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A laboratory assessment for the potential of entomopathogenic fungi to control *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

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Abstract
The pathogenecity of *Beauveria bassiana* and *Isaria fumosorosea* was evaluated by immersion bioassays against adults and 3rd instar grubs of *Rhyzopertha dominica* during years 2011-2013. Five concentrations i.e., 1.0- 5×10^8 spores/ml of each fungus were made by serial dilution. The results depicted that lowest LT_{50} values with highest concentration of *B. bassiana* 5×10^8 spores/ml were 3.60 days for both stages. Conversely, *B. bassiana* (LC_{50}) on 7th day post-treatment were 1.54×10^8 and 2.29×10^8 spores/ml both stages, respectively. The lowest LT_{50} values with highest concentration of *I. fumosorosea* 5×10^8 spores/ml were 3.58 and 3.04 days for both adults and grubs, respectively, while the LC_{50} value of *I. fumosorosea* on 7th day were 1.39×10^8 and 2.23×10^8 spores/ml for both stages, respectively. Comparison of LC_{50}, LT_{50} and percent mortalities indicated fungal virulence to *R. dominica.* The findings of the current research illustrate that these fungi can be used for the management of *R. dominica.*

Key Words: Pathogenecity, entomopathogenic fungi, *Rhyzopertha dominica*, bioassay, stored grain pest.

1. Introduction
Rhyzopertha dominica F. (Coleoptera: Bostrychidae) is one of the most notorious primary pest of stored wheat, legumes where it is able of damaging both cracked and sound grains of many cereals all over the world [1-5]. The larvae feed inside the grains and convert most of the grains into flour and irregular holes in the grains are seen easily in case of heavy infestation [6].
The lesser grain borer is also a secondary pest of grains in which the first instar larvae have been detected to enter grain through the intact kernel and during ordinary cleaning procedures it cannot be eliminated [7-8]. Residual synthetic grain protectants and fumigants are still considered best methods for the management of insect pests of stored foodstuffs [9]. Synthetic chemicals have been used since 1950s for the control of insect pests of stored harvest [10]. However the residual effects on stored products, food stuffs, the development of resistance and environmental setbacks have made it essential to evaluate alternative management tools for insect pests of stored commodities [10-12].
Plant-based insecticides, insect growth regulators and insect pathogens like entomopathogenic fungi, viruses, bacteria and protozoa are being evaluated as good alternatives to chemical-based insect control methods. Apart from other substitutes, different bio-control means like insect pathogenic fungi, viruses, bacteria and nematodes have got solemn consideration as they are least noxious to human beings, animals, more efficient and can also become the vital part of IPM programmes [13]. There are 100 or more genera which comprise of insect pathogens and most of them belong to class Deuteromycetes which are little toxic to mammals and environmentally safe [14-16]. As demonstrated by Wood and Thomas [17] the infected insects also play important role to enhance the residual effectiveness of mycopesticides through recycling of infection in the area.
The studies have shown efficacy of entomopathogenic fungi against the insect pests of stored products [18-20]. The ability of entomopathogenic fungi to manage insect pests of stored grains, particularly coleopterans has been confirmed in many studies during current years [21-22]. *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) has gained considerable importance in the IPM of stored product insects [23]. Attempts to manage *R. dominica*, *Sitophilus oryzae* (L.), and *Tribolium castaneum* (Herbst) with *B. bassiana* have been stated [24-26]. According to the findings of Dal-Bello *et al*. [27] it has been used for control of *S. oryzae* on

wheat with the application of suspension of *B. bassiana*. The present study was conducted to check the effectiveness of *B. bassiana and Isaria fumosorosea* against adults and immatures of *R. dominica*.

2. Materials and Methods

Insects were reared on dried healthy mature wheat grains in plastic containers (6×10 cm) under lab. conditions (25±5 ˚C, 75±5% R.H. and 16:8 h of illumination) during year 2013. The containers were covered with muslin cloth for proper ventilation. Newly emerged male and female adults were used in studies. The entomopathogenic fungi *B. bassiana* and *I. fumosorosea* were grown on Potato Dextrose Agar (PDA) for two weeks. Spores were separated from fifteen days old fungus culture and five different concentrations i.e., 1×10^8, 2×10^8, 3×10^8, 4×10^8 and 5×10^8 spores/ml of *B. bassiana* and *I. fumosorosea* were made in 0.05% Tween 80 solution by serial dilution while insects were treated by immersion method. There were six treatments including control and each treatment was replicated four times. The data for the effect of entomopathogenic fungi on adults and immatures was recorded after 24 hrs of application for consecutive seven days.

2.1 Statistical analysis

The mortality counts were corrected by using Abbott's formula[28], and the means were compared by means of SAS [29], under Completely Randomized Design using Duncan's Multiple Range Test (DMRT), while LC_{50} and LT_{50} values were calculated by using Probit analysis.

3. Results

3.1 Effect of *B. bassiana* on the adults of *R. dominica* with a range of conidial concentration over time

The pathogenecity of *B. bassiana* was checked against the adults of *R. dominica* which showed that the mortality of adults was obtained on the second day of treatment. Commutative mean percent mortality of *R. dominica* treated with *B. bassiana* with different concentrations was dose dependent and it increased with the increase in concentration. Minimum mortality 5% on 2nd day and maximum percent mortality 90 was recorded on 7th day after the application of concentrations 1×10^8 and 5×10^8 spores/ml of *B. bassiana*, respectively (Figure 1). The LC_{50} values of *B. bassiana* on the adults of *R. dominica* were 4.48×10^8, 2.95×10^8, 2.15×10^8 and 1.54×10^8 spores/ml on 4th, 5th, 6th and 7th day (Table 1). On the other hand the lethal time required to kill 50% population was 6.78 days at concentration of 2×10^8 spores/ml but it decreased up to 3.60 days at concentration of 5×10^8 spores/ml (Table 2).

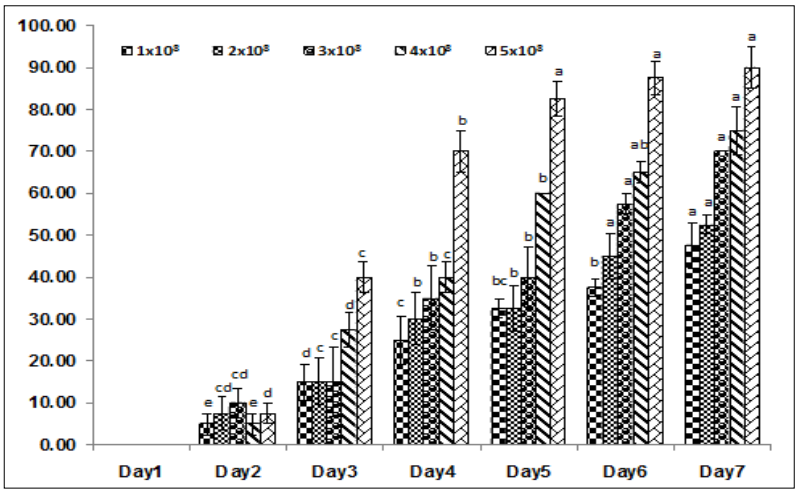

Fig. 1: Percent mortality of adults of *R. dominica* after exposure to different concentrations of *B. bassiana*. For each day the same letters are not significantly different (P < 0.05) according to Duncan's Multiple Range Test (DMRT)

Table 1: LC_{50} values of *B. bassiana* and *I. fumosorosea* against adults of *R. dominica*

Fungi	Days	LC 50	FD	Slope	D.F
Beauveria bassiana	4th	4.48×10^8	3.11×10^8-6.47×10^8	1.53±0.40	3
	5th	2.95×10^8	1.45×10^8-6.01×10^8	1.95±0.72	3
	6th	2.15×10^8	1.71×10^8-2.71×10^8	1.91±0.39	3
	7th	1.54×10^8	1.14×10^8-2.09×10^8	1.82±0.38	3
Isaria fumosorosea	4th	4.80×10^8	3.61×10^8-6.39×10^8	2.14±0.45	3
	5th	2.83×10^8	2.36×10^8-3.40×10^8	2.33±0.41	3
	6th	2.08×10^8	1.70×10^8-2.54×10^8	2.28±0.40	3
	7th	1.39×10^8	1.04-$\times10^8$$1.88\times10^8$	2.04±0.39	3

Table 2: LT_{50} values of *B. bassiana* and *I. fumosorosea* against adults of *R. dominica*

Fungi	Concentration	LT_{50}	F.D	Slope	D.F
Beauveria bassiana	2×10^8	6.78	5.65-8.13	3.12±0.52	5
	3×10^8	5.56	4.95-6.24	3.93±0.56	5
	4×10^8	4.82	4.39-5.28	4.50±0.57	5
	5×10^8	3.60	3.31-3.91	5.48±0.59	5
Isaria fumosorosea	2×10^8	6.37	5.66-7.17	4.68±0.74	5
	3×10^8	5.45	4.84-6.14	3.73±0.53	5
	4×10^8	4.49	4.09-4.92	4.41±0.54	5
	5×10^8	3.58	3.30-3.89	5.69±0.61	5

3.2 Effect of *B. bassiana* on 3rd instar grubs of *R. dominica* with a range of conidial concentration over time

The application of *B. bassiana* on 3rd instar grubs showed mortality on the second day of treatment at concentrations of 4×10^8 and 5×10^8 spores/ml. Commutative mean percent mortality of 3rd instar grubs of *R. dominica* was dose dependent and increased as concentration of *B. bassiana* increased. Minimum 10% mortality on 5th day and maximum mortality percentage 90 was obtained on 7th day after the application of concentrations 1×10^8 and 5×10^8 spores/ml of *B. bassiana* respectively (Figure 2). The LC_{50} values of *B. bassiana* on the 3rd instar grub of *R. dominica* were 3.80×10^8, 2.98×10^8 and 2.29×10^8 spores/ml on 5th, 6th and 7th day (Table 3). Conversely lethal time required to kill 50% population of immature of *R. dominica* was 4.02 days after treatment of concentration 4×10^8 spores/ml conversely it decreased up to 3.60 days at concentration of 5×10^8 spores/ml (Table 4).

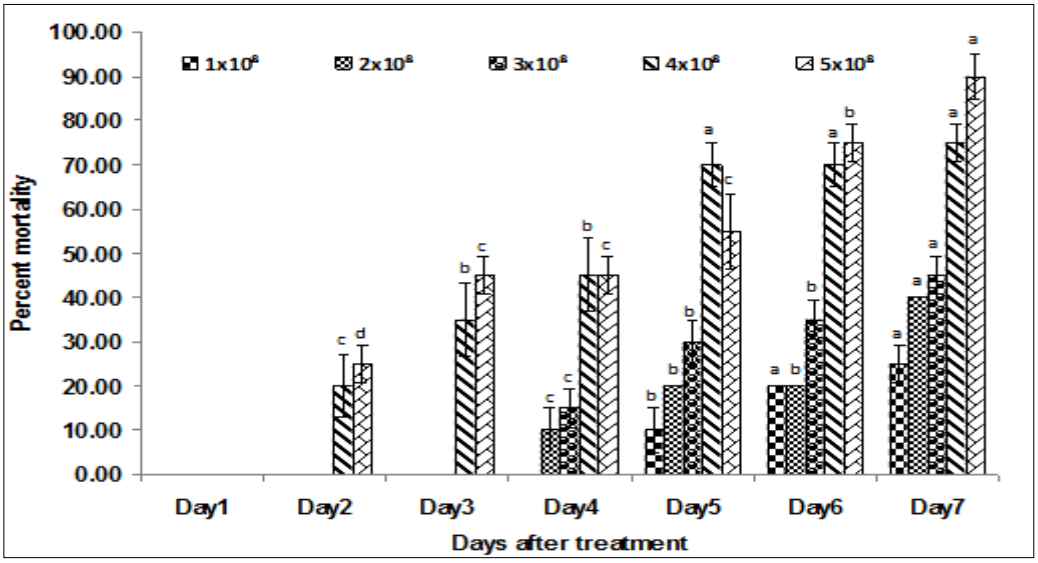

Fig 2: Percent mortality of 3rd instar grubs (immatures) of *R. dominica* after exposure to different concentrations of *B. bassiana*. For each day the same letters are not significantly different ($P < 0.05$) according to Duncan's Multiple Range Test (DMRT)

Table 3: LC_{50} values of *B. bassiana* and *I. fumosorosea* against immature of *R. dominica*

Fungi	Days	LC_{50}	FD	Slope	D.F
Beauveria bassiana	5th	3.80×10^8	2.88×10^8-5.02×10^8	2.49±0.64	3
	6th	2.98×10^8	2.18×10^8-4.08×10^8	1.91±0.56	3
	7th	2.29×10^8	1.78×10^8-2.94×10^8	2.49±0.58	3
Isaria fumosorosea	3rd	4.67×10^8	3.91×10^8-5.57×10^8	5.26±1.38	3
	4th	4.48×10^8	3.59×10^8-5.59×10^8	3.79±0.96	3
	5th	3.77×10^8	2.89×10^8-4.93×10^8	2.58±0.65	3
	6th	2.87×10^8	2.21×10^8-3.72×10^8	2.32±0.58	3
	7th	2.23×10^8	1.79×10^8-2.77×10^8	3.00±0.61	3

Table 4: LT_{50} values of *B. bassiana* and *I. fumosorosea* against immature of *R. dominica*

Fungi	Concentration	LT_{50}	F.D	Slope	D.F
Beauveria bassiana	4×10^8	4.02	3.41-4.74	3.29±0.58	5
	5×10^8	3.60	3.19-4.41	3.36±0.57	5
Isaria fumosorosea	2×10^8	6.37	5.66-7.17	4.68±0.74	5
	3×10^8	5.45	4.84-6.14	3.73±0.53	5
	4×10^8	4.60	3.87-5.47	3.20±0.60	5
	5×10^8	3.04	2.53-3.64	3.12±0.51	5

3.3 Effect of *I. fumosorosea* on the adults of *R. dominica* with a range of conidial concentration over time

The application of *I. fumosorosea* showed mortality of adults on the second day of treatment. Overall mean percent mortality of adults of *R. dominica* treated with *I. fumosorosea* with different concentrations was observed to increase with the strength of dose. Minimum percent mortality i.e., 2.5 on 2nd day and maximum 92% mortality was obtained on 7th day at concentrations of 1×10^8 and 5×10^8 spores/ml of *I. fumosorosea* respectively (Figure 3). The LC_{50} values *I. fumosorosea* on the adults of *R. dominica* were 4.80×10^8, 2.83×10^8, 2.08×10^8 and 1.39×10^8 on 4th, 5th, 6th and 7th day (Table 1), while lethal time required to kill 50% population was 6.37 days at concentration of 2×10^8 spores/ml but it decreased up to 3.58 days at concentration of 5×10^8 spores/ml (Table 2).

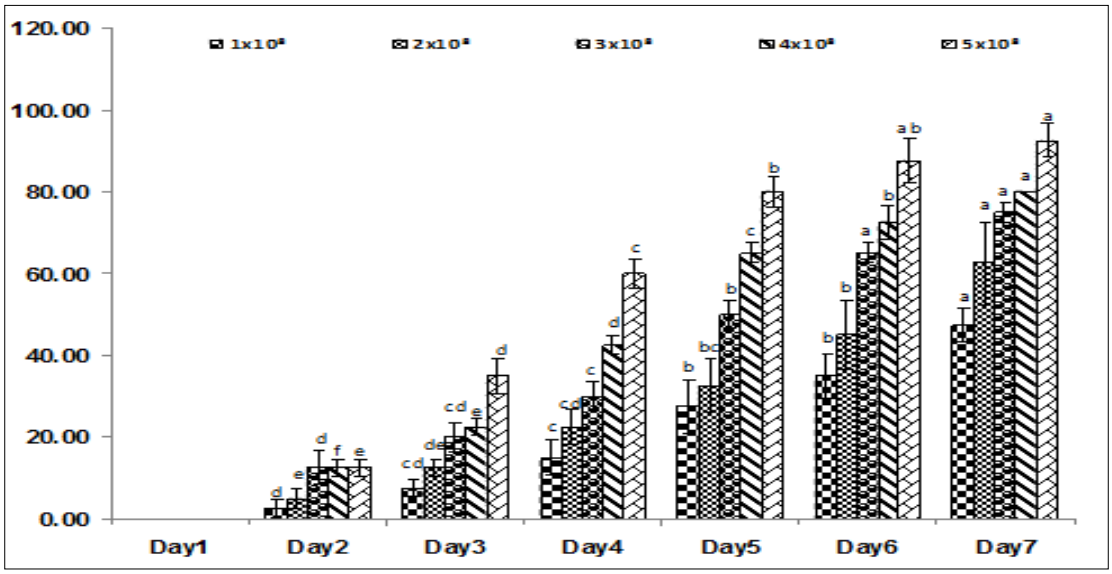

Fig. 3: Percent mortality of adults of *R. dominica* after exposure to different concentrations of *I. fumosorosea*. For each day the same letters are not significantly different (P < 0.05) according to Duncan's Multiple Range Test (DMRT)

3.4 Effect of *I. fumosorosea* on 3rd instar grubs of *R. dominica* with a range of conidial concentration over time

The application of different series of concentration of *I. fumosorosea* showed its effect on second day of treatment. Commutative mean percent mortality of 3rd instar grubs of *R. dominica* treated with *I. fumosorosea* with different concentrations was observed to be dose dependent and results were similar to adults. Minimum 5% mortality on 3rd day and maximum percent mortality of 90 was obtained on 7th day after application of concentrations of 2×10^8 and 5×10^8 spores/ml of *I. fumosorosea*, respectively (Figure 4). The LC_{50} values of *I. fumosorosea* on the 3rd instar grub of *R. dominica* were 4.67×10^8, 4.48×10^8, 3.77×10^8, 2.87×10^8 and 2.23×10^8 spores/ml on 3rd, 4th, 5th, 6th and 7th day (Table 3). In contrast to this lethal time required to kill 50% population was 6.37 days at concentration of 2×10^8 spores/ml but it decreased up to 3.04 days at concentration of 5×10^8 spores/ml (Table 4).

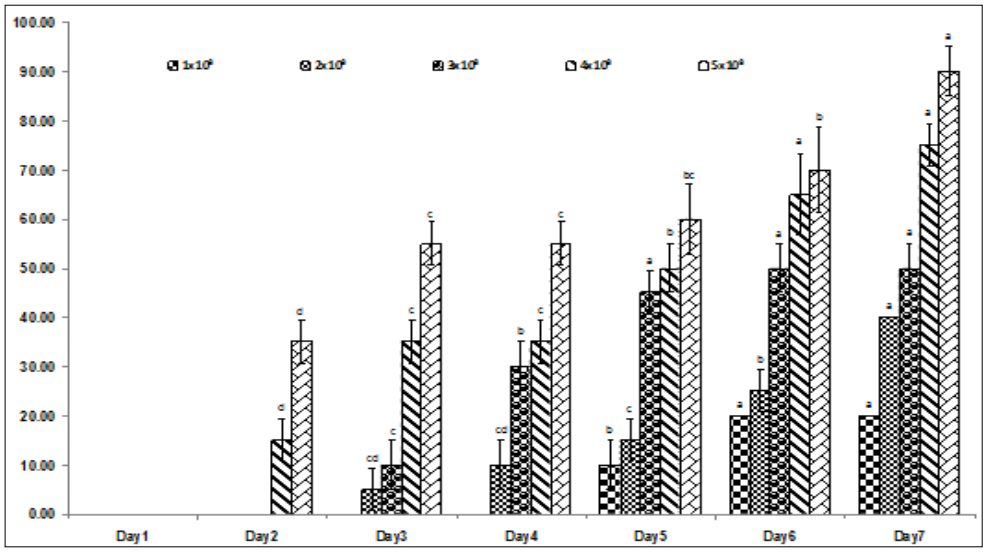

Fig. 4: Percent mortality of 3rd instar grubs (immatures) of *R. dominica* after exposure to different concentrations of *I. fumosorosea*. For each day the same letters are not significantly different (P < 0.05) according to Duncan's Multiple Range Test (DMRT)

4. Discussion

The efficacy of entomopathogenic fungi were evaluated against adults and immatures of *R. dominica*. The results showed that mortality of both adults and immature was dose dependent, mortality increased and time required for causing 50% mortality in population reduced as concentration of entomopathogenic fungi increased. Higher mortalities were obtained by the application of higher concentration of spores of *B. bassiana* and *I. fumosorosea* to adults and 3rd instar grubs of *R. dominica* after 7 days of treatment (Fig. 1-4). Numerous researchers have documented the potential of entomopathogenic fungi as a control measures against stored grain and other potential insect pests of different crops insect pests and prove as good alternatives to chemicals and fumigants [30-31]. According to the findings of Fargues *et al.* [32] several factors are involved in toxicity of insect pathogenic fungi against stored grain insects. Insecticidal efficacy of *B. bassiana* is very much manipulated by many factors for instance, insect's behavior, population density, age, nutrition and genetic information. Mode of entry of entomopathogenic fungi is mostly by contact method and *B. bassiana and I. fumosorosea* are also capable of penetrating through the insect cuticle, producing hydrolytic enzymes i.e., proteinases chitinases, and lipases [33-35] being helpful against many bruchids e.g., *R. dominica* [36-37].

The present study findings explain that the mortality of *R.*

dominica was dose dependent and increased as concentrations of entomopathogenic fungi were increased. The results are not in accordance with the findings of Akbar *et al*. [20] who reported that adults of *T. castaneum* exhibited very little susceptibility to *B. bassiana* at a concentration of 2.9×10^9 spores/ml. In another study it was demonstrated that adding 150 mg of conidia per kilogram of commercially formed, unformulated conidia of *B. bassiana* to cracked or whole oats caused in a 70 and 98% reduction, respectively, in the number of young ones produced by *Oryzaephilus surinamensis* which also support our results that entomopathogenic fungi can cause higher mortalities in stored grain pests up to 90 and 92% at 5×10^8 concentration of used fungi (Fig. 1 and 3).

Entomopathogenic fungi have the potential that these can be used commercially as microbial insecticides for safe and environment friendly pest control [38]. According to our findings both fungi proved to be virulent against *R. dominica* by immersion method as it has also been confirmed that different isolates from *M. anisopliae* and *B. bassiana* can provide good control of stored grain pest e.g., *Callosobruchus maculatus* by immersion bioassay [37]. It was recently verified that reduced atmospheric and grain moisture, could increase the efficacy of entomopathogenic fungi especially *B. bassiana* in storage facilities which strengths our results that this fungus can cause mortality in *R. dominica* and has the potential to be used against other stored grain pests [39-40]. The longevity of conidia of entomopathogenic fungi is generally more stable at cool and dry conditions and it supports our results that these entomopathogenic fungi cause the mortalities due to their persistence in dry environment and show the potential and abilities to be used against stored grain pests of different commodities [41]. As it has been pointed out that it is possible to control *Sitophilus zeamais* on maize by using formulated *B. bassiana* also support our results that entomopathogenic fungi can be used for the management of other stored grain pests of wheat like *R. dominica* [42].

The findings of current research also correlate and confirm the previous studies that stored grain pests can be managed safely by using entomopathogenic fungi, which is clear from our results that *B. bassiana and I. fumosorosea* are able to significantly reduce *R. dominica.* Comparisons of LT_{50} and LC_{50} values show that both tested fungi are virulent against the pest. LT_{50} values of *B. bassiana* and *I. fumosorosea* after 7 days of treatment against adults of *R. dominica* were almost similar 3.60 and 3.58 days, respectively (Table 2). Conversely the values of LC_{50} of *B. bassiana* and *I. fumosorosea* after 7 days of treatment against adults of *R. dominica* were 1.39×10^8 and 1.54×10^8 spores/ml, respectively (Table 1), while LT_{50} values after 7 days of treatment against 3rd instar grub of *R. dominica* were 3.60 and 3.04 days of *B. bassiana* and *I. fumosorosea*, respectively (Table 4).

5. Conclusion

In conclusion, these are safer ways to control insect pests of stored products and grains, and should be encouraged in IPM programmes of stored product and grain pests. It is compulsory to substitute conventional methods being used these insect pests with other secure and environment friendly methods for the storage of wheat and other stored commodities without any chemical pollution.

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