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A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae)

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A B S T R A C T

Corcyra cephalonica Stainton (Lepidoptera: Pyralidae) is a wide spread pest of stored grains and responsible for considerable damage in godowns and warehouses. *Beauveria bassiana* (Balsamo) Vuillemin, the most common and ubiquitous fungal entomopathogen is known to have high potential for the control of insects belonging to various orders. To evaluate the pathogenicity of *B. bassiana* against *C. cephalonica*, sorghum grains were treated with three conidial concentrations of *B. bassiana* i.e. 1.49×10^5, 4.05×10^6, 2.02×10^8 spores/ml. The highest concentration was found to be the most effective. It induced significantly higher larval mortality. Adverse effects such as reduced adult emergence and longevity of *C. cephalonica* were also observed when the grains were treated with *B. bassiana*. Significantly lesser number of adults emerged from pupae, when shorghum grains were treated with 2.02×10^8 spores/ml. The adverse effects were also exhibited as malformed adults that lived for a short period. Overall study showed that *B. bassiana* gave effective control of *C. cephalonica* and thus may play a pivotal role in the integrated pest management program.

1. Introduction

Insect pests have been damaging and causing heavy losses to stored grains quantitatively and qualitatively throughout the world especially in tropical and semitropical countries (Madrid et al., 1990; Tripathi et al., 2009). In countries without modern storage technologies, insect pests cause 10–40% damage in stored grains (Shaaya et al., 1991).Traditionally, fumigants and other synthetic insecticides have been commonly used for the protection of stored products from attack by storage insects. However, the continuous use of these insecticides has led to the development of resistance in insect pests as well as residual problem in stored grains (Michalaki et al., 2006). These adverse effects of synthetic insecticide application have increased public awareness regarding the human safety and possible environmental damage and focused the research towards other alternative strategies for the management of stored grain insect pests. The use of entomopathogenic fungi (EPF) and other microbial control agents is known to be a promising strategy with good potential to minimize the adverse effects of insecticides (Padin et al., 1997). The fungal biocontrol agents can be used in close proximity to foods and feed (Cox et al., 2003, 2004). EPF can be employed to treat empty stores to control residual pests before the new harvest is brought in or may be applied as direct admixture of conidia to grains either as preventive or curative treatments to bulk grains provided it does not decrease the quality and marketability of grains (Steenberg, 2005). Out of 700 entomopathogenic species of fungi reported from insects, only10 have been widely known as biocontrol agents (Hajek and St. Leger, 1994). Among these, one of the most frequently used fungi is *Beauveria bassiana* (Balsamo) Vuillemin. Its potential as a mycoinsecticide has been demonstrated on a large number of different insect hosts (Inglis et al., 1993; Feng et al., 1994; Padin et al., 1995; Cherry et al., 2005). *Beauveria bassiana* has potential for the control of different stored product pests. It is registered by the U.S. Environmental Protection Agency (EPA) for a wide range of insect control applications (Lord, 2001). Most of the research has been done on formulations of this fungus for the management of stored product insect pests (Akbar et al., 2004; Lord, 2007; Vassilakos et al., 2006). *Beauveria bassiana* has been demonstrated to control *Rhyzopertha dominica* (Fabricius) (Mahdneshin et al., 2009; Wakil et al., 2011), *Callosobruchus maculatus* (Fab.) and *Sitophilus granarius* (Linnaeus) (Shams et al., 2011), *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.) (Khashaveh et al., 2011). Several *B. bassiana*

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formulations (Boverosil, Mycotrol, ES, Mycotrol 22WP etc.) are commercially available and are registered for use in storage facilities (Khashaveh et al., 2011).

Corcyra cephalonica is a serious pest of some important stored food commodities such as rice, wheat, maize, sorghum, groundnut, cotton seeds, coffee, spices and cocoa beans, especially in the tropics (Cox et al., 1981; Allotey and Kumar, 1985; Allotey, 1991). The larvae of *C. cephalonica* cause considerable damage to stored food commodities while feeding, leaving silken threads wherever they move. The present study was carried out to evaluate the potential of *B. bassiana* as a biological control agent against *C. cephalonica*.

2. Materials and methods

This experiment with *B. bassiana* as a biocontrol agent against *C. cephalonica* was done at the Biocontrol Laboratory, Department of Zoology, Guru Nanak Dev University, Amritsar (Punjab), India. The adults of *C. cephalonica* were collected from stored wheat in the godowns. Then the culture was maintained at 25 ± 2 °C and 65 ± 5% RH in the laboratory on sorghum grains which is one of its preferred host (Muthukrishnan et al., 1996). Rearing was carried out on partially crushed sorghum grains in battery jars (15 cm $\times$ 10 cm) covered with muslin cloth to ensure proper ventilation. Ten adults in the ratio of 4:6 (M: F) were released in each battery jars half filled with crushed sorghum. After oviposition the dead adults were removed and jars were kept undisturbed for further development of *C. cephalonica*. From this culture 2nd instar larvae were used for experimental purpose.

Cultures of the fungus *B. bassiana* (NBAII-Bb-5a) were procured from National Bureau of Agriculturally Important Insects (NBAII), Bangalore and further maintained in the laboratory on Potato Dextrose Agar (PDA) at 30 °C. Three weeks old culture was used for experimental studies. Fungal suspension was prepared by adding 10 ml of distilled water and a drop of 0.01% Tween 80 solution to the slants and filtered through muslin cloth to remove mycelia. From this concentrated spore suspension, serial dilutions were made and spore count was obtained with the help of hemocytometer. Three concentrations i.e. C 1 = 1.49×10^5, C 2 = 4.05×10^6 and C 3 = 2.02×10^8 spores/ml were used for bioassay studies.

The bioassay studies are described as follows. Thirty ml of each spore suspension was sprayed on 120 g of partially crushed sorghum grains with the help of hand sprayer followed by thorough mixing. In control, grains were sprayed with same quantity of water having a drop of 0.01% Tween 80 solution. After air drying the treated grains were equally distributed in 6 plastic containers (4 cm $\times$ 6 cm) i.e. 20 g/container. Ten 2nd instar larvae were released in each container, which represents one replication. Similarly larvae were released in containers having untreated grains. The larvae were checked daily for larval mortality. Observations were also recorded on larval period, adult emergence, adult longevity and deformity in all the treatments and control. To verify that the larval death is due to fungus, the dead larvae were surface sterilized with 5% sodium hypochlorite and placed in autoclaved petri dishes having moist muslin cloth at the base. The petri dishes were kept in B.O.D. incubator at 25 ± 2 °C temperature. Observations were made on the mycelial growth of fungus on the dead larvae. The larval counts showing fungal growth were only considered for percent mortality due to fungus.

All the experiments were replicated six times with 10 larvae per replication and values were represented as their mean ± SE. To compare difference in the means, one way analysis of variance (ANOVA) with Tukey's test at $P \leq 0.05$ was performed. SPSS software for windows version 16.0 (SPSS Inc., Chicago) and Microsoft office Excel 2007 (Microsoft Corp., USA) were used to perform the statistical analysis.

3. Results

There was a significant difference in the mortality of *C. cephalonica* larvae when the highest concentration i.e. 2.02×10^8 spores/ml of *B. bassiana* was applied on sorghum grains ($F = 11.46$, $P < 0.001$). Relative to control six times higher mortality was recorded at the highest concentration. However, the lower doses of fungus (4.05×10^6 and 1.49×10^5 spores/ml) failed to induce significant mortality in the larvae feeding on treated grains as compared to control (Fig. 1a). The larval mortality started after 24 h of feeding on grains treated with the highest concentration whereas the first larval death was recorded after 48–72 h of exposure at lower doses. After death, the larvae became hard and stiff. The white mycelial growth appeared 24 h after death at the highest concentration, but at lower concentrations it took 2–3 days to grow on dead larvae (Fig. 2a–c). Larval mortality was also recorded on untreated grains. However, no fungal growth was observed on larvae died in control.

Treatment of sorghum grains significantly delayed the larval development of *C. cephalonica* as compared to control ($F = 62.50$, $P < 0.001$). The larvae took more time to pupate (6.79 days) when reared on grains treated with 2.02×10^8 spores/ml. Similarly the lower concentrations i.e. 4.05×10^6 and 1.49×10^5 spores/ml also significantly extended the development of larvae (Fig. 1b). Lesser number of adults emerged from pupae developed on treated sorghum as compared to control, but significant effect was observed only at 2.02×10^8 spores/ml of the fungus ($F = 11.46$, $P < 0.001$) (Fig. 3a). Longevity of adult male decreased significantly at all the concentrations of *B. bassiana* ($F = 23.00$, $P < 0.001$) (Fig. 3b). Significant influence of the fungus was also observed on female longevity at 4.05×10^6 and 2.02×10^8 spores/ml concentrations ($F = 9.77$, $P < 0.001$) (Fig. 3c).

Sublethal effects of *B. bassiana* on *C. cephalonica* were also manifested as emergence of deformed adults. As is evident from Fig. 3d, treatment of grains with 2.02×10^8 spores/ml of fungus induced morphological deformities in significantly higher number of adults (16.66%) as compared to adults emerged from larvae reared on untreated grains. The wings of these insects were underdeveloped and curled (Fig. 4a–b) as compared to normal adults (Fig. 4c). These malformed adults lived for only 1–2 days as compared to 5–6 days for normal adults.

4. Discussion

Efficiency of biocontrol of insect pests with entomopathogenic fungi is mainly due to their capacity to produce epizootics from the infected cadavers (Leathers et al., 1993). These cadavers act as a source of inocula and thus further disseminate microbial agent in the environment (Gottwald and Tedders, 1982; Lacey et al., 1994). Earlier Samodra and Ibrahim (2006) evaluated the dried conidia of eight isolates of entomopathogenic fungi against *C. cephalonica* in rice grains. Among these two isolates of *B. bassiana* (BbGc and BbPs) and one isolate of *Metarhizium anisopliae* (MaPs) gave high mortality to *C. cephalonica* larvae. Isolates of BbGc and BbPs formulated in kaolin and BbPs formulation in tapioca flour provided 100% larval mortality by 15th day of introduction. Even more than 90% mortality was recorded when rice grains were stored for 4 months. However, the pathogenicity of *M. anisopliae* against *C. cephalonica* larvae decreased rapidly upon storage at room temperature affording less than 60% control by the 2nd month of storage. Adane et al. (1996) also reported 88% mortality in *Sitophilus zeamais* (Motschulsky) due to treatment with *B. bassiana* at 10^4 conidia/ml within 8 days. There are reports indicating the effectiveness of *B. bassiana* and *M. anisopliae* against many coleopteran pests of stored grains (Mahdneshin et al., 2009; Khashaveh et al., 2011).