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Interaction Effects of Entomopathogenic Fungi on Lesser Grain Borer, *Rhyzopertha dominica* (F.) in Paddy

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ABSTRACT

The interaction effects of entomopathogenic fungi, *Beauveria bassiana, Metarhizium anisopliae* and *Lecanicillium lecanii* were tested against lesser grain borer, *Rhyzopertha dominica* at Post Harvest Technology Centre, Bapatla during the year 2011-12. The interactions of entomopathogenic fungi at 15 DAT, revealed that the grains treated with *Beauveria* + *Metarhizium* have recorded the highest adult mortality of 96.30% followed by *Beauveria* + *Metarhizium* + *Lecanicillium* (92.1%) when compared to *Beauveria* (89.2%), *Metarhizium* (84.6%) and *Lecanicillium* (62.2%) tested alone. At 180 DAT, *Beauveria* + *Metarhizium* + *Lecanicillium* has recorded least progeny adults and per cent weight loss of 119.7 and 17.7% followed by *Beauveria* + *Metarhizium* (122.00 and 18.8%) when compared to control (398.67 and 51.3%). Highest per cent reduction in progeny was observed with *Beauveria* + *Metarhizium* + *Lecanicillium* (69.9%) followed by *Beauveria*+ *Metarhizium* (69.40%), *Beauveria* alone (66.6%) and *Beauveria* + *Lecanicillium* (65.5%) when compared to control at 180 DAT. *Beauveria* + *Metarhizium* + *Lecanicillium* has recorded high per cent reduction in weight loss of 65.5% followed by *Beauveria* +*Metarhizium* (63.4%), *Beauveria* (61.8%) and *Beauveria*+ *Lecanicillium* (60.5%) when compared to control at 180 DAT.

Key words : *Beauveria bassiana*, Entomopathogenic fungi, Lesser grain borer, *Lecanicillium lecanii, Metarhizium anisopliae.*

Paddy is the most important staple food crop of India. About 65% of Indian population is dependent on rice for food stuff. After harvesting, unprocessed rice will be stored for various lengths of time at producers, wholesalers and millers level. While in storage, rice is at risk to infestation by a wide range of stored product insects like Rice moth (*Corcyra cephalonica* Stainton), Rice weevil (*Sitophilus oryzae* Linn.) and lesser grain borer (*Rhyzopertha dominica* Fabricius).In India, upto 12% of post harvest losses were caused by insect pests (Mohan, 2003). Lesser grain borer, *R. dominica* is a major insect pest of many stored grains, including rice (Arthur *et al.*, 2007) *etc.* The infestations of *R. dominica* cause loss of biomass (Swaminathan, 1977). Losses due to this pest have been estimated at 15% or more of total grains stored each year (Batta, 2005).

Application of insecticides is one of the preventing measures to reduce losses during storage period. The continuous use of chemical insecticides for control has also resulted in serious problems such as resistance to the insecticides, pest resurgence, elimination of economically beneficial insects, and toxicity to humans and wildlife. These problems and the demand for pesticide free foods have triggered efforts to find alternative management options (Padin *et al.*, 2002). Microbial pesticides are one such alternative to tackle insecticide problems. Several reports are available on efficacy of entomopathogenic fungi like *Beauveria bassiana* (Balsamo) Vuiillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Lecanicillium lecanii* (Zimmerman) on storage insect pests (Batta, 2005; Buba, 2010 & Hafez, 2011). *Dal Bello et al.* (2000) reported that the interaction of *B. bassiana* and *M. anisopliae* caused greater mortality of *S. oryzae* adults than the two fungi tested alone in storage. Zimmermann (2007a & b) reported that *B. bassiana* and *M. anisopliae* are considered to be safe with minimal risks to vertebrates, humans and the environment. In the present study, interaction effects of above three entomopathogenic fungi in mixtures against lesser grain borer, *R. dominica* in paddy were reported.

MATERIAL AND METHODS

The experiment was conducted at Post Harvest Technology Center, Bapatla during the year 2011-12. The fungal isolates of *B. bassiana, M. anisopliae and L. lecanii* were procured from Plant Pathology laboratory, Directorate of Oilseeds Research, Rajendranagar, Hyderabad, Andhra Pradesh. The paddy variety BPT 5204 (Sambamashuri) was procured from Rice Research Unit, Bapatla, Guntur District, Andhra Pradesh.

The pure cultures of all the three fungi were maintained and preserved on Potato Dextrose Agar (PDA) slants. Further, these cultures were mass multiplied by inoculating into the flask containing sterilized Potato Dextrose Broth (PDB) and were incubated at 32^0C in a incubator till the profused sporulation was attained. Then the mycelia mat along with spores was thoroughly macerated in a sterile pestle & mortar. The macerated material was then transferred to sterile conical flasks under aseptic conditions. The suspension of the fungi was mixed to the sterile talc powder at the rate of 1: 4 (250 ml/kg of carrier material). The population of the fungi in the talc powder formulation was determined by standard dilution technique by using MRBA. The population of the fungi was 2 x10^6, 1 x 10^9, and 2 x 10^7/g in *B. bassiana*, *M. anisopliae* and *L. lecanii* formulations, respectively.

Adults of lesser grain borer, *R. dominica* were collected from the stock culture of Entomology laboratory, Post Harvest Technology Centre, Bapatla and were transferred into 250 g of disinfested Paddy grains (BPT 5204) in a plastic jar of 1 L capacity. The released adults were allowed for 20 days to lay sufficient eggs in culture jars, later the adults were removed and the jars were kept for progeny adult emergence. The jars were regularly observed for adult emergence after 30 days of release. The newly emerged adults were used for experimental purpose.

The fungal formulations of 1.25 g each of *B. bassiana, M. anisopliae* and *L. lecanii*, 0.625 g each of *B. bassiana* + *M.anisopliae, B.bassiana* + *L. lecanii* and *M. anisoplea* + *L. lecanii* and 0.3125 g each of *B. bassiana* + *M. anisoplea* + *L. lecanii* were added to 250 g of paddy separately in each replication and mixed the grain thoroughly till all the dust distributed uniformly on the grain. Later the treated grain was kept in 0.5 L plastic jar, five pairs of freshly emerged adults (0-24 h old) were released and covered with muslin cloth for aeration. Three replications were maintained for each treatment. The experiment was conducted under ambient conditions. Observations were recorded on adult mortality at 7 DAT and 15 DAT. The progeny buildup and per cent weight loss was recorded from 30 DAT to 180 DAT at fortnight interval. To calculate the per cent corrected mortality the following Abbott's formula was used.

$$\text{Abbott's formula} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

The per cent weight loss was calculated by the following formula.

$$\text{Per cent weight loss} = \frac{(U.Nd) - (D.Nu)}{U\,(Nd+Nu)} \times 100$$

Where,

U = weight of undamaged grains

Nu = number of undamaged grains

D = weight of damaged grains

Nd = Number of damaged grains.

The adult mortality and per cent weight loss were transformed into arcsine values and the progeny build-up was transformed into square root values and was subjected to Complete Randomized Design (CRD) analysis.

RESULTS AND DISCUSSIONS

Effect on released adults

The data pertaining to the interaction effects of entomopathogenic fungi against the mortality of *R. dominica* were presented in Table 1. The data at 7 DAT indicated that among the different fungal treatments, *Beauveria* + *Metarhizium* was found superior in causing highest adult mortality (67.9%) which was on par with *Beauveria*+ *Metarhizium* + *Lecanicillium* (64.5%), *Beauveria* (64.1%), *Metarhizium* (56.7%) and *Beauveria* + *Lecanicillium* (53.3%). *Metarhizium* + *Lecanicillium* (46.7%) and *Lecanicillium* (39.3%) had produced less adult mortality, but were significantly different from control.

Similar trend was observed at 15 DAT with *Beauveria* + *Metarhizium* produced highest adult mortality of 96.3% which was on par with *Beauveria* + *Metarhizium* + *Lecanicillium*

Table 1. Interaction effects of entomopathogenic fungi against the mortality of *R. dominica* in paddy.

Treatments	Dosage (g/kg grain)	Mortality (%) 7 DAT	Mortality (%) 15 DAT
Beauveria bassiana	5	64.07 (53.27)[a]	89.17 (74.28)[ab]
Metarhizium anisopliae	5	56.67 (48.95)[ab]	84.63 (64.40)[bc]
Lecanicillium lecanii	5	39.26 (38.79)[c]	62.22 (52.20)[c]
Beauveria + Metarhizium	2.5 + 2.5	67.78 (55.65)[a]	96.30 (83.55)[a]
Beauveria + Lecanicillium	2.5 + 2.5	53.33 (46.95)[abc]	81.30 (64.69)[bc]
Metarhizium+ Lecanicillium	2.5 + 2.5	46.67 (43.10)[bc]	63.06 (52.61)[c]
Beauveria + Metarhizium+ Lecanicillium	1.67 + 1.67+1.67	64.44 (53.64)[a]	92.13 (76.65)[ab]
Control*		0.00	0.00
SE.m±		**±3.10**	**±4.87**
CD (0.05)		**9.30**	**14.61**

DAT- Days After Treatment
Means in a column with the same letter are not significantly different
The values in parentheses are Arc sine transformed values

(92.2%) and *Beauveria* (89.2%). The next best treatments were *Metarhizium* (84.6%) followed by *Beauveria + Lecanicillium* (81.3%), *Metarhizium + Lecanicillium* (63.1%) and *Lecanicillium* (62.2%) and were on par with each other. However, all the treatments were significantly different from control

The results are in agreement with the findings of Dal-Bello *et al*. (2001) who have reported that the fungal mix of *B. bassiana* strain ARSEF5500 + *M. anisopliae* strain ARSEF 2974 caused highest mortality (51.7%) of *S. oryzae* than the fungus tested alone. The results obtained in present study against the mortality of *R. dominica* at 15 DAT with *Beauveria*, *Metarhizium* and *Lecanicillium* alone was 89.2, 84.6 & 62.2, respectively whereas the interaction of *Beauveria + Metarhizium* fungus produced the mortality of 96.3% which has showed the synergistic effect, but *Beauveria + Lecanicillium* (81.3) and *Metarhizium + Lecanicillium* (63.1%) has produced the antagonistic effect when *Lecanicillium* was added to *Beauveria & Metarhizium*. Similar results were obtained with Mahmoud (2009) who reported 80, 92 & 100 per cent mortality of Olive fly, *Bactrocera oleae* with *B. bassiana , M. anisopliae* and *L. lecanii,* but in combination with *B. bassiana + M. anisopliae* showed synergistic effect, by causing 100% mortality, whereas antagonistic effect was recorded with *B. bassiana + L. lecanii* (72 %) and *M. anisopliae + L. lecanii* (62%) at 1 x 10^8 conidia/ml. Baker & Cook (1982) reported that the complex association in nature are more stable and attains successful biocontrol with a mixture of several bioagents than with a single alone.

Effect on progeny build-up

The data pertaining to the interaction effects of entomopathogenic fungi against the

Table 2. Interaction effects of entomopathogenic fungi against the progeny adult build up of lesser grain borer, *R. dominica.*

Treatments	Dosage (g/kg grain)	Progeny adult build-up (No.)					
		30 DAT	60 DAT	90DAT	120 DAT	150 DAT	180DAT
Beauveria bassiana	5	4.33	29.94	50.33	72.33	103.67	133.33
		(2.19)^{c}	(4.81)^{bc}	(7.11)^{b}	(8.51)^{bc}	(10.19)^{cd}	'(11.55)^{bc}
Metarhizium anisopliae	5	5.00	27.33	60.33	76.67	110.00	144.00
		(2.34)^{bc}	(5.21)^{bc}	(7.80)^{b}	(8.77)^{bc}	(10.50)^{c}	(12.00)^{c}
Lecanicillium lecanii	5	8.33	42.00	69.00	96.67	139.33	175.67
		(2.96)^{b}	(6.52)^{b}	(8.33)^{b}	(9.86)^{b}	(11.82)^{b}	(13.27)^{b}
Beauveria + Metarhizium	2.5 + 2.5	5.33	20.67	48.33	63.00	93.00	122.00
		(2.41)^{bc}	(4.60)^{c}	(6.98)^{b}	(7.97)^{c}	(9.67)^{cd}	(11.06)^{d}
Beauveria + Lecanicillium	2.5 + 2.5	5.67	29.94	52.00	74.33	99.33	137.67
		(2.44)^{bc}	(4.81)^{bc}	(7.23)^{b}	(8.65)^{bc}	(9.98)^{cd}	(11.74)^{bc}
Metarhizium+ Lecanicillium	2.5 + 2.5	6.67	38.33	57.33	77.67	106.67	138.67
		(2.68)^{bc}	(6.21)^{bc}	(7.60)^{b}	(8.83)^{bc}	(10.35)^{c}	(11.79)^{bc}
Beauveria + Metarhizium+	1.67 +	4.33	18.33^{c}	40.67	63.00	88.33	119.67
Lecanicillium	1.67+1.67	(2.18)^{c}	(4.32)	(6.38)^{b}	(7.97)^{c}	(9.42)^{d}	(10.95)^{d}
Control*		26.00	319.00	514.33	530.00	462.67	398.67
		(5.11)^{a}	(17.76)^{a}	(22.58)^{a}	(22.96)^{a}	(21.52)^{a}	(19.98)^{a}
SE.m±		**0.24**	**0.58**	**0.62**	**0.51**	**0.27**	**0.34**
CD (0.05)		**0.73**	**1.74**	**1.86**	**1.54**	**0.81**	**1.03**

DAT – Days After Treatment

The values in parentheses are square root transformed values

In each column values with similar alphabet do not vary significantly at 5%

progeny of *R. dominica* were presented in Table 2. The observations recorded on 30 DAT showed that *Beauveria + Metarhizium + Lecanicillium* and *Beauveria* were most effective & recorded equal progeny of 4.3 and were on par with *Metarhizium* (5.0), *Beauveria+ Metarhizium* (5.3), *Beauveria + Lecanicillium* (5.7) and *Metarhizium + Lecanicillium* (6.7) but was significantly different from *Lecanicillium* (8.3). All these treatments were significantly different from control (26.0) (Table 2). Highest per cent reduction in progeny was observed with *Beauveria + Metarhizium + Lecanicillium* (89.4%) followed by *Beauveria+ Metarhizium* (89.1%), *Beauveria* alone (88.8 %) and *Metarhizium* alone (86.6 %) when compared to control after 45 DAT. The data at 60 DAT showed less progeny with *Beauveria + Metarhizium + Lecanicillium* (18.3) which was on par with *Beauveria + Metarhizium* (20.7), *Metarhizium* (27.3), *Beauveria* (29.9) and *Beauveria+ Lecanicillium* (29.9). The highest progeny was recorded with *Lecanicillium* (42.0) and *Metarhizium + Lecanicillium* (38.3) which was on par with each other. All the treatments were significantly different from control (319.0) (Table 2).

At 90 DAT lowest progeny was recorded with *Beauveria + Metarhizium + Lecanicillium* (40.7) followed by *Beauveria + Metarhizium* (48.3), *Beauveria* (50.3), *Beauveria + Lecanicillium* (52.0), *Metarhizium + Lecanicillium* (57.3). The highest progeny were recorded with *Lecaniciilium* (69.0) followed by *Metarhizium* (60.3). All the treatments were on par with each other and were significantly different from control (514.3) (Table 2). Highest per cent

Table 3. Interaction effects of entomopathogenic fungi against weight loss (%) by lesser grain borer, *R. dominica.*

Treatments	Dosage (g/kg grain)	Weight loss (%)				
		60 DAT	90DAT	120 DAT	150 DAT	180 DAT
Beauveria bassiana	5	2.23 (8.59) cd	4.92 (12.82) cd	7.96 (16.37) c	13.90 (21.87) c	19.56 (25.63) bc
Metarhizium anisopliae	5	2.42 (8.91) cd	5.56 (13.64) bcd	10.33 (18.69) bc	14.54 (22.40) bc	21.28 (27.47) bc
Lecanicillium lecanii	5	3.60 (10.89) b	7.00 (15.30) bc	13.50 (21.53) b	18.19 (25.25) b	23.81 (29.18) b
Beauveria + Metarhizium	2.5 + 2.5	2.11 (8.36) d	4.83 (12.66) cd	7.59 (15.98) c	12.25 (20.47) c	18.75 (26.25) bc
Beauveria + Lecanicillium	2.5 + 2.5	2.56 (9.19) cd	5.07 (13.00) cd	8.41 (16.78) c	14.48 (22.34) bc	20.26 (26.75) bc
Metarhizium+ Lecanicillium	2.5 + 2.5	3.16 (10.21) bc	7.44 (15.83) b	12.17 (20.40) b	15.55 (23.22) bc	22.98 (28.65) b
Beauveria + Metarhizium+ Lecanicillium	1.67 + 1.67+1.67	2.04 (8.23) d	4.44 (12.13) d	7.66 (16.04) c	12.17 (20.42) c	17.68 (24.85) c
Control*		10.11 (18.51) a	20.36 (26.73) a	30.11 (33.10) a	43.42 (41.26) a	51.27 (45.77) a
SE.m±		**0.50**	**0.89**	**1.04**	**0.85**	**1.02**
CD (0.05)		**1.49**	**2.68**	**3.12**	**2.55**	**3.04**

DAT – Days After Treatment
The values in parentheses are square root transformed values
In each column values with similar alphabet do not vary significantly at 5%

reduction in progeny was observed with *Beauveria + Metarhizium + Lecanicillium* (92.1%) followed by *Beauveria+ Metarhizium* (90.6%), *Beauveria* alone (90.2%) and *Beauveria + Lecanicillium* (89.9%) when compared to control. Similar trend was observed at 120 DAT with less progeny adult build up was recorded in *Beauveria + Metarhizium + Lecanicillium* (63.0) and *Beauveria + Metarhizium* (63.0) which were on par with *Beauveria* (72.3), *Beauveria + Lecanicillium* (74.3), *Metarhizium* (76.7) and *Metarhizium + Lecanicillium* (77.7) and were significantly different from *Lecanicillium*. All the treatments were significantly different from control (530.0) (Table 2). Highest per cent reduction in progeny was observed with *Beauveria + Metarhizium + Lecanicillium* (87.8%) followed by *Beauveria+ Metarhizium* (87.4%), *Beauveria* alone (86%) and *Beauveria + Lecanicillium* (84.9%) when compared to control at 135 DAT . The results with regard to effect of treatments after 150 days of treatment showed less progeny build up with *Beauveria +Metarhizium + Lecanicillium* (88.3) which was on par with *Beauveria + Metarhizium* (93.0), *Beauveria+ Lecanicillium* (99.3) and *Beauveria* (103.7) & was significantly different from *Metarhizium + Lecanicillium* (106.7) and *Metarhizium* (110.0). All the treatments were significantly different from *Lecanicillium* (139.3) that showed least significance but was significantly different from control (462.7) (Table 2).

The observations recorded at 180 DAT indicated that *Beauveria +Metarhizium + Lecanicillium* was superior among all treatments with less progeny of 119.7 that was on par with *Beauveria +Metarhizium* (122.0) and was significantly different from all other treatments. *Beauveria* (133.3), *Beauveria + Lecanicillium* (137.7), *Metarhizium + Lecanicillium* (138.7). Highest progeny was recorded with *Lecanicillium* (175.7) followed by *Metarhizium* (144). All the treatments were significantly different from control (398.7) (Table 2). Highest per cent reduction in progeny was observed with *Beauveria + Metarhizium + Lecanicillium* (70%) followed by *Beauveria+ Metarhizium* (69.4%), *Beauveria* alone (66.6%) and *Beauveria + Lecanicillium* (65.5%) when compared to control.

Effect on per cent weight loss

At 45 DAT, *Beauveria + Metarhizium + Lecanicillium* has recorded high per cent reduction in weight loss of 79.05% followed by *Beauveria* (78.3%), *Metarhizium* (75.4%) and *Beauveria +Metarhizium* (74.1%) when compared to control. *Beauveria + Metarhizium + Lecanicillium* caused less per cent weight loss of 2.0% at 60 DAT which was on par with *Beauveria + Metarhizium* (2.1), *Beauveria* (2.3%), *Metarhizium* (2.4) and *Beauveria + Lecanicillium* (2.6%). *Metarhizium + Lecanicillium* (3.2%) and *Lecanicillium* (3.6%) are at par, but was significantly different from control (10.1%) (Table 3).

The observations recorded on 90 DAT showed less per cent weight loss with *Beauveria + Metarhizium + Lecanicillium* (4.4%) which was on par with *Beauveria + Metarhizium* (4.8%), *Beauveria* (4.9%), *Beauveria + Lecanicillium* (5.1%) and *Metarhizium* (5.6%) (Table 3). High per cent weight loss was observed in *Metarhizium + Lecanicillium* (7.4%) followed by *Lecanicillium* (7.0%) which were on par with each other and were significantly different from control (20.4%). *Beauveria + Metarhizium + Lecanicillium* has recorded high per cent reduction in weight loss of 78.2% followed by *Beauveria +Metarhizium* (76.3%), *Beauveria* (78.8) and *Beauveria + Lecanicillium* (75.7%) when compared to control.

The observations recorded on 120 DAT showed less per cent weight loss with *Beauveria+ Metarhizium* (7.6%) and *Beauveria + Metarhizium + Lecanicillium* (7.7%) which were on par with *Beauveria* (8.0%), *Beauveria + Lecanicillium* (8.4%) and *Metarhizium* (10.3%). High per cent weight loss was observed with *Lecanicillium* (13.5%) followed by *Metarhizium + Lecanicillium* (12.2) that were on par with *Metarhizium* (10.3%). All the treatments were significantly different from untreated control (30.1%) (Table 3). *Beauveria + Metarhizium + Lecanicillium* has recorded high per cent reduction in weight loss of 75.6% followed by *Beauveria +Metarhizium* (75.5%), *Beauveria* (73.9%) and *Beauveria+ Lecanicillium* (70.3%) when compared to control. *Beauveria + Metarhizium + Lecanicillium*, *Beauveria +Metarhizium* and *Beauveria* were found to be superior and caused less per cent weight loss of 12.2, 12.3 & 13.9% respectively, which were on par with *Beauveria + Lecanicillium* (14.5%), *Metarhizium* (14.5%) and *Metarhizium + Lecanicillium* (15.6%) at 150 DAT . High per cent weight loss was observed in *Lecanicillium* (18.2%). All treatments were significantly different from control (43.4%) (Table 3).

The observations recorded on 180 DAT showed less per cent weight loss with *Beauveria +Metarhizium + Lecanicillium* (17.8%) which was on par with *Beauveria + Metarhizium* (18.8%), *Beauveria* (19.6%), *Beauveria + Lecanicillium* (20.3%), *Metarhizium* (21.3%) and is significantly different from *Metarhizium + Lecanicillium* (23.0%) and *Lecanicillium* (23.8%). All treatments were significantly different from control (51.3%) (Table 3). *Beauveria + Metarhizium + Lecanicillium* has recorded high per cent reduction in weight loss of 65.5% followed by *Beauveria +Metarhizium* (63.4%), *Beauveria* (61.8) and *Beauveria+ Lecanicillium* (60.5%) when compared to control.

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