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Evaluation of entomopathogenic microbial isolates against fall army worm, *Spodoptera frugiperda* infesting maize

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Abstract
Investigations were carried out to explore entomopathogenic activity of microbial isolates against fall army worm, *Spodoptera frugiperda* infesting maize at University of Agricultural Sciences, Dharwad during *Kharif*, 2022-23 with five microbial isolates *viz.* DBT-64, DBT-80, DBT-90, AUUB-209, Neem leaf endophyte and *Metarrhizium rileyi* along with spinosad 45 SC (Standard check) and control. The results revealed that, among the isolates tested, *Streptomyces hyderabadensis* (DBT-64) and *Streptomyces xiamenensis* (DBT-80) at 40 ml/l has excelled over others by recording 76.67 and 73.33 percent mortality of *Spodoptera frugiperda* at 96 hours after treatments under laboratory condition. Further, median lethal concentration of theses microbial isolates was tested against *Spodoptera frugiperda*. The results revealed the lowest LC_{50} value was recorded in *Streptomyces hyderabadensis* (DBT-64) (29.52 mL/L) with lower and upper limit of 27.04 and 32.21 mL/L followed by *Streptomyces xiamenensis* (DBT-80) which recorded the LC_{50} value of 32.21 mL/L with lower and upper limit of 29.41 and 35.26 mL/L. Hence, microbial isolates exhibit potential insecticidal properties, they can be integrated as a component of IPM which is eco-friendly and reliable in sustainable agriculture.

Keywords: Microbial control, *Spodoptera frugiperda*, maize, lethal concentration, entomopathogen, fall army worm

Introduction
Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) is a noctuid polyphagous pest reported to attack 353 plant species belonging to 76 plant families (Montezano *et al*., 2018) [13]. It is a highly destructive and invasive pest, noticed for the first time in India on the maize crop in Karnataka during May 2018 (Sharanabasappa *et al.,* 2018) [18]. Adults can migrate up to 1,500-2,000 Kilometer per year in search of a warmer climate (Prasanna *et al.,* 2018) [17] and can travel 500 km in a single season to find oviposition sites and capacity to fly over 100 km per night (Anon., 2019) [2]. The characteristics like highly migratory, high fecundity, wide range of host plants and voracious feeding behaviour, without diapause made this pest a major destructive crop insect pest (Sharanabasappa *et al.,* 2021) [19]. The cumulative data published by the Department of Agriculture Cooperation and Farmers Welfare, GoI in 2019, indicated that Karnataka has the highest area affected with FAW (2,11,300 ha), followed by Telangana (24,288 ha), Maharashtra (5144 ha) and others (Rakshit, *et al.,* 2019) [18]. However, the pest has been reported to cause infestation ranging from 6.00 to 100 percent on maize in Northern Karnataka (Mallapur *et al*., 2018) [10]. This pest has developed resistance to many insecticides (41-143 Active ingredients) (Mota-Sanchez and Wise, 2019) [14].
Actinobacteria are Gram-positive, aerobic bacteria which are prolific producers of thousands of biologically active secondary metabolites (Manivasagan *et al.,* 2013b) [11]. Among them, out of more than 10,000 known antibiotics, 50-55 percent is produced by Streptomyces (Chater, 1993; Manivasagan *et al.,* 2014) [4, 11] which were effective against human pathogens, plant pathogens and plant herbivores. Many reports indicated that actinomycetes play an significant role in the biological control of insect pests but studies on lepidopteran pests are very meager and conducted only under laboratory conditions which includes, cotton leaf worm *Spodoptera littoralis* (Bream *et al*., 2001) [3], *Helicoverpa armigera* (Osman *et al*., 2007; Shivakumar, 2005) [15, 21], *Chilo partellus* (Vijayabharathi *et al.,* 2014) [25], *S. litura* and *P. xylostella* (Vijayabharathi *et al.,* 2014; Shivakumar *et al*, 2005; Srujana *et al.,* 2022; Suma *et al.,* 2022) [25, 21, 23, 26] and *S. frugiperda* (Suma *et al.,* 2022) [26].

Hence, there is a huge scope to utilize these microbial metabolites in insect pest control which are possessing entomopathogenic activity against many lepidopteran pests and the different method of application of these microbial isolates for effective control of *S. frugiperda* under laboratory conditions.

Hence, recently microbial control has attracted considerable attention because they are more specific, have low relative cost and are more eco-friendly. Among the biological control agents, actinobacteria are one of the most important microbial resources which can provide potential new bioactive compounds for use as insect control agents. Therefore, there is need to evaluate efficacy of the microorganisms for developing safe and eco-friendly alternatives to chemical insecticides as biocontrol agents under *in-vitro* condition.

Materials and Methods

Mass multiplication of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

The rearing of *S. frugiperda* was initiated in the Department of Entomology. Egg masses were collected from the maize (*Zea mays* L.) fields cultivated with hybrid (NK 6240) at Main Agricultural Research Station (MARS), UAS, Dharwad and were incubated. The larvae were reared in a circular insect breeding dish (Himedia, TCP030- 90 x 40 mm dia.) providing maize leaves as food under laboratory conditions at 27 ± 2 °C, 75 to 80% RH, L16:D08 photoperiod until pupation and the pupae were collected at regular intervals. After emergence, the adult moths were released in a cage (45 x 45 x 60 cm^3) and young maize plants of 15 days old were kept in the cage as an oviposition substrate. Ten percent (w/v) honey solution (Prakruthi®) fortified with multivitamin (MultipreX®) was provided as food for adult male and female moths. To prevent desiccation, egg masses laid by females on maize leaf were removed and placed in an insect breeding dish layered with moistened filter paper. This represents the F_1 generation and the neonate larvae from these egg masses were collected for further rearing up to third instars (5 days old larvae) for further experimentation.

Evaluation of entomopathogenic activity of microbial isolates

Five actinobacterial isolates were sourced from Microbial Genetics Laboratory, Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad (Karnataka-India) and were subjected for bioassay (Table 1) to know their bio-efficacy against fall army worm, *Spodoptera frugiperda*.

Preparation of actinomycetes culture for bioassay

Procedure for preparation of actinomycetes culture for bioassay was followed as per Srujana *et al.* (2022) [23]. Isolates were initially spotted on starch casein agar (SCA) medium and purified by four-way streaking method. Single pure colony was inoculated to the Starch Casein Broth (SCB) and incubated at 28±2 °C for 5-7 days on rotary shaker (190 rpm/min). Then the incubated flasks were taken and placed in dark room, after 10-12 days of incubation, culture broth were filtered and centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatant was subjected for the bio-efficacy studies against fall army worm.

Table 1: Details of microbial isolates used for the experiment

Sl. No.	Strain-ID	Scientific Name	Accession Number	Source of isolation	Concentration used (mL)
1.	DBT-64	*Streptomyces hyderabadensis*	ON573299	Soil	20, 25, 30, 35, 40
2.	DBT-80	*Streptomyces xiamenensis*	OM3989929	Soil	20, 25, 30, 35, 40
3.	DBT-90	Unidentified	-	Soil	20, 25, 30, 35, 40
4.	AUUB-209	*Streptomyces enissocaesilis*	OM792961	Soil	20, 25, 30, 35, 40
5.	Neem leaf Endophyte	*Streptomyces* sp.	LC516415	Neem (Leaves)	20, 25, 30, 35, 40

In vitro evaluation

The leaf dip bioassay method was followed as described by Tabashnik and Cushing (1987) [24]. Leaf discs of 9 cm of maize leaves were provided for *Spodoptera frugiperda* larvae. These leaves were dipped in aqueous solution of the test microbial isolates for about 60 seconds. After draining off excess fluid, leaves were dried under shade for 10 minutes and fed to third instar larvae of test insects. Each treatment was replicated thrice with ten larvae per treatment. Leaves dipped in distilled water alone served as a control (Mohapatra, 2011) [12] and leaves dipped in *Metarhizium rileyi* @ 2 g/l was treated as positive check. Spinosad 45 SC (Tracer®) @ 0.2 mL/L was used as a chemical check which is microbial originated insecticide derived fermentation of actinobacteria, *Saccharopolyspora spinosa*. The larval mortality was recorded at 24, 48, 72 and 96 hours after treatment. Larvae were considered dead if unable to move in a coordinated way when prodded with a fine-haired brush. The whole experiment was conducted under the ambient temperature of 27±2 °C and 55±10% RH with 16:8 (L: D) photoperiod. The corrected mortality was estimated using Abott's (1925) [1] and LC_{50} probit analysis was done according to Finney (1971) [5] using SPSS software.

Formula used for corrected mortality as per Abott's (1925) [1]:

Mc = (Mo-Me/100-Me) X 100

Where
Mc = Corrected mortality rate (%)
Mo = Observed mortality of treated larvae (%)
Me = Mortality rate of control (%)

Results and Discussion

The results pertaining to the entomopathogenic activity of different microbial isolates against third instar larvae of *Spodoptera frugiperda* was presented in Table 2.

During 24 hours after treatment, only 3.33 percent mortality was observed in DBT-64 and DBT-80 at 35 and 40 mL followed by and 6.67 percent in DBT-64 at 40 mL. No mortality was observed in any other treatment. While, spinosad registered 33.33 percent mortality.

At 48 hours after treatment, 30.33 and 23.33 percent in DBT-64 and DBT-80 at 40 mL. AUUB-209 and neem leaf endophyte recorded 20.00 and 16.67 percent mortality. While, *M. rileyi* recorded 10.00 and spinosad recorded 70.00 percent mortality and regarded as superior treatment (Table 3).

During 72 hours after treatment, highest mortality (60.00%) was recorded in DBT-64 (40 mL) which is statistically on par with *M. rileyi* which also recorded 60 percent mortality. followed by 56.67 percent in DBT-80 at 40 mL. While, 40.00 and 30.00 percent mortality was noticed in AUUB-209 and neem leaf endophyte (40 mL) which were statistically on par with each other. While, spinosad recorded highest mortality (90.00%) and regarded as superior treatment over microbial isolates.

96 hours after treatment, highest mortality (76.67%) was recorded in DBT-64 (40 mL) followed by 73.33 percent in DBT-80 at 40 mL. Mortality rate was increased as the concentration increased. While, 70.00 percent mortality was recorded in DBT-64 (35 mL) followed by AUUB-209 (40 mL) and neem leaf endophyte (40 mL) with 66.67 and 60.00 percent mortality, respectively. However, *M. rileyi* recorded 80.00 and spinosad recorded 100.00 percent mortality and regarded as superior treatments.

Absolutely no mortality was recorded in control and least mortality was observed in DBT-90 in all the concentrations but mortality rate increased as the concentration increased and same isolate could cause the maximum mortality to the tune of 53.33 percent @ 40 mL after 96 hours of post treatment (Table 2).

Median lethal concentration of microbial isolates against *Spodoptera frugiperda* at 96 HAT

Lethal concentration gives the exact concentration at which 50 percent of the test insects will show mortality response to the toxic substances and it also avoids the excess use of the toxic substance to control the insect pests. LC_{50} value was calculated for all five microbial isolates against *Spodoptera frugiperda* and the results were elucidated in Table 3.

Among five isolates evaluated, highest LC_{50} value was recorded in DBT-90 (51.38 mL) with 35.07 and 75.26 mL of lower and upper limits, respectively which was regarded as the inferior isolate as compared to others. Whereas, least concentration was recorded in DBT-64 isolate (29.52 mL) with lower and upper limit of 27.04 and 32.21 mL followed by DBT-80 (32.21 mL) with lower and upper limit of 29.41 and 35.26 mL, respectively. AUUB-209 recorded 39.29 mL with lower limit (33.29 mL) and upper limit (46.38 mL) followed by Neem leaf endophyte (49.15 mL) which had lower and upper limit of 49.15 and 35.11 mL, respectively (Table 3).

DBT-64 (*Streptomyces hyderabadensis*) and DBT-80 (*Streptomyces xiamenensis)* at 40 mL had emerged as a best treatment in bringing the mortality rate to the tune of 76.67 and 73.33, respectively at 96 HAT (Figure 1). Median lethal concentration of different microbial isolates revealed that, least LC_{50} value was recorded in DBT-64 (29.52 mL/L) and highest was noticed in DBT-90 (32.21 mL/L). Lower the LC_{50} value higher will be the efficacy which indicated that, superiority of the isolate DBT-64 *(Streptomyces hyderabadensis*) (Figure 2).

Present findings are in accordance with Srujana (2015) [22] who reported that actinobacterial isolates especially *Streptomyces* sp. are potent isolates possessing insecticidal activity against *Spodoptera litura* and *Plutella xylostella.* The secondary metabolites of new strain of Streptomyces gave displayed growth inhibition on the test pathogenetic insects, such *as S. exigua, Dendrolimus punctatus, P. xylostella, A. glycines* and *C. Pipiens* as observed by Huamei *et al.* (2008). The culture filtrates of six actinomycetes isolates showed mortality (>70%) on third instar larvae of *H. armigera* and nine isolates were recorded mortality of *Spodoptera litura* (38-77%) and *Chilo partellus* (100%) under lab condition (Gopalakrishnan *et al.,* 2012). Similarly, Vijayabharathi *et al.,* 2014 [25] recorded entmopthogenic acticvity of these microbial isolates against *Chilo partellu, Spodoptera litura* and *Plutella xylostella*. Suma *et al.* (2022) [26] revealed that, actinobacterial isolates DBT-64, recorded 78.85, 76.50 and 76.00 percent mortality of *P. xylostella, S. litura* and *S. frugiperda,* respectively followed by DBT-80 with 80.75, 75.25 and 77.50 percent mortality. Further, DBT-59 has recorded 79.50, 78.25 and 75.25 percent larval mortality of *P. xylostella, S. litura* and *S. frugiperda,* respectively at 72 HAT under *in vitro* conditions**.** The *B. bassiana* isolates were tested for pathogenicity and endophytic activity against Fall armyworm under laboratory conditions. The results showed that Bb TM isolate was capable of causing highest mortality of 66.67, 60.00 and 53.33 percent against first, second and third instar with LC_{50} values of 2.51 x 10^5, 2.05 x 10^6 and 4.56 x 10^7, respectively (Kiruthiga *et al.,* 2022) [9]. Hernandz (1988) [7] reported the larval mortality of *S. frugiperda* with 80 and 70 percent when treated with *Bt aizawai* and *Bt kurstaki* (3x 107 cells/ml) respectively. Polanczyk *et al.* (2000) [16] observed that *Bt aizawai* HD68 strains containing 3x10^8 cells/ml induced 84 percent larval death of *S. frugiperda*. Since, *S. frugiperda* is a recently invaded pest and the concept of employing actinobacteria in insect control is novel. There are no literatures available on the bio efficacy of actinobacterial isolates against *S. frugiperda.*

Several Streptomyces metabolites such as avermectin, emamectin, milbemycin and spinosyns have been established as potential protective agents against a variety of insect pests. They involved in disruption of nicotinic acetylcholine receptors. Their insecticidal activity, unique mode of action and lower environmental effect make them useful novel agents for modern integrated pest management.

Table 2: Bioefficacy of different microbial isolates against *Spodoptera frugiperda*

Treatments/ Isolates	Conc. (mL/L)	Larval mortality (%)			
		24 HAT	48 HAT	72 HAT	24 HAT
Streptomyces hyderabadensis (DBT-64)	20	0.00 (0.29)^{d}	0.00 (0.29)^{j}	6.67 (14.76)^{l}	10.00 (18.43)^{p}
	25	0.00 (0.29)^{d}	3.33 (10.51)^{i}	13.33 (21.41)^{j}	33.33 (35.26)^{k}
	30	0.00 (0.29)^{d}	10.00 (18.43)^{g}	30.00 (33.21)^{g}	66.67 (54.78)^{f}
	35	3.33 (10.51)^{c}	20.00 (26.56)^{d}	50.00 (45.00)^{d}	70.00 (56.79)^{e}
	40	6.67 (14.76)^{b}	30.00 (33.21)^{b}	60.00 (50.77)^{b}	76.67 (61.12)^{c}
Streptomyces xiamenensis (DBT-80)	20	0.00 (0.29)^{d}	0.00 (0.29)^{j}	3.33 (10.51)^{m}	6.67 (14.76)^{q}
	25	0.00 (0.29)^{d}	0.00 (0.29)^{j}	10.00 (18.43)^{k}	26.67 (31.09)^{l}
	30	0.00 (0.29)^{d}	6.67 (14.76)^{h}	33.33 (35.26)^{f}	53.33 (46.91)^{h}
	35	0.00 (0.29)^{d}	16.67 (24.08)^{e}	50.00 (45.00)^{d}	66.67 (54.78)^{f}
	40	3.33 (10.51)^{c}	23.33 (28.85)^{c}	56.67 (48.83)^{c}	73.33 (59.00)^{d}
DBT-90	20	0.00 (0.29)^{d}	0.00 (0.29)^{j}	0.00 (0.29)^{n}	0.00 (0.29)^{s}
	25	0.00 (0.29)^{d}	0.00 (0.29)^{j}	3.33 (10.51)^{m}	10.00 (18.43)^{p}
	30	0.00 (0.29)^{d}	0.00 (0.29)^{j}	6.67 (14.76)^{l}	23.33 (28.85)^{m}
	35	0.00 (0.29)^{d}	10.00 (18.43)^{g}	16.67 (24.08)^{i}	46.67 (43.10)^{j}
	40	0.00 (0.29)^{d}	16.67 (24.08)^{e}	20.00 (26.56)^{h}	53.33 (46.91)^{h}
Streptomyces enissocaesilis (AUUB-209)	20	0.00 (0.29)^{d}	0.00 (0.29)^{j}	3.33 (10.51)^{m}	6.67 (14.76)^{q}
	25	0.00 (0.29)^{d}	0.00 (0.29)^{j}	6.67 (14.76)^{l}	20.00 (26.56)^{n}
	30	0.00 (0.29)^{d}	3.33 (10.51)^{i}	10.00 (18.43)^{k}	26.67 (31.09)^{l}
	35	0.00 (0.29)^{d}	16.67 (24.08)^{e}	20.00 (26.56)^{h}	60.00 (50.77)^{g}
	40	0.00 (0.29)^{d}	20.00 (26.56)^{d}	40.00 (39.23)^{e}	66.67 (54.74)^{f}
Streptomyces sp.(NLE)	20	0.00 (0.29)^{d}	0.00 (0.29)^{j}	0.00 (0.29)^{n}	3.33 (10.51)^{r}
	25	0.00 (0.29)^{d}	0.00 (0.29)^{j}	3.33 (10.51)^{m}	13.33 (21.41)^{o}
	30	0.00 (0.29)^{d}	3.33 (10.51)^{i}	6.67 (14.76)^{l}	20.00 (26.56)^{n}
	35	0.00 (0.29)^{d}	13.33 (21.39)^{f}	20.00 (26.56)^{h}	50.00 (45.00)^{i}
	40	0.00 (0.29)^{d}	16.67 (24.08)^{e}	30.00 (33.21)^{g}	60.00 (50.77)^{g}
Metarhizium rileyi (2x10^{8} CFU)	2 g/ L	0.00 (0.29)^{d}	10.00 (18.43)^{g}	60.00 (50.77)^{b}	80.00 (63.44)^{b}
Spinosad 45 SC	0.2 mL/ L	33.33 (35.26)^{a}	70.00 (56.79)^{a}	90.00 (71.56)^{a}	100.00 (89.71)^{a}
Control	--	0.00 (0.29)^{d}	0.00 (0.29)^{j}	0.00 (0.29)^{n}	0.00 (0.29)^{s}
S.Em. ±		0.05	0.32	0.30	1.55
CD @ 1%		0.15	0.98	0.90	4.64
CV (%)		4.26	1.35	1.73	5.9

Values in parentheses are the arc sine transformed values. Means followed by the same letters in a column do not differ significantly (0.05) by DMRT. HAT: Hours after treatment

Table 3: Median lethal concentration of microbial isolates against *Spodoptera frugiperda* at 96 hours after treatment

Sl. No.	Treatments	LC_{50} (mL/L)	Fiducial limit		χ^2	R^2 value	Regression equation
			Lower	Upper			
1	DBT-64	29.52	27.04	32.21	3.28	0.93	Y = 6.34+7.7252x
2	DBT-80	32.21	29.41	35.26	2.14	0.96	Y= 7.58+8.4264x
3	DBT-90	51.38	35.07	75.26	3.39	0.83	Y = 20.08+16.197x
4	AUUB-209	39.29	33.29	46.38	5.72	0.80	Y = 20.61+16.699x
5	NLE	49.15	35.11	68.82	5.46	0.82	Y= 20.03+16.178x

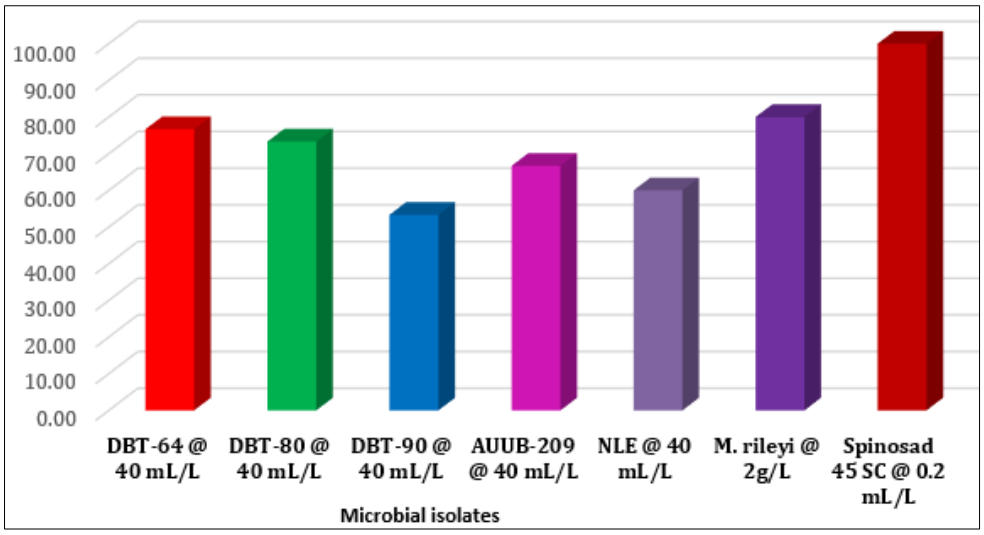

Fig 1: Larval mortality of *Spodoptera frugiperda* as influenced by different microbial isolates

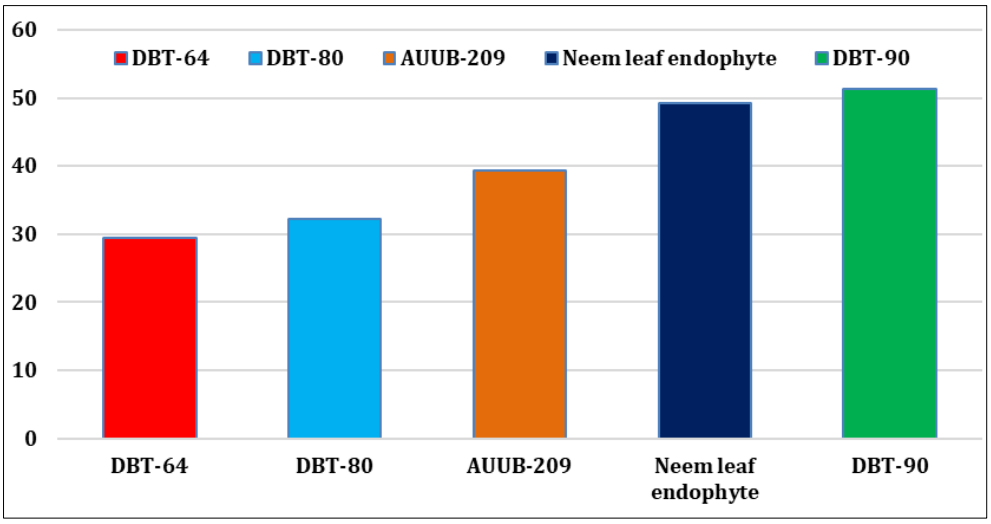

Fig 2: Lethal concentrations of different microbial isolates against *Spodoptera frugiperda*

Conclusion

Among the microbial isolates evaluated against *S. frugiperda*, DBT-64 (*Streptomyces hyderabadensis*) and DBT-80 (*Streptomyces xiaminensis*) were the potent microbial isolates possessing insecticidal activity against *S. frugiperda*.

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