# PATHOGENICITY OF THREE FUNGAL SPECIES INTERACTION AND THEIR COMPATIBILITY WITH VEGETABLE OILS TO LESSER GRAIN BORER RHYZOPERTHA DOMINICA F. IN TERMS OF PER CENT WEIGHT LOSS IN PADDY DURING STORAGE

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**Abstract:** The interaction effects of entomopathogenic fungi, *Beauveria bassiana* (2 x $10^6$ conidia/g), *Metarhizium anisopliae* (1 x $10^9$ conidia/g) and *Lecanicillium lecanii* (2 x $10^7$ conidia/g) @ 5g/kg grain and the compatibility of entomopathogenic fungi @ 5g/kg with two vegetable oils oils (2 ml/kg) *viz.* sunflower oil and groundnut oil were tested against lesser grain borer, *Rhyzopertha dominica* at Post Harvest Technology Centre, Bapatla during the year 2011-12. In the study of interaction effects, *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. In the study of compatibility of entomopathogenic fungi with edible oils, high per cent reduction in weight loss was recorded with *Beauveria* + Groundnut oil (64.88%) followed by *Metarhizium* + Groundnut oil (62.05%). The next better were recorded with *Beauveria* + Sunflower oil (60.03%), *Metarhizium* + Sunflower oil (56.50%), and *Lecanicillium* + Sunflower oil (51.61%) when compared to control at 180 DAT.

**Key Words:** Beauveria, Lecanicillium, Metarhizium, Rhyzopertha dominica, Sunflower oil.

**Introduction:** 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 producer's, wholesaler's and miller's 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). 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 (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. Vimala Devi and Prashanth (2009) reported that the effectiveness of entomofungal pathogens has been found to increase when formulated in oils. 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, efficacy of three entomopathogenic fungi alone, their interactions in mixtures, compatibility of the entomopathogenic fungi with the edible oils against lesser grain borer, *R. dominica* in paddy were reported.

**Material And Methods:** The experiment was conducted at Post Harvest Technology Center, Agricultural College, Bapatla, Guntur district, Andhra Pradesh 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 three entomopathogenic fungi, *B. bassiana, M. anisopliae and L. lecanii* were further tested for their purity by plating them on Martin Rose Bengal Agar medium. The pure cultures of these fungi were maintained and preserved on Potato Dextrose Agar (PDA) [(potato – 250 g, Agar- 16 g, Dextrose – 20 g)] slants at refrigerated condition for further studies. Further, these cultures were mass multiplied by inoculating into the flask containing sterilized Potato Dextrose Broth (PDB) under aseptic conditions in Laminar Air Flow (LAF) chamber. After inoculation, the flasks were incubated at 32°C in a bacteriological 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 and the populations of the fungi were 2 x$10^6$, 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, Agricultural College, 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.

**Interaction treatments of Entomopathogenic fungi against lesser grain borer:** 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.

**Compatibility treatments of Entomopathogenic fungi with the edible oils:** Sunflower oil (0.625 ml), Groundnut oil (0.625 ml), *B. bassiana* in sunflower oil (1.25 g + 0.625 ml), *B. bassiana* in Groundnut oil (1.25 g + 0.625 ml), *M. anisopliae* in Sunflower oil (1.25 g + 0.625 ml), *M. anisopliae* in Groundnut oil (1.25 g + 0.625 ml), *L. lecanii* in Sunflower oil (1.25 g + 0.625 ml) and *L. lecanii* in Groundnut oil (1.25 g + 0.625 ml) were added to 250 g of paddy separately in each replication and mixed the contents with grain thoroughly till all the contents were distributed on it. 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. Per cent weight loss of paddy grain due to damage by lesser grain borer was determined after excluding all insect stages, frass and dust from the grain. The per cent weight loss was calculated by the following formula (Adams and Schulten, 1978).The per cent weight loss was transformed into arcsine values and was subjected to Complete Randomized Design (CRD) analysis.

$$\text{Per cent weight loss} = \frac{(U.Nd) - (D.Nu)\ X100}{U(Nd + Nu)}$$

Where,U = weight of undamaged grains, Nu = number of undamaged grains,D = weight of damaged grains,Nd = Number of damaged grains.

**Results And Discussions: Interaction effects of entomopathogenic fungi against lesser grain borer in terms of 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. 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 1). 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. *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 1).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 1). *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. The present investigations are in agreement with El-Sebai (2011) who demonstrated that less per cent weight loss of 11% was observed in *B. bassiana* (1%w/w) compared to untreated wheat (56.33%) against *R. dominica.* Hafez (2011) recorded less per cent weight loss of 16.03% with *B. bassiana* (1%) against *T. confusum* in wheat.

Sabbour & Shadia (2007) reported 10 and 15 per cent weight loss with *B. bassiana* & *M. anisopliae*, respectively against *B. rufimanus* after 6 months of treatment.

**Compatibility of vegetable oils with entomopathogenic fungi against lesser grain borer, in terms of per cent weight loss:** At 45 DAT, high per cent reduction in weight loss was recorded with *Beauveria* + Groundnut oil (77.67%) followed by *Metarhizium* + Groundnut oil (75.00%). The next better were *Lecanicillium* + Groundnut oil (67.96%), *Beauveria* + Sunflower oil (53.64%) and *Metarhizium* + Sunflower oil (44.90%) when compared to control. Less per cent reduction in weight loss was recorded with Sunflower oil (11.16%) followed by Groundnut oil (24.27%) and *Lecanicillium* + Sunflower oil (28.16%) when compared with control.At 90 DAT *Beauveria* + Groundnut oil has recorded less per cent weight loss of 3.99 which was on par with *Metarhizium* + Groundnut oil (4.38%) and *Beauveria* + Sunflower oil (5.59%). The next best was *Lecanicillium* + Sunflower oil (6.53%), which was on par with *Metarhizium* + Sunflower oil (7.08%) and *Lecanicillium* + Groundnut oil (7.33%). High per cent weight loss was recorded with Sunflower oil (12.46%) followed by Groundnut oil (11.96%). All the treatments were significantly different from control (17.30%) (Table 2). High per cent reduction in weight loss was recorded with *Beauveria* + Groundnut oil (76.94%) followed by *Metarhizium* + Groundnut oil (74.68%). The next better treatments were recorded with *Beauveria* + Sunflower oil (67.69%), *Lecanicillium* + Sunflower oil (62.25%), *Metarhizium* + Sunflower oil (59.97%) and *Lecanicillium* + Groundnut oil (57.63%) when compared to control. Less per cent reduction in weight loss was recorded with Sunflower oil (27.97%) followed by Groundnut oil (30.87%) when compared to control. The data on 150 DAT showed that the grain treated with *Beauveria* + Groundnut oil and *Metarhizium* + Groundnut oil recorded less per cent weight loss of 12.11 which has shown significant results and were on par with *Metarhizium* + Sunflower oil (12.86%). The next better were *Beauveria* + Sunflower oil (15.11%) which was on par with *Lecanicillium* + Sunflower oil (15.96%) and were significantly different from control (35.84%). High per cent weight loss was recorded with sunflower oil (29.88%) which was not significantly different from control & was on par with Groundnut oil (25.93%) that was significantly different from control (Table 2).The observations on 180 DAT showed that the grain treated with *Beauveria* + Groundnut oil was found to be best and recorded the less per cent weight loss of 17.59 which was on par with *Metarhizium* + Groundnut oil (19.01%) and *Beauveria* + Sunflower oil (20.02%), *Metarhizium* + Sunflower oil (21.79%) and *Lecanicillium* + Sunflower oil (24.24%) (Table 2). High per cent weight loss was recorded with Sunflower oil (37.42%) followed by Groundnut oil (30.06%) and *Lecanicillium* + Groundnut oil (27.67%) which has shown less significance. All the treatments were significantly different from control (50.09%). High per cent reduction in weight loss was recorded with *Beauveria* + Groundnut oil (64.88%) followed by *Metarhizium* + Groundnut oil (62.05%). Less per cent reduction in weight loss was recorded with Sunflower oil (25.29%) followed by Groundnut oil (39.39%) and *Lecanicillium* + Groundnut oil (44.76%) when compared to control. Khalequazzaman *et al.* (2007) reported less per cent weight loss with Groundnut oil (1.5 & 0.80% at 30 & 60 days) and sunflower oil (3.7% & 9% at 30 & 60 days) against *C. chinensis*. Sabbour & Shadia (2007) reported less per cent weight loss of 17 and 22% with mustard and Nigella oil against Broad bean beetle, *B. rufimanus*.

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**Table 1. Interaction effects of entomopathogenic fungi against weight loss (%) by lesser grain borer, *R. dominica***

| Treatments | Dosage (g/kg) | Weight loss (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **45 DAT** | **60 DAT** | **75 DAT** | **90 DAT** | **105 DAT** | **120 DAT** | **135 DAT** | **150 DAT** | **165 DAT** | **180 DAT** |
| *Beauveria bassiana* | 5 | 1.33 (6.56) $^{b}$ | 2.23 (8.59) $^{cd}$ | 3.37 (10.53) $^{cd}$ | 4.92 (12.82) $^{cd}$ | 7.06 (15.41) $^{d}$ | 7.96 (16.37) $^{c}$ | 10.23 (18.67) $^{cd}$ | 13.90 (21.87) $^{c}$ | 15.90 (23.48) $^{bc}$ | 19.56 (25.63) $^{bc}$ |
| *Metarhizium anisopliae* | 5 | 1.50 (6.85) $^{b}$ | 2.42 (8.91) $^{cd}$ | 4.27 (11.91) $^{bc}$ | 5.56 (13.64) $^{bcd}$ | 7.83 (16.20) $^{cd}$ | 10.33 (18.69) $^{bc}$ | 12.21 (20.45) $^{bcd}$ | 14.54 (22.40) $^{bc}$ | 16.08 (23.63) $^{bc}$ | 21.28 (27.47) $^{bc}$ |
| *Lecanicillium lecanii* | 5 | 1.86 (7.73) $^{b}$ | 3.60 (10.89) $^{b}$ | 5.55 (13.62) $^{b}$ | 7.00 (15.30) $^{bc}$ | 12.27 (20.52) $^{b}$ | 13.50 (21.53) $^{b}$ | 15.60 (23.25) $^{b}$ | 18.19 (25.25) $^{b}$ | 19.87 (26.45) $^{b}$ | 23.81 (29.18) $^{b}$ |
| *Beauveria + Metarhizium* | 2.5 +2.5 | 1.58 (7.22) $^{b}$ | 2.11 (8.36) $^{d}$ | 2.75 (9.52) $^{d}$ | 4.83 (12.66) $^{cd}$ | 6.59 (14.85) $^{d}$ | 7.59 (15.98) $^{c}$ | 9.59 (18.02) $^{d}$ | 12.25 (20.47) $^{c}$ | 14.40 (22.30) $^{c}$ | 18.75 (26.25) $^{bc}$ |
| *Beauveria + Lecanicillium* | 2.5 + 2.5 | 1.78 (7.53) $^{b}$ | 2.56 (9.19) $^{cd}$ | 3.53 (10.76) $^{cd}$ | 5.07 (13.00) $^{cd}$ | 7.63 (15.98) $^{d}$ | 8.41 (16.78) $^{c}$ | 11.63 (19.92) $^{cd}$ | 14.48 (22.34) $^{bc}$ | 15.54 (23.17) $^{bc}$ | 20.26 (26.75) $^{bc}$ |
| *Metarhizium + Lecanicillium* | 2.5 + 2.5 | 2.13 (8.30) $^{b}$ | 3.16 (10.21) $^{bc}$ | 5.34 (13.30) $^{b}$ | 7.44 (15.83) $^{b}$ | 11.13 (19.46) $^{bc}$ | 12.17 (20.40) $^{b}$ | 13.37 (21.42) $^{bc}$ | 15.55 (23.22) $^{bc}$ | 17.64 (24.81) $^{bc}$ | 22.98 (28.65) $^{b}$ |
| *Beauveria + Metarhizium + Lecanicillium* | 1.67+1.67+1.67 | 1.28 (6.49) $^{b}$ | 2.04 (8.23) $^{d}$ | 2.66 (9.37) $^{d}$ | 4.44 (12.13) $^{d}$ | 6.35 (14.59) $^{d}$ | 7.66 (16.04) $^{c}$ | 9.56 (17.99) $^{d}$ | 12.17 (20.42) $^{c}$ | 13.03 (21.16) $^{c}$ | 17.68 (24.85) $^{c}$ |
| Control | | 6.11 (14.31) $^{a}$ | 10.11 (18.51) $^{a}$ | 16.35 (23.79) $^{a}$ | 20.36 (26.73) $^{a}$ | 21.22 (27.26) $^{a}$ | 30.11 (33.10) $^{a}$ | 39.17 (38.71) $^{a}$ | 43.42 (41.26) $^{a}$ | 48.78 (44.34) $^{a}$ | 51.27 (45.77) $^{a}$ |
| **SEm±** | | **0.79** | **0.50** | **0.75** | **0.89** | **1.11** | **1.04** | **0.91** | **0.85** | **1.08** | **1.02** |
| **CD (0.05)** | | **2.36** | **1.49** | **2.26** | **2.68** | **3.33** | **3.12** | **2.74** | **2.55** | **3.23** | **3.04** |

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



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**Table 2. Compatibility of vegetable oils with entomopathogenic fungi against weight loss (%) by *R. dominica***

| Treatments | Dosage (g +ml /kg) | Weight loss (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **45 DAT** | **60 DAT** | **75 DAT** | **90 DAT** | **105 DAT** | **120 DAT** | **135 DAT** | **150 DAT** | **165 DAT** | **180 DAT** |
| Sunflower oil | 2.5 ml/kg | 3.66 (10.97)[ab] | 4.36 (12.04)[b] | 11.76 (20.02)[b] | 12.46 (20.68)[b] | 18.67 (25.52)[a] | 23.19 (28.60)[b] | 24.02 (29.18)[b] | 29.88 (33.04)[ab] | 34.59 (35.99)[b] | 37.42 (37.69)[b] |
| Groundnut oil | 2.5 ml/kg | 3.12 (9.93)[ab] | 4.03 (11.57)[bc] | 9.99 (18.34)[b] | 11.96 (20.23)[b] | 16.20 (23.68)[ab] | 20.65 (27.01)[bc] | 21.22 (27.40)[bc] | 25.93 (30.60)[bc] | 26.93 (31.26)[c] | 30.06 (33.26)[bc] |
| *Beauveria* + Sunflower oil | 5 +2.5 | 1.91 (7.62)[bc] | 2.17 (8.47)[de] | 3.82 (11.26)[c] | 5.59 (13.68)[de] | 8.32 (16.47)[cd] | 11.30 (19.64)[d] | 13.17 (21.28)[de] | 15.11 (22.84)[de] | 16.58 (23.97)[de] | 20.02 (26.54)[e] |
| *Beauveria* + Groundnut oil | 5 +2.5 | 0.92 (5.47)[c] | 1.83 (7.43)[e] | 2.20 (8.47)[d] | 3.99 (11.48)[f] | 7.00 (14.89)[d] | 8.03 (16.18)[d] | 10.02 (18.31)[e] | 12.11 (20.29)[e] | 13.85 (21.74)[e] | 17.59 (24.70)[e] |
| *Metarhizium* + Sunflower oil | 5 +2.5 | 2.27 (9.00)[abc] | 3.24 (10.35)[bcd] | 4.95 (12.82)[c] | 7.08 (15.44)[cd] | 11.11 (19.37)[bcd] | 12.66 (20.78)[d] | 14.01 (21.93)[de] | 12.86 (21.00)[e] | 17.90 (25.03)[de] | 21.79 (27.77)[de] |
| *Metarhizium* + Groundnut oil | 5 +2.5 | 1.03 (5.82)[c] | 2.22 (8.55)[de] | 3.38 (10.49)[cd] | 4.38 (12.06)[ef] | 7.27 (15.39)[cd] | 8.93 (17.26)[d] | 10.77 (19.08)[de] | 12.11 (20.29)[e] | 16.30 (23.74)[de] | 19.01 (25.82)[e] |
| *Lecanicillium* + Sunflower oil | 5 +2.5 | 2.96 (9.82)[ab] | 2.73 (9.49)[cde] | 4.69 (12.51)[c] | 6.53 (14.81)[cd] | 10.17 (18.57)[bcd] | 11.70 (19.89)[d] | 13.55 (21.55)[de] | 15.96 (23.51)[de] | 19.18 (25.93)[de] | 24.24 (29.49)[cde] |
| *Lecanicillium* + Groundnut oil | 5 +2.5 | 1.32 (6.19)[c] | 3.38 (10.52)[bcd] | 5.12 (13.07)[c] | 7.33 (15.71)[c] | 12.09 (20.34)[bc] | 14.08 (22.04)[cd] | 16.57 (24.03)[cd] | 20.35 (26.82)[cd] | 23.38 (28.90)[cd] | 27.67 (31.74)[cd] |
| Control | | 4.12 (11.67)[a] | 7.90 (16.30)[a] | 16.39 (23.94)[a] | 17.30 (24.57)[a] | 23.45 (28.76)[a] | 32.47 (34.38)[a] | 33.64 (35.43)[a] | 35.84 (36.65)[a] | 45.95[a] (42.71) | 50.09 (45.05)[a] |
| **SEm±** | | **1.07** | **0.75** | **0.81** | **0.55** | **1.79** | **2.01** | **1.58** | **1.60** | **1.54** | **1.56** |
| **CD (0.05)** | | **3.18** | **2.22** | **2.40** | **1.64** | **5.31** | **5.98** | **4.70** | **4.77** | **4.58** | **4.63** |

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



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