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Evaluation of Indigenous Strains of Entomopathogenic Nematodes, in Combination with Low-Toxicity Insecticides for Control of Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae)

Indra Kumar Kasi[1], Kanchhi Maya Waiba[2], Gurveer Singh[3], Abhishek Bhat[3], Hemanth Kumar Kashyap[3], Elahe Rostami[4] and Robin[3]

[1]Dept. of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Himachal Pradesh (173 230), India
[2]Dept. of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University Varanasi, Uttar Pradesh (221 005), India
[3]Dept. of Entomology, Punjab Agricultural University, Ludhiana, Punjab (141 027), India
[4]Institute for Biological Research at Institute of Quality Standards & Testing Technology for Agro-Products, Fujian Academy of Agricultural Sciences (FAAS), China

Corresponding robin-2107008@pau.edu

0000-0001-9072-2813

ABSTRACT

A study was conducted during November, 2021 to evaluate the efficacy of local strains of *Steinernema feltiae* and *Heterorhabditis bacteriophora* in combination with low-toxicity insecticides at low and high dosages to control fifth-instar larvae in under laboratory condition. The experimental location in Department of Entomology, Nematology Laboratory, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India. The use of *S. feltiae*+Spinosad or chlorantraniliprole caused larvae mortality of over 90% at 96 h and *H. bacteriophora*+Spinosad or Chlorantraniliprole mortality caused of over 95% mortality at 96 h after treatment at the high dose and may be considered as a least toxic strategy to control fall armyworm. Our results showed that these strains of *H. bacteriophora* have high pathogenicity against *S. frugiperda* and have potential for biological control in integrated approaches causing even combination with low-toxicity insecticides at low and high dosages may be considered 90% mortality fifth instar larval of *S. frugiperda* at 96 h at the high dose as a least toxic strategy to control fall armyworm. The use of *S. feltiae*+Spinosad or chlorantraniliprole and *H. bacteriophora*+Spinosad or chlorantraniliprole caused larvae mortality of over 90% high dose and should be included as a least toxic strategy to control fall armyworm. Native strains of entomopathogenic nematodes are active against *S. frugiperda* shows potential alternative to the severe use of chemical insecticides to control fall armyworm population in corn plantations.

KEYWORDS: Chlorantraniliprole, *H. bacteriophora*, *S. feltiae*, Spinosad, *Spodoptera frugiperda*, Synergistic effect

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1. INTRODUCTION

Globally, fall armyworm; *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is one of the most severe pests in corn, *Zea mays* L. (Poaceae) (Belay et al., 2012). For instance, are reported multiple or cross-resistance in *S. frugiperda* organophosphates and *Bt* in fall armyworm (Zhu et al., 2015). Furthermore, there is a reported resistance in fall armyworm to Cry1Fa proteins of *Bt* from Florida, respectively (Blanco et al., 2010, Huang et al., 2014, Waiba et al., 2021a). Similarly, resistance to Cry1F (Monnerat et al., 2015, Santos-Amaya et al., 2016). Organophosphates and pyrethroids were reported in fall armyworm populations from Brazil (Carvalho et al., 2013). Also, the indiscriminate use of high-toxicity insecticides has harmful effects on humans and the environment (Grewal et al., 2005, Malhat et al., 2015).

For instance, methoxyfenozide, and Spinosad killed over 80% fall armyworm population from Florida (Belay et al., 2012). Furthermore, the entomopathogenic nematode *S. carpocapsae* (Weiser) (Nematoda: Steinernematidae) also has been used to control fall armyworm through low levels of host infectivity (from 1–28%) (Espky et al., 1994). As with other biological agents, adverse environmental conditions or the absence of enough fall armyworm larvae for the establishment and propagation of *S. carpocapsae* might be the major causes of lower % of mortality.

Entomopathogenic nematodes (EPNs) from the families, Heterorhabditidae and Steinernematidae are soil-inhabiting organisms that are obligate insect parasites in nature (Kaya and Gaugler, 1993, Kasi et al., 2020, Kasi et al., 2021b, Kasi et al., 2022). And which are caused in the intestine and infective juveniles (Bird and Akhurst, 1983, Arthurs et al., 2004, Kasi et al., 2021a). Nematodes locate their potential host by following insect cues (Lewis et al., 2006, Waiba et al., 2021b) After IJs locate a host, they infect it through an orifice such as the anus, mouth, or spiracles or by penetrating the cuticle (particularly in *Heterorhabditis* spp.). Once IJs enter the host, they shed their outer cuticle (Sicard et al., 2004), and begin ingesting hemolymph, which triggers the release of symbionts by defecation (in *Steinernema* spp.) or regurgitation (in *Heterorhabditis* spp.) (Molina et al., 1996, Martens et al., 2004, Vysali et al., 2021). The nematode–bacteria complex kills the host within 24–48 h through septicemia or toxemia (Dowds and Peters, 2002, Forst and Clarke, 2002). Bacteria recolonize the nematodes, which emerge as IJs from the depleted insect cadaver in search of new hosts (Poinar, 1990).

Additionally, differences among EPN species in host-seeking strategy and tolerance to environmental conditions such as temperature and desiccation can determine the field efficacy of EPNs (Martens et al., 2004, Sharma et al., 2022, Monika et al., 2022, Kasi et al., 2022). EPNs have been extensively used in the biological control of a variety of economically important pests occupying different habitats (Grewal et al., 2005). However, EPN formulations to desiccation and/or the addition of adjuvants to increase leaf coverage and persistence of the IJs have enhanced the use of EPNs against foliar pests (Williams and Walters 2000, Arthurs et al., 2004, Head et al., 2004). Susceptibility of *S. frugiperda* to EPNs has been frequently reported (Molina et al., 1996, Doucet et al., 1999, Garcia et al., 2008, Andalo et al., 2010, Kasi and Waiba 2022).

The use of insecticide combinations in the same spray tank is a common practice for control fall armyworm (Negrisoli et al., 2010). However, insecticide compatibility, synergistic or antagonistic effects, and optimum dosages need to be assessed by bioassays before their use in the field. Our objective was to evaluate the efficacy in combination with low-toxicity insecticides at low and high dosages to control fall armyworm larvae under laboratory conditions.

2. MATERIALS AND METHODS

2.1. *Entomopathogenic nematodes*

The native isolate was obtained from soil samples, collected from survey was carried out in Mid-hills of the North Himalayas, Rajgarh, Himachal Pradesh, India. Two strains of *S. feltiae* and *H. bacteriophora* were used in this study during the survey in November, 2021. This native isolated locally, from north India, isolate was obtained from soil samples, collected from Rajgarh, Himachal Pradesh, India using *G. mellonella* larvae as nematode traps. These isolates were cultured at 25±1° C temperature in an incubator on the last instar larvae of *G. mellonella*. Infective juveniles (IJs) that emerged during the first ten days were collected from white traps and stored at 4° C in distilled water for up to 14 days (Kaya and Gaugler, 1993). The nematodes were acclimatized at room temperature for about 30 min before being used in the experiments.

2.2. *Insects*

To establish the initial *S. frugiperda* colony, larvae were collected from areas of cornfields in the Deptt. of Vegetable Science, Dr. YSPUHF, Nauni, Himachal Pradesh, India. And then placed in test tubes of 15 cm in height and 1.5 cm in diameter, containing a young corn plant diet. After transforming into pupae, they were sexed and transferred to PVC tubes of 10 cm in diameter and 20 cm in height, with the tube extremities plugged with a voile-type fabric and sealed with elastic (Figure 1). The tubes were internally coated with filter paper so that the females could oviposition, just after the emergence of adults. The moths were fed daily with 10% honey isolates placed in the tube with cotton. The sterilized Petri dishes with an artificial diet and, when the

Figure 1: Lepidopteran insect adults egg Laing and egg hatching in cages

newly hatched caterpillars were later obtained, they were individualized in test tubes with the same diet (Figure 2).

Figure 2: After 1st instar larvae hatching *S. frugiperda* individual rearing in PVC tubes

2.3. Methodology

One larva was placed in a 20 ml plastic cup containing a young maize cob material diet (Natural feed). Low and high registered label dosages (converted to lab dosages) of 2 biological agents, 3 synthetic insecticides, and the biological agent+low-toxicity insecticides were used (Table 1). 15 larvae were treated topically with 200 μl of insecticide solution per dosage. The control was treated only with distilled water. Treated cups were held in a randomized complete block design with 4 replications (total n=40 larvae dosage^{-1}) in the lab at 18–20°C and photoperiod 12:12 h (L:D). Larval mortality was evaluated between 24 and 96 h after application. In separate assays insecticide dilutions of ¼x, ½x, x, ½y, and y were applied to 60 larvae treatment^{-1} to calculate the lethal dosages (LD_{50}) of chlorantraniliprole and Spinosad at 96 h (x=low dosage and y=high dosage equals maximum registered and described in Table 1) (Viteri et al., 2018, Kasi et al., 2022).

2.4. Data analysis

Abbott's formula (Fleming and Retnakarn, 1985), was used to correct the data for control larval mortality in the bioassays, and PROBIT analysis was conducted. Also, LSD (p<0.05) values were calculated to differentiate means among treatments.

3. RESULTS AND DISCUSSION

3.1. Bioassays with S. feltiae+S. frugiperda+Insecticides

In general, a higher mean percent mortality was noted at higher dosages (F=61.77; df=12; p<0.05) (Table 2). However, *S. feltiae* and Spinosad caused 80–97% of larvae mortality with low and high dosages at 96 h. The use of chlorantraniliprole and Spinosad resulted in % of mortality up to 97.50% at 96 h at the higher dosage (Figure 3). These results are different from those reported (Belay et

Table 1: Active ingredients and laboratory dosages to evaluate the efficacy of 2 biological agents, 3 synthetic insecticides, and least toxic combinations of insecticides to control fall armyworm [*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)] larvae

Insecticides	Commercial name* Active ingredient and %	Low dosage		High dosage	
		g or ml l^{-1}	PPM	g or ml l^{-1}	PPM
Bio agent (nematode)					
Steinernema feltiae (Sf)+rape seed oil 85%	Local strain+Addit	1.1 g+2.5 ml	1134+2500	2.2 g+2.5 ml	2268+2500
H. bacteriophora (Hb)+rape seed oil 85%	Local strain+Addit	1.1 g+2.5 ml	1134+2500	2.2 g+2.5 ml	2268+2500
Insecticides					
Chlorantraniliprole 18.4%	Coragen®	0.8 ml	800	0.16 ml	1600
Indoxacarb 14.5%	Syngenta	0.12 ml	120	0.24 ml	240
Spinosad 2.5%	MH Dow Success®	0.2 ml	200	0.4 ml	400
Combinations					
Chlorantraniliprole+SF+oil	---	0.8+1.1+2.5	---	0.16+2.2+2.5	---
Indoxacarb+SF+oil	---	0.12+1.1+2.5	---	0.24+2.2+2.5	---
Spinosad+SF+oil	---	0.2+1.1+2.5	---	0.4+2.2+2.5	---

Manufacturers: DuPont (Coragen), Bayer (Indoxacarb), Syngenta (Spinosad)

Table 2: % of mortality of fall armyworm [*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)] larvae in the fifth-instar at low and high dosages of 3 insecticides, and least toxic combinations evaluated from 24–96 h

Insecticides	24 h		48 h		72 h		96 h		Interaction
	Low	High	Low	High	Low	High	Low	High	
Bio agent (nematode)									
Steinernema feltiae (Sf)	15.00	22.50	27.50	40.00	40.00	52.50	52.50	60.00	(+)
Toxicity insecticides									
Chlorantraniliprole	4.50	7.50	6.50	12.50	8.50	12.50	18.50	25.00	(-)
Indoxacarb	0.00	0.00	4.50	7.50	9.00	15.00	15.50	22.50	(-)
Spinosad	4.00	10.00	8.50	15.00	12.50	20.00	17.50	27.50	(-)
Combinations									
Chlorantraniliprole+Sf	20.00	27.50	35.00	40.00	45.00	50.00	62.50	87.50	Synergistic
Indoxacarb+Sf	17.50	22.50	27.50	32.50	37.50	47.50	60.00	82.50	Synergistic
Spinosad+Sf	25.00	30.00	35.00	42.50	42.50	57.50	65.00	90.00	Synergistic
Mean	12.28	17.14	20.64	27.14	27.85	36.42	41.64	56.42	
LSD (p< 0.05)	4.15	5.96	5.23	6.91	4.96	6.14	6.15	7.37	

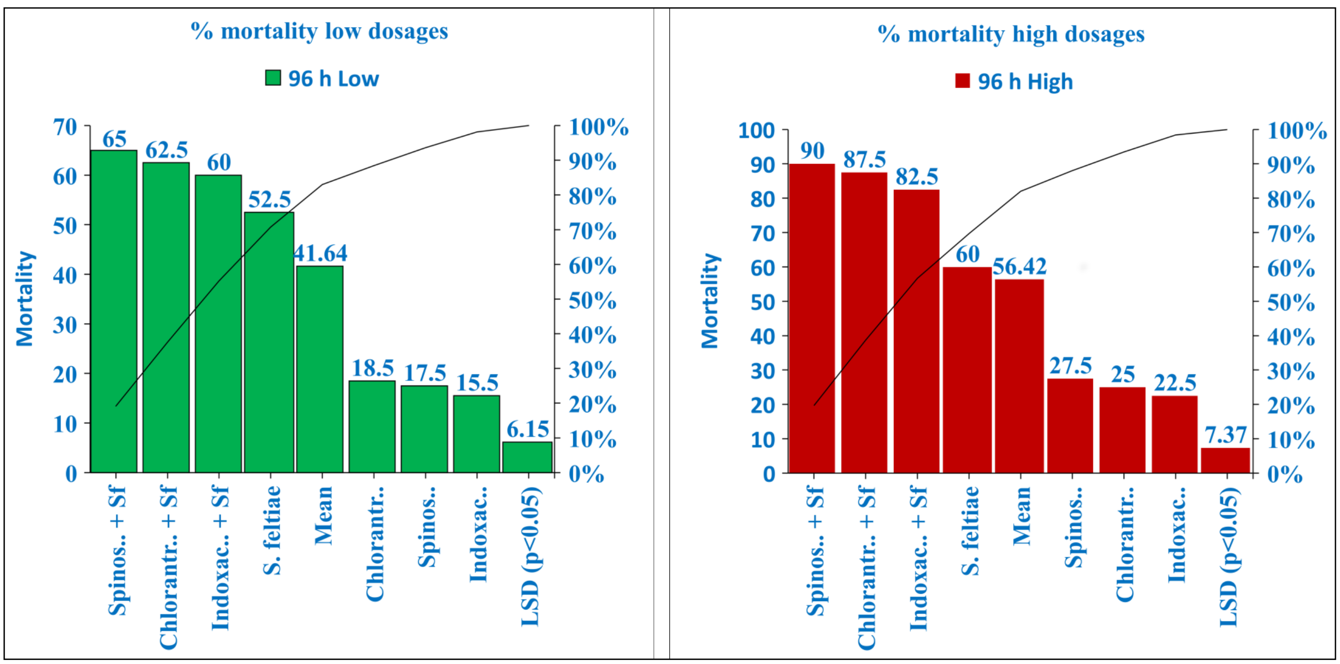

Figure 3: Mortality of fifth instar larvae of *S. frugiperda* exposed to entomopathogenic nematodes alone and in combination in the low toxicity insecticides

al., 2012), where spinosad and chlorantraniliprole caused larvae mortality over 80% at the same period. The LD_{50} was 1600 ppm for chlorantraniliprole and 400 ppm for Spinosad at 96 h in this study. Differences might be related to (1) the prolonged use of these active ingredients caused some level of resistance, (2) instar stage, third vs fifth used in this research, or (3) differences among fall armyworm populations on the island (Viteri et al., 2018). In contrast, indoxacarb caused 75 and 90% of larvae mortality in low and high doses, respectively, at 96 h (Figure 5).

The use of *S. feltiae* in combination with Spinosad and chlorantraniliprole produced higher % of mortality at 24 h compared with the use of these insecticides alone regardless of the dosage used (Table 2). Furthermore, the highest % of larval mortality (over 90%) were noted with high dosages at 72 h. Likewise, the combination of Spinosad+*S. feltiae* was effective (90.00% of dead larvae) with the high dose at 96 h compared with the lowest mortality caused by Spinosad

(17.50%), or *S. feltiae* (52.50%) applied alone (Figure 6 and 7), larvae exposed to 2 different modes of action [septicemia (*S. feltiae*)+lysed midgut epithelial cells (Spinosad), impaired regulation of muscles (chlorantraniliprole), or Indoxacarb neural transmission (spinetoram)] (Viteri et al., 2018), at the same time, caused their higher mortality. In fall armyworm populations from Florida (Viteri et al., 2018, Monika et al., 2022). However, further research is required to corroborate this synergistic effect.

3.2. Bioassays with H. bacteriophora+S. frugiperda+Insecticides

In general, a higher mean percent mortality was noted at higher dosages (F=67.09; df=12; p<0.05) (Table 3). However, *H. bacteriophora* and Spinosad caused 72.50–95% of larvae mortality with low and high dosages at 96 h. The use of chlorantraniliprole and Spinosad resulted in % of mortality up to 92.50% at 96 h at the higher dosage (Figure 4). These results are different from those reported,[3] where Spinosad and chlorantraniliprole caused larvae mortality over 95% at the same period. The LD_{50} was 400 ppm for Spinosad and 1600 ppm for chlorantraniliprole and at 96 h in this study. Differences might be related to (1) the prolonged used of these active ingredients caused some level of resistance, (2) instar stage, third vs fifth used in this research, or (3) differences among fall armyworm populations on the island. In contrast, indoxacarb caused 65 and 87.50% of larvae mortality in low and high doses, respectively, at 96 h (Figure 5).

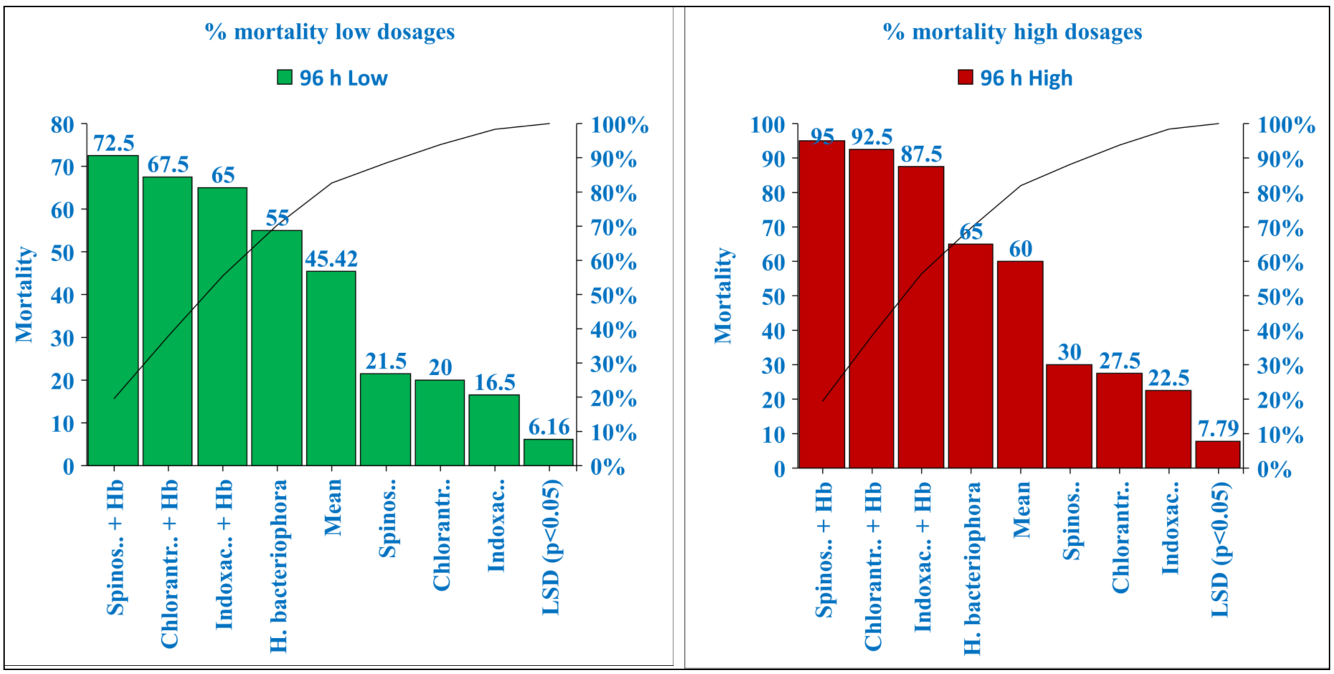

Figure 4: Mortality of fifth instar larvae of *S. frugiperda* exposed to entomopathogenic nematodes alone and in combination in the low toxicity insecticides

The use of *H. bacteriophora* in combination with Spinosad and chlorantraniliprole produced higher % of mortality at 24 h compared with the use of these insecticides alone regardless of the dosage used (Table 3). Furthermore, the highest % of larval mortality (over 90%) were noted with high dosages at 72 h. Likewise, the combination of Hb+Spinosad was effective (95.00% of dead larvae) with the high dose at 96 h compared with the lowest mortality caused by Spinosad (21.50%), or Hb (55.00%) applied alone, larvae exposed to 2 different modes of action [septicemia (*H. bacteriophora*)+lysed midgut epithelial cells (Hb), impaired regulation of muscles (Spinosad) (Figure 6 and 7), or abnormal neural transmission (spinetoram)] (Viteri et al., 2018, Kasi et al., 2021a, Kasi et al., 2022), at the same time,

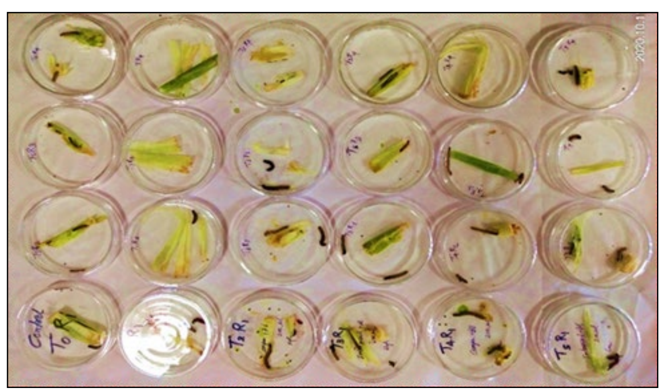

Figure 5: Bio-efficacy of EPN's against Spodoptera *S. frugiperda* (J.E. Smith)

Table 3: Percentage of mortality of fall armyworm [*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)] larvae in the fifth-instar at low and high dosages of 3 insecticides, and least toxic combinations among them evaluated from 24–96 h

Insecticides	24 h		48 h		72 h		96 h		
	Low	High	Low	High	Low	High	Low	High	Interaction
Bio agent (nematode)									
H. bacteriophora (Hb)	35.00	45.00	45.00	55.00	50.00	60.00	55.00	65.00	(+)
Toxicity insecticides									
Chlorantraniliprole	7.50	12.50	10.50	17.50	18.50	22.50	20.00	27.50	(-)
Indoxacarb	6.50	10.00	8.00	15.00	13.00	17.50	16.50	22.50	(-)
Spinosad	8.50	15.00	12.00	20.00	19.50	25.00	21.50	30.00	(-)
Combinations									
Chlorantraniliprole+Hb	45.00	75.00	57.50	87.50	62.50	87.50	67.50	92.50	Synergistic
Indoxacarb+Hb	50.00	72.50	55.00	77.50	60.00	82.50	65.00	87.50	Synergistic
Spinosad+Hb	57.50	77.50	62.50	85.00	67.50	90.00	72.50	95.00	Synergistic
Mean	30.00	43.92	35.78	51.07	41.57	55.00	45.42	60.00	
	4.23	5.55	7.12	8.06	8.15	9.81	6.16	7.79	

Figure 6: After treatment *Spodoptera frugiperda*

Figure 7: After nematode infected dead fall armyworm larvae dissected under microscopic condition and conformation Synergistic activity

caused their higher mortality. In fall armyworm population. However, further research is required to corroborate this synergistic effect.

4. CONCLUSION

S. frugiperda were suitable hosts for local strains *S. feltiae* strain and *H. bacteriophora*. In combination with low-toxicity insecticides at low and high dosages to control fifth-instar larvae in bioassays. The use of *S. feltiae*+Spinosad or chlorantraniliprole and *H. bacteriophora*+Spinosad or chlorantraniliprole caused larvae mortality of over 90% at 96 h at the high dose and should be included as a least toxic strategy to control fall armyworm.

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