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Petri-plate bioassay to estimate the toxicity of synthetic pyrethroid insecticide bifenthrin against *Callosobruchus chinensis* L. pulse beetle (Coleoptera: Chrysomelidae) under laboratory conditions

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Abstract
The current investigation was conducted to estimate the resistance level against different doses of synthetic pyrethroids *i.e.*, Bifenthrin 10% EC was carried out during (2021-22), at Regional Research Centre (soybean), Amravati, (M.S.). Observations on the percent mortality have been observed at 24 & 72 hours after treatments. The percent mortality in *C. chinensis* L. was increased with higher concentration at 24HAT was 66.6 percent in 150 ppm while lower concentration was 30 ppm 48 followed by 50 ppm was 56.5 percent followed by 70 ppm was 61.6 percent followed by 90 ppm was 70 percent compared with treatment control. After 72h the most superior treatment was 150 ppm at 86 percent mortality recorded while the least treatment was 30 ppm with 65 percent mortality found as compared with the treatment control (Distilled water).

Keywords: Toxicity, bioassay, synthetic pyrethroid, bifenthrin, test insect, mortality, *Callosobruchus chinensis* L., stored grain pest

1. Introduction
Bifenthrin, a non-alpha cyano pyrethroid insecticide, is used worldwide against a range of agricultural pests. It has moderate acute toxicity, as do most of the other pyrethroids recommended for public health. Etofenprox (a non-ester pyrethroid) is classified as unlikely to present acute hazard in normal use. Bifenthrin is classified by WHO as moderately hazardous (WHO 1998a) [8]. Bifenthrin has a very low vapor pressure (1.81 10Ð7 mmHg), a low water solubility (1 g/litre), and good stability to hydrolysis and photolysis (2 year at 50C under natural daylight). It is non-irritant to skin, virtually non-irritating to eyes on rabbits and presents no skin sensitization on guinea pigs (Tomlin 2000) [7]. It is non-irritant to skin, virtually non-irritating to eyes on rabbits and presents no skin sensitization on guinea pigs (Tomlin 2000) [7]. Because of these attributes, bifenthrin is potentially a good candidate insecticide for treatment of mosquito nets. It has moderate acute toxicity, as do most of the other pyrethroids recommended for public health. The results showed that cypermethrin caused toxicity to cabbage looper at lower, recommended, and higher doses as compared to bifenthrin and control, nonetheless it also revealed more toxic to predator lady bird beetles as compared to bifenthrin and control. So, as conclusion it can be affirmed that cypermethrin caused early toxicity to insects and also up to third trophic level as compared to bifenthrin which rendered harmless at third trophic level.

2. Materials and Methods
The experiment was carried out in the laboratory of Entomology section at Regional Research Centre, Amaravati (M.S.) during 2021-22. The materials were comprised of adults of pulse beetle, *Callosobruchus chinensis* L., Bifenthrin 10% EC, Distilled water (dH2O) 500 ml, filter papers, petri-plates, scissor, jars, glass gas chambers of 125 ml volume, syringe, falcon tubes, measuring cylinder 1000 ml, pipette, rubber bands, room heater (relay technique), muslin cloth, healthy, Uninfected green gram grains, etc.

2.1 Test insect
Nucleus culture of Pulse beetle, *Callosobruchus chinensis* L. was collected from the Department of Post-Harvest Technology, College of Agril. Engineering, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.).

The insect culture was maintained in sterilized plastic jars at 29±20C temperature and 80±5% relative humidity. The culture medium comprises of green gram seeds. Initially 1-2 days old adults were released on healthy, un-infested green gram seeds in plastic jars. The jars were covered with the muslin cloth for better aeration. The pure and sterilized jars with stock cultures were kept on iron shelf for free mating and oviposition. Emergence of adults from infested grains started from 25-28 DAIR (Days after insect release). These adults were further reared for multiplication up to nine generations. The adults from tenth susceptible generation were used as test insect for conducting the experiment.

2.2 Test material

The synthetic pyrethroid chemical Bifenthrin 10% EC bought from local market to carry out the experiment. The desired chemical was diluted at different concentrations using serial dilution method. The different concentrations were i.e., 30 ppm, 50 ppm, 70 ppm, 90 & 150 ppm respectively. Firstly, Stock A i.e., 10000 ppm (1%) 20.40 ml solution + 79.6 ml dH2O were prepared then transferred into Stock B 500 ppm, 5 ml+ 95ml dH2O then same amount of distilled water required to obtained these concentrations the serial dilution method was adopted. To form further concentrations and corrected calibration of solutions, the following formula used [11]:

$$V = \frac{C \times A}{a.i.\ (\%)}$$

Where

V= Quantity of required solution to be collected from stock solution

C= Required concentration of chemical

A= Total volume of the solution required (dH2O)

a.i. = Active ingredients in chemical

2.3 Analysis of Data

The mortality count of insects in three replications of each concentration was recorded and the average percent mortality in each concentration was calculated. The percent mortality in the control, if any, was corrected using Abbot's formula,

$$\text{Corrected mortality (\%)} = \frac{\text{(Mortality in treatment (\%) – Mortality in control}}{\text{100- Mortality in control (\%)}}$$

2.4 Preparation of stock and treatment solutions

With using calibration spray solution formula, for 1000 ppm concentration stock A, 10 ml chemical solution then added to 90 ml dH2O to get 100 ml solution and from stock B solution with 500 ppm 5ml chemical added in 95ml dH2O. Similarly, for 30 ppm solution, 6ml chemical solution then added to 94 ml dH2O then for 50 ppm solution, 10 ml chemical solution added to 90 ml. of dH2O followed by 70 ppm, 14ml chemical solution was added in 86ml. of dH2O followed by 90 ppm, total 18 ml chemical solution was added in 82 ml dH20. For higher concentration 150 ppm, 30ml chemical added in 70 ml distilled water respectively for experiment.

2.5 Toxicity

Whatman filter paper of 4.0cm diameter were placed in 4.0cm diameter petri plates and were impregnated with different doses, i.e., 30 ppm, 50 ppm, 70 ppm, 190 ppm and 150 ppm of the insecticide along with control and three replications. Air dried and 20 adult insects per petri dishes were released and kept in the laboratory under controlled environmental conditions, *i.e.*, 30±20C temperature and 65±5% relative humidity. Mortalities were observed at 24h & 72h in each petri-dish until end point mortality was released. After 3 days mortality rate decreased and 0% mortality was observed in each control petri-dish. The result from all replications form insecticide were statistically analysed using Probit analysis to calculate LC/LD50 values for regression method.

2.6 Toxicological impact of synthetic pyrethroid Bifenthrin 10% EC against *Callosobruchus chinensis* L.: Lab bioassay

For toxicity test, there were 6 treatments including control and 3 replications to carried out the experiment. 20 adult beetles were used as test insect per replications (including one control in treatment). The chemical solution of Bifenthrin 10% EC of different concentrations were sprayed with small handy spray on filter papers. In each filter paper was then placed at the bottom and upper side of a fibre petri-dish with same size. Test insects were released in each replicated fibre petri-dish and then covered with rubber band. As the test insects should not stay on the petri-dish, the inner side & upper side of the filter paper was coated with Vaseline. The treated filter paper with same size was petri-dish then dried at room temperature for one hour to two. To fasten the process the room heater used. 20 adult beetles/petri-dish, total 360 adult beetle required and labelled the plates. For this experiment then released test insects on dry treated filter paper petri-dishes properly. With different treatments the observation of dead beetle by lethal dose of different concentrations of synthetic pyrethroid then observed at 24 HAT & 72 HAT (hours after treatment) respectively, to find the mortality % in *C. chinensis* adult beetles. The data was analysed and calculated with using EPA Probit analysis program version 1.5.

Experimental details

1.	Year of Experiment	: 2020-21
2.	Type of experiment	: Laboratory
3.	No. of insecticides used	: 01
4.	No. of Concentration	: 05
5.	No. of replication	: 03
6.	Test insect	: *Callosobruchus chinensis* L.

3. Results and Discussion

In the present study of toxicity of chemical and mortality percent, the result showed that, in (given table no.3) the toxicity of Bifenthrin at higher concentration was found more superior than lower doses of concentrations and mortality percent has been observed promptly at 24 Hours after treatment in (1 day/s after insect release), in 30 ppm concentration the average mortality was 48 percent followed by next treatment 50 ppm *i.e.*, 56.5 percent followed by 70 ppm showed that 61.6 percent followed by, 90 ppm showed 70 percent next to 150 ppm showed 66.6 percent mortality has been found compared with control (water spray) was 33.3 percent found least respectively.

At 72 hours after treatment the toxicity of given synthetic pyrethroid Bifenthrin after 3 days after insect released revealed that, the higher concentration with 150 ppm found most superior than rest of the treatments with 86 percent mortality as followed by 90 ppm was 85 percent followed 70 ppm was 80 percent followed by 50 was 73.3 percent followed by lower concentration 30 ppm was 65 percent compared with control (water spray) was 35 percent respectively.

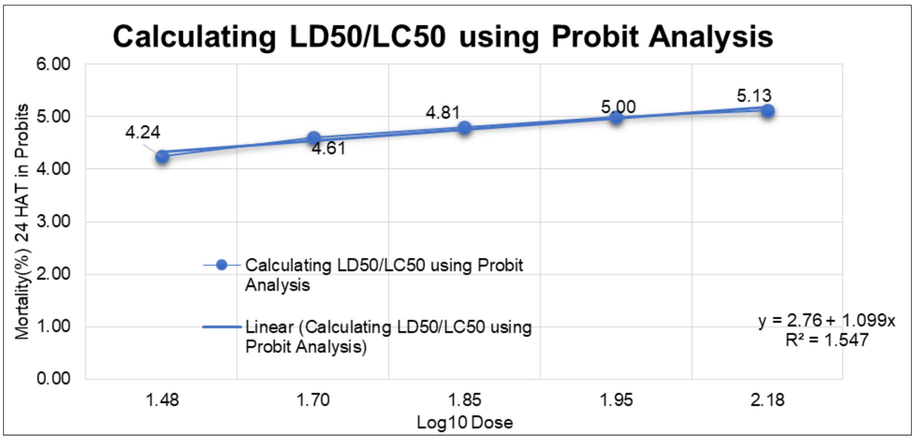

Fig 1: Regression model of toxicity of bifenthrin against *Callosobruchus chinensis* after 24 HAT

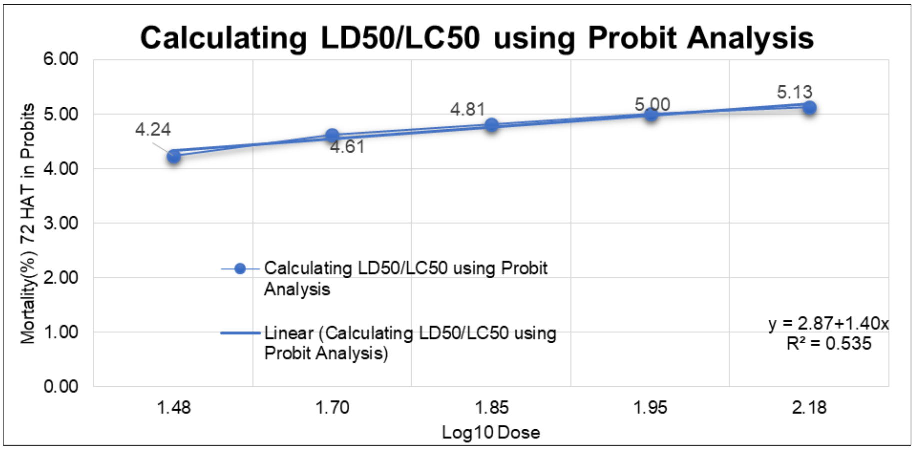

Fig 2: Regression model of toxicity of Bifenthrin against *Callosobruchus chinensis* after 72 HAT

In this present experiment, the toxicity of synthetic pyrethroid against *C. chinensis* L. after 24 hours (Table No. 4 & Fig.1) the mortality was gradually increased as per doses *viz.*, 30 ppm, 50 ppm, 70 ppm, 90 ppm and 150 ppm as 48%,56.5%, 61.6%, 70%, 66.6%, compared with control was observed 33.3% respectively.

The toxicity of Bifenthrin against *C. chinensis* L. after 72 hours (Table No. 5 & Fig.2) the mortality has been gradually increased as higher doses were 30 ppm, 50 ppm, 70 ppm, 90 ppm & 150 ppm was 65%, 73.3%, 80%, 85%, 86% compared with control was observed 35% respectively. The LC50 (ppm) with 95 percent fiducial limits was obtained 56.45 in between lower 24.08 to upper 91.16.

Table 1: Details of insecticide used in present investigation

Sr. no.	Common Name	Trade Name	Strength of insecticide	Source of supply
1	Bifenthrin	Imperial	10% EC	ADAMA India Pvt. Ltd., Gujrat

Table 2: Details of treatments

Sr. No.	Treatments	Concentrations
1	T_1	20 ppm
2	T_2	40 ppm
3	T_3	60 ppm
4	T_4	100 ppm
5	T_5	120 ppm
6	T_6 (Control)	Control

Table 3: Mortality response of synthetic insecticides Bifenthrin 10% EC on the adults of *C. chinensis* at 24 & 72 hours after treatment

Bifenthrin 10% EC	24 HAT	Bifenthrin 10% EC	72 HAT
Concentrations (ppm)	% Mortality	Concentrations (ppm)	% Mortality
30 ppm	48	30 ppm	65
50 ppm	56.5	50 ppm	73.3
70 ppm	61.6	70 ppm	80
90 ppm	70	90 ppm	85
150 ppm	66.6	150 ppm	86
Control (Distilled water)	33.3	Control (Distilled water)	35

Table 4: Toxicity of synthetic pyrethroid Bifenthrin on the adults of *C. chinensis* at 24 hours after exposure

Insecticides	Heterogeneity (χ^2)	Regression Equations (Y=a+b χ)	LC50 (ppm) (95% FL)	LC90 (ppm) (95% FL)	Slope b ($\pm$ SE)
Bifenthrin	1.547 (7.815)	2.76+1.099 χ	108.77 (40.097-1593.192)	1551.808 (369.022-5936.00)	1.099$\pm$ 0.50

Table 5: Toxicity of synthetic pyrethroid lambda cyhalothrin on the adults of *C. chinensis* at 72 hours after exposure

Insecticides	Heterogeneity (χ^2)	Regression Equations (Y=a+b χ)	LC50 (ppm) (95% FL)	LC90 (ppm) (95% FL)	Slope b ($\pm$ SE)
Bifenthrin	0.535 (7.815) *	Y=2.87+1.40 χ	32.802 (6.619-53.094)	268.710 (144.386-3147.392)	1.40 $\pm$ 0.46

(*Chi-square for heterogeneity-tabular value at 0.05 level)

4. Summary and Conclusion

The studies included development of homogenous population and determination of toxicity of bifenthrin against adult of *C. chinensis* L. after 24 and 72-hours treatment. In this experiment, serial dilutions of the desired insecticides were prepared using distilled water. Followed with, the distilled water was used as a control treatment and then was allowed to evaporate for 10 minutes in order to make sure it fully dried. Then, the filter paper that have been cooperated with the insecticides were placed in the petri dish with twenty adult insects were released for this experiment. Similarly, the procedure was repeated for all test concentrations and for all insecticides to assess the LC_{50} value and measured the toxicity of the bifenthrin insecticide. Mortality was assessed after 24 and 72 hours after. The mortality data was subjected to probit analysis (Finney, 1971) and LC_{50}, LC_{90}, heterogeneity (χ^2), intercept (a), slope of regression (b) and regression equation were calculated. The results revealed that bifenthrin exhibited the highest toxicity both at 24 and 72 hours. The LC_{50} values of bifenthrin was 150 ppm was 86% at 72 hours and was 66.6% at 24 hours.

Similarly, the results observed here are consistent with the earlier findings of Wilkin *et al*, (1994) [9] who found bifenthrin was very active against fi. *dominica* and *S. oryzae*, but not against *T. castaneum.* In addition, Collins (1990) [3] detected high levels of pyrethroid resistance in *T. castaneum.* Further, Reddy and Srivastava (2003) [10] showed better persistent toxicity of deltamethrin WP than bifenthrin WP on jute against *T. castaneum.* Though *S. oryzae* and *R. dominica* were found susceptible to bifenthrin, reports of high-level resistance to pyrethroids by Heather (1986) [5] and Collins *et al*, (1993) [2] suggest that these insects also have the potential to evolve resistance to bifenthrin. Since there is no single protectant that can control all species that attack stored grain, synergized combination of two products is inevitable. Successful control of R. dominica (Ali *et al*., 2003) [1]; *Sitophilus granaries* (L.) and *T. granarium* (Sokolov, 2004) [6] was achieved with a combination of bifenthrin and malathion. While, piperonyl butoxide synergised bifenthrin plus chlorpyriphos methyl prevented *R. dominica, T. castaneum, Oryzaephilus surinamensis* (L.) and *Cryptolestes ferrugineus* (Steph.) from producing live progeny for upto 7 months (Daglish *et al*., 2003) [4]. From the investigations it is clear that bifenthrin when used for prophylactic control is effective only against *S. oryzae*, *T. granarium* and *R. dominica.* Insensitivity of *T. castaneum* to bifenthrin suggests that a combination.

5. Acknowledgement

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