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Compatibility of *Streptomyces* sp., *Metarhizium anisopliae*, and Neem Seed Powder against Pigeon Pea Pod Borer Complex

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A B S T R A C T

Biopesticides such as *Streptomyces sp*. SAI-25, *Metarhizium anisopliae* and neem seed powder were previously demonstrated to have biocontrol potential against *Helicoverpa armigera*, the polyphagous insect pest of many crops. In the present investigation, the biopesticides, *Streptomyces sp.* SAI-25, *Metarhizium anisopliae*, and neem seed powder, were evaluated for their compatibility so that these can be used as consortia to manage pod borer complex. The results reveal that all the three biopesticides were compatible with each other and hence can be used in consortia to manage *H. armigera*.

Introduction

Pigeonpea, (*Cajanus cajan* L.) is the second most important grain legume crop after chickpea that occupies 2nd largest area among the various pulse crops grown in India. It is a staple diet and consumed as green peas as well as dry seeds (Tabo *et al.*, 1995). Globally, the area and production of pigeonpea has increased from 4.43 million hectares (mha) and 3.16 million tonnes (mt) in 2002 to 5.32 mha and 4.32 mt in 2012, respectively (FAOSTAT 2012). India is the largest producer and also the largest consumer of pigeonpea in the world. The yield levels of this crop are not very encouraging. Among the factors responsible for low yield, the damage caused by insect pests is one of the major factors in pigeonpea. It is attacked by several insect pests from seedling stage till harvesting. More than 250 insect pests are known to attack pigeonpea (Sharma *et al.*, 2008). However, Balikai and Yelshetty (2008) reported a total of 30 insect pests feeding on

pigeonpea. Out of these, one pest *Helicoverpa armigera* (Hubner) was recorded as major pest on this crop causing more than 51 percent damage, whereas, nine other insects viz., *Megalurothrips usitatus* (Bangall), *Empoasca kerri* (Pruthi), *Clavigralla gibbosa* (Spinola), *Riptortus pedestris* (Fb)., *Exelastis atomosa* (May.), *Melanagromyza obtuse* (Mlloch), *Cydia ptychora* (Meyr.), *Maruca testulalis* (Geyer) and *Etiella zinckenella* (Treit)., were recorded as moderate pests inflicting damage between 31 to 50 percent. As many as ten insect pests were recorded as minor pests on this crop, while ten were recorded as low important. Entomopathogenic fungi are often reported to cause high levels of epizootics in nature and environmentally safe. An attractive feature of these fungi is that the virulence caused by contact and the action is through penetration. These fungi are a heterogeneous group of over 100 genera with approximately 750 species, notified from different insects. Many of these are proved to be highly potential in pest management. The most considerable fungal species are *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella* spp., (Alves and Lopes 2008).

There are several bio-pesticides that are commercially available to farmers. A total of 175 registered bio-pesticide active ingredients and 700 products are available, globally, whereas in India so far only 12 bio-pesticides were registered, of which five were bacteria, three fungal, two viruses and two plant products.

Among various bio-products, *Bacillus thuringiensis* (Bt). *Trichoderma viridae*, *Metarhizium anisopliae*, *Beauveria bassiana*, Nuclear Polyhedrosis Virus (NPV) and neem are popularly used in plant protection (US Environmental Protection Agency, 2007). Hence this study is aimed to evaluate the compatibility of biopesticides including *Streptomyces sp.*, *Metarhizium anisopliae*, and neem seed powder against pigeon pea pod borer complex

Materials and Methods

The findings of the experiment on compatibility of *Streptomyces*, *Metarhizium anisopliae*, and neem seed powder against pigeonpea pod borer complex were conducted in the biocontrol laboratory at International crop research institute for the Semi-Arid Tropics (ICRISAT) Patancheru, Hyderabad during 2016-17.

Streptomyces (SAI-25) + *Metarhizium anisopliae* + Neem seed powder extract using GCY media

Streptomyces sp. SAI-25 Glucose casamino-acid yeast-extract (GCY) media was used for the dual culture assay. Composition of media was glucose 15g, Casamino acid 1.5g, Yeast extract 1.0g, KH2PO4 1.5g, MgSo47H2O 1.0g Agar 15g and distilled water 1lit. The media was prepared and autoclaved at 121^{o}C at 15lb for 15min. The autoclaved media was poured into Petri plates in laminar air flow and allowed to cool. A fungal disk (*Metarhizium anisipliae*) of 6 mm diameter was placed on one edge (1 cm from the corner) of the GCY agar plate and the SAI-25 was streaked on the other edge of the plate (1 cm from the corner) (Anjaiah *et al.*, 1998) and 100µl and 200µl concentration neem seeds powder extract also adding in each plates. Plates without *Streptomyces* and *Metarhizium anisopliae* were used as control. All plates were incubated at 27±1oC for until the pathogen completely covered the control plate. Inhibition of fungal mycelium (halo zone) around the *Streptomyces* sp. colony was noted as positive and the inhibition zone was measured at the end of 24 h and continued for 5 days, hence compatibility were tested in three replications.

Streptomyces (SAI-25) + neem seed powder extract using Streptomyces isolation agar media

Streptomyces isolation agar media was used for the dual culture assay. The media was prepared and autoclaved at 121^{o}C at 15lb for 15min. composition of Actinomycetes Isolation Agar (AIA) media was Sodium caseinate 2g, L-Asparagine 0.10g, Dipotassium sulphate 0.50g, Magnesium sulphate 0.10g, Ferrous sulphate 0.001g, Agar 15g and Distilled water 1lit.

The autoclaved media was poured into Petri plates in laminar air flow and allowed to cool. SAI-25 was streaked on *Streptomyces* isolation agar (AIA) and 100µl and 200µl concentration of neem seeds powder extract added in each plate of three replication and observation recorded upto the 5 day. All plates were incubated at 27±1^{o}C for until the pathogen completely covered the control plate.

Metarhizium anisopliae + Neem seed powder extract using Potato dextrose agar media

Potato dextrose agar media used for the dual culture assay. The media was prepared and autoclaved at 121^{o}C at 15 lb for 15 min. Composition of Potato Dextrose Agar (PDA) media was Dextrose 20g, Agar 15g and Distilled water 1 lit. The autoclaved media was poured into Petri plates in laminar air flow and allowed to cool. A fungal disk (*Metarhizium anisipliae*) of 6 mm diameter was placed on one edge (1 cm from the corner) of PDA plate and 100µl and 200µl concentration of neem seeds powder extract added in each plate of three replication and observation recorded upto the 5 day. Plates without neem powder concentration were used as control. All plates were incubated at 27±1^{o}C for until the pathogen completely covered the control plate.

Results and Discussion

The Compatibility of *Streptomyces, Metarhizium*, and neem seed powder against pod borer complex were conducted in the biocontrol laboratory at International crop research institute for the Semi-Arid Tropics (ICRISAT) Patancheru, Hyderabad during 2016-17.

Compatibility of *Streptomyces* and *Metarhizium anisopliae*

The Compatibility of Streptomyces and *Metarhizium anisopliae* revealed that a majority of the entomopathogenic fungus, *Metarhizium anisopliae* isolates, most of them accessioned and available in culture collections, were found compatible with the Streptomyces.

This study shows that the both *Streptomyces* and *Metarhizium anisopliae* are compatible and there is no inhibition found during compatibility of both *Streptomyces* and *Metarhizium anisopliae* upto the 5 day observations.

The growth phase of the mycelium continuing during compatibility including control (Table 1, Fig. 1).

Compatibility of *Streptomyces* + *metarhizium anisopliae* + Neem seed powder

Compatibility was tested in vitro through germination and growth assays. The Compatibility of *Streptomyces* + *metarhizium anisopliae* and Neem seed powder experiment was conducted in the In vitro study using dual culture technique with two entomopathogenic organisms and neem seed powder extract of two different concentrations 100µl and 200µl. *Streptomyces, metarhizium anisopliae and* Neem seed powder is being used as a biopesticide for many insect pests.

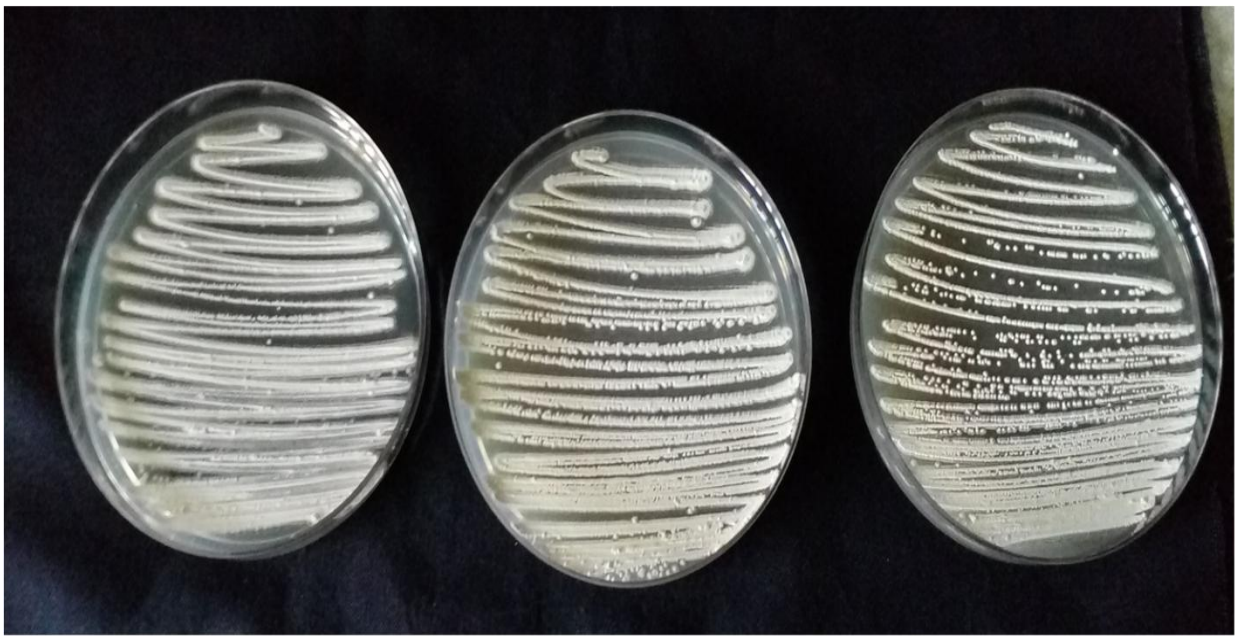
Fig.1 Compatibility of *Streptomyces* and *Metarhizium anisopliae*

Fig.2 Compatibility of *Streptomyces* + *metarhizium anisopliae* + Neem seed powder

Fig.3 Compatibility of *Metarhizium anisopliae* + Neem seed powder

Metarhizium anisopliae | ***Metarhizium anisopliae*** **+ Neem seed powder (100 µl)** | ***Metarhizium anisopliae*** **+ Neem seed powder (200 µl)**

Fig.4 Compatibility of *Streptomyces* + Neem seed powder



Streptomyces | ***Streptomyces*** **+ Neem seed powder (100 µl)** | ***Streptomyces*** **+ Neem seed powder (200 µl)**

Table.1 Compatibility of different Biopesticides

Sr. No	Treatments	Compatibility
1	*Streptomyces* + *Metarhizium anisopliae*	+
2	*Streptomyces* + *Metarhizium anisopliae* + Neem seed powder (100µl)	+
3	*Streptomyces* + *Metarhizium anisopliae* + *Neem* seed powder (200µl)	+
4	*Metarhizium anisopliae*	+
5	*Metarhizium anisopliae* + Neem seed powder(100µl)	+
6	*Metarhizium anisopliae* + Neem seed powder (200µl)	+
7	*Streptomyces*	+
8	*Streptomyces* + Neem seed powder(100µl)	+
9	*Streptomyces* + Neem seed powder (200µl)	+

+ Indicate that both are compatible

Compatibility studies between *Streptomyces* + *metarhizium anisopliae* + Neem seed powder revealed that there was no definite sign of suppression of any of the biopesticides, which significantly compatible growth of compound has been shown. It was concluded the compatibility *Streptomyces, metarhizium anisopliae and* Neem seed powder was Interestingly and significantly enhanced the growth of all the 3 biopesticides Compatibility. Studies among bacteria and fungus and neem seed powder showed that all were compatible with each other.

The compatibility between the neem derivatives with *M. anisopliae* can be effectively incorporated in the IPM programs for the effective control of pest population. The present findings of Hirose *et al.,* (2001) although the different neem derivatives tested in the present investigations inhibited the growth of *M. anisopliae* in poisoned media *in vitro*, the combined use of the fungus and neem derivatives cannot be completely ruled out. All the neem derivatives tested in this study have been combined at half recommended dose with entomopathogenic fungi for obtaining better control of pod borer in chickpea considering this, it is worth exploring the effect of these neem derivatives at sublethal dose with fungus for an enhance result over pest control (Table 1, Fig. 2).

Compatibility of *Metarhizium anisopliae* + Neem seed powder

Neem seed powder (azadirachtin) is an eco-safe popular botanical pesticide. The Compatibility of *Metarhizium anisopliae* + Neem seed powder, in order to assess the compatibility of botanical extract i.e., neem seed powder extract was mixed of different concentration 100µl and 200µl in PDA plates containing selected entomopathogenic microorganism such as *Metarhizium anisopliae* in three replications. This study revealed that neem seed powder was found to be compatible with fungus *Metarhizium anisopliae*. There were no signs of suppressing or inhibition appears upto the 5 day. Mohan *et al.,* 2007 reported that *Beauveria bassiana* is being used as a biopesticide for many insect pests. A biopesticde with a neem compatible isolate of *B. bassiana* will enable their simultaneous use in pest management. The effect of neem derivatives on the mycelial growth of *M. anisopliae* was conducted in *in vitro*. All the treatments showed significant mycelial growth, but less than control. Among neem derivatives tested, Neem soap 0.5% (w/v) (half recommended dose) was found more compatible with least growth inhibition percentage followed by Neem oil 2.5% (v/v) and NSKE 2.5% (w/v). The present findings

are in conformity with the findings of Hirose *et al.,* (2001) they also reported that neem oil less than 0.25% or neem derivatives less than 5% were relatively less toxic for mycelial growth and spores of *M. anisopliae.* The effect of neem derivatives on the mycelial growth of *M. anisopliae* was conducted in *in vitro*. All the treatments showed significant mycelial growth, but less than control. Among neem derivatives tested, Neem soap 0.5% (w/v) (half recommended dose) was found more compatible with least growth inhibition percentage followed by Neem oil 2.5% (v/v) and NSKE 2.5% (w/v) (Table 1, Fig. 3).

Compatibility of *Streptomyces* + Neem seed powder

Neem seed powder has minimal to no impact on non-target organisms, is compatible with other biological control agents and has a good fit into classical Integrated Pest Management programmes. Compatibility was tested in vitro using dual culture assay of *Streptomyces* + Neem seed powder, the neem seed powder was used with different concentrations such as 100µl and 200µl this concentration have been mixed with *Streptomyces* culture separately for the compatibility study. This result revealed that when neem seed powder of two different concentrations mixed with the *Streptomyce*s showing significant growth of *Streptomyces* and there is no suppression of growth was occurs of *Streptomyces* up to the 5 day observations. Both *Streptomyces* + Neem seed powder was showing compatible with each other (Table 1, Fig. 4).

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