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Indigenous entomopathogenic fungi as potential biological control agents of rose sawfly, *Arge rosae* L. (Hymenoptera: Argidae)

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Abstract: The rose sawfly, *Arge rosae* L. (Hymenoptera: Argidae), is one of the most destructive pests of roses grown both indoors and outdoors in Antalya province (south-western part of Turkey). Especially in greenhouse rose cultivation, growers depend heavily on synthetic pesticides to control this pest along with other arthropod pests in their crops. The aim of this study was to evaluate biocontrol potential of some indigenous soil-borne entomopathogenic fungi (EPF) against the pest. In pathogenicity assays, a total of 17 EPF isolates, belonging to three species [*Beauveria bassiana* (Bals.) Vuill. – 14, *Clonostachys rosea* (Link) Schroers – 2 and *Isaria farinosa* (Holmsk.) Fr. – 1] were tested against the 4th instar larvae of *A. rosae* under laboratory conditions. All the isolates were applied at a conidial suspension of 1×10^7 conidia/mL, using the spray method. The results from the assays showed that bio-efficacy of tested isolates increased significantly with elapsed time up to 9 days after inoculation. Of the 17 isolates tested, 5 isolates of *B. bassiana* (BbDm-1, BbKp-1, BbMp-1, BbSr-1 and BbMg-2) and 1 isolate of *I. farinosa* (IfGp-1) appeared to be most promising, causing mortalities between 76.7 and 86.7% 3 days post treatment. Probit analysis of mortality data showed that there were significant differences in the susceptibility of 4th instar larvae of *A. rosae* to tested EPF isolates. The above-mentioned six isolates had the lowest LT_{50} and LT_{95} values (ranged from 1.66 to 7.50 and 4.81 to 8.49 days, respectively), implying their high virulence and their biocontrol potential against the pest. Phylogenetic analysis based on the ITS region sequence revealed that all tested isolates showed high similarities (ranged from 99 to 100%) with other isolates of their respective fungal species in GenBank. Overall results suggested that the most virulent above-mentioned six isolates had significant potential as biological control agents against *A. rosae*.

Key words: *Arge rosae*, entomopathogenic fungi, molecular identification, pathogenicity, rose sawfly

1. Introduction

Roses (*Rosa* spp.) are woody perennial flowering plants in the family Rosaceae, and the genus *Rosa* L. has about 200 species and 18000 varieties (Sastry et al., 2019). They are important ornamental plants widely grown in parks and gardens all over the world. Besides their use for landscaping purposes, some species (*Rosa gallica* L., *R. centifolia* L. and *R. damascena* Mill.) have been used for many years in the production of rose oil and rose water in Turkey (Timor, 2011; Özçelik, 2013). However, rose oil and rose water productions have recently been done from only *R. damascena* (also known as "Isparta rose" in Turkey) by modern fabrication techniques in Isparta province (in the Mediterranean region of Turkey), where more than 80% of the total rose oil production in Turkey comes true. The province also meets approximately 50% of the rose oil production of the world (Timor, 2011). Roses are also grown in Turkey for cut flower production, and Antalya province, located on the Mediterranean coast of Turkey and neighbour to the province of Isparta, is the main production center of greenhouse-grown cut roses (Baris and Uslu, 2009).

The rose sawfly, *Arge rosae* L. (Hymenoptera: Argidae), is one of the most important insect pests of roses grown both indoors and outdoors in Turkey (Özbek and Çalmaşur, 2005; Demirözer and Karaca, 2011; Bolu et al., 2021). The species is narrowly oligophagous and considered to have two generations per year, although its complete biology has not been fully studied. It causes two types of damage: (i) the larvae feed on fresh leaves and often cause complete defoliation of rose plants during the 5 larval stages (approximately 25 days), and (ii) the females lay their eggs in young succulent shoots, resulting in elongate scars on the branches of rose plants. These scars can sometimes cause the stems to dry and consequently to shed leaves (Khosravi et al., 2015; Bolu et al., 2021).

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The current control of this pest in greenhouse-grown roses in Antalya province generally depends on the use of synthetic insecticides (generally pyrethroids). The most widely used synthetic pyrethroids include permethrin, cypermethrin, deltamethrin, fenvalerate, and alphacypermethrin. However, the desired levels of control have not been obtained from pesticide usage in recent years due to possible development of resistance to pesticides in the populations of this pest. In addition, most of the used pesticides, particularly pyrethroids and organophosphates, are highly toxic to the natural enemies present in greenhouses, and the effectiveness of predators and parasitoids has greatly diminished. As an environmentally friendly control approach, biological control is often considered to control many pests as an alternative method. Entomopathogenic fungi (EPF) are one of the main components of biological control (Mahr et al., 2008). Therefore, the purpose of this study was to evaluate the efficacy of 17 indigenous soil-borne EPF isolates, belonging to 3 species, against *A. rosae* as potential biological control agents.

2. Material and methods

2.1. Insect material

First instar larvae of *A. rosae* were collected from the infested greenhouse-grown roses in Antalya province and transported to the Entomology laboratory in the Plant Protection Department of Akdeniz University, Antalya (Figure 1). Rearing of insects was carried out on the foliage of rose (*R. damascena*) plants under controlled conditions (25 ± 2°C, 60 ± 5 RH, and a photoperiod of 16 : 8 (L : D) h) in a climate room. The insects were used in the assays when they reached the 4th instar larval stage (Smith, 1989; Bolu et al., 2021).

2.2. Indigenous EPF isolates

A total of 17 indigenous soil-borne EPF isolates were used in this study. Their code and species names, sampling sites, habitats, and geographic coordinates are presented in Table 1.

For the isolation of EPF from the collected soil samples, "*Galleria*-trap" method, which is one of the most used methods, was used (Zimmermann, 1986; Meyling, 2007). Therefore, a laboratory stock culture of the Great wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), was started with larvae from infested wax combs in the Plant Protection Department of Akdeniz University (Antalya), and subcultures were maintained on artificial medium having 100 mL pure water, 200 mL filtered honey, 200 mL honeycomb wax, 250 mL glycerin and 2 L wheat bran in complete darkness in an incubator at 26 ± 2°C and 60 ± 5% RH (Kaya and Stock, 1997). Fourth instar larvae were used in all isolation processes.

After thoroughly mixing each of the collected soil samples, their smaller portions (approximately 70–75 mL), were placed in 100 mL transparent polypropylene containers (one individual soil sample per container) and then moistened with sterile distilled water. After five *G. mellonella* larvae were added to each sample, the lid of each container was closed and perforated 20 times using a hot needle for ventilation. All the containers were then kept in complete darkness in an incubator at room temperature (22 ± 2 °C) for fungal growth. There were 3 replicates for each soil sample.

The contents of containers were inspected for dead *G. mellonella* larvae 3, 5, 7, and 10 days after the insect introduction. Dead larvae were removed and placed in a sterile Petri dish (disposable plastic 90 × 15 mm) lined with moist filter paper. All Petri dishes were sealed with Parafilm M (Bemis, Neenah, WI) and held at 25 °C in the dark until fungal growth was observed on the insect cadavers. During the inspections, the larvae, on which fungal growth was observed, were collected, and kept first in distilled water for 3 s and then in 1% sodium hypochlorite for surface sterilization for 10-20 s and lastly in 70% ethyl alcohol for 3 s. Then, they were passed through sterile pure water 3 times, and the excess water was absorbed by taking them on filter paper. Surface-sterilized insect cadavers were incubated in Petri dishes on moistened filter paper within an incubator adjusted to room temperature (25 ± 2 °C) for 7–14 days. After incubation, pure culture isolation was performed from the samples showing external fungal growth (Oudor et al., 2000; Padmaja and Kaur, 2001). Isolation was done using Sabouraud Dextrose Agar + 1% yeast extract and Potato Dextrose Agar medium (Meyling, 2007). To prevent bacterial contamination, 50 µg / mL ampicillin and 200 µg / mL streptomycin were added to the nutrient media (Eken, 2011).

2.3. Molecular identification and phylogenetic placement of EPF isolates

The genomic DNA of the tested EPF isolates was extracted through CTAB method described by Doyle and Doyle (1990). The PCR (polymerase chain reaction) was conducted in a gradient thermal cycler, using two primers, based on ITS-rDNA region gene sequences, ITS1 (5'-TCCGTAGGTGAACCTGCGG- 3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990).

The PCR products were sequenced with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, CA) and the ABI 3730XL Sanger sequencing device (Applied Biosystems, CA) in the Macrogen laboratory in Netherlands. The DNA sequences of EPF isolates were performed using the Bioedit program with ClustalW algorithm (Thompson et al., 1994; Hall, 1999).

Figure 1. First instar larvae of *A. rosae*, head capsules of which were black, and their legs were blackish, collected from the infested greenhouse-grown roses in Antalya province.

Molecular phylogenetic analyses were executed through the maximum likelihood (ML) method based on the Tamura 3-parameter model, using MEGA7 software (Biodesign Institute, Arizona) (Kimura, 1980; Tamura et al., 2011). The phylogenetic analysis was performed using the ITS region sequence of EPF isolates and the nucleotide sequence of the other isolates of the respective species retrieved from GenBank (Altschul et al., 1997).

2.4. Pathogenicity assays against the larvae of *A. rosae*

Pathogenicity assays were carried out under controlled conditions (25 ± 2 °C, 60 ± 5 RH, and a photoperiod of 16 : 8 (L : D) h) in the entomology laboratory of Plant Protection Department. All EPF isolates were assayed at a conidial suspension of 1×10^7 conidia/mL against the pest, using the spray method. Prior to assays, conidia viability of each isolate was determined using the method described by Goettel and Inglis, (1997), and isolates with a viability of above 95% were used for bioassay. For preparation of conidial suspensions, conidia from the actively growing 10-day-old culture of each isolate on PDA (Potato Dextrose Agar) medium at 26 ± 2 °C and 65 ± 5 RH were taken with a sterile loop and suspended in 10 mL sterile distilled water with a 0.03% Tween 80. All the prepared suspensions were filtered using a sterile 4-layer

Table 1. List of the indigenous EPF isolates used in the experiments.

Isolate name	Species	Sampling site	Habitat	Geographic coordinates
BbKm-1	*Beauveria bassiana*	Kumluca	Olive	N 36°19'17.1" E 30°20'23.0"
BbKm-2	*B. bassiana*	Kumluca	Orange	N 36°22'18.8" E 30°16'29.1"
BbKr-1	*B. bassiana*	Kemer	Forest	N 36°35'51.0" E 30°33'22.7"
BbDm-1	*B. bassiana*	Demre	Orange	N 36°14'39.7" E 29°58'45.0"
BbFn-3	*B. bassiana*	Finike	Orange	N 36°19'53.7" E 30°08'40.6"
BbKp-1	*B. bassiana*	Kepez	Forest	N 36°54'50.4" E 30°37'48.4"
BbDs-2	*B. bassiana*	Döşemaltı	Pomegranate	N 37°00'02.4" E 30°38'16.1"
BbMp-1	*B. bassiana*	Muratpaşa	Fig	N 36°53'07.2" E 30°44'30.4"
BbAk-1	*B. bassiana*	Aksu	Grassland	N 36°56'03.3" E 30°52'35.1"
BbSr-1	*B. bassiana*	Serik	Orange	N 36°55'33.8" E 31°07'20.7"
BbMg-1	*B. bassiana*	Manavgat	Olive	N 36°49'40.8" E 31°20'35.3"
BbMg-2	*B. bassiana*	Manavgat	Wheat	N 36°58'58.8" E 31°14'48.5"
BbKl-1	*B. bassiana*	Korkuteli	Pear	N 37°03'21.3" E 30°10'33.8"
BbGp-1	*B. bassiana*	Gazipaşa	Forest	N 36°12'52.0" E 32°23'45.0"
CrMg-1	*Clonostachys rosea*	Manavgat	Grassland	N 36°57'49.2" E 31°16'51.9"
CrKn-1	*C. rosea*	Konyaaltı	Grassland	N 36°53'52.7" E 30°37'50.8"
IfGp-1	*Isaria farinosa*	Gazipaşa	Olive	N 36°14'50.3" E 32°21'19.2"

cheesecloth to remove pieces of agar and mycelium and then vortexed for 3 min for homogenization. Lastly, the conidial suspensions were adjusted to 1×10^7 conidia/mL using a haemocytometer (Fancelli et al., 2013).

For each treatment, ten 4th instar larvae were placed in each Petri dish (9 cm in diameter) covered with 3-layer filter paper and then sprayed through a handheld sprayer from 30 cm distance, using 2 mL of conidial suspension of any EPF isolate. Larvae treated with sterile distilled water + 0.03% Tween 80 served as control. Each fungal treatment and control were replicated 3 times (10 larvae per each). After air drying, all treated larvae were carefully transferred to new dishes with clean rosa leaves through a fine camel-hair brush. The lids of the dishes were closed and then perforated using a hot needle for ventilation (20 times per each). All dishes were kept in the laboratory under the above-mentioned experimental conditions. Surviving larvae in each Petri dish were fed on clean rose leaves until the end of the experimental period. To obtain mortality data, dishes were examined daily under a stereomicroscope, and mortalities were recorded on the 3rd, 5th, 7th and 9th days of treatment. At each count, the larvae were touched using a sterilized fine forceps, and those displayed no observable motion were recorded as dead. All dead larvae were removed from the dishes and placed individually in moistened filter paper-lined new Petri dishes. They were then incubated at 25 ± 2 °C and 65 ± 5 RH in complete darkness, and lastly evaluated for up to 14 days under a stereomicroscope to observe fungal growth on larval cadavers. The presence of fungal outgrowth on dead larvae is an indication that the death of insects was caused by fungal agents. If a dead larva did not show fungal outgrowths of similar characteristics to those of the applied fungus as the treatment, its death was considered as caused by another factor, or factors, and was not included in the count.

2.5. Data analysis

Since no control mortality was detected in the pathogenicity tests, no adjustment was essential for the mortality values. All mortality data were arcsine-transformed prior to analysis and analyzed using the general linear model of the SPSS 23.0 Windows by one-way ANOVA (IBM Corp. 2015, USA). Tukey's HSD test at a significance level of $p < 0.05$ was used to define significant differences among the treatment means. The lethal time (LT_{50} and LT_{95}) values and the 95% confidence limits were also calculated using Probit analysis and Log-probit method (SPSS 23.0).

3. Results and discussion

3.1. Phylogenetic placement of tested EPF isolates

The accession numbers of the fungal isolates used in phylogenetic analysis are given in Table 2. After alignment analysis, the ITS region sequence for EPF isolates data set consisted of 460 aligned positions. All tested indigenous

Table 2. GenBank nucleotide accessions of indigenous isolates of tested EPF species along with other isolates of the respective or related species based on ITS region used for phylogenetic analysis.

Isolate name	Species	Accession no.	Isolate name	Species	Accession no.
BbKm-1	*Beauveria bassiana*	MT441868	MG562497	*B. bassiana*	MG562497
BbKm-2	*B. bassiana*	MT441869	SHU.M.161	*B. bassiana*	KU158472
BbKr-1	*B. bassiana*	MT441871	SHU.M.131	*B. bassiana*	KU158461
BbDm-1	*B. bassiana*	MT441872	EABb04	*B. bassiana*	KC753382
BbFn-3	*B. bassiana*	MT441875	SASRI BB444	*B. bassiana*	JX110368
BbKp-1	*B. bassiana*	MT441877	ARSEF 4622	*B. australis*	HQ880790
BbDs-2	*B. bassiana*	MT441879	ARSEF 4598	*B. australis*	HQ880789
BbMp-1	*B. bassiana*	MT441880	2718	*B. bassiana*	KU364353
BbAk-1	*B. bassiana*	MT441881	F19-N	*B. bassiana*	MG640376
BbSr-1	*B. bassiana*	MT441882	EABb 04/01	*B. bassiana*	DQ364698
BbMg-1	*B. bassiana*	MT441883	HHWG1	*B. brongniartii*	JX110385
BbMg-2	*B. bassiana*	MT441884	SASRI	*B. brongniartii*	JX110388
BbKl-1	*B. bassiana*	MT441885	CCCT 17.132	*C. rosea*	MN192944
BbGp-1	*B. bassiana*	MT441886	CCCT 17.128	*C. rosea*	MN192940
CrMg-1	*Clonostachys rosea*	MT441900	SFC101445	*I. farinosa*	MF186013
CrKn-1	*C. rosea*	MT441901	IHBF 2244	*I. farinosa*	MF326609
IfGp-1	*Isaria farinosa*	MT441902			

EPF isolates had 99%–100% homology with other fungal isolates of the respective species in the GenBank (Figure 2).

A review of the related literature revealed that our results were similar to some previous studies but different from some others. In a previous research, Gürlek et al. (2018) determined and characterized the molecular phylogenetic variety of 40 EPF isolates containing *B. bassiana* ones using gene of *β-tubulin* and *Bloc* sequences, and they found a close relationship between the isolates studied. In another study, Dhar et al. (2019) realised phylogenetic analysis of 13 Indian EPF isolates of *B. bassiana* through comparative 10 RAPD primers. Among the tested isolates, similarity was observed between only 3 isolates, and other isolates were found different in phylogenetic analysis. In a more recent study, Zhang et al. (2020) searched the genetic difference between the *B. bassiana* isolates obtained from 17 different insect hosts using phylogenetic analysis and found some genetic differences among virulent isolates.

3.2. Effectiveness of EPF isolates against *A. rosae*

All the isolates tested were pathogenic to the 4th instar larvae of *A. rosae*; however, significant differences were detected among the isolates tested in their mortality rates 3–9 days posttreatment ($p < 0.05$) (Table 3). Based on the mortality rates on the 3th day of treatment, six isolates (5 *B. bassiana* - BbDm-1, BbKp-1, BbMp-1, BbSr-1, and BbMg-2; 1 *I. farinosa* - IfGp-1) were more virulent than others, and caused mortalities, ranging from 76.7 to 86.7%. Of the 17 EPF isolates tested, isolates BbKm-1 and BbDs-2 caused the lowest mortalities by 23.3%. On the 9th day of treatment, except for 4 isolates (BbDs-2, CrKn-1, BbMg-1 and CrMg-1), all the isolates achieved 100% larval mortality.

For the EPF isolates tested at 1×10^7 conidia/mL, the time required for 50 and 95% mortality (LT_{50} and LT_{95}) of the 4th instar larvae of *A. rosae* varied between 1.66–7.50 days and 3.60–92.39 days, respectively (Table 4). The lowest LT_{50} and LT_{95} values were calculated for isolates BbDm-1, BbKp-1, BbMp-1, BbSr-1, BbMg-2 and IfGp-1, implying their high virulence and their biocontrol potential against *A. rosae*.

Our review of literature revealed that there has been only one study on the evaluation of EPF against the rose sawfly. In that study, Khosravi et al., (2014) assayed 4 *B. bassiana* isolates (IR-K-40, IRAN403C, SP566 and SPT22) at 5 different conidial concentrations against the 4th instar larvae of *A. rosae*. They reported that mortality caused by 3 isolates (SP566, IR-K-40 and SPT22) was low and not significantly different from each other, whereas isolate IRAN403C appeared the most promising for biological control of the pest with the lowest LT_{50} value (3.92 days) at a concentration of 2×10^8 conidia/mL. When their findings are compared with ours, except for 4 isolates (BbDs-2, BbMg-1, CrKn-1 and BbKl-1), all other isolates tested in this study had a lower LT_{50} value than their most

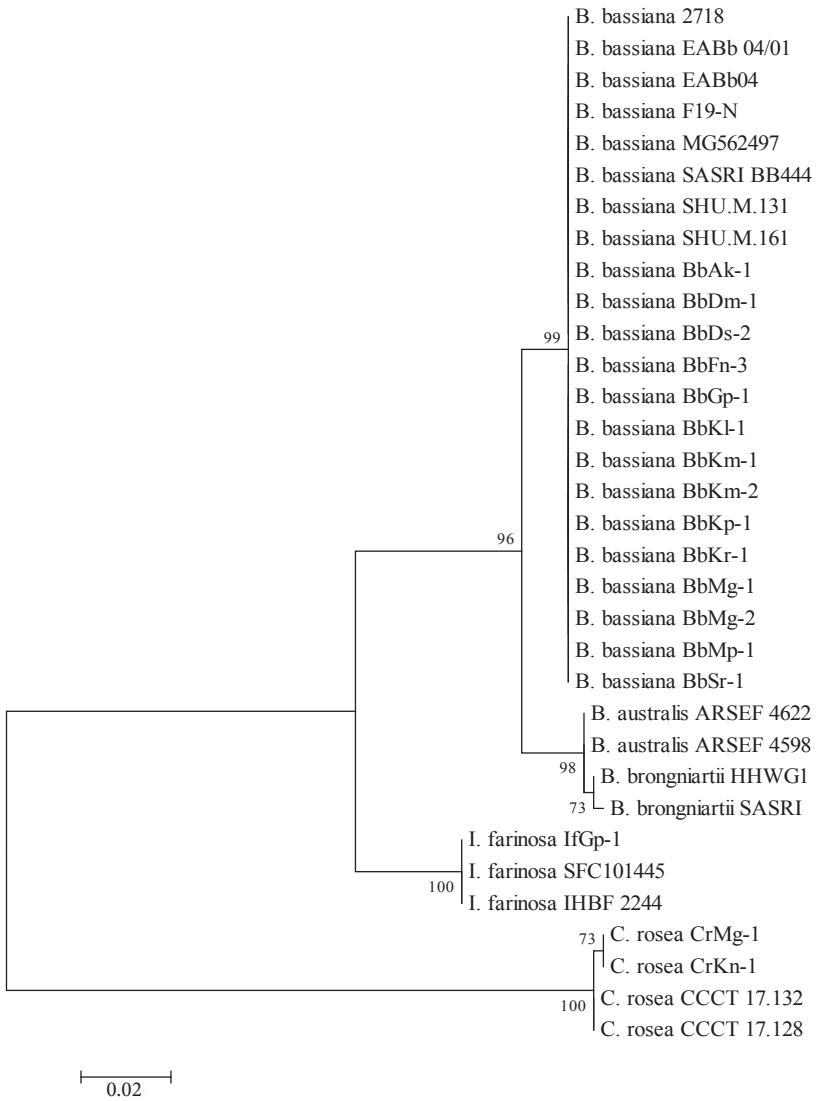

Figure 2. Maximum likelihood tree based on ITS region sequence, showing the phylogenetic relationship between the indigenous isolates of EPF species (*B. bassiana*, *C. rosea* and *I. farinosa*) and other isolates of the respective or related species in the GenBank.

virulent isolate IRAN403C although they were tested at a lower concentration (1×10^7 conidia/mL).

Our literature survey also revealed that there have been some studies on the pathogenicity of EPF against some hymenopteran pest species. For instance, Aslantaş et al. (2008) investigated the effectiveness of *B. bassiana* that could potentially be used in the control of the sour cherry slug *Caliroa cerasi* (L.) (Hymenoptera: Tenthredinidae), which causes serious damage to sour cherries in Turkey. The efficiency of *B. bassiana* on mature larvae of *C. cerasi* was tested at different conidial concentrations (1×10^6, 1.5×10^6, 1×10^7 and 1.5×10^7 conidia/mL) under laboratory conditions. Larvae sprayed directly with *B. bassiana* conidial suspensions and exposed to treated leaves resulted in 100% mortality within 2.90 and 2.77 days, respectively. Median lethal time (LT_{50}) and day to death showed the highest pathogenicity at a concentration of 1×10^7 conidia/mL for both direct spray and leaf exposure. The results of the study were similar to those of this study and showed that *B. bassiana* has a good potential for control of agricultural pests. In another study, Swiergiel et al. (2016) comparatively investigated the effectiveness of a commercial product ((BotaniGard) of *B. bassiana* strain GHA and an indigenous isolate (KVL 14–90) of *Metarhizium brunneum* Petch in the control of apple sawfly, *Hoplocampa testudinea* Klug (Hymenoptera: Tenthredinidae) both in laboratory and field conditions. They also investigated the persistence

Table 3. Percentage mortalities of the 4th instar larvae of *A. rosae* on the 3rd, 5th, 7th and 9th days of treatment in response to the EPF isolates tested at 1×10^7 conidia/mL.

Isolate name	Fungal species	Percent mortality (± SE)			
		3rd day	5th day	7th day	9th day
BbKm-1	*Beauveria bassiana*	$23.3 \pm 3.3^{bC**}$	80.0 ± 0.0^{bcB}	100^{aA}	100^{aA}
BbKm-2	*B. bassiana*	36.7 ± 3.3^{bB}	100^{aA}	100^{aA}	100^{aA}
BbKr-1	*B. bassiana*	33.3 ± 3.3^{bC}	76.7 ± 3.3^{cB}	100^{aA}	100^{aA}
BbDm-1	*B. bassiana*	86.7 ± 3.3^{aB}	100^{aA}	100^{aA}	100^{aA}
BbFn-3	*B. bassiana*	20.0 ± 0.0^{bC}	76.7 ± 3.3^{cB}	100^{aA}	100^{aA}
BbKp-1	*B. bassiana*	83.3 ± 3.3^{aB}	100^{aA}	100^{aA}	100^{aA}
BbDs-2	*B. bassiana*	23.3 ± 3.3^{bB}	46.7 ± 3.3^{fA}	50.0 ± 0.0^{cA}	50.0 ± 0.0^{dA}
BbMp-1	*B. bassiana*	76.7 ± 3.3^{aB}	100^{aA}	100^{aA}	100^{aA}
BbAk-1	*B. bassiana*	36.7 ± 3.3^{bC}	66.7 ± 8.8^{dB}	93.3 ± 3.3^{aA}	100^{aA}
BbSr-1	*B. bassiana*	76.7 ± 3.3^{aB}	86.7 ± 6.7^{bAB}	100^{aA}	100^{aA}
BbMg-1	*B. bassiana*	26.7 ± 3.3^{bC}	43.3 ± 3.3^{fB}	53.3 ± 3.3^{cAB}	60.0 ± 0.0^{cA}
BbMg-2	*B. bassiana*	80.0 ± 5.8^{aB}	93.3 ± 3.3^{abAB}	100^{aA}	100^{aA}
BbKl-1	*B. bassiana*	26.7 ± 3.3^{bC}	56.7 ± 3.3^{eB}	100^{aA}	100^{aA}
BbGp-1	*B. bassiana*	33.3 ± 3.3^{bB}	100^{aA}	100^{aA}	100^{aA}
CrMg-1	*Clonostachys rosea*	36.7 ± 3.3^{bB}	73.3 ± 3.3^{cA}	76.7 ± 3.3^{bA}	76.7 ± 3.3^{bA}
CrKn-1	*C. rosea*	30.0 ± 0.0^{bB}	53.3 ± 3.3^{eA}	53.3 ± 3.3^{cA}	56.7 ± 3.3^{cA}
IfGp-1	*Isaria farinosa*	80.0 ± 0.0^{aA}	100^{aA}	100^{aA}	100^{aA}

*Means in a column followed by the same lower-case letter are not significantly different and means in a row followed by the same upper-case letter are not significantly different (Tukey's HSD test; $P < 0.05$).

Table 4. LT_{50} and LT_{95} (days) values with 95% confidence limits of indigenous EPF isolates tested at 1×10^7 conidia/mL to the 4th instar larvae of *A. rosae*.

Isolate name	Species	LT_{50} (LCL-UCL)*	LT_{95} (LCL-UCL)	Regression equation (y = ax + b)
BbKm-1	*Beauveria bassiana*	3.76 (3.64–3.88)	6.02 (5.74–6.37)	y= –4.615 + 3.125x
BbKm–2	*B. bassiana*	3.15 (3.08–3.26)	3.99 (3.70–4.70)	y= –7.928 + 15.902x
BbKr-1	*B. bassiana*	3.58 (3.30–3.84)	6.29 (5.71–7.20)	y= –3.730 + 6.729x
BbDm-1	*B. bassiana*	2.04 (1.54–2.32)	3.60 (3.37–4.02)	y= –2.061 + 6.666x
BbFn-3	*B. bassiana*	3.88 (3.71–4.06)	6.17 (5.78–6.69)	y= –4.813 + 8.169x
BbKp-1	*B. bassiana*	1.66 (1.00–2.12)	4.29 (3.80–5.13)	y= –0.887 + 3.999x
BbDs-2	*B. bassiana*	7.50 (6.29–10.08)	92.39 (39.93–711.14)	y= –1.320 + 1.508x
BbMp-1	*B. bassiana*	2.35 (2.07–2.54)	4.03(3.77–4.46)	y= –2.609 + 7.028x
BbAk-1	*B. bassiana*	3.68 (3.14–4.14)	7.67 (6.55–9.97)	y= –2.931 + 5.171x
BbSr-1	*B. bassiana*	2.06 (0.76–2.79)	5.55 (4.48–9.50)	y= –1.208 + 3.830x
BbMg-1	*B. bassiana*	6.38 (5.83–7.08)	50.02 (33.21–95.09)	y= –1.481 + 1.840x
BbMg-2	*B. bassiana*	1.95(1.05–2.50)	4.75 (4.07–6.33)	y= –1.243 + 4.264x
BbKl-1	*B. bassiana*	4.03 (3.54–4.49)	7.20 (6.21–9.23)	y= –3.962 + 6.539x
BbGp-1	*B. bassiana*	3.19 (3.11–3.31)	4.04 (3.76–4.66)	y= –8.047 + 15.961x
CrMg-1	*Clonostachys rosea*	3.60 (2.55–4.34)	18.29 (12.47–43.63)	y= –1.297 + 2.330x
CrKn-1	*C. rosea*	5.97 (4.93– 7.47)	90.71 (37.69–899.98)	y= –1.080 + 1.392x
IfGp-1	*Isaria farinosa*	2.23 (1.90–2.45)	3.93 (3.68–4.36)	y= –2.335 + 6.693x

*95% confidence limits (CL); LCL, lower limit; UCL, upper limit.

of the tested entomopathogenic fungus isolates in the soil. The results of the study showed that the isolates tested in the laboratory showed a higher effect, and their effectiveness decreased over time in field conditions. Fungal density decreased to 25% after 49 days and to 0.4% after 55 weeks. After the application, the density of *B. bassiana* isolate was found to be higher than that of *M. brunneum* isolate in the soil. The study shows that the biological period of the pest and the time after application play an important role in the effectiveness of the control of entomopathogenic fungi. In a more recent study, Tozlu et al. (2017) investigated the potential use of a *B. bassiana* isolate (ET 10) in the control of Rose stem sawfly, *Syrista parreyssii* (Spinola) (Hymenoptera: Cephidae), which is one of the rose pests. The pathogenicity assays against the larval stages of the pest were performed at 3 different conidial concentrations (10^6, 10^7, and 10^8 conidia/mL) under laboratory conditions. The results of the study showed that tested *B. bassiana* isolate can be used effectively in the control of *S. parreyssii* at all tested concentrations, similar to this study.

4. Conclusion

Based on the data reported herein, the present study showed that indigenous isolates of *B. bassiana* and *I. farinosa* had a pathogenic activity against the 4th instar larvae of *A. rosae* under laboratory conditions; however, five isolates of *B. bassiana* (BbDm-1, BbKp-1, BbMp-1, BbSr-1 and BbMg-2) and 1 isolate of *I. farinosa* (IfGp-1) were more pathogenic than others. Therefore, these six isolates can be further evaluated against the pest under field conditions.

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