EFFECT OF ENTOMOPATHOGENIC FUNGUS, *PURPUREOCILLIUM LILACINUM* (SYN: *PAECILOMYCES LILACINUS*) ON *BLATTA ORIENTALIS* AND *SHELFORDELLA TARTARA* UNDER LABORATORY CONDITIONS

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ABSTRACT: Cockroaches are pests that can threat human health. In this study, the effects of the entomopathogenic fungus, *Purpureocillium lilacinum* TR1 (syn: *Paecilomyces lilacinus*), which has been reported from Turkey previously, on the nymphs of cockroaches [Oriental cockroach (*Blatta orientalis* Linnaeus) and Turkestan cockroach (*Shelfordella tartara* Sauss)] were researched under laboratory conditions. Experiments were carried out in Petri-dishes with 5 individual nymphs per replicate, and fungal isolate. Suspensions of *P. lilacinum* were applied at three different density (10^6, 10^7 and 10^8 cfu ml^{-1}) at spraying applications the surface of the plates with roaches (after putting in 5 roaches) and without roaches (before putting in 5 roaches). In the controls, sterile water with Tween 80 (0.01%) was applied to the surface of the plates with roaches and without roaches. Each plate contained moistened filter paper on the bottom and added to the bait (favored bait, banana fruit) placed on the center. The Petri plates were then placed in a growth chamber maintained at 25±2 °C and 60-65% R.H. in a dark laboratory. Mortalities were recorded on the 3rd, 6th, 9th, 10th and 12th days after application. The dead roaches‟ nymphs were examined under a microscope to determine whether mortality was because of *P. lilacinum*, and mycosis development was checked. The results of the study showed that although the effects at spraying applications the surface of plates with roaches were very high on the other hand it was very low on spraying applications without roaches. It was valid for both roach species (*B. orientalis* and *S. tartara*). The concentrations of 10^6 and 10^7 cfu ml^{-1} at the *B. orientalis* was found 100% effective; on the other hand the concentrations of 10^6 cfu ml^{-1} at the *S. tartara* was found 100% effective at the same applications 12th days after application. But all of them were dead at 12th days. The effect of the other applications (spraying applications the surface of the plates without roaches) was found below 40% for both roach species 12th days after application. It is hoped that this first study in laboratory conditions will serve to the future studies on the control of the pest.

KEY WORDS: Entomopathogenic fungus, *Blatta orientalis*, *Shelfordella tartara*, effect

Cocroaches, which generally prefer to live in high tempereture and humidity, are important industrial and urban pest. Having undergone any changes in their life cycle from the carbon age up to now, cochroaches include 3500 species among 28 families. One of the cochroaches species used in trial, Oriental cockroach (*Blatta orientalis* Linnaeus) is a major household pest in Turkey. It is also sometimes referred to as the "black beetle" or a "water bug" because of its dark black appearance and tendency to harbor in damp locations. Turkestan cockroach (*Shelfordella tartara* Sauss), also known as the rusty red cockroach, red runner cockroach or simply rusty red, red runner, or lat, is a primarily outdoor-dwelling cockroach native to an area from Northern Africa to Central Asia. This species damage people in different ways everywhere they are present. This damage can be

defined as direct consumption of food, causing uneasiness with their presence, polluting environment with their smell and waste and infesting some microorganisms causing illness in human by food. It is reported that cockroaches can transmit cholera, hansen disease, plague, typhoid, tuberculosis etc (Metcalf et al., 1962).

Cockroaches often deposits oothecae in cracks and crevices that can be very difficult to treat with insecticides or by the time the nymphs hatch from the oothecae the application is degraded or removed. In addition, the use of chemical insecticides against cockroaches poses a health risk because these agents have to be applied in living spaces. Therefore new methods are being developed to manage the roach. Predators, parasitoids and entomopathogens have an important role in biological control. Entomopathogens comprise nematodes, protozoa, rickettsia, fungi, bacteria and viruses, among which fungi have an important role in biological control. It is reported that entomopathogen fungi are very effective in biological control of cocroaches, and play an important role in population decrease especially in regions and seasons where relative humidity is high (Mohan et al., 1999).

Fungi enter the host through the cuticle and a contact with the insect can cause infection. Profuse mycosis develops on the cadavers of the insects that have died due to fungal infection. These mycotic cadavers perpetuate the lethal fungal infection in the insect population. Hyphomycetous fungi have been reported to infect cockroaches (Hywel-Jones, 1995; Kaakeh et al., 1996; Zukowski & Bajan, 1996). It should be easy to induce an entomopathogenic fungal infection articially in cockroaches because the development of profuse mycosis on the insects is favoured under the humid conditions prevailing in their ecological niches. A rapid spread of the infection in the insect population is therefore highly likely. A commercial formulation of *Metarhizium anisopliae* has been developed for cockroach control in the US by the Ecoscience Corporation (Andes, 1994; Kaakeh et al., 1996). *Beauveria bassiana*, an entomogenous fungus with a wide host range, occurs more commonly than *Metarhizium* spp. in the tropics. It has been reported to infect American and German cockroaches (Steinhaus, 1949; Zukowski & Bajan, 1996).

Fungi have much more diverse hosts than other microorganisms. *Purpureocillium lilacinum* (Thom) Luangsa-ard (syn: *Paecilomyces lilacinus*) is a soil-borne fungus which is an egg-parasite of plant pathogenic nematodes.

Commercial preparates of *P.lilacinum,* a successful biologic control agent for nematodes are also available. It is shown that this fungus is effective to some other pest groups, too.

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The use of chemical insecticides against cockroaches poses a health risk because these agents have to be applied in living spaces. Entomopathogenic fungi are receiving renewed interest as biocontrol agents in instances where chemical pesticides present a risk to human health (McCoy, 1990). Fungi enter the host through the cuticle and a contact with the insect can cause infection. Profuse mycosis develops on the cadavers of the insects that have died due to fungal infection. These mycotic cadavers perpetuate the lethal fungal infection in the insect population. Hyphomycetous fungi have been reported to infect cockroaches (Hywel-Jones, 1995; Kaakeh et al., 1996; Zukowski & Bajan, 1996). It should be easy to induce an entomopathogenic fungal infection articially in cockroaches

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Cockroaches often deposits oothecae in cracks and crevices that can be very difficult to treat with insecticides or by the time the nymphs hatch from the oothecae the application is degraded or removed. Therefore new methods are being developed to manage the roach. Predators, parasitoids and entomopathogens have an important role in biological control. Entomopathogens comprise nematodes, protozoa, rickettsia, fungi, bacteria and viruses, among which fungi have an important role in biological control. Fungi have much more diverse hosts than other microorganisms. *Purpureocillium lilacinum* (Thom) Luangsa-ard (syn: *Paecilomyces lilacinus*) is a soil-borne fungus which is an egg-parasite of plant pathogenic nematodes (Luangsa-Ard et al., 2011; Kepenekci, 2012; Kepenekci et al., 2014c). It was isolated firstly from insects in tropical regions. It has been recorded in many regions of the world specifically in warm regions (Domsch et al., 1980).

Commercial preparates of *P.lilacinum,* a successful biologic control agent for nematodes are also available. It is shown that this fungus is effective to some other pest groups, too. In Turkey, after Entomopathogenic Fungus, *Purpureocillium lilacinum* TR1 was determined and identificated (Kepenekci et al., 2013a), pre studies were done on the some important pests in Turkey and effective results were gathered. These results showed that detailed in *vivo* and in *vitro* studies had to be done (Kepenekci et al., 2013b,c, 2014 a,b,c).

The aim of this study is to investigate biological control possibilities of using *P. lilacinum* against *B.orientalis* and *S. tartara in vitro* conditions alternative to chemical control.

MATERIALS AND METHODS

Purpureocillium lilacinum TR1 was isolated from root-knot nematodes in the tomato plant roots in Sarıcakaya (Eskisehir) within the scope of the project "Determination of fungal and bacterial pathogens of root-knot nematodes, a problem for greenhouse vegetable growing in the cities of Burdur, Isparta and Eskisehir", which was carried out between 2002 and 2007. As a result of the study conducted to determine the fungal pathogens of root-knot nematodes in our country, the Turkey isolate of *P. lilacinus* was attained (Kepenekci et al., 2009). After entomopathogenic fungus, *Purpureocillium lilacinum* TR1 was determined and identificated based on morphometric and molecular data (Kepenekci et al., 2013a, 2015a). The culture of *Purpureocillium lilacinum* TR1 was provided by the Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection, Tokat (Turkey) and İnönü University, Faculty of Agriculture, Department of Plant Protection, Malatya (Turkey). The culture of Cockroaches was provided by the Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun (Turkey).

Mass-Culturing of *Purpureocillium lilacinum*

Purpureocillium lilacinum TR1 was subcultured on Potato Dextrose Agar with the help of sterilized bacteriological loop and the plates were closed by parafilm at 25±1°C for 14 days. The conidia were harvested using sterilized rubber loop attached to 1 ml borosilicate pipette at the angle of 45°. The scraped material was shifted into sterilized petri plates and stored at 4°C in refrigerator. The harvested fungal conidia were incorporated in to sterile 0.05% Tween-80 solution and the material was stirred for complete homogeneity. The serial dilutions were prepared and the number of conidia was measured to achieve the 10^6, $10^7$and 10^8cfu ml^{-1} concentration under haemocytometer. In this study, the effects of the entomopathogenic fungus, *P. lilacinum* TR1, which has been reported from Turkey previously, on the nymphs of Cockroaches (*B. orientalis* and *S. tartara*) were researched under laboratory conditions. Trials were performed on June 2017 at Faculty of Agriculture Ondokuz Mayıs University (Samsun, Turkey). Experiments were carried out in Petri-dishes with 5 individual nymphs per replicate and fungal isolate. Suspensions of *P. lilacinum* were applied at three different density (10^6, 10^7 and 10^8 cfu ml-1) at spraying applications the surface of the plates with roaches (after putting in 5 roaches) and without roaches (before putting in 5 roaches). Mortalities were recorded on the 2nd, 4th, 6th, 8th, 10th and 12th days after application. Mortalities for Cockroaches were evaluated in comparison to the control groups. The dead roaches nymphs were examined under a microscope to determine whether mortality was because of *P. lilacinum*, and mycelial development was checked. When necessary, these cadavers were placed in Petri dishes to follow up potential mycosis development.

Statistics

One-way ANOVA was used to compare the mortality of cockroaches. Means were compared at the P=0.05 level, and Tukey's test was used to separate means (SPSS, 1999). Arcsine transformation was carried out on mortality (%) before analyses.

RESULTS AND DISCUSSION

The results of the study showed that although the effects at spraying applications the surface of the plates with roaches were very high on the other hand it was very low on spraying applications without roaches. It was valid for both roache species (*B. orientalis* and *S. tartara*). The concentrations of 10^6 and 10^7 cfu ml^{-1} at the *B. orientalis* was found 100% effective; on the other hand the concentrations of 10^6 cfu ml^{-1} at the *S. tartara* was found 100% effective at the same applications 12th days after application. The effect of the other applications (spraying applications the surface of the plates with without roaches) was found below 40% for both cockroach species 12th days after application.

Studies were performed by using *Purpureocillium lilacinum* TR1 against stored product pests. In one of the studies, Tülek et al., (2015) reported that *P. lilacinum* was the most effective against *S. oryzae and then R. dominica* with the rate of 77.9 and 97.6 %, respectively in 25°C, 10^8 cfu ml^{-1} concentration and on 10^{th} day among trials performed against *Tribolium castaneum*, *T. confusum* (Coleoptera: Tenebrionidae), *Sitophilus oryzae*, *S. granarius* (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae). The same isolate was used against two important pests of potato [*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) ve *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomalidae)] and the effect was determined as 33.2 % for *L. decemlineata* and 43 % for *P. operculella* (Kepenekci et al., 2015a). In another

study, *P. lilacinum* TR1 isolate was used in three different concentrations (10^6, 10^7 ve 10^8 cfu ml $^{-1}$) and two different temperature (15 and 20°C) against *Acanthoscelides obtectus* Say, which is an important stored product pest in Turkey. The highest effect was recorded as 47.87% in 10^8 cfu ml^{-1} concentration. The researchers reported that the effect does not exeed 35% in 15°C (Kepenekci et al., 2015b). Additionally, Kepenekci et al., (2015c) has investigated the effectiveness of *P. lilacinum* TR1 against *Myzus cerasi* Fabricus (Hemiptera: Aphididae) adults and the highest effect was recorded as 83.64 % in 25°C and 10^8cfu concentration on 8th day.

CONCLUSION

In this study, the effects of the entomopathogenic fungus, *Purpureocillium lilacinum* TR1 (syn: *Paecilomyces lilacinus*), against the nymphs of cockroaches [Oriental cockroach (*Blatta orientalis* Linnaeus) and Turkestan cockroach (*Shelfordella tartara* Sauss)] were investigated under laboratory conditions.

It has been reported that the whole population was dead and the effect was very high in the first study performed on the effectiveness of *Purpureocillium lilacinum* against cochroaches in Turkey. It would be more suitable to perform more detailed studies especially to be used in applications for indoor spaces and packing houses.

#This study was presented in the 6th Entomopathogens and Microbial Control Congress and published as short summaries.

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Table 1. Mean adult mortality (%±SEM) of *cockroaches (Blatta orientalis and Sterfolla tartara)* exposed for 12th days with *Purpureocillium lilacinus* TR1 (isolated from Turkey) (10^6, 10^7 and 10^8cfu ml^{-1} concentration).

Cockroaches	Treatment	Doze (cfu ml^{-1})	Mortality (%)
Blatta orientalis	APR.*	10^6	100±0,00 a
		10^7	100±0,00a
		10^8	90,75±7,00a
	BFR*.	10^6	26,19±1,61b
		10^7	40,00±0,00b
		10^8	33,00±1,61b
	Control	Control	32,9±1,61b
Sterfolla tartara	APR.	10^6	100±0,00a
		10^7	73,79±1,61abc
		10^8	80,57±14,83ab
	BFR.	10^6	26,20±1,61bc
		10^7	13,94±11,72bc
		10^8	8,71±6,99c
	Control	Control	46,65±10,31bc

Blatta orientalis (F= 38,70; df: 6,19; P<0.05) *Sterfolla tartara* (F= 8,81; df: 6,19; P<0.05)
*AFR: after putting in roaches. * BFR : before putting in roaches.