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Evaluation of some entomopathogenic fungi against the fall webworm (Hyphantria cunea Durry, Lepidoptera: Arctidae)

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ABSTRACT

Fall webworm (*Hyphantria cunea* Durry, Lepidoptera: Arctidae) is an important pest infecting about 600 hosts. It is harmful especially in hazelnut orchards in the Black Sea Region and is becoming epidemic occasionally. It may cause damage in mulberry, cherry, apple, poplar, and willow beside hazelnut in the region. Due to having a polyphagous feeding behavior and a high reproduction power; fall webworm can spread rapidly and make difficult to manage. In the region, currently, mostly chemical control is applied against this pest. Due to adverse effects of the chemical control to the environment and to living organisms, it is inevitable to develop other alternative control methods for this pest. In this study, the effects of some entomopathogenic fungi isolates obtained from *Palomena prasina* which is another pest in hazelnut production areas, on *H. cunea* in laboratory conditions. Overall, $1x10^8$ conidia mL^{-1} of concentration obtained from 2 isolates of *Simplicillium lamellicola* (TR-01 and TR-02) and 4 isolates of *Lecanicillium muscarium* (TR-04, TR-05, TR-07 and TR-08) was used against 3rd period larva of the *H. cunea*. The experiment was conducted with four replications, 10 larvae individuals in each. Mortality of *H. cunea* were reported daily, over 12 days. At the end of 12th day, among the isolates of entomopathogenic fungi, the TR-05 isolate of the *L. muscarium* ranked the highest mortality by 93.9% rate. Effect of the other isolates of *L. muscarium* varied between 72.7% and 90.9%. The TR-01 isolate of the *S. lamellicola* showed effect of 57.6%, and the TR-02 isolate showed effect of 78.8% mortality. Effects of all the isolates used in the study were differed from the control ($P<0.05$). Based on LT_{50} and LT_{90} values, the most effective isolate was identified as TR-04 (5.64/day and 9.38/day, respectively). It can be concluded that, the isolates of *L. muscarium* was found quite effective and it could be a promising agent for controlling this pest in the field in the future.

1.Introduction

The Fall Webworm (*Hyphantria cunea* Drury) (Lepidoptera: Arctiidae) is a polyphagous pest that is native to the USA, Canada, and Mexico. It is also seen across Europe, Russia, Georgia, Iran, China, New Zealand, Korea, Japan, and Turkey (Yang et al., 2008). This pest is considered to have many hosts in the world with the ability to infect about 600 plant species including fruit trees, forest trees, ornamental plants, vegetables, and weeds (Waren and Tadic, 1970; Rezaei et al., 2006). The *H. cunea* is subject to in external quarantine applications worldwide and gives serious damage to agricultural areas and forests. This pest causes dehydration in all parts of its host plants. Since the pest is polyphagous and has a high reproductive rate, it can spread rapidly, making it difficult to control (Ecevit et al., 1994, Tuncer and Kansu, 1994, Yaman et al., 2001, Akkuzu and Mol, 2006, Saruhan et al., 2014). For this reason, highly effective insecticides are used for its control.

Currently, the farmers have different synthetic insecticides or only a microbial insecticide containing Bacillus thuringiensis var. kurstaki strain Pb-54 using option to reduce the loss of this insect in hazelnut orchards in Turkey. Unfortunately, alternative control

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methods are limited to manage this pest. Negative effects of the chemical control on human health and environment are well-known. Thus, alternative control methods to the chemical control are needed. Consequently, biological control in which entomopathogens are used might be an alternative control method. Entomopathogenic fungi are common natural enemies of arthropods worldwide, attracting attention as potential biological control agents. There are more than 700 species of entomopathogens in the fungi kingdom (Roy et al., 2006; Sandhu et al., 2012). Fungal entomopathogens such as *Beauveria bassiana* (Balsamo) Vuillemin, *Isaria farinosa*, *I. fumosorosea*, *Metarhizium anisopliae*, *Lecanicillium* spp. and *Simplicillium* spp. play an important role in the management of insect populations (Shah and Pell, 2003; Zimmermann, 2008; Gurulingappa et al., 2011).

Lecanicillium spp., formerly known as *Verticillium lecanii*, (Zare and Gams, 2001; Zimmermann, 2008;) are opportunistic and widely distributed ascomycete fungi of the order Hypocreales. Following a critical taxonomic review using rDNA sequencing to assess diversity within the taxon (Zare and Gams, 2001), the species was divided into a number of new taxonomic entities, including *L. lecanii*, *L. longisporum*, *L. attenuatum*, *L. muscarium* and *L. nodulosum* (Brodeur, 2012). *L. muscarium* is a well-known pathogen of arthropods. This species was isolated from aphids, scales, whiteflies, thrips and other insects (Askary and Yarmand, 2007; Kunimi, 2007; Goettel et al., 2008; Anand and Tiwary, 2009; Guclu et al., 2010; Saruhan et al., 2015). *Lecanicillium muscarium* is currently in the process of being made available as a commercial bioinsecticide, for example, Mycotal® (Koppert BV, Berkel en Rodenrijs, Netherlands), for use against whiteflies and thrips, and Verticilin® (Koppert BV, Berkel en Rodenrijs, Netherlands), for use against whiteflies and aphids (Goettel et al., 2008; Brodeur, 2012). The genus *Simplicillium* presently consists of three species: *Simplicillium lanosoniveum*, *Simplicillium obclavatum* and *Simplicillium lamellicola* (Nonaka et al., 2013). Some studies reported that *S. lamellicola* was used to control ticks (Polar et al., 2005), *Heterodera glycines* Ichinohe cysts and *Meloidogyne arenaria* eggs (Gams, 1988).

A number of studies on the use of some entomopathogenic fungi against the *H. cunea* (Sullivan et al., 2011; Iskender et al., 2012; Qin et al., 2012; Ajamhassani, 2013; Zibaee et al., 2013) were reported in the world. Three isolates of *B. bassiana* tested on larvae of *H. cunea* caused mortality between 90±5.77% and 96.6±3.33% (Iskender et al., 2012). In other study, the efficacy of *B. bassiana* strains FD and *Paecilomyces farinosus* strains SH9-4 on mature larvae of *H. cunea* were determined, and five days after inoculation, the corrected mortalities and LT_{50} values of *B. bassiana* and *P. farinosus* against the larvaes were detected to 92.4%, 94.9%, and 87.06 h, 92.34 h, respectively (Qin et al., 2012).

The aim of this study was to determine the pathogenicity of six isolates of entompathogenic fungi belonging to *L. muscarium* and *S. lamellicola* against 3rd period larvae of the *H. cunea* in laboratory conditions.

2. Material And Methods

2.1. Fungi Cultures

A total of six isolates of entomopathogenic fungi isolated from infected *Palomena prasina* (Heteroptera: Pentatomidae) in hazelnuts orchards in Black Sea region of Turkey were used in this study (Table 1) in 2015. The single-spore cultures of *S. lamellicola* (TR-01 and TR-02 isolates) and *L. muscarium* (TR-04, TR-05, TR-07 and TR-08 isolates) were stored at 4°C on Sabouraud dextrose agar (SDA) (Merck Ltd., Darmstadt, Germany) slants and also in cryogenic tubes containing 15% glycerol kept at –80°C, and deposited in the fungal culture collection of the Mycology Laboratory at the Ondokuz Mayis University, Faculty of Agriculture's Department of Plant Protection in Samsun, Turkey and in the USDA-ARS Entomopathogenic Fungal Culture Collection in Ithaca, NY.

2. 2. Insect cultures

The pupae of *H. cunea* were collected from various hazelnut production areas in the Çarşamba district of Samsun province. Firstly, fertile adults of *H. cunea* were obtained from the pupae brought to the laboratory, and needed eggs were produced from adult females. Same age 3rd instar larvae hatched from related eggs were used in the study.

2.3. Inoculum of entomopathogenic fungi isolates

The six entomopathogenic fungi isolates belonged to *S. lamellicola* (TR-01 and -02) and *L. muscarium* (TR-4, -5, -7 and -08) were incubated on potato dextrose agar (PDA; Oxoid Ltd., Basingstoke, UK) at 25±1 °C for 2 weeks to obtain conidia which were suspended in sterile distilled water, filtered through three layers of sterile cheesecloth, and diluted to a concentration of $1x0^8$ conidia mL^{-1} of each isolate plus 0.02% Tween 20.

2.4. Experimental design

Ten third instar *H. cunea* larvae were located in 1 L plastic ice-cream cups (disinfected by 70% ethanol) containing 4 fresh maple leaves. Bottoms of ice-cream cups were covered by filter paper that moisturized by sterile-distilled water. Conidial suspension ($1x10^8$ conidia mL^{-1}) of the each entomopathogenic fungus (TR-01, -02, -04, -05, -07 and -08) was applied to the 3rd instar *H. cunea* larvae (2 mL per ice-cream cup) using a Potter spray tower (Burkard, Rickmansworth,

Hertz UK). The spray tower was cleaned with 70% ethanol and sterile distilled water after each application

Table 1. Species, hosts and locations of isolates of entomopathogenic fungi used in this study.

Species / Isolate denomination	ARSEF accession numbers	Host	Location of collection
Simplicillium lamellicola / (TR-01)	ARSEF 11727	*Palomena prasina*	Giresun
Simplicillium lamellicola / (TR-02)	ARSEF 11728	*Palomena prasina*	Ordu
Lecanicillium muscarium / (TR-04)	ARSEF 11730	*Palomena prasina*	Ordu
Lecanicillium muscarium / (TR-05)	ARSEF 11731	*Palomena prasina*	Samsun
Lecanicillium muscarium / (TR-07)	ARSEF 11733	*Palomena prasina*	Ordu
Lecanicillium muscarium / (TR-08)	ARSEF 11734	*Palomena prasina*	Düzce

of the fungus suspension for the sterilization of the apparatus. Only sterile-distilled-water containing 0.02% Tween 20 was sprayed to control ice-cream cups. They were incubated at 25±1°C and 75±5% relative humidity (RH), 16:8 h light: dark photoperiod in a Binder incubator (Model KBWF 240; Germany). Polyethylene sheets were used along with rubber to cover open side of cups. All cups were inspected daily for twelve days. Fresh maple leaves were added when needed. Dead individuals on which the fungal sporulation observed were counted under a Leica EZ4 educational stereomicroscope at 40-70X magnification. Mortality was recorded daily basis and dead individuals were removed from cups. Evidence of *Lecanicillium* and *Simplicillium* on nymph cadavers were verified by microscopic inspection. The experiment was conducted once, with four replications (Saruhan et al., 2015).

2.5. Conidial germination assessment

The viability of conidia of the six isolates belonging to *S. lamellicola* and *L. muscarium* was determined. A spore suspension (100 µL) of each the isolate at $1x10^4$ conidia mL^{-1} was sprayed onto 6-cm-dia. Petri dishes. This dishes containing PDA were incubated at 25±1 °C for 24 h. Then, percentages of germinated conidia were counted using an Olympus CX-31 compound microscope (Olympus America Inc., Lake Success, NY) at 400X magnification. Conidia were considered as germinated when they produced a germ tube at least half of the conidial length. Germination ratios for each isolate were calculated after examining a minimum of 200 conidia from each of three replicate plates (Saruhan et al., 2015).

2. 6. Statistical analysis

The mortality was noted over 12 days fallowing each application. Dead individuals were counted under stereoscopic microscope and percent mortality was calculated. The mortality data was corrected by Abbott's Formula (Abbott, 1925). Fifty percent lethal time (LT_{50}) and ninety percent lethal time (LT_{90}) were determined using the probit analysis by SPSS (Ver. 21) program. The effects of mortality of the *H. cunea* was analyzed using two-way analysis of variance (ANOVA) (P=0.05), followed by a comparison of means using Duncan's multiple range test (SPSS).

3.Results

According to the results of *in vitro* tests to determine the insecticidal effects of entomopathogenic fungi, at the end of day 12, mortality rate of *S. lamellicola* isolate TR-01 was 57.58%, whereas TR-02 was more effective with mortality rate of 78.79%. Deaths in both isolates of *S. lamellicola* were rapid in the first three days and slow in the following days, with the most deaths occurring at the end of day 12. Among four isolates of *L. muscarium*, another entomopathogenic fungus used in the study, the most effective isolate was TR-05 with mortality rate of 93.94%. The mortality rates of the other isolates in this group were 90.91%, 75.76% and 72.73% for isolates TR-04, TR-07 and TR-08, respectively. The mortality rates in the isolates belonging to *L. muscarium* generally increased after day 5 and mortality in isolates of *S. lamellicola* also increased on day 12 in the group (Table 2).

Table 2. Mortality percentages on 3rd instar larvae of *Hyphantria cunea* by using the isolates of entomopathogenic fungi, *Lecanicillium muscarium* and *Simplicillium lamellicola*.

Isolates	Days						
	1	3	5	7	9	12	
TR-01	0.0	17,5	20.0	35.0	40.0	57,58	**b***
TR-02	0.0	27,5	32,5	45.0	55.0	78,79	**a**
TR-04	0.0	12,5	52,5	80.0	87,5	90,91	**a**
TR-05	0.0	10.0	37,5	57,5	65.0	93,94	**a**
TR-07	0.0	12.5	37,5	52,5	57,5	75,76	**a**
TR-08	0.0	22,5	45.0	50.0	60.0	72,73	**ab**
Control	0.0	02,5	07,5	12,5	15.0	17,50	**c**

* The same small letters within columns indicates no significant differences between means (P<0.05)

When the LT_{50} rates of the isolates used in the study were examined, the most effective isolate found as TR-04 (5.64/day). This was followed by TR-05 (6.88/day), TR-08 (7.38/day), TR-02 (7.64/day), TR-07 (7.65/day) and TR-01 (9.77/day). Based on LT_{90} rates, the most effective isolate was also TR-04 (9.38/day). This was followed by TR-05 (11.00/day), TR-07 (13.19/day), TR-08 (13.79/day), and TR-01 (16.84/day) (Table 3).

Table 3. Lethal time (LT_{50} and LT_{90}) values of 3rd instar larvae of *Hyphantria cunea* treated the isolates of entomopathogenic fungi, *Lecanicillium muscarium* and *Simplicillium lamellicola* (day).

Isolates	LT_{50} (95% confidence limit)		LT_{90} (95% confidence limit)		χ^2
TR-01	9,77(8,67-11,37)	**a***	16,84(14,45-21,11)	**a**	4,76
TR-02	7,64(5,91-10,09)	**ab**	13,82(11,01-21,73)	**ab**	7,83
TR-04	5,64(3,25-07,78)	**b**	9,38(07,38-15,95)	**ab**	18,01
TR-05	6,88(6,25-07,54)	**b**	11,00(10,02-12,43)	**b**	5,79
TR-07	7,65(6,15-09,61)	**ab**	13,19(10,83-18,94)	**ab**	7,09
TR-08	7,38(5,41-10,05)	**ab**	13,79(10,80-23,25)	**ab**	8,93

* The same small letters within columns indicates no significant differences between means (P<0.05)

4.Discussion

This study revealed that the six isolates belonging to both entomopathogenic fungi were effective at different levels against the 3rd instar larvae of *H. cunea*.

Various researchers also reported that *L. muscarium* was used effectively for different biological states of different pests in the world (Cuthbertson and Walters, 2005; Guclu et al., 2010; Luz et al., 2010; Saruhan et al., 2015).

All isolates of *L. muscarium* used in the study were found to be effective against the larvae of *H. cunea*. The TR-05 isolate of *L. muscarium* was the most effective isolate at the end of day 12, while TR-04 isolate showed a similar effect. Based on the distribution of the mortality rates by day of the larvae of *H. cunea*, TR-08 isolate caused a rapid death in the first days but the mortality rate decreased in the following. The larvae mortality rates of the TR-07, TR-05 and TR-04 isolates of *L. muscarium* used in the study were lower in the first days, while the mortality rate increased in the following. In fact, TR-05 isolate reached 50% mortality in three days. When the LT_{50} and LT_{90} rates of the *L. muscarium* isolates used were examined, TR-04 isolate (5.64 (min: 3.25 - max: 7.78 days)) had a faster effect and this was followed by other *L. muscarium* isolates. According to a previous study where some isolates of *L. muscarium* were tested against *Ricania simulans*, LT_{50} rates varied between 3.90 and 4.80 days (Guclu et al., 2010). Erper et al. (2016) investigated the activity of 4 isolates of *L. muscarium* against *P. prasina*, and found that LT_{50} rates ranged from 3.20 to 6.90 days. In another study, *L. muscarium* (TR-08) was used against *Aphis fabae* and the LT_{50} rate was 1.77 at 20°C and 1.93 days at 25°C (Saruhan et al., 2015). In a similar study, an isolate of *L. muscarium* was tested against some mosquito species and LT_{50} rates varied between 7.2 and 11.0 days (Luz et al., 2010).

Simplicillium lamellicola isolates used in the study had a relatively lower effect on the larvae of *H.*

cunea than isolates of *L. muscarium*. TR-02 (78.79%) isolate belonging to *S. lamellicola* showed a higher effect at day 12 than TR-01 (57.58%) isolate. The effect of *S. lamellicola* isolates on the larvae of *H. cunea* was shown to take longer than those of other *L. muscarium* isolates used in the study, with a mortality rate of 50% at days 6 to 11. In a study conducted by Ausique et al. (2017), *Simplicillium* sp. isolate ESALQ-1448 was tested against Aphids and identified that the LT_{50} rate was over 10 days. In another study, four different isolates of *S. lamellicola* were used against adult mosquitos and mortality rates were between 53.2% and 63.9% at the end of day 14 (Ishii et al., 2015). Iskender et al. (2012) found that three isolates of *B. bassiana* tested on *H. cunea* larvae caused mortality between 90±5.77% and 96.6±3.33%. Similarly, the efficacy of *B. bassiana* strains FD and *P. farinosus* strains SH9-4 on mature larvae of *H. cunea* were determined using crawling contact inoculation method, and 5 days after inoculation, the corrected mortalities and LT_{50} values of *B. bassiana* and *P. farinosus* against the larvae of *H. cunea* were detected to 92.4%, 94.9%, and 87.06 h, 92.34 h, respectively (Qin et al., 2012).

As a result, the isolates of *L. muscarium* (TR-04, TR-05, TR-07 and TR-08) and *S. lamellicola* (TR-01 and TR-02) used in the study were found to be effective against the larvae of *H. cunea*. It was determined that the most effective isolates are TR-04 and TR-05 isolates of *L. muscarium* in particular, and these isolates can be identified as the most promising isolates that can be used in controlling these pests in biological control or integrated pest management efforts in field conditions.

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