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
ozand@ziraat.sdu.edu.tr

Abstract: In this study, Entomopathogenic fungi *Perlomyces chlamidosporia*, *Fusarium subglutinans*, *Fusarium solani*, *Baveria bassiana* were investigated to the effect of larvae great wax moth *Galleria mellonella* (Lepidoptera: Pyralidae). *P. chlamidosporia, 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 ºC. $1x10^7$ spor / ml suspension for each isolates was used for each isolates 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 ^{0}C. 1, 24, 48 hours and 8, 13 days after the applications were eveluated 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 (*Galeria 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 larvaes do different levels of damage to honeycombs in the appropriate environtenmal 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-Shtayeh1 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 larvae

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* larvaes 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 larvaes from egg were removed from food environment before the pupal period for application.

The fungi were cultured on PDA medium and incubated at 25ºC under dark conditions for 10 days. Suspensions of spore from each isolate were prepared and spore concentrations ($1X10^7$) were prepared in distilled sterillized 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* larvaes (3-5 sn), after Tween 20 was added in suspensions. For the control experiment, sterillized water was applied to the test insects. The *G. mellonella's* larvaes were put in plastic petri dishes (12 cm) under controlled conditions (25ºC) and the plastic petri dishes were supplied with honey for nutrition purposes.

Petri dishes containing *G. mellonella's* larvaes 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.

Entomophage pathogen	Mortality of *G. mellonella*			
	24 h	48 h	8 d	13 d
Pochonia clamydosporia	0,2±0,2	0,6±0,4	1±0,3	1,4±0,5
Fusarium solani	0,6±0,4	0,6±0,4	1,2±0,7	3±0,8
Fusarium subglutinans (12)	0,2±0,2	0,2±0,2	1±0,3	2,8±07
Fusarium	0,6±0,2	1±0,4	1,6±0,7	2,8±0,5

subglutinans (8)				
Beauveria bassiana	0,4±0,2	0,6±0,6	1,6±0,5	2,8 ±0,6

Table 1: Mortality of *G. mellonella* larvae

It was observed 4% mortality of the larvae of *G. mellonella* infected with *F. subglutinans* 12 , 20% with *F. subglutinans* 8, while was determinated 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 *F. subglutinans* 12, *F. 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).

Entomophage pathogene	Mortality %	
	48h	13 d
Pochonia clamydosporia	12	28
Fusarium solani	12	60
Fusarium subglutinans (12)	4	56
Fusarium subglutinans (8)	20	56
Beauveria bassiana	12	56

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

Ahmad, M. (1994). Biological Control of Greater Wax Moth, Galleria mellonella L. *Journal of Apicultural Research* (Pakistan). Vol. 32(3), 319-323.

Allan, L. (2000). Wax Moth and its Control. Departmen of Agriculture Western Australia. http://www.agric.wa.gov.au/agency/pubns/farmnote/2000/f00697.htm.

Chandler, D., Hay, D., &Reid, A.P. (1997). Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils *Applied Soil Ecology* 5 133-141

Copping, L.G. (2004). The Manual of Biocontrol Agents.British Crop Protection Council, Alton, 702 pp

Delaware, M. (2000). New Jersey, Pensylvania, Westiginia and the USDA Cooperating. MAAREC Publication, 4.5.

Goettel, M.S., & Jaronsky, S.T. (1997). Safety and registration of microbial agents for control of grasshoppers and locust. *Mem. Entomol. Soc. Can.,* 171: 83-99.

Goettel, M.S., & Hajek A.E. (2000). Evaluation of non-target effects of pathogens used for management of arthropods. In: Wajnberg E,Scott JK, Quimby PC (eds) Evaluating indirect ecological effects of biological control. CABI Publishing, Wallingford, pp 81–97

Goettel, M.S., Hajek, A.E., Siegel, J.P., & Evans, H.C. (2001). Safety of fungal biocontrol agents.

HaeWoon, O., ManYoung, L., Young Duck, & Chang (1995). Developing Periods and Damage Patterns of Combs by The Greater Wax Moth, Galleria mellonella. *Korean Journal of Apiculture* 10: 1, 5-10.

James, R.R., & Elzen, G.W. (2001). Antagonism between *Beauveria bassiana* and imidacloprid when combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) control. *J. Econ. Entomol*., 94: 357–61

Kumova, U., & Korkmaz, A. (2002). Peteklerin Büyük Bal Mumu Güvesi (Galleria mellonella L.) ' ne Karşı Korunması Üzerine Bir Araştırma. Türkiye 3. Arıcılık Kongresi, Adana.

Mohammed S. Ali-Shtayeh1, Abdel-Basit B.M, & Mara'i Rana M. J. (2002). Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area *Mycopathologia* 156: 235–244, 2002.

Ritter, W., Perschill, F., Vogel R. 1992. Comparision of the effect of various methods fort he control of wax moths. *Allgemeine Deutsche Imkerzeitung*. 26: 1, 11-13. CAB Abstracts 1993-1994, AN:930234018

Sanfort, M.T. (2003). Controlling Wax moth, one of a series of the Entomology and nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. EDIS Web Site at http://edis.ifas.ufl.edu

Shah, P. A., & Pell, J. K. (2003). Entomopathogenic fungi as biological control agents. *Appl Microbiol Biotechnol*, 61:413–423

Tuınınga, A. R., Mıller J. L., Morath S. U., Danıels T. J., Falco R. C., Marchese M., Sahabı, S., Rosa, D., & Stafford K. C. (2009). Isolation of Entomopathogenic Fungi From Soils and *Ixodes scapularis* (Acari: Ixodidae) Ticks: Prevalence and Methods. *J. Med. Entomol*. 46(3): 557-565

Tutkun E., Çakmakçı, L., & Boşgelmez A. (1987). Bal Arısı Kolonilerinde Bacillus thrugiensis Preparatlarının Büyük Mum Güvesi (G. mellonella) Larvalarına Karşı Kullanım Olanakları Üzerinde Araştırmalar. TÜBİTAK, Tarım ve Ormancılık Araştırma Grubu, Tarımsal Mikrobiyoloji Ünitesi Proje no: Tarmik-8-34 s.

Yacobson, B., Navarro, S., Donahaye, E. J., Azrielli, A., Sloyevsky, Y., & Ephrati, H. (1997). Control of Beeswax moths using carbondioxide in flexible plastic and metal structure. In: Proc. Int. Conf. Controlled atmosphere and fumigation in grain storages 21-26 April 1996, Nicosia Cyprus pp: 169-174.

Zeinat K.M., S.A. El-Sayed, Radwan T.E.E., & Ghada, S. Abd El-Wahab (2009). Potency Evaluation of *Serratia marcescens* and *Pseudomonas fluorescens* as Biocontrol Agents for Root-knot Nematodes in Egypt. *Journal of Applied Sciences Research,* 4(1): 93-102