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Naturally existing *Beauveria* on the surface of stored wheat kernels, and their pathogenicity on *Rhyzopertha dominica* and *Sitophilus oryzae* adults

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Abstract

Entomopathogenic fungi have been investigated to control stored product pests, as an alternative strategy to chemical insecticides. Although many studies evaluated isolates from various sources, few studies surveyed fungi naturally infecting stored product pests, revealing predominantly *Beauveria* isolates. This study aimed to reveal the amount of *Beauveria* carried on the surface of stored wheat kernels, and their pathogenicity against *Rhyzopertha dominica* and *Sitophilus oryzae* adults. Sixteen wheat samples from different storage facilities in four cities were examined for existence of *Beauveria*. One-hundred g of wheat was washed in 100 mL of 2% Tween80 solution. After increasing concentration of possible fungi by centrifugation, the liquid was spread on medium with dodine and monitored at 25±2°C. Nine of the isolates were tested for pathogenicity at 500 ppm (w/w) at 25±2°C, 65±5% r.h. in darkness with five replicates. While only four samples did not have *Beauveria*, others had 17-2992 cfu/100 g wheat. Six samples had 17-50, four samples 150-858, one sample 1625 and one had 2992 cfu/100 g wheat. Mortalities against *R. dominica* adults ranged between 5-86% and 32-100% in 7 and 14 days, respectively. Mortality of *S. oryzae* ranged from 3-45% and 8-83% in 7 and 14 days, respectively. This study demonstrated that wheat kernels can naturally carry *Beauveria* with various levels of pathogenicity. Potential naturally occurring entomopathogenic fungi can be isolated directly from stored commodities to be evaluated as biological control agents for stored product pest control.

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

1. Introduction

Cereals are important for human consumption and livestock in the world. After harvesting they are usually stored for various lengths of time. During storage, they need to be protected against insect and mite pests. Unless suppressed, the populations of these pests cause reduction in the weight and value as well as decline of seed germination (Moino et al., 1998; Padin et al., 2002; Haq et al., 2005; Stejskal et al., 2015). The use of synthetic insecticides to suppress pest populations has been commonly practiced (Athanassiou & Palyvos, 2006); however, its negative effects such as pest resistance to the chemicals (Arthur, 1996), residue accumulation in grains (Ferizli et al., 2005), and

detrimental effects on humans and the environment (Michalaki et al., 2007) have directed researchers to seek nontoxic and environmentally friendly methods to suppress stored product pests. One alternative that has been considered is the use of entomopathogenic fungi (Moino et al., 1998; Michalaki et al., 2007; Sewify et al., 2014; Wakil & Schmitt, 2014) due to their organic nature and low hazard to human and the environment (Moore et al., 2000). The potential of entomopathogenic fungi as bioinsecticides against insect pests of stored products has been reported in many literature (Cherry et al., 2005; Wakil & Ghazanfar, 2010; Shams et al., 2011; Barra et al., 2013; Khashaveh & Chelav, 2013; Sewify et al., 2014). The potential of entomopathogenic fungi in combination with diatomaceous earth has also been reported (Athanassiou & Steenberg, 2007; Athanassiou et al., 2008; Wakil et al., 2011; Riasat et al., 2011, 2013; Shafighi et al., 2014). There are few studies investigated the natural occurrence of entomopathogenic fungi in stored product pests (Odour et al., 2000; Wakefield et al., 2005; Wakil et al., 2014; Er et al., 2016). Most commonly, *B. bassiana* has been found and most of the literature evaluated this species as a biocontrol agent. In this study wheat kernels are examined for existence of *Beauveria* as source of inoculum and the pathogenicity of obtained isolates was tested against adults of two coleopteran pests of stored grains, *Rhyzopertha dominica* and *Sitophilus oryzae*.

2. Materials and Methods

Insect cultures

Rhyzopertha dominica and *Sitophilus 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 L capacity with 250 g 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.

Wheat samples

Sixteen wheat samples from different storage facilities in four cities in Turkey were taken as described in Er et al. (2016) and examined for existence of *Beauveria*. Eight samples were from Osmaniye, 5 from Hatay, 2 from Adana and one from Kahramanmaraş.

Processing samples and isolation of fungi

Each sample is mixed and 100 g of it was used for fungus isolation. Wheat kernels were washed by placing in 100 mL of 2% Tween 80 solution and agitating. After removing the kernels, particles in the liquid were sedimented in two 50 mL capacity tubes by using a fixed angle centrifuge at 8000 g for 10 mins. Sediments in both tubes were combined and filled to 5 mL. After increasing concentration of possible fungi by centrifugation, 200 µL of the liquid was spread on oat meal agar supplemented with 650 µL/L dodine and 1% streptomycin + penicillin. The experiment was conducted with three replicates. Growth of fungi was monitored at 25±2ºC and number of *Beauveria* colonies in each petri dish was recorded. Selected *Beauveria* samples are subcultured and purified on potato dextrose agar at the same conditions. Nine *Beauveria* isolates were used for spore production following mass production procedure described by Barış (2016). One-hundred g of rice was soaked overnight with tap water and the excess water was drained. The rice supplemented with 1.5 g 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 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 darkness. Wheat kernels without spores were used as control. The experiment had five replicates.

3. Results

Among the 16 samples, 12 had *Beauveria* colonies in at least one of the Petri plates, while only four samples did not have *Beauveria* colonies. The mean number varied between 17 and 2992 cfu/100 g wheat. Six samples had 17-50, four samples 150-858, one sample 1625 and one had 2992 cfu/100 g wheat. When these fungi were tested, *R. dominica* adult mortalities ranged between 5-86% and 32-100% in 7 and 14 days, respectively. *S. oryzae* mortalities were lower and recorded as 3-45% and 8-83% in 7 and 14 days, respectively.

4. Discussion

This study demonstrated that stored wheat kernels can naturally carry *Beauveria* at various levels, but in rather low concentrations. These results support the findings of previous survey studies for natural fungal infections on stored product pests (Odour et al., 2000; Wakefield et al., 2005; Wakil et al., 2014; Er et al., 2016). In all these studies, a low percentage of insects were found to be naturally infected by *Beauveria*. The reason could be the existence of low *Beauveria* inoculum in their habitats as shown in this study.

Testing the isolates in the study resulted in various levels of mortalities in both insect species. Such variation in the pathogenicity of isolates within the same species was commonly reported (Moino et al., 1998; Kassa et al., 2002; Wakefield et al., 2005; Sewify et al., 2014). The efficacy of the isolates against *S. oryzae* adults was lower than that against *R. dominica* adults. Similarly, Moino et al. (1998) and Sewify et al. (2014) found that *R. dominica* is more susceptible than *S. oryzae*. All the findings together suggest that diversity of *Beauveria* isolates obtained in this study is similar to those reported earlier in terms of their pathogenicities. Therefore, naturally occurring potential entomopathogenic fungi can be isolated directly from stored commodities to be evaluated as biological control agents for stored product pest control.

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Pulses Protein Quality Control at Different Storage Conditions for Further Protein Extraction – A Review

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Abstract

The storage conditions are of extreme importance with regards to grains (cereal & pulses) components (carbohydrates, lipids, proteins) preservation and quality for industry (that may interfere to whole process and quality of the final product). In addition, the vegetarian consumers' interest of protein supplement (capsules)