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IN VITRO EFFICACY OF LEAF EXTRACT OF *IRESINE HERBSTII* AND *ISARIA FUMOSOROSEA* TO CONTROL *APHIS GOSSYPII* GLOVER

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Abstract

The present study was conducted to evaluate the water plant extract of *Iresine herbstii* and conidial suspension of entomopathogenic fungus *Isaria fumosorosea* against *Aphis gossypii* under laboratory conditions. The results showed that all tested concentrations of plant extract and *I. fumosorosea* were effective against nymphs and adult of *A. gossypii* compared with control treatment. Corrected mortality level was increased with increased concentration, where the highest mortality rates 80.78 and 90.00% of adult stage, were caused with 20 mg/ml of plant leaf extract and 1×10^8 conidia/ml-1 of *I. fumosorosea*, respectively compared with 20.47 % at control treatment 3 days post-treatment. The developmental period of times from 1st nymphal stage to reach the adult stage were 9.00 and 9.50 days for individuals treated with either 20 mg/ml plant leaf extract or 1×10^8 conidia/ml of *I. fumosorosea*, compared with 6.75 and 6.50 days for 1st instar nymphs exposed to sterile water as a control treatment. Results were also showed that both leaf plant extract of *I. herbstii* and the entomopathogenic fungus *I. fumosorosea* has reduced the number of offspring produced per female, compared with untreated aphids. Based on these results, both water plant extracts of *I. herbstii* and the conidial suspension of *I. fumosorosea* may be used as promising natural alternatives to synthetic insecticides against the cotton aphids.

Keywords **:** Plant extract, spore suspension *Iresine herbstii*, *Isaria fumosorosea*, *Aphis gossypii*

Introduction

Aphis gossypii Glover (Homoptera: Aphididae) is one of the most important aphid insect pests worldwide. It has been recorded infesting a broad range of hosts plants belong to 88 plant families including field crops (Cereals, Pulses, and Oilseeds), vegetable crops, fruits, ornamental plants, weeds, and wild plants(Mifsud *et al*., 2011; Blackman and Eastop, 2000). *A. gossypii* causes two kinds of damages which include: direct damages as a result of their feeding on sap-sucking and honeydew production which resulted in curling and deformation of young leaves and twigs, and indirect damages by transmitting several plant viruses such as Cucumber mosaic virus (CMV) (Pitrat and Lecoq, 1980). The widespread use of chemical insecticides led to raised several negative impacts (Prakash *et al*., 2008), including environmental contamination and human health (Tscharntke *et al.*, 2005). In addition, *A. gossypii* has developed resistance against a number of insecticides from different chemical groups (Seyedebrahimi *et al.*, 2016). Therefore, there is a need for adopting another alternative safety and eco-friendly methods to control *A. gossypii* such as Botanical insecticides and entomopathogenic fungi (Birgücü *et al*., 2015; Mohammed *et al.*, 2018). Several plants belonging to different plant families contain a number of chemical compounds have high pesticide activity, similar to those of chemical pesticides such as (saponins, tannins, alkaloids, alkenyl phenols, di, and triterpenoids) (Rattan, 2010). *Iresine herbstii* belongs to the family Amaranthaceae and commonly known as blood leaf. It is considered a medicinal plant where its leaves can be used as wound healing and anticancer agent (Sebold, 2003). *I. herbstii* like other plants of Amaranthaceae family are contained polyphenolic compounds, glycosylated polyhydroxy and polymethoxy derivatives of a flavylium (2-phenylbenzopyrylium) salt (Meskin *et al*., 2004), which may have a role in the mechanism of plant resistance to pest attack (Ponmozhi, 2011). Biological control agents against serious insect pests have gained increased in recent years as an alternative method to conventional insecticides. Among the EPF, *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*) (Hypocreales: Cordycipitaceae) is one of the most important biocontrol agents that had been tested against many insect species (Zimmermann, 2008). Several studies have been confirmed the potential effects of *I. fumosorosea* to control aphid species worldwide such as *Myzus persicae*, *A. gossypii* and *Aulacorthum solani* (Jandricic *et al.*, 2014). This study was aimed to evaluate the above bio-control agents as an alternative method to insecticides, to be more environmentally friendly and less costly by testing the efficacy of different concentrations of the leaf extract of *I.herbstii* and various conidial suspensions of *I. fumosorosea* on some aspects of life performance of *A. gossypii* under laboratory condition.

Materials and Methods

Insect collection and rearing

Adults of cotton aphid *A. gossypii* were collected from *Cestrum nocturnum* at College of Agriculture, Al Qasim Green University, Babil, Iraq. Aphids were reared on *C. nocturnum* in 45x45 cm cages under greenhouse conditions (25±2 ºC and humidity 65±5% RH with 12 h daily photoperiod) for several generations.

Preparation of Plant Extracts

The leaves of *I. herbstii* were collected from gardens of Agriculture College, Al Qasim Green University. The plant was chosed because it is repellant and never infected by *A. gossypii*. The leaves were washed with clean tap water and dried at room temperature for 3 days. Leaves were grinded by the electric miller and then 20g of the powdered leave were placed in a 500ml conical flask contained 200 ml of distilled water. The contents were mixed using Magnetic stirrer for 10 min. The mixture was left for 24 h, in a

reciprocating shaker for continuous agitation at 150 revs/min and filtered using filter papers (Whatman No.1). The filtrate was concentrated using rotary evaporator. The residues were collected and used for the experiment. Finally, The concentrated was kept in a glass container at - 4°C in a refrigerator (Harbone, 1984). A stock solution was prepared by dissolving 2 g of the extract with 97 ml of sterile water of a final concentration of 20 mg/ml, The above process was repeated several times to obtain other dilutions: 10 mg/ml, 5 mg/ml.

Entomopathogenic fungus

Isaria fumosorosea was obtained from the Laboratory of Entomology, Plant Protection Department, University of Baghdad which was originally isolated from insect samples. The isolate was cultivated on Potato Dextrose Agar (PDA) at 25 ± 1 °C for 15 days. Aerial conidia were harvested from the upper surface of the culture by scraped and diluted in a 200 ml conical flask containing 100 ml of sterile distilled water with 0.03% Tween-80. The conidial suspension was vortexed for 10 min and then filtered through layers of sterilized cheesecloth. Conidial concentrations were determined using a hemacytometer. The stock conidia suspension was diluted to produce the following concentrations: $1x10^4$, $1x10^6$ and $1x10^8$ conidia/ml.

Bioassay Test

Twenty adult aphids were placed on the filter paper into 9 cm diameter Petri dishes using a hairbrush. Aphids were sprayed directly using 1cc insulin syringe with plant extracts and conidia suspensions concentrations. Aphids in control treatment were sprayed with 0.03% sterile aqueous Tween 80. Four plates replicates were tested for each treatment. The leaves of the *C. nocturnum* were placed in Petri dishes for aphid and they were replaced with fresh leaves whenever needed. Petri dishes had a1-cm hole covered with nylon mesh on the lid for ventilation. The dishes were surrounded using Adhesive tape to avoid the adults escaping of the dishes. Then, plates were incubated at (25ºC±2 and70%±10 R.H.). Mortality was recorded after 1, 2 and 3 days post-treatment.

Effect of plant leaf extract of *I. herbstii* and *I. fumosorosea* on developmental period and fecundity of individual aphids

This experiment was conducted to determine the effect of both bio-control agents on the Developmental period of the immature stages and fecundity of adult females. Where 20 one day old first-instar nymphs (neonate aphids) were collected and treated with the types of extracts and conidial concentration as in previous experience. Neonate aphids treatment has been pursued to reach the adult stage. The Developmental period of each nymphal stage was recorded to reach the adult stage. To determine the fecundity of adult females, emerged adults were selected (4 adults per replicate and three replicates per treatment) and placed onto leaves of *C. nocturnum*. The number of nymphs produced by these adults was recorded.

Statistical analysis

The data obtained were analyzed using GenStat package 3 (3rh edition) using randomized complete block design with two factors. The percentage effects of the extract plant and spore suspension were calculated and corrected by Abbott's formula (Abbot 1925). Angular transformation was used for Mortality statistical analysis except for developmental period and female fecundity. The treatment means were compared by least significant difference (L.S.D) at 5% level of significance ($P \leq 0.05$).

Results and Discussion

Results in the table (1) showed the effectiveness of different concentrations of the water plant extracts of *I. herbstii* and the conidial suspension of *I. fumosorosea* on the adult of *A. gossypii* compared with control treatment. The data indicated that the mean adult mortality levels were increased with increasing the plant extract and fungal concentrations. Also, the mortality increased with increasing the time interval. The highest concentration 20 mg/ml of the extract plant caused 59.14, 72.11 and 80.78 % compared with 9.22, 20.47and 20.47% in control treatment 1, 2 and 3 days post-treatment, respectively. The mortality observed was low on day 1-2 post-treatment in all fungal concentrations of *I. fumosorosea*, but it was increased gradually and the highest mortality level was obtained on day 3. For example, the highest corrected mortality was 90.00% at 1×10^8 conidia/ml 3days post-treatment, compared with 33.97% after 1 day. Aphid mortality in control treatment was 20.47 % after 3 days. These results are in agreement with findings of Ali *et al.* (2018) who reported the efficiency of the water plant extracts of *Azadirachta indica* and the entomopathogenic fungi (EPF); *Beauveria bassiana* or *Metarhizium anisopliae* against *Sitobion avenae*. Birgücü *et al.* (2015) investigated the effects of the extracts obtained from many plants against *A. gossypii* where the extract of *Ocimum basilicum* caused 40.67% which was the highest mortality at the 24th h post-treatment. None of the plant extracts had a significant impact on *A. gossypii* at the 72nd and 168th h post-treatment. Mweke *et al.* (2018) tested the efficacy of several entomopathogenic fungi were against *Aphis craccivora*. They found that *M. anisopliae*, *B. bassiana*, and *Isaria sp.* were the highest virulent pathogenicity of *Aphis craccivora*. Also, Asi *et al*. (2009) found the mortality observed was low on day 1-2 after treatment in all fungal isolates, but it was increased gradually on day 3-4 after treatment. The pesticidal activities of *I. herbstii* have been attributed to the presence number of active compounds such as polyphenolic and glycosylated compounds (Meskin *et al*., 2004). Extracts of this plant might have controlled aphids by acting as a toxic, repellent, antifeedant growth, impair their feeding and reduces the fertility of *A. gossypii.* The life cycle of entomopathogenic fungi involves four steps: adhesion, germination, differentiation, and penetration. Fungi can infect insects via several pathways including the gut (ingestion), and through direct penetration of the integument. Mycelial penetration through the integument by conidia which have evolved both physical and enzymatic mechanisms. Conidia attach to the cuticle, germinate and penetrate the cuticle, this can occur between 24 to 48 h under ideal conditions (Wraight *et al*., 2000). Host death often occurs due to a combination of fungal toxins, physical obstruction of blood circulation, nutrient depletion and organ invasion. (Shahid *et al*., 2012).

Table 1: Corrected mortality of adults *A. gossypii* exposed to different concentrations of the plant leaf extracts of *I. herbstii* and conidial suspension of *I. fumosorosea.*

Treatment	Days			Mean
	1	2	3	
Control (0)	9.22	20.47	20.47	16.72
I. herbstii 5 mg/ml	36.22	47.89	50.83	44.98
I. herbstii 10 mg/ml	45.00	60.11	63.81	56.31
I. herbstii 20 mg/ml	59.14	72.11	80.78	70.68
I. fumosorosea 1×10^4 conidia/ml	24.16	42.05	60.64	42.29
I. fumosorosea 1×10^6 conidia/ml	29.36	53.94	76.72	53.34
I. fumosorosea 1×10^8 conidia/ml	33.97	58.98	90.00	60.98
	33.87	50.79	63.32	
L.S.D 0.05	Conc.	D.	Conc .× D.	
	6.6	4.3	11.5	

Figure 1 showed that the effects of the plant extracts of *I. herbstii* and the spore suspension of *I. fumosorosea* on the developmental time of *A. gossypii*. The developmental period of time from first stage to adulthood was significantly increased in treated individuals, compared with untreated aphids. It was ranged between 6.75 and 6.50 days in control treatment compared with 9.00 and 9.50 days for individuals treated with concentration of 20 mg/ml and 1×10^8 conidia/ml, respectively. These results are similar to those found by Bayhan *et al*. (2006) who reported that *A. gossypii* nymphs exposed to plant extracts have longer developmental times than the aphid nymphs submitted to the control treatment. The increasing developmental period of immature stages that treated with extracts may be attributed to decreased larval efficiency of food conversion movement of food through the gut, inhibition of digestive enzyme production or IGR activity which affected negatively on growth and increased developmental period, or due to the interference with the disturbance of the endocrine system and other physiological systems (Al Sharook and Girjees, 1993). These results are in agreement with Mondy *et al.* (1998) who also reported that larvae of *Lobesia botrana* ingestion of the fungus *Botrytis cinerea* led to increases the survival and development of larvae.

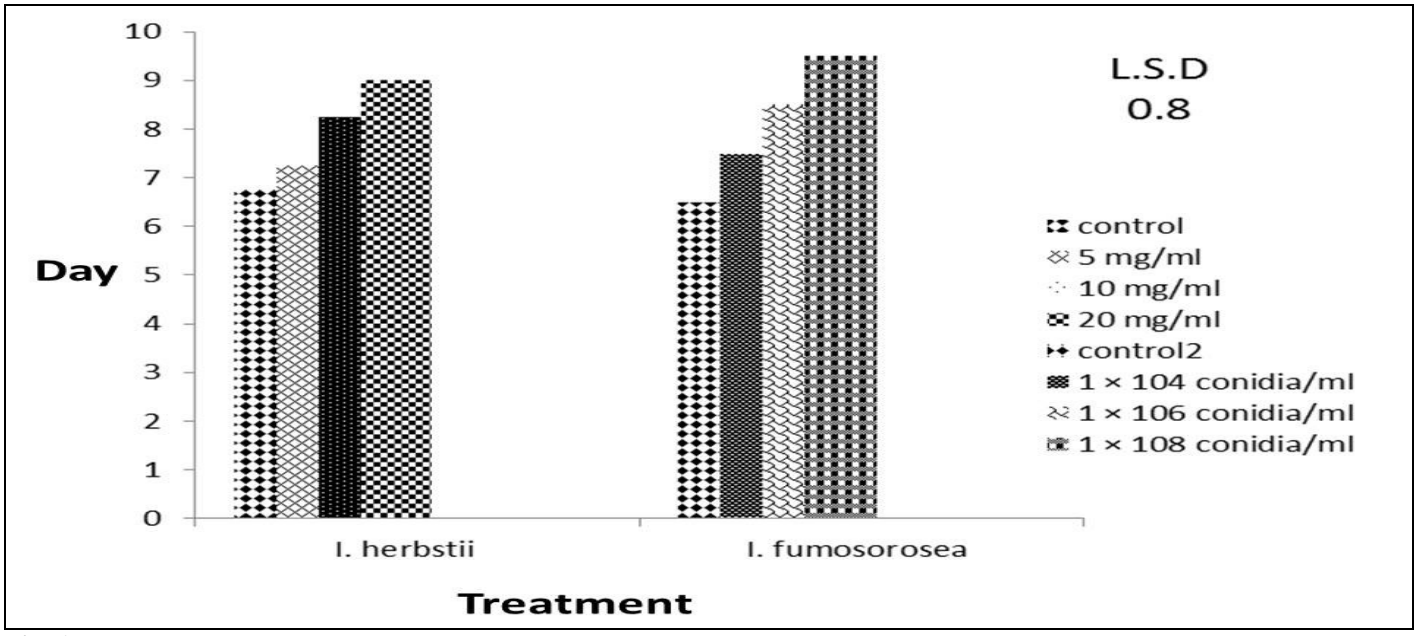

Fig. 1 : Development (days) of *A. gossypii* treated with different concentrations of the plant extracts of *I. herbstii* and the spore suspension of *I. fumosorosea*

Table 2 showed the effects of water extract plant of *I. herbstii* and the conidial suspension of *I. fumosorosea* was significantly reduced the female fecundity of *A. gossypii* compared to untreated aphids. Where productivity decreased as the concentration increased, the average numbers of offspring produced by adult female of *A. gossypii* significantly reduced from 8.0 nymph/female in the control treatment to 6.0 and 5.00 nymph/per female treated exposed to 20 mg/ml and 1×10^8 conidia/ml, respectively. Results of the study coincided with Pavela *et al.* (2004) observed that the effects of low concentrations of azadirachtin, on the fertility of cabbage aphid *Brevicoryne brassicae* L. The study concluded that the fertility of aphids also decreased significantly after increased concentrations of azadirachtin. The negative effects of extracts of some plants on fecundity and production of offspring from treated adult *A. gossypii* was lower than that of the control aphid (Bayhan *et al.*, 2006). Baverstock *et al.* (2006) also reported the fecundity of pea aphids, *Acyrthosiphon pisum*, infected with *B. bassiana* and *Pandora neoaphidis*, reduced within 24 h of infection.

Table 2 : Fecundity (nymph per-female) of adult *A. gossypii* treated with different concentration of water extract plant of *I. herbstii* and the spore suspension of *I. fumosorosea*.

Treatment	Concentration	Fecundity nymph_ Female
control	0	8
I. herbstii	5 mg/ml	6.67
	10 mg/ml	6.00
	20 mg/ml	6.00
I. fumosorosea	1×10^4 conidia/ml	6.67
	1×10^6 conidia/ml	5.67
	1×10^8 conidia/ml	5.00
L.S.D 0.05	0.9	

Conclusions

The laboratory experiments represented here indicate that both water plant extract plant of *I. herbstii* and entomopathogenic fungus *I. fumosorosea* could be an appropriate biological control agent for controlling *A. gossypii*. In addition, both *I. herbstii* and *I. fumosorosea* increased the period of time of immature stages to become adult and decrease fecundity of exposed adults. However, further studies under more realistic conditions are needed to generate more information on their efficacy. Also, there is a need for further research about the combination effects of *I. herbstii* and *I. fumosorosea* to control *A. gossypii* and other aphid species.

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