The Pharma Innovation Journal 2022; 11(3): 1879-1882

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(3): 1879-1882

www.thepharmajournal.com
Received: 02-01-2022
Accepted: 12-02-2022

Shyamkant Munje Munje
Associate Professor, Department of Agricultural Entomology, Regional Research Centre Soybean, Amravati, Maharashtra, India

Sachin Pawar
M.Sc. Scholar, Department of Agricultural Entomology, Post-Graduation Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Krishi Nagar, Akola, Maharashtra, India

DB Undirwade
Post-HoD and Professor, Department of Agricultural Entomology, Post-Graduation Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Krishi Nagar, Akola, Maharashtra, India

US Kulkarni
Post-Associate Professor, Department of Agricultural Entomology, Post-Graduation Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Krishi Nagar, Akola, Maharashtra, India

AN Warghat
Ph.D. Scholar, Department of Agricultural Entomology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India

Corresponding Author:
Shyamkant Munje Munje
Associate Professor, Department of Agricultural Entomology, Regional Research Centre Soybean, Amravati, Maharashtra, India

Petri-plate Bioassay to estimate the toxicity of biological insecticide Spinosad against *Callosobruchus chinensis* L. pulse beetle (Chrysomelidea-Coleoptera) under laboratory conditions

Shyamkant Munje Munje, Sachin Pawar, DB Undirwade, US Kulkarni and AN Warghat

Abstract
The present investigation entitled "Determination of toxicity of biological insecticides Spinosad against *Callosobruchus chinensis* L. pulse beetle (Chrysomelidea- Coleoptera) under laboratory conditions" was conducted in insect toxicology laboratory of Department of Agricultural Entomology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, during the year of 2020-21 with objective to develop homogeneous population and from that to evaluate relative toxicity Spinosad 45% SC, against pulse beetle, *C. chinensis* on the basis of per cent adult mortality at 24, 48 and 72 hours after treatment (HAT). The bioassay was conducted by impregnated filter paper method with five concentrations of each four treatments including control and replicated thrice Spinosad 45% SC showed best efficiency at both LC_{50} and LC_{90} and it was more toxic than other insecticides at 24, 48 and 72 hours of exposure. The LC_{50} value of Spinosad 45% SC to the adult of *C. chinensis* were 0.011, 0.009 and 0.007 ppm while LC_{90} value were 0.035, 0.029 and 0.019 ppm at 24, 48, 72 hours after treatment (HAT), respectively. The log concentration Probit (Lcp) line slope (b) values of Spinosad 45% SC were 2.625, 2.638 and 3.084 at 24, 48 and 72 HAT, respectively. The insect mortality and toxicity of insecticides increase marginally at 48 hours and then at 72 hours after exposure.

Keywords: Toxicity, Spinosad, bioassay, *Callosobruchus chinensis* L., laboratory condition, pulse beetle

Introduction
Pulses, the "wonderful gift" of nature, play an important role both as an indispensable constituent of Indian diet and economy. Pulse crop because of their high protein content (approximately 21-25%) (Tiwari and Shing, 2012) [12] of which lysine is of great importance and serve as best mean of solving malnutrition problems in the vegetarian Indian diet. Pulses also contain carbohydrates (50-60%) and several vitamins like riboflavin, thiamine, niacin and folic acid; in addition, they also contain a quantity of fibres and minerals play an important role in the diet of common people of developing countries like India (Chakraborty and Mondal, 2015) [4]. Undoubtedly; the pulses have been considered as poor mans' meat for underprivileged people who cannot afford animal protein. Keeping in view large benefits of pulses for human health the United Nation has proclaimed 2016 as the international year of pulses. The total World area under pulses is about 689.9 lakh hectares with production of 689.9 lakh tonnes at 999 kg/ha yields level. India is the World's largest producer and consumer of pulses, accounting about 28.34% (195.5 lakh tonnes tones) total global production with an area of 42.6% (294.3 lakh hectares) with productivity of 664 kg/ha. Major pulse crops grown in India are green gram, cowpea, chickpea, black gram, cowpea, pigeon pea, lentil, horse gram etc. In India, the prominent total pulses producing states combining both *Kharif* and *Rabi/Summer* are Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Andhra Pradesh, and Karnataka (Anonymous, 2019) [2]. Pulses are generally stored for about a year at farmer, trader and government levels in various types of storage structures until the harvest of next crop. During the post-harvest period, particularly in storage, a sizeable loss of pulses is observed in the terms of quality and quantity. According to IGMRI, Hapur (UP), annual storage losses have estimated 14 million tonnes worth up to Rs. 7,000 crores in India in which insect alone accounts for 1,300 crores. The major loss caused by storge insect pest is not always by consumption but also by the amount of contamination and according to World Bank

report (1999), post-harvest losses in India accounts to 12-16 million metric tonnes of food grains each year. If this storage loss could be prevented it would be sufficient to feed India's one-third of poor population (Anonymous, 1999) [3]. The post-harvest annual losses in pulses due to different insects amount to around 20–25 per cent (Maneepun, 2003) [8]. During sever infestation the post-harvest seed losses due to the pulse beetle can reach even up to 100 per cent. (Srinivasan *et al*. 2010) [11]. Decreasing the postharvest losses, particularly in developing countries, could be a sustainable solution for increasing food supply; eliminate hunger and improving the livelihoods of farmers (Kumar and Kalita, 2017) [6].

Materials and Method

The present investigation entitled on "Determination of toxicity of biological insecticides Spinosad against *Callosobruchus chinensis* L. pulse beetle (Chrysomelidea-Coleoptera) under laboratory conditions" was carried out on the Insect toxicology laboratory, Department of Entomology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, during the year 2020-21 with a view to study the toxicity of biological insecticide Spinosad on pulse beetle and to work out the effective treatment for management of pulse beetle.

Mass culturing of pulse beetle

A test sample of 250 g of green gram will be taken in plastic jars (45x15 cm) and 10 pairs of adults will be released for oviposition and the jars were covered with muslin cloth and tightly secured by rubber bands. Mating and oviposition allowed for ten days and then adults will be removed. The host grain containing eggs will be left undisturbed until the new adults emerge. Same procedure will be followed up to fifth generation and the subsequent homogenous and susceptible population will be used for further experimental purpose.

Preparation of insecticidal solutions

A biological insecticide Spinosad was used to carry out bioassay in present study. In the Table 1. A stock solution of 1% of 100 ml was prepared for each insecticide by dissolving their respective formulations in distilled water. For example, 35.71 ml of Spinosad 45% EC was taken into 100 ml volumetric flask and the volume made to 100 ml by adding distilled water. Similarly, stock solutions of other insecticides were also prepared giving due consideration to the actual toxicant in the formulation. From 1% stock solution desired concentrations of all insecticides were prepared using distilled water. The insects were exposed initially to a wide range of concentrations for insecticide and later on the basis; the test concentrations for insecticide were selected in such a manner that the per cent mortality varies around 10% at lower concentration to around 95% at higher concentration. The treatments were replicated thrice.

Bioassay procedure: impregnated filter paper method

The adult beetles of *C. chinensis* L. of 3 to 5 days old were subjected to the bioassay with the test insecticides by impregnated filter paper dip technique. For each solution of the insecticides, 600 µl were applied by using pipette to Whatman no.1 filter paper which about 9 cm diameter. In this experiment, serial dilutions of the insecticides were prepared using distilled water. After that, the distilled water was used as a control purpose and then was allowed to evaporate for 10 minutes in order to make sure it fully dried. Then, the filter paper that have been cooperate with the insecticides were placed in the petri dish with 20 adult insects were released for this experiment. Similarly, the procedure was repeated for all test concentrations and for Spinosad to assess the LC_{50} value and measure the relative toxicity of the insecticide. Mortality was assessed after 24, 48 and 72 hours after treatment and the LC_{50} values were calculated by probit analysis method (Finney, 1971) [5].

Experimental details

1. Year of Experiment: 2020-21
2. Types 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.

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

Analysis of Data

The mortality count of insects in three replications of each concentration was recorded and the average per cent mortality in each concentration was calculated. The per cent mortality in the control, if any, was corrected using Abbot's formula (1925) [1].

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

For Calibration of Insecticide Solution data

$$\text{Volume of insecticide (mL)} = \frac{\text{Total volume (L)} \times \text{Percentage of insecticide required (\%)}}{\text{Formulation of insecticide (\%)}} \times 100$$

Result and Discussion

The result obtained from the present studies of toxicity of Spinosad molecules against the pulse beetle *Callosobruchus chinensis* L. under laboratory condition are presented below,

Table 2: Mortality response of Spinosad on the adults of *C. chinensis* at 24, 48 and 72 hours after exposure.

Spinosad		Spinosad		Spinosad	
Concentration (ppm)	% Mortality 24h	Concentration (ppm)	% Mortality 48h	Concentration (ppm)	% Mortality 72h
0.004	13.33	0.01	20	0.004	30.00
0.007	26.66	0.02	31.66	0.012	41.66
0.01	43.33	0.03	48.33	0.020	60.00
0.013	56.66	0.04	65.00	0.028	78.33
0.016	66.66	0.05	76.66	0.036	91.66
Control	00	Control	00	Control	10.00

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

Insecticides	Heterogeneity ($\chi2$)	Regression Equations ($Y=a+b\chi$)	LC50 (ppm) (95% FL)	LC90 (ppm) (95% FL)	Slope b (+ SE)
24hr	0.132	$Y=10.108+2.625\chi$	0.011 (0.009-0.016)	0.035 (0.022-0.141)	2.625 ± 0.688
48hr	0.726	$Y=10.342+2.638\chi$	0.009 (0.007-0.012)	0.029 (0.019-0.091)	2.638 ± 0.664
72hr	1.954	$Y=11.567+3.084\chi$	0.007 (0.005-0.009)	0.019 (0.014-0.039)	3.084 ± 0.731

At 24,48 and 72 Hours after Exposure

The results of mortality response and relative toxicity of Spinosad against the adults of *C. chinensis* are given in Table 2 and Table 3. The highest mortality (66.66%) was observed with Spinosad at 0.016 ppm concentration with LC_{50} values being 0.011 ppm. Based on LC_{50} values, Spinosad was found to be relatively more toxic insecticide compared with control. With respect to LC_{50} values, the relative toxicity (Table 3) of the Spinosad was (1.6363). On the basis of LC_{90} values, the order of toxicity of Spinosad remained the same as in LC_{50} values. The LC_{90} value of the Spinosad was 0.035.

The insect mortality increased marginally at 48 hours. The highest mortality (76.66%) was observed with Spinosad at 0.016 ppm concentration. The LC_{50} values were 0.009 ppm (Table 5) for Spinosad. With respect to LC_{50} values, the relative toxicity (Table 5) of Spinosad was (1.1111). The data clearly indicates the superior performance of Spinosad over others. The calculated X^2 values indicated that the *C. chinensis* adult population used in the study was homogeneous. On the basis of LC_{90} values, the order of toxicity of insecticides remained the same as in LC_{50} values. The LC_{90} value of Spinosad was 0.029.

At 72 hours, the highest mortality (91.66%) was observed with Spinosad at 0.016 ppm. Mortality of 76.66. The LC_{50} values were 0.007. With respect to LC_{50} values, the toxicity of Spinosad (1.1428). On the basis of LC_{90} values, the order of toxicity of insecticides retained the same as in LC_{50} values. The LC_{90} value of Spinosad was 0.019 ppm. Thus at 24, 48 and 72 hours the most toxic insecticide was Spinosad.

Thus, at both 24 and 48 hours, the most toxic insecticide was Spinosad. The toxicity was more at 48 hours compared to 24 hours after treatment. This is in accordance to the findings of Lokare *et al*. (1999) [7] who have confirmed that the toxicity increases with the increase in the period of exposure. The toxicity was slightly more at 48 hours as compared to 24 hours after treatment and toxicity at 72 hours was slightly more as compared to 48 hours after treatment with consequent decrease in LC50 and LC99.9 values this is accordance to the findings of Ramesh Babu *et al*. (2018) [9] who have confirmed that the toxicity increases with the increase in the period of exposure with consequent decrease in LC50 and LC99.9 values. Similarly, Sanon *et al*. (2010) [10] who identified the effectiveness of Spinosad in controlling *Callosobruchus maculates* which exhibited high mortality and decreased in the number of eggs laid by females. After 6 months of storage, the number of insects emerging from cowpea seeds was reduced by > 80% by Spinosad treatment.

Summary and Conclusion

The studies included development of homogenous population and determination of relative toxicity of different insecticides against adult of *C. chinensis* after 24, 48 and 72 hours after the treatment. In this experiment, serial dilutions of the insecticides were prepared using distilled water. After that, the distilled water was used as a control purpose and then was allowed to evaporate for 10 minutes in order to make sure it fully dried. Then, the filter paper that have been cooperate with the insecticides were placed in the petri dish with 20 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 measure the relative toxicity of the selected insecticides. Mortality was assessed after 24, 48 and 72 hours after. The mortality data was subjected to probit analysis (Finney, 1971) [5] and LC50, LC90, heterogeneity ($\chi2$), intercept (a), slope of regression (b) and regression equation were calculated. The results revealed that Spinosad exhibited the highest toxicity both at 24, 48and 72 hours. The LC50 values of Spinosad was 0.011 ppm was 0.009. at 48 hours and 0.007 ppm.

Conclusion

Spinosad unique and non-cross resistant mode of action will make it a valuable new tool in stored grain resistance management programs. In anticipation of Spinosad widespread use, additional baseline studies on the natural variation in susceptibility of stored product pest species to Spinosad are needed. These results will prove invaluable in future years to help separate emerging resistance issues from the normal background of genetic or geographic variation already known to exist among non-selected, susceptible insect species. Studies that generate dose-response curves and lethal concentration estimates on different pest/grain. if utilized a standard set of experimental protocols and evaluation conditions (temperature, humidity, life stage, and exposure period) that facilitated comparison of results across studies. Spinosad will represent a valuable new tool in the limited arsenal of grain protectant products, both for organic and non-organic grain. It's a set of broad pest spectrum, low mammalian toxicity, persistence, and sound environmental profile will be unique among existing grain protectant products and can positively impact global food security.

References

1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology.

1925;18:265-267.
2. Anonymous. Pulse India. All India pulses and grains association publication, 2019 July-September, 4(2).
3. Anonymous. World Development Report. Published for the World Bank, Oxford University Press, 1999.
4. Chakraborty S, Mondal P. Studies on the biology of pulse beetle (*Callosobruchus chinensis* L.) infesting cowpea. International Journal of Current Research. 2015;7(12):2351223515.
5. Finney DJ. Probit analysis, Cambridge University Press, London, 1971, 109.
6. Kumar D, Kalita P. Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. Foods. 2017;6:8.
7. Lokare RU, Chandele AG, Kharbade SB. Avermectins, a class of compounds: implications for use in arthropode pest control. Annual Review of Entomology. 1999;36:91-117.
8. Maneepun S. Traditional processing and utilization of legumes. In: Shanmugasundaram S (Ed.), Report of the APO Seminar on Processing and Utilization of Legumes, Japan, 9–14 October 2000. Asian Productivity Organization, Tokyo, 2003, 53-62.
9. Ramesh Babu S, Sai Ram Kumar DV, Madhumathi T. Toxicity of newer insecticide molecules against lesser grain borer, Rhyzopertha dominica (Fabricius) (Bostrichidae: Coleoptera) J of Entomology and Zoology Studies. 2018;6:2340-2344.
10. Sanon A, Niango BM, Binso-Dabire CL, Pittendrigh BR. Effectiveness of Spinosad (Naturalytes) in controlling the cowpea storage pest, Callosobruchus maculatus (Coleoptera: Bruchidae). J of Economic Entomology. 2010;103(1):203-209.
11. Srinivasan T, Duraimurugan P, Singh SK, Chattopadhyay C. Bruchids infestation in pulses and its management. Indian Farming. 2010;60:1316.
12. Tiwari BK, Singh N. Pulse chemistry and technology. Royal Society of Chemistry, Cambridge, Royal Society of Chemistry, 2012, 310.