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Laboratory Evaluation of Insecticide Toxicity on the Larval Ectoparasitoid, *Habrobracon hebetor* (Say) (Braconidae : Hymenoptera) of Maize Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera : Noctuidae)

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Abstract

Different insecticides are recommended for field use in maize ecosystem against *Spodoptera frugiperda* (J. E. Smith) and other sucking pests. Toxicity of four fold and two fold of the field recommended concentration were determined against the adults of a larval parasitoid, *Habrobracon hebetor* (Say). Emamectin benzoate 5 per cent SG, Spinetoram 11.7 per cent SC, Spinosad 45 per cent SC, Chlorantraniliprole 18.5 per cent SC, Imidacloprid 17.8 per cent SL were used. The higher dose of field recommended concentration (FRC) of spinetoram and spinosad gave 100 per cent mortality in the test insect after 48 hours of application, while at lower and recommended dose rates 100 per cent mortality was recorded after 72 hours of application. Meanwhile, insecticide treatments with emamectin benzoate, chlorantraniliprole and imidacloprid at different doses, were ranked harmless with less than 50 per cent mortality at all the interval of observation after their application. The FRC of all the above insecticides are also tested on *H. hebetor.* Except spinetoram and spinosad all the insecticides tested are safe to the test insect. Spinetoram and spinosad were harmful to *Habrobracon hebetor* (Say).

Keywords : *Habrobracon hebetor*, Insecticides, Mortality, *Spodoptera frugiperda*

Habrobracon hebetor is most widely used gregarious, polyphagous ectoparasitoid which parasitises many lepidopteran larvae. *H. hebetor* females first paralyse the later-instar larvae of their host in a 'wandering' phase by injecting paralytic venom and ovipositing variable numbers of eggs on or near the surface of paralyzed host (Ghimire and Phillips, 2010). It is an important natural enemy and the most promising biological agent of many important lepidopteran pests of stored products *i.e.,* rice moth, *Corcyra cephalonica* as well as field crops like *Spodoptera litura, Helicoverpa armigera*, *Spodoptera frugiperda* (Sreelatha *et al.,* 2019). Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera : Noctuidae) is a serious pest of maize all around the world. This pest also has been reported recently from India in 2018 (Behera and Mohan, 2021) and become notorious pestiferous insect with high dispersal ability, wide host range and high fecundity that makes it one of the most severe pest causing economic loss to farmers (Shylesha *et al*., 2018).

Insecticides are still the front line defence and vital component of the integrated pest management strategy (Akkanna and Naik, 2019). Bio-control agent alone cannot manage a pest species effectively and quickly. So the effective combination of conventional chemical and biological control strategies is critical for the success of an integrated pest management (IPM) programme (Pedigo, 1999 & Adan *et al.,* 2011). Commonly used synthetic insecticides such as organophosphates, carbamates and pyrethroids are broad-spectrum toxicants that cause significant non-target mortality to natural enemies, especially adult parasitoids (Hill & Foster, 2000 and Haseeb *et al.,* 2005). Moreover, predators and parasitoids commonly are more sensitive to toxicants than their prey (Croft, 1990). Thus, should chemical insecticides be incorporated into an IPM program, they should be used only when necessary and when it is least disruptive to the biological control approach (Wang *et al.,* 2008). Biological control offers environmentally friendly and sustainable solutions to a variety of insect pest problems. However, effective plant protection that relies solely on biological control and is hard to achieve without the help of conventional chemical insecticides (Kanzaki and Tanaka, 2010).

Mortality is one of the major parameter that is used to determine the effect of an insecticide against natural enemies. As part of an IPM program, the utilization of selective pesticides is a reasonable strategy because it favours the conservation of natural enemies in the agro-ecosystem (Carvalho *et al*., 2003). In this regard, the availability of highly selective insecticides is essential (Croft, 1990).

The objective of this study was to assess the acute effects of some insecticides which are recommended and used in the management of *S. frugiperda* and other sucking pests in the maize ecosystem on the larval ectoparasitoid, *H. hebetor.* The findings provide an estimate of population-level effects and help to determine how compatible these pesticides are with *H. hebetor* in IPM programs.

Material and Methods

Raising of Insect Cultures

Adults of *H. hebetor* were obtained from insect colonies maintained at the ICAR-National Bureau of Agricultural Insect Resources, Bengaluru. The colony was maintained in the laboratory at 26±1°C, 60±5 per cent RH, using last instar larvae of *Corcyra cephalonica* (Lepidoptera : Pyralidae) as host. Adult parasitoid wasps were reared for several generations in the laboratory. Newly emerged female parasitoids (<24 h old) were used to the conduct experiments. Adult wasps were provided with cotton swab soaked in 30 per cent honey solution for feeding of the parasitoid.

Chemical Compounds

The insecticides used in this experiment were Emamectin benzoate 5 per cent SG, Spinetoram 11.7 per cent SC, Spinosad 45 per cent SC, Chlorantraniliprole 18.5 per cent SC, Imidacloprid 17.8 per cent SL and Azardirachtin 1 per cent EC. Information about the insecticides is listed in Table 1.

Table 1

Insecticides screened for toxicity to *H. hebetor*

Chemical name	Formulation	Dose/ lit of water
Spinetoram	11.7% SC	0.5 ml
Emamectin benzoate	5% SG	0.4 g
Chlorantraniliprole	18.5% SC	0.4 ml
Imidacloprid	17.8% SL	0.3 ml
Spinosad	45% SC	0.3 ml
Azardirachtin	1% EC	2 ml

SC - Solution Concentrates; EC - Emulsion Concentrates; SG - Soluble Granule; SL - Soluble Liquid

The stock solution of each formulated insecticide was prepared at a concentration that reflected four fold and two fold of the field recommended concentration (FRC) in maize. The FRC of Emamectin benzoate, Spinetoram, Spinosad, Chlorantraniliprole and Imidacloprid were 0.4g, 0.5ml, 0.3ml, 0.4ml, 0.3ml / litre of water, respectively. The stock solution of each insecticide were prepared at a concentration of 160 ppm for Emamectin benzoate, 464 ppm for Spinetoram, 1080 ppm for Spinosad, 592 ppm for Chlorantraniliprole and 427 ppm for Imidacloprid. Aliquot was taken from the stock solution mixed with distilled water and prepared five concentrations of each insecticidal concentration by serial dilution. For Emamectin benzoate (80, 40, 20, 10, 5 ppm / 100 ml), Spinetoram (240, 120, 60, 30, 15 ppm / 100ml), Spinosad (540, 270, 135, 67.5, 33.75 ppm / 100ml), Chlorantraniliprole (296, 148, 74, 37, 18.5 ppm / 100ml) and Imidacloprid (213.6, 106.8, 53.4, 26.7, 13.35 ppm / 100ml). Control was treated with distilled water alone that was used to assess the natural mortality in the test insects.

Bioassay

Bioassays studies were performed with young adults (24 h post eclosion) according to the standard methods to test the side-effects of pesticides on braconidae, method No. 5.1.6 developed by the lOBC/WPRS working group 'Pesticides and Beneficial Organisms' with some modifications. Uncontaminated fresh maize leaves were collected from the unsprayed field, washed thoroughly with running water and shade dried. Further, the maize leaves were cut into pieces approximately (2cm x 10cm) size. The test concentration of insecticides was prepared by using distilled water and the leaves were dipped in the insecticide solution for 10 seconds by using forceps. Then the leaves were allowed to dry completely on filter paper. The treated leaves were placed in a test tubes (2cm x 15cm) containing 10 adults per test tube and triplicate was maintained for each concentration along with control. The mouth of the test tube was plugged with non absorbent cotton to prevent the escape of the parasitoids. After one hour of exposure to the treatment cotton swab dipped in 30 per cent honey solution was provided as a food for the parasitoids. The vials were placed in the growth chamber at 26±1°C, 60±5 per cent RH. The numbers of dead and live wasps were counted after exposure period of 24, 48 and 72 hours. Those parasitoids that appeared extremely lethargic or unable to maintain equilibrium at this time also were recorded as dead. The insecticides were categorized as four evaluation categories based on eco-toxicological tests defined by the International Organization for Biological Control (IOBC) such as 1) harmless (< 50 %); 2) slightly harmful (50-79 %); 3) moderately harmful (80-99%) and 4) harmful (> 99%) (Hassan *et al*., 1985).

Statistical Analysis

Data obtained were subjected to ANOVA ($p < 0.05$) after checking for normality. Per cent means were compared by Tukey's test, admitting significant differences at $p < 0.05$. For all the analyses, OPSTAT software (COBS & H CCS HAU, Hissar) was used.

RESULTS AND DISCUSSION

The data on per cent mortality of newly emerged adults of *H. hebetor* at 24, 48 and 72 hours of treatment application with different insecticides at different doses was analysed statistically and presented in Table 2 and Fig.1. All the treatments with different doses of insecticides gave significant mortality of adult parasitoid after different time intervals as compared to untreated check. It is observed from the data, that the doses (two fold and four fold above and below the field recommended concentration) of Spinetoram and Spinosad, ranked as highly toxic, with mortality 100 per cent in treatments after 72 hours of observation in both the insecticides at all the concentration even at lower concentrations. Whereas, same insecticides at 24 hours of observation were less harmful to moderately harmful to the test insects. At 48 hours of observation higher dose above the field recommended concentration were harmful with 100 per cent mortality and below FRC are moderately harmful with (80 - 86.66%) in Spinetoram and (83.3 - 86.66%) mortality in Spinosad. At 24 hours of observation above the FRC were moderately harmful with (83.3 - 90%) mortality and below FRC were harmless to slightly harmful (43 - 70%) in Spinetoram. In case of Spinosad the rank varies from slightly harmful to moderately harmful (53 - 83%) at 24 h of observation.

All the doses of Emamectin benzoate, Chlorantranili prole, Imidacloprid were proved to be safe / harmless with less than 50 per cent mortality of *Habrobracon hebetor* at 24, 48 and 72 hours of observation. Finally, minimum toxicity (0.000 ± 0.000) to *H. hebetor* was, however, found in the untreated control.

The data on per cent mortality of *H. hebetor* adults were recorded after 24, 48 and 72 h of treatment with the different insecticides which had been applied at field recommended concentrations (FRC) analysed statistically and presented in Table 2. Field recommended concentrations of the chemicals significantly affected the adults of parasitoids at 12h, 24h and 48h (Table 2). The per cent mean mortality related to pesticide treatments and the control group as shown in Fig. 2.

Utilisation of biological control agents in IPM is very important. It is also necessary to take into consideration, the adverse impact of chemical pesticides on natural enemies used in fields. For this

TABLE 2

Percentage mortality in *Habrobracon hebetor* at different time intervals of the different concentration of insecticide applications

Insecticide	Dose/lit (gm or ml)	Treatments	Mean Mortality (%) After 24 hrs of treatment application	Mean Mortality(%) After 48 hrs of treatment application	Mean Mortality(%) After 72 hrs of treatment application
Spinetoram	2 ml	T1	90 ± 5.774 a (74.970 ± 7.854)	100 ± 0.00 a (89.960 ± 0.00)	100 ± 0.00 a (89.960 ± 0.00)
	1 ml	T2	86.66 ± 3.33 ab (68.830 ± 2.710)	100 ± 0.00 a (89.960 ± 0.00)	100 ± 0.00 a (89.960 ± 0.00)
	0.5 ml	T3	83.333 ± 3.33 b (66.120 ± 2.710)	86.66 ± 3.33 b (68.830 ± 2.710)	100 ± 0.00 a (89.960 ± 0.00)
	0.25 ml	T4	70 ± 5.774 d (56.977 ± 3.656)	83.333 ± 3.33 bc (66.120 ± 2.710)	100 ± 0.00 a (89.960 ± 0.00)
	0.125 ml	T5	43.333 ± 3.33 f (41.140 ± 1.920)	80 ± 000 c (63.410 ± 0.00)	100 ± 0.00 a (89.960 ± 0.00)
Emamectin benzoate	1.6 g	T6	30 ± 5.774 g (32.990 ± 3.659)	33.33 ± 3.33 d (35.207 ± 2.007)	50 ± 5.774 b (44.983 ± 3.328)
	0.8 g	T7	20 ± 5.774 hi (26.060 ± 4.271)	30 ± 5.774 de (32.990 ± 3.659)	43.333 ± 6.667 c (41.053 ± 3.952)
	0.4 g	T8	16.667 ± 3.33 ij (23.843 ± 2.707)	20 ± 0.00 gh (26.550 ± 0.007)	36.667 ± 8.819 d (36.917 ± 5.444)
	0.2 g	T9	6.667 ± 3.33 lm (12.287 ± 6.143)	10 ± 5.774 i (14.993 ± 7.855)	13.333 ± 3.333 h (21.137 ± 2.707)
	0.1 g	T10	3.333 ± 3.33 mn (6.143 ± 6.143)	3.333 ± 3.33 j (6.143 ± 6.143)	3.333 ± 3.333 ij (6.143 ± 6.143)
Chlorantraniliprole	1.6 ml	T11	23.333 ± 3.33 h (28.767 ± 2.217)	26.66 ± 3.33 ef (30.983 ± 2.217)	33.333 ± 3.333 de (35.207 ± 2.007)
	0.8 ml	T12	16.667 ± 3.33 ij (23.843 ± 2.707)	23.333 ± 6.667 fg (28.277 ± 4.923)	26.667 ± 3.333 fg (30.983 ± 2.217)
	0.4 ml	T13	13.333 ± 3.33 jk (21.137 ± 2.707)	20 ± 5.774 gh (26.060 ± 4.271)	23.333 ± 3.333 g (28.767 ± 2.217)
	0.2 ml	T14	6.667 ± 3.33 lm (12.287 ± 6.143)	10 ± 0.00 i (18.430 ± 0.00)	16.667 ± 3.333 h (23.843 ± 2.707)
	0.1 ml	T15	3.333 ± 3.33 mn (6.143 ± 6.143)	3.333 ± 3.333 j (6.143 ± 6.143)	3.333 ± 3.333 ij (6.143 ± 6.143)
Imidacloprid	1.2 ml	T16	13.333 ± 3.33 jk (21.137 ± 2.707)	26.667 ± 3.333 ef (30.983 ± 2.217)	36.667 ± 3.333 d (37.213 ± 2.007)
	0.6 ml	T17	10 ± 00 kl (18.430 ± 0.00)	16.667 ± 3.333 h (23.843 ± 2.707)	30 ± 00 ef (33.200 ± 0.00)

Insecticide	Dose/lit (gm or ml)	Treatments	Mean Mortality (%) After 24 hrs of treatment application	Mean Mortality(%) After 48 hrs of treatment application	Mean Mortality(%) After 72 hrs of treatment application
	0.3 ml	T18	6.667 ± 3.33 ^{lm} (12.287 ± 6.143)	16.667 ± 3.333 ^{h} (23.843 ± 2.707)	26.667 ± 3.333 ^{fg} (30.983 ± 2.217)
	0.15 ml	T19	3.333 ± 3.33 ^{mn} (6.143 ± 6.143)	3.333 ± 3.333 ^{j} (6.143 ± 6.143)	6.667 ± 3.33 ^{i} (12.287 ± 6.143)
	0.075 ml	T20	00 ± 00 ^{n} (0.00 ± 0.00)	00 ± 00 ^{j} (0.00 ± 0.00)	3.333 ± 3.33 ^{ij} (6.143 ± 6.143)
Spinosad	1.2 ml	T21	83.333 ± 3.33 ^{b} (66.120 ± 2.710)	100 ± 0.00 ^{a} (89.960 ± 0.00)	100 ± 0.00 ^{a} (89.960 ± 0.00)
	0.6 ml	T22	76.667 ± 3.33 ^{c} (61.197 ± 2.210)	100 ± 0.00 ^{a} (89.960 ± 0.00)	100 ± 0.00 ^{a} (89.960 ± 0.00)
	0.3 ml	T23	66.667 ± 3.33 ^{d} (54.763 ± 2.007)	86.667 ± 3.333 ^{b} (68.830 ± 2.710)	100 ± 0.00 ^{a} (89.960 ± 0.00)
	0.15 ml	T24	56.667 ± 3.33 ^{e} (48.827 ± 1.923)	83.333 ± 3.333 ^{bc} (66.120 ± 2.710)	100 ± 0.00 ^{a} (89.960 ± 0.00)
	0.075 ml	T25	53.333 ± 3.33 ^{e} (46.903 ± 1.923)	83.333 ± 3.333 ^{bc} (66.120 ± 2.710)	100 ± 0.00 ^{a} (89.960 ± 0.00)
Distilled water	Control	T26	00 ± 00 ^{n} (0.00 ± 0.00)	00 ± 00 ^{j} (0.00 ± 0.00)	00 ± 00 ^{j} (0.00 ± 0.00)

Values within column followed by the different letters are significantly different. ANOVA with Tukey range test ($p < 0.05$).
Figures in the parentheses are arcsine transferred values; Numbers with same alphabets are statistically on par

reason, our study evaluated the effects of some insecticides used in Maize fields on larval ectoparasitoid, *H. hebetor* under laboratory condition.

The effects of different insecticides on adult parasitoids showed at different times, that the mean mortality of parasitoid increased with time. With each pesticide treatment, mortality increased with the increase of time - from 12 to 72 h (Fig. 1 and Fig. 2) these results are onpar with the results of Rasool *et al.*, 2005. They studied the impact of pesticides on the parasitoid *H. hebetor*. They reported that as time increased, mortality also increased due to insecticides, and this may indicate a direct relationship between these two parameters.

The present investigations undertaken showed that Spinosad and Spinetoram are highly toxic on natural enemies than the Emamectin benzoate, Chlorantraniliprole, Imidacloprid and Azardirachtin. Similar results were mentioned by Abbes *et al.* (2015) who reported the non-target effects of Spinetoram and Spinosad cause 100 per cent mortality of *Bracon nigricans* and also reported that Chlorantarniprole is the safest among the four tested insecticides (Spinetoram, Spinosad, Cyantraniliprole). However, Kovalankov (2002) reported that Spinosad exhibited marginal to excellent selectivity but was highly toxic to *Bracon mellitor*. Emamectin benzoate is less toxic or slightly harmful to the *H. hebetor* under laboratory conditions similar results were given previously by Khan *et al.* (2009). Rafiee-Destjerdi *et al.* (2009) reported that pyrethroid pesticides (such as Imidacloprid) are less toxic to adults of *H. hebetor*. It is also confirmed in case of *B. brevicornis* adults, toxicity recorded in Imidacloprid, was low (32.43%

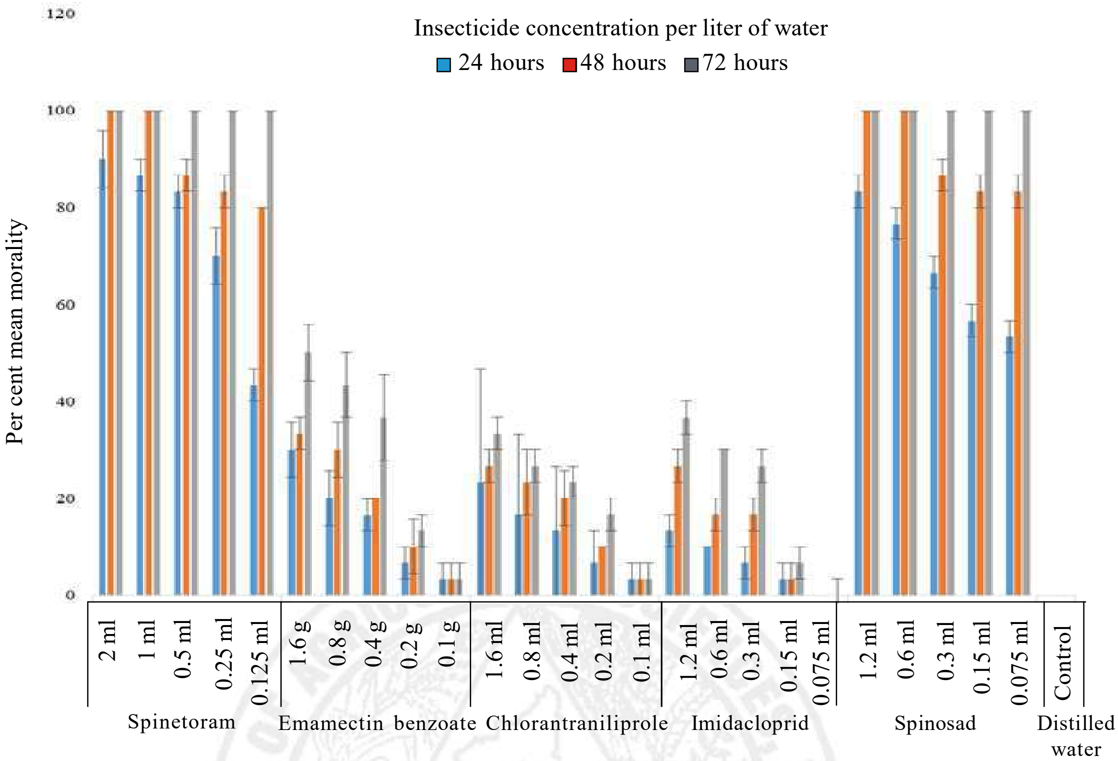

Fig. 1: Per cent mortality in adults of *Habrobracon hebetor* at 24, 48 and 72 h time -intervals at different concentration of insecticide applications

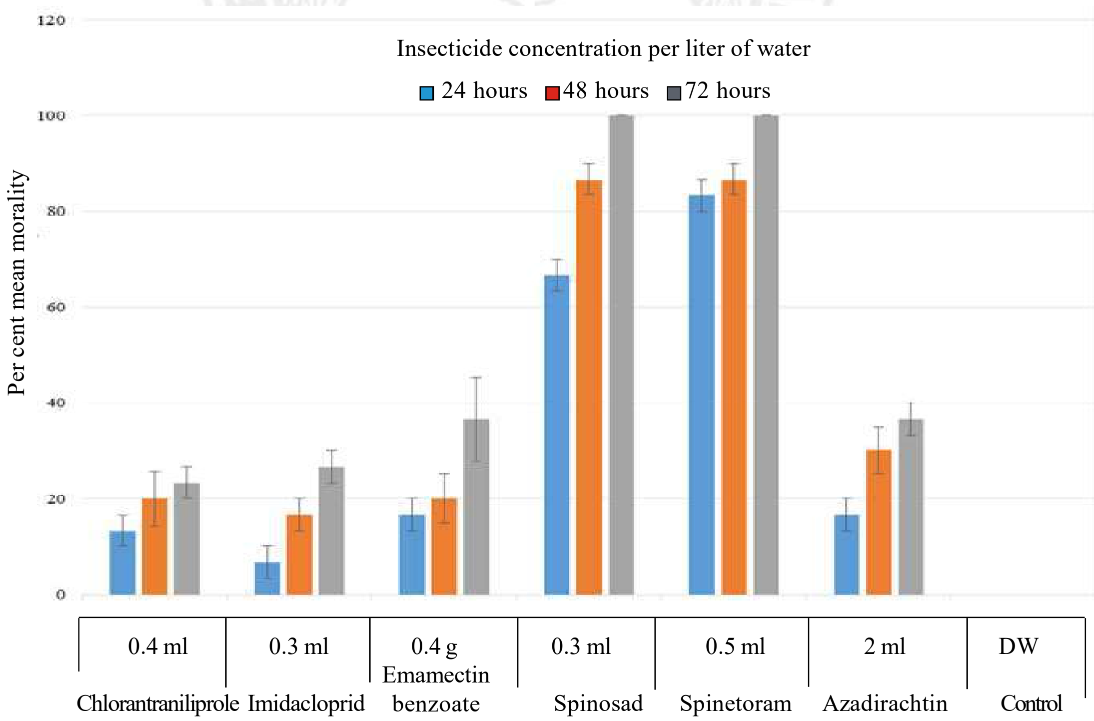

Fig. 2 : The percent mortality of parasitoid adults exposed to FRC of insecticides and control treatments at 24, 48 and 72 h after application

TABLE 3

Per cent mortality in *Habrobracon hebetor* after different time intervals of the field recommended concentration (FRC) of different insecticide applications

Treatment	Dose/ litre of water	Percent mortality at different times after application		
		24 hours	48 hours	72 hours
Chlorantraniliprole	0.4 ml	13.33 ± 3.33 [c] (21.137 ± 2.707)	20 ± 5.77 [c] (26.060 ± 4.271)	23.33 ± 3.33 [c] (26.060 ± 4.271)
Imidacloprid	0.3 ml	6.66 ± 3.33 [d] (12.287 ± 6.143)	16.66 ± 3.33 [c] (23.843 ± 2.707)	26.66 ± 3.33 [c] (30.983 ± 2.707)
Emamectin benzoate	0.4 g	16.66 ± 3.33 [c] (23.843 ± 2.707)	20 ± 5.132 [c] (26.550 ± 0.007)	36.66 ± 8.81 [b] (36.917 ± 5.444)
Spinosad	0.3 ml	66.66 ± 3.33 [b] (54.763 ± 2.007)	86.66 ± 3.33 [a] (68.830 ± 2.710)	100 ± 00 [a] (89.960 ± 0.000)
Spinetoram	0.5 ml	83.33 ± 3.33 [a] (66.120 ± 2.710)	86.66 ± 3.33 [a] (68.830 ± 2.710)	100 ± 00 [a] (89.960 ± 0.000)
Azadirachtin	2 ml	16.66 ± 3.33 [c] (23.843 ± 2.707)	30 ± 4.89 [b] (32.990 ± 3.569)	36.66 ± 3.33 [b] (37.213 ± 2.007)
Control	DW	00 ± 00 [e] (0.00 ± 0.00)	00 ± 00 [d] (0.00 ± 0.00)	00 ± 00 [d] (0.00 ± 0.00)

Values within column followed by the different letters are significantly different. ANOVA with Tukey range test ($p < 0.05$).
Figures in the parentheses are arcsine transferred values; Numbers with same alphabets are statistically on par

mortality). Azardirachtin was found to be the safest (20.08% mortality) (Saha *et al.,* 2017). Finally, minimum toxicity (0.000 ± 0.000) to *H. hebetor* was, however, found in the untreated control.

According to the results, Spinosad and Spinetoram used at various doses, had the most adverse effect on *H. hebetor* adults and the Emamectin benzoate, Chlrantraniliprole, Imidacloprid and Azardirachtin treatments had lower toxicity. The results of our study showed that among the studied insecticides, the Spinosad and Spinetoram treatments were associated with the highest mortality. Spinosad and Spinetoram could be avoided and Emamectin benzoate can be used as compatible chemical insecticide for the management of fall army worm, *S. frugiperda* along with biological control agents in integrated pest management (IPM) programs.

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