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Toxicity of novel insecticides against pulse beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) through jute cloth disc impregnation method

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Abstract
A study was conducted to evaluate the toxicity of novel insecticides *viz.*, spinosad 45 SC, spinetoram 11.7 SC, emamectin benzoate 5 SG, chlorfenapyr 10 SC, permethrin 25 EC and azadirachtin 10000 ppm against pulse beetle, *Callosobruchus maculatus* (Fab.) by jute cloth disc impregnation method under laboratory conditions during the year 2020-21 in the Department of Entomology, Agricultural College, Bapatla. Results of the study were compared with check treatments malathion 50 EC and deltamethrin 2.8 EC. Among tested insecticides the lowest values of LC_{50}, LC_{75} and LC_{90} were recorded for emamectin benzoate (0.00006, 0.0002 and 0.0006%) and chlorfenapyr (0.00006, 0.0002 and 0.0008%). Emamectin benzoate and chlorfenapyr recorded highest relative toxicity of 233.33 and 191.00 times than malathion and 41.67 and 83.00 times than deltamethrin at LC_{50} and LC_{75} whereas at LC_{90}, emamectin benzoate and chlorfenapyr were 156.50, 117.38 times toxic than malathion and 151.50, 113.63 times toxic than deltamethrin, respectively.

Keywords: Pulse beetle, callosobruchus maculatus, novel insecticides, jute disc, toxicity

1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops extensively grown in India during *rabi* season. It plays a vital role to address the Indian national food and nutritional security due to its high protein content and is being categorised as "smart-food" for its critical role to food basket. India is the largest producer of chickpea with a share of about 70% in area and 67% in the production of chickpea in the world [1]. During 2020-21, a total of 119.11 lakh tonnes of chickpea was produced in India from an area of 99.96 lakh ha with a productivity of 1192 kg ha^{-1} [2]. The total loss of chickpea produce at national level during harvest and post-harvest handling was 8.41% with an estimated monitory loss of Rs. 2453 Crore which includes 1.18% loss in storage [3]; where in the storage losses were mainly attributed to bruchids (*Callosobruchus* spp.) which cause substantial losses even up to 100 per cent and render the grain unsuitable for food or seed within 4-6 months [4].

The use of insecticides to control insect infestations is the most commonly followed method of grain protection due to their less cost and easy method of application. Chemical methods mostly rely on the use of synthetic insecticides and fumigants for the past several years; however, their continued usage has led to a number of problems including insecticide resistance in pulse beetle to malathion, deltamethrin [5], toxic residues in food grains, and environmental pollution. Also, insect pest tolerance to pesticides is an indicator of the species evolution demonstrating how they can survive and physiologically adapt under chemical stress [6]. In view of these problems together with the upcoming WTO regulations, there is a need to restrict their use globally and implement safe alternatives of conventional insecticides or suitable new insecticides molecules which are effective against insecticide-resistant insect species to protect stored grains from insect infestations [7].

In India, most of the latest insecticide groups registered in the resent past are safer, highly selective and fit to well in integrated pest management [8]. Spinosad is currently registered in several countries as a grain protectant at a maximum labeled use rate of 1 ppm (1 mg *a.i.* kg^{-1} of grain) and with the maximum residue level (MRL) or tolerance on grains set at 1 or 1.5 ppm [9]. Spinosad, spinetoram and emamectin benzoate being derivatives of soil microorganisms can be more practical alternative to the malathion and deltamethrin. Both of these compounds are highly toxic to bruchids and have relatively low mammalian toxicity. Chlorfenapyr is a natural product isolated from *Streptomyces fumanus*, it is commercially developed and has wide range of activities against major pests associated with both in filed and stored products [10]. Permethrin is a III generation synthetic pyrethroid acts through contact mode of action. Neem is envisaged as an eco-friendly pesticide having rich source of bioactive chemicals with a greater potential for development as successful pest control agent which can affect insects in several ways *viz.*, they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants, anti-feedants or ovipositional deterrents, also retard or accelerate development or interfere with the life cycle of the insects [11]. Keeping these facts in mind the present investigation has been undertaken to evaluate the toxicity and bio efficacy of novel insecticide molecules having novel mode of action against pulse beetle, *Callosobruchus maculatus* (Fabricius) (Chrysomelidae: Coleoptera) under laboratory conditions.

2. Materials and Methods

2.1 Rearing of the test insect

The insect culture was developed on chickpea by introducing few pairs of pulse beetles collected from the post-harvest technology centre, Bapatla, Andhra Pradesh by following the procedure given by Andrewartha, 1961 [12]. The mother culture of *C. maculatus* was maintained under laboratory conditions on locally available chickpea variety, JG-11. The grains were disinfested by fumigating with aluminium phosphide tablets @ 3 tablets per ton for seven days to ensure that they were free from insects and mites. Then the grains were well aerated to remove phosphine residues. About 500 g of chickpea grains were taken in a plastic jar measuring 45 x 15 cm and ten pairs of *C. maculatus* adults were released into it for oviposition. The mouth of the container was covered with muslin cloth and tightly fastened with rubber bands. Adults were removed after five days and released into another jar containing chickpea grains, thus a succession of the insect culture was maintained by utilizing the eggs laid staggerly to ensure constant supply of test insects of known age for conducting experiments. The jars were kept undisturbed under laboratory conditions (31±2^{0}C temperature and 70±5% relative humidity) till the emergence of adults. The newly emerged adults were transferred into fresh grains and used for the multiplication of culture as well as for conducting the experiment.

2.2 Test insecticides

The formulations of newer insecticides as mentioned below were tested for their toxicity and bio efficacy against *C. maculatus* through jute cloth disc impregnation method.

S. No.	Insecticide	Commercial formulation used
1.	Spinosad	Tracer 45 SC
2.	Spinetoram	Largo 11.7 SC
3.	Emamectin benzoate	Safeclaim 5 SG
4.	Chlorfenapyr	Intrepid 10 SC
5.	Permethrin	Permasect 25 EC
6.	Azadirachtin	Nimbecidine 10000 ppm
7.	Malathion (Check)	Cythion 50 EC
8.	Deltamethrin (Check)	Decis 2.8 EC

2.3 Preparation of test concentrations of insecticides

One per cent stock solution was prepared in 100 ml volumetric flask for each insecticide by dissolving respective formulations in distilled water. Twenty grams of emamectin benzoate 5 SG was taken into 100 ml volumetric flask and the volume was made up to 100 ml by adding distilled water. Similarly, the stock solutions of other insecticides were also prepared by giving due consideration to the actual toxicant in the formulation. From the stock solution the desired concentration of all the insecticides were prepared separately by serial dilution technique in the laboratory using distilled water as a solvent.

2.4 Bioassay through jute cloth disc impregnation method

The adult beetles of *C. maculatus* of one day old were subjected to the bioassay with the test insecticide concentrations by following jute cloth disc impregnation method [13]. Two ml of insecticidal solution was found to be sufficient for complete impregnation of the jute cloth disc of nine cm diameter. After impregnation, the jute cloth discs were air dried and transferred to petri plates. One day old beetles were collected from the culture and were starved for two hours. The starved beetles were then transferred on to treated jute cloth discs in petri plates @ 20 beetles per petri plate. Three replications were maintained for each test insecticide concentration. The beetles were confined to the treated surface for 24 hours. Simultaneously, a control was maintained where in jute cloth disc was impregnated with distilled water alone. Initially, a preliminary test with a broad range of concentrations was conducted for each test insecticide and depending on the mortality of the test insect, narrow range concentrations were tested to obtain adult mortality in the range of 10 to 90 per cent. Eight concentrations of insecticides were fixed based on the preliminary test of the bioassay to subject to probit analysis.

2.5 Observations recorded

The adult mortality data were recorded at 24, 48 and 72 hours after treatment (HAT) by considering moribund insects as dead. Mortality at 72 hours after treatment was considered as the endpoint for the assessment of the toxicity of test insecticides. Then, the mean per cent mortality was calculated from the mortality count of adult insects in three replications of each concentration. The per cent mortality of *C. maculatus* in each treatment was subjected to Abbot's correction [14] by using the per cent mortality of adults in the control.

$$\text{Corrected mortality (\%)} = \frac{T - C}{100 - C} \times 100$$

T- Per cent mortality in treatment; C- Per cent mortality in control

Relative toxicity of the test insecticides was calculated by using the following formula

$$\text{Relative Toxicity of test insecticide} = \frac{\text{LC value of malathion/deltamethrin}}{\text{LC value of test insecticide}}$$

2.6 Statistical Analysis

The corrected per cent mortality was subjected to probit analysis [15] by using SPSS (Statistical Product and Service Solutions) 21.0 version software for calculating LC_{50}, LC_{75},

LC_{90}, heterogeneity (χ^2), intercept (a), the slope of the regression line (b), regression equation and fiducial limits (at 95% C.L) for each insecticide.

3. Results and Discussion

3.1 Toxicity of Novel Insecticides on Jute Cloth Disc Surface against *C. maculatus*

Comparing the toxicity of all insecticides, it was evident that all the insecticides were variably toxic to pulse beetle, *C. maculatus*. The LC_{50} and LC_{75} values after 72 hours period of exposure revealed that emamectin benzoate and chlorfenapyr had lower values (0.00006 and 0.0002%) followed by spinetoram (0.0012 and 0.0041%), deltamethrin (0.0025 and 0.0166%), azadirachtin (0.0076 and 0.0331%), permethrin (0.0078 and 0.0363%), malathion (0.0140 and 0.0382%), and spinosad (0.0141 and 0.0396%), whereas LC_{90} values revealed that emamectin benzoate (0.0006%) had lower value followed by chlorfenapyr (0.0008%), spinetoram (0.0121%), deltamethrin (0.0909%), malathion (0.0939%), spinosad (0.1002%), azadirachtin (0.1239%), and permethrin (0.1446%) (Table 1).

The chi-square test values of all insecticides used in bio assay were less than that of table value (12.592) ($p< 0.05$) revealing the homogeneity of the test insects with good fitness of the data. The slope (b) values of log dose - probit (ldp) lines of spinosad, spinetoram, emamectin benzoate, chlorfenapyr, permethrin, azadirachtin, Malathion and deltamethrin were 1.64, 1.28, 1.33, 1.15, 1.05, 1.09, 1.62 and 0.83 respectively indicating that the difference between concentrations was varied at different mortality levels. The regression coefficient values (R^2) values of bio assay of insecticides were also higher than 0.938 with spinosad (0.938), spinetoram (0.980), emamectin benzoate (0.972), chlorfenapyr (0.961), permethrin (0.942), azadirachtin (0.957), malathion (0.957), and deltamethrin (0.949) revealing the fact that 93 per cent reliability of dosages tested (Table 1 and Figure 1-8).

3.2 Relative toxicity of test insecticides in comparison with checks against *C. maculatus* at 72 HAT

The relative toxicity of test insecticides in comparison with commonly used insecticides in storage *viz*, malathion and deltamethrin (checks) were calculated at LC_{50}, LC_{75} and LC_{90} at 72 HAT (Table 2).

The comparison with malathion revealed the relative toxicity in decreasing order as emamectin benzoate and chlorfenapyr (233.33) > spinetoram (11.67) > azadirachtin (1.84) > permethrin (1.79) > spinosad (0.99) at LC_{50} level, emamectin benzoate and chlorfenapyr (191.00) > spinetoram (9.32) > azadirachtin (1.15) > permethrin (1.05) > spinosad (0.96) at LC_{75} level and emamectin benzoate (156.50) > chlorfenapyr (117.38) > spinetoram (7.76) > spinosad (0.94) > azadirachtin (0.76) > permethrin (0.65) at LC_{90} level at 72 HAT.

Similar trend was followed in comparison with deltamethrin, which revealed that the relative toxicity in decreasing order as emamectin benzoate and chlorfenapyr (41.67) > spinetoram (2.08) > azadirachtin (0.33) > permethrin (0.32) > spinosad (0.18) at LC_{50} level, emamectin benzoate and chlorfenapyr (83.00) > spinetoram (4.05) > azadirachtin (0.50) > permethrin (0.46) > spinosad (0.42) at LC_{75} level and emamectin benzoate (151.50) > chlorfenapyr (113.63) > spinetoram (7.51) > spinosad (0.91) > azadirachtin (0.73) > permethrin (0.63) at LC_{90} level at 72 HAT.

Similar studies were conducted by Duraimurugan *et al.* (2014) [16], who reported the LC_{50} value of spinosad 45 SC was 0.00019 per cent whereas 0.0037 per cent was reported by Mondal *et al.* (2018) [17] against *C. chinensis*. Babu *et al.* (2020) [6] reported LC_{50} and $LC_{99.9}$ values of spinosad 45 SC, emamectin benzoate 5 SG and chlorfenapyr 10 SC were 0.0002, 0.0004 and 0.0365 per cent and 0.001, 0.0031, 0.4643 per cent, respectively at 72 HAT (hours after treatment) against pulse beetle, *C. maculatus* whereas Babu *et al*. (2018) [18] reported that 0.0085, 1.9150 per cent and 0.0555, 0.4678 per cent at 72 HAT, respectively against *R. dominica* and the relative toxicity of chlorfenapyr 10 SC was 7.45 and 7.34 times and emamectin benzoate 5 SG was 1.82 and 1.79 times toxic than malathion and deltamethrin at $LC_{99.9}$ level. Thorat and Salokhe (2018) [19] reported that chlorfenapyr 10 SC insecticide was most effective on glass surface with LC_{50} value of 0.007273 against adults of *T. castaneum*.

The current research results of toxicity of novel insecticide molecules against pulse beetle through jute cloth disc impregnation method indicated that emamectin benzoate was found to be highly toxic to *C. maculatus* with its low LC values followed by chlorfenapyr and also with high relative toxicity compared to malathion and deltamethrin which may be due to their novel mode of action where the emamectin benzoate acts through contact as well as by ingestion and kills the insect by causing rapid excitation by activation of nicotinic acetylcholine receptors of the nervous system and also affects GABA receptor functioning whereas chlorfenapyr acts through contact and ingestion and works by uncoupling the oxidative phosphorylation from electron transport process in mitochondria and interferes with formation of ATP which is essential for muscle contraction causing death in insects [20]. These results are similar with Babu *et al*. (2018) [18] reported chlorfenapyr and emamectin benzoate were relatively best at $LC_{99.9}$ when compared with malathion and deltamethrin.

Table 1: Toxicity of different insecticides against *C. maculatus* on jute cloth disc surface at 72 HAT

S. No.	Treatments	LC values (%) (95% FL)			Heterogeneity (χ^2)	Slope b (± SE)	Regression equation Y = a + bX
		LC_{50}	LC_{75}	LC_{90}			
1.	Spinosad 45 SC	0.0141 (0.0122-0.0164)	0.0396 (0.025-0.0506)	0.1002 (0.0748-0.1460)	11.28	1.64 (±0.11)	Y = -3.48+1.64X
2.	Spinetoram 11.7 SC	0.0012 (0.0010-0.0015)	0.0041 (0.0034-0.0051)	0.0121 (0.0091-0.0175)	3.85	1.28 (±0.10)	Y = -1.41+1.28X
3.	Emamectin benzoate 5 SG	0.00006 (0.00005-0.00007)	0.0002 (0.0001-0.0003)	0.0006 (0.0004-0.0009)	5.61	1.33 (±0.10)	Y = 0.26+1.33X
4.	Chlorfenapyr 10 SC	0.00006 (0.00005-0.00007)	0.0002 (0.0001-0.0003)	0.0008 (0.0006-0.0012)	9.65	1.15 (±0.07)	Y = 0.31+1.15X
5.	Permethrin 25 EC	0.0078 (0.0060-0.0097)	0.0363 (0.0283-0.0489)	0.1446 (0.0992- 0.2354)	9.56	1.05 (±0.08)	Y = -1.97+1.05X
6.	Azadirachtin 10000 ppm	0.0076	0.0331	0.1239	7.49	1.09	Y = -2.03+1.09X

		(0.0060-0.0094)	(0.0262-0.0436)	(0.0874-0.1938)		(±0.08)	
7.	Malathion 50 EC (Check)	0.0140 (0.0121-0.0161)	0.0382 (0.0320-0.0472)	0.0939 (0.0723-0.1318)	8.99	1.62 (±0.11)	Y = -3.47+1.62X
8.	Deltamethrin 2.8 EC (Check)	0.0025 (0.0019-0.0032)	0.0166 (0.0115-0.0274)	0.0909 (0.0495-0.2199)	6.09	0.83 (±0.08)	Y = -1.17+0.83X

Table 2: Relative toxicity of different insecticides in comparison with Malathion and deltamethrin against *C. maculatus* at 72 HAT

S. No.	Treatments	Malathion 50 EC			Deltamethrin 2.8 EC		
		LC_{50}	**LC_{75}**	**LC_{90}**	**LC_{50}**	**LC_{75}**	**LC_{90}**
1.	Spinosad 45 SC	0.99	0.96	0.94	0.18	0.42	0.91
2.	Spinetoram 11.7 SC	11.67	9.32	7.76	2.08	4.05	7.51
3.	Emamectin Benzoate 5 SG	233.33	191.00	156.50	41.67	83.00	151.50
4.	Chlorfenapyr 10 SC	233.33	191.00	117.38	41.67	83.00	113.63
5.	Permethrin 25 EC	1.79	1.05	0.65	0.32	0.46	0.63
6.	Azadirachtin 10000 ppm	1.84	1.15	0.76	0.33	0.50	0.73

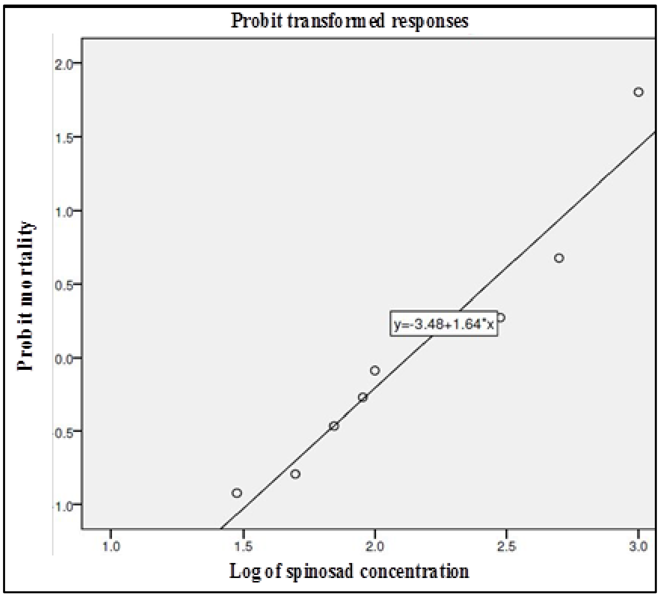

Fig 1: Median lethal concentration mortality response of c. maculatus treated with spinosad

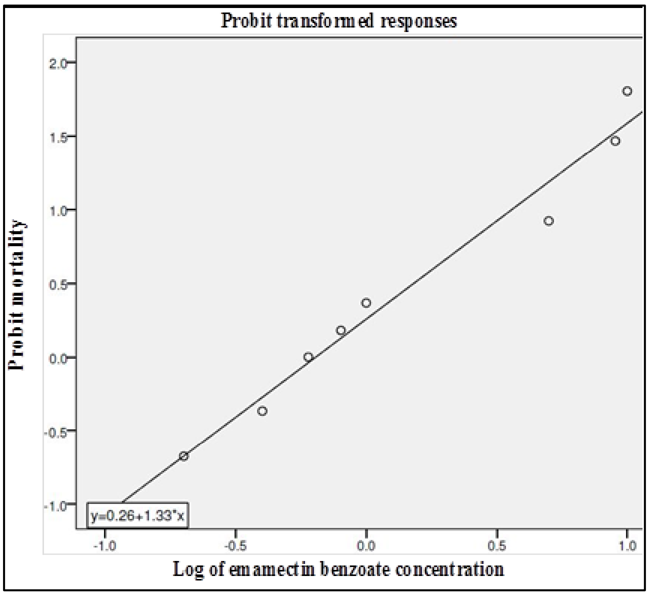

Fig 3: Median lethal concentration mortality response of c. maculatus treated with emnamectin benzoate

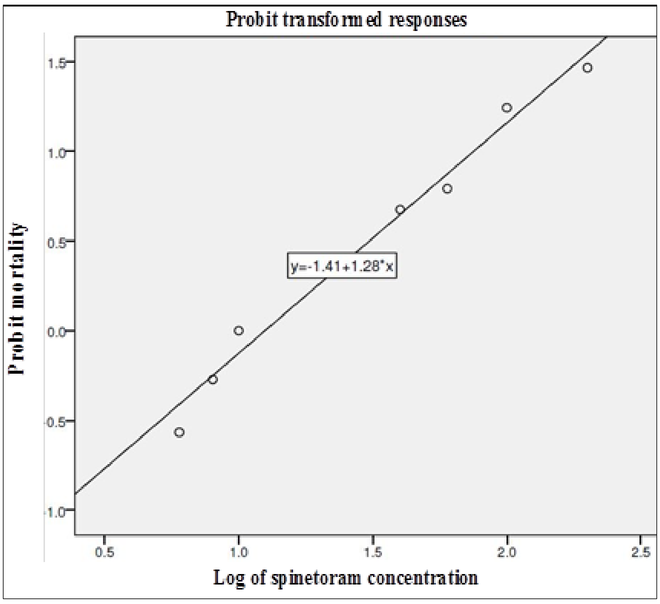

Fig 2: Median lethal concentration mortality response of c. maculatus treated with spinosad

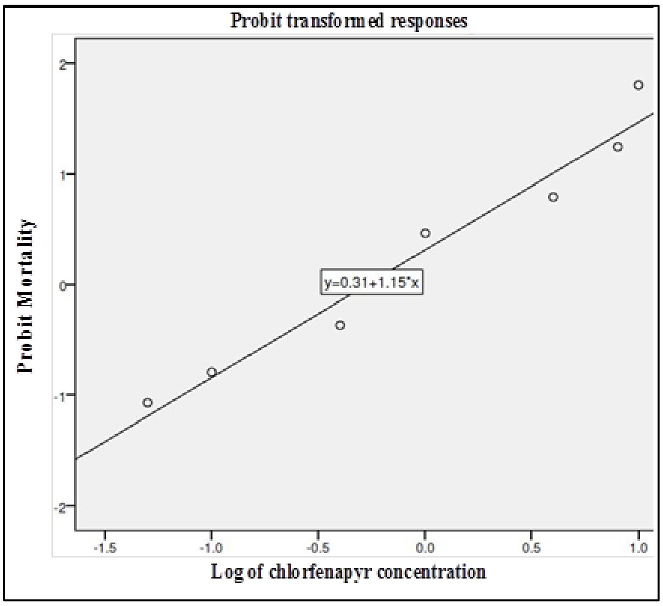

Fig 4: Median lethal concentration mortality response of c. maculatus treated with chlortenapyr

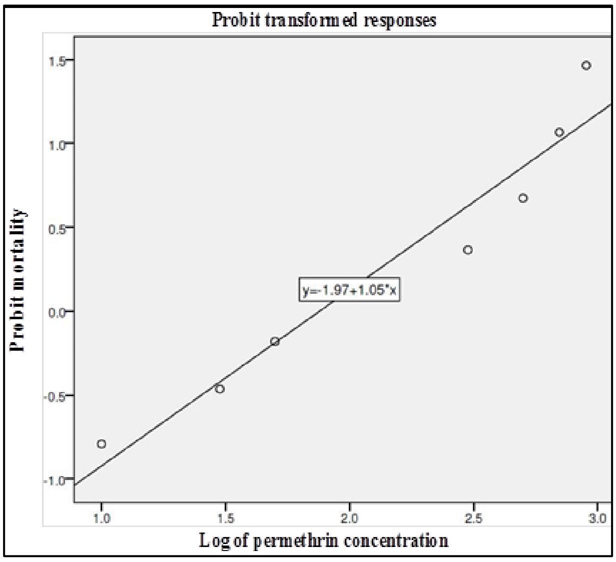

Fig 5: Median lethal concentration mortality response of c. maculatus treated with permethrin

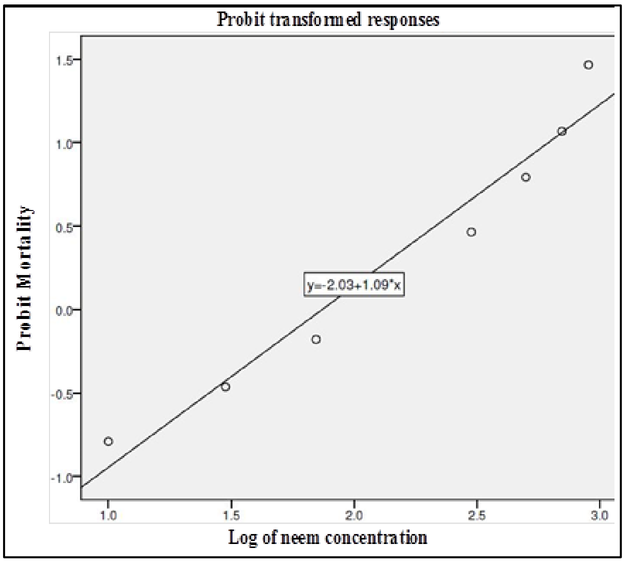

Fig 6: Median lethal concentration mortality response of c. maculatus treated with neem

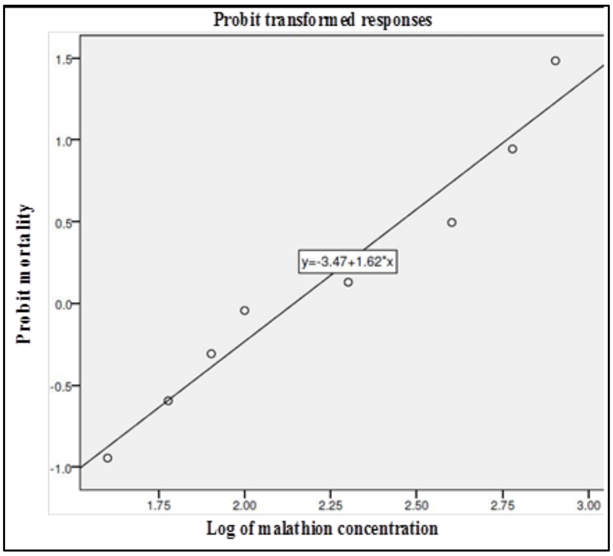

Fig 7: Median lethal concentration mortality response of c. maculatus treated with Malathion (check)

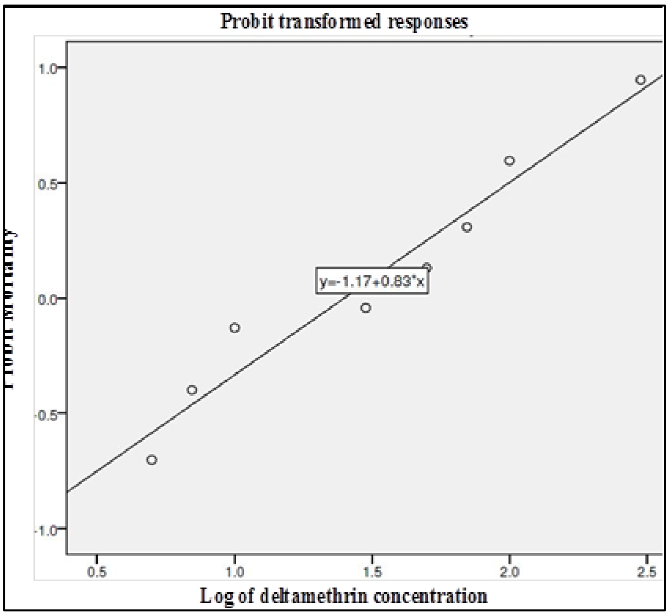

Fig 8: Median lethal concentration mortality response of c. maculatus treated with deltamethrin (Check)

4. Conclusion

In the present study, six insecticides were evaluated for their toxicity against pulse beetle, *C. maculatus* along with malathion and deltamethrin as checks and found that, among microbial derived insecticides, emamectin benzoate and chlorfenapyr were effective with least LC_{50}, LC_{75} and LC_{90} values followed by spinetoram and spinosad. They were also found more relatively toxic when compared with the conventional and commonly used insecticides in storage insect pest management, malathion and deltamethrin. The treatments, permethrin and azadirachtin recorded least toxicity with highest LC_{90} values.

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