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Original article (Özgün makale)

Indigenous Turkish entomopathogenic fungi as potential biological control agents of the Rose Aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae)

Derya BAKİ [1]*, Hilal Şule TOSUN[2], Fedai ERLER[2]

Gül Yaprakbiti, *Macrosiphum rosae* L. (Hemiptera: Aphididae)'nin potansiyel biyolojik mücadele etmenleri olarak yerel entomopatojen funguslar

Öz: Gül yaprakbiti *Macrosiphum rosae* L. (Hemiptera: Aphididae), Türkiye'nin yanı sıra dünyanın birçok yerinde güllerin (*Rosa* spp.) (Rosaceae) önemli zararlılarından biridir. Bu çalışmada 5 yerel entomopatojen fungus izolatı [3 *Beauveria bassiana* izolatı (BbDm-1, BbKm-1, BbMp-1), 1 *Isaria farinosa* izolatı (IfGp-1) ve 1 *Purpureocillium lilacinum* izolatı (PlKa-1)] laboratuvar koşullarında *M. rosae*'nin nimflerine ve erginlerine karşı test edilmiştir. İzolatların 10 günlük PDA (patates dekstroz agar) ortamında kültüründen elde edilen 3 farklı konsantrasyondaki ($1x10^7$, $1x10^8$ ve $1x10^9$ konidia/ml) spor solüsyonları *M. rosae*'nin hem nimflerine hem de erginlerine karşı püskürtülerek test edilmiştir. Sonuçlar, ölüm oranının konsantrasyona bağımlı olduğunu ve izolatların etkinliğinin artan spor konsantrasyonuyla arttığını göstermiştir ($P < 0.05$). Test edilen 5 izolattan, 2 *B. bassiana* izolatı (BbDm-1 ve BbKm-1) en yüksek konsantrasyonda (1×10^9 konidia/ml) uygulamadan 7 gün sonra sırasıyla nimflerde %100 ve %83.3 ve yetişkinlerde %96.7 ve %80.0 ölümlere neden olmuştur. Elde edilen sonuçlar, bu iki *B. bassiana* izolatının *M. rosae*'nin kontrolünde kullanılma potansiyeline sahip olduğunu göstermektedir.

Anahtar kelimeler: *Beauveria bassiana*, Entomopatojenik fungus, *Isaria farinosa*, *Macrosiphum rosae*, *Purpureocillium lilacinum*

Abstract: The rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae), is one of the important pests of roses (*Rosa* spp.) (Rosaceae) in many parts of the world, including Türkiye. In the present study, the pathogenic activity of five indigenous entomopathogenic fungal (EPF) isolates [three isolates of *Beauveria bassiana* (BbDm-1, BbKm-1, BbMp-1), one isolate of *Isaria farinosa* (IfGp-1) and one isolate of *Purpureocillium lilacinum* (PlKa-1)], were tested for their activity against nymphs and adults of *M. rosae* under laboratory conditions. Spore suspensions of three different concentrations (1×10^7, 1×10^8 and 1×10^9 conidia/ml) obtained from a 10-day old culture of the isolates on PDA (Potato Dextrose Agar) medium were tested by spray application against both nymphs and adults of *M. rosae*. The mortality rate was dose-dependent and increased with spore concentration of the isolates ($P < 0.05$). Of the five isolates tested, two *B. bassiana* isolates, BbDm-1 and BbKm-1, were the

[1] Tarım ve Orman Bakanlığı, Antalya Zirai Karantina Müdürlüğü, Antalya, Türkiye
[2] Akdeniz Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Antalya, Türkiye
* Corresponding author (Sorumlu yazar): derya.baki@hotmail.com
ORCID ID (Yazar sırasıyla): 0000-0003-1285-169X, 0000-0001-8360-2610, 0000-0002-7216-9871
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most pathogenic and caused mortalities of 100% and 83.3% in nymphs, and 96.7% and 80.0% in adults, respectively, at the highest concentration (1 x 10^9 conidia/ml), 7 days after treatment. Overall results suggest that these two *B. bassiana* isolates have the potential to be used in the control of *M. rosae*.

Keywords: *Beauveria bassiana*, Entomopathogenic fungi, *Isaria farinosa*, *Macrosiphum rosae*, *Purpureocillium lilacinum*

Introduction

Roses (*Rosa* spp.) (Rosaceae) are among the perennial ornamental plants widely grown across the world, with 200 known species and 18,000 varieties (Sastry et al. 2019). Roses are also used in the perfume and cosmetics industries, in the pharmaceutical industry due to their antibacterial and antioxidant properties, and in the food industry as additives, in addition to their use for landscaping purposes (Timor 2011; Özçelik 2013).

Rose plants are infested by many insect pests, especially rose aphids, thrips, whiteflies, and some hymenopteran and lepidopteran larvae. These pests cause considerable damage to rose plants, especially to buds, leaves and flowers. Therefore, rose yields are reduced by infestations of these insect pests. The rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) is one of the most important pests of roses in different parts of the world, including Türkiye. This species damages the aesthetic appearance of rosebushes by sucking on the sap of their flowers and foliage. Chemical, biological and cultural control methods are applied for the control of this insect (Budak et al. 2022). Especially in cutrose production systems in Türkiye, it is generally controlled by the use of synthetic chemical insecticides, with adverse effects on non-target organisms. The use of entomopathogenic fungi (EPF) in the control of arthropod pests is considered environmentally friendly, being safe for non-target organisms (Sáenz-de-Cabezón et al. 2003; İzgi & Güven 2016).

The aim of the present study was to evaluate the pathogenic activity of five indigenous Turkish entomopathogenic fungal isolates [three isolates of *Beauveria bassiana* (BbDm-1, BbKm-1, BbMp-1), one isolate of *Isaria farinosa* (IfGp-1) and one isolate of *Purpureocillium lilacinum* (PlKa-1)] against nymphs and adults of *M. rosae* under laboratory conditions.

Materials and Methods

Insect culture

The *M. rosae* used in the experiments were obtained from a laboratory culture that originated with field-collected adults from rose-growing areas in Antalya Province, Turkiye. Rearing was done in wire cageson the foliage of potted industrial oil rose plants (*Rosa damascena* Mill.) in a climate control room set to 25 ± 1°C, 60 ± 5 R.H. and 16 : 8 (L / D) hour photoperiod. Subsequent cultures were maintained to obtain

known age *M. rosae*. In this study, 0-48 h old nymphs and adults of *M. rosae* were used.

Indigenous entomopathogenic fungal isolates

Five local EPF isolates belonging to three fungal species, which had beenisolated from soil samples collected from agricultural areas in Antalya Province, Turkiye and maintained in the EPF Collection of the Plant Protection Department of Akdeniz University, Antalya, were tested in this study. Molecular identification of the isolates was made by Baki (2021) using the PCR (Polymerase Chain Reaction) method. Along with their accession numbers in the NCBI GenBank, species and code names, habitats, geographic coordinates and sampling sites of the isolates are presented in Table 1.

Table 1. List of the indigenous (Antalya Province, Turkiye), soil-borne entomopathogenic fungal isolates used in bioassays against the rose aphid, *Macrosiphum rosae*

Isolate (code) name	Species name	Origin	Vegetation	Latitude and longitude	NCBI accession number
BbDm-1	*Beauveria bassiana*	Demre	Orange	N 36°14'39.7" E 29°58'45.0"	MT441872
BbKm-1	*Beauveria bassiana*	Kumluca	Olive	N 36°19'17.1" E 30°20'23.0"	MT441868
BbMp-1	*Beauveria bassiana*	Muratpaşa	Fig	N 36°53'07.2" E30°44'30.4"	MT441880
IfGp-1	*Isaria farinosa*	Gazipaşa	Olive	N 36°14'50.3" E 32°21'19.2"	M T441902
PlKa-1	*Purpureocillium lilacinum*	Konyaaltı	Apple	N 36°53'53.5" E 30°37'51.8"	OM267784

Bioassays

In order to determine the pathogenicity of the EPF isolates against *M. rosae*, trials were conducted in the Entomology Laboratory of the Plant Protection Department of Akdeniz University in Antalya (Figure 1). The EPF isolates were grown in PDA (Potato Dextrose Agar) medium in an incubator set at 25±2°C for 10-14 days in a dark environment. Ten mL of sterile distilled water containing 0.1% Tween 80 was added on the developing fungi and conidia were obtained by scraping with a Dirigalski spatula (Fancelli et al. 2013). Spore solutions were passed through four layers of sterile cheese-cloth into 50 mL sterile glass flasks and agar and mycelial fragments were removed. The obtained spore solutions were homogenized by vortexing for 2-3 minutes (Abebe 2002). From the preliminary dose-testing studies, three different doses, which were determined to be highly effective, were used in the efficacy testing of the EPF isolates. Spore suspensionss of the isolates were prepared by counting on a Thoma Lam device and final concentrations of the fungal isolates

were adjusted to 1×10^7, 1×10^8 or 1×10^9 conidia/mL (Fancelli et al. 2013; Gabarty et al. 2014).

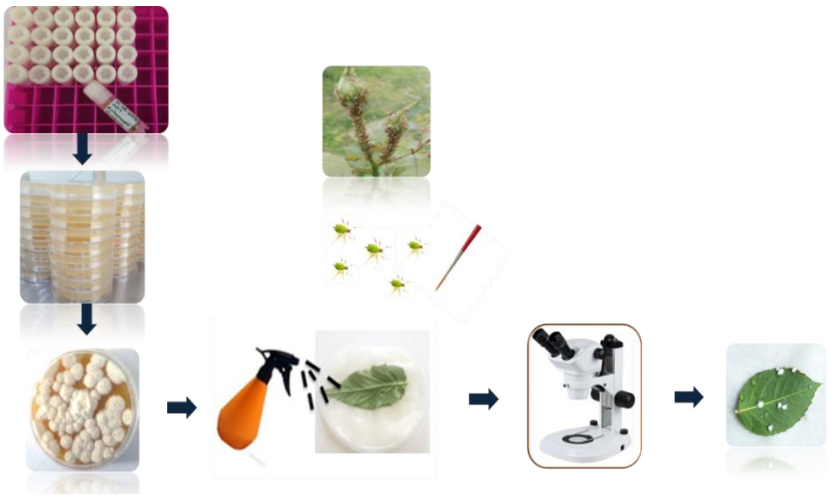

Figure 1. The sequence of steps involved in bioassay tests of local Turkish EPF isolates against the rose aphid, *Macrosiphum rosae*

Bioassay tests of EPF isolates on *M. rosae* were carried out in a climate room at a temperature of 25 ± 1°C and a relative humidity of 60 ± 5%. Firstly, three layers of blotting paper were placed in Petri dishes (9 cm in diameter), and then the rose leaves were placed on them with the lower side up. By spraying, spore solutions were applied on the leaves with a hand spray device three times from a distance of 10-20 cm. After the leaves were air-dried, 10 nymphs or 10 adults of *M. rosae* obtained from the culture were placed on them with the help of a fine-tipped brush. The experiment was carried out with 3 replications. Treatments were checked daily, with final mortality counts made on the 3rd, 5th and 7th days after application, and with the live and dead aphids recorded separately. To confirm that the deaths were caused by the applied EPF isolates, fungal re-isolation was performed from dead individuals and thus Koch's postulates were confirmed. As the control, water containing 0.1% Tween 80 was sprayed on the leaves before the aphids were placed on them . The experiments were established according to a randomized plot design.

Data analysis

In all cases, no control mortality was observed and, therefore, no correction was necessary for the mortality data. All values were subjected to arcsine transformation before analysis. Data were analyzed by one-way ANOVA by using the general linear model of SPSS 23.0 Windows (IBM Corp. 2015, New York, USA). Differences between treatment means were compared at a significance level of $P < 0.05$ by using Tukey's multiple comparison test.

Results and Discussion

Results showed that there were significant differences in the pathogenicity of EPF isolates against nymphs and adults of *M. rosae* at the different concentrations used ($P < 0.05$) (Figure 2) (Table 2 and 3). Of the five isolates tested, two *B. bassiana* isolates (BbDm-1 and BbKm-1) were the most pathogenic, and caused mortalities of 100% and 96.7% in nymphs, and 86.7% and 83.3% in adults, respectively, at the highest concentration (1×10^9 conidia/ml), 7 days after treatment.

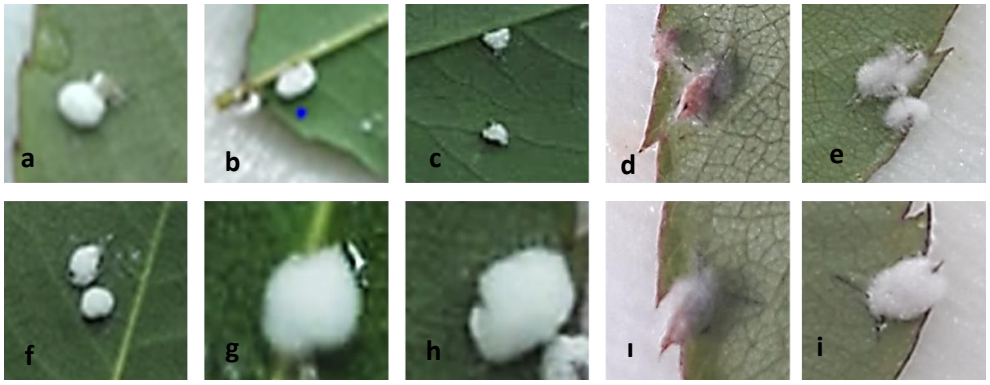

Figure 2. Infected nymphs (a: BbDm-1, b: BbKm-1, c: BbMp-1, d: PlKa-1 and e: IfGp-1) and adults (a: BbDm-1, b: BbKm-1, c: BbMp-1, d: PlKa-1 and e: IfGp-1) of *Macrosiphum rosae* following the application of local EPF isolates from Antalya Province, Turkiye

Table 2. Mean mortality (%) of *Macrosiphum rosae* nymphs exposed to different concentrations (1×10^7, 1×10^8 and 1×10^9 conidia/mL) of local EPF isolates from Antalya Province, Turkiye

Fungal species	**Isolate name***	**Concentration (conidia/ml)****	**Percent mortality (± SE)**		
			3rd day***	**5th day**	**7th day**
Beauveria bassiana	BbDm-1	1×10^7	80.0±0.0 AbII	83.3±3.3 AaI II	90.0±0.0 AbI
		1×10^8	86.7±3.3 AabI	93.3±3.3 AaI	96.7±3.3 AabI
		1×10^9	93.3±3.3 AaI	96.7±3.3 AaI	100.0±0.0 AaI
	BbKm-1	1×10^7	80.0±5.8 AaI	83.3±3.3 AaI	86.7±8.8AaI
		1×10^8	83.3±3.3 AaI	90.0±0.0 AaI	93.3±3.3 AaI
		1×10^9	90.0±0.0 AaI	93.3±3.3 AaI	96.7±3.3 AaI
	BbMp-1	1×10^7	43.3±3.3 BaI	46.7±3.3 BbI	56.7±3.3 BaI
		1×10^8	50.0±0.0 BaII	53.3±3.3 BabI II	60.0±0.0 BaI
		1×10^9	53.3±3.3 BaII	60.0±0.0 BaI II	66.7±3.3 BaI
Purpulalicum lilicanium	PlKa-1	1×10^7	26.7±3.3 BaI	30.0± 0.0CbI	36.7±3.3 BbI
		1×10^8	33.3±3.3 CaI	36.7± 3.3BabI	46.7±3.3 BabI
		1×10^9	36.7±3.3 CaII	43.3±3.3 BaI II	53.3±3.3 BaI
Isaria farinosa	IfGp-1	1×10^7	33.3±3.3 BaII	40.0±0.0 BCaI II	46.7±3.3 BaI
		1×10^8	36.7±3.3 BCaI	43.3±6.7 BaI	50.0±5.8 BaI
		1×10^9	46.7±3.3 BCaI	53.3±6.7 BaI	56.7±3.3 BaI

* The differences between the means with different capital letters in different isolates on the same day are statistically significant ($P < 0.05$; Tukey test).

** The differences between the means with different lower case letters on the same day and in the same isolate are statistically significant ($P < 0.05$; Tukey test).

*** The differences between the means with different roman numerals on different days in the same isolate are statistically significant ($P < 0.05$; Tukey test).

Table 3. Mean mortality (%) of *Macrosiphum rosae* adults exposed to different concentrations of local EPF isolates from Antalya Province, Turkiye

Fungal species	Isolate name*	Concentration (conidia/mL)**	Percent mortality (± SE)		
			3rd day***	5th day	7th day
Beauveria bassiana	BbDm-1	1×10^7	70.0±0.0 AbII	76.7±3.3 AabI II	80.0±0.0 AaI
		1×10^8	76.7±3.3 AaI	80.0±0.0 AaI	83.3±3.3 AaI
		1×10^9	80.0±0.0 AaI	83.3±3.3 AaI	86.7±3.3 AaI
	BbKm-1	1×10^7	66.7±3.3 AaI	70.0±0.0 AaII	73.3±3.3 AaI
		1×10^8	70.0±0.0 AbI	73.3±3.3 AabI II	80.0±0.0 AaI
		1×10^9	76.7±3.3 AaI	80.0±0.0 AaI	83.3±3.3 AaI
	BbMp-1	1×10^7	40.0±5.8 BaI	43.3±3.3 BaI	50.0±0.0 BaI
		1×10^8	43.3±6.8 BaI	46.7±3.3 BaI	53.3±3.3 BaI
		1×10^9	46.7±3.3 BaI	50.0±0.0 BaI	56.7±3.3 BaI
Purpulalicum lilicanium	PlKa-1	1×10^7	23.3±3.3 BbI	26.7± 3.3CaI	33.3±6.7 BaI
		1×10^8	30.0±0.0 BabII	33.3± 3.3CaI II	40.0±0.0 CaI
		1×10^9	36.7±3.3 BaI	43.3±6.7 BaI	46.7±3.3 BaI
Isaria farinosa	IfGp-1	1×10^7	26.7±3.3 BbII	30.0±0.0 CbII	43.3±3.3 BaI
		1×10^8	33.3±3.3 BabII	40.0±0.0 BCaI II	46.7±3.3 BCaI
		1×10^9	40.0±0.0 BaII	46.7±3.3 BaI II	50.0±0.0 BaI

* The differences between the means with different capital letters in different isolates on the same day are statisticall significant ($P < 0.05$; Tukey test).

** The differences between the means with different lower case letters on the same day and in the same isolate ai statistically significant ($P < 0.05$; Tukey test).

*** The differences between the means with different roman numerals on different days in the same isolate are statisticall significant ($P < 0.05$; Tukey test).

The results of the present study showed that some indigenous Turkish EPF isolates tested have the potential tobe used in the control of *M. rosae*. Sayed et al. (2019) reported that a *B. bassiana* isolate had the potential to be effective in the control of rose aphids, as did this study. In another study, Eidy et al. (2016) investigated the effects of *B. bassiana* and *Verticillium lecanii* (Zimm.) Viegas [previously known as *Lecanicillium lecanii* (Zimm.)] (Sordariomycetes: Hypocreales) on *M. rosae* adults under laboratory conditions. Laboratory bioanalysis studies were performed with five different concentrations (10^4, 10^5, 10^6, 10^7 and 10^8 conidia/mL) of *B. bassiana* and *V. lecanii* on adult *M. rosae*. They conducted the study in a completely randomized design with six replications. LC_{50} values for *V. lecanii* and *B. bassiana* were 1.38 x 10^4 and 2.66 x 10^5 conidia/mL, respectively. LT_{50} values ranged from 1.80 to 3.05 days for the *V. lecanii* concentrations and from 2.30 to 3.16 days for the *B. bassiana* concentrations, in adult aphids. Their results showed that *V. lecanii* and *B. bassiana* had the potential to be used in the control of *M. rosae*, which approximate the results obtained from the current study.

The EPF isolates used in this study have also been tested against rose sawfly, *Arge rosae* (L.) (Hymenoptera: Argidae), which causes damage to roses (Baki et al. 2021). In bioassay analyses, a total of 17 isolates belonging to 3 fungal species [*B. bassiana* – 14, *Clonostachys rosea* (Link) Schroers – 2 and *I. farinosa* – 1] were tested under laboratory conditions against 4th instar larvae of *A. rosae* at a conidial suspension of 1 x 10^7 conidia/mL, using the spray method. The results of that study

showed that the efficacy of the tested EPF isolates, similar to the results of this study, increased significantly up to 9 days after the bioassay. Five isolates of *B. bassiana* (BbDm1, BbKp-1, BbMp-1, BbSr-1 and BbMg-2) and one isolate of *I. farinosa* (IfGp-1) caused mortalities between 76.7% and 86.7%, at three days post-treatment.

In another study, Khosravi et al. (2014) tested four *B. bassiana* isolates (IR-K-40, IRAN403C, SP566 and SPT22) at five different conidial concentrations against the 4th instar larvae of *A. rosae*. While the pathogenicity of three tested isolates (SP566, IR-K-40 and SPT22) was low, they reported that the IRAN403C *B. bassiana* isolate could potentially be used effectively in the biological control of the pest, with the lowest LT_{50} value of 2×10^8 conidia/mL (3.92 days). These two studies showed that EPF isolates can be used against *A. rosae*, another pest of roses. The results of these two studies were similar to those of the current study and showed that *B. bassiana* has good potential for the control of important rose pests.

Budak et al. (2022) investigated the potential of four different commercial vegetable oils (aloe vera (*Aloe barbadensis*), tea tree (*Melaleuca alternifolia*), eucalyptus (*Eucalyptus globulus*) and garlic (*Allium sativum*)), using different doses, for the control of rose aphid. They reported that the toxic effect of each vegetable oil on rose aphid increased with the dose and time. Alghamdi (2018) determined the contact effect of the essential oils of four plants (*Moringa oleifera* L., *Eruca sativa* L., *Raphanus sativus* L., *A. sativum* L.) against rose aphid at 1%, 2% and at 4% concentrations, after 12, 24, 48, and 72 hours of exposure. Arugula oil gave the highest value against the pest with a mortality reaching 97.5% at all concentrations, followed by garlic oil (%) 80.6%, radish oil (69.2%) and moringa oil (63.3%). Mortality rates increased with both increasing essential oil concentrations and trial times.

In conclusion, *B. bassiana* has been widely investigated for its bio-control potential against many important insect pests, due to its multitude of variants and widespread occurrence across the world. Overall results suggest that two *B. bassiana* isolates (BbDm-1 and BbKm-1) have the potential to be used in the control of *M. rosae*. However, further studies under field conditions are required to validate the laboratory results.

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