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Insecticidal activity and sublethal effects of *Beauveria bassiana* (Bals.-Criv.) Vuill. isolates and essential oils against *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)

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Insecticidal activity and sublethal effects of *Beauveria bassiana* (Bals.-Criv.) Vuill. isolates and essential oils against *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)

Abstract: The cotton aphid, *Aphis gossypii* Glover, 1877, is a polyphagous species and one of the most important pests of cucumber crops in Iran. In this study, virulence of three *Beauveria bassiana* (Bals.-Criv.) Vuill isolates, IRAN 108, IRAN 429C and LRC 137, as well as insecticidal activity of two essential oils extracted from *Matricaria chamomilla* L. and *Cuminum cyminum* L. were evaluated against adult stage of *A. gossypii* under laboratory conditions. The data for life table were analyzed using the age-stage, two-sex life table theory. Results showed that all isolates were pathogenic on aphid, but their virulence was varied in different isolates. The lowest calculated LC_{50} was belonged to IRAN 429C (3.9×10^4conidia ml^{-1}). The lowest LT_{50} was obtained at concentration of 10^8 and 10^7 conidia ml^{-1} for IRAN 429C (2.9 and 3.55 days, respectively). *M. chamomilla* essential oil had the lowest LC_{50} and LT_{50} values (19 µl l^{-1} air and 11.4 h), respectively. Longevity and population growth parameters, including the intrinsic rate of increase (r_m), gross reproduction rate (*GRR*), net reproductive rate (R_0), generation time (*T*) and finite rate of population increase (λ), were affected negatively by both agents. According to the results obtained in this study, both entomopathogenic fungi and essential oils could be used as an alternative to chemical insecticides in aphid IPM programs.

Key word: entomopathogenic fungi; biological control; integrated pest management; essential oil; sublethal dose

Insekticidna aktivnost in subletalni učinki izolatov entomopatogene glive *Beauveria bassiana* (Bals.-Criv.) Vuill. in eteričnih olj na bombaževo uš (*Aphis gossypii* Glover, 1877, Hemiptera: Aphididae)

Izvleček: Bombaževa uš (*Aphis gossypii* Glover, 1877) je polifagna vrsta in je eden izmed najpomembnejših škodljivcev kumar v Iranu. V raziskavi so bili preučevani virulenca izolatov entomopatogene glive (*Beauveria bassiana* (Bals.-Criv-) Vuill.) IRAN 108, IRAN 429C and LRC 137 in insekticidna aktivnost dveh eteričnih olj ekstrahiranih iz vrst *Matricaria chamomilla* L. in *Cuminum cyminum* L. na odrasle osebke bombaževe uši v laboratorijskih razmerah. Podatki preživetja so bili analizirali glede na starost, spol in razvojne faze škodljivca. Podatki so pokazali, da so bili vsi izolati patogeni za uši, vendar se je virulenca med izolati razlikovala. Najmanjša izračunana vrednost LC_{50} je pripadala izolatu IRAN 429C ($3,9 \times 10_4$ konidijev ml^{-1}). Najmanjša vrednost LT_{50} je bila dosežena pri koncentracijah 10^8 in 10^7 konidijev ml^{-1} za izolat IRAN 429C (2,9 in 3,55 dni). Eterično olje prave kamilice je imelo najmanjše vrednosti LC_{50} in LT_{50} (19 µl l^{-1} zraka in 11,4 h). Preživetje in parametri rasti populacije kot so potencialna rast populacije (r_m), bruto reprodukcija (*GRR*), neto reprodukcija (R_0), čas med dvema zaporednima generacijama (*T*) in končna velikost povečanja populacije (λ) so bili negativno prizadeti pri obeh obravnavanjih. Glede na rezultate pridobljene v tej raziskavi, bi kot alternativo kemičnim insekticidom v programih intergriranega upravljanja z listnimi ušmi lahko uporabili oboje, entomopatogene glive in eterična olja.

Ključne besede: entomopatogene glive; biološki nadzor; integrirano upravljanje s škodljivci; eterična olja; subletalna doza

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1 INTRODUCTION

Aphids are considered as an important pest of agricultural products all around the world and due to their specific biological characteristics, including the multiplicity and interference of generations and type of nutrition, as well as their resistance to some common chemical pesticides. So, different control strategies have been used against them (Abramson et al., 2006). *Aphis gossypii*, which is commonly known as cotton or melon aphid, is one of the most important pests of the plants in the families Rutaceae, Malvaceae and Cucurbitaceae all around the world and is recorded from more than 100 plant families (Van emden & Harrington, 2017). It is a cosmopolitan and polyphagous species distributed in tropical, subtropical and temperate regions, which causes direct damage by feeding on phloem sap and disrupting the plant growth, and indirectly through virus transmission and honeydew production (Martin et al., 2003). More than 50 plant viruses, both non-persistent and persistent, are transmitted by *A. gossypii* (Martin et al., 2003; Van emden & Harrington, 2017). To control this pest, various methods such as host-plant resistance, cultural practices, biological control, chemical control and integrated management have been used (Lowery & Smirle, 2003; Van emden & Harrington, 2017).

The occurrence of resistance in aphid populations to commonly used insecticides makes many of the pesticides inefficient and ineffective in aphid control programs, which is contributed to overall increases in the application of pesticides, continue to affect food and resource productivity, increase environmental exposure to chemical pesticides and etc. So, the use of anti-resistance strategies and/or using alternative methods in integrated pest management (IPM) programs are an urgent necessity (Van emden & Harrington, 2017; Wakil et al., 2017). *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae, Hypocreales) is a well-known entomopathogenic fungus with a broad host range infecting many arthropods including Coleoptera, Lepidoptera, Hemiptera, Formicidae and Acarina on diverse crops and it is one of the most widely used entomopathogenic fungi in biological control programs (Sowjanya Sree & Varma, 2015; Lacey, 2016). Its cosmopolitan existence, rich diversity, growing naturally in soils throughout of the world, as an endophyte inside different plants and as a pathogen acting against different insect species make it a suitable mycoinsecticde in IPM programs (Vega et al., 2008; Ragavendran et al., 2017).

Essential oils or volatile oils as commonly defined are aromatic oily liquids consisting of mixtures of volatiles mono- and sesquiterpenoids and phenyl propanoids, characterized by a strong odor and lower density than that of water (Bakkali et al., 2008; Norris et al., 2015). They have well proven antibacterial, antifungal, antiviral, antiparasitic, antioxigenic, anti-inflamatory, acaricidal and insecticidal properties (Shahriari et al., 2019; Srivastava et al., 2015). Because of their low persistence in the environment, low mammalian toxicity, non-phytotoxicity and diverse mode of actions, activities and applications, they are a very good and promise candidates in IPM programs (Liao et al., 2016; Sapindal et al., 2018). Over 3000 essential oils have been identified of which about 300 have commercial importance in the pharmaceutical, agricultural, food, health, cosmetics and perfume industries (Bakkali et al., 2008). Good insecticidal and acaricidal potential have been demonstrated in essential oils and during the different studies, their contact, fumigant, antifeedant, repellent, ability to delay development and fertility and oviposition inhibition activities has been identified (Marimuthu et al., 1997; Isman, 2000; Koul et al., 2008; Tripathi et al., 2009; Marcic, 2012; de Oliveira Cruz et al., 2013; Germinara et al., 2017). In addition, their effects in preventing the development of resistance in insect pests has been documented (Liao et al., 2016).

The aim of the present study was to assay and compare toxicity and sublethal effects of three indigenous isolates of *Beauveria bassiana*: IRAN 429C, IRAN 108 and LRC 137 and two essential oils, *Matricaria chamomilla* and *Cuminum cyminum* against *A. gossypii.*

2 MATERIALS AND METHODS

2.1 APHID COLONY

The initial colony of cotton aphid was collected from cucumber gardens in research fields of Agricultural Faculty, Urmia University, Iran and identified at species level based on morphological characteristics. It was reared and maintained on cucumber plants under laboratory conditions at 27 ± 2 °C, 65 ± 5% RH and a 16:8 (L:D) h photoperiod.

2.2 ESSENTIAL OILS

Dried parts of *M. chamomilla* plants except wooden stem and *C. cyminum* seeds were grounded into powder. 50 g of herbal powder was extracted with 600 ml of distilled water using a clevenger-type apparatus and hydro-distillation for 3 hours (Hassanpouraghdam et al., 2009). Extracted oil was dried with anhydrous sodium sulfate and stored in dark capped tubes at 4 °C until analysis.

2.3 FUNGAL ISOLATES

Three isolates of *Beauveria bassiana* were purchased from the Iranian Plant Protection Research Institute, Tehran, Iran (Table 1). All the isolates were grown on Sabouraud Dextrose Agar (SDA) medium and incubated at 25 ± 2 °C, 16:8 (L:D) h for 14 days to complete the sporulation. The conidia were directly harvested by scrapping off the colony surfaces with sterile scalpel and suspended in 15 ml of distilled water containing Tween 80. The suspension was filtered through a three-layered cheesecloth to remove fungal mycelia and substrate materials. Five concentrations form 10^4 to 10^8 conidia ml^{-1} were prepared from stock suspensions after which conidial concentration were determined based on haemocytometer (improved Neubauer) counts. Distilled water containing 0.05 % Tween 80 was used as control. The viability of spores for each isolate were determined prior to bioassays by spreading the dilute suspension (1 × 10^5 spore ml^{-1}) over the surface of potato dextrose agar (PDA, 39 g l^{-1}, Merck, Germany) medium. The plates were incubated at 25 ± 1 °C for 24 h and were viewed under a 400 x microscope magnification. While the length of germ tube was longer than the spore width, the spore was considered germinated. The germination rate was > 90 % in all bioassays.

2.4 INSECTICIDAL ACTIVITY

The bioassay was conducted to assess LC_{50} values. Filter paper (2 cm diameter) was soaked in different concentrations of essential oils and placed on the top of a glass bottles (305 ml), each containing 20 adult insects (< 24 h old). In order to prevent direct contact between insects and the essential oil, a cloth mesh was used to isolate filter paper. The main concentrations of essential oils tested on *A. gossypii* were 9.8, 14.8, 21.7, 32.1, and 47.4 µl l^{-1} air for *M. chamomilla* and 19.7, 27.3, 37.6, 51.9, and 71.7 µl l^{-1} air for *C. cyminum* for 24 h, respectively. In the control, the filter papers were soaked with distilled water. The mortality was evaluated 24 h after exposure. The experiment had three replications for each treatment. Insects that did not show any movement when touched with the brush are considered dead.

For the test of fungi, twenty adult aphids (< 24 h old) were immersed for 30 seconds into five different spore concentrations (1 × 10^4 to 1 × 10^8 conidia ml^{-1}) of the fungal isolates. In control treatments, aphids were immersed into a 0.05 % Tween 80 solution. Each treatment had three replications and mortality data was assessed daily up to 10 days. Again, insects that did not show any movement when touched with the brush are considered dead. Dead aphids were transferred into new petri dishes containing a moist filter paper and incubated at 25 °C to observe the possible development of mycelium/conidia of the treated fungi (Kassa, 2002).

Bioassays was done to determine the median effective time to cause mortality of 50 % of the test insects (LT_{50} value), that for this study the aphids immersed for 30 seconds into five different spore concentrations (1 × 10^4 to 1 × 10^8 conidia ml^{-1}) of the fungal isolates. Also, for this test in essential oils, LC_{50} value of both oils were used. Ten adult aphids (< 24 h old) were introduced to each glass bottles (305 ml). Each treatment had three replications and the mortality was evaluated every 2, 7, 12, 18 and 24 h to obtain the end point of mortality.

Table 1: Details of Beauveria bassiana isolates used in bioassay experiments

Accession Number	Substrate	Location
IRAN 429C	*Chilo suppressalis*	Iran
IRAN 108	Soil	Iran
LRC 137	*Leptinotarsa decemlineata*	Canada

2.5 SUBLETHAL EFFECTS

The sublethal effects test was carried out in a similar manner except that 50 adult aphids (< 24 h old) were immersed for 30 seconds in conidial suspensions containing 2.2 × 10^3 and 3.9 × 10^4 conidia ml^{-1} for IRAN 429C and IRAN 108 fungal isolates, respectively. This two fungal isolates were selected for sublethal studies, because of their higher efficacy to infect the aphids in previous tests. In control treatments, aphids were immersed in 0.05 % Tween 80 solution. Mortality was recorded daily from the time of emergence of the insects until last insect's life.

To determine the sublethal effects of the *M. chamomilla* and *C. cyminum* essential oils, 50 adult aphids (< 24 h old) were exposed to an LC_{30} of each essential oil (13.52 and 27.87 µl l^{-1} air, respectively) for a period of 24 h. The live insect were transferred individually after 24 h to a plastic Petri plates (6 cm in diameter). Treated aphids were examined daily and the nymphs were counted and removed until the death of the last adult aphid. Aphids were considered dead if they didn't move when contacted with a needle.

3 DATA ANALYSIS

The experiments were conducted under completely randomized design (CRD) with three replicates of each treatment. Mortality data were corrected by Abbott's formula (Abbott, 1925). The data obtained from lethal concentration assays were subjected to probit analysis for calculation of LC_{50}, LC_{30} and LT_{50} values. All statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, USA). The life history raw data of *A. gossypii* were analyzed according to the age-stage, two-sex life table theory and the method described by Chi (1988) using TWOSEX-MSChart software (Chi & Liu, 1985; Chi, 2016). The intrinsic Rate of Increase (r) estimated by using iterative bisection method from the Euler-Lotka formula:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

with age indexed from 0 (Goodman, 1982). The life table parameters (the age-specific survival rate (l_x); the age specific fecundity (m_x)) and the population parameters (the net reproductive rate (R_0), finite rate of increase (λ), and mean generation time (T)) were calculated according to Chi (1988) method:

The net reproductive rate (R_0):

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

mean generation time (T):

$$T = \frac{\ln R_0}{r}$$

and finite rate of increase (λ):

$$\lambda = e^r$$

The means and standard errors of the population parameters were estimated by using the bootstrap method (Efron & Tibshirani, 1993; Huang & Chi, 2012) embeded in the TWOSEX-MSChart (Chi, 2016). The paired bootstrap test was used to compare differences (Efron & Tibshirani, 1993). Survival, fecundity and reproductive value curves were constructed using SigmaPlot (12.3).

4 RESULTS

4.1 FUMIGANT TOXICITY OF ESSENTIAL OILS

Estimated values of LC_{50} of the essential oils are summarized in Table 2. The results showed that the essential oils of *M. chamomilla* and *C. cyminum*, had good aphicidal activity against the adults of cotton aphids after 24 h at very low concentrations. LC_{50} values for *M. chamomilla* and *C. cyminum* were 19 and 37.36 µl l^{-1} air, respectively. The results obtained from LT_{50} showed that *M. chamomilla* oil affected the insects faster than *C. cyminum* oil (Table 3).

4.2 SUBLETHAL EFFECTS OF ESSENTIAL OIL

In the studies dealing with sublethal effects of essential oils on adult aphids, there were significant differences in the life table parameters such as intrinsic rate of increase (r), net reproductive rate (R_0), gross reproduction rate (*GRR*), finite rate of increase (λ) and female adult longevity among essential oils treatments and the controls. The survival rate of female adults was significantly lower in the *M. chamomilla* and *C. cyminum* than control group ($p < 0.001$) (Table 4).

Gross reproduction rate (*GRR*) in control group was higher than both of the essential oil treatments ($p < 0.001$). Intrinsic rate of increase (r) was 0.44, 0.26, and 0.26 in control and *M. chamomilla* and *C. cyminum* es-

Table 2: LC_{30} and LC_{50} values with confidence intervals of *Matricaria chamomilla* and *Cuminum cyminum* essential oils on *Aphis gossypii* adults after 24 hours

Plant species	LC_{30} (95 % C.I.) (µl l^{-1} air)	LC_{50} (95 % C.I.) (µl l^{-1} air)	Slope±S.E.	χ^2 (df)
M. chamomilla	13.52 (11.54-15.26)	19 (17.01-21.1)	3.54 ± 0.38	1.39 (3)
C. cyminum	27.87 (24.54-30.75)	37.36 (34.11-40.91)	4.11 ± 0.45	1.03 (3)

Table 3: LT_{50} and LT_{90} values of *Matricaria chamomilla* and *Cuminum cyminum* essential oils on *Aphis gossypii* adults

Plant species	LT_{50} (95 % C.I.) (hours)	LT_{90} (95 % C.I.) (hours)	Slope ± SE	χ^2 (df)
M. chamomilla	11.4 (9.82-13.19)	35.17 (27.70-50.13)	2.62±0.3	5.8 (3)
C. cyminum	13.88 (12.2-15.85)	36.38 (29.04-51.58)	3.06±0.37	3.05 (3)

sential oils, respectively. Intrinsic rate of increase significantly decreased between control and both essential oils ($p < 0.001$). The finite rate of increase in population (λ) for control and essential oils was 1.7, 1.3, and 1.3, respectively. This parameter significantly decreased in both essential oils compared with the control ($p < 0.001$). The age-specific survival rate (l_x), fecundity (m_x), and age-specific net maternity ($l_x m_x$) of *A. gossypii* are presented in Fig. 1. The beginning of oviposition in both treatments was delayed in compared with control. Also, these result showed that *C. cyminum* caused more declined faster decline in age-specific fecundities in compared with *M. chamomilla*. The mean oviposition days of *A. gossypii* were reduced significantly in *M. chamomilla* and *C. cyminum* essential oil treatments than the controls. There were no significant differences in the intrinsic rate of increase (r), gross reproduction rate (*GRR*) and finite rate of increase (λ) between essential oil treatments (Table 4).

4.3 LETHAL EFFECTS OF FUNGAL ISOLATES

All the tested fungal isolates were infective to adult aphids at the used conidial concentrations and the mortality rate of the aphids was correlated with conidium concentration. Based on the results, there was a linear relationship between the conidial concentrations of each fungal isolate and mortality of the aphids. The values of LC_{30} and LC_{50}, confidence intervals and slope of different isolates of *B. bassiana* against adult aphids are presented in Table 5. IRAN 429C and LRC 137 isolates caused the highest and lowest mortality rates in the adult aphids, re-

Table 4: Life table parameters (mean ± SE) of *Aphis gossypii* adults treated with two essential oils and in the control treatment

Parameter	*M. chamomilla*	*C. cyminum*	Control
r (day^{-1})	0.2685 ± 0.003 a	0.2691 ± 0.006 a	0.4487 ± 0.011 b
R_0(offspring/individual)	10.24 ± 0.34 a	13.22 ± 0.34 b	58.68 ± 1.29 c
GRR	16.96 ± 1.18 a	16.33 ± 0.46 a	62.68 ± 1.43 b
T (day)	8.66 ± 0.16 a	9.59 ± 0.17 b	9.07 ± 0.21 b
λ (day^{-1})	1.3080 ± 0.008 a	1.3088 ± 0.008 a	1.56± 0.018 b
Oviposition days	5.48 ± 0.22 a	7.44 ± 0.21 b	17.48 ± 0.37 c
Female adult longevity (day)	8.52 ± 0.28 a	11.72 ± 0.21 b	23.66 ± 0.25 c

r: intrinsic rate of increase; R_0: net reproductive rate; *GRR*: gross reproduction rate; T: mean generation time; λ: finite rate of increase. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared with paired bootstrap test ($P < 0.05$). The mean followed by different lower case letters indicate significant differences between three varieties.

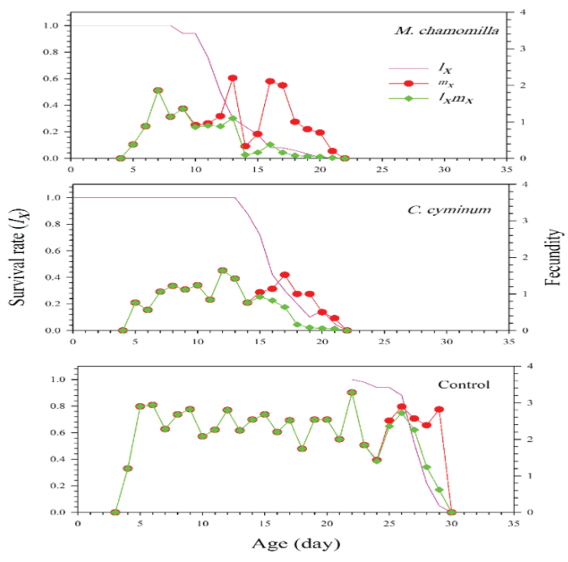

Figure 1: Age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *Aphis gossypii* treated with the essential oils

spectively. The lowest LC_{50} (3.9×10^4 conidia ml^{-1}) was calculated for IRAN 429C. Based on LC_{50} values, IRAN 429C was the most virulent isolate, followed by IRAN 108 and LRC 137 (Table 5). Low mortality was observed in control treatments and no signs of fungal growth were seen on dead insects. The results of LT_{50} indicated that IRAN 429C, had the lowest LT_{50} values followed by IRAN 108 and LRC 137, respectively (Table 6). For concentrations of 10^4, 10^5 and 10^6 conidia ml^{-1}, LT_{50} wasn't calculated because there weren't reached 50 % mortality rate at the end of the experiment.

4.4 SUBLETHAL EFFECTS OF FUNGAL ISOLATES

The fungal isolates, IRAN 429C and IRAN 108 were selected to evaluate their sublethal effects because of their higher virulence against adult aphids. The life table parameters of *A. gossypii* affected by fungal isolates are summarized in Table 7. The intrinsic rate of increase (r) in the control, IRAN 429C and IRAN 108 treatments was 0.44, 0.31, 0.31, respectively (Table 8). There wasn't a significant difference between the used fungal isolates ($p > 0.05$), but significant differences were found between fungal isolates and the control treatment ($p < 0.001$).

Gross reproduction rate (GRR) showed significant decrease in fungal isolates in compare with control ($p < 0.001$). Net reproductive rate (R_0) was significantly higher in control than that of fungal isolates ($p < 0.001$). The finite rate of population increase (λ) for control, IRAN 429C and IRAN 108 were 1.56, 1.36 and 1.36, respectively. This parameter significantly decreased in both fungal isolates compared with control ($p < 0.001$). The oviposition period for untreated aphids was significantly higher than that treated aphids ($p < 0.001$). There wasn't significant differences in the intrinsic rate of increase (r) and finite rate of increase (λ) among the two fungal isolates ($p > 0.05$). The age-specific survival rate (l_x), fecundity (m_x), and age-specific net maternity ($l_x m_x$) of *A. gossypii* are presented in Fig. 2. The beginning oviposition in Iran 429C was delayed in compared with control. Also, these result showed that IRAN 429C caused more declined in age-specific fecundities in compared with IRAN 108. At the end-point, the results showed that sublethal doses of the essential oils had a better performance against aphids than that of fungal isolates (Table 9).

5 DISCUSSION

Organophosphates, carbamates and pyrethroids are three main groups of chemical insecticides, which

Table 5: LC_{30} and LC_{50} values with confidence intervals of *Beauveria bassiana* isolates on *Aphis gossypii* adults

Isolates	LC_{30} (95% C.I.) (conidia/ml)	LC_{50} (95% C.I.) (conidia/ml)	Slope ± S.E.	χ^2 (df)
IRAN 429C	2.2×10^3 (7.2×10^2-5.1×10^3)	3.9×10^4 (1.9×10^4-7.4×10^4)	0.41 ± 0.03	5.85 (4)
IRAN 108	3.9×10^4 (9.5×10^3-1×10^5)	4.3×10^5 (1.5×10^5-1.1×10^6)	0.50 ± 0.03	6.93 (4)
LRC 137	7.4×10^4 (6.2×10^3-3.5×10^5)	1.4×10^6 (2.9×10^5-1×10^7)	0.41 ± 0.03	12.6 (4)

Table 6: LT_{50} and LT_{90} values of *B. bassiana* isolates at 10^8 conidia mL^{-1} on *Aphis gossypii* adults

Isolates	LT_{50} (95 % C.I.) (days)	LT_{90} (95 % C.I.) (days)	Slope±S.E.	χ^2 (df)
IRAN 429C	2.90 (2.62-3.18)	9.51 (8.43-11.02)	2.48 ± 0.16	4.75 (8)
IRAN 108	3.84 (3.47-4.22)	15.71 (13.17-19.75)	2.09 ± 0.15	2.44 (8)
LRC 137	4.64 (4.17-5.14)	23.34 (18.32-32.44)	1.82 ± 0.15	0.80 (8)

Table 7: LT_{50} and LT_{90} values of *B. bassiana* isolates at 10^7 conidia mL^{-1} on *Aphis gossypii* adults

Isolates	LT_{50} (95 % C.I.) (days)	LT_{90} (95 % C.I.) (days)	Slope ± SE	χ^2 (df)
IRAN 429C	3.55 (3.19-3.91)	14.90 (12.52-18.68)	2.05 ± 0.15	1.04 (8)
IRAN 108	6.21 (5.60-6.97)	30.64 (23.17-45.16)	1.85 ± 0.16	2.51 (8)
LRC 137	4.78 (4.34-5.25)	20.76 (16.79-27.57)	2.0 ± 0.15	2.57 (8)

Table 8: Life table parameters (mean ± SE) of *Aphis gossypii* adults treated with two *B. bassiana* isolates and the control treatment

Parameter	IRAN 108	IRAN 429C	Control
r (day^{-1})	0.3142 ± 0.007 a	0.3103 ± 0.006 a	0.4487 ± 0.011 b
R_0(offspring/individual)	26.52 ± 0.74 a	19.7 ± 0.90 b	58.68 ± 1.29 c
GRR	32.23 ± 1.18 a	28.76 ± 0.98 b	62.68 ± 1.43 c
T (day)	10.43 ± 0.22 a	9.60 ± 0.18 b	9.07 ± 0.21 b
λ (day^{-1})	1.36 ± 0.010 a	1.36 ± 0.008 a	1.56 ± 0.018 b
Oviposition days	11.08 ± 0.23 a	8.88 ± 0.4 b	17.48 ± 0.37 c
Female adult longevity (day)	17.28 ± 0.2 a	13.18 ± 0.36 b	23.66 ± 0.25 c

r: intrinsic rate of increase; R_0: net reproductive rate; *GRR*: gross reproduction rate; *T*: mean generation time; *λ*: finite rate of increase. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared with paired bootstrap test ($p < 0.05$). The mean followed by different lower case letters indicate significant differences between three varieties.

Table 9: Comparison of life table parameters (mean ± SE) of *Aphis gossypii* treated with fungal isolates and essential oils

Parameter	Treatment			
	Fungi		Essential oil	
	IRAN 108	IRAN 429C	*M. chamomilla*	*C. cyminum*
r (day^{-1})	0.3142 ± 0.007 a	0.3103 ± 0.006 a	0.2685 ± 0.003 b	0.2691 ± 0.006b
R_0(offspring/individual)	26.52 ± 0.74 a	19.7 ± 0.90 b	10.24 ± 0.34 c	13.22 ± 0.34 d
GRR	32.23 ± 1.18 a	28.76 ± 0.98 b	16.96 ± 1.18 c	16.33 ± 0.46 c
T (day)	10.43 ± 0.22 a	9.60 ± 0.18 b	8.66 ± 0.16 c	9.59 ± 0.17 b
λ (day^{-1})	1.36 ± 0.010 a	1.36 ± 0.008 a	1.3080 ± 0.008 b	1.3088 ± 0.008b
Oviposition days	11.08 ± 0.23 a	8.88 ± 0.4 b	5.48 ± 0.22 c	7.44 ± 0.21 d
Female adult longevity (day)	17.28 ± 0.2 a	13.18 ± 0.36 b	8.52 ± 0.28 c	11.72 ± 0.21 d

r: intrinsic rate of increase; R_0: net reproductive rate; *GRR*: gross reproduction rate; *T*: mean generation time; *λ*: finite rate of increase. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared with paired bootstrap test ($p < 0.05$). The mean followed by different lower case letters indicate significant differences between three varieties.

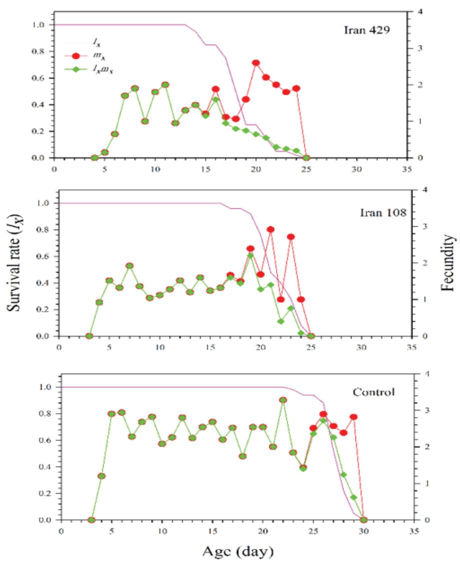

Figure 2: Age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *Aphis gossypii* treated with the fungal isolates

are commonly used in aphid control. The long-term use of these insecticides caused resistance development in aphid populations which make them difficult to control as well as frequent environmental and health risks (Sadeghi et al., 2009; Asadi et al., 2018). In recent years, the insecticidal properties of essential oils and their main compounds have been investigated on various pests, some of which had promising results (Al-Jabr, 2006). The results of the present study indicates that this biological agents had a significant lethal effect on the tested pests. Also, given the LC_{50} values of these two essential oils on adult insects, it was recognized that chamomile essential oil performed better compared to other essential oil in this pest. Meanwhile, IRAN 429C isolate cause the highest mortality in adult insects compared to the other two isolates. The toxicity of plant essential oils may be due to the fact that the seeds or leaves of these plants include compounds that have anti-nutrition or toxic activity or disrupt the molting which is often fatal for insects (Champagne et al., 1989). High level of insecticidal activity of *Eucalyptus globules* L. essential oil against *Aphis gossypii* has been reported (Mareggiani et al., 2008). In another study, the insecticidal activities of *Azadirachta indica* Adr. Juss., *Eucalyptus camaldulensis* Dehn. and *Laurus nobilis* L. essential oils were evaluated against *A. gossypii* (Ebrahimi et al., 2013). According to the results, *A. indica* and *E. camaldulensis* have a greater insecticidal activity compared to *L. nobilis*. Also, fertility and life span of the treated aphids were significantly decreased. Besides, it was reported that the essential oils of *Origanum syriacum* var. *bevanii* L., *C. cyminum* L., *Pimpinella anisium* L. and *E. camaldulensis* Dehn. were effective in fumigant assays against melon aphids and green peach aphids (Isman, 2000). Therefore, the results of this study are consistent with the findings of this researcher on the control of melon aphids by essential oils such as *C. cyminum*. In an experiment, Al-Jabr (2006) proved the toxicity and repelling effect of chamomile essential oil on *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrioidae). Also, El-Khyat et al. (2017) studied the insecticide activity of three essential oils, including chamomile on *Ephestia cautella* (Walker, 1863) and concluded that all of the three tested essential oils had a significant insecticidal effect on the pest. Among these, the most repelling essential oil was chamomile. The study results of AL-Jabr (2006) and El-Khyat et al. (2017) have been compatible with the results of the present study on the control potential of chamomile essential oil on insects. These reports are consistent with the present study in terms of the insecticidal activity of essential oils on the Aphididae family.

Entomopathogenic fungi are considered as one of the most promising alternatives in biological control of insect pests (Kaaya & Hassan, 2000). Gurulingappa et al. (2011) were studied the effects of endophytic *B. bassiana* and *Lecanicillium lecanii* isolates on mortality, survival and reproduction of *A. gossypii*. Results showed that the tested fungi significantly reduced the rate and period of reproduction and increased mortality of *A. gossypii*. Feng et al. (1990) compared pathogenicity of *B. bassiana* and *Verticillium lecanii* R. Zare & W. Gams, isolates against six species of cereal aphids. Although both fungal species were pathogenic on aphids, but *B. bassiana* was more virulent than that of *V. lecanii*. In a study, virulence of six *B. bassiana* isolates was studied on Russian wheat aphid based on LC_{50} and LD_{50} indices (Feng & Johnson, 1990). Although all isolates infect the aphids, but their virulences were very different and only one isolate showed significant virulence on aphids with the lowest LC_{50}. Similar results were obtained in pathogenicity assessment of indigenous isolates of *B. bassiana* on adult insects of Russian wheat aphids. In an experiment, fertility of *Aphis craccivora* Koch, 1854 were studied under the influence of *B. bassiana* isolate (Zaki, 1998). Result showed that by increasing in concentration of fungal conidia, the fertility rate was decreased which is in accordance with our finding. Our results were in agreement with those obtained by Kim (2007) who examined the effect of *Lecanicillium attenuatum* Zare & W. Gams CS625 on the reproduction of the cotton aphid. They found net reproduction rate of aphid nymphs was reduced and the reduction was corresponded well with spore concentration.

Based on the results obtained in this and previous studies, both entomopathogenic fungi and essential oils are good alternative candidates to chemical pesticides in aphid control programs. Further studies are needed to evaluate the insecticidal activities of these promise biological control agents directly under greenhouse and field conditions.

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