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BIO-EFFICACY OF ENTOMOPATHOGENIC FUNGUS *Beauveria bassiana* (BALS.) AGAINST *Trogoderma granarium* (EVERTS) AND *Tribolium castaneum* (HERBST)

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Stored grains are attacked by several insect pests. *Tribolium castaneum* (Herbst) and *Trogoderma granarium* (Everts) are notorious stored grain insect pests. The present study was conducted to evaluate the insecticidal and growth inhibitory effects of an entomopathogenic fungus *Beauveria bassiana* against two geographical populations of *Trogoderma granarium* and *Tribolium castaneum* collected from Bahawalpur and Faisalabad districts of Punjab, Pakistan in 2015-2016. Homogenous insect cultures of these populations were reared at optimum growth conditions (30±2°C and 65±5% RH). *B. bassiana* (Racer^{TM}) was applied at contraction of 0.3×10^8, 0.6×10^8 and 0.9×10^8 conidia kg^{-1} of each diet (broken wheat grains and whole grains). The 3rd instar larvae of both insects were released on fungus treated food medium and data regarding percent mortality were recorded after 7, 14 and 21 days. The results indicate that *T. castaneum* larvae of Bahawalpur district showed maximum mortality 57.35% at 0.9×10^8 conidial concentration of *B. bassiana* (Racer^{TM}) followed by 50.61% larval mortality of Faisalabad district insect population. The least larval mortality of *T. granarium* Faisalabad and Bahawalpur district was 4.47 and 4.48%, respectively at 0.3×10^8 conidia. In growth inhibition bioassays, maximum pupal and adult inhibition of *T. castaneum* of Bahawalpur strain were 57.77% and 45.97%, respectively, whereas 40.67% and 38.67% inhibition in pupal and adult emergence were recorded in case of Faisalabad strain of *T. granarium.* Overall results show that the mortality and growth inhibition of test insects were found to be directly related to contraction and exposure time.
Keywords: Concentration, percent mortality, pupal inhibition, adult inhibition, conidia.

INTRODUCTION

Grain products are generally infested by many insect pests (Haq *et al*., 2005). Losses in different stored food commodities due to insect pest infestations have been reported from 5-15% that decreases the viability, quality and commercial value of stored products (Padin *et al*., 2002; Thompson and Reddy, 2016).

The red flour beetle, *Tribolium castaneum* is considered as one of the major insect pest of stored food commodities in warehouses, processing plants, stores and flour mills (Campbell *et al*., 2003). Beside the physical damage to stored grain products, the adults of *T. castaneum* excrete carcinogenic quinones which are liable for allergic reactions in human beings (Lu *et al.*, 2010). The Khapra beetle, *Trogoderma granarium* is also an important primary insect pest of stored cereals (Ahmedani *et al*., 2007).

Synthetic insecticides (phosphine fumigant, deltamethrin, cypermethrin, malathian, etc. are being used for the control of stored grains insect pests. Use of these fumigants has become increasingly limited due to development of resistance in stored grain insect pests. Moreover, residual effects of these insecticides have also been recorded in food commodities (Arnaud *et al*., 2002; Cox, 2004).

Entomopathogenic fungi (EPF) have been found very effective control agents for the management of stored grain insect pests due to their target specific and eco-friendly nature (Roy *et al*., 2006; Patil *et al*., 2014; Saruhan *et al*., 2015; Storm *et al*., 2016). The use of naturally occurring EP fungi for the effective control of insect pests of stored grain products is of increasing interest these days (Cox *et al*., 2004; Lord, 2005; Kubilay, 2016). The EPFs have been investigated with success under both laboratory and field trials against numerous insect pest species of stored grains (Batta, 2004; Vassilakos *et al*., 2006; Sabbour and Abd El-Aziz, 2012). EPFs such as *Paecilomyces farinosus*, *Verticillium lecanii*, *Beauveria bassiana* and *Metarhizium anisopliae* have been potentially used for the control of *S. oryzae*, *T. castaneum* and other stored grain insect pests (Bello *et al*., 2001; Padin *et al*., 2001; Khashaveh *et al*., 2011; Shafighi *et al*., 2014; Kubilay *et al*., 2016). Similarly, the IMI389521 isolate of *Beauveria bassiana* isolate has been found effective against large grain borer, *Prostephanus truncatus* (Horn) (Nboyine *et al*., 2015) and *Ephestia kuehniella* (Shakarami *et al*., 2016).

So, present study was carried to check the toxic and growth inhibition potential of a *B. bassiana* formulation (Racer^{TM}) against *T. castaneum* and *T. granarium* populations of Faisalabad and Bahawalpur districts.

MATERIALS AND METHODS

***Collection and rearing of test insects*:** Populations of *Tribolium castaneum* and *Trogoderma granarium* were collected from grain markets located in Faisalabad and Bahawalpur districts of Province Punjab, Pakistan in 2015-2016. Insect culture was maintained in sterilized plastic jars (1.0 kg capacity). Broken wheat grains were used for rearing of *T. castaneum* (Imura, 1991) whereas whole wheat grains were used for rearing of *T. granarium*. After releasing the collected insects into jars, the jars were placed in cooled incubator (SANYO, MIR-254) under optimum laboratory conditions (30±2°C and 65±5% RH). Adult beetles were sieved out by sieves (60mm mesh size for *T. castaneum*, 40mm mesh size for *T. granarium*) after 5 days. Sieved wheat grains along with insect eggs were again shifted into jars (1.0 kg capacity) and placed in incubators at optimum conditions for getting homogenous population.

Bioassay 1: Entomocidal effect of *Beauveria bassiana* (Racer™) against *Tribolium castaneum* and *Trogoderma granarium*: The formulation of *B. bassiana* (Racer ™) (1 g of Racer ™ containing 10^8 conidia) was applied at three concentrations of 0.3 x 10^8, 0.6 x 10^8 and 0.9 x 10^8 conidia kg^{-1} of diet (broken and whole wheat grains) against *T. castaneum* and *T. granarium*. The sterilized food treated with each of contraction of *B. bassiana* (Racer™) was fed to thirty larvae of each of the both insects. Three replicates of 50 g from each treated commodity were taken into separate sterilized small plastic jars. Three jars with untreated food were used as control. All the treatments were kept in incubator at (30±2°C and 65±5% RH). Mortality was recorded after regular exposure periods (7, 14 and 21 days) (Bello *et al.*, 2001).

Bioassay 2: Growth regulatory effect of *Beauveria Bassiana* against *Tribolium castaneum* and *Trogoderma granarium*: The *B. bassiana* formulation was applied at three concentration of 0.3 x 10^8, 0.6 x 10^8 and 0.9 x 10^8 conidia kg^{-1} of diet. Thirty larvae of each of these two test insect pests were released in separate plastic jars. 50 g each of the treated diet was used in each plastic jar for *T. castaneum* and *T. granarium.* Data regarding growth regulation were recorded after regular intervals till the emergence of adult stage.

***Statistical Analysis*:** Abbott's formula has been applied to compute the percent corrected mortality from experimental data (Abbot, 1925) that is given below:

$$\text{Corrected mortality (\%)} = \frac{\text{Mo (\%) - Mc (\%)}}{\text{100 - Mc (\%)}} \times 100$$

Where; Mo= Observed mortality, Mc=Mortality in control treatment, For calculation of pupal or adult inhibition used the following formula:

$$\text{Pupal or adult inhibition} = 100*(1-t/c)$$

Where t= No. of pupae (in case of pupae inhibition) or adults (in case adult inhibition) in treated grains, c= is the no of pupae or adult in control treatment.

The data was analyzed statistically using Statisita-8 software following Completely Randomized Design and Tuckey-HSD test was used for multiple comparisons of means.

RESULTS

Results shows that the larval mortality of *T. castaneum* and *T. granarium* of Bahawalpur strain after 7 days (d) was 15.53 % and 14.56 % at highest concentration (0.9 x 10^8 conidia kg^{-1} of wheat) of *B. bassiana* (Racer ™) which reached 57.35% and 54.41% after 21 days of exposure (Table 1). While lowest larval mortality rates of *T. castaneum* and *T. granarium* have been recorded as 5.57% and 4.49 %, respectively, at lowest concentration (0.3 x 10^8 conidia kg^{-1} of wheat) of *B. bassiana* (Racer™) after 7 days of exposure. The mortality effect was found concentration and time dependent.

Table 1. Percent mean mortality of grubs of *Tribolium castaneum* and *Trogoderma granarium* collected from Bahawalpur using different conidial concentrations of *Beauveria bassiana* (Racer ™).

Time (Days)	Contraction (conidia/kg of wheat grains)	Mean Mortality ± SE	
		T. castaneum	*T. granarium*
7	0.3 x 10^8	5.57±1.12f	4.49±1.56f
	0.6 x 10^8	11.10±1.21ef	8.89±1.11f
	0.9 x 10^8	15.53±1.23ef	14.56±1.11f
14	0.3 x 10^8	25.56±2.11e	17.23±1.13e
	0.6 x 10^8	31.10±2.22de	26.30±1.13de
	0.9 x 10^8	35.56±2.34cd	29.71±1.13d
21	0.3 x 10^8	41.64±2.43bc	37.38±1.21c
	0.6 x 10^8	43.87±2.43b	41.52±1.23b
	0.9 x 10^8	57.35±3.11a	54.41±1.43a

Similarly, the data shows that larval mortality rates of *T. castaneum* and *T. granarium* of Faisalabad strain after 7 d have been noted as 12.21 % and 8.83 % at highest concentration (0.9 x 10^8 conidia kg^{-1} of wheat) of *B. bassiana* (Racer ™) which reached 50.61 and 46.25% after 21 days of exposure (Table 2). While lowest larval mortality rates of *T. castaneum* and *T. granarium* were recorded as 3.54% and 4.47%, respectively at lowest concentration (0.3 x 10^8 conidia kg^{-1} of wheat) of *B. bassiana* (Racer™) on wheat after 7 days of exposure. The mortality of two target insect pests was found concentration and time dependent.

***Growth inhibition description*:** Results showed that the pupal inhibition values of Bahawalpur strains of *T. castaneum* and *T. granarium* were 24.98% and 26.68% at lowest concentration (0.3 x 10^8 conidia kg^{-1} of wheat grains) of *B. bassiana* (Racer ™) which reached 56.76 % and 54.32% at highest conidial concentration (0.9 x 10^8 conidia kg^{-1} of wheat grains) (Fig. 1). The growth inhibitory effect was found concentration and time dependent.

Table 2. Percent mean mortality of grubs of *Tribolium castaneum* and *Trogoderma granarium* collected from Faisalabad using different concentrations of *Beauveria bassiana* (Racer ™).

Time (Days)	Contraction (conidia/kg of wheat grains)	Mean Mortality ± SE	
		T. castaneum	*T. granarium*
7	0.3 x 10^8	3.54±1.01g	4.47±1.28f
	0.6 x 10^8	8.89±1.01fg	5.54±1.11f
	0.9 x 10^8	12.21±1.01f	8.83±1.11f
14	0.3 x 10^8	24.43±1.11e	20.31±1.22e
	0.6 x 10^8	28.89±1.11de	24.80±1.22de
	0.9 x 10^8	32.23±1.11cd	28.17±1.22d
21	0.3 x 10^8	35.90±1.12c	35.01±0.33c
	0.6 x 10^8	41.63±1.12b	40.52±1.23b
	0.9 x 10^8	50.61±1.12a	46.25±1.23f

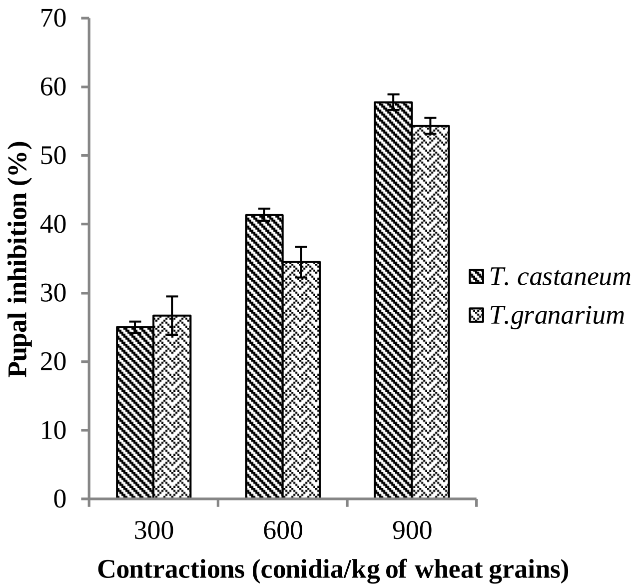

Figure 1. Pupal inhibition of *Tribolium castaneum* and *Trogoderma granarium* collected from Bahawalpur district using different concentrations of *Beauveria bassiana* (Racer ™).

In case of Faisalabad strains, it has been observed that showed that the pupal inhibition values of *T. castaneum* and *T. granarium* were 20.75% and 18.54% at lowest concentration (0.3 x 10^8 conidia kg^{-1} of wheat grains) of *B. bassiana* (Racer ™) which reached 52.87% and 47.57% at highest conidial concentration (0.9 x 10^8 conidia kg^{-1} of wheat grains (Fig. 2). The growth inhibitory relationship was directly related to conidial contraction

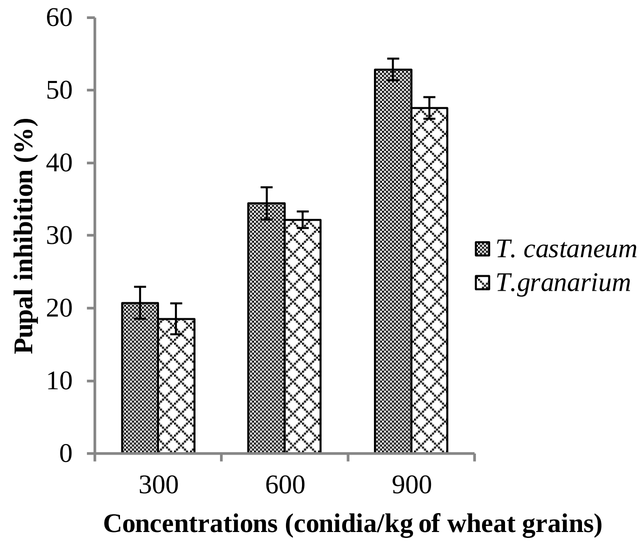

Figure 2. Pupal inhibition of *Tribolium castaneum* and *Trogoderma granarium* collected from Faisalabad district using different concentrations of *Beauveria bassiana* (Racer ™).

On the other hand, the adult emergence inhibitions of Bahawalpur strains of *T. castaneum* and *T. granarium* have been observed as 21.09% and 20.23% at highest concentration (0.3 x 10^8 conidia kg^{-1} of wheat) of *B. bassiana* (Racer ™) which reached 45.97% and 40.67% at highest conidial concentration (Fig. 3). The growth inhibitory relationship was directly related to contraction of conidia.

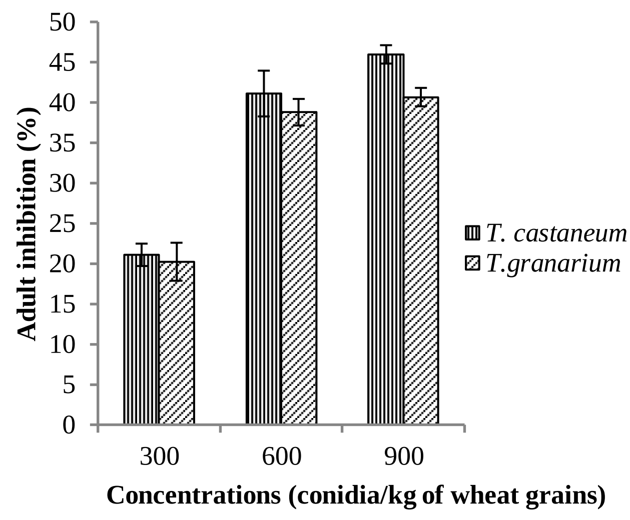

Figure 3. Adult emergence inhibition of *Tribolium castaneum* and *Trogoderma granarium* collected from Bahawalpur district using different

concentrations of *Beauveria bassiana* (Racer ™).

Results (Fig. 4) showed that the adult emergence inhibition rates of Faisalabad strains of *T. castaneum* and *T. granarium* have been noted as 19.13% and 17.19% at lowest concentration (0.3 x 10^8 conidia kg^{-1} of wheat) of *B. bassiana* (Racer ™) which reached 44.20% and 38.67% at highest conidial concentration. The growth inhibitory relationship was directly related to contraction of fungal conidia.

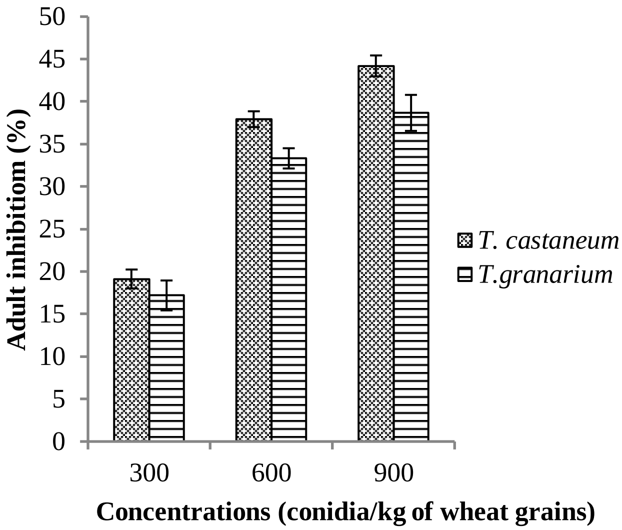

Figure 4. Inhibition of adult emergence of *Tribolium castaneum* and *Trogoderma granarium* collected from Faisalabad using different concentrations of *Beauveria bassiana* (Racer ™).

DISCUSSION

The overall results of present study revealed that the larval mortality rates of *T. castaneum* and *T. granarium* were found significantly high, and it was also found both time and concentration dependent exposed to the treatments of *B. bassiana* (Racer™). The effectiveness of *B. bassiana* (Racer™) is directly associated to the moisture content (Lord, 2007). The low mortality of these two target insect pests after the application of *B. bassiana* was due to the high temperature experienced in the current study, in agreement with Vassilakos *et al.* (2006) found higher efficacy of *B. bassiana* at 26°C as compared to 30°C. Our results were closely related with Khashaveh *et al.* (2011) who found concentration and time depended effect of *B. bassiana* for *T. castaneum*, *O. surinamens is* and *S. granarius.* The results of current study at low contraction of *B. bassiana* were found similar with Akbar *et al.* (2004) who used a formulation of *B. bassiana* (containing 9.4×10^{10} conidia per 2000 gram on adults of *T. castaneum*.

Our findings were similar with Khashaveh *et al.* (2011) who used commercially product of *B. bassiana* strain PPRI 5339 (BbWeevil™, 1 gram of product containing 2.9×10^9 conidia) and found mortality of *T. castaneum* up to 64% and around 55% at 1000 and 750 grams of commercial product of *B. bassiana*. The slight difference in findings may be due to relative concentration. The cadavers of larvae produced by an infective agent (*B. bassiana*) play a very major role in the secondary cycling of infection and boost the effectiveness and persistence of a bio-pesticide (Thomas *et al*., 1995).

Results regarding growth inhibition revealed that the main effect (conidial concentration) was found significant at P<0.001. Both of pupal and adult inhibitions were found dependent fungal conidial concentrations. The pupal inhibition was found 57.77% at highest conidial concentration. Our findings are partially in agreement with Buda and Peciulyte (2008) they found inhibition up to 50% at highest contraction of fungal conidia (2.23 x 10^8 conidia of *B. bassiana*. Similar results regarding pupal inhibition were also reported by Arooni-Hesari (2015).

Results regarding pupal inhibition were up to 52% which was similar with Quesada-Moraga *et al.* (2006) who found pupal mortality of *Ceratitis capitata* from 52-70%. Riasat *et al*. (2011) carried out a similar study but found pupal inhibition slightly higher (60.33%) at the contraction of *B. bassiana* (2.23×10^9 conidia kg^{-1} of wheat) against *R. dominica* while current study revealed pupal inhibition up to 45.97% in case of *T. castaneum*. The difference in results might be due to the usage of less contraction application, use of *B. bassiana* without surfactant (tween solution) and difference in insect species.

***Conclusion*:** The present study is novel approach involving the use of a fungal formulation and its efficacy against two different target stored grain insect pests mortality results were found to be directly related to concentration and exposure time. Maximum larval and pupal inhibition has been noticed as 57.77% and 45.97%, respectively, for *T. castaneum* larvae (Bahawalpur strain), while least 40.67% and 38.67% have been observed in case of *T. granarium* larvae (Faisalabad strain). Thus, it is concluded that the fungal species applied in this study for biocontrol will be very influential even without dilution in surfactant for integrated pest management program against local insect pest species.

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