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A Laboratory Evaluation for the Potential of Entomopathogenic Fungi against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae)

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A B S T R A C T

The red flour beetle is a very important primary insect pest of wheat worldwide. The experiments were performed to check the virulence of *Beauveria bassiana* and *Isaria fumosorosea* by insect dipping method on the adults and 2nd instar of *Tribolium castaneum*. Conidia were taken from 15 days old fungi and subsequently four concentrations i.e., 2×10^8, 3×10^8, 4×10^8 and 5×10^8 spores/ml of both fungi were prepared in 0.05% Tween 80 solution. Minimum 12.5% and maximum 32.5% mortality of adult insects was recorded on 7th day after the treatment at 2×10^8 and 5×10^8 spores/ml concentrations of *B. bassiana*, respectively while on larval stages, minimum 2.5% on 5th day and maximum 80.0% mortality was observed on 7th day post treatment of *B. bassiana*, respectively. On the other side, minimum 7.5 and maximum 22.5 mortality percentage was noted on 7th day post application of *I. fumosorosea*, respectively while on immatures minimum 5% on 6th day and highest 70% mortality was noted on 7th day post infection with 2×10^8 and 5×10^8 spores/ml of *I. fumosorosea*, correspondingly. This study showed the effectiveness of insect pathogenic fungi against the important stored grain insect pest and proved to be a positive management strategy.

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Introduction

Cereals are the utmost vital food crop for majority of the people of the world. Grain losses in their store houses because of insect damage hinders food accessibility for the people (Cogburn, 1980). Storage and maintenance of agricultural produce are very significant activities after harvesting. Substantial quantity of grains is being damaged after harvesting because of absence of adequate storing and processing services (Singh and Satapathy, 2003). Stored food products are infested by more than 670 insect pests, primarily consisting of beetles and lepidopterans thus causing quantitative and qualitative losses (Rajendran, 2002). Around 20-25% of grains are spoiled annually owing to insect pests (Rajashekar et al., 2010). In most of the developed countries like America, Canada, United Kingdom, Australia etc. there is zero thresholds for insect-pests of stored products (White, 1995; Pheloung and Macbeth, 2002).

Tribolium castaneum (Herbst.) (Coleoptera: Tenebrionidae) is a diverse insect pest of stored products and plant based processed materials. Control of this insect relies mostly on the use of chemical insecticides and fumigants. Due to the constant use of insecticides it has developed resistance against different insecticides belonging to various groups (Haliscak and Beeman 1983; Beeman and Wright 1990; Zettler and Cuperus 1990; Arthur 1992).

The use of chemicals for the protection of grains is a very old practice. Insecticides have been in use for years for efficient control of insects (Salem et al., 2007). Themassive and indiscriminate use of insecticides however has resulted in increased resistance in insect pests along with hazardous residual effects (Norman, 2000, Philips and Throne, 2010). Moreover, their detrimental effects on non-target species, handling hazards and the ecological consequences warrants further development of novel approaches in pest management (Salem et al., 2007; Mahdi and Rahman, 2008). Therefore, it is worthy to sort out natural supplements to insecticides which are readily available, cheap and less detrimental, having least mammalian toxicity (Talukder and Miyata, 2002: Udo, 2005; Phillips and Throne, 2010).

Beauveria bassiana, *Metarhizium anisopliae* and *Isaria fumosorosea* an alternate to chemical insecticides are being used to manage a number of insects of stored grains (Murad et al., 2006). The conidia of fungus cause death of insect after penetration into its cuticle. Insect pathogenic fungi are naturally occurring organisms, safer to environment and less toxic to mammals (Cox and Wilking, 1996). *B. bassiana* and *I. fumosorosea* has proved its achievement against numerous stored products insects in laboratory and field conditions (Hidalgo et al., 1998; Smith et al., 1998, Rice and Cogburn, 1999; Bourassa et al., 2001; Moore et al., 2000; Dal-Bello et al., 2001; Padin et al., 2002; Stathers, 2002; Wakefield et al., 2002; Akbar et al., 2004) and used as registered food protectants in America (Moore et al., 2000). It is also stated that *B. bassiana* is used to manage *T. castaneum*, *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (Rice and Cogburn, 1999; Padin et al., 2002). The current research was carried out to investigate the efficacy of *B. bassiana and I. fumosorosea* against *T. castaneum* on both adult and immature stages.

Materials and Methods

Tribolium castaneum were raised on sound wheat in jars (6×10 cm) under rearing conditions of 25 ± 5°C, 75 ± 5% R. H. and 16: 8 h L: D. The jars were covered with cloth for aeration. Eclosed adults and 2nd instar grubs were used in experiments. *B. bassiana* and *I. fumosorosea* were obtained from Insect Microbiology lab at Department of Entomology, Bahauddin Zakariya University and grown for two weeks on Potato Dextrose Agar (made from natural ingredients in the lab). Conidia were scrapped from 15 days old fungal plates and four concentrations i.e., 2×10^8, 3×10^8, 4×10^8 and 5×10^8 of each fungus were made in 0.05% Tween 80 solution by serially diluting the stock solution while insects were treated by dipping method.

Ten adults and immatures of *T. castaneum* per replication were treated with fungi having strengths of (2×10^8, 3×10^8, 4×10^8 and 5×10^8 spores/ml) by dipping up to five seconds. The treated insects were placed on filter paper to absorb the extra liquid. After treatment insects were transferred into individual petri dishes and provided with clean and sterilized wheat grains as food. The control groups of insects were treated with Tween 80 solution and reared under the same given conditions as described earlier. Five treatments with control were used and these treatments/concentrations were repeated four times. The data for fungal treatment was recorded at daily basis for seven days and cadavers were shifted to petri dishes with wet filter paper for fungal sporulation. The data regarding toxicity of different concentrations of two insect pathogenic fungi on *T. castaneum* was corrected by using Abbott's formula (Abbott, 1925) and data were statistically analysed by using SAS (SAS, 2002). The means for corrected mortality of adults and immatures of treated insects by application of *B. bassiana* and *I. fumosorosea* were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The pathogenicity of *B. bassiana* was evaluated against adults of *T. castaneum* which displayed the mortality on the third day after infection. Mortality of *T. castaneum* infected with *B. bassiana* was concentration dependent which augmented with the application of higher concentrations. Minimum 12.5% and maximum 32.5% mortality was noted on 7th day after the application with 2×10^8 and 5×10^8 spores/ml fungus, respectively (Figure 1). The results showed that *B. bassiana* at these concentrations was not effective to control *T. castaneum*. On the other hand, the virulence of *I. fumosorosea* was evaluated against *T. castaneum* adults which showed similar results as that of treatment with *B. bassiana*. Cumulative mean percent mortality of *T. castaneum* treated with *I. fumosorosea* amplified with the increase in concentrations. Minimum i.e., 7.5% mortality and maximum 22.5 % mortality was noted on 7th day after infection with concentrations 2×10^8 and 5×10^8 of *I. fumosorosea*, correspondingly (Figure 2). The infection with *B. bassiana* on 2nd instar immatures exhibited mortality on the third day at concentrations of 4×10^8 and 5×10^8 spores/ml. Minimum 2.5% mortality on 5th day and maximum mortality percentage i.e., 80.0 was noted on 7th day after the infection with 2×10^8 and 5×10^8 spores/ml of *B. bassiana*, correspondingly (Figure 3). The LC_{50} values on the 2nd instar *T. castaneum* were 4.36×10^8 and 3.31×10^8 spores/ml on 6th and 7th day post infection (Table 1). Contrary, lethal time LT_{50} was 6.41 and 5.09 days after the application of 4×10^8 and 5×10^8 spores/ml (Table 2).

Table 1. Lethal doses (LD_{50} and LD_{90} values of *Beauveria bassiana* and *Isaria fumosorosea* against 2nd instar grubs of *Tribolium castaneum*

Fungi	Days	LD_{50}	F.D (Fiducial limits)	Slope	D.F
Beauveria bassiana	6th	4.36×10^8	3.68×10^8-5.18×10^8	4.88±1.27	2
	7th	3.31×10^8	2.75×10^8-3.99×10^8	3.57±1.03	2
Isaria fumosorosea	7th	4.18×10^8	3.26×10^8-5.38×10^8	3.10±1.05	2

Table 2. Lethal times (LT_{50} and LT_{90}) values of *Beauveria bassiana* and *Isaria fumosorosea* against 2nd instar grubs of *Tribolium castaneum*

Fungi	Concentration	LT_{50}	F.D (Fiducial limits)	Slope	D.F
Beauveria bassiana	4×10^8	6.41	5.18-7.93	3.57±0.81	5
	5×10^8	5.09	4.42-5.86	4.26±0.80	5
Isaria fumosorosea	4×10^8	7.50	5.88-9.69	3.96±1.02	5
	5×10^8	6.58	5.41-8.00	4.16±0.96	5

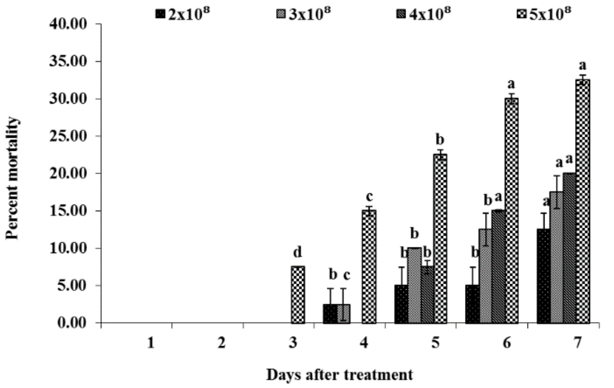

Figure 1. Percent mortality of *Tribolium castaneum* adults after infection with *Beauveria bassiana*.
(For each day the similar alphabets are not statistically different ($P<0.05$) according to DMRT)

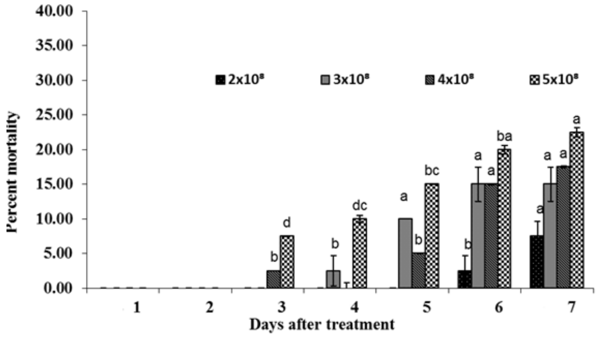

Figure 2. Percent mortality of *Tribolium castaneum* adults after infection with *Isaria fumosorosea*.
(For each day the similar alphabets are not statistically different ($P<0.05$) according to DMRT)

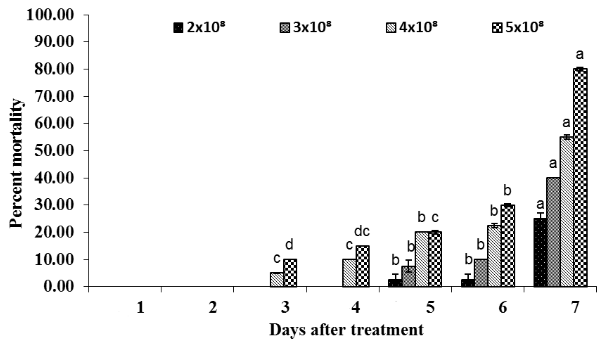

Figure 3. Percent mortality of *Tribolium castaneum* immatures after infection with *Beauveria bassiana*.
(For each day the similar alphabets are not statistically different ($P<0.05$) according to DMRT)

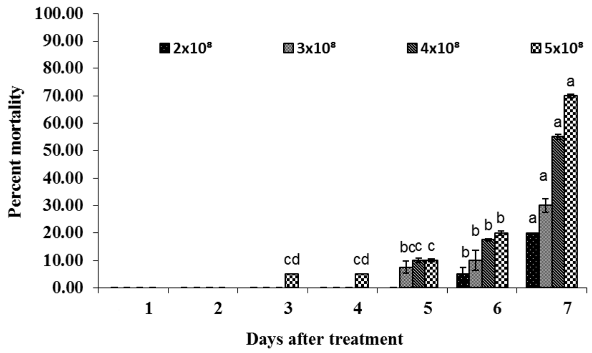

Figure 4. Percent mortality of *Tribolium castaneum* immatures after infection with *Isaria fumosorosea*.
(For each day the similar alphabets are not statistically different ($P<0.05$) according to DMRT)

The application of *I. fumosorosea* on 2nd instar exhibited mortality on the third day after infection with 5×10^8 spores/ml concentration. Over all mean percent mortality of 2nd instar grubs of *T. castaneum* positively correlated with the increase of dose of *I. fumosorosea*. Minimum 5.0 and maximum 70.0 % mortality was noted on 6th and 7th day after infection with 2×10^8 and 5×10^8 spores/ml (Figure 4). The *I. fumosorosea* LC_{50} on the 2nd instar *T. castaneum* was 4.18×10^8 on 7th day (Table 1). On the other hand, LT_{50} was 7.5 days after treatment with 4×10^8 spores/ml concentration. Conversely, it was decreased to 6.58 days after infection with 5×10^8 spores/ml concentration (Table 2).

Our results clearly showed that with increasing concentration of insect pathogenic fungi produces higher mortalities in 2nd instars grubs of *T. castaneum* on wheat which is also supported with the results of Athanassiou and Steenberg (2007), who applied *B. bassiana* with diatomaceous earth against *Sitophilus granaries*. In other study Michalaki et al. (2006), checked *M. anisopliae* against *T. confusum* and results showed that mortality was increased by using high dose rate up to 8×10^{10} conidia /kg with wheat also supports our results which showed mortality up to 70 and 80 % in 2nd instars grubs by using concentration of 5×10^8 of *I. fumosorosea* and *B. bassiana*, respectively.

Our findings explained that the mortality of *T. castaneum* was concentration dependent and it augmented as concentrations increased but not very high in adults of this insect. The findings of current study are in conformity with the results of Akbar et al. (2004), who showed that *T. castaneum* displayed insufficient infectivity to *B. bassiana*. This supports our results on adults where maximum 32.5 and 22.5% mortality was recorded on adults of *T. castaneum* by using concentrations of *B. bassiana* and *I. fumosorosea.*

Fungi have the potency to be used on commercial basis as microbial control as friendly pest mangement (Throne and Lord, 2004). The findings of the current experiment clearly depicted that mortality was low up to 32.5 and 22.5% by using higher concentrations 5×10^8 of *B. bassiana* and *I. fumosorosea*, respectively as reported by Rice and Cogburn 1999, Padin et al. 2002 on the adults of *T. castaneum*. Nevertheless, still lower concentration of *B. bassiana* has revealed to have a reasonably improved control on other insects such as *R. dominica* etc. (Lord 2001).

Conclusion: The study showed the potential of insect pathogenic fungi for the management of stored grain insect pests. Both insect pathogenic fungi have potential against *T. castaneum* and should be mass produced and used on commercial scale.

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