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Effects of some entomopathogenic fungi on the aphid species, *Aphis gossypii* Glover (Hemiptera: Aphididae)

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Abstract

Background: Effects of the entomopathogenic fungi *Beauveria bassiana*, *Verticillium alfalfae*, and *Trichoderma viride*; secondary metabolites of MS1 (*B. bassiana*) and MS2 (*V. alfalfae*); and Dimethoate active substances on the aphid species, *Aphis gossypii* Glover (Hemiptera: Aphididae), were tested.

Main body of the abstract: Fungus isolates were prepared as 10^7 conidia ml^{-1} of spore suspensions and applied on the 2nd instar nymphs of *A. gossypii*. After the applications, evaluations were made on the 1st, 3rd, 5th, and 7th days by counting the live individuals. Obtained data were 100 and 93% mortality rate at MS1 (*B. bassiana*) and MS2 (*V. alfalfae*), and secondary metabolites were recorded in the 3rd day count results. On the 5th day counts, the highest mortality rates after secondary metabolites were statistically at the same group with *B. bassiana*, *T. viride*, and dimethoate. On the 7th day, counting results of all experiment groups were analyzed statistically and were found effective.

Short conclusion: Obtained results showed that the fungal secondary metabolites might be useful when utilized as a biocontrol agent against the aphids.

Keywords: *Aphis gossypii*, Entomopathogenic fungi, Biological control, Secondary metabolite

Background

Aphids are one of the most important insect groups that cause economic damages in the agricultural fields. Of them, the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a cosmopolitan species, widely spread in tropical, subtropical, and temperate regions of the world (Leclant and Deguine 1994). Due to its wide host range and as vector of many plant viruses, it is an important pest. Nowadays, synthetic chemical insecticides are generally used to pest control (Wang et al. 2002). However, the widespread usage of chemical insecticides causes pest resistance, non-target organisms, and negative effects on the environment (Antwi and Reddy 2015). However, these negative effects on human health, environment, and non-target organisms could be reduced with IPM implementations (Lopes et al. 2009). Using the entomopathogenic microorganisms as biological control agents is an important application in the field (Lacey and Shapiro-Ilan 2008). It is estimated that 1000 entomopathogenic fungi (EPF) (Shang et al. 2015) and more than 100 mycoinsecticides are used as biological agents worldwide (Jaronski 2010). It has been recorded that fungi are effective in pre-adult and adult periods in insects that belong to orders of Lepidoptera, Hemiptera, and Diptera (Herlinda 2010). EPF used as controlling agents of aphids and some other pests are as follows: *Beauveria bassiana* (Kılıç and Yıldırım 2008), *Metarhizium anisopliae* (Inanlı et al. 2012), *Lecanicillium lecanii* (Ujjan and Shahzad 2012), *Isaria fumosorosea* (Jandricic et al. 2014), *Paecilomyces* (Shi and Feng 2004), and *Nomuraea rileyi* (Devi et al. 2003). EPF usually attack the pest through penetrating the insect cuticle and secrete toxins. Furthermore, they produce secondary metabolites

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effective on their hosts, such as pathogenic fungi (Sentürk and Abacı-Günyar 2019). The secondary metabolites produced by the fungi have low molecular weights and are beneficial bioactive compounds. These compounds are important in the field of agriculture, medicine, and several industrial sectors (Demain and Fang 2000).

In this study, the effects of *B. bassiana*, *V. alfalfae*, and *T. viride* isolates, and the secondary metabolites of MS1 (*B. bassiana*) and MS2 (*V. alfalfae*) on the 2nd instar nymphs of *A. gossypii* were investigated.

Materials and methods

Production of host plants and aphids

Cotton seed, Flash (*Gossypium hirsutum* L.) (ProGen®), variety was used by sowing the seeds in 1.5-l plastic pots that measured 170 mm × 140 mm. When the cotton plants had 5–6 leaves, the pots were transferred to a climate chamber (25 ± 1 °C, 60 ± 5% RH, and 16:8 h light-dark period conditions for aphid production). The aphid production was accomplished by infesting the cotton plants with the aphids.

Preparation of fungi cultures and spore suspensions

B. bassiana, *V. alfalfae*, and *T. viride* were defined morphologically and isolated from the soil of a wheat field at the National Plant Protection Institute (INPV-National Institute of Plant Protection of Constantine, Constantine, Algeria) following the method of Vinayaga Moorthi et al. (2015) and Abdelaziz et al. (2018). To isolate the fungi, 1 g of the soil was diluted in 9 ml of sterile distilled water; then, 100 μl from the 10^{-3}, 10^{-4}, and 10^{-5} dilutions of these suspensions was planted on potato dextrose agar (PDA: 200 g potatoes, 20 g glucose, and 20 g agar), supplemented with chloramphenicol (10 mg l^{-1}). Petri dishes were incubated at 28 °C for 2 weeks.

Preparation of entomopathogenic fungal secondary metabolites

According to the method of Gurulingappa et al. (2011), secondary metabolite preparation was carried out at 4 steps. EPF were first placed in a 250-ml flask containing 100 ml of PDB and incubated at 28 °C for 21 days. The suspension was filtered in a Whatman no. 1 paper. Then, ethyl acetate was used for extraction purposes; later, the solvent was evaporated, using an evaporator. At last, extract was mixed by a sterile water to recuperate of secondary metabolites of MS1 (*B. bassiana*) and MS2 (*V. alfalfae*).

Application of entomopathogenic fungi and secondary metabolites

Fungus and secondary metabolite suspensions were used in the experiments by diluting with Tween 80 (0.05%) sterile distilled water containing 10^7 conidia ml^{-1}. The experiments were carried out with 5 replicates. Blotting paper and untreated cotton leaves were placed on the floor of the Petri dishes. Cells with a size of 5 × 4 cm and 4 cm space were placed on the leaf surface, and 5 individuals in the 2nd instar nymphs of *A. gossypii* were transferred to this space. Fungus suspensions were then sprayed on the nymphs 3 times, using a hand sprayer from about 20 cm distance. The control group contained only Tween 80. After the applications, Petri dishes were incubated at 25 ± 1 °C, 60 ± 5% RH, 16:8 h light-dark period. Live individuals were counted and recorded on the 1st, 3rd, 5th, and 7th days of the experiment. Experiments were carried out with 5 replications.

Analysis of the data

One-way ANOVA was applied to the data obtained, and the data were evaluated using IBM SPSS® Statistics (version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) package statistics program. The difference between the means was determined by using the Tukey (1949) multiple comparison test ($P < 0.05$), and the mortality rates (%) were calculated by using an Abbott formula (Abbott 1925).

$$\text{Abbott's corrected mortality}\% = \frac{(\text{living in control} - \text{living in treatment})}{\text{living in control}} \times 100$$

Results and discussion

The highest mortality rate (53.33%) was recorded at the secondary metabolite of MS2 (*V. alfalfae*) on the 1st day counts of the experiment, while the mortality rate for *V. alfalfae* group was not determined. On the 3rd day counts, 100 and 93% mortality rates were recorded for MS1 (*B. bassiana*) and MS2 (*V. alfalfae*) secondary metabolites, respectively, showing that they were statistically at the same group. On the 5th day counts, the highest mortality rates after secondary metabolites were statistically at the same group with *B. bassiana* and *T. viride* and dimethoate. On the 7th day counts, all experiment groups were recorded statistically in the same group and were found effective (Table 1).

The results in Table 1 show that the 2nd instar nymphs of *A. gossypii*, treated with EPF and secondary metabolites, were highly affected and mortality rates ranged 80–100% on the 7th day of the experiment. The results revealed that the secondary metabolites of MS1 (*B. bassiana*) and MS2 (*V. alfalfae*) had the most efficient pathogenicity (100%), followed by *T. viride* (93.33%), dimethoate (90.00%), *B. bassiana* (80.00%), and *V. alfalfae* (73.33%).

According to the literature, applications of *I. fumosorosea* strain (Ifu13a) (Bugti et al. 2018), *L. lecanii* (Mohammed et al. 2018), and *L. lecanii* 41185 isolates were found effective by 100% on *A. gossypii* individuals at 10^8 conidia

Table 1 Mortality rates resulting from applications of EPF and secondary metabolites at a concentration of 10^7 conidia ml^{-1} to 2nd instar nymphs of *Aphis gossypii*

Fungus isolates	1 DAA	3 DAA	5 DAA	7 DAA
Beauveria bassiana	13.33 ± 7.30ab	63.33 ± 19.93ab	80.00 ± 14.97ab	80.00 ± 14.97a
Verticillium alfalfae	0.00 ± 0.00b	23.33 ± 8.79b	46.67 ± 15.66b	73.33 ± 13.47a
MS1 (*B. bassiana*)	43.33 ± 15.41ab	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
MS2 (*V. alfalfae*)	53.33 ± 20.13a	93.33 ± 7.30a	100.00 ± 0.00a	100.00 ± 0.00a
Trichoderma viride	23.33 ± 10.46ab	66.67 ± 10.83ab	86.67 ± 7.30ab	93.33 ± 4.62a
Dimethoate	10.00 ± 10.95ab	33.33 ± 16.65b	66.67 ± 14.61ab	90.00 ± 7.48a

The means followed by the same letters within columns are not significantly different from each other according to Tukey's HSD ($P < 0.05$)

ml^{-1}concentration (Vu et al. 2007). Similar studies reported the mortality rate of 100% for *B. bassiana* (IBCB 66) and *M. anisopliae* (IBCB 121) isolates applied to *A. gossypii* individuals (Loureiro and Moino 2006). Tesfaye and Seyoum (2010) recorded the mortality rates of 73.33–93.33% for isolates of *Beauveria* and *Metarhizium*. In other studies reported, *Beauveria* ARSEF 5493 isolate was found effective (Jandricic et al. 2014).

EPF are an important regulatory factor in biological control of insects. Earlier studies have been conducted with different species or isolates of EPF against different host species, which showed different pathogenicity. For instance, as a result of the application of *B. bassiana* (BB-72 and BB-252) and *L. lecanii* (V-4) isolates to *Myzus persicae* individuals, 95, 91, and 87% mortality rates were recorded (Nazir et al. 2018). In another study, *B. bassiana* had been reported to have an effect over 75%, as a result of application of BAU004, BAU018, and BAU019 isolates to the same aphid species (Al-alawi and Obeidat 2014). Another study was conducted on *Aphis craccivora* (Koch) individuals; 77.50 to 100% mortality rates were reported in application of *B. bassiana*, *M. anisopliae*, *V. lecanii*, *Hirsutella thompsonii*, and *Cladosporium oxysporum* isolates at the concentration of 10^8 conidia ml^{-1} (Saranya et al. 2010). According to Ekesi et al. (2000), mortality rates at the 4 different concentrations of *B. bassiana* CPD 11, and *M. anisopliae* CPD 4 and 5 isolates were recorded as 58–91, 64–93, and 66–100%, respectively. In a study with other aphids and as a result of the application of *C. oxysporum* isolate at 10^8 conidia ml^{-1} concentration against *Aphis fabae* individuals, the mortality rate was 67.90% (Bensaci et al. 2015). Mortality rate recorded was (86%) for the applications of *V. lecanii* IBCB 473 isolate on *Cinara atlantica* individuals at a concentration of 10^8 conidia ml^{-1} (Loureiro et al. 2004). Mortality rates were reported as 95.83, 63.98, and 51.83%, respectively, as a result of the application of *B. bassiana*, *C. cladosporioides*, and *V. alfalfae* isolates to *Metopolophium dirhodum* (Walker) individuals (Abdelaziz et al. 2018).

Conclusion

The obtained results showed that the fungal secondary metabolites might be useful when utilized as a biocontrol agent against the aphids. Among them, *B. bassiana* and *V. alfalfae* were the most promising ones. However, the present work indicated the potentiality of *V. alfalfae*, as a new resource of secondary metabolite, which may suggest that these metabolites could be used in the selection of candidates of aphid biological control.

Abbreviations

PDA: Potato dextrose agar; DAA: Days after application; ANOVA: Analysis of variance; MS1: Secondary metabolite—*Beauveria bassiana*; MS2: Secondary metabolite—*Verticillium alfalfae*

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Authors' contributions

ABE, OA, AKB, MMS, AO, and İK collaborated in the creation of the manuscript. ABE carried out the experiments, recorded the data, interpreted the results, and wrote the manuscript. AKB supervised the manuscript. All authors read and approved the final manuscript during the present study.

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