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In Vitro Antagonistic Activity of Entomopathogenic Fungi Against *Phytophthora infestans*

Işılay AKÇA[1], Şerife ACAR[1], Zeliha Çağla TARAKÇI[1], Ali SEVİM[1*]

**Corresponding Author: ali.sevim@ahievran.edu.tr*

[1] Department of Plant Protection, Faculty of Agriculture, Kırşehir Ahi Evran University / akcaisilay5@gmail.com
[1] Department of Plant Protection, Faculty of Agriculture, Kırşehir Ahi Evran University / srfe42.acar@gmail.com
[1] Department of Plant Protection, Faculty of Agriculture, Kırşehir Ahi Evran University / z.cagla8640@gmail.com
[1] Department of Plant Protection, Faculty of Agriculture, Kırşehir Ahi Evran University / ali.sevim@ahievran.edu.tr

Abstract: Potato downy mildew is a disease caused by a fungus called *Phytophthora infestans* (Mont.) de Bary, which is mainly seen in potatoes and tomatoes, but also in other culture and wild plants belonging to the Solanaceae family. This fungus is quite important both from an economic and historical point of view. In case of an epidemic, it can cause full crop deterioration or serious losses in potatoes. Although cultural and chemical control methods are generally used in the control of the disease, there is no specific biological control method in this regard. Entomopathogenic fungi (EPFs) are generally used as biological control agents in the control of insect pests. Recently, there are many studies showed that these fungi can live endophytically on various cultivated plants and provide beneficial properties to the plants they live with. In this study, it was aimed to determine the *in vitro* antagonistic activities of different entomopathogenic fungi previously isolated from potato fields and identified by molecular methods against *P. infestans*. Earlier work determined that these fungi had a lethal effect on *Leptinotarsa decemlineata* (Say, 1824). Antagonistic activity tests were performed according to the "direct opposition method" and percentage (%) activity values were calculated. As a result of the tests, the most effective isolates were found to be *Beauveria* sp. SK-14 (75.23%) and *Metarhizium* sp. SK-24 (76.23%). It is thought that the results obtained will contribute to the biocontrol of diseases and pests in potatoes.

Keywords: Entomopathogenic fungi, Potato downy mildew, Antagonistic activity, Biological control

Phytophthora infestans'a Karşı Entomopatojenik Fungusların *In Vitro* Antagonistik Aktivitesi

Öz: Patates mildiyösü *Phytophthora infestans* (Mont.) de Bary adı verilen bir fungus tarafından oluşturulan başta patates ve domates olmak üzere Solanaceae familyasına ait diğer kültür ve yabani bitkileri de görülen bir hastalıktır. Bu fungus hem ekonomik hem de tarihsel açıdan oldukça önemlidir. Salgın durumunda patateste tam mahsul bozulmasına veya ciddi kayıplara neden olabilmektedir. Hastalığın mücadelesinde genellikle kültürel ve kimyasal mücadele yöntemleri kullanılmakla beraber bu konuda spesifik bir biyolojik mücadele yöntemi bulunmamaktadır. Entomopatojenik funguslar ise zararlı böceklerle mücadelede biyolojik mücadele etmeni olarak yaygın bir şekilde kullanılmaktadır. Son zamanlarda ise bu fungusların çeşitli kültür bitkileri üzerinde endofitik olarak yaşayabildiğini ve birlikte yaşadığı bitkilere faydalı özellikler sağladığına dair birçok çalışma bulunmaktadır. Bu çalışmada ise daha önceden patates tarlalarından izole edilen ve moleküler yöntemlerle tanımlanan çeşitli entomopatojenik fungusların *P. infestans*'a karşı *in vitro* antagonistik etkilerinin belirlenmesi amaçlanmıştır. Daha önce yapılan çalışmada bu fungusların *Leptinotarsa decemlineata* (Say, 1824) üzerinde öldürücü etkiye sahip olduğu belirlenmiştir. Antagonistik aktivite testleri "direct opposition method" yöntemine göre yapılmış ve yüzde (%) aktivite değerleri hesaplanmıştır. Testler sonucunda en etkili izolatların *Beauveria* sp. SK-14 (%75.23) ve *Metarhizium* sp. SK-24 (%76.23) olduğu tespit edilmiştir. Elde edilen sonuçların patatesteki hastalık ve zararlılarının biyolojik mücadelesine katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: Entomopatojenik fungus, Patates mildiyösü, Antagonistik aktivite, Biyolojik mücadele

Introduction

Potato downy mildew caused by *Phytophthora infestans* (Mont) de Bary (Mildiyö) is one of the best-known, the most studied and still one of the most important crop loss factors of all potato diseases in potato production all over the world (Kassa & Hiskias, 1996; Jones, 1998). Worldwide losses in potato production due to this disease are estimated to exceed $5 billion per year, and therefore this pathogen is considered a threat to global food security (Latijnhouwers et al., 2004; Sesli et al., 2020). In our country, this disease is widely seen in all regions of Turkey, including the microclimate areas of the Central Anatolia region. Cultural and chemical control methods are widely used against this disease. In the cultural control, various methods such as removing diseased piles, removing them from the fields and preferring various storage practices are used (T.C. Ministry of Agriculture and Forestry, 2017). In the chemical control, the use of various fungicides such as metalaxly is recommended. However, it has been recently reported that some fungicide-resistant strains have been developed due to the use of fungicides (Matson et al., 2015). In addition, the side effects of various chemicals used in agriculture and forestry on the environment, human and animal health have been known issue for a long time. For this, it is preferred to use safer methods such as biological control will bring solutions in the long term.

Insect pathogenic fungi are a considerable agent in the natural control of insect pests and some arthropods, and these fungi often cause epizootics covering large areas in insect pest populations. These fungi have been used as microbial control agents for over 200 years. In general, many insect orders are susceptible to fungal diseases, and entomopathogenic fungi have good potential as microbial control agents against insect pests (Roberts, 1989). Many entomopathogenic fungi infect their hosts directly from the cuticle and therefore do not need to be eaten by their hosts. This feature makes entomopathogenic fungi a leading candidate in the control of insect pests that feed on plant sap or animal blood. Today, there are many commercial preparations from entomopathogenic fungi around the world, and they are used against various pests in both agriculture and forestry (Goettel et al., 2005). So far, 700 species of entomopathogenic fungi belonging to at least 90 genera have been described, and some species, such as *Beauveria bassiana* (Bals.) Vull. (Böcekküfü), *Metarhizium anisopliae* (Metch) Sorok (Böceksaran), *Isaria fumosorosea* (Wize) Brown (Hoşnut izarya) (=*Paecilomyces fumosoroseus*) and *Verticillium lecanii* (Zimm.) (Şamdan küfü), are commercially produced and used in many countries in the control of many pests (Rath, 2000; Sesli et al., 2020). For example, *B. bassiana* is used in Brazil against the banana root borer *(Cosmopolites sordidus* (Germar)), in China against the pine caterpillar *(Dendrolimus* spp.), and in Europe against aphids and European corn borer (*Ostrinia nubilalis* Hub.) (Goettel et al., 2005; Sevim et al., 2015).

In addition to the use of entomopathogenic fungi as microbial control agents against harmful insect species, recent studies have also shown that these fungi can live endophytically and epiphytically with plants (Vega et al., 2008). The term endophyte was first introduced by the German scientist Heinrich Anton De Bary (1884) and is now defined as bacteria or fungi that live within plant tissues and do not cause any visible symptoms in the plant (Wilson, 1995). Fungal endophytes of many plants have been identified in studies so far, and most of these plants include agriculturally important crops such as wheat, bananas, and tomatoes (Watts et al., 2023). These fungal endophytes are also known to protect plants against insects, pathogens, and nematodes (Vega, 2018). Apart from this, today, fungal endophytes have become a point of interest in biotechnology due to their potential to be used as genetic vectors, secondary sources of metabolites and use as biological control agents (Ting et al., 2021; Larran et al., 2007; Hubbard et al., 2013).

So far, some studies have determined that various entomopathogenic fungi showed antifungal activity against fungal disease agents. Of these, it has been determined that various entomopathogenic fungi, especially *B. bassiana*, showed anti-fungal activity against *Rhizoctonia* sp. (Dalindiren) and *Botrytis cinerea* (Pers.) & F (Kurşuni küf), which cause disease in potatoes (Barra-Bucarei et al., 2019; Tomilova et al., 2020; Sesli et al., 2020). In this study, the *in vitro* antagonistic effects of various entomopathogenic fungi previously isolated from potato fields against *P. infestans* was investigated. It is thought that the results obtained are useful in the biological control of potato downy mildew disease.

Material and Metod

Antagonistic Activity of Entomopathogenic Fungal İsolates

The *in vitro* antagonistic activity of entomopathogenic fungal isolates (24) obtained from potato fields against *P. infestans* was determined. The entomopathogenic fungal isolates were both morphologically and molecularly identified in our previous study (Keçili et al., 2022). The antagonistic avtivities of the entomopathogenic fungal isolates were determined according to the "direct opposition method" defined by Dennis and Webster (1971). Briefly, a disc of P. *infestans* mycelium actively growing on PDA medium with a diameter of 5 mm was taken and placed at a distance of 1 cm away from the approximate edge of the 120 mm PDA medium. For entomopathogenic fungal isolates, an actively growing mycelium disc of the same size (5 mm) was taken and placed 1 cm away from the opposite side of the petri dish. These petri dishes were then be left to incubate at 28°C and in the dark. Only *P. infestans* mycelium disc was present in the control group. To calculate the percentage of inhibition, the radial growth of fungi in both the control group and the inhibition tests were measured by caliper on day 10. All testes were repeated three times on different occasion. The percentage of inhibition was calculated using the following formula: (Royse & Ries, 1977; Landum et al., 2016).

$$I\ (\text{Inhibition percentage}) = \left(\frac{\text{R1 (colony radius in control)} - \text{R2 (colony radius in test)}}{\text{R1}}\right) \times 100$$

As a result of the experiments, the interaction between the two fungi was expressed on a scale from 1 to 4 (Dharmaputra, 2003). 1; growth inhibition of *P. infestans* after contact with entomopathogenic fungi, 2; *P. infestans* interbreeding with entomopathogenic fungi, but growth is slow and at varying rates, 3; Mutual inhibition in the range of less than 0.2 cm and 4; mutual inhibition greater than 0,2 cm.

Statistical Analysis

The percentage data calculated from the antagonistic activity tests was analyzed using Minitab 17 software. The difference among the entomopathogenic fungal isolates in terms of percentage (%) inhibition against *P. infestants* was determined ANOV followed by Tukey test to make multiple comparision. All data was tested using Levene statistics with respect to variance homogeneity.

Results

Based on ANOVA analysis, all isolates caused different antagonistic activities against *P. infestans* (F=3,47, df=23, p<0,05). The highest activities were obtained from *Beauveria* sp. SK-14 (75,23%) and *Metarhizium* sp. SK-24 (76,23%) (Figure 1). The other antagonistic activitiy values were ranged from 48,4% to 70,23%. All activity values, isolates and scale values were given on table 1.

Discussions

The use of microbial populations as biological control agents is not only effective against insect pests. but also safe for the environment. human health. and non-target organisms. In this sense. the controlling of pest populations with entomopathogenic fungi (such as *Beauveria* and *Metarhizium)* is an alternative method to chemical insecticides and one of the most desirable agricultural practices (Sharma & Sharma. 2021). Recently. it has been shown that these fungi can live endophytically with various agriculturally important plants (Quesada Moraga, 2020). These entomopathogenic fungal endophytes have also been shown to protect plants against both insect pests and various plant pathogens (Mantzoukas & Eliopoulos. 2020; Bamisile et al. 2021). In a previous study. various entomopathogenic fungi were obtained from potato fields and found to have good lethal effects on *L. decemlineata* (Keçili et al., 2022). In this study. the antagonistic effects of 24 previously isolated entomopathogenic fungal isolates against *P. infestans*, one of the most important disease agents of potatoes, were examined.

Table 1. The *in vitro* antagonistic activity of the entomopathogenic fungal isolates against *P. infestans* and their scale values.

No	Species	Isolate	The percentage inhibition ± standard error	Scale value
1		SK-1	62.30 ± 3.72	1
2		SK-5	67.07 ± 2.06	1
3		SK-8	69.45 ± 3.94	1
4		SK-12	56.25 ± 2.49	3
5	*Beauveria* sp.	SK-14	75.23 ± 3.80	4
6	(Böcekküfü)	SK-16	66.93 ± 3.17	1
7		SK-17	54.35 ± 1.89	1
8		SK-28	62.49 ± 9.44	4
9		SK-40	60.72 ± 11.79	3
10		SK-45	63.33 ± 2.72	4
11		SK-3	64.20 ± 0.43	4
12		SK-9	61.33 ± 3.94	4
13		SK-10	48.40 ± 18.9	4
14		SK-15	60.89 ± 1.27	4
15		SK-21	61.71 ± 1.09	1
16		SK-22	66.49 ± 3.92	4
17	*Metarhizium* sp.	SK-24	76.23 ± 3.16	4
18	(Böceksaran)	SK-27	59.81 ± 7.53	4
19		SK-29	56.44 ± 1.83	4
20		SK-37	64.63 ± 3.08	4
21		SK-42	62.14 ± 1.55	4
22		SK-47	62.93 ± 2.25	4
23		SK-49	63.56 ±0.11	4
24		SK-50	70.23 ± 0.69	4

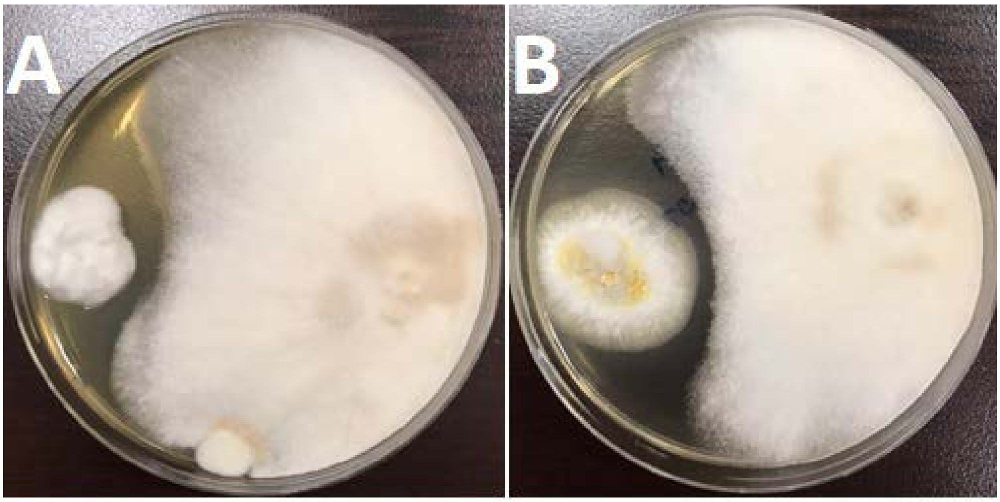

Figure 1. The antagonistic activity pictures of the most effective isolates against *P. infestans*. A; *Beauveria* sp. SK-14 (75.23%). B; *Metarhizium* sp. SK-24 (76.23%).

Many previous studies have shown that entomopathogenic fungal endophytes suppress various plant pathogens and reduce disease symptoms in plants. For example. *B. bassiana and M. brunneum* Petch. (Böcek Saran) can live endophytically in wheat and increase plant growth by suppressing *Fusarium culmorum* (WG) Smith Sacc (Başak küfü). In the same study, it has been also shown that they reduced the disease symptoms in wheat (Jaber, 2018; Sesli et al., 2020). Jaber & Alananbeh (2018) investigated the entophytic colonization of *B. bassiana* and. *M. brunneum* in sweet peper (*Capsicum annuum* L.) and showed that both fungal entomopathogens significantly inhibited all three *Fusarium* species in this pepper. Lozano-Tovar et al. (2017) investigated the antifungal activity of two entomopathogenic fungal isolates (*B. bassiana* and *M. brunneum*) against the olive pathogens *Verticillium dahliae* Kleb. (Avcı şamdan küfü) and *Phytophthora megasperma* Drechs. (Mildiyö) (Sesli et al., 2020). They found that the entomopathogenic fungus *M. brunneum* produces antifungal compounds that reduce the number of the pathogen propagules in the soil and the severity of *Verticillium* wilt. Kang et al. (2018) also investigated the biocontrol potential of *Isaria javanica* (Bally) Samson & Hywel-Jones (Gürbüz izarya) against aphids and plant fungal pathogens (*Fusarium oxysporum* (Fo) (Sebze küfü) and *Colletotrichum gloeosporioides* Penz. & Sacc. (Ağuantraknozu), *Phytophthora capsica,* Leonian (Biber mildiyösü) and *Rhizoctonia solani* Kühn. (Hırçın dalindiren) (Sesli et al., 2020). They found that *I. javanica* was pathogenic to aphids and had the antifungal activity against these four plant pathogens. Therefore. they suggested that this entomopathogenic fungus can be considered as a dual biocontrol agent against aphids and fungal diseases in red pepper. There are many more similar studies in the literature.

Our study is the first to include antifungal activity of entomopathogenic fungi against *P. infestans*. Our results suggest that the two most effective isolates identified in this study (*Beauveria* sp. SK-14 and *Metarhizium* sp. SK-24*)* can be used simultaneously for controlling both the potato beetle and *P. infestans.* However. to fully prove this. *in vivo* plant trials need to be conducted.

Author contributions

All authors have equal contribution

Conflict of interest

The authors declare that there is no competing interest

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Işılay AKÇA, Şerife ACAR, Zeliha Çağla TARAKÇI, Ali SEVİM).

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