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Bioefficacy of Actinomycetes Secondary Metabolites against *Maruca vitrata* (Geyer) in Pigeonpea

M. Keerthana, B. Ratna Kumari*, S.R. Koteswara Rao, Jaba Jagdish[1] and Rajan Sharma[1]

Acharya N G Ranga Agricultural University, Lam, Guntur
[1]International Crop Research Institute for Semi-Arid Tropics, Patancheru, Hyderabad-502 324, India
**E-mail: b.ratnakumari@angrau.ac.in*

Abstract: Secondary metabolites of seven actinomycetes isolates were tested for their insecticidal activity against second instar larvae of *Maruca vitrata* using diet impregnation and detached flower bioassay. In diet impregnation bioassay mortality rates recorded by KAI-26, MMA-32, SAI-13, CAI-93, CAI-21, SAI-25 and KG-13 were 90.90%, 90.90%, 81.81%, 63.63%, 54.54%, 51.51% and 18.18%, respectively. In detached flower bioassay mortality rates shown by MMA-32, KAI-26, SAI-13, SAI-25, KG-13, CAI-21 and CAI-93 were 94.44%, 94.44%, 88.88%, 72.22%, 44.44% and 33.33%, respectively. Isolates which showed highest efficacy in both diet impregnation and detached flower bioassay were further evaluated in glass house. Glass house studies revealed that KAI-26, MMA-32 and SAI-13 maintained the same performance with 90.90% mortality. The effective isolates against *M. vitrata* were KAI-26, MMA-32 and SAI-13.

Keywords: Actinomycetes, Secondary metabolites, *Maruca vitrata*, Bioassay, Mortality

Pigeonpea (*Cajanus cajan* L. Millsp.) is an important grain legume crop widely cultivated in tropical and subtropical regions for its high protein content and resilience to environmental stresses. Despite its agronomic importance, productivity is severely constrained by insect pests, among which the legume pod borer, *Maruca vitrata* (Geyer), is one of the most destructive (Shejulpatil et al., 2020). *Maruca vitrata* (Geyer), a widespread tropical insect pest, causes extensive damage to leguminous crops like pigeon pea, cowpea, mung bean, and soybean, posing a serious threat to yields in tropical and subtropical regions worldwide. The larvae feed destructively on flowers, buds, and developing pods, making it a major global pest of grain legumes with severe agricultural and economic consequences (Sharma and Franzmaan 2000).

Actinomycetes are gram-positive, filamentous bacteria that form spores and thrive in diverse environments including soil, rhizosphere, actinorhizal plants, hypersaline soils, limestone, freshwater, marine ecosystems, sponges, volcanic caves, deserts, air, insect guts, earthworm castings, goat faeces and as endophytes (Selim et al., 2021). Some prokaryotic bacteria, particularly *Streptomyces* species, synthesize bioactive secondary metabolites (Berdy 2005), which include antibiotics and anticancer agents that have significantly advanced medicine and pharmaceutical research. Bioactive secondary metabolites offer a sustainable alternative to synthetic insecticides, as they are less toxic and more biodegradable. These metabolites, derived from actinomycetes, can be integrated into pest management strategies as novel biopesticidal formulations (Aggarwal et al., 2016).

The present study investigates the bioefficacy of secondary metabolites derived from actinomycetes against *M. vitrata* in pigeonpea. This research aims to identify potential biocontrol agents capable of reducing pest incidence while promoting sustainable pigeonpea production. This work contributes to developing novel, environmentally safe pest management approaches and expanding the use of microbial metabolites in crop protection.

MATERIAL AND METHODS

The research work was conducted at Microbiology department and Insect rearing unit, International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru. Whole cultures of 20 actinomycetes isolates were screened against second instar larvae of *M. vitrata*. The isolates which showed highest, medium and lowest mortality were selected for further extracellular metabolite extraction.

Extra-cellular metabolite extraction: Approximately 2–4 loopfuls of actinomycetes cultured on Actinomycetes Isolation Agar (AIA) plate were inoculated into 3 liters of starch casein broth and incubated in a shaker incubator at 120 rpm and 28±2°C for eight days. The extracellular metabolites (ECM) were extracted according to the method described by Khattab et al. (2016). On the eighth day, the broth was centrifuged at 10,000 rpm for 10 minutes. The supernatant and cell mass (pellet) were separated using muslin cloth. The pH of the supernatant was adjusted to 3 to ensure proper extraction, as some lipophilic compounds in the culture filtrate may contain ionizable groups (such as carboxylates) that could hinder organic phase partitioning unless suppressed by acidic conditions.

The supernatant was subjected to solvent partitioning using a separating funnel. Ethyl acetate, in a volume equal to culture filtrate, was added to the filtrate for extraction. The partitioning process was repeated three times to ensure maximum recovery of compounds. The mixture was vigorously shaken for one minute and then allowed to settle until clear separation of the organic (ethyl acetate) and aqueous (culture filtrate) phases occurred. The ethyl acetate layer was carefully separated and pooled. To eliminate any residual moisture or cell debris, anhydrous sodium sulphate was added to the combined organic phase. The solution was then filtered and concentrated through evaporation.

The organic fraction was concentrated using a rotary evaporator at 40°C to form a thin film. The residue adhering to the flask walls was dissolved in methanol (using 10–15 ml of methanol per 3 liters of broth culture) and then transferred to glass screw-cap tubes. These samples were stored at 4°C for subsequent analysis and use.

Diet impregnation bioassay: About 200 ul of ECM was added to the cell well plate containing about 2 ml of dried artificial diet. Later pre-starved second instar larva was added to each individual well. Two replications were used for each treatment (sample) and each replication consisted of 12 larvae. Mortality was observed on 3rd, 5th and 7th DAT. Moribund larvae were also considered as dead. Percentage of mortality for each test isolate was computed. Mortality in methanol control was also recorded and corrected mortality (Abbott 1925) per each treatment was calculated.

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

Detached flower bioassay: About 10 ml of 3% boiled agar was prepared and poured into plastic cups placed at an angle of 45°. After solidification, flower buds which were dipped in ECM for 2-5 min were kept in the cups containing agar. Ten second instar larvae of *M. vitrata* which were pre-starved for 1-2 hours released into each cup containing treated flowers. Two replications were used for each treatment (sample) and each replication consisted of 10 larvae. The insect mortality was recorded on 3rd, 5th and 7th day after treatment (DAT). Moribund larvae were also considered as dead. Percentage of mortality for each test isolate was computed. Mortality in methanol control was also recorded and corrected mortality (Abbott 1925) per each treatment was calculated.

Glass house experiment: Isolates that showed highest efficacy in both diet impregnation assay and detached flower bioassay were selected for glasshouse evaluation on *M. vitrata* in pigeonpea and the larval mortality was recorded. Methanol and water were used as negative controls. Fifty days after seedling emergence, plants were sprayed with secondary metabolite extracts (5ml/plant) and infested with 2nd instar larvae and covered with net bags. Four replications were used for each treatment (sample), and each replication consisted of ten larvae. The larval mortality was recorded on 3rd, 5th and 7th day after treatment. Per cent mortality for each test isolate was computed.

Statistical analysis: The 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).

RESULTS AND DISCUSSION

Diet impregnation bioassay: The 3rd day evaluation revealed significant variation in treatment efficacy, with KAI-26 demonstrating superior insecticidal activity (78.78%

Diet impregnation bioassay

Detached flower bioassay

Glass house experiment

mortality). SAI-13 was moderately effective (45.45% mortality), followed by SAI-21 (27.27%) and SAI-25 (22.72%). MMA-32 exhibited limited impact (22.72% mortality), while CAI-93 and KG-13 displayed no detectable mortality at this early evaluation stage.

The bioassay results at 5th DAT revealed a progressive increase in larval mortality across all treatments. MMA-32 and KAI-26 emerged as the most effective isolates, both achieving 81.81% mortality. SAI-13 followed with substantial efficacy (68.18%), while CAI-21 and CAI-93 demonstrated moderate activity (54.54%). SAI-25 showed lower insecticidal potential (36.36%) and KG-13 remained the least effective, with only 9.09% larval mortality. The bioassay results at 7th DAT revealed significant differences in treatment efficacy. KAI-26 and MMA-32 demonstrated the highest larval mortality, 90.90%, followed by SAI-13 with 81.81% mortality. Intermediate efficacy was observed in CAI-93 (63.63%), CAI-21 (54.54%) and SAI-25 (51.51%). In contrast, KG-13 showed markedly lower effectiveness, producing only 18.18% mortality.

High levels of larval mortality observed following exposure to ECM from the selected isolates may be attributed to their production of insecticidal metabolites, with these bioactive compounds potentially enhancing the susceptibility of *M. vitrata* to treatment. Another possible explanation for the mortality is decreased feeding, as either the whole culture (WC) or the produced metabolites could possess antifeedant properties or demonstrate contact toxicity, thereby reducing larval survival. The variation in mortality rates could be attributed to differences in the effective dosage against *M. vitrata*. The above results coincided with Lakshmi et al. (2025) who evaluated the ECM of three isolates against second instar larvae of fall armyworm. Highest mortality was reported in KG-13 (85.19%) followed by CAI-17 (81.48%) and CAI-134 (70.37%).

Detached flower bioassay: At 3 DAT, mortality rates showed clear stratification among treatments, with MMA-32 demonstrating the highest efficacy (61.11%), followed by SAI-13 and KAI-26 (55.55%). SAI-25 exhibited moderate activity (44.44%), while CAI treatments showed limited effectiveness, particularly CAI-93 (16.66%) and KG-13 (0%) displayed no measurable mortality at this early stage. By 5th DAT, mortality patterns became more pronounced, with three treatments SAI-13, MMA-32 and KAI-26, reaching high equivalent efficacy (83.33%). There was an improvement observed in KG-13, from 0% to 66.66% mortality. In contrast, the CAI treatments continued to demonstrate poor performance, with CAI-21 remaining at 33.33% and CAI-93 showing only minimal improvement to 27.77% larval mortality.

At the 7th DAT, treatment efficacy reached its peak, with MMA-32 and KAI-26 achieving the highest mortality rates (94.44%). SAI-13 maintained its strong performance (88.88%), while SAI-25 and KG-13 showed comparable efficacy (72.22%). Notably, the CAI treatments remained the least effective, with CAI-21 reaching only 44.44% and CAI-93 plateauing at 33.33% mortality. The above results align with the findings of Vijayabharathi et al. (2014), who conducted a detached leaf bioassay for the assessment of insecticidal activity of the ECM of 15 isolates on lepidopteran pests. Among them, 100% mortality was reported in BCA-546, BCA-659, CAI-13, CAI-87, CAI-132, CAI-133, CAI-155 and SAI-25 isolates.

Glass house experiment: On 3rd DAT, there was a significant difference in the efficacy of the treatments. Among

Table 1. Efficacy of secondary metabolites (extracellular) of actinomycetes on 2nd instar larvae of *M. vitrata* in diet impregnation bioassay

Treatment	Mean percent mortality at different intervals after treatment					
	3 DAT		5 DAT		7 DAT	
	Mortality (%)	Corrected	Mortality (%)	Corrected	Mortality (%)	Corrected
SAI 13	50	45.45^{bc}	70.833	68.18^{de}	83.33	81.81^{de}
SAI-25	29.16	22.72^{ab}	41.66	36.36^{c}	54.16	51.51^{cd}
MMA-32	25	22.72^{ab}	83.33	81.81^{de}	91.66	90.90^{e}
KAI-26	79.16	78.78^{c}	83.33	81.81^{e}	91.66	90.90^{e}
CAI 21	33.33	27.27^{b}	58.33	54.54^{cd}	58.33	54.54^{cd}
CAI-93	0	0^{a}	58.33	54.54^{cd}	66.66	63.63^{de}
KG-13	0	0^{a}	16.66^{b}	9.09^{b}	25	18.18^{bc}
MC	8.33^{ab}		8.33^{ab}		8.33^{ab}	
NC			0^{a}			

Means followed with the same letters do not differ significantly (0.05) by DMRT (Number of treated larvae, N = 12). DAT = Days after treatment, MC = Methanol, NC= Normal control

Table 2. Efficacy of secondary metabolites (extracellular) of actinomycetes on 2nd instar larvae of *M. vitrata* in detached flower bioassay

Treatment	Mean percent mortality at different intervals after treatment					
	3 DAT		5 DAT		7 DAT	
	Mortality (%)	Corrected	Mortality (%)	Corrected	Mortality (%)	Corrected
CAI-21	35	27.77^{b-d}	40	33.33^{c}	50	44.44^{cd}
CAI-93	25	16.66^{a-c}	35	27.77^{bc}	40	33.33^{bc}
SAI-13	60	55.55^{cd}	85	83.33^{d}	90	88.88^{ef}
SAI-25	50	44.44^{b-d}	70	66.66^{d}	75	72.22^{de}
MMA-32	65	61.11^{d}	85	83.33^{d}	95	94.44^{f}
KAI-26	60	55.55^{cd}	85	83.33^{d}	95	94.44^{f}
KG-13	5	0.00^{a}	70	66.66^{d}	75	72.22^{de}
MC	10.00^{ab}		10.00^{ab}		10.00^{ab}	
NC	(0.00)^{a}					

Means followed with the same letters do not differ significantly (0.05) by DMRT (Number of treated larvae, N = 12). DAT = Days after treatment, MC = Methanol, NC= Normal control

Table 3. Efficacy of secondary metabolites (extracellular) of actinomycetes on 2nd instar larvae of *M. vitrata* on pigeonpea under glasshouse conditions

Treatment	Mean percent mortality at different intervals after treatment					
	3 DAT		5 DAT		7 DAT	
	Mortality (%)	Corrected	Mortality (%)	Corrected	Mortality (%)	Corrected
KAI-26	41.66	39.39^{c}	91.66	90.90^{b}	91.66	90.90^{b}
MMA-32	32.91	26.66^{c}	79.16	77.27^{b}	91.66	90.90^{b}
SAI-13	32.08	25.45^{c}	91.66	90.90^{b}	91.66	90.90^{b}
MC	8.33^{a}		8.33^{a}		8.33^{a}	
NC	0.0^{a}					

Means followed with the same letters do not differ significantly (0.05) by DMRT (Number of treated larvae, N = 12). DAT = Days after treatment, MC = Methanol, NC= Normal control

the tested treatments, highest efficacy was recorded in KAI-26 with 39.39% larval mortality. The rest of the two treatments, MMA-32 and SAI-13, exhibited 26.66% and 25.45% larval mortality, respectively. On 5th DAT, there was a rapid improvement in the efficacy of treatments. The larval mortality in KAI-26 and SAI-13 increased to 90.90%, Whereas MMA-32 recorded 79.16% mortality. On 7th DAT, KAI-26 and SAI-13 maintained the same performance, whereas the larval mortality in MMA-32 increased to 90.90%.

The results of the current study align with the findings of Pedaveeti et al. (2022), who assessed the insecticidal effects of three actinobacterial extracts, namely DBT-80, DBT-64 and DBT-59, against second instar larvae of *S. frugiperda* under greenhouse conditions on maize. The mortality percentages recorded 96 hours after treatment were 80.50, 79.50 and 78.25, respectively.

CONCLUSIONS

Among the seven tested isolates KAI-26, MMA-32 and SAI-13 were highly effective isolates in diet impregnation bioassay, detached flower bioassay and glasshouse screening. KAI-26 was identified as a *Streptomyces albus* strain, whereas MMA-32 was a strain of *Streptomyces roseoviolaceus* and SAI-13 was a *Streptomyces* species. The effective isolates against *M. vitrata* were KAI-26, MMA-32 and SAI-13. Among them KAI-26 isolate was quick in action. Future work may be concentrated on dose optimization and development of formulations and compound identification that helps to identify mode of action.

AUTHOR'S CONTRIBUTION

This study was conducted as a part of master's research work in ICRISAT under the supervision of Rajan Sharma and Jaba Jagdish. Study was conceptualized by B. Ratna Kumari. Methodology, data collection and analysis were carried out by M. Keerthana. The first draft of manuscript was written by M. Keerthana and all the authors reviewed the manuscript.

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