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BIOEFFICACY OF CRUDE FILTRATES OF TWO SPECIES ENTOMOPATHOGENIC FUNGI TO CONTROL THE *APHIS FABAE* SCOPOLI (HEMIPTERA: *APHIDIDAE*) UNDER LABORATORY CONDITIONS

Mohammed Hadi Ibrahim[1], Najeha Mohamed Barry[2], Ayad Ismael Khaleel[3]*, Ahmed Saeed Mohmed[1] and Rasha Abdulrazaq Jwada[4]

[1]Department of corp, Faculty of Agriculture, Al Qasim Green University, Babylon, Iraq.
[2]Technical college Al-mussaib, Al-Furat Al-Awsat Technical University, Iraq.
[3*]Department of Horticulture, Faculty of Agriculture, Al Qasim Green University, Babylon, Iraq.
[4]Department of Plant Protection, Faculty of Agriculture, University of Kufa, Najaf, Iraq.

Abstract

This study was conducted to evaluate different concentrations of the crude filtrates *Isaria fumosorosea* and *Trichoderma harzianum* on the mortality percentage of nymphs and adults of *Aphis fabae*. The results showed that 100% concentration of *I. fumosorosea* and *T. harzianum* filtrates had a higher mortality effect on nymphs and adults of *A. fabae*. The mortality rates were of the nymphs and adult of the crude filtrates of *I. fumosorosea* reached 90.00% and 65.32% at 100% concentration Compared with 13.83% and 9.22% in control treatment respectively after 72 h., while the mortality rates were of the nymphs and adult when used 100% concentration of the crude filtrates of *T. harzianum* amounted to 71.81% and 53.88% Compared with 13.83% and 9.22% in control treatment respectively.

Key words: filtrate, *Isaria fumosorosea*, *Trichoderma harzianum*, *Aphis fabae*, plant protection.

Introduction

In Iraq, pests are one challenge facing the country in agricultural development. The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is considered one of the most important pests Because can cause more than 200 host plants globally and can damage all plant parts (Barnea *et al.*, 2005). In addition to transferring many viral diseases such as beet yellows virus (BYV) and brome mosaic virus (BMV) (Stanković *et al.*, 2015). In recent years, synthetic pesticides usages have shown to have many negative effects in the agricultural systems and The appearance of resistance of the aphids against chemical compounds (Ahmad *et al.*, 2003). So, some researchers began looking for alternative safe methods of environmental system and active for using in control pest, Among several factors of friendly environmental, Entomopathogenic fungi (EPF) such as *Isaria fumosorosea* and *Trichoderma harzianum* have been successfully developed as biological control agents against a number of different pests, including aphids (Zimmermann, 2008 and Kalaf *et al.*, 2013). The common method of attacking the host pest by their fungi are through the body wall (Lnglis *et al.*, 1997). *I. fumosorosea* and *T. harzianum* have produced a variety of enzyme and toxins such as protease, chitinase, chitosanase, lipase,beauvericin and beauverolides (Ali *et al.*, 2010; Binod *et al.*, 2007; Weng *et al.*, 2019) which play a role in mortality the host by dissolving their vital structures, such as the peritrophic membrane and cuticle of insects primarily composed of chitin and protein, acts as a physicochemical barrier to environmental hazards and predators (Charnley, 2003).

The objective of the present study was to evaluate different concentrations of the crude filtrates of *I. fumosorosea* and *T. harzianum* as a biocontrol agent on the mortality percentage of nymphs and adults of *A. fabae*.

***Author for correspondence :** E-mail: engayad78@gmail.com

Materials and Methods

Insect collection and rearing

Nymphs and adults of *A. fabae* were collected from the fields College of Agriculture, Al-Qasim Green University planted by of broad bean. The aphids were reared on broad bean plants in 45×45 cm cages under greenhouse conditions (25±2°C and humidity 65±5% RH with 12 h. daily photoperiod) for several generations.

Preparation of the crude filtrates entomopathogenic fungi

The entomopathogenic fungus *I. fumosorosea* was obtained from the Laboratory of insect Department of the Plant Protection University of Baghdad which was originally isolated from insect samples, while *T. harzianum* obtained from Laboratory of Plant Diseases Department of the Plant Protection University of Kufa which was originally isolated from soil samples. Both isolates were cultured on Potato Dextrose Agar (PDA) at 25±1°C for 15 days before the initiation of the experiment. Two discs (7 mm diameter for each disc) were separately cut from each growing species, placed in a sterile flask containing 400 ml of potato dextrose broth (PDB) and incubated at 27°C for 28 days. After completing incubation, the mycelia separated from the filtrate by filter paper. According to (Hanson, 2008), then sterilized using the Millipore sterile filter through a Millipore 0.45 µm syringe filter. The crude filtrate output is considered a stock solution 100% then prepared Serial concentrations 75, 50%. While, the control treatment included Sterile distilled water only.

Bioassay Test

Ten nymphs and adults aphid were counted and placed on the filter paper in sterile Petri dishes 9 cm diameter using a hairbrush pen. Aphids were sprayed directly using 1cc insulin syringe with the crude filtrate of fungi concentrations, in addition to the control treatment previously mentioned above, four dishes for each treatment as replicates as well as the control treatment. The leaves of the broad bean were prepared in Petri dishes For feeding aphids and old leaves were exchanged for fresh leaves whenever needed. Petri dishes had a

Table 1: Effect of crude filtrates concentration of *I. fumosorosea* and *T. harzianum* on Mortality percentage of *A. fabae*

Treatment		*I. fumosorosea*			*T. harzianum*		
Conc.	TP	IS		Conc. × TP	IS		Conc. × TP
		Nymph	Adult		Nymph	Adult	
0	24	9.22	4.61	6.92	9.22	4.61	6.91
	48	13.83	9.22	11.53	13.83	9.22	11.53
	72	13.83	9.22	11.53	13.83	9.22	11.53
50	24	29.89	26.74	28.31	33.30	26.67	29.99
	48	45.14	39.38	42.26	42.25	36.67	39.46
	72	63.62	50.42	57.02	60.16	45.00	52.58
75	24	42.28	33.26	37.77	35.06	30.10	32.58
	48	53.88	45.00	49.44	53.85	42.27	48.06
	72	71.71	55.26	63.48	63.61	50.92	57.27
100	24	45.17	35.21	40.19	39.40	33.98	36.69
	48	60.00	50.77	55.39	56.94	45.14	51.04
	72	90.00	65.32	77.66	71.81	53.88	62.84
L.S.D (P≤0.05)		7.15		5.05	7.02		4.96
IS		44.88	35.37	Conc.	41.11	32.31	Conc.
L.S.D (P≤ 0.05)		2.06			2.02		
Conc. × IS	0	12.29	7.68	9.99	12.29	7.68	9.99
	50	46.22	38.84	42.53	45.24	36.11	40.68
	75	55.95	44.51	50.23	50.84	41.10	45.97
	100	65.06	50.44	57.75	56.05	44.34	50.20
L.S.D (P≤ 0.05)		4.13		2.92	4.05		2.86
				TP			TP
TP × IS	24	31.64	24.96	28.30	29.25	23.84	26.54
	48	43.21	36.09	39.65	41.72	33.33	37.52
	72	59.79	45.06	52.42	52.35	39.75	46.05
L.S.D (P≤ 0.05)		3.57		2.52	3.51		2.48

Conc = Concentration, TP= Time period, IS= Insect Stages.

1cm 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 and 70%±10 RH). Daily observations were continued. Mortality was recorded after 24, 48 and 72 hours post-treatment.

Statistical analysis

The data obtained were analyzed using GenStat package 3 (3rd edition) by randomized complete block design with three factors The percentage effects of the crude filtrates were calculated and corrected by Abbott's formula (Abbot, 1925). Angular transformation was used for Mortality statistical analysis. The treatment means were compared by least significant difference (L.S.D) at significance $P \leq 0.05$. The effect Concentrations, Time period and insect stages of both filtrates on *A. fabae* were compared by t-test unequal variances at $P \leq 0.05$.

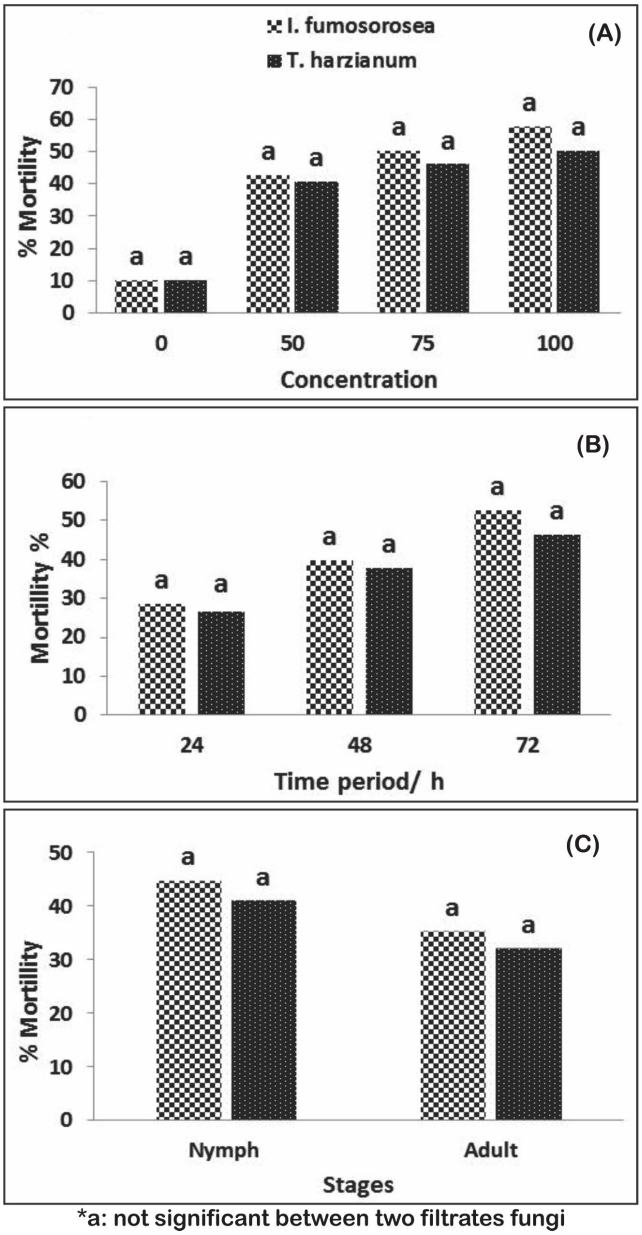

Fig. 1: Compared between percentage mortality of *A. fabae* exposed to crude filtrates concentrations of *I. fumosorosea* and *T. harzianum*. (A) Concentration, (B) Time period, (C) Insect Stages.

Results and Discussion

The results in (Table 1) show that the different concentrations of crude filtrates of *I. fumosorosea* and *T. harzianum* a significantly higher in the mortality percentage of nymphs and adults of *A. fabae*. Higher mortality was observed at higher concentration of crude filtrates of *I. fumosorosea* and *T. harzianum* recorded 45.17, 35.21% and 39.40, 33.98% mortality of nymphs and adults of *A. fabae* respectively Compared with 9.22, 4.61% and with 9.22, 4.61% in control treatment respectively at 24h. The mortality then dramatically increased at 48-27 h., which was at 72 h. 90.00, 65.32% and 71.81, 53.88% mortality of nymphs and adults of *A. fabae* at concentration 100% respectively while no increased mortality in the control treatment. The interactions between fungi concentrations and time period were significantly different ($P \geq 0.05$) and the same of interactions between fungi concentrations and insect Stages as well the interactions between time period and insect stages and for both crude filtrates fungi.

The statistical analysis of data to compare the results using the t-test, un paired t-test revealed that in the comparisons between concentration, time period and insect Stages of the crude filtrates of *I. fumosorosea* and *T. harzianum*, no-significant differences were observed ($P \leq 0.05$) (Fig. 1).

Discussion

The aim of this study was to evaluate the biocontrol efficacy of crude filtrates of *I. fumosorosea* and *T.harzianum* against *A. fabae*. Results of the study coincided with Farooq and Freed, (2018) observed that the toxic crude proteins from entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *I. fumosorosea* effected on survival *of Musca domestica.* Also these results are in agreement with findings of Kalaf *et al.,* (2013) who reported the efficiency of *T. harzianum* and *T. viride* exudates against *Schizaphis graminum* where the mortality rate was higher in third day 48.37% compared with first day 32.06%. Binod *et al.,* (2007) investigated the effects of culture filtrate containing chitinase from *T.harzianum* against *Helicoverpa armigera* where it reduced the feeding rate and body weight of the larvae and increased larval and pupal mortality. The insect integument is composed of enzymes and chitin with associated lipids and phenolic

compounds which serve as a barrier against invading microorganisms (St. Leger, 1991), So Cultural filtrates of entomopathogenic fungi have several enzyme activities, such as chitinase, protease and lipase, enzymes are important factors involved in the initiation of infection process by entomopathogenic fungi (Samuels and Paterson, 1995) and are similar as pesticidal compounds (Yoon *et al*., 2013) where have different effects for pests as insecticidal or reducing digest food (Kim *et al.*, 2013). In general, nymphs were more sensitive than adults to both crude filtrates of *I. fumosorosea* and *T. harzianum*, may be back to contain thin exoskeletons at this stage of development which it makes more vulnerable to crude filtrates and therefore facilitate the penetration into the cuticle of the insect.

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