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Compatability Response of Certain Entomopathogenic Fungi Consortia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigates the compatibility and development of consortia among four entomopathogenic fungi: *Beauveria bassiana*, *Lecanicillium lecanii*, *Metarhizium anisopliae*, and *Metarhizium rileyi*, aiming to enhance control measures against *S. litura*. Through visual observation of fungal interactions in petri dishes, different combinations were assessed for compatibility, revealing varying degrees of intermingling and inhibition. Partial compatibility was noted between *Beauveria bassiana*, *Lecanicillium lecanii*, Metarhizium anisopliae which can be grouped to a consortia.

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1. INTRODUCTION

The tobacco caterpillar. Spodoptera litura (Fabricius) is a regular polyphagous pest of field and horticultural crops (Murthy et al., 2006). This pest is next to Helicoverpa armigera (Hubner) in economic importance, both at national and global level. Spodoptera litura has emerged as a serious pest causing enormous losses to many economically important cultivated crops such as cotton, soybean, groundnut, tobacco, vegetables etc (Qin et al., 2004). It infests more than 112 crop plants belonging to 44 families in the world, of which 40 cultivated plants as well as 24 wild plants are known from India. The major host plants known from India are tobacco, cotton, cabbage, groundnut, sunflower, castor, lucerne, chilli, potato, soybean, cauliflower, tomato, beans etc. S. litura is primarily a defoliator but also feeds on buds, flower and pods of legumes (Krishnamurthy et al., 2005). Several synthetic pesticides have been tested and recommended for control of S. litura, which have caused serious concern due to their adverse impacts like health hazard, resistance build-up, residue problem, environment pollution etc.

Most of insect pest are becoming resistant to chemical pesticides due to its indiscriminate and excessive use which also affect the natural enemies of insect pest adversely and disturb the ecosystem. In such conditions bioagents play an important and effective role. Biopesticides offer several advantages over the chemical pesticides viz. safety, targeted activity to the desired pests, effective in lower quantities thereby offering lower exposure and quick decomposition to leave residues and allowing field no re-entrv immediately after application and amenability to use in rotation with chemical pesticides as part of IPM programs. If the two entomopathogens complement each other, or act synergistically, a beneficial effect can be obtained. One of the limitations of fungi as microbial control agents is that, each species and strains within a species are usually efficacious in a narrow window of climatic conditions. Theoretically, this issue could partly be addressed through the development of an appropriate co-formulation of two or more fungi or fungal strains with different host ranges and ecological tolerances (Wang et al., 2002). So in the current study, we tried to check the interaction between various entomopathogenic fungi to record the compatibility to form a consortia against damaging pests in agriculture. In the current study, we have investigated the compatibility between various entomopathogenic fungi to develop a consortia.

2. MATERIALS AND METHODS

Four entomopathogenic fungi that were reported to cause mortality in S. litura viz., Beauveria bassiana. Lecanicillium lecanii. and Metarhizium anisopliae. Metarhizium rilevi were selected to study the compatibility and to develop a consortia. All the four fungi were first arown separately in petriplates and inoculated for coculturing as per the procedure described by Upamanya et al., 2020. The four fungi were divided into two groups, fast-growing and slowgrowing fungi. The fungi that covered the whole petriplate within 5 days of inoculation were considered fast-growing and fungi that took more than 5 days after inoculation were considered as slow-growing. Beauveria bassiana, Lecanicillium Metarhizium lecanii. and anisopliae were considered as fast-growing and Metarhizium rileyi was considered to be slow-growing fungi. The M. rileyi was inoculated first in all the treatment combinations (Table 1) containing it by placing a 5mm disc on petriplate lined with potato dextrose agar (PDA) and incubated at 28±2°C for 5 days and the other two fast-growing fungi were inoculated at an equidistance after 5 days in a similar way under aseptic conditions in laminar airflow. In fourth combination where all fastgrowing fungi were present, all the three fungi were inoculated at a same time in an equal distance and the growth was observed for 14 days.

The compatibility between the fungi was studied by visually observing the interaction between the colonies after 14 days as per the procedure described by Mohammad et al.,2011 (Fig. 7). All the combinations were replicated thrice.

3. RESULTS AND DISCUSSION

3.1 Combination 1 (Beauveria bassiana + Metarhizium anisopliae + Metarhizium riyeli)

The *M.rileyi* was inoculated on potato dextrose agar and after 5 days of inoculating *M riyeli*, *B. bassiana* and *M. anisopliae* was inoculated at an equal distance in a petriplate. After 12 days and a zone of inhibition was observed around *M. riyeli* and no intermingling was observed between *M. riyeli* and other two fungi. The treatment is found to be incompatible by observing visually.

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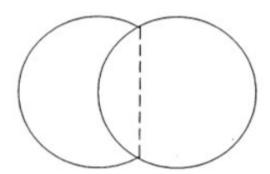


Fig. 1. Mutual intermingling (Compatible)

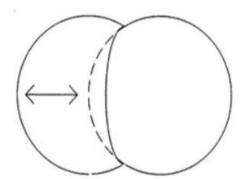


Fig. 3. Invasion/Replacement (Early Stage)

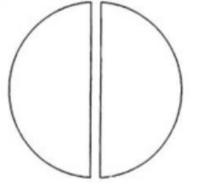


Fig. 5. Inhibition/deadlock (at touching point)

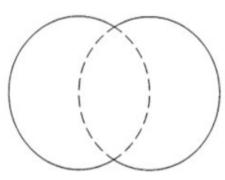


Fig. 2. Partial intermingling (Partial Compatible)

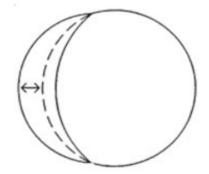


Fig. 4. Invasion/Replacement (Final Stage)

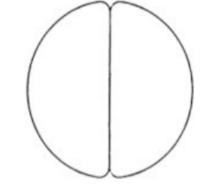


Fig. 6. Inhibition/deadlock (at distance)



Fig. 7. Petriplate showing inoculation for Combination 1

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Fig. 8. Petriplate showing inoculation for Combination 2



Fig. 9. Petriplate showing inoculation for Combination 3



Fig. 10. Petriplate showing inoculation for Combination 4

Table 1. Treatment combinations

Combination – 1	Beauveria bassiana + Metarhizium anisopliae + Metarhizium riyeli
Combination - 2	Beauveria bassiana + Metarhizium riyeli + Lecanicillium lecanii
Combination – 3	Beauveria bassiana + Metarhizium anisopliae + Lecanicillium lecanii
Combination - 4	Metarhizium anisopliae + Metarhizium riyeli + Lecanicillium lecanii

3.2 Combination 2 (Beauveria bassiana + Metarhizium riyeli + Leacanicillium lecanii)

After 5 days of inoculating the *M. riyeli* on PDA, *L. lecanii* and *B. bassiana* were inoculated at the same time in an equal distance and after 12 days no intermingling of *M. riyeli* with other two fungi was observed visually and a zone of inhibition was observed around the *M. riyeli*.

3.3 Combination 3 (Beauveria bassiana + Metarhizium anisopliae + Leacanicillium lecanii)

B. bassiana, M. anisopliae and *L. lecanii* were inoculated at the same time at an equal distance in a petriplate and after 12 days partial intermingling of three fungi was observed visually as described by Mohammad et al.,2011. The treatment was noted to be partially compatible (Fig. 7).

3.4 Combination 4 (Metarhizium anisopliae + Metarhizium riyeli + Leacanicillium lecanii)

M. anisopliae and *L. lecanii* were inoculated after 5 days of growing the *M. riyeli*. No intermingling of *M. riyeli* was observed with other two fungi with a zone of inhibition present around the *M. riyeli*.

It was found that there is a partial compatibility between the combination of *Beauveria bassiana* + *Metarhizium anisopliae* + *Leacanicillium lecanii* and in all other combinations, where *Metarhizium rileyi* is present, a clear zone of inhibition was observed around the fungi with no intermingling and they are observed to be incompatible for forming a consortia.

The current findings are in accordance with Sumalatha et al.,2011 who evaluated the compatibility of L.lecanii with other entomopathogenic fungi and reported that the B. bassiana is compatible with L. lecanii. Mohammad et al.,2011 divided six fungal isolates with the ability to compost an oil palm industrial waste into fast and slow-growing fungal groups and inoculated them at different times and observed the growth for a few days. Based on the visual observation they reported that Trichoderma viride and Penicillium sp. (Tv/P), T. viride and Basidiomvcete M1 (Tv/M1). Trichoderma reesei and Panus tigrinus M609RQY (Tr/M6) were compatible.

The current findings are in accordance also with Upamanya et al. 2020 where they reported that there is a compatibility of B. bassiana with M. anisopliae and Trichoderma harzianum. They formed two combinations of compatible biocontrol agents with combination-I containing T. harzianum +B. bassiana; T. harzianum + M. anisopliae; B. bassiana + M. anisopliae and T. harzianum +B. bassiana+ M. anisopliae and the combination-II containing T. asperellum +B. bassiana; T. asperellum + M. anisopliae; B. bassiana + M. anisopliae and T.asperellum +B. bassiana+ M. anisopliae and used these combinations to control the insect pest and diseases in brinjal crop.

Karthiba et al.,2010 by dual culture technique, have streaked the Pseudomonas strain on one side of a petri dish and placed a mycelial disc of a seven-day-old culture of B. bassiana on the opposite side perpendicular to bacteria and found that there is no growth hindrance of any of the organisms and the combination effectively reduced the leaf folder and sheath blight incidence in rice plants. Thakkar A and Saraf M (2015) have noted the compatibility between antagonistic fungi and bacteria by co-culturing at 30°C them on PDA after inoculating them 2 cm apart from each other and reported that Trichoderma citrinoviride was compatible with Pseudomonas aeruginosa, Bacillus cereus and can be used as an consortia against Macrophomina phaseolina and Sclerontinia sclerotiorum Murthy, 1983.

4. CONCLUSION

The fungal combination of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Leacanicillium lecanii* were found to be partially compatible under laboratory conditions, and further studies are required to understand the efficacy of the consortia in terms of causing mortality in insect populations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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