Determination of Biological Effect of Entomopathogen Fungus on Galleria mellonella (Lepidoptera: Pyralidae)

Ş. Evrim Arıcı
University of Suleyman Demirel,
Faculty of Agriculture, Department of Plant Protection Isparta/TURKEY
evrima@ziraat.sdu.edu.tr

Mehmet Sedat Sevinç
University of Suleyman Demirel,
Faculty of Agriculture, Department of Plant Protection Isparta/TURKEY
medantinc@mynet.com.tr

İsmail Karaca
University of Suleyman Demirel,
Faculty of Agriculture, Department of Plant Protection Isparta/TURKEY
ikaraca@sdu.edu.tr

Ozan Demirözer
University of Suleyman Demirel,
Faculty of Agriculture, Department of Plant Protection Isparta/TURKEY
ozandi@ziraat.sdu.edu.tr

Abstract: In this study, Entomopathogenic fungi Perlomyces chlamidospora, Fusarium subglutinans, Fusarium solani, Baveria bassiana were investigated to the effect of larvae great wax moth Galleria mellonella (Lepidoptera: Pyralidae). P. chlamidospora, F. solani, two isolates of F. subglutinans (8, 12), and B. bassiana were cultured for 10 days potato dextrose agar (PDA) medium and incubated in dark conditions at 25 ± 1 °C. 1x10^7 spores/ml suspension for each isolate was used for each isolate and Tween 20 was added in. By dipping method suspensions of spore were experimented on Larvae of G. mellonella (Lepidoptera: Pyralidae). Larvae of G. mellonella were cultured in 12-cm plastic petrie dishes at 25 ± 1 °C. 1, 24, 48 hours and 8, 13 days after the applications were evaluated the death larvae of G. mellonella. The mortality rate of G. mellonella was observed between 50-70% but statistical difference between isolates was not determined. The results obtained in the application used the types of Entomopathogenic fungus can be used biological control for G. mellonella.

Introduction

Great wax moth (Galleria mellonella) is economical pest of honey bees (A. mellifera) and they are widespread in particularly low altitude and temperate reason region which beekeeping land (Allan, 2000), and they known that an important pest which beekeepers fall into trouble to protect their honeycombs all over the world (Sanford, 2003). Great Wax Moth’s mature individuals, pupae, and egg stages don’t damage to effervesced honeycombs but their larvae do different levels of damage to honeycombs in the appropriate environment conditions (temperature, humidity, food). Great wax moth develops at above 4 °C temperature and %70 humidity, commonly eliminates possibility of re-use of honeycomb by opening the tunnels in stored effervesced honeycombs. (HaeWoon et al. 1995, Ritter et al., 1992). Such as chemical (aluminum phosphide, methyl bromide, ethylene dibromide, paradichlorobenzene (naphthalene), sulfur), physical applications (cold-hot) and biological insecticides (Bacillus thuringiensis) control methods used in different ways to protect effervesced honeycombs from great wax moth in some countries (Tutkun et al. 1987; Ritter et al. 1992; Ahmad, 1994; Yacobson et al. 1997; Delaware, 2000; Kumova &Korkmaz 2002).
Many chemical substances which using to Great wax moth reduce the chance of marketing or eliminate it because of residue on honey or wax. Today from this perspective, residue and ease of application of insecticide to pest has taken into consideration and lead to new ways is inevitable (Allan, 2000). Biological control is one of the most suitable alternative method for chemical control in different ecosystems and an environmentally friendly alternative that involve the use of natural enemies and pathogens to control pests. A number of entomopathogenic fungi have been identified biological control agents to control insects. These fungal agents are virulent, infect insect by contact persist in the environment for a long time and have one of the largest host list. Recently it was studied on safety of entomopathogenic fungi (Goettel & Jaronski 1997; Goettel & Hajek 2000; Goettel et al. 2001; Ali-Shtayeh et al. 2002, Shah & Pill 2003, Copping, 2004, Tuininga et al 2009¸ Zeinat et al. 2009).

The aim of the present study was to determine of possible effects of entomopathogenic Fusarium subglutinans, Fusarium solani, Beauveria bassiana and Pochonia clamydosporia on the Galeria mellonella’s larva.

Materials and Methods

Galeria mellonella and entomopathogenic fungi two isolates of Fusarium subglutinans (8, 12), Fusarium solani, Beauveria bassiana and Pochonia clamydosporia were used in this study.

Galeria mellonella were taken to climate room in 25±1 ºC temperature and % 65±5 humidity for reproduction of their number. Food from mix of honey, glycerin, dry wax and wheat bran were taken into the jar which closed with tulle for air circulation and G. mellonella’s larvae were taken into this jars to be mature individuals. The adults from pupaes were taken into the jars which prepared by same way to laying egg. The larvae from egg were removed from food environment before the pupal period for application.

The fungi were cultured on PDA medium and incubated at 25°C under dark conditions for 10 days. Suspensions of spore from each isolate were prepared and spore concentrations (1×10⁷) were prepared in distilled sterilized water with the help of haemocytometer. By dipping method suspensions of spore were experimented on last instar stage larvae and newly hatched G. mellonella’s larvae (3-5 sn), after Tween 20 was added in suspensions. For the control experiment, sterilized water was applied to the test insects. The G. mellonella’s larvae were put in plastic petri dishes (12 cm) under controlled conditions (25°C) and the plastic petri dishes were supplied with honey for nutrition purposes.

Petri dishes containing G. mellonella’s larvae were sealed with Parafilm to reduce moisture loss. Samples were then incubated at 25 0C and a photoperiod of 16 hours light for 13 days. For each petri dish had five larvae of G. mellonella five replicants and experiment for each entomopathogenic fungi consisted of five replicate according to completely randomized design. For each experiment daily mortality had been recorded for 13 days. Obtained data from bioassays were analyzed by SPSS version 16.0.1. Data were analyzed by ANOVA and treatment means separated by TUKEY test (P <0.05) to select isolates for a new assay with several cultivars.

Results and Discussion

In this study G. mellonella larvae found to be infected by entomopathogenic fungi and was observed mortality of G. mellonella’s larvae (Table 1). Although a statistical difference was found between the isolates and the control group, no statistical difference was determined between the isolates.

<table>
<thead>
<tr>
<th>Entomophage genus</th>
<th>Mortality of G. mellonella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Pochonia clamydosporia</td>
<td>0,2±0,2</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>0,6±0,4</td>
</tr>
<tr>
<td>Fusarium subglutinans (12)</td>
<td>0,2±0,2</td>
</tr>
<tr>
<td>Fusarium</td>
<td>0,6±0,2</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Entomophage pathogene</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48h</td>
</tr>
<tr>
<td>Pochonia clamydospora</td>
<td>12</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>12</td>
</tr>
<tr>
<td>Fusarium subglutinans (12)</td>
<td>4</td>
</tr>
<tr>
<td>Fusarium subglutinans (8)</td>
<td>20</td>
</tr>
<tr>
<td>Beauveria bassiana</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 2:** Mortality percentage of *G. mellonella* ‘s larvae

In conclusion; fungal mycel of entomopathogens used in this study had grown on death larvae of *G. mellonella*. Entomopathogenic fungi can used for biological control and in pest management.

**References**


---

The mortality of *G. mellonella* larvae was observed 4% mortality of the larvae of *G. mellonella* infected with *Fusarium subglutinans* 12, 20% with *Fusarium subglutinans* 8, while was determined 12% mortality of larvae of *G. mellonella* infected with *Fusarium solani*, *B. bassiana*, and *P. clamidosporium* in 48 h (Table 2). It was recorded in 13 d 28% mortality of larvae of *G. mellonella* infected with *P. clamidosporium*, 60% with *F. solani*, while was observed 56% mortality of *G. mellonella* larvae with *Fusarium subglutinans* 12, *Fusarium subglutinans* 8 and with *B. bassiana*.

Some researcher indicated similarly results (James & Elzen 2001; Shah & Pill 2003; Mohammed et al. 2009). In addition same researcher used larvae of *G. mellonella* to determine the relative abundance of entomopathogenic nematodes, and fungi (Chadler et al. 1997; Ali-Shtayeh et al. 2001; Tuininga et al. 2009).


