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To Test the Residual Toxicity of the Selective Nicotinic Receptor Antagonist-Spinosad on the *Callosobruchus chinensis* L. pulse Beetle (Coleoptera: Chrysomelidae) in a Lab Setting

A.N. Warghat[1*], A.R. Tayde[2] and S.S. Munje[3]
[1]Ph.D. Scholar, Department of Entomology,
NAI, SHUATS, Prayagraj (Uttar Pradesh), India.
[2]Assistant Professor, Department of Entomology,
NAI, SHUATS, Prayagraj (Uttar Pradesh), India.
[3]Associate Professor, Department of Entomology,
AICRP-Regional Research Centre (Soybean), Amravati,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola (Maharashtra), India.

(Corresponding author: A.N. Warghat)*
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ABSTRACT: The study was conducted in the Department of Entomology lab at the AICRP (Soybean)-RRC, Amravati Dr. P.D.K.V., Akola (Maharashtra State), Regional Research Centre for (Soybean) in Akola between 2020 and 2021. The study's findings, To Test the Residual Toxicity of the Selective Nicotinic Receptor antagonist-Spinosad on the *Callosobruchus chinensis* L. pulse beetle (Coleoptera: Chrysomelidae) in a Lab Setting, were well-detailed, and it was evident that the study of rearing insects of the pulse beetle, *Callosobruchus chinensis* L. The goal of the laboratory tests was to create a homogeneous population. From there, Spinosad 45% SC relative toxicity against the pulse beetle, *C. chinensis*, was assessed based on the percentage of adult mortality of resistant species classified as Susceptible (S_5) and Resistant Population (R_1) and count the probit analysis of Susceptible (S_6) and Resistant Population (R_2). The bioassay, which was carried out using the impregnated filter paper method with five concentrations of each of the four treatments, including the control, and reproduced three times with Spinosad 45% SC, revealed that the higher concentrations of Spinosad had greater toxicity than the lower doses and Mortality percentage was noticed right away at 24 HAT in a 30 ppm concentration, where the average mortality was (8.33%), followed by the next treatment at 50 ppm, which was (10.00%), followed by 70 ppm, which showed that (13.33%), next to 90 ppm, which showed (15.00%), next to 150 ppm, which showed (16.67%) mortality, respectively. The average mortality was 8.33% at 48 HAT in a 30 ppm concentration, followed by 50 ppm treatment, or (11.67%), then 70 ppm revealed that (15.00%), then 90 ppm showed (18.33%), and finally 150 ppm showed (21.67%). Mortality was discovered to be 1.67 times higher than the water spray control. The average mortality at 72 HAT 30 ppm concentration was (35.00%), followed by the next treatment at 50 ppm, which was (38.33%), then 70 ppm showed that (45.00%), then 90 ppm showed (50.00%), and finally 150 ppm showed (53.33%), respectively. Mortality was also found when compared to the control (water spray), which was (8.33) found least frequently. The Probit analysis of the mortality % and extract concentration at 24, 48, and 72 hours after treatment revealed a linear connection when probit regression lines of the Spinosad resistant (S6) and (R2) populations were generated. According to the analysis, the regression line equations for (T5) 150 ppm were Y = 0.773x + 3.219 24 hours after treatment. The tested pesticides' computed X2 values were lower than the table value (0.951), indicating that the adult population was homogenous. The regression line equations were computed as Y= 0.979x + 2.067 for (T5) 150 ppm at 48 hours post-treatment. The tested pesticides computed X^2 values were lower than the table value (0.951), indicating that the adult population was homogenous. The regression line equations for (T5) 150 ppm at 72 HAT were derived as Y= 0.773x + 3.221. The tested pesticides' computed X^2 values were lower than the table value (0.951), indicating that the adult population was homogenous. This study suggests that eco-friendly management can help break down pest resistance in food grains, allowing them to be stored for longer periods without contamination.

Keywords: Resistant species, population, stored grain pest, probit analysis, residual toxicity, Spinosad, Agro chemical.

INTRODUCTION

India is the world's leading producer of pulses, which are farmed for their nutritional value as a source of protein, carbs, and minerals. A lack of protein, amino acids, vitamins, and minerals causes around 840 million individuals globally to be undernourished. Pulses

include important nutrients like calcium, iron, and dietary fiber, to name just a few (Ofuva and Akhidue 2005). About 60 million tonnes of pulses are produced each year for the global market. In 2010, 723 million acres were used to grow pulses, yielding 644,08,000 metric tons at a productivity of 890 kg per hectare. In terms of global production and consumption, India leads the market.

In 2010, India's pulse crop was over 17.29 million metric tons. in particular, the procedure for creating new records. India makes for roughly 32.24% of the world's output of pulses out of a total of 171 producing nations. Despite the 16.47 million tons of pulses that were gathered in 2016 (Directorate of Economy and Statistics; (Anonymous, 2016), the total area sown was just 2.52 million acres. In Maharashtra, pulses are produced on 4316 000 ha, producing 3849 000 t with a productivity of 891.8 kg/ha.

Pulses are a crucial category of crops globally, particularly for the dietary needs of low-income populations. India leads the world in pulse production, with an area of 23.63 million hectares and a yield of 14.76 metric tons (Anonymous, 2015a). Among these pulses, the Pigeon pea, scientifically known as *Cajanus cajan* (L.), holds significant importance. India holds the top position globally in the production of Pigeon pea, cultivating it over an area of 4.09 million hectares and producing 2.75 million tons. In the state of Maharashtra, Pigeon pea is grown over an area of 10.97 lakh hectares, yielding 9758 hundred tonnes (Anonymous, 2015b). A study by (Gujar and Yadav 1978) in India documented a loss in seed weight ranging from 32.2 to 55.7 per cent. In extreme cases, pests can cause up to 100 per cent damage (Pruthi and Singh 1950). It's a well-established fact that the components of food are essential for the survival and reproductive abilities of insects. Preventing damage to stored products from insect pests is a crucial aspect of Indian agriculture. Insecticidal protectants are commonly used to shield stored grain from insect damage. However, these chemicals have become less effective due to the development of resistance in pests like *Callosobruchus chinensis*. Additionally, the toxic residues from these chemicals can pose risks to human health and the environment. As a result, plant powders (botanicals) are being used as grain protectants. These botanicals have insecticidal properties against stored grain insect pests (Bakkali *et al.,* 2008) and are safer for both human health and the environment (Adhe *et al.,* 2018). Pulses, often referred to as nature's "Marvelous gift", are integral to both the Indian diet and its economy. These crops are high in protein content (around 21-25%) (Tiwari and Singh 2012), including essential amino acids like lysine, making them an effective solution for malnutrition in the predominantly vegetarian Indian diet. Pulses also provide carbohydrates (50-60%), various vitamins such as riboflavin, thiamine, niacin, and folic acid, as well as dietary fibers and minerals. These nutritional components make pulses a crucial part of the diet for people in developing countries like India (Chakraborty and Mondal 2015). The primary pulse crops cultivated in India include green gram, cowpea, chickpea, black gram, pigeon pea, lentil, and horse gram. The states that contribute significantly to the total pulse production in India, across both Kharif and Rabi/Summer seasons, are Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Andhra Pradesh, and Karnataka (Anonymous, 2019). In recognition of the significant health benefits of pulses, the United Nations declared 2016 as the International Year of Pulses. Globally, pulses are cultivated on approximately 689.9 million hectares of land. The average yield is about 999 kg per hectare. Reducing postharvest losses, especially in developing nations, could be a viable strategy for increasing food availability, eradicating hunger, and enhancing the livelihoods of farmers (Kumar and Kalita 2017). India holds the title for being the largest producer and consumer of pulses in the world. It contributes to approximately 28.34% (195.5 million tonnes) of the total global pulse production. Furthermore, it utilizes about 42.6% (294.3 million hectares) of the world's pulse cultivation area, yielding an average productivity of 664 kg per hectare. According to (Maneepun, 2003), approximately 20-25% of the annual harvest of pulses is lost due to various insect infestations after harvesting. The primary damage from storage pests is not just from consumption, but also from the contamination they cause. A 1999 World Bank report stated that India loses 12-16 million metric tonnes of food grains each year due to post-harvest losses. If these losses could be avoided, it would be enough to feed about a third of India's population living in poverty (Anonymous, 1999).

For many different causes, including but not limited to weather conditions, water limitations, a lack of seeds and fertilizers, insect-pest damage to stored goods and agricultural fields, disease, and so on, the state's output is low compared to the rest of the region. The storage of pulses, which is affected by a wide variety of storage pests, is one of the main factors lowering production in the state. In India, a person consumes about 45 gm of pulses daily, although the WHO recommends 80 gm. And by 2018, there will be a nearly 38-million-ton surge in demand for pulses.

MATERIALS AND METHODOLOGY

A. Experimental details

Experiment Conducted: 2020-21
Experiment Setting: Laboratory
Number of Insecticides Utilized: 01
Number of Concentrations Used: 05
Number of Replications Conducted: 03
Insect Used for Testing: *Callosobruchus chinensis* L.

Table 1: Details of insecticide used in present investigation.

Sr. No.	Common Name	Trade Name	Strength of insecticide	Source of supply
1.	Spinosad	Tracer	45% SC	Dow Agro Chemicals, New Delhi

B. Analysis of Data

In each concentration, the number of insect deaths was observed across three separate trials. The average percentage of mortality was then determined for each concentration. If there was any mortality in the control group, it was adjusted using Abbot's formula from 1925.

Each concentration's average percent mortality was derived using the insect mortality counts in three replications of each concentration. Using Abbot's formula (1925), the percent mortality in control was adjusted, if necessary.

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

Insect mortality was recorded 24, 48, and 72 hours after release. Insects unable to move or immobile were regarded as dead. The following formula was used to calculate percent mortality:

$$\text{Per cent mortality} = \frac{\text{Number of dead pulse beetle}}{\text{Total number of pulse beetle released}} \times 100$$

Abbott's formula was used to evaluate the corrected mortality and probit analysis was used to calculate LC_{50} values.

Abbott's formula,

$$P = \frac{P' - C}{100 - C} \times 100$$

Where, the observed mortality (%) was denoted by P', P denotes the corrected mortality (%) and C represents the mortality of control.

Abbott's formula,

$$P_{corr} = \frac{P_{exp} - P_{con}}{1 - P_{con}} \times 100$$

Where, mean treatment response of the experiment is denoted by $P_{exp,}$ P_{corr} represents the mean experimental treatment response corrected for control response and P_{cont} denotes the mean control response.

C. Large-scale breeding of pulse beetles

A 250g sample of green gram will be placed in plastic jars (45x15 cm) and 10 pairs of adult beetles will be introduced for egg-laying. The jars will be covered with muslin cloth and secured tightly with rubber bands. The beetles will be allowed to mate and lay eggs for ten days, after which the adults will be removed. The grain, now containing the eggs, will be left undisturbed until new adults emerge. This process will be repeated up to the fifth generation, and the resulting homogeneous and susceptible population will be used for further experiments.

D. Formulating insecticides mixtures

In this study, Spinosad, a biological insecticide, was utilized for a bioassay. As shown in Table 1, a 1% stock solution of 100 ml was created for each insecticide by dissolving their respective formulations in distilled water. For instance, 35.71 ml of Spinosad 45% EC was added to a 100 ml volumetric flask and the volume was topped up to 100 ml with distilled water. In a similar manner, stock solutions of other insecticides were prepared, taking into account the actual toxicant in the formulation. Desired concentrations of all insecticides were prepared from the 1% stock solution using distilled water. Initially, insects were exposed to a broad spectrum of concentrations for each insecticide. Based on these initial results, test concentrations for each insecticide were chosen such that the mortality rate ranged from approximately 10% at the lower concentration to around 95% at the higher concentration. Each treatment was replicated three times.

E. Bioassay procedure: impregnated filter paper method

Adult beetles of *C. chinensis* L., aged between 3 to 5 days, were used in the bioassay with the test insecticides, employing the impregnated filter paper dip technique. For each insecticide solution, 600 µl was applied to a Whatman no.1 filter paper, approximately 9 cm in diameter, using a pipette. In this study, a series of dilutions of the insecticides were made using distilled water. This distilled water was also used as a control in the experiment. The solutions were then left to evaporate for 10 minutes to ensure they were completely dry. Then, the filter paper, which had been treated with the insecticides, was placed in a petri dish. For the experiment, 20 adult insects were then introduced into the petri dish. The same procedure was carried out for all test concentrations and for Spinosad to determine the LC_{50} value and evaluate the relative toxicity of the insecticide. The mortality rate was recorded at 24, 48, and 72 hours post-treatment, and the LC_{50} values were computed using the probit analysis method (Finney, 1971).

RESULTS

The bioassay, which was carried out using the impregnated filter paper method with five concentrations of each of the four treatments, including the control, and reproduced three times with Spinosad 45% SC, revealed that the higher concentrations of Spinosad had greater toxicity than the lower doses and Mortality percentage was noticed right away at 24 HAT in a 30 ppm concentration, where the average mortality was (8.33%), followed by the next treatment at 50 ppm, which was (10.00%), followed by 70 ppm, which showed that (13.33%), next to 90 ppm, which showed (15.00%), next to 150 ppm, which showed (16.67%) mortality, respectively.

The average mortality was 8.33% at 48 HAT in a 30 ppm concentration, followed by 50 ppm treatment, or (11.67%), then 70 ppm revealed that (15.00%), then 90 ppm showed (18.33%), and finally 150 ppm showed (21.67%). Mortality was discovered to be 1.67 times higher than the water spray control. The average

mortality at 72 HAT 30 ppm concentration was (35.00%), followed by the next treatment at 50 ppm, which was (38.33%), then 70 ppm showed that (45.00%), then 90 ppm showed (50.00%), and finally 150 ppm showed (53.33%), respectively. Mortality was also found when compared to the control (water spray), which was (8.33) found least frequently.

The Probit analysis of the mortality % and extract concentration at 24, 48, and 72 hours after treatment revealed a linear connection when probit regression lines of the Spinosad resistant (S_6) and (R_2) populations were generated. According to the analysis, the regression line equations for (T_5) 150 ppm were Y = 0.773x + 3.219 24 hours after treatment. The tested pesticides' computed X^2 values were lower than the table value (0.951), indicating that the adult population was homogenous. The regression line equations were computed as Y= 0.979x + 2.067 for (T_5) 150 ppm at 48 hours post-treatment. The tested pesticides' computed X^2 values were lower than the table value (0.951), indicating that the adult population was homogenous. The regression line equations for (T_5) 150 ppm at 72 HAT were derived as Y= 0.773x + 3.221. The tested pesticides' computed X^2 values were lower than the table value (0.951), indicating that the adult population was homogenous.

DISCUSSION

The data and lab bioassay have been calculated to count the LD_{50} or LC_{50} values of Susceptible strain (S_5) and Resistance Population (R_1) against Spinosad using serial dilution method following the observation of mortality at 24 HAT (Hours After Treatment) by Filter impregnation method (Spinosad 45% SC).

The toxicity of selected test insecticide spinosad and mortality responses against the adults of *Callosobruchus chinensis* L., the result showed that, the insect mortality increased marginally at 24 hours. The highest mortality (16.67 %) was observed with spinosad at 150 ppm concentration with LC_{50} values being (3698.09 ppm). In (Table 2 and Fig. 1) the toxicity of Spinosad 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 DAIR (day/s after insect release), in 30 ppm concentration the average mortality was (8.33 %) followed by next treatment 50 ppm *i.e*., (10.00 %) followed by 70 ppm showed that (13.33 %) followed by, 90 ppm showed (15.00 %) next to 150 ppm showed (16.67 %) mortality has been found compared with control (water spray) was (1.67) found least respectively.

In the present study of toxicity of selected test insecticide spinosad and mortality percent against the adults of *Callosobruchus chinensis* L., the result showed that the insect mortality increased marginally at 48 hours. The highest mortality rate (21.67 %) was observed with spinosad at 150 ppm concentration with LC_{50} values being (986.89 ppm). In (Table 2 and Fig. 2) the toxicity of spinosad at higher concentration was found more superior than lower doses of concentrations and mortality percent has been observed promptly at 48 Hours after treatment in 2 DAIR (day/s after insect release), in 30 ppm concentration the average mortality was (8.33 %) followed by next treatment 50 ppm i.e., (11.67 %) followed by 70 ppm showed that (15.00 %) followed by, 90 ppm showed (18.33 %) next to 150 ppm showed (21.67 %) mortality has been found compared with control (water spray) was (1.67) found least respectively. Final mortality was calculated with Abbot's formula and the corrected mortality data were analysed by probit analysis design.

In the present study of toxicity of selected test insecticide spinosad and mortality percent against the adults of *Callosobruchus chinensis* L., the result showed that the insect mortality increased marginally at 72 hours. The highest mortality (21.67 %) was observed with spinosad at 150 ppm concentration with LC_{50} values being (200.38 ppm). In (given Table 2 and Fig. 3) the toxicity of spinosad at higher concentration was found more superior than lower doses of concentrations and mortality percent has been observed promptly at 72 Hours after treatment in 3 DAIR (day/s after insect release), in 30 ppm concentration the average mortality was (35.00 %) followed by next treatment 50 ppm *i.e*., (38.33 %) followed by 70 ppm showed that (45.00 %) followed by, 90 ppm showed (50.00 %) next to 150 ppm showed (53.33 %) mortality has been found compared with control (water spray) was (8.33) found least respectively. Final mortality was calculated with Abbot's formula (Abbott, 1925) and the corrected mortality data were analysed by probit analysis designed by Finney (1964).

When probit regression lines of the Spinosad resistant (S_6) and (R_2) populations were calculated, they showed a linear relationship between mortality percentage and extract concentration at 24, 48 and 72 hours after treatment. From the analysis, the regression line equations at 24 hours after treatment were Y = 0.773x + 3.219 for (T_5) 150 ppm, The calculated X^2 values in selected insecticides tested were less than that of table value (0.951) suggesting that the adult population was homogeneous. At 48 hours after treatment, the regression line equations were calculated as Y= 0.979x + 2.067 for (T_5) 150 ppm. The calculated X^2 values in selected insecticides tested were less than that of table value (0.951) suggesting that the adult population was homogeneous. At 72 HAT, the regression line equations were calculated as Y= 0.773x + 3.221 for (T_5) 150 ppm. The calculated X^2 values in selected insecticides tested were less than that of table value (0.951) suggesting that the adult population was homogeneous.

Comparing all regression lines at 24, 48 and 72 hours after treatment. In the Table 3 the regression lines for treatment (T_5) and 150 ppm showed higher probit mortality in every case. The higher dosage of treatment was more effective at providing immediate control of *C. chinensis* L, but its effectiveness decreased over time. In contrast, the resistance to chemicals increased with time. The difference among different dosages and extracts were not significant statistically. As per Begum *et al.* (2017); Mondal *et al.* (2018) mortality percentage was found directly proportional to the level of doses and time after treatments. Higher concentrations

resulted in a greater percentage of *C. chinensis* L. mortality, whereas lower concentrations were compared to the treatment control (Distilled water).

Table 2: Mortality response of selected Insecticide of Spinosad 45% SC at 24, 48 and 72 HAT.

Sr. No.	Concentrations (ppm)	% Mortality Spinosad at 24 HAT	% Mortality Spinosad at 48 HAT	% Mortality Spinosad at 72 HAT
T_1	30 ppm	8.33	8.33	35.00
T_2	50 ppm	10.00	11.67	38.33
T_3	70 ppm	13.33	15.00	45.00
T_4	90 ppm	15.00	18.33	50.00
T_5	150 ppm	16.67	21.67	53.33
T_6	Control	1.67	1.67	8.33
Probability level	-	**NS**	**NS**	**NS**

N.S. not significant (α = 0.05)

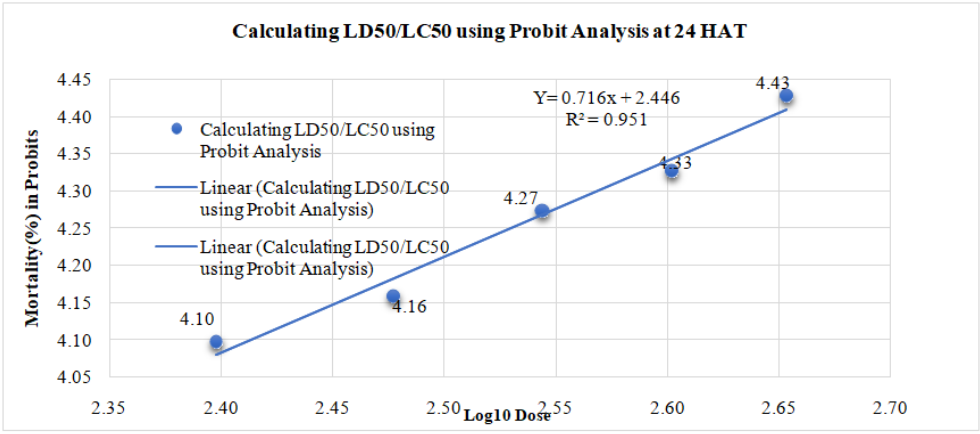

Fig. 1. Regression model of toxicity of Spinosad against *Callosobruchus chinensis* L. after 24 HAT.

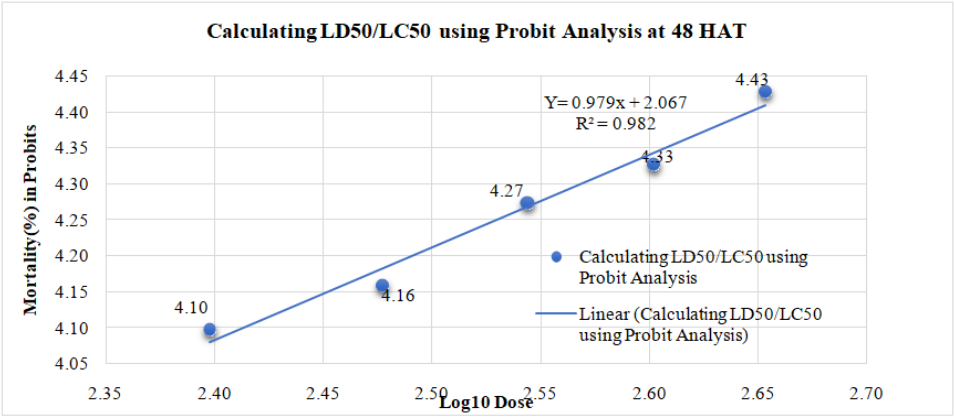

Fig. 2. Regression model of toxicity of Spinosad against *Callosobruchus chinensis* L. after 48 HAT.

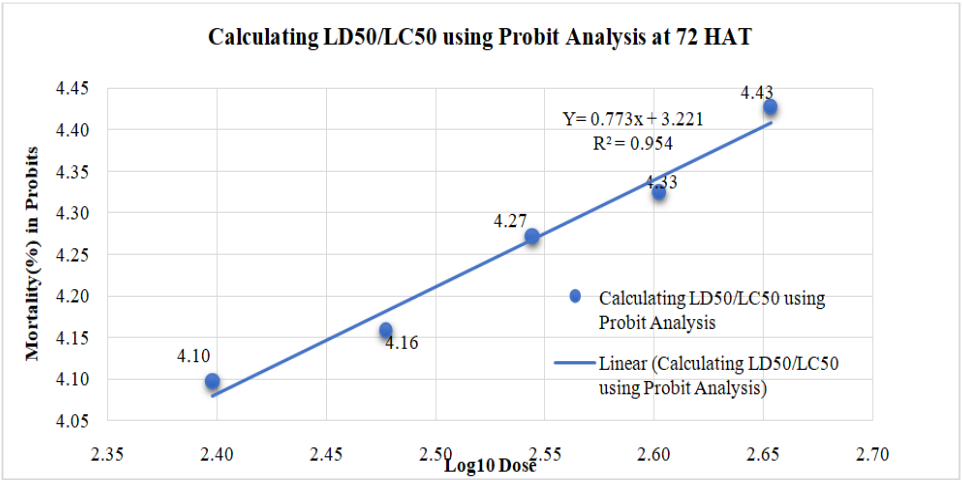

Fig. 3. Regression model of toxicity of Spinosad against *Callosobruchus chinensis* L. after 72 HAT.

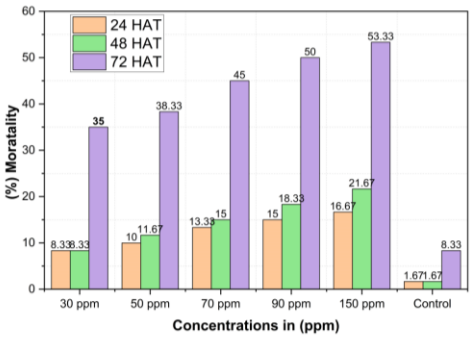

Fig. 4. Mortality response of selected Insecticide of Spinosad 45% SC at 24, 48 and 72 HAT.

Table 3: **Toxicity of Spinosad 45% SC on the adults of *C. chinensis* at 24, 48 and 72 hours after exposure.**

Chemical exposure	Heterogeneity (x^2) [df = 3]	Regression Equations (Y=mx+c)	LC_{50} (ppm) (95% FL)	LC_{90} (ppm) (95% FL)	Slope b (+ SE)
Spinosad 45% SC at 24 HAT	0.951 (0.988)*	Y = 0.715x + 2.446	3968.09 (826.85-16539.76)	228281.99 (51041.08-1020994.58	0.715 ± 0.332
Spinosad 45% SC at 48 HAT	0.982 (0.993)*	Y = 0.978x + 2.069	986.89 (347.19-2805.21)	20079.03 (7063.90-57074.34)	0.978 ± 0.231
Spinosad 45% SC at 72 HAT	0.954 (0.990)*	Y = 0.773x + 3.219	200.38 (69.03-581.69)	9112.06 (3138.96-26451.34)	0.773 ± 0.236

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

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Conflict of Interest. None.

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