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Research Article

In vitro Entomopathogenic Efficacy of *Beauveria bassiana* (Ascomycota: Hypocreales) Against *Corcyra cephalonica* (Lepidoptera: Pyralidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract

Background and Objective: Rice meal moth *Corcyra cephalonica* (*C. cephalonica*) and Red flour beetle *Tribolium castaneum* (*T. castaneum*) are the most common and cosmopolitan stored grain insect pests responsible for significant losses to stored wheat all over the world. The present study encompassed the laboratory determination of pathogenicity and virulence of entomopathogenic fungus, *Beauveria bassiana* formulation (Racer BB™), against rice meal moth, *Corcyra cephalonica* larvae and red flour beetle, *Tribolium castaneum* adults. **Materials and Methods:** Three concentrations of *B. bassiana* i.e., 3×10^7, 6×10^7 and 9×10^7 conidia kg^{-1} of wheat grains were used. Mortality data was recorded at different post-treatment time intervals and were subjected to the analysis of variance followed by the separation of treatment means by least significant difference (LSD) test at standard significance level (α = 0.05). **Results:** Maximum larval and adult mortality was recorded, respectively for *C. cephalonica* (79.67%) and *T. castaneum* (70.00%) at higher concentration (9×10^7 conidia kg^{-1} wheat) of entomopathogenic fungus at 21 days post-treatment. Remaining concentrations of the fungus gave mortalities less than 50% for the treated stored grain pests. **Conclusion:** This study suggested that *B. bassiana* had the ability to be used as an effective biocontrol agent for the control of stored grain insect pests such as *C. cephalonica* (rice meal moth) and *T. castaneum* (red flour beetle).

Key words: *Beauveria bassiana*, *Corcyra cephalonica*, entomopathogenic fungi, stored grain pests, *Tribolium castaneum*, wheat

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Grain storage is an essential and common activity that permits food grains to be utilized from weeks to months and years after crop harvest[1]. As production of grain crops is seasonal and their consumption is continuous throughout the year, their safe storage is inevitable in order to maintain their grains quality and quantity[2]. However, cereals, pulses and oilseed crops are normally infested by various insect pests which cause substantial quantitative and qualitative losses[3,4]. The most common insect pests of stored grain crops in Indo-Pak regionarerice meal moth (*Corcyra cephalonica* Staint.), red flour beetle (*Tribolium castaneum* Herbst), lesser grain borer (*Rhyzopertha dominica* Fab.) and angoumois grain moth (*Sitotroga cerealella* Olivier)[5,6]. Estimated about 10-40% losses were recorded in stored grain crops only due to the infestation of insect pests in developing countries[7,8].

Among major stored grain insect pest species, *C. cephalonica* (Lepidoptera, Pyralidae) is a serious pest of wheat, rice, maize, groundnut, cocoa beans, sorghum and cotton[9,10]. Larval stage of these moths is the most damaging stage causing direct damage by feeding and indirect damage by spoiling stored grains with their fecal matter and silken cocoons[11]. Similarly, *T. castaneum* (Coleoptera, Tenebrionidae) is a cosmopolitan and polyphagous stored grain insect pest of stored grain crops[8,12,13]. The adults of this insect pestare not only responsible for the physical damage to grains but they also cause allergic diseases in human beings upon consumption of such infested grains[14]. Losses from 5-15% in grain weight incurred by *C. cephalonica* and *T. castaneum* have been reported during the storage of cereals, pulses and oilseed crops[15,16].

As insect pests are one of the most significant and contemporary issues of storage sector all over the world, a number of synthetic chemicals have been registered and recommended for the control of stored grain pests in these countries including Indo-Pak region[17,18]. Although playing a vital role in lowering the insect pest infestations in stored grains, most of these synthetic pesticides are highly persistent and toxic[16]. Moreover, the indiscriminate and extensive use of these grain protectants (pesticides) have resulted in the development of pesticide resistance, resurgence of secondary pests and other environmental and human health hazards[19-21].

Hence, there is a need to search for some alternative approaches for the control of these stored grain insect pests such as the use of biological control agents which have been as environment-friendly and safe pest control strategies[22,23]. For instance, a number of fungal species have been reported very effective against a wide variety of insect pests[24-27]. The entomopathogenic fungi have contact mode of action and usually grows through insect cuticle and penetrates into insect body, proliferates, produces toxins and finally causes death of the insect pest[24,28,29]. Many scientists have reported that optimum temperature for different entomopathogenic fungi ranges between 20-30°C[30,31], therefore, their utilization for the control of stored grain insect pests is feasible and effective. *Beauveriabassiana* is one of the most widely used and effective entomopathogenic fungi against a large number of insect pest species including stored grain insects and mites[11,32]. The present study was aimed to evaluate the pathogenicity of a commercial formulation of *B. bassiana* against two major stored grain insect pests i.e., *C. cephalonica* and *T. castaneum* under laboratory conditions.

MATERIALS AND METHODS

The present study was conducted at the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad, Punjab, Pakistan during spring, 2017. Populations of *T. castaneum* and *C. cephalonica* were collected from the grain market of Faisalabad (Punjab, Pakistan) and were brought to the laboratory under cool conditions. Both insect pests were reared in sterilized clear plastic jars (1 L) in a controlled incubator (Sanyo MIR-254, Japan) at 30±2°C and 60±5% relative humidity. Sterilized wheat grains and wheat flour were used as a culture media for *C. cephalonica* and *T. castaneum*, respectively. The adults of *C. cephalonica* and *T. castaneum* were released on wheat grains (500 g) and fresh wheat flour (500 g), respectively. Jars were then covered with fine muslin cloth for aeration and tightened up by rubber bands to avoid the escape of insects. The larvae of *C. cephalonica* and adults of *T. castaneum* emerged from these stock colonies were used for further experiments.

Bioassays were conducted under laboratory conditions following complete randomized design (CRD) according to the protocol described by Dal Bello *et al.*[33]. The commercial formulation of entomopathogenic fungus *Beauveria bassiana* (Racer®BB1.15% WP, AgriLife™, Hyderabad, India) was evaluated against these insect pests. This formulation had been derived from two naturally occurring soil strains i.e., ATCC and NCIM 1216. Bioassays were conducted using three different concentrations of *B. bassiana* as independent treatments i.e., T1 = 3×10^7, T2 = 6×10^7 and T3 = 9×10^7 conidia kg^{-1} of wheat grains with three replications for each treatment. Sterilized food was supplied to both insect pests during the experimentation. Samples of 50 g of treated commodity for each concentration were taken into three separate sterilized plastic jars. Three jars with untreated food were set-up as control. Thereafter, one hundred active adults of *T. castaneum* and larvae of *C. cephalonica* were released in each jar separately and jars tops were closed with muslin cloth to ensure proper aeration. The treatments were kept in an incubator at 30±2°C and 60±5% relative humidity. With weekly intervals, the mortality of test insects was recorded at 7, 14 and 21 days post-release of insect pests. Dead insects having fungal growth on their body were removed from the jars and counted and considered dead as a result of infection caused by *B. bassiana*.

Statistical analysis: Of data was performed using software Statistic V. 8.1 (Analytical Software)[34]. Mortality data recorded for different concentrations at different post-treatment time intervals were subjected to the analysis of variance (one-way ANOVA), followed by the separation of treatment means by least significant difference (LSD)test at standard significance level ($\alpha = 0.05$).

RESULTS

According to statistical analysis, there was significant difference for all treatments as compared to control treatment for all observation times (Table 1 and 2). After 7 days interval, a significant difference ($p>0.05$) was observed for each treatment in reference to control treatment. Maximum mortality of *C. cephalonica* was observed in treatment 3 (9×10^7 conidia kg^{-1} of grains). After 21 days, cumulative mortality percentages of *C. cephalonica* larvae were recorded as 79.67for treatment 3, 49.33 for treatment 2 and 36.67 for treatment 1 (Fig. 1).

Similar trend of mortality was found in case of *T. castaneum* adults. At all the time intervals, mortality of *T. castaneum* adults was significantly higher as compared to control treatment. Maximum mortality was observed for treatment 3 (9×10^7 conidia kg^{-1} of grains) suggested that higher was the *B. bassiana* conidial concentration, greater will be efficiency. Maximum mortality of *T. castaneum* for all treatments was observed at 21 days post-treatment. Cumulative mortality percentages of *T. castaneum* adults after 21 days were 70.03 for treatment 3, 49.67 for treatment 2 and 29.67 for treatment 1 (Fig. 2). Among both insect pest species, *C. cephalonica* larvae were more susceptible to *B. bassiana* formulation rather than *T. castaneum* adults because the highest mortalities of *C. cephalonica* larvae at all the exposure times and concentration rates were observed for this species (Fig. 1 and 2). Average mortality was recorded as 55 and 49% for *C. cephalonica* and *T. castaneum*, respectively.

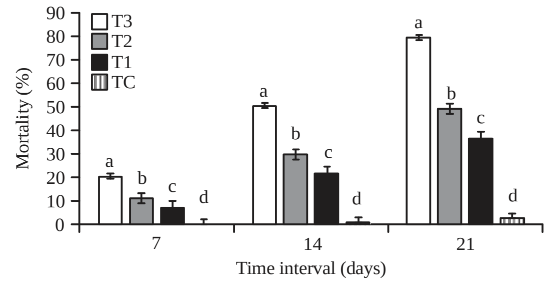

Fig. 1: Mortality ± SEM (%) of *Corcyra cephalonica* 3rd instar larvae recorded for three different concentrations of *Beauveria bassiana* at time intervals. T1 = 3×10^7 conidia kg^{-1} grains, T2 = 6×10^7 conidia kg^{-1} grains, T3 = 9×10^7 conidia kg^{-1} grains, TC = Control grains food. For each time interval, columns bearing different alphabet letters are significantly different from other treatments
(LSD test at p = 0.05)

Table 1: Analysis of variance for percent mortality in *C. cephalonica* larvae bioassayed against different concentrations of *B. bassiana* at different exposure periods

	Days		
	7	14	21
D.F	3.000	3.000	3.000
SS	667.667	1292.670	1158.920
MSS	222.556	430.889	386.306
CV	13.450	8.070	7.320
p-value	>0.01	>0.05	>0.05

One-way ANOVA (p<0.05) taking concentration as factor

Table 2: Analysis of variance for percent mortality in *T. castaneum* adults against different concentrations of *B. bassiana* at different exposure periods

	Days		
	7	14	21
D.F	3.000	3.00	3.000
SS	704.333	930.00	899.000
MSS	234.778	310.00	299.667
CV	11.780	6.19	6.150
p-value	>0.01	>0.05	>0.05

One-way ANOVA (p<0.05) taking concentration as factor

DISCUSSION

Fungal mycelial growth on the tested insect body demonstrated that the target insects died due to the pathogenicity incurred by *B. bassiana*. In both bioassays,

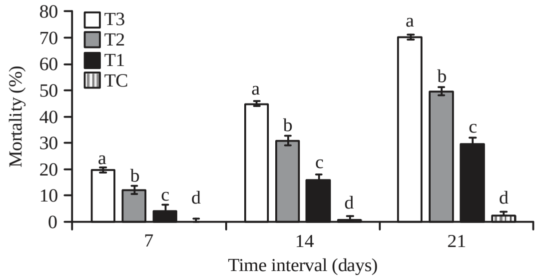

Fig. 2: Mortality ±SEM (%) of *Tribolium castaneum* adults recorded for three different concentrations of *Beauveria bassiana* at time intervals. T1 = 3×10^7 conidia kg^{-1} grains, T2 = 6×10^7 conidia kg^{-1} grains, T3 = 9×10^7 conidia kg^{-1} grains, TC = Control grains food. For each time interval, columns bearing different alphabet letters are significantly different from other treatments
(LSD test at $p = 0.05$)

mortality was found directly proportional to the conidial concentration of *B. bassiana* formulation and to the exposure time intervals. The highest conidial concentration of *B. bassiana*, i.e., 9×10^7 conidia kg^{-1} grains, proved most effective against both tested insect pests than other lower concentrations. Larvae of *C. cephalonica* were more susceptible to *B. bassiana* infection as compared to adults of *T. castaneum*. It is most probably due to the more delicate and less sclerotized larval cuticle of *C. cephalonica* as compared to hard and rigid body wall of *T. castaneum* adults with only the intersegmental membranes as penetration sites rendering the larvae more susceptible to *B. bassiana* infection and proliferation[35].

The application of *B. bassiana* formulation caused mortality more than 79% in *C. cephalonica* larvae and about 70% in adults of *T. castaneum*. Similar results for *B. bassiana* causing maximum mortality in the larvae of *C. cephalonica* at a concentration of 2.02×10^8 conidia mL^{-1} [11]. However, this mortality recorded for both insect pests is less than observed for a *B. bassiana* isolate bioassayed against *S. oryzae*, *R. dominica* and *T. casteanesum*[36]. In another study conducted in which isolation of *B. bassiana* (BbGc and BbPs) and *Metarhizium anisopliae* (MaPs) caused mortality among larvae of *C. cephalonica* as a factor of dose and time[37]. Likewise, the efficacy of *B. Bassiana* isolates (ITCC No. 6628, ITCC No. 6645 and B. NCIPM) against *C. cephalonica* and found effective with mortality percentage of 31-98 achieved within 3 weeks of application[38]. Similar results are reported by Khashaveh *et al.*[39], who revealed that *B. bassiana* is very efficient against *T. castaneum, S. granarius* and *O. surinamensis* exhibiting an average mortality of 64.99, 88.33 and 78.31%, respectively after 15 days of exposure to a concentration of 1 g kg^{-1} wheat or 2×10^9 conidia g^{-1} wheat which is higher than the application rates of 3×10^7 to 9×10^7 conidia kg^{-1} of wheat grains used in this study. High dose rates used by Khashaveh *et al.*[39] would probably be responsible for a high mortality of test insects within a short exposure period. Hence, the results of this study revealed that the effectiveness of biocontrol fungi such as *B. bassiana* can be achieved even at lower conidial concentration by providing proper conditions. Although Padin *et al.*[15] recorded similar findings that *B. bassiana* produced maximum mortality (55%) in *T. castaneum* (adults) under laboratory conditions after 21 days of inoculation, there was no significant difference found in this study regarding the percent weight loss among treated and untreated wheat grains infested by *T. castaneum*[15]. However, the results of this study corroborate the findings of many previous studies which have revealed the effectiveness of various isolates of *B. bassiana* against various insect pests species either alone or in combination of diatomaceous earth and synthetic insecticides either under laboratory or field conditions[15,20,23,27,40-46].

CONCLUSION

In brief, *B. bassiana* has the ability to be used as an effective entomopathogenic fungus and to be utilized for the control of stored grain insect pests such as *C. cephalonica* (rice meal moth) and *T. castaneum* (red flour beetle). In grain storage, application of commercial formulations of *B. bassiana* Racer® BB 1.15% WP could be recommended as an alternate and environmental-friendly grain protectant.

SIGNIFICANCE STATEMENTS

Application of microbial biocontrol agents against stored grain insect pests can be an effective and environment-friendly alternate strategy. Most of entomopathogenic fungal formulations such as of *Beauveria bassiana* are more selective to target pests and less toxic to humans and hence can be efficiently utilized in post-harvest pest management programs. This laboratory study demonstrated the effectiveness of a commercial formulation of *B. bassiana* (Racer® BB 1.15% WP) against two most economic stored grain insect pests, i.e., rice meal moth (*C. cephalonica*) and Red flour beetle (*T. castaneum*).

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