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Effect of passing *Beauveria bassiana* through alkane based media on the adult mortalities of *Rhyzopertha dominica* and *Sitophilus oryzae*

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Abstract

Entomopathogenic fungi have been investigated for management of stored product pests as alternatives to chemical control. *Beauveria bassiana* is commonly considered and thus increasing its efficacy has also been studied. The purpose of this study is to evaluate the effect of passing two *B. bassiana* cultures (wild and single-spore cultures) through n-hexadecane and n-octocasane based media on *Rhyzopertha dominica* and *Sitophilus oryzae* adult mortalities. For each Petri plate, 2 ml of 10% alkane was spread, let to evaporate and fungus was inoculated. After sporulation, spores for pathogenicity tests were produced by solid fermentation method on rice. Pathogenicity tests were conducted by application of 500 ppm (w/w) spores in wheat on 20 adults at 25±2°C, 65±5 r.h. in darkness with five replications. The efficacy of wild culture towards *R. dominica* adults was enhanced in both treatments. Mortality in 7 days increased from 35% to 55 and 69% when n-hexadecane and n-octocasane were used, respectively. Similarly, these treatments increased 14-day mortalities from 65% to 77 and 87%, respectively. Treatment of single-spore culture, however, either showed no change or reduced mortality. Passing both cultures through both alkane based media did not statistically affect the activity against *S. oryzae*. This study illustrated that increasing the virulence of *B. bassiana* is possible for *R. dominica* and increase depends both on the starting fungus culture and alkane used. Starting with a wild fungus culture with a wider genetic diversity, and using n-octocasane can produce a better enhancement.

Keywords: microbial control, biological control, virulence, entomopathogen.

1. Introduction

Cereals are produced throughout the world as nutrition for both humans and livestock. These commodities generally require storage for at least a short time and need to be protected against

insect and mite pests. Unprotected stored grains usually lead to quantitative and qualitative loss of grain and reduction of seed germination (Moino et al., 1998; Padin et al., 2002; Haq et al., 2005; Stejskal et al., 2015). Although synthetic insecticides have been used to control stored product pest populations (Athanassiou & Palyvos, 2006), they have various negative consequences such as residue accumulation in products (Ferizli et al., 2005), hazardous effects to humans and the environment (Michalaki et al., 2007), and pest resistance (Arthur, 1996). Therefore, there have been increasing efforts to find environmentally friendly and nontoxic ways to control these pests. Entomopathogenic fungi have been one of the considered alternatives (Moino et al., 1998; Michalaki et al., 2007; Sewify et al., 2014; Wakil & Schmitt, 2014) because they are natural and safer for humans and the environment (Moore et al., 2000). Bioinsecticide potential of entomopathogenic fungi against various insect pests of stored products have been established with a number of studies (Cherry et al., 2005; Wakil & Ghazanfar, 2010; Shams et al., 2011; Barra et al., 2013; Khashaveh & Chelav, 2013; Sewify et al., 2014). They have also been considered potential in combination with diatomaceous earth (Athanassiou & Steenberg, 2007; Athanassiou et al., 2008; Wakil et al., 2011; Riasat et al., 2011, 2013; Shafighi et al., 2014). Enhancing the pathogenicity of a potential fungal isolate would increase its value as biocontrol agent and has been the subject of many studies (Ortiz-Urquiza et al., 2015). One way of doing this is the modification of culturing media by using alkanes as carbon source (Crespo et al., 2002; Pedrini et al., 2011; Barra et al., 2015). In this study, the effect of passing two *B. bassiana* cultures (wild and single-spore cultures) through n-hexadecane and n-octocasane based media was tested to increase their efficacy against *Rhyzopertha dominica* and *Sitophilus oryzae* adults.

Materials and Methods

Insect cultures

Rhyzopertha dominica and *Sitophius oryzae* cultures have been maintained in our laboratory. Starting insects had been originally obtained from surrounding storage facilities. Durum wheat with 12% moisture content was used for the cultures. Glass jars of 1 Lt capacity with 250 gr of wheat were used. Adults of mixed sex were placed into the jars and kept for three days for oviposition. After removing the adults, the cultures were incubated for the emergence of new generation adults. One week old adults were used for the bioassays. All the cultures were maintained at 26±2 ºC and 65±5% relative humidity in darkness.

Fungus cultures and spore production

In the study, two *B. bassiana* cultures were used; one wild culture (151138) and another one that was obtained after single-spore selection (5-4) (Er et al., 2016). The fungi were grown on potato dextrose agar and their spores were suspended in %0.02 Tween 80. After determination of concentration by using Neubauer hemacytometer, spore concentration was adjusted to 10^6 spores/ml by dilution. 200 µl of spore suspension was spread on deficient media agar (DMA) containing alkane (Crespo et al., 2002). 10% n-hexadecane and 10% n-octacosane were prepared using hexane and 2 ml of required alkane was spread on DMA and evaporated prior to spore inoculation. In order to see any effect of the solvent hexane, DMA with only hexane was also tested. These cultures were kept at 25±2ºC for 14 days and grown fungi were used for spore production following mass production procedure described by Barış (2016). 100 g of rice was soaked overnight with tap water and the excess water was drained. The rice supplemented with 1.5 gr of $CaSO_4$ and $CaCO_3$ was sterilized in a polyethylene bag (25 cm x 38 cm). After cooling, it was inoculated with 10 ml of spore suspension ($2x10^7$ spores/ml) and sealed. Following fungal growth at 25±2°C, 12/12 photoperiod for 14 days the culture was dried at 25±2°C. Spores were separated from the substrate by using a 500 µm sieve.

Pathogenicity tests

Centrifuge tubes of 50 ml capacity each with 40 g of wheat were used for the tests. Wheat in each tube was mixed with 20 mg of spores producing a final concentration of 500 ppm (w/w) by shaking for 5 minutes. Twenty adults were released in each tube and kept at 25±2ºC, 65±5% relative humidity in constant darkness. Wheat kernels without spores were used as control. The experiment had five replicates.

Results

The efficacy of wild culture (151138) of *B. bassiana* towards *R. dominica* adults was enhanced when the fungus was passed through both n-hexadecane and n-octocasane based media. Mortality in 7 days increased from 35% to 55 and 69% when n-hexadecane and n-octocasane were used, respectively. Similarly, these treatments increased 14-day mortalities from 65% to 77 and 87%, respectively. Treatment of single-spore culture (5-4) of *B. bassiana*, however, either showed no change or reduced mortality. Passing both *B. bassiana* cultures through both alkane based media did not statistically affect their efficacies against *S. oryzae* adults. Using hexane alone did not change the effects of the *B. bassiana* to either of the species.

Discussion

This study illustrated that increasing the virulence of *B. bassiana* against *R. dominica* adults is possible by passing the fungus through media having n-hexadecane or n-octocasane as carbon source. Increase in mortality was reported by Crespo et al. (2002), Pedrini et al. (2011) and Barra et al. (2015) when fungi were grown on media containing these alkanes. In the case of *S. oryzae*, this procedure did not enhance the efficacy. This may be due to differences in the cuticular components of two insect species. In the previous studies, host insects were treated with spores that had been harvested directly from media with alkanes. However, in the present study the fungi were passed through media with alkanes and then cultured by mass production procedure using rice as substrate. Therefore, the results of this study indicate that the increase in virulence against *R. dominica* adults was due to a selection of fungi that can use n-hexadecane or n-octocasane as carbon source. This was also supported by the mortality levels caused when single-spore culture (5-4) of *B. bassiana* was used after passing through the alkanes. Starting with a wild fungus culture with a wider genetic diversity, and using n-octocasane can produce a better enhancement.

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Bio-nanosilver synthesized by the entomopathogenic nematode-symbiotic bacterium as bio-insecticide for the red flour beetle (*Tribolium castaneum)*

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Abstract

Biological control can be another important way to manage post-harvest insect pests. Some organisms that showed biological control activity against some soil pests are insect-parasitic nematodes. There are two different species of nematodes, steinernematids and heterorhabditids, who carry within their bodies insect-pathogenic bacteria. *Xenorhabdus* spp are bacteria which infest steinernematids and *Photorhabdus* spp. bacteria infect