

**PATHOGENICITY OF THE ENTOMOPATHOGENIC FUNGUS,  
PURPUREOCILLIUM LILACINUM TR<sub>1</sub> AGAINST THE BLACK  
CHERRY APHID, MYZUS CERASI FABRICUS  
(HEMIPTERA: APHIDIDAE)**

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**ABSTRACT:** *Myzus cerasi* (Fabricus) as a cosmopolitan species chooses plants from such families as Cruciferae, Plantaginaceae, Rosaceae, Rubiaceae and Scrophulariaceae as hosts. This pest species, which is widespread in our country, causes curling and distortion of the leaves by establishing large colonies on growing shoots. Besides, they cause the growth of black sooty fungi on account of the honeydew they secrete. It is known that this species can transmit several viruses including *Bean yellow mosaic virus* (BYMV), *Celery mosaic virus* (CeMV) and *Onion yellow dwarf virus* (OYDV) in a non-persistent manner. In this study, it is searched the effects of entomopathogenic fungi, [*Purpureocillium lilacinum* TR<sub>1</sub> (syn.: *Paecilomyces lilacinus*)] on the black cherry aphid (BCA) adults in three conidial suspension ( $10^6$ ,  $10^7$  and  $10^8$  cfu ml<sup>-1</sup>) and at two different temperatures (15 and 25°C) in laboratory conditions. The data for mortality was recorded after 2, 4, 6, 8 and 10 days intervals. At the end of the study highest effect was found at 25 °C  $10^8$  cfu ml<sup>-1</sup>. The mortality rate were found respectively after 6 and 8 days %73,48, %83,64 and the data was remained stable after 10 days. It will be appropriate that the results obtained from this first study performed in laboratory conditions should also to be tried in the field conditions for the control of *M. cerasi*. It is hoped that the study will be helpful in the control strategies of this aphid species that will be put forward in a future time. It is seen that after having obtained hopeful results from this study, making similar works on the other aphid species are also necessary.

**KEY WORDS:** Entomopathogenic fungi, *Purpureocillium lilacinum*, Black Cherry Aphid, *Myzus cerasi*.

Black cherry aphid (BCA) sucks sap from the foliage of cherries of *Prunus cerasus*, *P. avium* and *P. padus* during late spring and early summer. BCA feeds on the undersides of the leaves and the shoot tips. After the feeding, leaves become severely crumpled and curled and damaged leaves may dry up and turn brown. They are excreted honeydew then foliage becomes sticky with the sugary and a black sooty mould may develop (Anonymous, 2014).

*Myzus cerasi* is widespread in our country causes the growth of black sooty fungus on account of the honeydew they secrete. This aphid is considered to be the most important vector of plant viruses throughout the world. (Kennedy et al., 1962; Namba & Sylvester, 1981; Blackman & Eastop, 1984, 2000).

Chemical pesticides have been the main control methods for these pests in crop production both world and Turkey. *M. cerasi* has got natural enemies but, these do not adequately control high populations. Several mycopenicides have been developed and used in several countries. An entomopathogenic fungus can act as a parasite of insects and kills or seriously disables them. Naturally

occurring entomopathogenic fungi (EPFs) are considered to be one of the best alternative to existing chemicals (Hajek & Leger, 1994). Entomopathogenic fungi such as *Lecanicillium* sp. (Jung et al., 2006), *Beauveria bassiana* (Quesada et al., 2006; Wakil et al., 2011a), *Metarhizium anisopliae* (Wright et al., 2004), *Paecilomyces* spp. (Shia & Feng, 2004) and *Nomuraea rileyi* (Devi et al., 2003; Wakil et al., 2011b) are being used for the control of aphids, mites and other insect pests.

*Purpureocillium lilacinum* (Thom) Luangsaard, Hywel-Jones, Houbraeken and Samson (syn: *Paecilomyces lilacinus*) (Sordariomycetes: Hypocreales) is a soil fungus with a good potential for biological control. This species has been described as being as efficient as the commonly used nematicides (Dube & Smart, 1987; Schenck, 2004; Mendoza et al., 2007); it is also a controller of insects (Posada et al., 1998; Suh et al., 2002; Gökçe & Er, 2005; Wakil et al., 2012) and others arthropods (Fiedler & Sosnowska 2007; Shin et al., 2011; Angelo et al., 2012). According to Bellows (2001), Headrik & Goden (2001) and other authors, the use of entomopathogenic fungi is an excellent method for the biological control of insects. Entomopathogenic fungi have been used successfully to control aphids, according to Milner (1997) *Verticillium lecanii* isolates controlled aphids on chrysanthemum and whitefly on cucumber and tomato.

After Entomopathogenic Fungus, *Purpureocillium lilacinum* TR1 was determined and identified (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 *in vivo* and in *in vitro* studies had to be done (Kepenekci, et al., 2013b,c; Kepenekci et al., 2014a,b).

*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).

In diagnosis of *P. lilacinum* TR1, DNA extraction was performed using DNeasy Blood and Tissue Kit (Qiagen, Germany), and 123 bp bands were attained using primer set specific to species. By the same study, this fungus was defined in details (morphologically and morphometrically) (Kepenekci et al., 2013a). Also, cDNA sequences of PCR products attained using general ITS primers were derived, and these cDNA sequences were compared with NCBI data. cDNA sequences of the isolate diagnosed molecularly were entered in GenBank and their access number were received. The isolate was stored in GenBank.

In this paper, we evaluated the control potential of *P. lilacinum* TR1 against adults of *Myzus cerasi* in the laboratory.

## MATERIALS AND METHODS

### Fungi Sources

The culture of *Purpureocillium lilacinum* TR1 was provided by the Plant Protection Central Research Institute, Ankara, Turkey. It was firstly isolated from the eggs of root-knot nematode [*Meloidogyne incognita* (Kofoid & White)] collected from the greenhouse in Sarıcakaya (Eskişehir, Turkey) (Kepenekci et al., 2009; Kepenekci et al., 2013a).

## **Insect Sources**

*Myzus cerasi* adults were obtained from the laboratory colony maintained at the entomology division, Plant Protection Central Research Institute in Ankara, Turkey. *M. cerasi* adults were collected from the plums orchard from Ankara (Turkey).

## **Mass-Culturing of *Purpureocillium lilacinum***

The fungi was subcultured on Potato Dextrose Agar with the help of sterilized bacteriological loop and the plates were closed by parafilm at  $25\pm 1^\circ\text{C}$  for 14 days. The conidia were harvested using sterilized rubber loop attached to 1 ml borosilicate pipette at the angle of  $45^\circ$ . The scraped material was shifted into sterilized petri plates and stored at  $4^\circ\text{C}$  in refrigerator. The harvested fungal conidia were incorporated in to sterile 0.05% Tween-80 solution and the material were stirred for complete homogeneity.

The serial dilutions were prepared and the number of conidia was measured to achieve the  $10^6$ ,  $10^7$  and  $10^8$  cfu  $\text{ml}^{-1}$  concentration under haemocytometer.

## **Effect of Temperature and Fungi Concentration on Mortality of *Myzus cerasi* Adults**

### **Detached leaf-disc bioassay**

The detached leaf disc method was adopted and the healthy cherry leaf discs (5cm in diameter) were placed in 9cm petri-plates (Wakil et al., 2012). The leaf discs were dipped in *P. lilacinum* conidial suspension ( $10^6$ ,  $10^7$  and  $10^8$  cfu  $\text{ml}^{-1}$ ) for treatments. The leaf discs were air-dried in the clean bench and at room temperature for 1 hour. The leaf discs treated with 0.05% Tween-80 served as control. Each petri plate with leaf discs was provided with moistened filter paper on the bottom with 1cm hole on lid covered with fine mesh for aeration. Ten black cherry aphid were released in each petri plate using camel's hair brush and the petri plates were then placed in growth chamber maintained at 15 and  $25^\circ\text{C}$  and  $>70\%$  relative humidity at 16L:8D photoperiod. The data for mortality was recorded after 2, 4, 6, 8 and 10 days intervals. Each treatment and bioassay was repeated independently for three times. Dead individuals were removed and considered as dead if they did not move when prodded with needle. The dead *M. cerasi* were examined under microscope to determine whether mortality was because of entomopathogenic fungus, and micelle development was checked. When required, these cadavers were placed in petri dishes to follow up potential mycosis development (Fig. 1).

### **Statistics**

One-way ANOVA was used to compare the mortality of *M. cerasi*. 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**

The data generally showed that all concentration were effective against the *M. cerasi* adults (Figs. 2, 3). When we look overall results of this study, at  $25^\circ\text{C}$ ,  $10^8$  cfu concentration had the highest effect on the 8<sup>th</sup> day. This effect cannot show any changes on the tenth day and remained constant. This effect is 2.5 times of a lower concentration ( $10^7$  cfu) (to 83,64% from 33,25%) (Figs. 2, 3).

When it was compared to effect of the concentrations used ( $10^6$ ,  $10^7$  ve  $10^8$  cfu) according to temperature ( $15^\circ\text{C}$  and  $25^\circ\text{C}$ ), at the end of the 2<sup>nd</sup> and 4<sup>th</sup> days at  $15^\circ\text{C}$  in  $10^6$  cfu concentration, the effect did not reach 10% (1,14% and 6,69% ). At the end of the 6<sup>th</sup> and 8<sup>th</sup> days, effect increased slightly and at tenth day remained constant (13,01%, 19,99% and 19,99%) ( $F= 6,78$ ;  $df=5,17$ ;  $P<0.003$ ). At  $10^7$

concentration, at the same temperature on 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> days, the effect increased in a small amount (4,53, 13,01 and 16,35%), at the end of the 8<sup>th</sup> day, the effect was recorded as 19,99%. At the end of the tenth day, effect has emerged to 23,17% with very little an increase. At this concentration, between 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> days, statistically a significant difference wasn't found ( $F= 12,28$ ;  $df=5,17$ ;  $P<0.000$ ). The situation is similar in the highest concentration ( $10^8$ cfu), but on the 8<sup>th</sup> day mortality reached the highest value (39,85%) and *M. cerasi* death has not been observed on the 10<sup>th</sup> day and the death rate has remained stable. At this concentration, between 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> day, statistically a significant difference wasn't found ( $F= 8,44$ ;  $df=5,17$ ;  $P<0.001$ ). At the end of the experiment, in  $10^6$  ve  $10^7$  concentrations (the 10<sup>th</sup> day counts  $10^7$  concentrations excluding) effect cannot over of 20%.

Analyzing trial established at 25°C, *P. lilacinum* has caused more deaths in adults of *M. cerasi*. When we look at the concentrations applied,  $10^6$ cfu was found to have minimal influence again (mortality didn't rise above 30%) and mortality above of 20% was recorded on the 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days (23,17%, 26,52% and 29,99%). At this concentration, between 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> days, statistically a significant difference wasn't found ( $F= 18,20$ ;  $df=5,17$ ;  $P<0.000$ ). At  $10^7$ cfu concentration, effect has outpaced over an amount of 30% on the only 10<sup>th</sup> day (33,25%). At this concentration, between 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> days, statistically a significant difference wasn't found ( $F= 9,03$ ;  $df=5,17$ ;  $P<0.001$ ). At  $10^8$  concentration after from 4<sup>th</sup> day mortality in *M. cerasi* increased significantly. At the end of the 8<sup>th</sup> day mortality rate reached to 83,64% and at the 10<sup>th</sup> day remained constant. At this concentration, with 2<sup>nd</sup> and 4<sup>th</sup>, between 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days statistically a significant difference wasn't found ( $F= 44,39$ ;  $df=5,17$ ;  $P<0.000$ ). At this temperature the end of the experiment, concentrations of  $10^6$  and  $10^7$  (counts of the 10<sup>th</sup> day except at concentration of  $10^7$ ) mortality didn't rise above 30% (Fig. 2B).

When it was evaluated according to the time (2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days) effects of the application concentrations ( $10^6$ ,  $10^7$  and  $10^8$ cfu), in the counting of the 2<sup>nd</sup> day; at 15°C at  $10^6$  and  $10^7$ cfu concentrations mortality didn't rise above 5% (1,14% and 4,53%) (Fig. 3A), at the same concentrations at 25°C mortality didn't rise above 10% (4,53 and 9,99%) (Fig. 3B). At 15°C and 25°C, at  $10^8$ cfu, deaths, in the end of the 2<sup>nd</sup> day, was recorded as 26,52% and 15,72% ( $F= 3,98$ ;  $df=3,11$ ;  $P<0.053$ ) (Fig. 3). At the end of the 4<sup>th</sup> day at concentration of  $10^8$ cfu the death rate was found (33,25% and 22,45%) higher than other concentrations in 15°C and 25°C. Same day, in other concentrations mortality of *M. cerasi* increased with the temperature but it did not exceed 20%. At end of the 4<sup>th</sup>day counts statistically a significant difference wasn't found ( $F= 6,67$ ;  $df=3,11$ ;  $P<0.014$ ) (Fig. 3). In the 6<sup>th</sup> and 8<sup>th</sup> days at 15°C, at concentrations of  $10^6$  and  $10^8$ cfu mortality rates remained constant (13,01 and 33,25) (Fig. 3A); at 25°C at concentrations of  $10^6$  and  $10^7$ cfu mortality rates remained virtually unchanged. But it increased in  $10^8$ cfu from 33,25% to 73,48% (Fig. 3B) ( $F= 45,07$ ;  $df=3,11$ ;  $P<0.000$  and  $F= 79,01$ ;  $df=3,11$ ;  $P<0.000$ ). At the end of the 10<sup>th</sup> day at 15°C at concentrations of  $10^6$  and  $10^7$ cfu, death rates remained unchanged (19,99%), in  $10^8$  was recorded as 39,85% (Fig. 3A). At the end of the same day at 25°C at concentrations of  $10^6$  and  $10^7$ cfu mortality was recorded as 33,25% and 29,99%. At  $10^8$  mortality was found as 83,64% ( $F= 15,13$ ;  $df=3,11$ ;  $P<0.001$ ) (Fig. 3B). At 25°C, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days, concentrations of  $10^6$  and  $10^7$ , a statistically significant difference wasn't found (Fig. 3B). At the end of the trial, at the same temperature effect increased in parallel to the increase of time exposure. This rise

was recorded as the highest at  $10^8$  concentration and on 6<sup>th</sup> day (from 33,25% to 73,48) (Fig. 3B).

The data regarding the effect of different treatments on the aphid in cherry leaf discs at different intervals showed highly significant difference (Fig. 2, 3). The maximum mortality 83,64% of aphid was recorded in 8<sup>th</sup> day and  $10^8$ cfu ml<sup>-1</sup> concentration of 25°C. The lowest effect on aphid was recorded  $10^6$ cfu ml<sup>-1</sup> concentration 2<sup>nd</sup> and 4<sup>th</sup> day;  $10^7$ cfu ml<sup>-1</sup> concentration 2<sup>nd</sup> day; 1,14%, 6,69% and 4,53% of 15°C, respectively (Fig. 2A, 3A). It is observed that the effectiveness of all the treatments showed an increasing trend up to 10 days of post application.

## DISCUSSION

The entomopathogenic fungi are extensively evaluated for the control of aphid on various crops as Steinkraus (1999) controlled cotton aphid by applying aerial conidia of *Neozygites fresenii*.

The significant control of aphid on cotton seedlings was also observed by the application of *Colletotrichum orbiculare* (Russo et al., 1997) which is in line with the present work. Similarly, Kim et al. (2008) tested the effectiveness of the commercial formulation of *Lecanicillium longisporum* (Vertalec) for the control of cotton aphid and reported the noteworthy reduction in the aphid number compared to untreated control.

The pathogenicity of different isolates of *Beauveria bassiana*, *Paecilomyces* spp. and *Lecanicillium attenuatum* were evaluated against cotton aphid (Kim & Kim, 2008) where mortality reached up to 100% after 5 days when treated either with conidia or blasto-spores of the fungi.

Wakil (2012) tested the efficacy of *Paecilomyces lilacinus* ( $2.3 \times 10^9$  conidia ml<sup>-1</sup>), *Azadirachta indica* (10ml L<sup>-1</sup>) and the formulation of diatomaceous earth (PyriSec) (DE) (3g L<sup>-1</sup>) for the control of cotton aphid, *Aphis gossypii* (Insecta: Homoptera: Aphididae) both under laboratory and semi-natural conditions. All the tested treatments gave significant control of aphid; however, *P. lilacinus* in combination with Neem showed the best control of aphids in detached leaf bioassay and semi-natural conditions. The applications of *P. lilacinus* and DE showed weak knock down effect on the insect pest. Furthermore, an increasing trend in mortality of aphids was observed in all the treatments with an increase in the time intervals. The results of the study clearly indicated that the *P. lilacinus* may give effective control of the aphids in combination with other eco-friendly agricultural practices.

According to Özçelik et al. (2013) at  $10^8$ cfu concentration, while *Isaria farinosa* caused 47% mortality of green peach aphid, *Myzus persicae* in 75% humidity, *Purpureocillium lilacinum* caused 96% mortality of same aphids in 95% humidity. Similarly in our study, the highest effect was found at 25°C,  $10^8$  cfu ml<sup>-1</sup>. In the another study, Satar & Koç (2004), emphasized that at 25°C *Fusarium subglutinans* caused significantly mortality.

The data generally showed that all fungal concentration had higher effect at 25°C than 15°C against the *M. cerasi* adults. At 15°C, no entomopathogenic fungi caused over than 40% mortality. Ansari et al. (2004) also found that mortality depend on the concentration of conidial suspension, exposure time and temperature. The another study relevant to effect of different conidial concentrations *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae* against *Brevicoryne brassicae* L. showed that aphids mortality increased with increase in spore concentration and exposure time (Asi et al., 2009). In another study, virulence of *Beauveria bassiana* against *Myzus persicae*

was examined, and the results showed that aphids had higher mortality rates at 28 and 21°C than those at 16 and 11°C (Yinquan et al., 2000). These results were similar to our study.

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Figure 1. *Myzus cerasi* adults infected by entomopathogenic fungus, *Purpureocillium lilacinum* TR<sub>1</sub> (syn: *Paecilomyces lilacinus*).

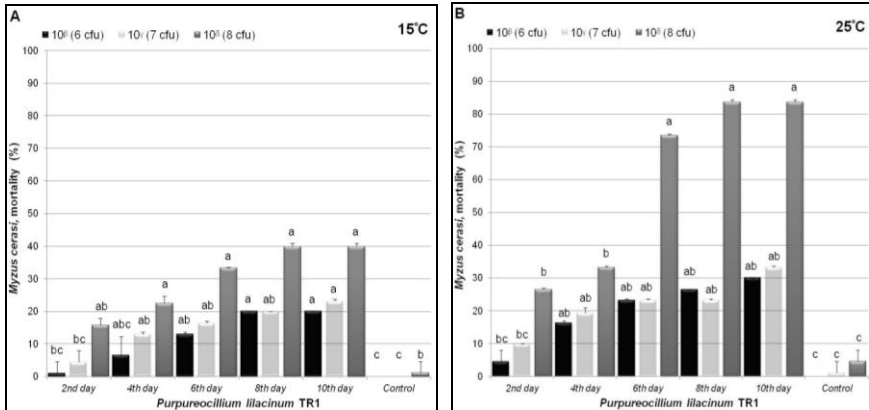


Figure 2. Mortality (%) of *Myzus cerasi* adults following application of entomopathogenic fungi *Purpureocillium lilacinus* TR1 (isolated from Turkey) at 10<sup>6</sup>, 10<sup>7</sup> and 10<sup>8</sup>cfu ml<sup>-1</sup> concentration at different temperatures [15°C (A) and 25°C (B)]. Data are expressed as mean±SEM. The same letter above the error bars indicates no significant difference (P>0.05; Tukey test).

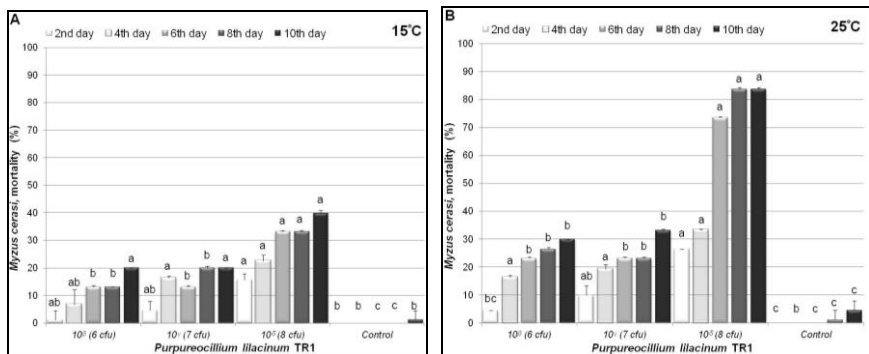


Figure 3. Mean adult mortality (%±SEM) of *Myzus cerasi* exposed for 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days on detached leaf discs treated with *Purpureocillium lilacinus* TR1 (isolated from Turkey) (10<sup>6</sup>, 10<sup>7</sup> and 10<sup>8</sup>cfu ml<sup>-1</sup> concentration) at different temperatures [15°C (A) and 25°C (B)] (means followed by the same letters are not significantly different at P=0.05).