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Research Article

Field Evaluation of Native Fungus, *Beauveria bassiana* (Bals.) Vuillemin Against some Piercing-Sucking Insects on the Grapevine

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Abstract

Background and Objective: The Taify cultivar of grapevine (*Vitis vinifera* L.) is the second important economical fruit after pomegranate at high altitudes of the Taif region in Saudi Arabia. The grapevine trees are infested with different piercing-sucking insect pests especially aphids, whiteflies and thrips. The purpose of this study was to evaluate the ability of an indigenous endophytic entomopathogenic fungus, *Beauveria bassiana* to control the important piercing-sucking insect pests on grapevines. **Materials and Methods:** This investigation was carried out through 5, 10 and 15 day intervals between sprays for controlling *Aphis illinoisensis*, *Bemisia tabaci* and *Frankliniella occidentalis* with a concentration of 6×10^6 conidia mL^{-1} under field conditions. **Results:** The higher infestation in the untreated control was by aphids followed by whitefly and thrips. At the end of the experiment in the treated trees, aphid and whitefly reduction percentages with 5 day intervals of sprays (98.5 and 96.12%, respectively) were not significantly different from 10 day intervals (95.17 and 91.81%, respectively) while these reductions were significantly higher than the reduction occurred by 15 day intervals of sprays (65.93 and 44.51%, respectively). Meanwhile, the 3 intervals of sprays did not differ significantly in the thrips reduction occurred by them with a range from 93.62-96.46%. **Conclusion:** This indigenous *B. bassiana* as 6×10^6 conidia mL^{-1} with 10 day intervals of the spray-on grapevine can suppress the piercing-sucking insect pests. This also will participate in grapevine organic production and furthermore, it could replace the chemical treatment.

Key words: Entomopathogenic fungi, *Vitis vinifera*, aphid, whitefly, thrips, *Aphis illinoisensis*, *Bemisia tabaci*, *Frankliniella occidentalis*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Grapevine (*Vitis vinifera* L. cv. Taify) is considered as the second important economical fruit after pomegranate at the Taif region in Saudi Arabia. This cultivar is consumed as table grapes, grape juice, or raisins and is reported as the best quality in its chemical composition comparing to other cultivars cultivated in Saudi Arabia¹. The grapevine trees are infested with various insect pests especially piercing-sucking insects such as aphids, whiteflies, thrips, scale insects, mealybugs and leafhopper. The cotton aphid, *Aphis gossypii* Glover and the grapevine aphid, *Aphis illinoisensis* (Shimer) (Hemiptera: Aphididae) are among the aphid pests infesting grapevines. The second aphid infests the young terminal shoots, stems and the lower surface of youthful leaves while fruit clusters infestation causes the dropping of some grape berries². Moreover, the grapevine cultivar significantly affects the mean adult longevity and mean post-reproductive period while developmental and mortality rates, mean fecundity and population growth parameters were not significantly different among grapevine cultivars³. The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is considered as one of the 24 species included in the defined species complex of *B. tabaci*⁴. It infests a wide range of over 600 species of host plants including grapevines and caused direct damage by feeding on the phloem sap while indirect damage occurred by the production of the large amounts of honeydew, which induces the saprophytic fungi growth on leaves and fruits⁵. Thrips (Thysanoptera: Thripidae) are reported as pests on table grapes in different areas of the world. Many thrips species cause damage to grapes, such as *Frankliniella occidentalis* (Pergande), *F. cestrum* Moulton, *Drepanothrips reuteri* (Uzeli) and *Thrips tabaci* (Lindeman). The first species feeds on young leaves, causing silvery and depressed lesions in the leaf tissue, while fruit damage results from the oviposition and feeding of the insect during the blooming and early post-blooming periods⁶.

Endophytic fungi are present entirely within various tissues of the host plant and do not cause apparent disease symptoms. They may have mutualistic relationships with their plant hosts⁷. Various endophytic fungi protect the host plant from different insect pests by producing some toxic compounds or by modifying the defense response of the host plant to enhance pest and pathogen resistance⁸. Some of these fungi could be isolated from plant tissues and used as biocontrol agents against insect pests. Also, they can be isolated from the soil or infected insects⁹. The endophytic entomopathogenic fungi (EEPF) could be used in two

strategies for pest control; spray and/or plant inoculation. The inoculation methods are foliar spray and seed treatment, which both resulted in negative impacts on different herbivores (41 and 53%, respectively)¹⁰. *Beauveria bassiana* Balsamo (Vuillemin) has been recognized as an endophyte that happens normally in a various range of plant species¹¹. Recently, there is an increasing interest in isolation, mass propagation and use of endophytic EPF including *B. bassiana* for controlling insect pests and plant diseases¹².

It is conceivable to discover individual isolates or pathotypes which represent a significantly restricted host range¹³. The indigenous species are preferred as biocontrol agents when both indigenous and exotic species/isolates have the same potentiality advantages because they are already adapted and tolerant to the local climatic conditions and have less non-target effects in a given environment when released. Thus, they are more successful and maintain control for a long time, than other strategies¹⁴. Different EPF formulations are used in pest control as concentrated suspensions and wettable powders for spray application or as beads and granules for soil treatment¹⁵. Liquid formulations of EPF contained oils leads to increase the adhesion and retention on leaves and can be atomized into small droplets and also providing the prolonged humidity needed for spore germination¹⁶.

Therefore, this study aimed to evaluate an indigenous isolate of *B. bassiana* against the important piercing-sucking insect pests; *A. illinoisensis*, *B. tabaci* and *F. occidentalis* on grapevine under field conditions. This evaluation was carried out at 3 different spray strategies; 5, 10 and 15 day intervals to obtain which one is effective for suppressing these pests in the field.

MATERIALS AND METHODS

Study area: The study was carried out at Taif Governorate, Saudi Arabia during April and May, 2020.

Fungus isolate, propagation and suspension preparation:

An indigenous isolate of endophytic *B. bassiana* named Bb-Taif1 which was isolated from grapevine leaves tissues at the Taif region¹⁷ was used in this study. The isolate was cultured on Sabouraud Dextrose Agar (SDA). Aerial conidia were mass propagated 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). Conidia were first dehydrated in desiccators containing silica gel for 7 days under room

temperature conditions to obtain pure conidia. Then, the colonized substrates were sieved in a 100-mesh sieve under the agitation of 250 rpm. The harvested conidia were adjusted to 6×10^6 spores mL^{-1} where this concentration achieved LC_{90} for *A. illinoisensis* after 3 days of treatment in the laboratory¹⁷. Also, 0.02% of Tween 80 was added to disperse the conidia uniformly. The suspension was stored at 4°C until used.

Field experiment: The experiment was carried out on a farm at Taif Governorate, Saudi Arabia which is cultivated with Taify grapevine trees. The tree's height is about 2 m and also 2.5 m interface between trees. The experiment was conducted with a Randomized Complete Block Design (RCBD). Four trees were used for each treatment. All the 3 treatments were treated with the same conidial concentration (6×10^6 spores mL^{-1}) while the control (untreated trees) was treated with water. In treatment (1), 5 day intervals between sprays were adopted with a total of 6 sprays (6th, 11th, 16th, 21th, 26th of April and 1st of May, 2020). In treatment (2), 10 day intervals between sprays were adopted with a total of 3 sprays (6th, 16th and 26th of April). In treatment (3), a 15 day interval between sprays was adopted with a total of 2 sprays (6th and 21st of April). The application was carried out with a backpack sprayer, equipped with a hollow cone spray nozzle where the conidial suspension was manually shaken just before spraying. Furthermore, to limit the negative effects caused by UV radiation, the conidial suspensions and the water for control were maintained with 1% glycerol and 1% canola oil¹⁸. For the same purpose, the treatments were carried out in the early morning.

Estimation of pests infestation: The counting of aphids, whiteflies and thrips was done on the upper and lower surfaces of leaves every 5 days, started on the same day of 1st spray days (6th of April) and continued till 6th of June. Randomly 3 leaves from each treated or untreated tree (Control) were inspected with a total of 12 leaves for each treatment or control. For each pest reduction estimation, each tree was considered as 1 replicate (total 4 replicates).

Data analysis: The percentages of reduction for the three investigated pests were calculated from the infestation data according to the equation of Henderson and Tilton¹⁹. One-way ANOVA was conducted for infestation (individuals/leaf) and reduction (%). Means were compared by Duncan's test ($p = 0.05$), using SPSS program ver. 23²⁰.

RESULTS AND DISCUSSION

Data presented in Table 1 show the infestation and reduction of aphids. The initial infestation at the beginning of the experiment on untreated trees was 20.17 individuals/leaf and was not significantly different from all trees those treated after this date ($p = 0.476$). After 5 days of 1st spray, aphid infestation in all treatments (ranged from 8.75-9.34 individuals/leaf) did differ significantly from that in the control (26.59 individuals/leaf), without a significant difference among all treatments in aphid reduction (ranged from 63.4-68.64%). After 10 days of 1st spray, treatment (1) received 2 sprays while treatments (2) and (3) received 1 spray only resulted in a significant difference in the aphid reduction in treatment (1) (89.17%) and both of treatments 2 (68.27%) and 3 (63.0%). After 15 days of 1st spray, treatment (1) received 3 sprays while treatment (2) received 2 sprays but treatments (3) received only 1 spray. This achieved an insignificant difference in the aphid reduction between treatment 1 (93.63%) and 2 (93.02%) but both of them were significantly different from treatment 3 (46.78%). The same last significant differences continued until the end of the experiment where treatment (1) was treated with 6 sprays while treatment (2) had 3 sprays but treatment (3) had 2 sprays only (15 day interval). The infestation reached 49.58 individuals/leaf in the control at the last investigation (after 30 days). At this time, the aphid reduction reached 98.5, 95.17 and 65.93% in treatments 1, 2 and 3, respectively. This result confirms that the aphid reduction with 5 day intervals of EEPF *B. bassiana* spray did not differ significantly with 10 day intervals but both of them were significantly different from aphid reduction occurred with 15 day intervals of spray. This result is in agreement with the previous finding where after 3 sprays (10 day interval) of indigenous *B. bassiana* with 4.6×10^6 conidia mL^{-1} on rose plants, the reduction percentage of rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) reached 91.58%²¹. Also, 93.33% mortality for sugarcane woolly aphid, *Ceratovacuna lanigera* was achieved after 10 days of treatment with *B. bassiana*²². Field evaluation of *B. bassiana* for *Myzus persicae* Sulzer (Hemiptera: Aphididae) control on cabbage at 3 different times with an equivalent of (1×10^{13} viable conidia ha^{-1}) achieved the higher aphid reduction between 4th and 5th weeks after the 1st spray, with a range of 57-65% and 76-83% for oil dispersions and unformulated conidia, respectively²³. A positive effect was reported on aphid populations on cotton plants after 1 week of exposure to

B. bassiana inoculated plants, while after 2 weeks of exposure, the aphid population growth was negatively affected²⁴.

Regarding whitefly control, Table 2 indicated its infestations and reduction percentages where the initial infestation was 10.25 individuals/leaf which did not significantly differ from all trees treated after this date ($p = 0.928$) as the result for aphid infestation. After 5 days of 1st spray, there was no significant difference among treatments 1, 2 and 3 in infestation rates (6.25, 7.08 and 6.33 individuals/leaf, respectively) and significantly different from that in the control (11 individuals/leaf). At this period, there was no significant difference among treatments 1, 2 and 3 in whitefly reduction (45.19, 35.41 and 39.81%, respectively). These lower reductions compared to the aphid reductions at the same time may be related to the pathogenicity of this isolate or because the whitefly behavior where the 2nd, 3rd and 4th nymphal instars are immobile and remain flattened on the leaves and therefore they are not exposed to more conidia. The same differences between infestation and reduction rate were observed on the 10th day of the investigation. After 15 days of 1st spray, there was no significant difference in the aphid reduction between treatment 1 (87.40% with 3 sprays) and 2 (87.14% with 2 sprays) but both of them did differ significantly from treatment 3 (45.16%) which received 1 spray only. In the same context of aphid reduction, the same last significant differences for whitefly reduction continued until the end of the experiment. The whitefly infestation reached 21.25 individuals/leaf in the control at the end of the experiment while the reduction reached 96.12, 91.81 and 44.51% in treatments 1, 2 and 3, respectively. This obtained result is the same result for aphid control which stated that the whitefly reduction with 10 day intervals of spray was not significantly different from 5 day intervals while both of them were significantly higher than the reduction occurred by 15 day intervals of spray. Different investigations recorded high impacts for EEPF on *B. tabaci*. For example, after 15 days of eggplant colonization with 2 different isolates of endophytic fungus, *Cordyceps fumosorosea* resulted in a significant reduction of *B. tabaci* incidence²⁵. Also, the transient endophytic colonization by *Metarhizium brunneum* and *B. bassiana* following the spraying of conidia onto the plant achieved an increase in nymphs' mortality of *B. tabaci*²⁶.

For *F. occidentalis* control, the infestation rate at the beginning of the experiment (ranged from 5.92-6.75 individuals/leaf) was lower than other investigated

insect pests (Table 3). After the 1st spray, all investigations from the beginning till the end of the experiment, there was no significant difference among treatments 1, 2 and 3 in infestation rates but they were significantly different from that in the control. Also, there was no significant difference among treatments 1, 2 and 3 in thrips reduction in all investigation dates. This result indicates that 5, 10 and 15 day intervals were not significantly different in their impact on thrips reduction. This may be due to the lower number of thrips per leaf. Moreover, the thrips infestation in the control was slightly increased at the experiment end compared to aphid and whitefly where it reached 10.75 individuals/leaf while the reduction reached 96.46, 94.56 and 93.62% in treatments 1, 2 and 3, respectively. In the same context, using *B. bassiana* to control *F. occidentalis* on rose plants achieved a significant reduction of this pest²⁷. Other EPF is effective to control thrips on grapevines or other plants. For example, the treatment of *Metarhizium anisopliae* (1×10^7 conidia mL⁻¹) with methiocarb (100 mL/100 L) on Niagara table grape achieved 95.5% efficacy control for *F. occidentalis* comparing to 54.9% with only methiocarb treatment⁶. Onion inoculation with *B. bassiana* had negative effect against onion thrips, *Thrips tabaci* Lindeman (Thysanoptera:Thripidae)²⁸.

In general, it was demonstrated that *B. bassiana* as an EPF has a high effect on various sucking insect pests²⁹. For examples on the grapevine, the use of conidial suspension of *B. bassiana* on grapevine leaves to control the grape Phylloxera, *Phylloxera vastatrix* (Phylloxeridae:Hemiptera) gave a lethality rate of 70% (2.5×10^7 conidia mL⁻¹) and 94.7% (3.2×10^9 conidia mL⁻¹) after six days and it was similar to that achieved by chemical preparation¹⁸. The low endophytic colonization rates of *B. bassiana* in grapevine plants in the field resulted in significant negative effects on the infestation of *Planococcus ficus* and grape leafhopper, *Empoasca vitis*³⁰. Recently, biological pest control with EEPF plays an important role in the sustainable management of insect pests. EPF has several advantages over conventional insecticides, including cost-effectiveness, absence of harmful side-effects for beneficial organisms, high yield, fewer chemical residues in the environment and increased biodiversity in ecosystems³¹. Moreover, many investigations have proven the effectiveness of indigenous isolates of EPF including *B. bassiana* whether isolated from soil, infects insect, or from plant tissues (EEPF) against native insect pests^{32,33}. Furthermore, EEPF, *B. bassiana* can be combined with other natural enemies such as the predator, *Chrysoperla carnea* and the parasitoid, *Aphidius colemani* in IPM programmes^{34,35}.

Table 1: Population densities and reduction (%) of grapevine aphid, *Aphis illinoisensis* on grapevine leaves treated with *Beauveria bassiana* over three different periods interval

Treatments/days interval	April, 6		April, 11		April, 16		April, 21		April, 26		May, 1		May, 6	
	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction
Control (untreated plants)	20.17±0.8 ^A	-	26.59±1.1 ^A	-	31.52±1.2 ^A	-	34.67±2.0 ^A	-	40.67±2.3 ^A	-	45.58±1.8 ^A	-	49.58±2.6 ^A	-
Treatment 1/5 days	21.25±1.3 ^A	8.75±0.64 ^B	8.75±0.64 ^B	68.51±1.7 ^A	3.50±0.63 ^C	89.17±1.0 ^A	2.34±0.73 ^C	89.17±1.0 ^A	93.63±1.7 ^A	1.58±0.61 ^C	96.36±1.2 ^A	1.42±0.45 ^C	97.08±0.7 ^A	98.50±0.7 ^A
Treatment 2/10 days	21.50±1.0 ^A	8.83±0.74 ^B	8.83±0.74 ^B	68.64±1.9 ^A	10.50±0.56 ^B	68.27±2.1 ^B	2.58±0.61 ^C	68.27±2.1 ^B	93.02±0.2 ^A	3.33±0.8 ^{BC}	92.19±1.5 ^A	1.25±0.49 ^C	97.46±0.8 ^A	95.17±1.3 ^A
Treatment 3/15 days	19.42±0.9 ^A	9.34±.89 ^B	9.34±.89 ^B	63.40±1.2 ^A	11.17±0.71 ^B	63.00±1.7 ^B	17.75±1.2 ^B	63.00±1.7 ^B	46.78±1.9 ^B	6.33±0.68 ^B	83.68±1.1 ^B	10.08±0.5 ^B	76.92±0.7 ^B	65.93±2.4 ^B
F-values	0.85	100.55	<0.001	3.24	208.16	65.93	140.08	65.93	314.66	199.72	23.36	415.05	207.19	112.89
p-value	0.476	<0.001	0.087	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Infestation: Number of aphid individuals per leaf (Mean±SE), in each column, means bearing different letters are significantly different according to Duncan test (p = 0.05)

Table 2: Population densities and reduction (%) of the whitefly, *Bemisia tabaci* on grapevine leaves treated with *Beauveria bassiana* over three different periods interval

Treatments/days interval	April, 6		April, 11		April, 16		April, 21		April, 26		May, 1		May, 6	
	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction
Control (untreated plants)	10.25±0.5 ^A	-	11.00±0.5 ^A	-	14.17±1.0 ^A	-	15.50±1.0 ^A	-	16.42±0.9 ^A	-	18.67±1.7 ^A	-	21.25±1.0 ^A	-
Treatment 1/5 days	10.75±0.6 ^A	6.25±0.58 ^B	6.25±0.58 ^B	45.19±4.4 ^A	3.25±0.73 ^B	78.30±2.7 ^A	2.00±0.52 ^C	78.30±2.7 ^A	87.40±2.4 ^A	1.00±0.73 ^C	93.90±1.7 ^A	1.08±0.48 ^C	94.68±1.8 ^A	96.12±3.0 ^A
Treatment 2/10 days	10.33±0.5 ^A	7.08±0.62 ^B	7.08±0.62 ^B	35.41±6.4 ^A	3.42±0.72 ^B	75.50±6.2 ^A	2.00±0.49 ^C	75.50±6.2 ^A	87.18±1.6 ^A	1.58±0.5 ^{BC}	90.28±3.2 ^A	1.04±0.52 ^C	94.04±2.7 ^A	91.81±3.0 ^A
Treatment 3/15 days	10.58±0.6 ^A	6.33±0.77 ^B	6.33±0.77 ^B	39.81±4.6 ^A	4.33±0.87 ^B	70.16±7.0 ^A	8.75±1.01 ^B	70.16±7.0 ^A	45.16±2.0 ^B	4.83±0.74 ^B	71.32±2.2 ^B	7.67±1.14 ^B	59.92±6.6 ^B	44.51±3.0 ^B
F-values	0.15	11.65	0.857	37.87	37.87	0.535	63.87	0.535	135.53	113.44	23.44	55.99	21.55	88.66
p-value	0.928	<0.001	0.457	<0.001	<0.001	0.603	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Infestation: Number of whitefly individuals per leaf (Mean±SE), in each column, means bearing different letters are significantly different according to Duncan test (p = 0.05)

Table 3: Population densities and reduction (%) of the western flower thrips, *Frankliniella occidentalis* on grapevine leaves treated with *Beauveria bassiana* over three different periods interval

Treatments/days interval	April, 6		April, 11		April, 16		April, 21		April, 26		May, 1		May, 6	
	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction	Infestation	Reduction
Control (untreated plants)	6.08±1.38 ^A	-	7.17±1.27 ^A	-	7.67±1.33 ^A	-	9.41±0.95 ^A	-	9.17±0.93 ^A	-	10.83±0.8 ^A	-	10.75±0.8 ^A	-
Treatment 1/5 days	5.92±1.16 ^A	6.42±1.04 ^A	2.50±0.78 ^B	63.28±3.4 ^A	0.92±0.45 ^B	87.73±5.9 ^A	1.17±0.53 ^B	87.48±0.6 ^A	0.42±0.29 ^B	0.42±0.29 ^B	96.18±2.2 ^A	0.25±0.25 ^B	97.53±2.4 ^A	96.46±3.5 ^A
Treatment 2/10 days	6.42±1.04 ^A	6.75±1.20 ^A	2.17±0.67 ^B	71.95±6.7 ^A	2.67±0.80 ^B	66.19±7.4 ^A	1.25±0.55 ^B	87.23±5.6 ^A	0.83±0.58 ^B	0.83±0.58 ^B	90.27±5.8 ^A	0.42±0.42 ^B	95.87±4.1 ^A	94.56±3.2 ^A
Treatment 3/15 days	6.75±1.20 ^A	0.095	6.73	68.22±7.4 ^A	2.08±0.75 ^B	76.69±6.6 ^A	2.67±0.91 ^B	74.94±5.4 ^A	0.93±0.48 ^B	0.93±0.48 ^B	90.62±3.3 ^A	0.75±0.41 ^B	93.16±2.8 ^A	93.62±4.8 ^A
F-values	0.095	0.963	6.73	0.501	11.18	2.59	26.59	2.51	47.39	47.39	0.66	92.72	0.47	86.12
p-value	<0.001	<0.001	<0.001	0.622	<0.001	0.129	<0.001	0.136	<0.001	<0.001	0.542	<0.001	0.642	0.88

Infestation: Number of thrips individuals per leaf (Mean±SE), in each column, means bearing different letters are significantly different according to Duncan test (p = 0.05)

The findings of the present study revealed that use of an indigenous fungus, *B. bassiana* suppressed piercing-sucking insect pests such as aphids, whiteflies and thrips. This also will participate in grapevine organic production and furthermore, it could replace the chemical treatment. It could be recommended that use this isolate with a concentration of 6×10^6 spores mL^{-1} to control the investigated insect pests. Other studies can be carried out for determination the efficacy of this isolate on other insect pests on grapevines and other economic crops.

CONCLUSION

Aphid and whitefly reduction percentages with 5 day intervals of sprays were not significantly different from 10 day intervals while these reductions were significantly higher than the reduction occurred by 15 day intervals of sprays. Meanwhile, 5, 10 and 15 day intervals of sprays did not differ significantly in the thrips reduction occurred by them. Further investigations can be carried out on the efficacy of this EEPF under field conditions on other pests such as chewing insect pests.

SIGNIFICANCE STATEMENT

Using this indigenous endophytic *B. bassiana* as 6×10^6 conidia mL^{-1} with 10 day intervals of spray-on grapevine can suppress the piercing-sucking insect pests especially aphids, whiteflies and thrips. This also could participate in grapevine organic production and it could replace the chemical treatment.

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