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Suitability of five plant species extracts for their compatibility with indigenous *Beauveria bassiana* against *Aphis gossypii* Glov. (Hemiptera: Aphididae)

Samy Sayed[1,2*], Sayed-Ashraf Elarnaouty[2] and Esmat Ali[3,4]

Abstract

Background: The cotton aphid, *Aphis gossypii* Glov. (Hemiptera: Aphididae), is a major insect pest on a wide range of plants that causes high damage and transmits plant viruses. This study was carried out to evaluate an indigenous isolate, *Beauveria bassiana* (*Bb*), and extracts of 5 plant species: *Psiadia penninervia*, *Pulicaria crispa*, *Euryops arabicus*, *Salvia officinalis*, and *Ochradenus baccatus* against *A. gossypii*, as individual and combined treatments to estimate their compatibility under laboratory conditions. Also, the antifungal activity of these plant extracts against *B. bassiana* was evaluated.

Results: LC_{50} value was 8.64×10^4 spores/ml of *Bb* against *A. gossypii*, while LC_{50} values of the tested 5 plant extracts on *A. gossypii* were 103.64, 879.92, 747.90, 783.28, and 262.42 μg/ml for *P. penninervia*, *P. crispa*, *E. arabicus*, *S. officinalis*, and *O. baccatus*, respectively. Both *P. penninervia* and *O. baccatus* extracts had the highest antifungal activities against *Bb* and were significantly different from the other 3 plant extracts. After 24 h of treatment with the combination of *Bb* and each extract, no effect for these combinations on *A. gossypii* mortality was recorded. Meanwhile, 5 days after treatment, the combined treatments between *Bb* and each plant extract achieved a significant increase in mortality than that of the single treatment with *Bb* or plant extract, except for *P. penninervia* extract, which did not achieve a significant mortality increase when combined with *B. bassiana* than that of its single treatment.

Conclusion: *P. penninervia* extract was not compatible with *B. bassiana*, but the other tested 4 plant extracts were compatible with *B. bassiana*. These 4 plant extracts could be used to control aphids in combinations with *B. bassiana*. Further laboratory and field investigations are needed to examine the effects of these plant extracts on other insect pests or associated beneficial insects.

Keywords: Cotton aphid, *Aphis gossypii*, Bioassay, Plant extracts, *Beauveria bassiana*, Compatibility

* Correspondence: samy_mahmoud@hotmail.com
[1]Department of Science and Technology, University College-Ranyah, Taif University, B.O. Box 11099, Taif 21944, Saudi Arabia
[2]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza 12613, Egypt
Full list of author information is available at the end of the article

Background

The cotton aphid, *Aphis gossypii* Glov. (Hemiptera: Aphididae), attacks more than 92 plant families including field, vegetable, fruit, and ornamental crops. The important effect of *A. gossypii* on plants is the transmission of plant viruses. Also, it causes high damage in plants such as necrosis, chlorosis, stunting, wilting, flower and fruit abortion, leaf distortion, and defoliation (Ebert and Cartwright 1997). There are many eco-friendly methods as alternatives to chemical pesticides for insect pest control such as using of natural and biodegradable compounds, predators, parasitoids, and entomopathogenic microorganisms (Ghodke et al. 2013).

Plants protect themselves against herbivorous and microbial attacks through producing secondary metabolites such as phenols, flavonoids quinones, terpenoids, alkaloids, and tannins. Extracts or essential oils of medicinal or aromatic plants are commonly used for pest control due to their efficacy against different life stages of many insect pests (Ahmed et al. 2020). These compounds have antimicrobial activities against a wide range of microorganisms (González-Lamothe et al. 2009). There is an expanding request in the search for new active products and substances for pest control with decreasing negative impacts on the environment (Rodríguez-González et al. 2019). Recently, botanical pesticides and plant extracts have shown an important role for pest control due to their low cost without residual effects, friendly to the environment, high availability, and highly toxic against many insect pests such as aphids. Moreover, they are not likely to cause pesticide resistance among diseases and pests due to their molecule complexity (Bedini et al. 2020). Most of the synthetic pesticides are harmful to many biocontrol agents. Thus, integrated pest management (IPM) combining biocontrol agents and biopesticides is gaining importance and is proved to be an ecologically safe management strategy under which biocontrol agents may be combined with plant-derived extracts (Kalita and Hazarika 2018).

Entomopathogenic fungi (EPF) are considered an important microbial control agent of insect pests. *Beauveria bassiana* (*Bb*) has a virulence against various insect pests. Naturally, it grows in the soil and causing a disease called white muscardine in different arthropod species. Many fungal species or isolates showed a high ability for controlling aphids throughout spraying (Jandricic et al. 2014). Most of the synthetic pesticides do not have compatibility with EPF, but the effect of EPF could be enhanced when applying them with low rates of insecticides. The interaction between these control agents is synergistic, additive, or antagonistic. Synergistic interactions would enhance the efficacy of EPF while reducing the adverse effects of insecticides (Islam and Omar 2012). Many factors are limitations of the EPF as other biological control agents. Among these factors are the environmental conditions and their compatibility with synthetic pesticides or fungicides (Jaber et al. 2018). Plant-derived insecticides are more effective when they are combined with microbial or synthetic insecticides than in the case of their individual application (Isman 2006). The combination of pesticides and EPF contributes to the selection of suitable products for IPM programs (Neves et al. 2001). This combined application can improve the effect of pest control by reducing environmental pollution hazards and minimizing the applied amounts and build-up of pest resistance (Usha et al. 2014).

The compatibility of botanical extracts and EPF depends on the quantitative and qualitative variations in the composition of secondary metabolites, which may have a negative effect on EPF (Ribeiro et al. 2012). Using incompatible botanicals may inhibit the pathogenicity and development of EPF and therefore affecting IPM. Moreover, interactions between botanicals and EPF can be negative or positive. On the other hand, compatible botanicals may enhance EPF and achieve more control efficiency with other benefits such as minimizing the conventional insecticide amount and decreasing insecticide resistance and pollution risks (Quintela and McCoy 1998). Thus, it is important to carry out assessments on other parameters of EPF to determine the compatibility with botanicals in order to arrange the suitable products for best IPM programs in pest control (Ribeiro et al. 2012). The combination between neem and *B. bassiana* caused a higher mortality rate for *B. tabaci* nymphs than individual treatments of both (Islam et al. 2010). Efficacy of 4 EPF including *B. bassiana* was tested alone or in combination with neem oil (1:1) against different sucking insect pests achieving a higher mortality than each individual treatment indicating good compatibility among them (Halder et al. 2013). The combined application of *B. bassiana* or *Metarhizium brunneum* with plant extracts of *Inula viscosa* or *Calotropis procera* had an additive effect on mortality of all stages of *B. tabaci* Gennadius (Jaber et al. 2018). Other plant extracts (eucalyptus or neem) when combined with *B. bassiana* did not achieve a significant increase in mortality of the wheat aphid, *Sitobion avenae* Fab., compared to that of *B. bassiana* only. Meanwhile, the combination of Neem with *B. bassiana* achieved the lowest mortality rate (54%) after 5 days of treatment, which was significantly different from the highest mortality rate of 87% with the mixture of eucalyptus extract and *B. bassiana* (Ali et al. 2018).

The present study was carried out to evaluate an indigenous isolate, *B. bassiana*, and extracts of 5 plant species, widely distributed in the Taif region, Saudi Arabia, against the cotton aphid, *A. gossypii*, under laboratory conditions.

Methods

Plant extracts

Five plants from 3 different families, i.e., *Psiadia penninervia* (*Pp*), *Pulicaria crispa* (*Pc*), and *Euryops arabicus* (*Ea*) (Asteraceae); *Salvia officinalis* (*So*) (Lamiaceae); and *Ochradenus baccatus* (*Ob*) (Resedaceae), were used in this study. These tested plants were morphologically identified by the Herbarium Unit at the Biology Department, Faculty of Science, Taif University, Saudi Arabia. Fresh leaves of these plant species were collected from Al-Shafa, Taif region. Extractions were carried out according to Sayed et al. (2020a). Five grams of fine powder from each plant was extracted with 100 ml 95% methanol at 35 °C for 2 days in a thermostat water bath shaker. After cooling, each extract was centrifuged at 7000 rpm for 15 min and filtered 3 times with Whatman filter paper No. 1. Then, the supernatant was passed through a Buchner funnel in a rotary vacuum evaporator at 30 °C. Each pellet was dissolved in an aqueous solution of dimethylsulfoxide 1% (DMSO) and adjusted to a final concentration of 1000 μg/ml. The plant extracts were stored at 4 °C until they were used for the bioassays.

Fungus isolate

A Saudi Arabian indigenous isolate of *B. bassiana* [Accession numbers: LC338054 for internal transcript spacer (ITS) and LC338058 for cytochrome oxidase I (COI)] was chosen to be used in this study because it had a high virulence against *Macrosiphum rosae* in laboratory and field experiments (Sayed et al. 2019) and *Aphis punicae* in laboratory experiments (Sayed et al. 2020b). The propagation of this isolate was carried out according to Sayed et al. (2019) to obtain a sufficient amount for the experiments with a final concentration of 1×10^7 spores/ml.

Insects

Newly adults of the cotton aphid, *A. gossypii*, were obtained from cultural rearing on cucumber plants.

Antifungal activity of plant extract

All tested plant extracts were estimated for their antifungal activity against the tested *B. bassiana* isolate. Ten milliliters of liquid media of potato dextrose agar (PDA) was placed in each Petri dish (10 cm in diameter). After 30 min, 1 ml of fungus suspension with a concentration of 1×10^8 spores/ml was impregnated on the medium surface. Ten microliters of each plant extract was dropped on a piece of filter paper (5 mm in diameter). Then, the Petri dishes were incubated at 37 °C for 2 days. The formation of the inhibition zone (diameter in millimeters) represented the antifungal activity of each extract against the fungus isolate. Ten replications were carried out for each plant extract.

Fungal bioassay

The fungal isolate suspension was prepared in 6 concentrations of 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, and 1×10^7 spores/ml with 0.02% Tween 80 for homogeneity. Three milliliters from each concentration was sprayed by a fine sprayer machine (Thomas Scientific, USA) on 300 cm^2 (10 μl/cm^2). This area contained 3 young leaves of cucumber plant where each leaf had 20 aphid individuals (3 replicates for each concentration). In control, aphids were sprayed with distilled water with 0.02% of Tween 80. Then, each leaf was kept in a separate Petri dish with moistened cotton tissues for humidity maintenance. The aphid individuals were investigated for mortality on the 5th day after treatment that is indicated by gentle probing with a fine brush.

Plant extract bioassay

The stock for each extract was diluted to obtain 4 concentrations of 125, 250, 500, and 1000 μg/ml. The DMSO solvent (1%) was used as the control. For each plant extract/concentration, 3 ml was sprayed as previously described in the fungal bioassay. After 24 h, the aphids were investigated for mortality.

Bioassay of the combination between fungus and plant extracts

From the resulted data of fungus bioassay, the concentration of 1×10^5 spores/ml was used to be combined with a low concentration of each extract (125 μg/ml). The mixture of each plant extract and fungus was prepared from 1.5 ml of plant extract (250 μg/ml with 2% DMSO) and 1.5 ml of fungus suspension (2×10^5 spores/ml with 0.04 Tween 80) to obtain 3 ml of mixture with a final concentration of 125 μg/ml (plant extract) and 1×10^5 spores/ml (fungus). Therefore, 11 treatments were carried out: 1×10^5 spores/ml (fungus alone), 5 treatments with 125 μg/ml (for each plant extract), and 5 treatments of mixtures of fungus with the 5 tested plant extract. Three control groups were used in this experiment: control 1—aphids were sprayed with distilled water with 0.02% of Tween 80 to correct mortality of fungus alone; control 2—aphids were sprayed with DMSO solvent (1%) to correct mortality of each plant extract alone; and control 3—aphids were sprayed with DMSO solvent (1%) and Tween 80 (0.02%) to correct mortality of mixtures. The spray was applied as previously described in the fungal bioassay. Each treatment or control was repeated 3 times, where each replicate contained 20 aphid individuals. Then, all Petri dishes were investigated after 24 h and 5 days for mortality. All

treatments were carried out under the controlled conditions of 25 ± 2 °C, 65 ± 5% RH, and 16:8 h (L:D).

Statistical analysis

Mortality rate in each treatment was corrected according to Abbott's formula (Abbott 1925). The median lethal concentration (LC_{50}), slope, intercept, and chi-square (χ^2) were estimated using probit analysis of mortality versus concentration. In order to compare the LC_{50} values of plant extracts, analyses of relative median potency (RMP) was used. Meanwhile, one-way ANOVA with Duncan's test was used to compare among corrected mortalities of combination and individual treatments on 1 and 5 days after application. The statistical analysis was carried out using version 23 of the SPSS software program.

Results

Pathogenicity of *B. bassiana* against *A. gossypii*

Regarding the pathogenicity of *B. bassiana* against *A. gossypii* after 5 days of treatment, the mortality rate in the control was 5%. After correction of mortality rates in the treatments, the probit analysis indicated that the LC_{50} value was 8.64×10^4 spores/ml with 95% confidence limits 5.11×10^4 and 1.50×10^5 (intercept = − 2.732 ± 0.114, slope = 0.24 ± 0.01, χ^2 (df) = 47.68 (16), and $P < 0.0001$).

Toxicity of tested plant extracts on *A. gossypii*

No mortality was in the control after 24 h. LC_{50} values of the tested 5 plant extracts on *A. gossypii* are presented in Table 1. *P. penninervia* extract had the highest toxicity (LC_{50} = 103.64 μg/ml) among all the tested plants followed by *O. baccatus* (262.42 μg/ml), *E. arabicus* (747.90 μg/ml), *S. officinalis* (783.28 μg/ml), and *P. crispa* (747.90 μg/ml). Relative median potency (RMP) analyses (Table 2) showed that LC_{50} value of *P. penninervia* was significantly different form all other 4 plant extracts. Also, the LC_{50} value of *O. baccatus* had significant differences from all other 4 plant extracts. Meanwhile, there was an insignificant difference among the LC_{50} values of the other 3 plant species extracts, i.e., *E. arabicus*, *S. officinalis*, and *P. crispa.*

Antifungal activity of tested plant extracts against *B. bassiana*

With the lower concentration of plant extracts (125 μg/ml), the results indicated that there were significant differences among the tested 5 plant extracts in their antifungal activities against *B. bassiana* (F = 12.02, df = 4, 45, $P < 0.0001$). Both *O. baccatus* and *P. penninervia* extracts had the highest antifungal activities, without a significant difference between both of them (14.1 and 12.9 mm of inhibition zone, respectively). These 2 plant extracts were significantly different from the other 3 plant extracts. The other 3 plant extracts had a non-significant difference among them (*E. arabicus* = 9.5 mm, *S. officinalis* (8.4 mm), and *P. crispa* (8.8 mm)) (Fig. 1). According to this result, *E. arabicus*, *S. officinalis*, and *P. crispa* extracts may be more compatible with *B. bassiana* than *P. penninervia* and *O. baccatus* extracts.

Compatibility of tested plant extracts and *B. bassiana* against *A. gossypii*

The mortality rates after 1 day were 1.67, 0, and 6.67% for control groups 1, 2, and 3, respectively, indicated in the "Methods" section. Corrected mortality rates after 24 h of treatments for individual and combined application are shown in Fig. 2. It was stated that the mortality rates caused by the individual treatments of both *P. penninervia* (60 ± 2.89%) and *O. baccatus* (33.33 ± 4.41%) extracts were significantly different from the other 3 plant extracts and *B. bassiana* (ranged from 4.58 to 10% without significant differences). However, the mixture of each plant extract and *B. bassiana* was non-significantly different from that of individual treatments for either of them after 24 h of application. The mortalities after 5 days were 5, 6.67, and 11.67% for control groups 1, 2, and 3, respectively, indicated in the "Methods" section. The corrected mortalities after 5 days of treatments are indicated in Fig. 3. The mortality rates caused by individual treatments of plant extracts were in the same context after 24 h of treatments where the mortality rates of *P. penninervia* (69.6%) and *O. baccatus* (53.57%) extracts were significantly different from the other 3 plant extracts, those that had no significant differences among them (ranged from 17.68 to 32.21% mortality). After 5 days of *B. bassiana*-alone treatment, the mortality rate was 49.12%, while it was 4.58% after 24 h of

Table 1 LC_{50} values (μg/ml) for the extracts of 5 plant species against *A. gossypii* adults

Plant extract	LC_{50}	Confidence interval limits	Intercept ± SE	Slope ± SE	χ^2 (df)
P. penninervia	103.64	80.38–122.08	− 6.81 ± 0.65	3.38 ± 0.29	22.22 (10)
P. crispa	879.92	734.59–1120.04	− 5.41 ± 0.36	1.84 ± 0.13	16.15 (10)
E. arabicus	747.90	658.14–872.58	− 4.78 ± 0.33	1.66 ± 0.13	9.70 (10)
S. officinalis	783.28	636.12–1045.71	− 4.73 ± 0.33	0.71 ± 0.06	20.67 (10)
O. baccatus	262.42	211.31–317.84	− 5.71 ± 0.34	1.02 ± 0.06	46.68 (10)

Table 2 Relative susceptibilities (with limits) of *A. gossypii* adults to 5 plant extracts

Plant extract	*P. penninervia*	*P. crispa*	*E. arabicus*	*S. officinalis*
P. crispa	**12.50** (8.38–20.05)	–	–	–
E. arabicus	**10.17** (9.70–15.88)	0.81 (0.65–1.02)	–	–
S. officinalis	**10.49** (7.17–16.44)	0.84 (0.67–1.05)	0.97 (0.77–1.21)	–
O. baccatus	**3.79** (2.82–5.29)	**0.30** (0.23–0.39)	**2.69** (2.10–3.54)	**2.77** (2.16–3.66)

Values of relative median potency (RMP) analyses: values indicate the comparison of the plant in the row versus the plant in the column. Value > 1 indicates less susceptibility. Value < 1 indicates more susceptibility. Each bold value indicates significant values (95% CI did not contain 1)

treatment. The combination between *B. bassiana* and each plant extract achieved a significant increase in mortality than that of the individual treatment with *B. bassiana* or plant extracts, except for *P. penninervia* extract, where it did not achieve a significant mortality increase when combined with *B. bassiana* (75.47%) than that of its individual treatment (69.64%). This indicated that *P. penninervia* extract was not compatible with *B. bassiana*, unlike the other 4 plant extracts that showed their compatibility with *B. bassiana.*

Discussion

The LC_{50} value of tested *B. bassiana* isolate was 8.64 × 10^4 spores/ml against *A. gossypii*. Contextually, the same isolate had an LC_{50} value of 6.46 × 10^4 spores/ml against the rose aphid, *Macrosiphum rosae* (Sayed et al. 2019). Other investigations were in the same context for *B. bassiana* isolates such as 4.5 × 10^4 spores/ml against *A. gossypii* (Nirmala et al. 2006) and 6.57 × 10^5 spores/ml against *Aphis craccivora* Koch (Saranya et al. 2010). In general, there was a high variation in the virulence of EPF species/isolates even on the same host. For example, Jandricic et al. (2014) evaluated 20 isolates of *B. bassiana* against 1st nymphs of *A. gossypii* and recorded high variations among these isolates in their pathogenicity (1.38 to 56.9% mortality).

The result related to the toxicity of tested plant extracts on *A. gossypii* showed that the plant species from the same family are different in their efficacy. This toxicological variability due to the variation in the chemical composition (Sayed et al. 2020a) tested the same 5 plant extracts on *A. craccivora* and found that *P. penninervia* extract had the highest toxicity, followed by *O. baccatus*, and both of them were significantly different from all the other 4 plant extracts. Other investigations revealed that 7 plant extracts achieved 100% mortality after 24 h against the pea aphid, *Acyrthosiphon pisum* (Khan et al. 2017).

In this study, both of *O. baccatus* and *P. penninervia* extracts had the highest antifungal activities than the other 3 plant extracts (*E. arabicus*, *S. officinalis*, and *P. crispa*). This high antifungal activity for both plant extracts may be due to their components where all 5 tested plants are variables in minor components of phenols

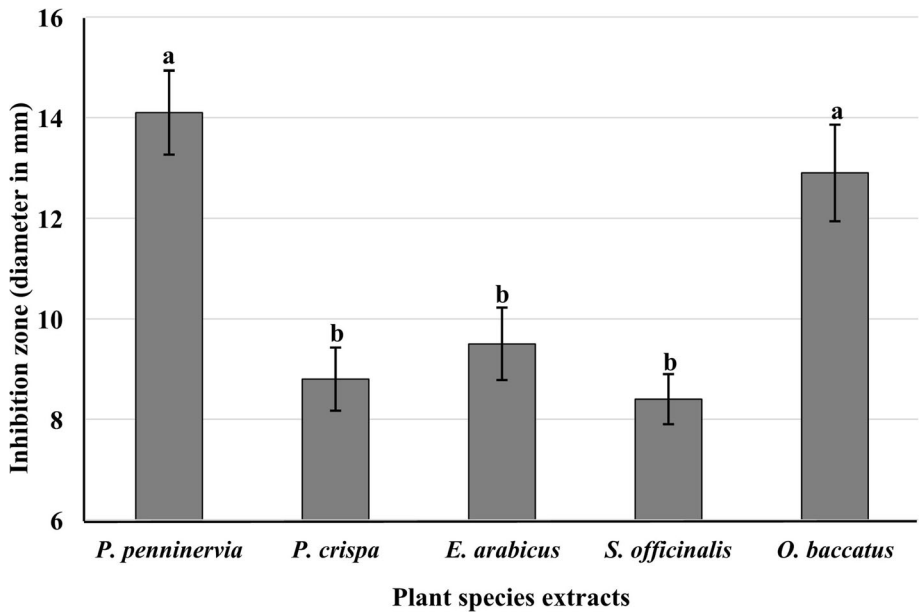

Fig. 1 Effect of 5 plant extracts on *Beauveria bassiana* as an inhibition zone in millimeter diameter (mean ± SE)

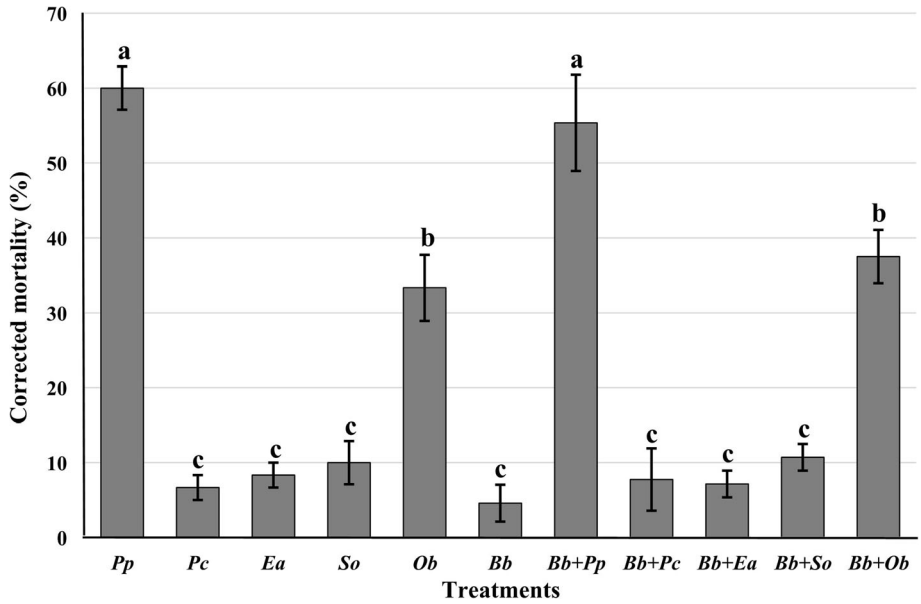

Fig. 2 Corrected mortality rates (% ± SE) of 5 plant extracts and *Beauveria bassiana* alone and in combination with *Aphis gossypii* after 24 h of treatment

and flavonoids (Sayed et al. 2020a). Sahayaraj et al. (2011) tested some commercial botanicals and other 6 plant extracts against 3 EPF including *B. bassiana* and found high variations among them in growth-inhibiting activity of all 3 fungi. In vitro evaluation, leaf and seed extracts of neem were less harmful to *B. bassiana* than the emulsible neem oil. Therefore, neem oil at high concentrations was not compatible with *B. bassiana*, inhibiting vegetative growth of conidia significantly. Meanwhile, both leaf and seed extracts were compatible in all concentrations of *B. bassiana* (Depieri et al. 2005). Also, *B. bassiana* colony growth was reduced by chinaberry, neem,

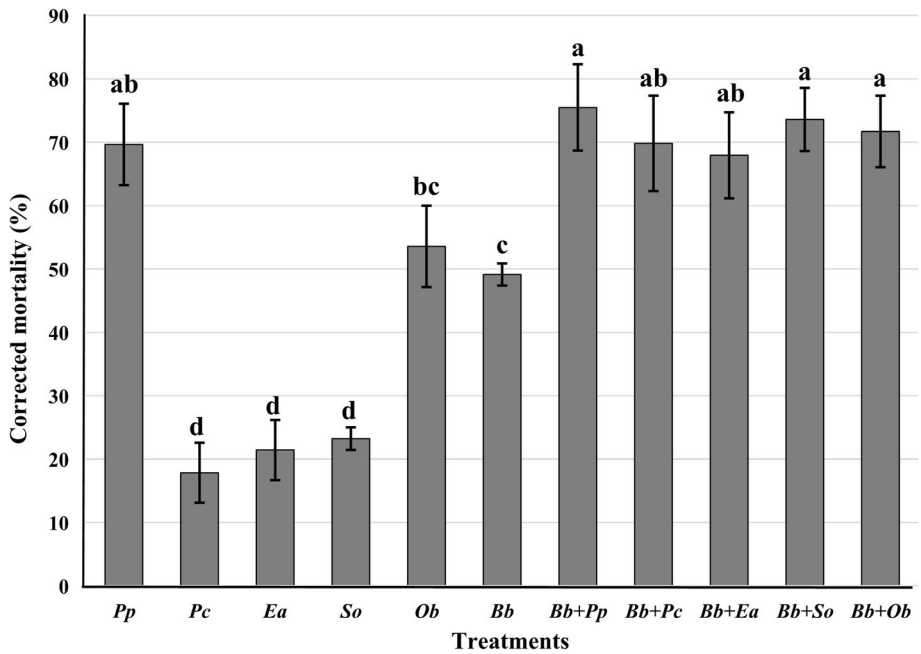

Fig. 3 Corrected mortality rates (% ± SE) of 5 plant extracts and *Beauveria bassiana* alone and in combination with *Aphis gossypii* after 5 days of treatment

lantana, and Mexican Sunflower leaf extracts, but the highest colony growth was shown with chinaberry extract (Afandhi et al. 2020).

In this study, the combination of each plant extract with *B. bassiana* did not significantly differ in the mortality of *A. gossypii* than individual treatments after 24 h of application. This result may be due to most EPF need more than 24 h to achieve mortality for insects. After 5 days of *B. bassiana*-alone treatment, the mortality rate was 49.12%, while after 24 of treatment, it was 4.58%. In this regard, an indigenous isolate, *B. bassiana*, from Algeria with a concentration of 10^7 conidia/ml achieved (13.33%) mortality rate for *A. gossypii* after 24 h of treatment, while on the 7th day, 80% of mortality was achieved (Bayındır Erol et al. 2020). In this study, *P. penninervia* extract was not compatible with *B. bassiana*, unlike the other 4 plant extracts that have shown their compatibility with *B. bassiana*. In general, plant extracts had variable antimicrobial activities because of their major or minor components such as phenols, flavonoids, tannins, and anthocyanins. In this way, 18 of the 43 extracts from 18 plant species indicated a high antimicrobial activity, but after removal of tannins, this activity of 16 of the 18 plants was lost (Jelager et al. 1998). In this context, some plant-derived insecticides had a growth-regulating action which enhances the establishment and penetration of EPF conidia through the cuticle of insects (Filotas et al. 2005). This means that such plants could support the virulence of EPF, when they are combined together. Therefore, the present results showed that the mortality rate of aphids was increased after 5 days than after 1 day with the combinations of the 4 plant extracts with *B. bassiana*.

Generally, there was a relation between the results of antifungal activity of plant extracts on *B. bassiana* and the results of *A. gossypii* mortality, when each plant extract was combined with *B. bassiana*. These results indicated that *P. penninervia* extract was not compatible with *B. bassiana* because it had a high toxicity on *B. bassiana* and also did not achieve a significant increase in *A. gossypii* mortality when combined with *B. bassiana*. In contrast, the extracts of *E. arabicus*, S. *officinalis*, and *P. crispa* had a low toxicity on *B. bassiana* and also achieved a significant increase in *A. gossypii* mortality. Meanwhile, *O. baccatus* extract had a high toxicity on *B. bassiana*, but it achieved a significant increase in *A. gossypii* mortality.

Conclusion

P. penninervia extract was not compatible with *B. bassiana*, but the other 4 tested plant extracts, *E. arabicus*, *S. officinalis*, *P. crispa*, and *O. baccatus*, were compatible with *B. bassiana*. These findings recommended them to be used against aphids in combination with *B. bassiana*. Further laboratory experiments could be carried out to determine the efficacy of these compatible extracts with *B. bassiana* on other biocontrol agents. Moreover, field experiments should be carried out for these extracts to be evaluated on insect pests and associated beneficial insects.

Abbreviations

IPM: Integrated pest management; EPF: Entomopathogenic fungi; *Bb*: *Beauveria bassiana*; *Pp*: *Psiadia penninervia*; *Pc*: *Pulicaria crispa*; *Ea*: *Euryops arabicus*; *So*: *Salvia officinalis*; *Ob*: *Ochradenus baccatus*; PDA: Potato dextrose agar; LC_{50}: Median lethal concentration; χ^2: Chi-square; RMP: Relative median potency

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Authors' contributions

SS designed the study and performed the statistical analysis. EA prepared the plant extracts. SS and S-AE performed the experiments. SS and S-AE wrote the first draft of the manuscript. SS proofread the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed in this study are available in this published 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.

Author details

[1]Department of Science and Technology, University College-Ranyah, Taif University, B.O. Box 11099, Taif 21944, Saudi Arabia. [2]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza 12613, Egypt. [3]Department of Biology, Faculty of Science, Taif University, B.O. Box 11099, Taif 21944, Saudi Arabia. [4]Department of Horticulture, Faculty of Agriculture, Assuit University, Assuit, Egypt.

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