different experimental groups. In first experimental set, cross mating of freshly emerged treated males and females was considered, whereas in second set treated males were allowed to cross mate with normal females. In third set, both treated females and male flies were allowed to crossmate. All set of experiments were repeated in the form of triplicate along respective control groups. After 5 days, flies were removed and subsequently, the laid eggs (Figure, 3) were counted under a dissection microscope for all experimental sets and obtained result were compared with that of control groups (Figure 4).

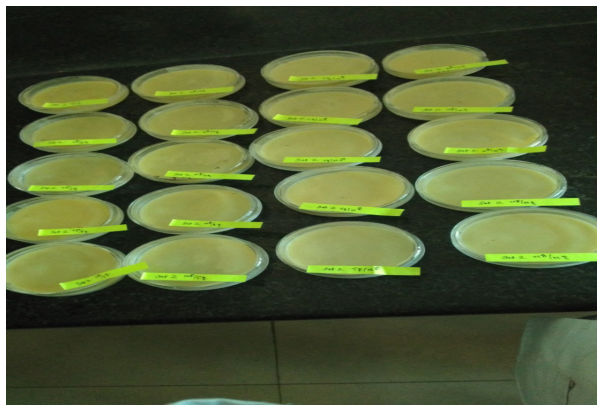


Figure 4: Experimental set for the assessment of reprotoxicity of Pinoxaden.

Data Analysis: The obtained data was considered for analysis of mean and statistical significance of data, by Z-test. The fecundity in each experimental set was compared with that of control. Obtained data was statistically analysed and results were compared, in pesticide exposed groups and control stocks (Figure 5,6,7).

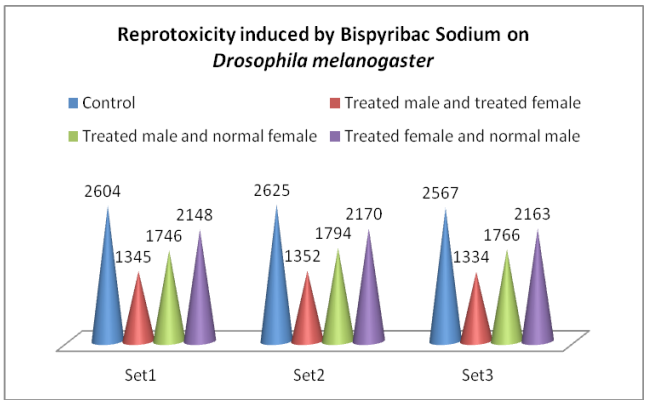


Figure 5: Graphical depiction of reprotoxicity induced by Sodium bispyribac on Drosophila melanogaster, at sublethal concentration LC_{20} , for 24 hours. Fecundity was drastically reduced when both male and female flies were exposed to Sodium bispyribac.

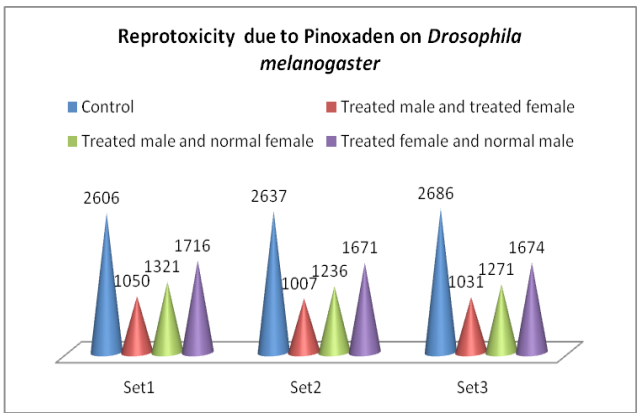


Figure 6: Reprotoxicity induced by Pinoxaden on Drosophila melanogaster, at sublethal concentration LC_{20} , for 24 hours. Male fruit flies were more susceptible than female fruit flies

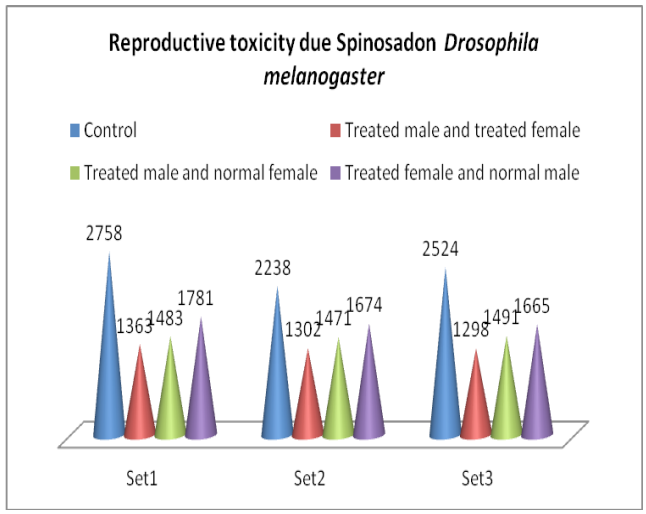


Figure 7: Effects of Spinosad on reproductive potential of Drosophila melanogaster , at sublethal concentration LC_{20} , for 24 hours.

Results

Present research exploration deals with adverse consequences of Bispyribac Sodium, Pinoxaden and Spinosad at $LC_{20,}$ on fecundity of *Drosophila*. Experiments were executed in three different sets, along with respective 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 third set, both treated male and female fruit flies were allowed to crossmate with each other. Each experimental set had been repeated in the form of triplicate, along with respective control. In the concerned research execution, it was observed that selected pesticides significantly reduced the fecundity or egg production of *Drosophila*, in comparison to normal population, which is clearly indicating, hazardous impact induced by selected pesticide. Furthermore, it was reported that average fecundity decreases was more predominant when treated males were crossed with treated females as compared to groups, in which treated male were crossed with normal female. Obtained results clearly indicate selected pesticides induced, detrimental effects on male fruit flies than female *Drosophila* as indicated by average decreased fecundity.

Table 1: Statistical analysis of reprotoxic effects of Bispyribac Sodium on Fecundity of Drosophila melanogaster.

Type	Set -1	Set-2	Set-3	Mean	Z value
Control	2604	2625	2567	130	54.15
Treated male and Treated female	1345	1352	1334	67	
Treated male And normal female	1746	1794	1766	88	29.29
Treated female and normal male	2148	2170	2163	109	13.23

Table 2: Statistical analysis of consequences of Pinoxaden on fecundity of Drosophila melanogaster, which indicated significant affected egg laying capacity .

Type	Set -1	Set-2	Set-3	Mean	Z value
Control	2606	2637	2686	132	71.63
Treated male and Treated female	1050	1007	1031	51	
Treated male And normal female	1321	1236	1271	64	54
Treated female and normal male	1716	1671	1674	85	30

Table 3: Statistical analysis of reproductive toxicity of Spinosad on fecundity of Drosophila melanogaster.

Type	Set -1	Set-2	Set-3	Mean	Z value
Control	2758	2238	2524	131	58.52
Treated male and Treated female	1363	1302	1298	66.05	
Treated male And normal female	1483	1471	1491	74	48.28
Treated female and normal male	1781	1674	1665	84	32.58

Discussion

The procured results obtained, after executing present experimental work for reproductive toxicity indicated that selected synthetic agro-chemical pesticides *viz*: Bispyribac Sodium, Pinoxaden and Spinosad, induced statistically significant reprotoxicity in *Drosophila melanogaster*. Complete experimental set had been repeated in the form of triplicate and the fecundity rate of treated flies was calculated over control flies. In all pesticide exposed groups, there was statistically significant reduction in egg production. Furthermore, for all synthetic chemicals, the decreased fecundity was predominantly expressed, when exposure of pesticides was given to treated male fruit flies, which could be due to abnormality induced by selected agro-chemicals, at the time of spermatogenesis. Other abnormalities recorded were difficulty in movement, posture maintenance complication, deformed wing structure, in pesticide exposed groups, as compared to normal flies. In scientific literature, the particular observations are well documented by various explorations conducted on different pesticides, including a study, on the effects of chlorpyrifes using *Drosophila melanogaster*

indicated that sublethal concentration of concerned pesticide increased the expression hsp70 expression, which had become possible reason for complete non hatchability, in pesticide exposed groups than control[11], whereas, another exploration indicated that mercury affected the embryonic development of *Drosophila melanogaster* , and concluded that there was excessive decrease in egg production[12]. Furthermore, a study analyzed that synthetic pyrethroid cypermethrin had hazardous effects on reproductive system of *Drosophila melanogaster* and further concluded that males flies were more susceptible to cypermethrin over treated females. Hsp70 activation was more in testis of males while no such expression of hsp70 gene was seen in female ovaries[13]. Similarly, dichlorovos exposed fruit flies had statistically significant reduced reproductive potential [14]. Furthermore, it had been observed that that the male flies were more susceptible to pesticide, as indicated by reduced fecundity and they concluded that over expression of hsp in the testicular lobes of male flies could be the possible reason, as indicated by molecular analysis. In same test model, it had been observed that in pesticide treated males[15] have down-regulated seminal proteins encoding gene that is Acp70 A and Acp 36. Furthermore, reduced the fecundity and delayed emergence of larvae, had been observed in sodium nitroprusside treated *Drosophila melanogaster* [16]. In *Rhyzopertha dominica* species, it had been reported that Spinosad pesticide, increased their egg laying capacity of females[17]. Furthermore, it had been reported that females treated with Spinosad protrude their ovipositor due to increased involuntary contraction of muscular tissue. Similarly, parathion treatment to *Drosophila melanogaster* induced statistically significant decrease in fecundity, when concerned pesticide exposure was given to one day old flies for continuation of 24 hrs. Another exploration indicated that the impacts of Pinoxaden on egg production of *Bombus terrestris* and concluded that concerned chemical decreased fecundity significantly in exposed groups[18]. Similarly, a study on *Lissorhoptrus oryzophilus* concluded that thiamethoxam exposure reduces first instar larvae and egg production in weevil fly[19]. Furthermore, another report indicated that at sub lethal, LC_{10} concentrations of thiamethoxam had significant consequences on developmental of fourth instar larvae, pupation, adult oviposition of aphid predator, *Hippodamina variegate*[20]. It had been further concluded that in same test organism, males flies were more susceptible to parathion than females[21]. Similarly, in *Apis mellifera,* at sub lethal concentration, thiamethoxam reduced reproductive success[22], whereas in same insect, neonicotinoid pesticides thiamethoxam and clathianidin induced severe consequences on reproductive anatomy and physiology[23]. The concerned chemicals, induce alternation of ovaries and spermathecae of queen, therefore affect storage of sperms in spermathecae and ultimately fertilization. Furthermore, during present study also, same conclusion has been snapped that selected pesticides, effect fecundity of *Drosophila melanogaster,* at sublethal concentration, additionally, effects become more pronounced on treated male fruit flies.

Concluding remarks:

The main conclusion snapped from these series of experiments is that the selected chemicals impacted badly, the fecundity of *Drosophila melanogaster*, when exposure is given at sublethal concentration for duration of 24 hrs. The experiment was repeated thrice and in all experimental sets, it was observed that the egg production by the treated flies were lower than the control population, and additionally, the conducted experiment revealed that males fruit flies were more susceptible to selected pesticides. Furthermore delayed hatchability and molting, was observed in treated flies, the reason could be the correlation between developmental time and exposed concentration that disturbed the early development of the eggs. Therefore, alternatives of pesticides should be implemented and strategies of Integrated pest management should be practisized.

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Conflict of Interest

None Declared

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