

# COMPATIBILITY RESPONSE OF CERTAIN ENTOMOPATHOGENIC FUNGI CONSORTIA

Abstract :

This study investigates the compatibility and development of consortia among four entomopathogenic fungi: *Beauveria bassiana*, *Lecanicilliumlecanii*, *Metarhiziumanisopliae*, and *Metarhiziumrileyi*, 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*, *Lecanicilliumlecanii*, *Metarhiziumanisopliae* which can be grouped to a consortia.

Keywords: *entomopathogenic fungi, compatibility, visual observation, consortia*

## 1. INTRODUCTION

The tobacco caterpillar, *Spodopteralitura* (Fabricius) is a regular polyphagous pest of field and horticultural crops (Shankar *et al.*, 2006). This pest is next to *Helicoverpaarmigera* (Hubner) in economic importance, both at national and global level. *Spodopteralitura* 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 Rao *et al.*, 1983). 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 no residues and allowing field re-entry 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. lituraviz.*, *Beauveria bassiana*, *Lecanicilliumlecanii*, and *Metarhiziumanisopliae*, *Metarhiziumrileyi* were selected to study the compatibility and to develop a consortia. All the four fungi were first grown separately in petriplates and inoculated for co-culturing as per the procedure described by Upamanya *et al.*, 2020. The four fungi were divided into two groups, fast-growing and slow-growing 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*, *Lecanicilliumlecanii*, and *Metarhiziumanisopliae* were considered as fast-growing and *Metarhiziumrileyi* 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  $28\pm 2^{\circ}\text{C}$  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 fast-growing 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.

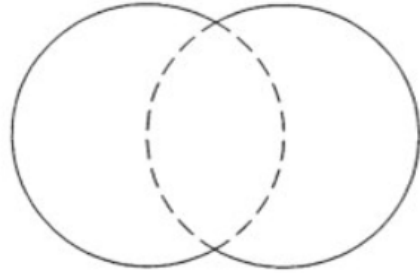
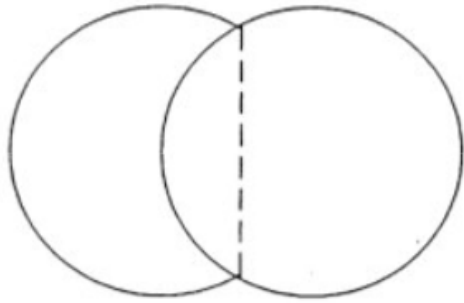


Fig 1 Mutual intermingling (Compatible) fig 2 : Partial intermingling (Partial Compatible)

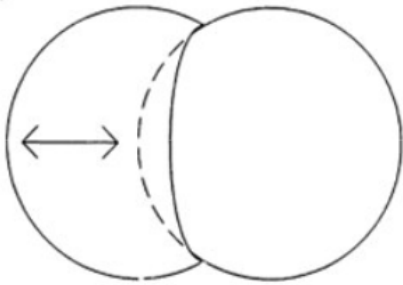


Fig 3 : Invasion/Replacement (Early Stage)

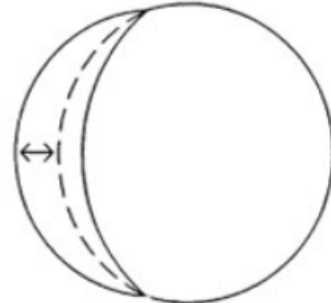


fig 4 : Invasion/Replacement (Final Stage)

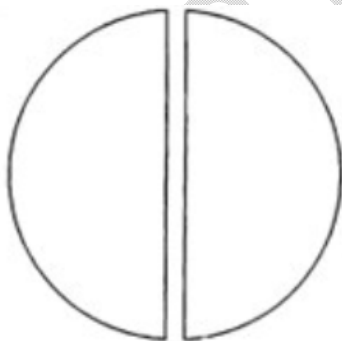


Fig 5 : Inhibition/deadlock (at touching point)

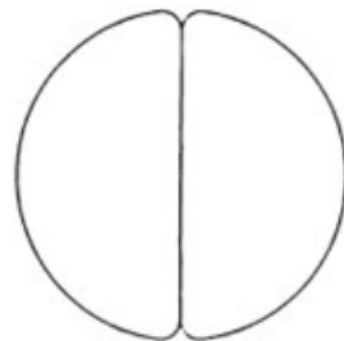


fig 6 : Inhibition/deadlock (at distance)

### 3. RESULTS AND DISCUSSION

#### 3.1. Combination 1 (*Beauveria bassiana* + *Metarhiziumanisopliae* + *Metarhiziumriyeli*)

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.

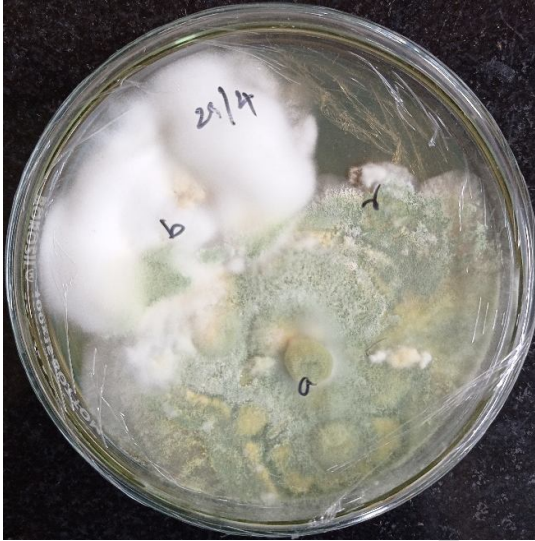


Fig :7 Petriplate showing inoculation for Combination 1

### 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*.



Fig 8:Petriplate showing inoculation for Combination 2

### 3.3 Combination 3 (*Beauveria bassiana* + *Metarhiziumanisopliae*+ *Leanicilliumlecanii*)

*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 (fig7).



Fig 9:Petriplate showing inoculation for Combination 3

### 3.4 Combination 4 (*Metarhiziumanisopliae* + *Metarhiziumriyeli*+ *Leanicilliumlecanii*)

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



Fig. 10.

It was found that there is a partial compatibility between the combination of *Beauveria bassiana* + *Metarhiziumanisopliae*+ *Leanicilliumlecanii* and in all other combinations, where *Metarhiziumrileyi* 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.* (2017) 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 Basidiomycete M1 (Tv/M1), *Trichoderma reesei* and *Panustigrinus* 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.

Karthibaet *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 *Macrophominaphaseolina* and *Sclerotiniasclerotiorum*.

**Table -1: Treatment combinations**

Combination – 1	<i>Beauveria bassiana</i> + <i>Metarhiziumanisopliae</i> + <i>Metarhiziumriyeli</i>
Combination - 2	<i>Beauveria bassiana</i> + <i>Metarhiziumriyeli</i> + <i>Lecanicilliumlecanii</i>
Combination – 3	<i>Beauveria bassiana</i> + <i>Metarhiziumanisopliae</i> + <i>Lecanicilliumlecanii</i>
Combination - 4	<i>Metarhiziumanisopliae</i> + <i>Metarhiziumriyeli</i> + <i>Lecanicilliumlecanii</i>

#### 4. CONCLUSION

The fungal combination of *Beauveria bassiana*, *Metarhiziumanisopliae*, and *Lecanicilliumlecanii* 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.

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## REFERENCES

1. Krishnamurthy Rao, B.H., Subba Ratnam, G.V and Thakkar, A and Saraf, M.2015. Development of microbial consortia as a biocontrol agent for effective management of fungal diseases in *Glycine max* L. Archives of Phytopathology and Plant Protection, 48(6): 459–474.
2. Sumalatha, J., Rahman, S.J., Rahman, S.M.A.S and Prasad, R.D. 2017. Compatibility of entomopathogenic fungi *Verticillium lecanii* with other bio pesticides in laboratory

- conditions. The Pharma Innovation Journal. 6 (9): 264-266.
3. Upamanya, G.K., Bhattacharyya, A and Dutta, P. 2020. Consortia of entomo-pathogenic fungi and bio-control agents improve the agro-ecological conditions for brinjal cultivation of Assam. 3 Biotech. 10: 1-19.
  4. Murthy, K.S.R.K. 1983. Losses due to insect pest in Andhra Pradesh. In: Proceedings of the National Seminar on Crop Losses due to Insect Pests. 1-15.
  5. Wang, C.S., Li, Z.Z and Butt, T.M. 2002. Molecular studies of co-formulated strains of the entomopathogenic fungus, *Beauveria bassiana*. Journal of Invertebrate Pathology. 80 (1): 29-34.
  6. Qin, H. G., Z. X. He, S. J. Huang, J. Ding and R. H. Luo. 2004. The correlations of the different host plants with preference level, life duration and survival rate of *Spodopteralitura* Fabricius. Chinese Journal of Eco-Agriculture. 12: 40-42.
  7. Shankara, Murthy M., Thippaiah, M and Kitturmmath, M.S. (2006). Effect of neem formulations on larvae of tobacco cutworm, *Spodopteralitura* (Fab.). Insect Environment. 12: 84-85.
  8. Karthiba, L., Saveetha, K., Suresh, S., Raguchander, T., Saravanakumar, D and Samiyappan, R. 2010. PGPR and entomopathogenic fungus bioformulation for the synchronous management of leaf folder pest and sheath blight disease of rice. Pest

Management Science: formerly Pesticide Science. 66(5): 555-564.

9. Mohammad, N., Alam, Z., Kabashi, N.A. and Adebayo, O.S., 2011. Development of compatible fungal mixed culture for the composting process of oil palm industrial waste. *African Journal of Biotechnology*, 10(81), pp.18657-18665.

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