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Research Article

Efficacy of secondary metabolites of promising entomopathogenic actinomycetes against fall armyworm, *Spodoptera frugiperda* (Lepidoptera : Noctuidae)

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ABSTRACT: Biopesticides are the most reliable strategies to protect crops at the initial stages of pest attack. The present investigation was conducted to identify effective entomopathogenic actinomycetes for management of Fall Armyworm (FAW). Of 24 actinomycetes that were previously screened against 2nd instar larvae of FAW, three isolates were identified as effective, *viz.,* KG-13, CAI-134 (*Nocardiopsis* sp.), and CAI-17 (*Streptomyces albus*) which showed mortality more than 50 %. These three promising isolates were selected for further evaluation using their Intracellular Metabolites (ICM) and Extracellular Metabolites (ECM) in both laboratory and greenhouse conditions. Under laboratory through diet impregnation bioassay, KG-13 caused maximum mortality in 2nd instar larvae of FAW, with both ECM (85.19%) and ICM (70.37%). Larval prolongation (22.57 ± 1.98 days) and larval pupal intermediates were observed in the ECM of CAI-17. In the evaluation of chitinase activity, the highest zone of inhibition was recorded in KG-13 (34.67 mm), followed by CAI-17 (25.33 mm) and CAI-134 (19.67 mm). ECM of KG-13, CAI-17 and ICM of KG-13 were noticed with 66.66, 53.33 and 53.3 % mortality in greenhouse studies. This study shows the impact of promising actinomycetes secondary metabolites on the 2nd instar FAW and their effectiveness in both laboratory and greenhouse conditions. Comparatively, less mortality was noticed in the greenhouse than in laboratory conditions, whereas *Bt* var. *kurstaki* (73.33%) and Emamectin (73.33%) in greenhouse studies showed considerablemortality than actinomycetes metabolites.

KEYWORDS: Actinomycetes, diet impregnation bioassay, fall armyworm, spinosad, secondary metabolites

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ABBREVIATIONS: ECM: Extracellular metabolites; EPNs: Entomopathogenic Nematodes; EPBs: Entomopathogenic Bacteria; DAT: Days after treatment; GABA: Gamma-aminobutyric acid; ICM: Intracellular metabolites; WC: Whole culture.

INTRODUCTION

Crop losses occur due to abiotic and biotic stresses, in which biotic stresses have a major role. Among the biotic stresses, insect pests cause great losses to the crop. Insect pests not only lessen crop yield but also affect the quality of the produce. One of the invasive pests reported in India (2018) was the Fall Armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). It is native to the tropical and subtropical Americas. In India, FAW was first noticed in Karnataka during the Kharif season in maize and disseminated all over the country (Sharanabasappa *et al*., 2018). It is a devouring, polyphagous pest that feeds on a wide range of host plants. The word "armyworm" refers to the large-scale invasive behaviour of the larval stage. It attacks a total of 353 plant species of 76 plant families with a large number of taxa from families Poaceae (106 taxa), Asteraceae (31 taxa) and Fabaceae (31 taxa) (Montezano *et al.,* 2018) causing major economic damage in maize, sorghum, cotton and soybean and reported as threat to rice, wheat, sugarcane, vegetable and fodder crops (Hardke *et al.,* 2015). The most serious damage caused by this pest is continuous feeding of shoots that reduces the photosynthetic area of the plant. Usually, chemical/synthetic insecticides are preferred to control the pest due to their quick action, ready availability in the market and easy application, but FAW larvae are exposed less to insecticide as they remain feeding inside the plant shoots. Also, repeated application of chemicals leads to many problems like environmental pollution, death of non-targeted organisms, resistance development in the pest, pest resurgence, *etc*. So, there is a need to develop more reliable strategies which are environmentally safe, cost-effective, and safe to beneficial insects and mammals. Due to their eco-friendliness and low production costs, the use of bacteria with insecticidal characteristics has emerged as one of the most sustainable strategies for agricultural production. Among bacteria, actinobacteria have proved themselves to be a source of novel and very powerful agents having considerable potential for the biocontrol of insect pests. Actinomycetes are filamentous gram-positive bacteria belonging to the phylum actinobacteria and are ubiquitous (Ventura *et al*., 2007). They also exhibited vast metabolic and physiological properties, such as secretion of extracellular enzymes that lead to the development of a wide range of secondary metabolites (Schrempf, 2001). The production of novel secondary metabolites is the distinct characteristic feature of actinomycetes because of their very vast genome and capacity to grow in different environmental conditions. They are useful novel agents for contemporary integrated pest control programmes because of their insecticidal spectrum, distinct mechanism of action, and reduced environmental impact (Kirst, 2010). Among actinomycetes, mainly *Streptomyces,* were the source of around 61 % of all bioactive microbial metabolites (Moncheva *et al*., 2002). Different metabolites extracted from different species of genus *Streptomyces*, such as spinosad, avermectins, emamectin, milbemycin and polynactins have different modes of action against insect pests (Copping & Menn, 2000). It is possible to use compounds generated from these microbes to control insect pests of agriculturally significant crops since they are generally not poisonous to mammals.

The present study was aimed at evaluating the insecticidal efficacy of secondary metabolites extracted from three promising actinomycetes isolates against 2nd instar larvae of FAW. Isolates were KG-13, CAI-134, which belong to *Nocardiopsis* sp., and CAI-17, which is a strain of *Streptomyces albus*.

MATERIALS AND METHODS

Actinomycetes cultures were collected from the Biocontrol laboratory, International Crops Research Institute for Semi-Arid Tropics (ICRISAT) Patancheru. They were previously isolated from washings of herbal vermicomposts at ICRISAT and soils of Karnataka. They were subcultured in Actinomycetes Isolation Agar for culture maintenance. Later, they were inoculated into starch casein broth for the preparation of whole culture as per the procedure given by Vijayabharathi *et al.* (2014). Larvae were reared on a sorghum semi-synthetic diet (Jaba *et al*., 2020). Research was conducted at the Biocontrol laboratory, ICRISAT, Patancheru.

Sample preparation

Pure cultures were used for metabolite extraction. Fermented broths were subjected to centrifugation at 10000 rpm for 10 min to separate supernatant and cell mass. Supernatant and cell mass were used for extracting extra and intracellular metabolites (Plate 1).

Extraction of extracellular metabolites

The supernatant was partitioned thrice with solvent ethyl acetate and shaken vigorously for a minute and left undisturbed. Then, ethyl acetate (organic phase) was separated from the culture filtrate (aqueous phase). The organic phase was collected separately and combined with anhydrous sodium sulphate. Later, the organic phase was filtered and subjected to evaporation. Organic fraction was concentrated by film evaporation on rotary evaporator at 40ºC. Residue was collected with the help of methanol (Khattab *et al*., 2016).

Extraction of intracellular metabolites

Biomass was suspended in a small amount of methanol and crushed with the help of a pestle and motor. Further, methanol was added to crushed cells in the ratio of 1:1 (W/V) and kept on a shaker at 120 rpm for 2 days, and it was filtered using filter paper. The methanol mixture was evaporated using a rotary evaporator at 40ºC and obtained metabolite stored at 4ºC for further use (Kumar *et al.,* 2014).

Metabolite assay under laboratory conditions

About 2 mL of the artificial diet of *S. frugiperda* was poured into a 12-well plate and allowed to dry. About 200 µL of the metabolite was added to the diet in each well and air dried for 3 hrs. The 2nd instar larvae were pre-starved for 4 hours prior to release (one each) into the treated 12-well plates. Three replications were used for each treatment (sample), and each replication consisted of 12 larvae (Plate 2). The diet treated with methanol was denoted as Methanol Control (MC). Emamectin benzoate 5% SG (SPAR) was used as a positive control. Diet treated with water was denoted as Normal Control (NC). The insect mortality was recorded from the 3rd to 7th Day After Treatment (DAT). Moribund larvae were also considered dead. The %age of mortality for each test isolate was computed.

$$\% \text{ mortality} = \frac{\text{Dead larvae}}{\text{Total no.of larvae}} \times 100$$

Corrected mortality (Abbott, 1925) per each treatment was calculated using the following formula.

Corrected larval mortality (%) =

$$\frac{(\%Mortality\ in\ treatment - \%Mortality\ in\ control)}{(100 - \%Mortality\ in\ control)} \times 100$$

Assessment of chitinase activity

Chitinase degradation activity was evaluated for the prominent isolates. Discs were placed on the media plate to absorb the culture and also to restrict the over spreading on the media. About 20 µl of culture was poured on the disc, and they were incubated for at least 5 days for the observation of the maximum zone of inhibition (Plate 3). Based on the length of the zone of inhibition showed by the test isolate, chitinase degrading capability was recorded. Further, the correlation between chitinase activity and mortality % was done to know whether mortality % was dependent on the chitinase degradation.

Evaluation of metabolites under greenhouse conditions

A pot mixture (1000 g) was prepared by mixing black soil, sand and compost at 3:2:2 and placed in 20 cm diameter plastic pots. Sorghum seeds (variety: SPV 104) were surface sterilized with sodium hypochlorite (2.5% for 5min), and it was rinsed with sterilized water (8 times) before being transferred into respective pots (five seeds/pot) and watered as required. Each pot was sown with five seeds, and they were thinned to two after five days of sowing. Light irrigation was given at frequent intervals as per crop requirement. The greenhouse was maintained at 25±2°C under natural lighting conditions.

Isolates that showed the highest efficacy in diet impregnation assay were selected for greenhouse evaluation on *S. frugiperda* in sorghum, and the larval mortality was recorded. In addition, *Bacillus thuringiensis var. kurstaki* and *Metarhizium anisopliae* and Emamectin benzoate 5% SG (Spar) formulations were used as positive controls. Methanol and water were used as negative controls. Twenty-one days after seedling emergence, plants were sprayed with secondary metabolites (5mL/plant) and infested with 2nd instar larvae (pre-starved for 4 h) to each treated plant (5 larvae/plant) and covered with mesh to restrict the movement larvae. Three replications were used for each treatment (sample), and each replication consisted of five larvae. The larval mortality was recorded from the 3rd to 7th DAT.

Statistical analysis

The experiment was conducted in a Complete Random Design (CRD) in both laboratory and greenhouse conditions. Larval mortality % was observed at the 3rd, 4th, 5th, 6th and 7th day after treatment and data was subjected to Analysis Of Variance (ANOVA) after arcsine transformation (arc sine) and the means were separated by Duncan's Multiple Range Test (Duncan, 1955). The analysis was done in the software IBM SPSS 21.0.

RESULTS

Metabolite assay under laboratory conditions

Metabolites of three promising isolates, *viz.*, CAI-17, CAI-134 and KG-13 were evaluated for insecticidal activity through diet impregnation assay on 2nd instar larvae of FAW. Results about mean mortality % are shown in Table 1 and Figure 1.

On the 3rd DAT, the highest larval mortality resulted in ECM of KG-13 (59.26 %) followed by Emamectin benzoate 5% SG (51.85 %). ICM of KG-13 expelled 48.15 % larval load, which was statistically on par with Emamectin treatment. A mortality % of 40.74 was observed in ECM of CAI-134 followed by ECM of CAI-17 showed 37.04 % mortality. ICM of CAI-17 and CAI-134 registered 18.52 %. Larval mortality of 10.0 % was noticed in MC, so corrected mortality was used for each treatment.

Emamectin benzoate registered with 81.48 % of mortality, and ECM of KG-13 showed larval mortality of 74.07 % on 4th DAT. ECM of CAI-134 registered with 62.96 %. ICM of KG-13 showed 51.85 %, followed by ECM of CAI-17 observed with a mortality of 48.15 % and were statistically on par with each other. ICM of CAI-17 and CAI-134 observed mortality %ages of 33.33 and 25.93, respectively.

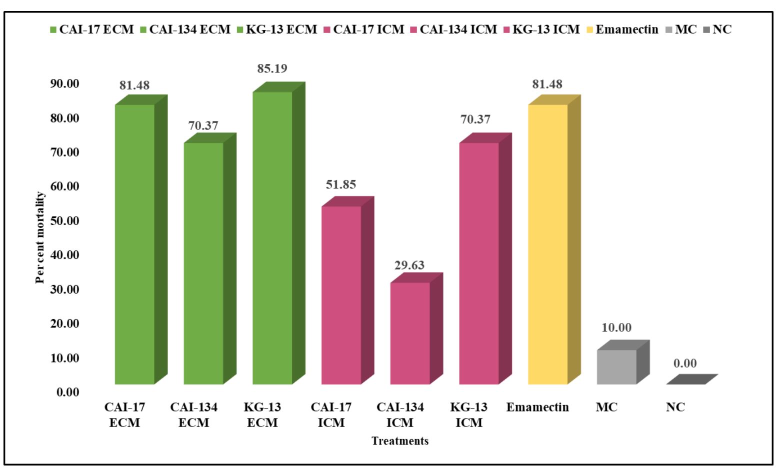


Figure 1. Per cent mean mortality of 2nd instar larvae of *S. frugiperda* treated with secondary metabolites of promising actinomycetes in diet impregnation bioassay.

Table 1. Efficacy of secondary metabolites of actinomycetes on 2nd instar larvae of *S. frugiperda* in diet impregnation bioassay

S. No.	Treatment	Mean per cent mortality at different intervals after treatment									
		3 DAT		4 DAT		5 DAT		6 DAT		7 DAT	
		% mortality	Corrected mortality	% mortality	Corrected mortality	% mortality	Corrected mortality	% mortality	Corrected mortality	% mortality	Corrected mortality
1	**CAI-17 ECM**	43.33	37.04 (37.51)c	53.33	48.15 (43.96)de	83.33	81.48 (64.54)ef	83.33	81.48 (64.54)de	83.33	81.48 (64.54)de
2	**CAI-134 ECM**	46.67	40.74 (39.51)c	66.67	62.96 (52.54)ef	73.33	70.37 (57.05)de	73.33	70.37 (57.05)d	73.33	70.37 (57.05)d
3	**KG-13 ECM**	63.33	59.26 (50.36)d	76.67	74.07 (59.42)fg	86.67	85.19 (67.40)f	86.67	85.19 (67.40)e	86.67	85.19 (67.40)e
4	**CAI-17 ICM**	26.67	18.52 (25.50)b	40.00	33.33 (35.28)cd	56.67	51.85 (46.08)c	56.67	51.85 (46.08)c	56.67	51.85 (46.08)c
5	**CAI-134 ICM**	26.67	18.52 (25.50)b	36.67	25.93 (30.63)bc	36.67	29.63 (33.00)b	36.67	29.63 (33.00)b	36.67	29.63 (33.00)b
6	**KG-13 ICM**	53.33	48.15 (43.96)cd	56.67	51.85 (46.08)e	66.67	62.96 (52.54)cd	73.33	70.37 (57.05)d	73.33	70.37 (57.05)d
7	**Emamectin benzoate**	56.67	51.85 (46.08)cd	83.33	81.48 (64.54)g	83.33	81.48 (64.54)ef	83.33	81.48 (64.54)de	83.33	81.48 (64.54)de
8	**MC**	10.00 (18.44)ab		10.00 (18.44)ab		10.00 (18.44)ab		10.00 (18.44)ab		10.00 (18.44)ab	
9	**NC**	0.0 (0.29)a									
S.Em±		**4.48**		**5.08**		**4.50**		**4.27**		**4.27**	
LSD (5% level)		**13.44**		**15.22**		**13.49**		**12.79**		**12.79**	

*Values in parentheses were arcsine-transformed values. Means allotted with same letters do not differ significantly (0.05) by DMRT (No. of treated larvae N = 12). DAT=Days after treatment, MC= methanol, NC= Normal control, ECM= Extracellular metabolite, ICM= Intracellular metabolite

The highest mortality on the 5th DAT was noticed in the ECM of KG-13 (85.19 %) followed by Emamectin benzoate and ECM of CAI-17 registered with 81.48 % larval mortality and all the three were statistically on par with each other. CAI-134 ECM was observed with 70.37 % mortality. ICM of KG-13, CAI-17 and CAI-134 expelled larval load of 62.96, 51.85, and 29.63 %, respectively.

On 6th DAT, mortality rate increased in only ICM of KG-13 (70.37%), and mortality % in remaining treatments remained same. Similarly, no change in mortality % on 7th DAT was observed.

Influence of metabolites on survived larvae

The larval durations in treated plates were statistically on par with the control, *i.e.*, 19.38±0.75, but in the case of the larvae treated with ECM of CAI-17 showed prolongation of larval duration up to 22.57±1.98 days. Also, the larvae treated with the ECM of CAI-17 showed larval-pupal intermediates, and larvae failed to pupate (Table 2, Figure 2).

Correlation between chitinase activity and mortality %

In evaluating chitinase activity for prominent isolates, the zone of inhibition for KAI-90, KG-13, CAI-17 and CAI-134 were 11.33, 34.67, 25.33 and 19.67mm, respectively. Chitinase activity showed a positive correlation with larval mortality %. Chitinase activity showed a positive correlation with larval mortality % (Table 3, Figure 3). Hence, more chitin degrading capable isolates showed high mortality %, *i.e.*, KAI-90 showed a high zone of inhibition with 25.33 mm in which high larval mortality (66.67%) was recorded. KAI-90 with a less zone of inhibition (11.33 mm) showed low larval mortality (33.33%).

Evaluation of metabolites under greenhouse conditions

ECM of isolates which showed a mortality % of greater than 80 and ICM isolates that showed highest mortality (70%) in diet impregnation bioassay were considered as promising isolates, and they were further evaluated in greenhouse conditions. In addition, *Bt var. kurstaki*, *M. anisopliae* and Emamectin benzoate 5% SG (Spar) were also used in the evaluation for comparative studies. Results pertaining to mean mortality % are shown in Table 4 and Figure 4.

On the 3rd DAT, *Bt var. kurstaki* treated plants showed a mortality of FAW up to 63 %, followed by Emamectin benzoate with 60 % mortality. Among isolates, KG-13 ECM showed comparatively high mortality (46.6%). CAI-17 ECM, KG-13 ICM and *M. anisopliae* resulted in 33.33 % mortality.

Bt var. kurstaki and Emamectin benzoate 5% SG resulted in 73.3 % mortality, followed by 66.66 % in KG-13 ECM,

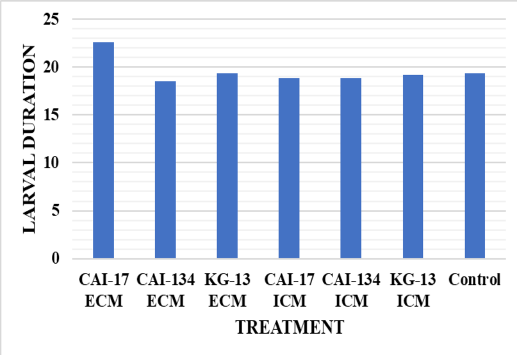


Figure 2. Larval duration of survived *S. frugiperda* treated with extracellular and Intracellular metabolites of promising actinomycetes isolates.

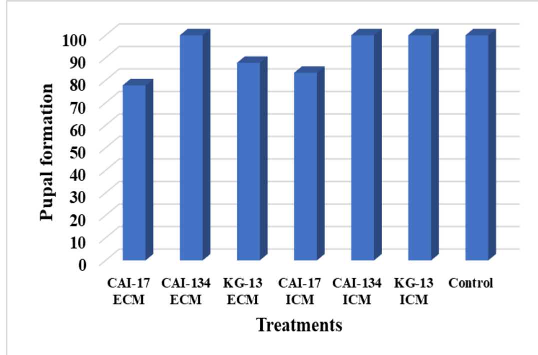


Figure 3. Per cent pupation in survived *S. frugiperda* treated with extracellular and intracellular metabolites of promising actinomycetes isolates.

Table 2. Fate of survived *S. frugiperda* larvae treated with Extracellular metabolite and Intracellular metabolite of promising actinomycetes isolates in terms of larval duration and pupal formation

S. No.	Treatment	Larval duration	Pupal formation
1	**CAI-17 ECM**	22.57±1.98**	77.77
2	**CAI-134 ECM**	18.50 ±0.57	100
3	**KG-13 ECM**	19.38±0.74	93.77
4	**CAI-17 ICM**	19.83±0.74	100.0
5	**CAI-134 ICM**	18.83±0.54	100.0
6	**KG-13 ICM**	19.17±0.51	100.0
7	**Control**	19.38±0.75	100.0

Means ± SD followed by *: significantly different ($P< 0.05$), **: highly significantly different ($P< 0.01$)

Table 3. Correlation between chitinase activity of promising actinomycetes isolates and per cent mortality of *S. frugiperda* larvae

Isolate	Chitinase activity (in mm)	Mortality per cent
KAI--90	11.33	33.33
KG-13	34.67	66.67
CAI-17	25.33	56.67
CAI-134	19.67	53.33

	Correlation coefficient	Regression equation	R^2 value
Chitinase (x) Vs Mortality per cent	0.963*	21.281+1.372 X	0.926 (92.6 %)

X- Chitinase activity
*Correlation is significant at 0.05 level (2 tailed)

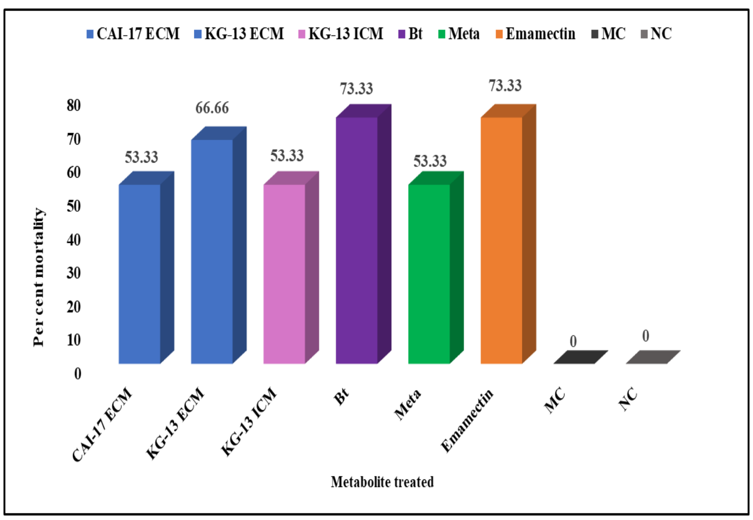


Figure 4. Per cent mean mortality of 2nd instar larvae of *S. frugiperda* treated with secondary metabolites of promising actinomycetes on sorghum in greenhouse.

Table 4. Evaluation of secondary metabolites of promising actinomycetes against 2nd instar of *S. frugiperda* on Sorghum in greenhouse

S. No.	Treatment	Mean per cent mortality at different intervals after treatment				
		3 DAT	**4 DAT**	**5 DAT**	**6 DAT**	**7 DAT**
1	**CAI-17 ECM**	33.33 (35.28)^{b}	40 (39.25)^{b}	53.33 (46.93)^{b}	53.33 (46.93)^{b}	53.33 (46.93)^{b}
2	**KG-13 ECM**	46.66 (43.11)^{bc}	66.66 (54.76)^{cd}	66.66 (54.76)^{bc}	66.66 (54.76)^{bc}	66.66 (54.76)^{bc}
3	**KG-13 ICM**	33.33 (35.28)^{b}	53.33 (46.93)^{bc}	53.33 (46.93)^{b}	53.33 (46.93)^{b}	53.33 (46.93)^{b}
4	***Bt var. kurstaki***	63 (52.56)^{c}	73.33 (58.94)^{d}	73.33 (58.94)^{c}	73.33 (58.94)^{c}	73.33 (58.94)^{c}
5	***M. anisopliae***	33.33 (35.28)^{b}	53.33 (46.93)^{bc}	53.33 (46.93)^{b}	53.33 (46.93)^{b}	53.33 (46.93)^{b}
6	**Emamectin (Spar)**	60 (50.79)^{c}	73.33 (58.94)^{d}	73.33 (58.94)^{c}	73.33 (58.94)^{c}	73.33 (58.94)^{c}
7	**MC**	0.0 (0.29)^{a}				
8	**NC**	0.0 (0.29)^{a}				
S.Em±		**4.96**	**4.96**	**5.70**	**5.70**	**5.70**
LSD (5% level)		**15.05**	**15.05**	**17.30**	**17.30**	**17.30**

*Values in parentheses were arcsine-transformed values. Means allotted with same letters do not differ significantly (0.05) by DMRT. DAT=Days after treatment, MC= methanol, NC= Normal control, ECM=Extracellular metabolites, ICM= Intracellular metabolites

a) 

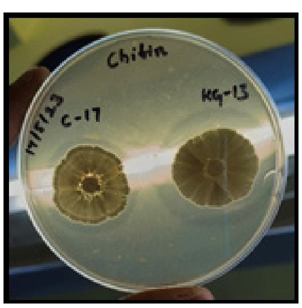
b) 

Plate 1. (a). View of Whole culture, ECM, ICM of isolate CAI-17; (b). Extra and intracellular metabolites stored in screwcap tubes.

a) 

b) 

Plate 2. (a). Untreated plate and treated plate (KG-13); (b). Larval- pupal intermediates observed in KG-13 and CAI-17 ECM treated larvae.

a) 

b) 

Plate 3. (a). Zone of inhibition in CAI-17 and KG-13; (b). Pots covered with mesh after larval release.

CAI-17 showed 40 % and KG-13 ICM and *M. anisopliae* registered 53.33 % on 4 DAT.

On 5th DAT, only CAI-17 ECM resulted in 53.33 %. The remaining isolates showed no increase in mortality rate. On the 6th and 7th DAT, the mortality rate remained unchanged.

In greenhouse evaluation, among all treatments, formulations of *Bt var. kurstaki* and Emamectin benzoate showed comparatively highest mortality (73.33%) followed by KG-13 ECM with 66 % mortality whereas CAI-17 ECM, KG-13 ICM and *M. anisopliae* resulted in 53.3 % mortality.

DISCUSSION

Among three isolates that were used for the production of ECM and ICM that were evaluated against 2nd instar larvae FAW, KG-13 ECM showed the highest mortality (85.19 %) followed by CAI-17 (81.48 %). KG-13 ICM and CAI-134 ECM showed 70.37 % mortality. ICM of CAI-17 and CAI-134 eliminated larval load of 51.85 and 29.63 %, respectively. Results are in line with the work of Vijayabharathi *et al*.. (2014), who assessed ECM and ICM of 15 actinomycetes isolates against *S. litura*. Mortality results were between 0.00 and 100.0. ECM and ICM of isolate CAI-13 recorded 83 and 52 % larval mortality, followed by ECM and ICM of isolate BCA-656 that recorded 63 and 13 % mortality. Isolate BCA-508 ECM and ICM recorded mortality of 59 and 13 %, respectively.

Comparatively, in all the promising isolates, ECM exhibited more larval mortality % than ICM and WC. This investigation was similar to the findings of Vijayabharathi *et al.* (2014), who demonstrated that the insecticidal efficacy relation among WC, ECM and ICM was ECM>ICM>WC. These results were satisfied with the justification of Kekuda *et al*. (2010), *i.e.*, most of the metabolites which shown insecticidal, herbicidal, antimicrobial properties were extracellular that were generally released into the culture media and it was supported with the review of Omura (2011), *i.e.*, most of the metabolites in his research were isolated and characterized from bacteria, fungus and actinomycetes isolates were extracted from extracellular metabolites.

In the bioassay with ECM and ICM, only ECM registered satisfactory larval mortality %. But in case of KG-13, ICM also showed considerable mortality %. Osman *et al*. (2007) isolated 15 *Streptomyces* and evaluated the insecticidal activity of both pellets and culture filtrate against cotton leafworm. Some pellets (intracellular metabolites) of *Streptomyces* isolates showed more efficacy than culture filtrates.

The effect of ECM and ICM of the isolates that were reported with the highest larval mortality may be due to the production of metabolites with insecticidal properties, and the compounds present in the metabolite might have resulted in susceptibility to the test insect (FAW). Mortality observed in the bioassays might also be due to restricted feeding. WC/Metabolite may act as an antifeedants, or it may have a contact mode of action. This mode of action might be similar to EPNs. EPNs that enter the insects through larval natural openings like spiracles, mouth and anus. After entry, EPNs release EPBs like *Photorhabdus* and *Xenorhabdus* into the haemocoel. The poisons released by these EPBs affect the immune systems of insects and cause detrimental effects in insects (Prasad & Singh, 2023). This was also supported with the work of Hazaa *et al*. (2017) who treated Emamectin (*Streptomyces* extract) on *Galleria mellonella* (Linnaeus) larvae and inferred that the compound blocked the mechanical, olfactory function of sensilla and restricted larval movement which lead to reduction in feeding behaviour or completely ceased feeding and eventually died. Soliman *et al.* (2021) also found larvae of *G. mellonella* that were treated with the purified extract of *Streptomyces* sp. with many deformities in the external body and its associated sensilla. Also, the extract resulted in the closure of the spiracles of the larvae, which led to the inability to breathe.

Out of 85 isolates, the crude extract (ECM) of 24 isolates against green aphid resulted in contact toxicities. Among them, 8 isolates showed mortality greater than 60 % (Chen *et al.*, 2018).

Actinomycetes' secondary metabolites exhibit insecticidal activity with different modes of action. Generally, the mode of action is dependent on the compound present in the metabolite, such as spinosad, which acts on GABA receptors and nicotinic acetylcholinic receptors; avermectins act as agonists for GABA-gated chloride channels, and milbemycin stimulates GABA and binds receptor sites of inhibitory motor neurons. Some compounds act as insect growth regulators (Bream *et al*., 2001). So, to depict and confirm the mode of action of any metabolite, compound identification is crucial.

Influence of metabolites on survived larvae

These results were in line with Bream *et al*. (2001), who investigated morphometric effects of different actinomycetes secondary metabolites against *S. littoralis.* Out of 41 strains, only three strains showed a significant effect on larval prolongation. The larvae treated with the secondary metabolites of strains 9, 15, 18, and 22 restricted 60 % of the larvae pupal formation stage, and they remained in larval-pupal intermediates. This was due to the blockage of eclosion hormone production or the presence of ecdysteroid similar compounds, and it was concluded that secondary metabolites might have an ecdysteroid kind of structural similarity.

Correlation between Chitinase activity and mortality %

Chitinase activity showed a positive correlation with larval mortality %. Hence, more chitin degrading capable isolates showed high mortality %, *i.e.*, KAI-90 showed a high zone of inhibition with 25.33 mm in which high larval mortality (66.67%) was recorded. KAI-90 with a less zone of inhibition (11.33 mm) showed low larval mortality (33.33%). This was by Kaur *et al*. (2014), who evaluated the insecticidal activity of ethyl acetate extract of DH16 against *S. litura*. About 40 % larval mortality and extension of larval period were noticed. The larvae which were fed on a diet treated with extract showed significant larval prolongation in comparison with the control. All the larvae formed into pupae after 2 weeks in the control diet, whereas only 6.66% pupation was noticed in larvae fed on incorporated diet even after 45 days, which was significantly lower than the control and which occurred due to production of insecticidal compounds and chitinase enzymes.

The positive correlation of chitinase activity with mortality % was supported by findings of Veliz *et al*. (2017), who demonstrated that chitinases affect insect growth in terms of feeding rate and body weight. It was observed that a decrease in larvae weight when contacted with chitinase ultimately led to death. These symptoms were attributed to the weakening of the peritrophic membrane that lines the gut epithelium of the larvae, the main component of which is chitin.

Evaluation of metabolites under greenhouse conditions

Present results are in partial support with the findings of Pedaveeti *et al*. (2022) who evaluated the insecticidal activity of three actinobacterial extracts, *viz.,* DBT-80, DBT-64 and DBT-59 against second instar larvae of *S. frugiperda* under greenhouse conditions on maize and mortality % recorded at 96 hours after treatment were 80.50, 79.50 and 78.25 per, respectively.

Mortality obtained from greenhouse studies was low when compared to laboratory studies. Similar findings were reported by Vijayabharathi *et al.* (2014), who evaluated promising actinomycetes isolates in a greenhouse against *H. armigera*. Different mortality rates were recorded in their study in which ECM isolates of BCA-667, BCA - 689, BCA-546 and BCA -508 recorded 68, 72, 75, 77 and 79 % of mortality in the greenhouse, whereas the same ECM isolates resulted in 100 % mortality in laboratory studies. This might occur due to less persistence of metabolite on the leaf surface, or it may require any carrier materials or adhesive materials for better efficacy. Insufficient dosage can also be attributed as one of the reasons.

CONCLUSION

Among screened 24 actinomycetes, only three isolates were proven effective against 2nd instar larvae of fall armyworm. In the results of metabolite assay, ECM of prominent isolates showed higher mortality than ICM, *i.e.*, ECM of KG-13 showed 85.19 %, and Emamectin showed a mortality of 81.48 %, which were statistically on par with each other. In the ECM of CAI-17, larval prolongation and larval-pupal intermediates were observed, which might be due to the insect growth regulation activity present in the metabolites. Chitinase activity and larval mortality % were positively correlated, which was revealed by mortality reported in larvae treated with promising isolates. KG-13 and CAI-17 were effective against *S. frugiperda,* but KG-13 caused the highest mortality, than CAI-17. Comparatively, less mortality of FAW was noticed in greenhouse conditions than in laboratory conditions.

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REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol*, **18**(2): 265-267. https://doi.org/10.1093/jee/18.2.265a

Bream, A. S., Ghazal, S. A., El-Aziz, Z. K. A., and Ibrahim, S. Y. 2001. Insecticidal activity of selected actinomycetes strains against the Egyptian cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Meded Fak Landbouwkd Toegep Biol Wet Univ Gent*, **66**(2a): 503-544. https://www.researchgate.net/publication/11041777

Chen, Y., Shafi, J., Li, M., Fu, D., and Ji, M. 2018. Insecticidal activity of endophytic actinomycetes isolated from *Azadirachta indica* against *Myzus persicae*. *Arch Biol Sci*, **70**(2): 349-357. https://doi.org/10.2298/ABS170729052C

Copping, L. G., and Menn, J. J. 2000. Biopesticides: A review of their action, applications and efficacy. *Pest Manag Sci*, **56**(8): 651-676. https://doi.org/10.1002/1526-4998(200008)56:8%3C651::AID-PS201%3E3.0.CO;2-U

Duncan, D. B. 1955. Multiple range and multiple 'F' tests. *Biomet*, **11**: 1-42. https://doi.org/10.2307/3001478

Hardke, J. T., Lorenz, G. M., and Leonard, B. R. 2015. Fall armyworm (Lepidoptera: Noctuidae) ecology in southeastern cotton. *J Integr Pest Manag*, **6**(1): 1-8. https://doi.org/10.1093/jipm/pmv009

Hazaa, M. A., Sayed, R. M., and Abdalla, R. S. 2017. Toxicological and ultrastructure studies in control greater wax moth, *Galleria mellonella* (L.), using gamma radiation and/or emamectin benzoate. *Egypt J Biol Pest Co*, **27**(2): 195-203. https://www.researchgate.net/publication/318755919

Jaba, J., Sathish, K., and Mishra, S. P. 2020. Biology of fall army worm *Spodoptera frugiperda* (JE Smith) on artificial diets. *Indian J Entomol*, **82**(3): 543-546. http://dx.doi.org/10.5958/0974-8172.2020.00135.2

Kaur T., Vasudev A., Sohal S. K., & Manhas R. K. 2014. Insecticidal and growth inhibitory potential of *Streptomyces hydrogenans* DH16 on major pest of India, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *BMC Microbiol*, **14**: 1-9. https://doi.org/10.1186/s12866-014-0227-1

Kekuda, T. P., Shobha, K. S., and Onkarappa, R. 2010. Potent insecticidal activity of two *Streptomyces* species isolated from the soils of the Western Ghats of Agumbe, Karnataka. *J Nat Pharm*, **1**(1): 30-32.

Khattab, A. I., Babiker, E. H., and Saeed, H. A. 2016. *Streptomyces*: Isolation, optimization of culture conditions and extraction of secondary metabolites. *Int Curr Pharm J*, **5**(3): 27-32. http://dx.doi.org/10.3329/icpj.v5i3.26695

Kirst, H. A. 2010. The spinosyn family of insecticides: Realizing the potential of natural products research. *J Antibiot*, **63**(3): 101-111. https://doi.org/10.1038/ja.2010.5

Kumar, P. S., Duraipandiyan, V., and Ignacimuthu, S. 2014. Isolation, screening and partial purification of antimicrobial antibiotics from soil *Streptomyces* sp. SCA 7. *Kaohsiung J Med Sci*, **30**: 435-446. https://doi.org/10.1016/j.kjms.2014.05.006

Moncheva, P., Tishkov, S., Dimitrova, N., Chipeva, V., Antonova-Nikolova, S., and Bogatzevska, N. 2002. Characteristics of soil actinomycetes from Antarctica. University of Toronto. https://hdl.handle.net/1807/1178

Montezano, D. G., Spencht, A., Sosa-Gómez, D. R., Roque-Specht, V. F., Sousa-Silva, J. C., Paula-Moraes, S. V., Peterson, J. A ., and Hunt, T. E. 2018. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the America. *Afri Entomol*, **26**(2): 286-300. https://hdl.handle.net/10520/EJC-112bc26060

Osman, G., Mostafa, S., and Mohamed, S. H. 2007. Antagonistic and insecticidal activities of some *Streptomyces* isolates. *Pak J Biotechnol*, **4**(1-2): 65-71. https://pjbt.org/index.php/pjbt/article/view/199

Pedaveeti, S., Kambrekar, D., Mallapur, C., and Krishnaraj, P. 2022. Insecticidal properties of actinobacterial extracts against selected lepidopteran insect pests under laboratory and greenhouse condition. *J Farm Sci*, **35**(1): 108-115. https://doi.org/10.61475/jfm.v35i01.113

Prasad, D., and Singh, R. P. 2023. Entomopathogenic Nematodes: Mode of Action and Application Methods. *Biotica Res Today*, **5**(2): 199-203.

Schrempf, H. 2001. Recognition and degradation of chitin by *Streptomyces*. *Anton Leeuw*, **79**: 285-289. https://doi.org/10.1023/A:1012058205158

Sharanabasappa, D., Kalleshwaraswamy, C. M., Asokan, R., Swamy, H. M. M., Maruthi, M. S., Pavithra, H. B., … Goergen, G. 2018. First report of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. *Pest Manag Horticul Ecosys*, **24**(1): 23-29. https://hdl.handle.net/10568/103519

Soliman, M. A. W., Hamza, A. F., Zahran, N. F., and Bassioni, G. 2021. Microbiological study and insecticidal potential of purified extract from *Streptomyces* sp. on the larvae of *Galleria mellonella*. *J Plant Dis Prot*, **128**: 1565-1574. https://doi.org/10.1007/s41348-021-00508-0

Veliz, E. A., Martínez-Hidalgo, P., and Hirsch, A. M. 2017. Chitinase-producing bacteria and their role in biocontrol. *AIMS Microbiol*, **3**(3): 689-705. https://doi.org/10.3934/microbiol.2017.3.689

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., and Van Sinderen, D. 2007. Genomics of actinobacteria: Tracing the evolutionary history of an ancient phylum. *Microbiol Molecular Biol Rev*, **71**(3): 495-548. https://doi.org/10.1128/mmbr.00005-07

Vijayabharathi, R., Kumari, B. R., Sathya, A., Srinivas, V., Abhishek, R., Sharma, H. C., and Gopalakrishnan, S. 2014. Biological activity of entomopathogenic actinomycetes against lepidopteran insects (Noctuidae: Lepidoptera). *Can J Plant Sci*, **94**(4): 759-769. https://doi.org/10.4141/cjps2013-298