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Efficacy of indigenous entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, isolates against the rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) in rose production

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Abstract

The rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae), is an important insect pest of rose plants because it damages the leaves and flowers. The entomopathogenic fungus, *Beauveria bassiana* Balsamo (Vuillemin), is environmentally safe in comparison with the chemical pesticides. The indigenous fungal isolates can be the best options in biological control because they are more adapted to the local environment. In the present study, four genetically different isolates of *B. bassiana* (isolates 1, 2, 3, and 4) were used to estimate LC₅₀ for the rose aphid. Also, the same isolates were used to control the aphid's infestation on rose plants in the field, and the efficacy was compared with a commercial strain (Naturalis®) with two concentrations for each isolate (2.3×10^6 and 4.6×10^6 conidia/ml). Bioassay results indicated that isolate 1 differed significantly (LC₅₀ = 6.46×10^4) than the other three ones (LC₅₀ = 1.46×10^5 , 1.52×10^5 , and 1.71×10^5). In field trials, the concentration of 4.6×10^6 conidia/ml, for indigenous as well as commercial strains, achieved the highest reductions of rose aphid infestation and the highest extraction of rose oil. Thus, this fungus can be recommended in organic rose production. Further investigations are needed for the improvement in utilization of these isolates with regard to UV radiations in the field.

Keywords: *Macrosiphum rosae*, Biological control, *Beauveria bassiana* isolates, *Rosa damascena*

Background

Rose plant, *Rosa damascena trigintipetala* Mill., is cultivated in Taif, Saudi Arabia, where a special local variety called Taify rose is well known for its oil contents and its suitability for growth under local climate. Rose plants are infested by many insect pests especially rose aphids, thrips, whiteflies, and some lepidopteran larvae. These insect pests cause high damages to rose plants especially to buds, leaves, and flowers. Therefore, the rose yields are reduced by infestations with these insect pests (Karlik and Tjosvold 2003).

Rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae); green peach aphid, *Myzus persicae*; cotton aphid, *Aphis gossypii* Glov.; and potato aphid, *Macrosiphum*

euphorbiae (Thomas), are the important aphid pest species that infest and damage rose plants (Karlik and Tjosvold 2003). *M. rosae* also attacks the photinia, pyracantha, and many of fruit trees, which are closely related to rose plants. Aphid infestation deformed leaves and new bloom stems and also is considered as a vector of several plant viruses that impact rose plants (Chau and Heinz 2004). The infestation of aphids on rose plants may affect the composition of plant biochemicals (Singh et al. 2014). Sayed and Montaser (2012) and Sayed and Alghamdi (2017) reported high infestations with *M. rosae* on Taif's rose.

Entomopathogenic fungus (EPF), *Beauveria bassiana* Balsamo (Vuillemin), can be one of the best bio-control agents of the rose aphid. There is an increasing interest in utilization and mass propagation of EPF for controlling insect pests (Inglis et al. 2001). *B. bassiana* is the causing

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agent of the white muscardine disease of various insect species (Tanada and Kaya 1993) that causes epizootics under various climatic conditions among field and soil-borne insect pests. This fungus represents the first example describing a microorganism as the bio-agent of a contagious disease and contains various genotypes and probably comprises a complex of many species. Consequently, it is thinkable to find individual isolates or pathotypes which represent a substantially restricted host range (Inglis et al. 2001).

Many of EPF are being developed for controlling a large number of agricultural pests and some of them such as *B. bassiana* and *Metarhizium anisopliae* (Metschnikoff) Sorokin are already available as commercial products for controlling various pest species (Amatuzzi et al. 2017). Moreover, EPF can be used in combination with the parasitoids and predators, as biological control agents, for controlling the aphids. This method requires an effective time management in order to prevent the antagonistic interactions (Martins et al. 2014 and Seiedy et al. 2015).

The isolation of indigenous isolates or strains of EPF from different hosts and locations provides a control program with available specific tools for controlling various indigenous pests (Zayed 2003). Four isolates of *B. bassiana* were isolated from Taif region (Saudi Arabia) and genetically identified (Sayed et al. 2018).

The present study aimed to evaluate such isolates against the rose aphid, *M. rosae*, under laboratory and field conditions. The indigenous isolates and a commercial *B. bassiana* were studied for the aphid control throughout the infestation rates of aphid on Taif's rose. Also, their effect on rose flowers and rose oil yields were evaluated.

Materials and methods

Fungus isolates and their mass production

Four genetically different indigenous isolates of *B. bassiana* (Sayed et al. 2018) were used in this study. These isolates were previously isolated from Taif region. Each isolate was cultured twice on potato dextrose agar (PDA) (Acumed, Lansing, USA). Aerial *B. bassiana* conidia were mass produced in polypropylene bags containing cooked parboiled rice as semi-solid medium (100 g of commercially available rice and 100 ml of distilled water were added to the flask) (Leite et al. 2003). In order to obtain pure conidia, batches of fungus-containing substrate, they were first dehydrated in desiccators containing silica gel for 7 days under room temperature conditions. Subsequently, the colonized substrate was sieved (100 - mesh sieve) under agitation (250 rpm). Then, the harvested conidia were concentrated to 2.3×10^7 spores/ml and stored at 4 °C until used.

Bioassay

Insects and fungus isolates

Rose plant leaves infested with rose aphid, *M. rosae*, were collected on the same day of the experiment from the rose field at Taif during March, 2018. Each fungal isolate was prepared as a suspension in five concentrations (1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 spores/ml). Each conidial suspension was vortexed for 5 min to achieve suspension homogeneity. In order to disperse the conidia uniformly in the suspension, 0.02% Tween 80 was added (Selvaraj et al. 2012).

Experiment method

Totally, 100 aphid individuals were used for each concentration (5 replicates, each contained 20 individuals). The bioassay was carried out by dropping 1 μ l from each concentration directly on the insect body by a micropipette as direct drop application (Eidy et al. 2016). Petri dishes were filled with a thick layer (about 3 mm) of 0.1% agar. Twenty aphid individuals (1 day old) were transferred by a camel hairbrush on rose leaf into each Petri dish. Each aphid adult (in control) was treated with 1 μ l of distilled water with Tween 80 (0.02%). Rose fresh leaves were changed daily for feeding aphids. All treatments were maintained at (26 ± 1 °C, $70 \pm 5\%$ RH and a photoperiod of 16:8 h (L:D)) in an incubator. The aphid individuals were investigated daily for mortality till the 3rd day.

Field applications

The field experiment was conducted in a rose farm at Alhada, Taif Governorate, Saudi Arabia, by selecting 70–90-cm tall and wide rose plants. These rose plants were pruned at early February 2017. The vegetative and flowering buds appeared by the end of February.

The experimental site was consisted of plots, measuring 4 m \times 4 m and in a randomized complete block design (RCBD). Three meters long space was kept between plots and blocks. Each treatment was replicated three times. Each of the four isolates and the commercial *B. bassiana* (Naturalis®) at the concentration 2.3×10^7 spores/ml was applied by two different applications of 0.1 and 0.2% v/v (2.3×10^6 and 4.6×10^6 conidia/ml, respectively), but the control was treated with water only. A 10-day interval between sprays was adopted with a total of five sprays (from the 3rd of March to the 12th of April), and applications were carried out with a backpack sprayer, equipped with a hollow cone spray nozzle, with a pressure equivalent to 2.88 kgf/m².

Aphid counts

The counting of aphids started 10 days after the first spray (3rd of March, 2017). The investigation was carried out on leaves. Randomly, 10 leaves (upper and lower surfaces) from each treated or untreated plant (Control) were

inspected (Sayed and Montaser 2012). This inspection continued for 10 days after each spray until the last fungus spray (22nd of April, 2017).

Estimation of rose yield

Numbers of flowers per plant and total weight of flowers in treatment and control plants were estimated by the end of the season.

Extraction of rose oil

The Soxhlet extraction method was used for obtaining the rose oil. Flowers were filled in a thimble placed in a cylinder. The apparatus was fitted into a flask containing 95% pure *N*-hexane as a solvent (Moates and Rehynolds 1991). The yield of rose petals of each treatment was used for oil extraction. The flask containing *N*-hexane was heated until boiling. Hexane was vaporized and condensed into thimble. It dissolves the compounds of petals' volatiles. By this way, the organic components were obtained in the flask along with hexane. Then, the oil amounts of each treatment were measured in microliters by a micropipette.

Statistical analysis

The LC₅₀ value for each isolate was calculated, using Probit analysis with SPSS program (SPSS 2015). The LC₅₀ values, 95% confidence intervals (lower bound–upper bound), intercept, slope, and chi-square of each isolate were subjected to one-way ANOVA test and means were compared by Duncan's test ($P = 0.05$). In order to correct the mortality data in the treatments with that in the control, Abbott's formula was used (Abbott 1925). The aphid percentage of reduction was analyzed for the five treatments started from day 10 after the first spray. One-way ANOVA was conducted for all parameters, and means were compared by Duncan's test ($P = 0.05$), using SPSS program, version 23 (SPSS 2015).

Results and discussion

The LC₅₀ values for the four tested indigenous isolates of *B. bassiana* against *M. rosae* after 3 days of treatment are shown in Table 1. The LC₅₀ value of isolate 1 (6.46×10^4) was significantly higher than the other 3 ones (1.46×10^5 ,

1.52×10^5 , and 1.71×10^5 for isolates 2, 3, and 4, respectively) ($F = 8.141$, $P = 0.002$). There were insignificant differences among the lowest bound of 95% confidence intervals of LC₅₀ values ($F = 2.4$, $P = 0.106$), while these differences were significant in the upper bound ($F = 2.863$, $P = 0.03$). The differences in intercepts, slopes, and χ^2 were also insignificant ($F = 0.088$, 0.149 , and 1.734 , $P = 0.965$, 0.929 , and 0.200 , respectively). This result is in correspondence with previous findings of many studies such as of Selvaraj et al. (2012) who found that the LC₅₀ value of *B. bassiana* was 1.5×10^4 spores/ml to coriander aphid, *Hyadaphis coriandri*. Liu et al. (1999) reported that LC₅₀ values for six isolates of *B. bassiana* against *M. persicae* ranged from 1.2×10^4 to 1.55×10^6 spores/ml. Eidy et al. (2016) recorded LC₅₀ value of *B. bassiana* on *M. rosae* as 2.66×10^5 spores/ml. Moreover, Saranya et al. (2010) recorded that the LC₅₀ value of *B. bassiana* was 4.5×10^4 spores/ml against the aphid, *Aphis craccivora* adults. Nirmala et al. (2006) revealed that 6.57×10^5 spores/ml was the LC₅₀ value for a *B. bassiana* strain against *A. gossypii* nymphs. In this sense, the LC₅₀ values of *B. bassiana* on aphid differed according to the isolate virulence and the host species. For examples, Akmal et al. (2013) found that LC₅₀ values of *B. bassiana* on the aphids *Brevicoryne brassicae* and *Schizaphis graminum* were 6.28×10^5 and 6.76×10^6 spores/ml, respectively. Moreover, the virulence of various isolates of *B. bassiana* varied against whitefly nymphs on different host plants (Zafar et al. 2016).

Under field experiments, *M. rosae* was the single aphid species recorded on rose plants. The data presented in Table 2 revealed that the aphid's populations in the control increased gradually through the experimental period and provided the highest aphid density values during the last investigation on 22nd of April (32.9 aphid individuals per leaf).

After 10 days of 1st spray, aphid individuals were significantly decreased on leaves in all treatments (13.57–15.67 aphid individuals per leaf for a concentration of 2.3×10^6 /ml) and (6.97–8.7 aphid individuals per leaf for the concentration of 4.6×10^6 /ml) comparing to the control (18.67 aphid individuals per leaf). The aphid infestation rates on leaves decreased gradually during the experimental period in all treatments with at commercial and

Table 1 Values of LC₅₀ with 95% confidence intervals of the four tested indigenous isolates of *Beauveria bassiana* on rose aphid, *Macrosiphum rosae*

Isolates	LC ₅₀ (spores/ ml)	95% confidence intervals		Intercept	Slope ± SE	χ^2 (df = 3)
		Lower bound	Upper bound			
1	6.46×10^4 ^A	3.83×10^4 ^A	1.08×10^5 ^A	4.0788 ^A	0.853 ± 0.076 ^A	6.0498 ^A
2	1.46×10^5 ^B	9.38×10^4 ^A	2.20×10^5 ^B	4.1862 ^A	0.814 ± 0.077 ^A	4.8008 ^A
3	1.52×10^5 ^B	7.53×10^4 ^A	3.25×10^5 ^B	4.0524 ^A	0.784 ± 0.073 ^A	7.811 ^A
4	1.71×10^5 ^B	9.72×10^4 ^A	3.31×10^5 ^B	4.2958 ^A	0.824 ± 0.067 ^A	5.8834 ^A

Means within each column bearing different letters are significantly different according to the Duncan test ($P = 0.05$)

Table 2 Population dynamics of aphid, *Macrosiphum rosae*, on rose plants leaves treated with the four tested indigenous and one commercial *Beauveria bassiana*

Concentrations	Treatments	No. of aphids per leaf (mean \pm SD)				
		13 Mar.	23 Mar.	02 Apr.	12 Apr.	22 Apr.
Control (spray with water)		18.67 \pm 1.91 ^A	27.73 \pm 3.40 ^A	29.70 \pm 3.06 ^A	31.53 \pm 1.70 ^A	32.90 \pm 3.61 ^A
0.1% v/v (2.3 \times 10 ⁶ /ml)	Naturalis	13.57 \pm 1.37 ^B	12.47 \pm 1.10 ^B	9.33 \pm 0.71 ^B	6.83 \pm 1.01 ^B	6.50 \pm 0.87 ^B
	Isolate 1	15.67 \pm 1.72 ^{AB}	11.77 \pm 2.10 ^B	10.53 \pm 2.47 ^B	8.70 \pm 2.19 ^B	9.17 \pm 2.35 ^B
	Isolate 2	15.40 \pm 1.80 ^{AB}	12.23 \pm 1.40 ^B	11.33 \pm 2.35 ^B	8.63 \pm 1.90 ^B	8.00 \pm 1.25 ^B
	Isolate 3	13.63 \pm 1.80 ^B	13.00 \pm 3.00 ^B	9.73 \pm 2.95 ^B	8.73 \pm 1.72 ^B	7.40 \pm 1.73 ^B
	Isolate 4	13.87 \pm 2.28 ^B	12.57 \pm 3.27 ^B	9.70 \pm 1.50 ^B	9.37 \pm 2.00 ^B	7.40 \pm 1.95 ^B
0.2% v/v (4.6 \times 10 ⁶ /ml)	Naturalis	7.30 \pm 1.78 ^C	4.60 \pm 0.75 ^C	2.43 \pm 0.70 ^C	1.17 \pm 1.07 ^C	0.47 \pm 0.42 ^C
	Isolate 1	7.73 \pm 2.55 ^C	4.97 \pm 1.10 ^C	2.50 \pm 1.25 ^C	1.37 \pm 1.35 ^C	0.83 \pm 0.42 ^C
	Isolate 2	8.70 \pm 2.82 ^C	4.70 \pm 1.25 ^C	2.63 \pm 0.35 ^C	1.50 \pm 1.37 ^C	0.47 \pm 0.57 ^C
	Isolate 3	7.70 \pm 2.13 ^C	5.00 \pm 1.73 ^C	3.13 \pm 1.96 ^C	1.07 \pm 1.05 ^C	0.67 \pm 0.61 ^C
	Isolate 4	6.97 \pm 2.31 ^C	3.87 \pm 1.17 ^C	2.97 \pm 1.16 ^C	1.07 \pm 0.57 ^C	0.60 \pm 1.04 ^C
F values		11.930	34.582	52.370	100.884	97.629
P		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Means within each column bearing different letters are significantly different according to the Duncan test ($P = 0.05$)

indigenous *B. bassiana* but this decrease was slightly observed at the low fungal concentration (Table 2).

Regarding the percent aphid reduction, these values varied and significantly influenced according to the fungus concentration and the investigation time (Table 3). This percentage differed significantly between the two-tested concentrations in all the investigated times but there are no significant differences among all isolates with the same concentration except for the lower concentration on the 22nd of April. After three sprays in the present study, the aphid reduction percentages reached (< 90%) with the highest concentration but this ratio was not reached by the lowest concentration even at the end of the

experiment. In addition, this value (< 95%) was achieved after four sprays with the highest concentration (Table 3). In general, different applications were carried out to investigate the efficacy of various isolates of *B. bassiana* for controlling various species of aphids on different crops (Michereff et al. 2011 and Ujjan and Shahzad 2012). Obtained results revealed that the aphid population in the control increased gradually through the experimental period and gave the highest aphid density values during April. This finding coincides with the results of Sayed and Montaser (2012) who found that the numbers of aphid individuals of *M. rosae* in untreated rose plants during the experiment ranged from 34.75 to 47.1 individuals/leaf.

Table 3 Aphid percentage reduction of aphid, *Macrosiphum rosae*, on rose plants leaves treated with the four tested indigenous and one commercial *Beauveria bassiana*

Concentrations	Treatments	Aphid percentage reduction (mean \pm SD)				
		13 Mar.	23 Mar.	02 Apr.	12 Apr.	22 Apr.
0.1% v/v (2.3 \times 10 ⁶ /ml)	Naturalis	27.32 \pm 7.32 ^A	55.05 \pm 3.97 ^A	68.57 \pm 2.39 ^A	78.33 \pm 3.19 ^A	80.24 \pm 2.65 ^A
	Isolate 1	16.07 \pm 9.23 ^A	57.57 \pm 7.57 ^A	64.53 \pm 8.32 ^A	72.41 \pm 6.95 ^A	72.14 \pm 7.13 ^B
	Isolate 2	17.50 \pm 9.66 ^A	55.89 \pm 5.05 ^A	61.84 \pm 7.90 ^A	72.62 \pm 6.04 ^A	75.68 \pm 3.79 ^{AB}
	Isolate 3	26.97 \pm 9.65 ^A	53.13 \pm 10.83 ^A	67.23 \pm 9.93 ^A	72.31 \pm 5.44 ^A	77.51 \pm 5.27 ^{AB}
	Isolate 4	25.72 \pm 12.22 ^A	54.69 \pm 11.81 ^A	67.34 \pm 5.05 ^A	70.30 \pm 6.34 ^A	77.51 \pm 5.92 ^{AB}
0.2% v/v (4.6 \times 10 ⁶ /ml)	Naturalis	60.89 \pm 9.52 ^B	83.41 \pm 2.72 ^B	91.81 \pm 2.37 ^B	96.30 \pm 3.39 ^B	98.58 \pm 1.26 ^C
	Isolate 1	58.57 \pm 13.68 ^B	82.09 \pm 3.98 ^B	91.58 \pm 4.20 ^B	95.67 \pm 4.28 ^B	97.47 \pm 1.26 ^C
	Isolate 2	53.39 \pm 15.08 ^B	83.05 \pm 4.51 ^B	91.14 \pm 1.18 ^B	95.24 \pm 4.36 ^B	98.58 \pm 1.73 ^C
	Isolate 3	58.75 \pm 11.40 ^B	81.97 \pm 6.25 ^B	89.45 \pm 6.60 ^B	96.62 \pm 3.33 ^B	97.97 \pm 1.86 ^C
	Isolate 4	62.68 \pm 12.39 ^B	86.06 \pm 4.21 ^B	90.01 \pm 3.90 ^B	96.62 \pm 1.80 ^B	98.18 \pm 3.16 ^C
F values		9.062	14.543	15.294	19.716	25.781
P		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Means within each column bearing different letters are significantly different according to Duncan test ($P = 0.05$)

The same observation was recorded in the study of Sayed and Alghamdi (2017).

The results showed that both commercial and indigenous isolates were effective against rose aphid. Filho et al. (2011) demonstrated that the numbers of adults and nymphs of *M. persicae* per leaf were significantly reduced in plots treated with two *B. bassiana* isolates ranging from 57 to 60%. Further field studies using screened cages with isolate CG 864 (formulated as oil dispersion) reduced the population of aphids by 85–87% than the untreated plants, while a reduction of 71% was demonstrated in the plants treated with the aqueous conidial suspension, 20 days after the first spray. Furthermore, in a commercial cabbage field (without cages), the fungus was sprayed at three different times, each with an equivalent of 1×10^{13} viable conidia per ha. In this experiment, the aphid reduction per leaf was the most evident between 4th and 5th weeks after the first spray, resulting in 76–83% and 57–65% control for unformulated conidia and oil dispersions, respectively. Generally, *B. bassiana* as an EPF is effective on various sucking insect pests (Dara 2017). Different investigations stated that *B. bassiana* showed high efficacies for controlling different aphid species such as *Aphis craccivora* Koch. infesting cucumber plants (Zaki 1998) and the cabbage aphid *Brevicoryne brassicae* (Pacheco et al. 2017). Moreover, Murphy et al. (1998) used *B. bassiana* to control the western flower thrips (WFT) on commercial roses and stated that significantly fewer WFT were detected in the *B. bassiana* ES plots than in the untreated (control) plots. Obtained results indicated that the aphid reductions, with a concentration of 4.6×10^6 conidia/ml were about 98% for all indigenous and commercial isolates. Loureiro and Moino (2006) found that *B. bassiana* applied at 10^6 spores/

ml caused 100% mortality in the aphid *M. persicae* while Ramegowda et al. (2007) recorded that 93.33% mortality was achieved on sugarcane woolly aphid, *Ceratovacuna lanigera*, after 10 days of treatment.

The data presented in Table 4 represent the effect of the four indigenous and one commercial *B. bassiana* on rose plant flowers' formation and oil yield. Number of flowers per plant was not affected by these applications. It ranged between 756 and 885 flowers per plant. The weight of flowers per plant was significantly differed between the control and the highest concentration of all tested isolates but with insignificant differences between the control and the lowest concentration of all tested isolates. The current findings revealed that the amount of oil extracted from flowers per plant was highly affected by these applications. But among the same concentration treatments, there were insignificant differences. The findings coincide with previous results on quantification of lipids in the rose inflorescence as its level was significantly lowered in highly infested plants with aphids relative to lower aphid infested plants (Singh et al. 2014). In general, due to the infestation of aphids on rose plants, the biochemical composition of plant was affected (Singh et al. 2014). Teulon et al. (1999) reported a high reduction in the value of the medicinal components of the plant and economic loss to growers due to the infestation of insect pests, especially aphids during spring and summer seasons. Moreover, applications of effective control strategies of various pests and diseases especially biocontrol agents under organic management practices in rose production are very important for the production of high flower yields and also the oil quality (Chalova et al. 2017). Virulence of the four tested indigenous isolates in the bioassay indicated that one isolate was higher in its efficacy with lower LC_{50} value than

Table 4 Application effect of the four tested indigenous and one commercial *Beauveria bassiana* on production of rose plants

Concentrations	Treatments	No. of flower/plant (mean \pm SD)	Weight of flower/plant (gm) (mean \pm SD)	Oil amount/plant (microliter) (mean \pm SD)
Control (spray with water)		756.33 \pm 87.05 ^A	773.00 \pm 60.75 ^A	565.00 \pm 66.01 ^A
0.1% v/v (2.3×10^6 /ml)	Naturalis	819.33 \pm 33.29 ^A	931.67 \pm 64.93 ^{AB}	728.67 \pm 74.23 ^B
	Isolate 1	796.00 \pm 80.52 ^A	949.67 \pm 115.98 ^{AB}	706.67 \pm 49.36 ^B
	Isolate 2	752.67 \pm 113.68 ^A	920.67 \pm 159.36 ^{AB}	721.67 \pm 63.71 ^B
	Isolate 3	843.00 \pm 61.02 ^A	947.33 \pm 153.51 ^{AB}	724.00 \pm 31.24 ^B
	Isolate 4	803.67 \pm 59.41 ^A	893.33 \pm 71.74 ^{AB}	692.00 \pm 20.66 ^B
0.2% v/v (4.6×10^6 /ml)	Naturalis	813.67 \pm 105.40 ^A	998.67 \pm 105.19 ^B	855.33 \pm 41.40 ^C
	Isolate 1	825.33 \pm 90.63 ^A	988.00 \pm 98.96 ^B	826.00 \pm 51.26 ^C
	Isolate 2	885.67 \pm 27.75 ^A	1006.00 \pm 34.00 ^B	876.33 \pm 55.18 ^C
	Isolate 3	803.67 \pm 73.92 ^A	978.00 \pm 108.59 ^B	816.67 \pm 56.15 ^C
	Isolate 4	783.67 \pm 25.15 ^A	1012.33 \pm 95.97 ^B	853.33 \pm 26.10 ^C
F values		0.763	1.312	10.035
P		0.662	0.284	< 0.001

Means within each column bearing different letters are significantly different according to the Duncan test ($P = 0.05$)

the other three isolates while all four isolates approximately had the same effect on aphid reduction in the field. This difference in efficacy between laboratory and field assessment might be due to the effect of the environmental conditions under which the tests were carried out.

Conclusions

In conclusion, the concentration 4.6×10^6 conidia/ml of the commercial or indigenous isolates achieved higher reductions of *M. rosae* infestation on rose plants and resulted in the highest extraction of rose oil. Moreover, the use of *B. bassiana* could participate in organic production of rose and prevent the pollution with chemical pesticides in rose oil. Therefore, using the indigenous isolates of *B. bassiana* for controlling aphids on rose plants is recommended. Further studies on the efficacy of the EPF under field conditions are still needed.

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Availability of data and materials

The datasets generated and analyzed during the current study are available in the text.

Authors' contributions

All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

This study does not contain any individual person's data.

Competing interests

The authors declare that they have no competing interests.

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References

- Abbott WS (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267
- Akmal M, Freed SH, Malik MN, Gul HT (2013) Efficacy of *Beauveria bassiana* (Deuteromycotina: Hypomycetes) against different aphid species under laboratory conditions. *J Zool* 45(1):71–78
- Amatuzzi RF, Cardoso N, Poltronieri AS, Poitevi, CG, Dalzoto P, Zawadeneak MA, Pimentel IC (2017) Potential of endophytic fungi as biocontrol agents of *Duponchelia fovealis* (Zeller) (Lepidoptera: Crambidae). *Braz J Biol, Epub November 09, 2017*. <https://doi.org/10.1590/1519-6984.166681>
- Chalova VI, Manolov IG, Manolova VS (2017) Challenges for commercial organic production of oil-bearing rose in Bulgaria. *Biol Agric Hort* 33(3):183–194
- Chau A, Heinz KM (2004) Biological control of aphids on ornamental crops. In: Heinz KM, van Driesche RG, Parrella MP (eds) *Biocontrol in protected culture*. Ball publishing, Batavia, pp 277–295
- Dara SK (2017) Biology and management of sucking pests of canola. Integrated management of insect pests on canola and other Brassica oilseed crops. Reddy G. CABI 68-76
- Eidy M, Dastjerdi HR, Zargazadeh F, Golizadeh A, Mahdavi V (2016) Pathogenicity of the Entomopathogenic Fungi *Beauveria bassiana* (Balsamo) and *Verticillium lecanii* (Zimmerman) against aphid *Macrosiphum rosae*, Linnaeus (Hemiptera: Aphididae) under laboratory conditions. *Jordan J Biol Sci* 9(1):25–29
- Filho MM, Oliveira SOD, De Liz RS, Faria M (2011) Cage and field assessments of *Beauveria bassiana*-based Mycoinsecticides for *Myzus persicae* Sulzer (Hemiptera: Aphididae) control in cabbage. *Neotrop Entomol* 40:470–476
- Inglis DG, Goettel MS, Butt TM, Strasser H (2001) Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N (eds) *Fungi as biocontrol agents: Progress, problems, and potential*. CAB International, Wallingford, pp 23–69
- Karlik JF, Tjosvold SA (2003) Integrated pest management (IPM) for roses. In: Roberts AV (ed) *Encyclopedia of rose science*. Elsevier Science, Amsterdam
- Leite LG, Batista FA, Almeida JEM, Alves SB (2003) Produção de fungos entomopatogênicos. Ribeirão Preto, Pinto AS, 92p
- Liu YQ, Liu SS, Feng MG (1999) Effect of *Beauveria bassiana* on the fecundity of the green peach aphid, *Myzus persicae*. *Acta Phytophylacica Sin* 26(1):30–34
- Loureiro EDS, Moino JA (2006) Pathogenicity of hyphomycete fungi to aphids *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Neotrop Entomol* 35(5):660–665
- Martins ICF, Silva RJ, Alencar JRDC, Silva KP, Cividanes FJ, Duarte RT, Agostini LT, Polanczy RA (2014) Interactions between the entomopathogenic fungi *Beauveria bassiana* (Ascomycota: Hypocreales) and the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae) on *Myzus persicae* (Hemiptera: Aphididae). *J Econ Entomol* 107(3):933–938
- Michereff M, Oliveira S, de Liz RS, Faria M (2011) Cage and field assessments of *Beauveria bassiana*-based Mycoinsecticides for *Myzus persicae* Sulzer (Hemiptera: Aphididae) control in cabbage. *Neotrop Entomol* 40(4):470–476
- Moates GK, Rehnolds J (1991) Comparison of rose extract produced by different extraction techniques. *J Essential Oil Res* 4:289
- Murphy BC, Morisawa TA, Newman JP, Tjosvold ST, Parrella MP (1998) Fungal pathogen controls thrips in greenhouse flowers. *Calif Agric* 52:32–36
- Nirmala R, Ramanujan B, Rabindra RJ, Rao NS (2006) Effect of entomofungal pathogens on mortality of three aphid species. *J Biol Control* 20:89–94
- Pacheco JC, Poltronieri AS, Porsani MV, Zawadeneak MAC, Pimentel IC (2017) Entomopathogenic potential of fungi isolated from intertidal environments against the cabbage aphid, *Brevicoryne brassicae* (Hemiptera: aphididae). *Biocontrol Sci Techn* 25(4):496–509
- Ramegowda GK, Vidya M, Patil RK, Puttannavar MS, Lingappa S (2007) Laboratory and field evaluation of some entomopathogenic fungi against sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner (Homoptera: Aphididae). *J Biol Control* 21:173–176
- Saranya S, Ushakumari R, Jacob S, Philip BM (2010) Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). *J Biopest* 3(1):138–142
- Sayed SM, Alghamdi A (2017) Field evaluation of two indigenous coccinellids species released for controlling the rose aphid, *Macrosiphum rosae* (L.) on rose plants. *Egypt J Biol Pest Control* 27(2):217–221
- Sayed SM, Ali EF, El-Arnaouty SA, Mahmoud SF, Amer SA (2018) Isolation, identification and molecular diversity of indigenous isolates of *Beauveria bassiana* from Taif region, Saudi Arabia. *Egypt J Biol Pest Control* 28:47 6
- Sayed SM, Montaser MM (2012) Preliminary molecular characterization and utilization of native *Orius* sp. (Hemiptera: Anthracoridae) for controlling aphids infesting taif's rose. *Arch Phytopathol Plant Protec* 45(3):373–380
- Seiedy M, Heydari S, Tork M (2015) Orientation of *Hippodamia variegata* (Coleoptera: Coccinellidae) to healthy and *Beauveria bassiana*-infected *Aphis fabae* (Hemiptera: Aphididae) in an olfactometer system. *Turk J Zool* 39:53–58
- Selvaraj K, Kaushik HD, Gulati R (2012) Evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against coriander aphid, *Hyadaphis coriandri* (Das) (Aphididae: Homoptera). *J Biol Control* 26(1):55–58
- Singh P, Dhal MK, Sagar SK (2014) Experimental investigation on nutritional variation in plant foliage of rose (*Rosa damascene*): effect of pest infestation. *Int J Sci Res Publication* 4(5):1–12
- SPSS (2015) IBM SPSS Statistics for Windows (Version 23.0). IBM Corp, Armonk Chicago, IL
- Tanada Y, Kaya HK (1993) *Insect pathology*. Academic Press, London

- Teulon DAJ, Eastop VF, Stufkens MAW, Harcourt SJ (1999) Illustrated multiple entry key for apterous aphids of economic importance in New Zealand. Crop Info Confidential Report No. 612. Crop and Food Research, Lincoln, New Zealand Pp 140
- Ujjan AA, Shahzad S (2012) Use of entomopathogenic fungi for the control of mustard aphids (*Lipaphis serysimi*) on canola (*Brassica napus* L.). Pak J Bot 44:2081–2086
- Zafar J, Freed S, Khan BA, Farooq M (2016) Effectiveness of *Beauveria bassiana* against cotton whitefly, *Bemisia tabaci* (Gennadius) (Aleyrodidae: Homoptera) on different host plants. Pak J Zool 48(1):91–99
- Zaki FN (1998) Efficiency of the entomopathogenic fungus, *Beauveria bassiana* (Bals), against *Aphis craccivora* Koch and *Bemesia tabaci*, Gennadius. J Econ Entomol 122(1–5):397–399
- Zayed A (2003) Pathogenicity of two *Beauveria bassiana* indigenous isolates towards the greater wax moth *Galleria mellonella* L. larvae in Egypt. Efflatounia 3:10–14

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