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EFFICACY OF ENTOMOPATHOGENIC FUNGI *VERTICILLIUM LECANII* AND *ISARIA FUMOSOROSEA* AGAINST *MYZUS PERSICAE* UNDER LABORATORY CONDITIONS

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Abstract

A series of laboratory experiments were conducted to study the effect of Iraqi entomopathogenic fungi *Verticillium lecanii*, and *Isaria fumosorosea* entomopathogenic on the mortality level of larval and adult stages of Green peach aphids *Myzus persicae*. The results showed that first and second instar nymphs were more susceptible to infection with both fungal species where the mortality levels were (96.9 and 93.9%) and (90 and 89.8%) respectively, compared to adult stage which was less susceptible to infection. The evaluation of effect of aphid host plant (cucmber, green pepper, eggplant) on the efficacy of *V. lecanii* and *I. fumosorosea* against third instar nymphs of *M. persicae* was showed that there was a significant effect of host plant species on the corrected mortality of *M. persicae* caused by *V. lecanii* and *I. fumosorosea*, with the highest rate of mortality recorded against *M. persicae* feeding on cucumber compared to green pepper and eggplant which was 95.5 and 87.5% respectively.

Key words: *Myzus persicae*, *Verticillium lecanii*, *Isaria fumosorosea*, green pepper, cucumber

Introduction

The green peach aphids (*Myzus persicae*) (Hemiptera: Aleyrodidae), is one of the most devastating and serious insect pests of protected vegetable crops in Iraq (Mohammed *et al.,* 2018). All feeding stages of *M. persicae* damage crops both directly, through the direct removal of phloem sap and indirectly by secreting large amounts of honeydew, encouraging the growth of sooty mould and it is a vector of several economically important plant viruses (Blackman and Eastop, 2007).

Although chemical control is still the main way of *M. persicae* worldwide, but its use results in insecticide residues, high levels of insecticide resistance and high costs (Bielza, 2008, Gao *et al.,* 2012). Secondary, there are increasing awareness of environmental concerns related to the use of insecticides (Koureas *et al.,* 2012). Both reasons have encouraged the development of alternative methods of *M. persicae* control such as biological control including the use of entomopathogenic fungi in the greenhouses.

Several entomopathogenic fungi including *Beauveria bassiana*, *Lecanicillium muscarium*, and *Metarhizium anisopliae*, play an important role in biological control of aphid species worldwide (Kim *et al.,* 2007). More recently, an Iraqi isolate *Lecanicillium lecanii* (Sordariales: Chaetomiaceae) has been shown a significant against both *M. persicae* and *Aphis gossypii* under laboratory and greenhouse conditions (Mohammed *et al.,* 2018). Worldwide, there are 38 entomopathogenic fungal species or varieties have been developed and used as microbial control agents against insect pests including aphid species (Faria and Wraight, 2007). However, there are no commercial products based on entomopathogenic fungi registered to control insect pests in Iraq.

One of the most important aspects on the potential use of entomopathogenic fungi to infect host insects is insect developmental stages (Kim and Roberts, 2012). The differences in susceptibility of may relate to biochemical composition and/or physiological characteristics of the cuticle (Tang and Hou, 1998, Kirkland *et al.,* 2004). The susceptibility of different developmental stages of aphid species such as *M. persicae* (Mohammed and Hatcher, 2016) and *A. gossypii* (Kim and Roberts, 2012) to fungal infection has been studied, but there are a few information on the efficacy of Iraqi isolates of entomopathogenic fungi against different developmental stages of *M. persicae*. Thus, such information may help develop management strategies for the use of entomopathogenic against *M. persicae* populations in field and greenhouse conditions.

Host plant species often differ in their complex direct and indirect defence mechanisms against aphids (Cory and Hoover, 2006). Direct defence includes morphological and chemical strategies which are the traits or densities of the aphids (Wu and Baldwin, 2010). These direct effects of plants on the density or traits of aphids may then propagate to entomopathogenic fungi indirectly influence. The most direct way that a plant affects entomopathogenic fungi is through the leaf surface (phylloplane). Surface waxes are an important plant characteristic influencing the efficacy of entomopathogenic fungi (Inyang *et al*., 1999). For instance, the efficacy of the fungus *Pandora neoaphidis* against the pea aphid *Acyrthosiphon pisum* was higher on plants with reduced wax bloom as a result of increased adhesion and germination of the fungal conidia on the aphid cuticle (Duetting *et al*., 2003).

The secondary metabolites can also modify the physiology and growth of insect herbivores, affecting their susceptibility to fungal infection (Ali *et al*., 1998). For example, inadequate insect host plant often leads to increased insect susceptibility to entomopathogens (Santiago-Alvarez and Ortiz-Garcia, 1992) by reducing aphid performance, and the use of host plants that induce nutritional stress in aphids can substantially enhance the efficacy of entomopathogenic fungi. Naranjo *et al*. (2003) reported that *Bemisia tabaci* reared on preferred plants were more vigorous and less susceptible to fungal infection than when reared on non-preferred plants.

The objectives of this study were to evaluate the susceptibility of different instar nymphs and adults of *M. persicae* to infect with Iraqi entomopathogenic fungal isolates, and to investigate the effect of different aphid host

plants on the efficacy of *V. lecanii* and *I. fumosorosea* against *M. persicae*.

Materials and Methods

Host plants

Three host plants were used: cucumber (*Cucumis sativus*), green pepper (*Capsicum annuum*) and eggplant (*Solanum melongena).* The plants were grown in a constant temperature room at 20°C, 50-65% relative humidity and a photoperiod of 12:12 (L:D) h. All experiments were performed using plants with 7-10 leaves (6-8 weeks old).

Rearing *M. persicae*

M. persicae, was collected initially from cucumber and eggplant plants in greenhouses at Faculty of Agriculture, University of Kufa in 2017. The aphids were identified under a light compound microscope using polyphagous aphid keys (Blackman and Eastop, 2000). *M. persicae* was reared on cucumber plants 5–6 weeks old in 45 × 45 × 45 cm cages at 23 ± 2°C and with 16:8 h daily photoperiod for several generations. Plants were replaced every two weeks with healthy 5–6 week-old plants. From the original colony of *M. persicae* above, separate colonies were reared on each of the three host plants for two generations on the same crop prior to the experiment start for the insects to adapt to each host plant in the same conditions.

Source and preparation of entomopathogenic fungi

V. lecanii and *I. fumosorosea* were obtained from the Plant Protection Department, University of Kufa, which were originally isolated from aphids. These fungal isolates were cultivated on potato dextrose agar (PDA) or Sabouraud's agar at 25°C. Aerial conidia of each isolate were harvested from 10-d-old cultures by adding 12 ml of 0.02% Tween 80 to culture agar plates and gently scraping the surface of the cultures with a sterile inoculating loop to dislodge the conidia from the surface of the agar plates. The conidial suspension of each isolate was pipetted from the plate and filtered through three layers of cheesecloth. The number of conidia in the suspension was determined using a haemocytometer (Neubauer improved, Superior Marienfeld, Germany). The resulting suspension of each isolate was diluted to the desired concentrations with 0.02% Tween 80 (BDH Chemicals Ltd., Poole, UK) as required. The viability of the conidia of each fungal isolate was determined by spraying 0.1 ml of 1 × 106 conidia ml−1 on a sterile Petri dish with 1.5% Sabouraud dextrose agar (SDA). The dishes were sealed with parafilm and incubated at 20 °C, 90 ± 2% RH and a photoperiod of 16:8 (L:D) h. After 24 h, the number of germinated spores per 100 spores of each plate was assessed under the microscope (400× magnification). Germination was considered positive when the length of the germ tube was at least half the spore length. The viability of each fungal isolate exceeded 93%.

Effect of *M. persicae* developmental stages on the efficacy of *V. lecanii* and *I. fumosorosea*

This experiment was carried out in a growth chamber at 20°C, 65 ± 2% RH and a photoperiod of 12:12 h (L:D). The method described by Mohammed and Hatcher (2016) was used to obtain a uniform age of different developmental stages of *M. persicae* (adults, fourth instars, third instars, second instars and first instars). Adults of *M. persicae* were transferred from a stock culture onto 8- week-old cucumber plants (20 adults per plant) using two 3-cm-diameter clip-cages and allowed to produce nymphs for 10, 7, 5, 3 or 1 days before bioassay to allow all five developmental stages to be available for experimental use on the same treatment date. The adults were then removed and the offspring counted (25 nymphs per plant) and allowed to develop on the plants for 9, 6, 4, 2 or 0 additional days before beginning the experiment in the growth chamber at 20°C, 65 ± 2% RH and a photoperiod of 12:12 (L:D) h. One milliliter of conidia suspension (10^7 conidia ml^{-1}) of either *V. lecanii* and *I. fumosorosea* was sprayed onto cucumber leaves which had either 25 first instar nymphs, second instar nymphs, third instar nymphs, fourth instar nymphs or adults using handheld sprayer. The control group was sprayed with 0.02% sterile aqueous Tween 80. Dead aphids were counted after 1, 3, 5 and 7 days.

The effect of aphid host plant on the efficacy of *V. lecanii* and *I. fumosorosea*

The goal of this laboratory experiment was to determine the effect of three different host plants on the efficacy of either *V. lecanii* and *I. fumosorosea* against *M. persicae*. The plants of each species, each of which had been infested with 25 third instar aphid nymphs, were sprayed once with 1 ml of fungal concentration (1×10^8 spores ml^{-1}) of either *V. lecanii* and *I. fumosorosea.* Plants in the control treatment were sprayed with 0.02% sterile aqueous Tween 80 only. After treatment, the plants were kept at room temperature for 1 h to dry and then transferred to a growth chamber at 20°C, 65 ± 3% RH and a photoperiod of 12:12 (L:D) h. To prevent aphids moving within or between treated plants, they were kept in 3-cm-diameter clip-cages post-spraying. Seven replicates per each host plant and the same number in the control were used. Dead aphids were counted after 1, 3, 5 and 7 days.

Statistical analysis

Statistical analyses of all experiments were conducted using GenStat (version 16). Data were transformed using arcsine square root transformation when it was necessary to meet the assumption of normality. Aphid mortality data were corrected for natural death in the control using Abbott's formula (Abbott, 1925). The effect of aphid developmental stage on the efficacy of either *V. lecanii* and *I. fumosorosea* was analysed using two-factor repeated measurement analysis ANOVA. The effect of aphid host plant on the mortality of *M. persicae* was analysed using two-factor repeated measurement analysis ANOVA.

Results and Discussion

Effect of *M. persicae* developmental stages on the efficacy of *V. lecanii* and *I. fumosorosea*

The developmental stage of *M. persicae* had a significant effect on the aphid mortality when aphids were treated with *V. lecanii* or *I. fumosorosea*. In case of *V. lecanii*, first instars and second instars were high susceptible for infection, compared with adults, fourth instars and third instars 7 days post-treatment ($P \leq .001$) (Table 1). In case of *I. fumosorosea*, the highest mortality occurring to first instars and second instars compared to adults, fourth instars and third instars 7 days post-treatment (Table 2). Aphid mortality was much lower in control treatments and ranged from 2% to 5%. The lower susceptibility of adults of *M. persicae* may be explained by changes in cuticle biochemical composition

during development such as the presence of toxic compounds, which may inhibit spore germination (Kirkland *et al.,* 2004). These results are in agreement with previous studies, indicating highest mortality caused by entomopathogenic fungi in young nymph stage of aphid species and other insects as compared with adult stages (Nazemi *et al.,* 2014).

Table 1: Corrected mortality (±SE) of first, second, third and fourth instar nymphs and adults of *M. persicae* sprayed with 1×10^7 conidia ml^{-1} of *V. lecanii* after 1, 3, 5 and 7 days of application.

Developmental stage	Corrected mortality (±SE)			
	1 day	3 days	5 days	7 days
First instar nymph	22 ± 0.9	53.5 ± 2.9	80.1 ± 4.3	90 ± 3.6
Second instar nymph	21.4 ± 0.6	46.7 ± 1.4	77.5 ± 3.2	89.9 ± 3.4
Third instar nymph	9.2 ± 0.3	43.4 ± 0.8	76.9 ± 2.5	81.1 ± 2.7
Fourth instar nymph	5.5 ± 0.1	38.1 ± 1.1	65.8 ± 1.9	74.3 ± 3.2
Adult	4.1 ± 0.1	35 ± 2.3	55.4 ± 1.7	74.3 ± 4.1
$L.S.D_{(0.05)}$ for developmental stage = 3.01; $L.S.D_{(0.05)}$ for days= 1.41 $L.S.D_{(0.05)}$ for interaction = 3.98				

Table 2: Corrected mortality (±SE) of first, second, third and fourth instar nymphs and adults of *M. persicae* sprayed with 1×10^7 conidia ml^{-1} of *I. fumosorosea* after 1, 3, 5 and 7 days of application.

Developmental stage	Corrected mortality (±SE)			
	1 day	3 days	5 days	7 days
First instar nymph	5.4 ± 0.2	64.5 ± 1.6	75.9 ± 2.5	96.9 ± 2.9
Second instar nymph	6.8 ± 0.1	54.9 ± 2.1	73.3 ± 1.3	93.9 ± 3.8
Third instar nymph	8 ± 0.3	40.9 ± 1.4	65.8 ± 0.6	94 ± 4.3
Fourth instar nymph	4 ± 0.1	39.2 ± 2.3	62.8 ± 2.5	79.9 ± 2.1
Adult	1.2 ± 0.1	32.4 ± 0.9	55.4 ± 1.9	72.6 ± 2.2
$L.S.D_{(0.05)}$ for developmental stage = 3.53; $L.S.D_{(0.05)}$ for days=1.81 $L.S.D_{(0.05)}$ for interaction = 4.97.				

The effect of aphid host plant on the efficacy of *V. lecanii* and *I. fumosorosea*

Mean percentages (±SE) of aphid mortality in control treatments were very low: 1.2 ± 0.8 % (eggplant) to 3.8 ± 1.2 % (green pepper) after 7 days. There was a significant effect of host plant species on the corrected mortality of aphids caused by *V. lecanii* ($P \leq 0.001$), with the highest mortality recorded against third instar nymphs of *M. persicae* feeding on cucumber, compared with the lowest mortality recorded against aphids feeding on eggplant (Table 3).

Table 3: Cumulative mortality (Mean ± SE). of *M. persicae* fed on cucumber, green pepper or eggplant and treated with 1×10^7 conidia ml^{-1} of *V. lecanii*.

Host plant	Corrected mortality (±SE)			
	1 day	3 days	5 days	7 days
Cucumber	0.8 ± 0.2	47.4 ± 1.6	74.7 ± 2.5	87.5 ± 2.9
Green pepper	0.8 ± 0.1	33.8 ± 2.1	67.8 ± 1.3	85.7 ± 3.8
Eggplant	0 ± 0.3	31.2 ± 1.4	65.2 ± 0.6	82.1 ± 4.3
$L.S.D_{(0.05)}$ for host plant = 1.24 ; $L.S.D_{(0.05)}$ for days = 1.38 $L.S.D_{(0.05)}$ for interaction = 2.42.				

Meanwhile, there was a significant effect of host plant species on the corrected mortality of aphids caused by *I. fumosorosea* ($P \leq 0.001$), with the highest mortality recorded against third instar nymphs of *M. persicae* feeding on cucumber, compared with the lowest mortality recorded against aphids feeding on eggplant (Table 4). The time after fungal application and the interaction between host plant species and time after treatment were also significantly different ($P \leq 0.001$).

Table 4: Cumulative mortality (Mean ± SE). of *M. persicae* fed on cucumber, green pepper or eggplant and treated with 1×10^7 conidia ml^{-1} of *I. fumosorosea*.

Host plant	Corrected mortality (±SE)			
	1 day	3 days	5 days	7 days
Cucumber	2.4 ± 0.2	44.9 ± 1.6	79.9 ± 2.5	95.5 ± 2.9
Green pepper	1.6 ± 0.1	39.8 ± 2.1	67.8 ± 1.3	92.8 ± 3.8
Eggplant	0 ± 0.3	27.1 ± 1.4	55.6 ± 0.6	90.4 ± 4.3
$L.S.D_{(0.05)}$ for host plant = 1.45 ; $L.S.D_{(0.05)}$ for days = 1.22 $L.S.D_{(0.05)}$ for interaction = 2.32.				

The significant lower mortality of *M. persicae* feeding on eggplant compared to those aphids feeding on cucumber may have two possible reasons. First, it could be a result of the direct effects of eggplant on aphid nutritive values by increasing their fresh weights and performance, which may be decreased their susceptibility to both *V. lecanii and I. fumosorosea*. Naranjo *et al*. (2003) found that *Bemisia tabaci* reared on preferred plants were more vigorous and less susceptible to fungal infection than when reared on non-preferred plants. In addition, Mohammed (2016) reported the lower efficacy of *Lecanicillium muscarium* against *M. persicae* feeding on green pepper and bean compared to those aphids feeding on rye and coriander. The second possible reason of the lower mortality of *M. persicae* feeding on eggplant might be due to the high concentrations of tomatine in eggplant which sequestered by the aphids. Tomatine produced by some plant species of Solanaceae are hypothesised to alter many steps of the infection cycle by entomopathogens depending upon their concentrations (Cory and Hoover, 2006). These results are in agreement with Santiago-Álvarez *et al*. (2006) found that nymphs of *B. tabaci* reared on cotton and green pepper were less susceptible to infection by *B. bassiana* than were nymphs reared on cucumber and marrow. They suggested that the decreased susceptibility might be due to the high concentrations of gossypol and tomatine in cotton and green pepper which sequestered by the whiteflies.

It can be concluded that both Iraqi fungal isolates *V. lecanii* and *I. fumosorosea* were high virulent to all development stages *M. persicae* in the laboratory conditions when applied at a rate of 1×10^7 conidia ml^{-1} . In addition, *V. lecanii and I. fumosorosea* caused a high mortality level against *M. persicae* feeding on all tested host plants, especially cucumber. These results suggested that it might be useful to use these entomopathogenic fungi as an alternative to synthetic insecticides for the control of *M. persicae*. However, further studies are required to be carried out under field and semi-field conditions to establish the sensible application dosage for these entomopathogenic fungi, the cost-effectiveness of using them as a part of integrated pest management.

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References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18(2): 265–267.

Ali, M.; Felton, G.; Meade, T. and Young, S. (1998). Influence of interspecific and intraspecific host plant variation on the susceptibility of heliothines to a baculovirus. Biological Control, 12: 42-49.

Bielza, P. (2008). Insecticide resistance management strategies against the western flower thrips, Frankliniella occidentalis. Pest Management Science, 64: pp.1131–1138.

Blackman, R.L. and V.F. Eastop (2000). Aphids on the world's crop: An identification and information guide. Chichester: Wiley.

Blackman, R.L. and Eastop, V.F. (2007). Taxonomic issues. In: van Emden, H.F., and R. Harringto (eds) Aphids as crop pests. CABI, Wallingford, 1–22.

Cory, J.S. and Hoover, K. (2006). Plant-mediated effects in insect–pathogen interactions. Trends in Ecology and Evolution, 21: 278-286.

Duetting, P.S.; Ding, H.; Neufeld, J. and Eigenbrode, S.D. (2003). Plant waxy bloom on peas affects infection of pea aphids by Pandora neoaphidis. Journal of Invertebrate Pathology, 84(3): 149-158.

Faria, M.R. and Wraight, S.P. (2007). Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control, 43: 237–256.

Gao, Y.L.; Lei, Z.R. and Reitz, S.R. (2012). Western flower thrips resistance to insecticides: detection, mechanisms, and management strategies. Pest Management Science, 68: 1111–1121.

Inyang, E.N.; Butt, T.M.; Beckett, A. and Archer, S. (1999). The effect of crucifer epicuticular waxes and leaf extracts on the germination and virulence of *Metarhizium anisopliae* conidia. Mycological Research, 103(4): 419-426.

Kim, J.J., and Roberts, D.W. (2012). The relationship between conidial dose, moulting and insect developmental stage on the susceptibility of cotton aphid, Aphis gossypii, to conidia of Lecaniicillium attenuatum, an entomopathogenic fungus. Biocontrol Science and Technology, 22: 319–331.

Kirkland, B.H.; Cho, E.M. and Keyhani, N.O. (2004). Differential susceptibility of Amblyomma maculatum and *Amblyomma americanum* (Acari: Ixodidea) to the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Biological Control, 31: pp.414–421.

Koureas, M.; Tsakalof, A.; Tsatsakis, A. and Hadjichristodoulou, C. (2012). Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicology Letter, 210: 155–168.

Mohammed, A.A. (2016). Interactions between the entomopathogenic fungus *Lecanicillium muscarium* and the parasitoid *Aphidius colemani* for the control of green peach aphid *Myzus persicae* under laboratory and field conditions. Ph.D. thesis, University of Reading, UK.

Mohammed, A.A.; Kadhim, J.H. and Kamaluddin, Z.N.A. (2018). Selection of highly virulent entomopathogenic fungal isolates to control the greenhouse aphid species in Iraq. Egyptian Journal of Biological Pest Control, 28.

Mohammed, A.A. and Hatcher, P.E. (2016). Effect of temperature, relative humidity and aphid developmental stage on the efficacy of the mycoinsecticide Mycotal against Myzus persicae. Biocontrol Science and Technology, 26: 1379–1400.

Naranjo, S.; Ellsworth, P. and Canas, P. (2003). Population dynamics, sampling and damage of Bemisia tabaci in agricultural systems. 3rd International Bemisia Workshop. Barcelona (Spain), 17-20.

Nazemi, A.H.; Moravvej, G.; Karimi, J. and Talaei-Hassanlouei, R. (2014). Pathogenicity of *Lecaniicillium longisporum* (Ascomycota: Hypocreomycetidae) on the aphid *Cinara pini* (Hemiptera: Lachnidae) in laboratory conditions Journal of Crop Protection, 3(2): 159-171.

Santiago-Álvrez, C., and R. Ortiz-Garcia (1992). The influence of host plant on the susceptibility of *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) larvae to Spodoptera littoralis NPV (Baculoviridae, Baculovirus). Journal of Applied Entomology, 114: 124-130.

Santiago-Álvrez, C.; Maranhão, E.A.; Maranhão, E. and Quesada-Moraga, E. (2006). Host plant influences pathogenicity of *Beauveria bassiana* to *Bemisia tabaci* and its sporulation on cadavers. BioControl, 51: 519-532.

Tang, L.C. and R.F. Hou (1998). Potential application of the entomopathogenic fungus, Nomuraea rileyi, for control of the corn earworm, *Helicoverpa armigera*. Entomologia Experimentalis et Applicata, 88(1): 25–30.

Wu, J. and Baldwin, I.T. (2010). New insights into plant responses to the attack from insect herbivores. Annual Review of Genetics, 44(1): 1-24.