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

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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 hand, 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.

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).
**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. The infection with *B. bassiana* and *I. fumosorosea* 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 25% 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 LC50 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 LT50 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 (LD50 and LD90) values of *Beauveria bassiana* and *Isaria fumosorosea* against 2nd instar grubs of *Tribolium castaneum*

| Fungi               | Days | LD50 (Fidalucial 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 (LT50 and LT90) values of *Beauveria bassiana* and *Isaria fumosorosea* against 2nd instar grubs of *Tribolium castaneum*

| Fungi               | Concentration | LT50 | F.D (Fidalucial 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   |
The application of *I. fumosorosea* on 2nd instar exhibited mortality on the third day after infection with 5×10^6 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 2×10^6 and 5×10^6 spores/ml (Figure 4). The *I. fumosorosea* L.C50 on the 2nd instar *T. castaneum* was 4.18×10^6 on 7th day (Table 1). On the other hand, L.T50 was 7.5 days after treatment with 4×10^6 spores/ml concentration. Conversely, it was decreased to 6.58 days after infection with 5×10^6 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 granarius*. 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 8×10^11 conidia/kg with wheat also supports our results which showed mortality up to 70 and 80 % in 2nd instars grubs by using concentration of 5×10^6 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 management (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 5×10^6 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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