Journal of Stored Products Research 77 (2018) 205–210

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Rhyzopertha dominica adult mortalities after exposure to indigenous *Beauveria bassiana* isolates from stored-grain pests: Effects of certain factors in sampling process

Mehmet Kubilay Er [a, *], Ali A. Işikber [a], Fadime Aydin [b], Ceyda Ücük [b], Hasan Tunaz [a]

[a] Department of Plant Protection, Faculty of Agriculture, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey
[b] Department of Plant Protection, Graduate School of Natural and Applied Sciences, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey

ARTICLE INFO

Article history:
Received 28 November 2017
Received in revised form
17 April 2018
Accepted 1 May 2018

Keywords:
Stored-product pests
Biological control
Microbial control
Coleoptera
Hypocreales

ABSTRACT

In search for potential entomopathogenic fungi for microbial control of *Rhyzopertha dominica*, 75 *Beauveria bassiana* isolates previously recovered from stored-grain insects sampled in Adana and Mersin provinces of Turkey were tested and the mortality levels were used to evaluate possible effects of three sampling factors on encountering potential isolates. In the bioassays, twenty adults were exposed to 1000 ppm spores mixed into wheat kernels and adult mortalities after 14 days were used for statistical analysis. The relations between mortalities and 1) species of host insects from which isolates were obtained, 2) collection season −summer or autumn− of host insects from storage facilities, and 3) time of host insect's death −before or after collection− were evaluated. Considerable variations in mortalities were detected (13.67%–100%) depending on isolates. Amongst twelve Adana isolates, isolate 155657 caused the highest mortality. Eleven Mersin isolates were found to be effective against *R. dominica*. There was no correlation between *R. dominica* mortality and insect species from which isolates were obtained. Statistically, the frequency of potential isolates was higher in Adana samples collected in summer months compared to that in autumn months; however, sampling season did not have a significant effect in Mersin. The frequency of potential *B. bassiana* isolates was significantly higher when isolation was made from hosts that died in laboratory after sample collection compared to those isolated from hosts already dead at the time of collection. This study demonstrated that stored-grain insects are a good source of finding various *B. bassiana* isolates with different efficacy levels. At least for stored-grain insects, specifically sampling targeted species does not significantly increase the chance of finding highly potential isolates. However, retaining alive sampled insects can increase the likelihood of encountering isolates with high efficacy.

1. Introduction

Due to various shortcomings of the use of synthetic insecticides, entomopathogenic fungi have been under investigation as alternatives and/or complementary agents to suppress pest insects. Although the development of insecticides based on entomopathogenic fungi remains below expectations, a considerable progress has been achieved for certain pests of crops produced under cover and in greenhouses, and to some extent for those grown on open fields and forests. The evaluation of entomopathogenic fungi to control stored product pests attracted researchers' attention much later and their potential has been established through a number of studies (Adane et al., 1996; Hidalgo et al., 1998; Rice and Cogburn, 1999; Sheeba et al., 2001; Padin et al., 2002; Cherry et al., 2005; Wakil and Ghazanfar, 2010; Shams et al., 2011; Barra et al., 2013; Khashaveh and Chelav, 2013; Sewify et al., 2014). Amongst these studies, as a common primary pest of stored-grains, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) is a frequently targeted pest species. In order to determine the potential of entomopathogenic fungi for the control of *R. dominica*, *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson isolates (Barra et al., 2013), *M. anisopliae* (Metschnikoff) (Moino et al., 1998; Mahdneshin et al., 2009; Wakil and Ghazanfar, 2010; Sewify et al., 2014) and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Moino

* Corresponding author. Department of Plant Protection, Faculty of Agriculture, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey.
E-mail address: mker@ksu.edu.tr (M.K. Er).

https://doi.org/10.1016/j.jspr.2018.05.003
0022-474X/

et al., 1998; Rice and Cogburn, 1999; Mahdneshin et al., 2009; Sewify et al., 2014) isolates were evaluated by bioassays. Although the mortality results varied considerably depending on pathogens, experimental procedures and conditions, they all considered at least one isolate as potential for the control of *R. dominica*. Application of entomopathogenic fungi in combination with diatomaceous earth was also found promising against *R. dominica* (Lord, 2001, 2005; Vassilakos et al., 2006; Batta, 2008; Shafighi et al., 2014; Riasat et al., 2011; Wakil et al., 2011, 2012; Wakil and Schmitt, 2014; Eissa et al., 2014).

Because of the fact that one of the important factors for the efficacy is the characteristics of the pathogen itself, discovering a suitable isolate with desired features is vital through screening of available candidates. Although most of the entomopathogenic fungi tested against *R. dominica* in previous studies were from other habitats, insect pest populations in storage ecosystems were emphasized as good sources for finding entomopathogenic fungi with high potential against stored product pest by Kassa et al. (2002), Wakil et al. (2014) and Er et al. (2016). Some studies indicated that initial sampling procedure can have a role on finding good candidates in such survey studies. Oduor et al. (2000) found that sampled dead insects was more fruitful to isolate entomopathogenic fungi. Results of Cherry et al. (2005) indicated that sampling targeted species recovers more virulent isolates. There have not been any reports confirming these findings. Because changes in ambient conditions (temperature and relative humidity) and pest populations are possible in storage facilities, sampling time can also have a role on encountering entomopathogens.

In this study, *B. bassiana* isolates previously obtained from stored-grain pests collected from grain storages in Adana (46 isolates) and Mersin (29 isolates) provinces of Turkey (Er et al., 2016) were tested for their efficacies against *R. dominica* adults and the mortality levels resulted were used to evaluate possible effects of above mentioned three sampling factors (host species, sampling time, and sampling of dead or alive hosts) likely to have a role on encountering potential isolates for developing a microbial control agent.

2. Materials and methods

2.1. Fungal cultures

Details of tested *B. bassiana* isolates are presented in Table 1. All the isolates were deposited as lyophilized samples in the entomopathogenic fungal culture collection in Department of Plant Protection, University of Kahramanmaraş Sütçü İmam, Turkey. They were identified to species previously by Er et al. (2016), based on their morphological and molecular characteristics. The fungi were grown on potato dextrose agar (PDA) (Merck 1.10130) at 25±2 °C in darkness until sporulation was completed. The cultures were left in the same conditions with their lids open to reduce moisture for two days. The conidia were harvested by vacuuming from the surface of the colonies and collected within small vials as described by Athanassiou and Steenberg (2007). An autoclavable vacuum filter system (Nalgene) with a short tube connected to the top was used. Spores accumulated on a fitted membrane filter were transferred into a small vial. The gathered conidia (1.3×10^{11} conidia/gr) were kept at +4 °C in a refrigerator on silica gel until their use for a maximum of two days.

2.2. Insect culture

Rhyzopertha dominica cultures have been maintained in our laboratory for the last three years, and starting insects had been originally obtained from surrounding storage facilities. Durum wheat with 11–13% moisture content was used for *R. dominica* cultures. Adults of mixed sex were placed into glass jars of 1 Lt capacity having 250 gr of wheat, and kept for three days for oviposition at 26 ± 2 °C and 65 ± 5% relative humidity in continuous darkness. After removing the adults, the cultures were incubated for the emergence of new generation adults under the same conditions. One week old adults were used for testing *B. bassiana* isolates.

2.3. Experimental procedure

Prior to the experiments, fungal conidia were checked for germination abilities for each isolate. A dilute suspension of conidia within sterile distilled water including 0.2% Tween 80 was spread on PDA and incubated for 24 h at 25±2 °C in darkness. Thereafter, a minimum of 100 conidia were examined under a phase contrast light microscope for germination, three times for each isolate. Those spores with a germination tube equal or longer than the spore length were considered germinated. All tested isolates in this study had 98–100% germination at the time of experiments.

Centrifuge tubes of 50 ml capacity were used for bioassays. Each tube had 40 gr of wheat homogenously mixed with 40 mg of spores by shaking for 5 min, producing a final concentration of 1000 ppm (w/w) *B. bassiana* spores in each tube. Twenty *R. dominica* adults were placed in each tube and closed with cheesecloth for aeration. For control units, centrifuge tubes with only wheat without conidia were used. The experiment was set with four replications at 26 ± 2 °C and 65 ± 5% relative humidity in darkness. Insect mortalities after 14 days were used for statistical analysis.

2.4. Statistical analysis

All adult mortalities were corrected according to Abbott's formula (Abbott, 1925). Control mortalities varied from 1.25% to 12.5%. The isolates were divided into two groups according to the mortalities they caused. Those induced equal or over 80% mortalities were considered as “potential” isolates, and the others as non-potential isolates to be developed as microbial control agents. This seemingly lower mortality level was chosen for potential isolates as all were wild cultures open for efficacy improvement. The mortalities in each group were subjected to ANOVA and Duncan multiple comparison test at $P < 0.1$ after arcsine transformation (Zar, 1996). After evaluation of the mortalities, their relations to certain factors in sampling process were evaluated. The factors were 1) the species of the host insect from which the isolate was obtained, 2) the collection season – summer or autumn – of the host insect from storage facilities, and 3) the time of host insect's death – before or after collection. Chi square tests were used to find any effects of these factors on the efficacy of obtained *B. bassiana* isolates against *R. dominica* adults. Yates correction for continuity was used in the case of $v = 1$ (Zar, 1996). All the analyses were conducted by using SPSS version 24. In the analysis of the first factor, *Sitophilus* species and *Tribolium* species were pooled separately under genus, and species with zero frequencies had to be omitted.

3. Results

Adult *R. dominica* mortalities caused by the *B. bassiana* isolates from Adana were illustrated in Table 2. Considerable variations in mortalities were detected (13.67%–100%) depending on isolates. Thirty four isolates resulted in less than 80% mortality with statistically significant variations ($F = 6.96$; $df = 33, 102$; $P < 0.01$) resulting in overlapping 12 groups. Twelve isolates brought about 80% or higher mortalities and amongst them statistically significant