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COMPATIBILITY OF ENTOMOPATHOGENIC FUNGUS *METARHIZIUM RILEYI* WITH BIORATIONALS

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ABSTRACT

In this study, *Metarhizium rileyi* isolates (*M. rileyi* NIPHM, *M. rileyi* MTCC 4254 and *M. rileyi* MTCC 10395) were evaluated for their compatibility with other entomopathogenic fungi viz. *Metarhizium anisopliae* NBAIR (Ma-35), commercial formulation of *M. anisopliae*, *M. anisopliae* (Local), *Beauveria bassiana* ITCC 7126, *B. bassiana* (Local) and commercial formulation of *Verticillium lecanii* and neem based formulations- (azadirachtin 0.03%EC) and (azadirachtin 0.15%EC) along with synthetic insecticide spinetoram 11.7%SC. Neem-based formulations viz., azadirachtin 0.03% EC 1.5 ml/ l and 0.15%EC 5 ml/ l reduced *M. rileyi* MTCC 4254 growth by 44.77% and 53.73% over control, respectively; *M. rileyi* MTCC 4254 was more compatible with *M. anisopliae* NBAIR (Ma-35) and commercial formulation of *M. anisopliae* with less reduction in growth (24.3% and 24.9%, respectively); but it was least compatible with commercial formulation of *V. lecanii*. *M. rileyi* MTCC 4254 recorded 71.64% reduction with spinetoram 11.7% SC (0.4 ml/ l). Thus, *M. rileyi* was compatible with *M. anisopliae* NBAIR (Ma-35), *M. anisopliae* followed by azadirachtin 0.03% EC, and these could be used as components in IPM.

Key words: *Metarhizium rileyi*, *M. anisopliae*, *Beauveria bassiana*, *Verticillium lecanii*, azadirachtin, spinetoram, compatibility, reduction in growth, IPM

Entomopathogens are microbial control agents used for crop pest management. Entomopathogenic fungi (EPF) like *Metarhizium anisopliae*, *Beauveria bassiana, Verticillium lecanii* and *Metarhizium rileyi* are widely used against pest in agriculture fields and greenhouses. These are ecofriendly and have an important role in plant protection for sustainable IPM (Grewal and Joshi, 2021). Synthetic pesticides traditionally used have negative effects, and efforts have been made to reduce their use. *Metarhizium rileyi* (Farlow) Kepler, Rehner and Humber, formerly known as *Nomuraea rileyi* (Kepler et al., 2014), is a fungus with a specific host range that is used as a biocontrol agent for the management of lepidopteran pests. These secrete secondary metabolites that act as immunosuppressive compounds which lead to fungal infection (Constanza et al., 2019). Botanicals can also be used as an alternative because they pose little risk to humans and they can be readily combined with many other bioagents (Mohan et al., 2007). Azadirachtin is less harmful to the environment and prevents development of insect resistance (Isman, 2006). In IPM the compatibility of such fungi with botanicals and pesticides is very important (Neves et al., 2001) and can improve effectiveness with less pollution risks by reducing the amount of pesticides used (Usha et al., 2014). Compatibility studies on these are important to deploy such biopesticides and biocontrol agents (Rashid et al., 2010). Therefore, compatibility of entomopathogenic fungi with botanicals/ other microbials is necessary (Sahayaraj et al., 2011). This study assesses the compatibility of *M. rileyi* with neem formulations, insecticides and other entomopathogenic fungi.

MATERIALS AND METHODS

Two *M. rileyi* isolates, viz. MTCC 10395 and MTCC 4254, were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India, and one isolate, *M. rileyi* NIPHM, was procured from the National Institute of Plant Health Management, Hyderabad, India. These isolates were grown and maintained on Sabouraud maltose agar with yeast extract (SMAY) (mycological peptone 1%, maltose 4%, agar 2%, yeast extract 1% and chloramphenicol 0.5%), and refrigerated till further use. Compatibility study of *M. rileyi* with azadirachtin and insecticide was carried out using the poison food technique with some modifications (Reddy et al., 2021). Azadirachtin 0.03%EC (@ 1.5 ml/ l, @ 2.5 ml/ l), azadirachtin 0.15% EC (@ 5 ml/ l, @ 10 ml/ l) and spinetoram 11.7%SC (@ 0.2 ml/ l, @ 0.4 ml/ l) were added individually to the sterilized growth culture media and poured into the petri plate after proper agitation and allowed to solidify. This supplemented growth media plate was inoculated with 1 mm fungal disc of fully-grown *M. rileyi*. A control plate with a pure culture of *M. rileyi* was used. These plates were incubated at 25±2°C for ten days. *M. rileyi* compatibility studies with entomopathogenic fungi were carried out using the dual culture technique with some modifications (Sumalatha et al., 2017). The autoclaved media was poured into petri plates and allowed to cool. After solidification the plates were inoculated with *M. rileyi* at one side of the plates and test entomopathogenic fungi was kept at opposite side in individual plates. A pure culture of *M. rileyi* on growth media was used as control. These plates were incubated at a 25± 2°C for ten days, and % inhibition was recorded. Data given represent the mean and± SD (standard deviation) by applying one-way ANOVA done in SPSS 16.0 statistical software.

RESULTS AND DISCUSSION

Ten days after incubation, *M. rileyi* MTCC 4254 recorded maximum radial growth (3.7± 0.14) with 44.77% growth reduction over control on SMAY media supplemented with azadirachtin 0.03%EC at 1.5 ml/ l concentration (Table 1); *M. rileyi* MTCC 10395 recorded minimum radial growth (2.2± 0.14) and maximum growth % reduction (62.71%) over control when media was supplemented with azadirachtin 0.15%EC (10 ml/ l) concentration. Results showed that azadirachtin 0.03%EC at lower concentration (1.5 ml/ l) was safe and compatible with *M. rileyi*. This may be due to the effect of concentrations of neem derivatives on the growth of *Metarhizium* spp. Hirose et al. (2001) also observed that neem derivatives containing <5% or neem oil containing <0.25% were less toxic to *M. anisopliae* mycelial growth and spores. Neem oil at lower concentrations is compatible and synergistic when combined with entomopathogenic fungi such as *B. bassiana* and *Metarhizium* spp. However, beyond such concentrations, spore viability or colony growth is delayed and suppressed, indicating that neem oil can cause loss of potency or inhibition of entomopathogenic agents (Togbé et al., 2014). Dev et al. (2021) evaluated *M. rileyi* Farlow (Samson) impregnated with azadirachtin against *Helicoverpa armigera* (Hubner) and reported *M. rileyi* blended with azadirachtin @ 10^6 conidia/ ml and @ 10^8 conidia/ ml caused the highest mortality of 86.21% and 89.66% of 2nd and 3rd instar larvae of *H. armigera,* respectively. Sahayaraj et al. (2011) used liquid and dual plate bioassays to investigate the compatibility of commercial botanicals (Biospark, Exodos, and Phytophrate) with *Isaria fumosorosea*, *Beauveria bassiana* and *Lecanicillium lecanii* in vitro. They found that commercial botanicals significantly reduced the mycelial growth of *B. bassiana*, *L. lecanii* and *I. fumosorosea*. Mohan et al. (2007) screened 30 isolates of *Beauveria bassiana* for compatibility with commercial formulation of neem oil (Margoside®) at field recommended dose (0.3% v/v) and reported 23 isolates were compatible with neem. In neem sensitive isolates growth was decreased but not totally inhibited.

In vitro compatibility study of *M. rileyi* with insecticide spinetoram 11.7% SC recorded 71.64% growth reduction over control in *M. rileyi* MTCC 4254 (Fig. 1). Variation in radial growth, growth % reduction over control and sporulation of *M. rileyi* (Farlow) Samson was recorded by Matcha et al. (2021). They reported that fungicides carbendazime,

Table 1. *M. rileyi* growth on SMAY supplemented with azadirachtin @ 0.03% and 0.15% EC)

Treatment	Control**	Conc. of azadirachtin @ 0.03% EC (ml/ l)	*M. rileyi* growth on SMAY supplemented with (azadirachtin @ 0.03% EC) (cm) (Mean± S.D)* (after 10 incubation)		Conc. of azadirachtin @ 0.15% EC (ml/ l)	*M. rileyi* growth on SMAY supplemented with Indo neem (azadirachtin @ 0.15% EC) (cm) (Mean± S.D)* (After ten days incubation)	
			Radial growth	Growth % reduction over control		Radial growth	Growth % reduction over control
M. rileyi NIPHM	6.4± 0.14	1.5	3.3± 0.07	48.43	5	2.9± 0.42	54.68
	6.4± 0.14	2.5	3.2± 0.21	50.00	10	2.7± 0.70	57.81
M. rileyi MTCC 4254	6.7± 0.21	1.5	3.7± 0.14	44.77	5	3.1± 0.56	53.73
	6.7± 0.21	2.5	3.5± 0.42	47.76	10	2.95± 0.21	55.97
M. rileyi MTCC 10395	5.9± 0.42	1.5	2.9± 0.56	50.84	5	2.35± 0.49	60.16
	5.9± 0.42	2.5	2.8± 0.56	52.54	10	2.2± 0.14	62.71

*Values mean± SD; **Control- Growth media without supplementation of azadirachtin

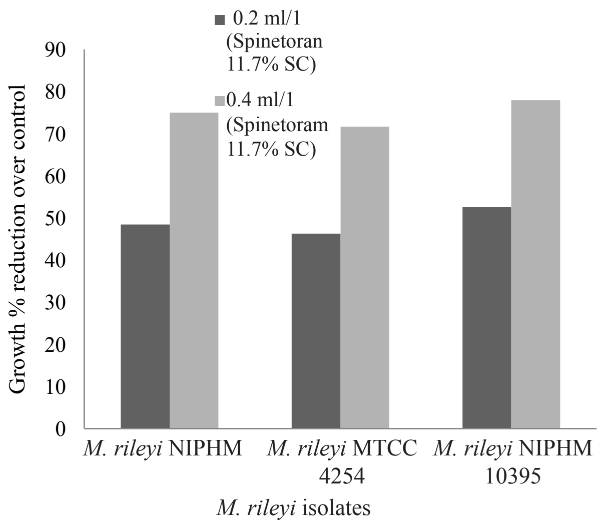

Fig. 1. Growth % reduction of *M. rileyi* with spinetoram

propiconazole showed complete growth inhibition (100%), insecticide Emamectin benzoate 5 SG showed a maximum inhibition of 77.37% while Azoxystrobin and Tebuconazole recorded lower growth inhibition of 8.17% and 12.36% respectively. The compatibility studies of *Metarhizium* (*Nomuraea*) *rileyi* rice bran oil formulation with four insecticides recorded higher % inhibition of 34.67 by novaluron and lower % inhibition of 23.56 was recorded by lufenuron (Saheb et al., 2021). The difference in radial growth or % inhibition observed could be attributed to the inherent variability of chemicals to biological agents. This could be due to the inconsistent interaction between fungus and insecticides. Compatibility study of *M. rileyi* with other entomopathogenic fungal strains revealed that *M. anisopliae* NBAIR (Ma-35) recorded maximum radial growth (5.9± 0.36) with minimum growth % reduction (24.3%) over control followed by the commercial formulation of *M. anisopliae* with 24.9% growth reduction over control against *M. rileyi* MTCC 4254. However, it was least compatible with *V. lecanii* (Fig. 2). This variation in compatibility may be due to difference in fungal growth parameters. Limited published information is available regarding the compatibility of *M. rileyi* with other entomopathogenic fungi to substantiate the complete results of the present study. The current study concluded that neem formulation, (azadirachtin 0.03% EC) at lower dose 1.5 ml/ l was safe for *M. rileyi*. Further, *M. rileyi* was more compatible with *M. anisopliae* and was least compatible with commercial formulation of *V. lecanii*. However, further field studies will enable better understanding of compatibility studies, which can lead to the use of these formulations for ecofriendly IPM.

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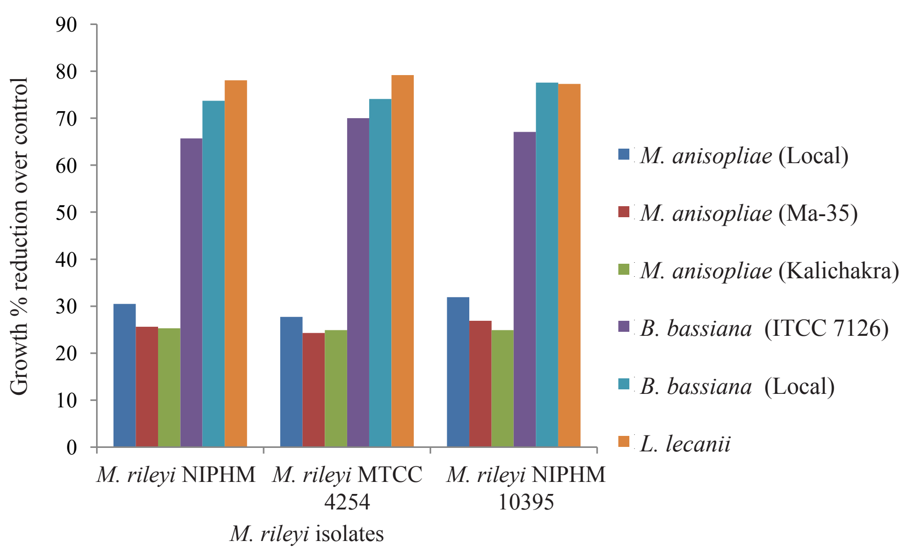

Fig. 2. Growth % reduction of *M. rileyi* with entomopathogenic fungi

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