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LETHAL EFFECTS OF CRUDE SECONDARY METABOLITES OF *TRICHODERMA HARZIANUM* AGAINST *MYZUS PERSICAE* (HOMOPTERA: APHIDIDAE)

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Abstract

The present study aimed to revealing and identifying some of compounds produced of filtrate *Trichoderma harzianum* by using gas chromatography mass spectrometry (GC-MS) technique, and then study the effect of this filtrate on some aspects of life performance of *Myzus persicae* in the in the vitro. GC-MS analysis of filtrate *T. harzianum* showed the presence of many compounds such as Oxalic acid, Hexadecanoic acid, 2 Methylenecyclododecanone, 1, 2-ethanediyl ester, -8 Cyclohexadecen-1 -one and 1 -Octadecyne. The results also showed the mortality of nymphs and adults of *M. persicae* increased with increasing concentrations to reach 39.15 and 36.22 respectively, compared with 12.29 and 6.1 % in control treatment after 3d . The developmental period of the immature stages has increased from 6.6 days in the control treatment compared with 9.0 days in 100% concentration while the fecundity of adult females reduced to reach 17.00 nymph at concentration 100% compared with 28.33 nymph in the control treatment.

Key words: GC-MS Analysis, Secondary Metabolites, *Trichoderma harzianum*, *Myzus persicae*

Introduction

The green peach aphid, *Myzus persicae* (Homoptera: Aphididae) is a major insect pests, which attack more than 800 plant species worldwide (Van Emden and Harrington, 2017). *M. persicae* destroys crops both in the greenhouse and field by transmitting vector viral diseases and their damage includes necrosis, wilting, stunting, flower and fruit abortion, and leaf distortion lead to plant death (Singh and Joshi, 2020). At present, farmers complain of this pest has developed multiple resistances to many chemical insecticides as a result to overuse of insecticides because they often have limited information about pest outcrop and suitable methods of control (Zhang *et al.,* 2015). Therefore, Management of insect pests using entomopathogenic fungi (EPF) as a biological aphid control method for organic cultivation of vegetables under protected conditions to be best biocontrol tools. As well as, its effectiveness, environment-friendly and target only the harmful pest (Manfrino *et al.,* 2014). *Trichoderma harzianum* is reported to control aphids and other pests, because it contains cuticle degrading enzymes like protease; lipase and chitinase which degrade the insect cuticle and thereby releases several mycotoxins and last decade, the enhancement of indirect plant defense barriers against aphids was observed in plants colonized by *T. harzianum* (Coppola *et al.,* 2017).T he current study aimed to detect and identify some chemical compounds produced in the fungal crude filtrate obtained from *T. harzianum* , and the effect these compounds on some aspects of life performance of *M. persicae* in the in the *vitro*.

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Materials and Methods

Preparation of Fungal Filtrate

T. harzianum was obtained from the Laboratory of Plant Diseases, Department of the Plant Protection, University of Kufa (originally isolated from local soil samples). Isolates was cultured on potato dextrose agar at 25 ± 1°C for 15 days before the initiation of the experiment. Two discs (7 mm diameter) were cut from growing pathogen, placed in a sterile flask containing 400 mL of potato dextrose broth, and incubated at 27°C for 28 days. After completing incubation, the mycelia were separated by filtration. The filtrate was sterilized using the Millipore sterile filter (Millipore 0.45 µm syringe filter).

The filtrate was considered a stock solution (100%) and then diluted to prepare 75 and 50% concentrations in sterile distilled water. The control treatment was sterile distilled water only.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To identify compounds of the filtrate *T. harzianum* submitted the filtrate to the gas chromatography-mass spectrometer technique (GC-MS). This analysis was performed by Instrument of Gas Chromatography–Mass spectrometry Shimadzu's in laboratories of Ministry of Science and Technology - Baghdad.

Insect Collection and Rearing

Green peach aphids were collected from eggplant plants grown in greenhouses, *M. persicae* was reared on healthy eggplant. All the potted plants were maintained in plastic cages (26×25×21 cm^3) at 25 ± 2°C and 65 ± 5% relative humidity (RH) in a 16 Light/8 Dark (L/D) photoperiod.

Bioassay Test

Ten adults and nymphs were placed separately on the filter paper into 9 cm diameter Petri dishes using a hairbrush. Aphids were sprayed directly using 1cc insulin syringe with the crude filtrates of *T. harzianum* concentrations. The same number was used for controls as well, where sprayed with 0.03% sterile aqueous Tween80. three plates replicates were tested for each treatment. The treated adults and nymphs eggplant leaves were fed on. Petri dishes had a1-cm hole covered with nylon mesh on the lid for ventilation. The dishes were sealed by adhesive tape to avoid the adults and nymphs escape. The treated plates were incubated at (25 ± 2ºC and 65 ± 5% ± 10 R.H.). Mortality was recorded after 1, 2 and 3 days post-treatment.

To calculate 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 filtrates of *T. harzianum* concentrations as in previous experience. Neonate aphids treatment has been pursued to reach the adult stage. The developmental period of nymphal stage was recorded to reach the adult stage. To determine the fecundity of adult females, emerged adults were selected (3 adults per replicate and three replicates per treatment) and placed onto leaves of eggplant. 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 three and one factor. The percentage effects of the filtrates of *T. harzianum* 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

GC-MS analysis of filtrates of *T. harzianum*

Analyzing the results of the mass spectrum of GC-MS of the filtrate of *T. harzianum* indicated the most important chemical compounds separated from the filtrate Fig. 1 where 19 of the chemical compounds that belong to specific groups appeared and several compounds appeared mainly within these groups, including Oxalic acid, Hexadecanoic acid, 2 Methylenecyclododecanone, 1, 2-ethanediyl ester, -8 Cyclohexadecen -1 -one and 1-Octadecyne, table 1. The results were consistent with (Gupta *et al.*, 2019) which indicated that the filtrate of *T. harzianum* contained Kojic acid, acetic acid, volatile alcohols and citric acid.

Effect of filtrates of *T. harzianum* on the mortality

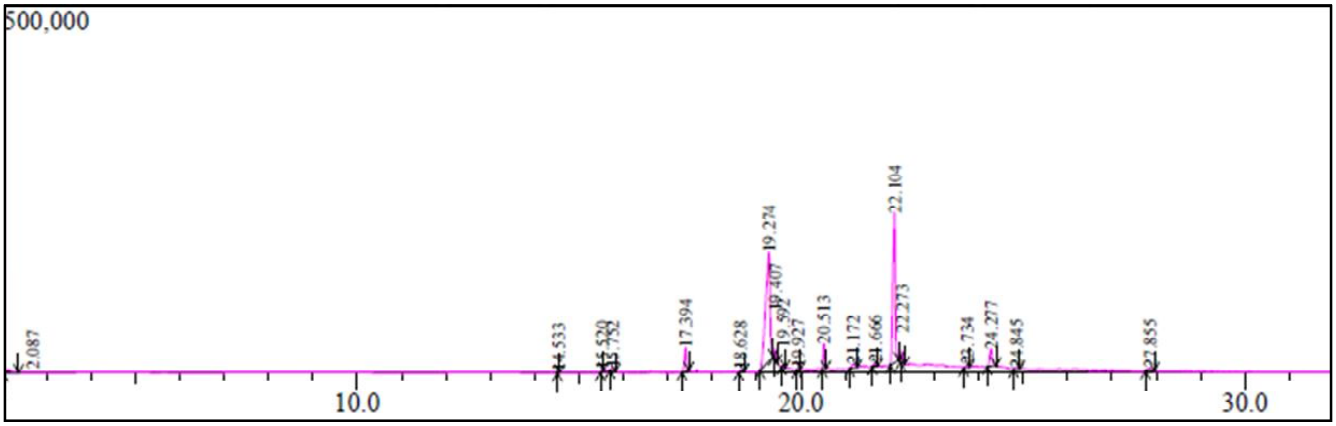

Fig. 2: GC-MS Chromatogram of the filtrate of *T. harzianum*.

Table 1: Compounds found in the filtrate of *T. harzianum*.

	Name of compound	Retenti-on time	Peak area	Peak Height
1	Oxalic acid	2.087	50144	4422
2	Hexadecanoic acid	17.394	103172	32574
3	2Methylenecyclododecanone	19.274	1028060	147102
4	1,2-ethanediyl ester	20.513	79683	34968
5	8-Cyclohexadecen-1-one	22.104	778889	203641
6	1-Octadecyne	24.277	100585	23917

of *M. persicae*

Table 2 shows that the filtrate of *T. harzianum* concentrations played significant role on control of adult and nymph of *M. persicae* during different time periods. The highest mortality recorded at high concentration 100% in both adult and nymph stages to reach 39.15 and 36.22 % respectively, compared with 12.29 and 6.1% in control treatment after 3 d of exposure. The significant difference was observed toward the decrease of mortality in adults compared with in nymphs.

Results and statistical analyses from Experiment 2 are summarised in table 3. The period of development increased with an increase in the concentration of filtrate of *T. harzianum* compared with control treatment, it increased from 6.6 days in control treatment to about 9.0days at a concentration 100%. While, Female productivity decreased as the concentration increased

Table 2: Effects of filtrate of *T. harzianum* concentrations on mortality of adult and nymph of *M. persicae* at different time periods.

Conc-entr-ation	Mortality(%)							
	Nymph				Adult			
	Time periods/days				Time periods/days			
	1	3	3	Mean	1	3	3	Mean
0	0.00	6.15	12.29	6.15	0.0	6.1	6.1	4.1
50	23.85	26.56	30.99	27.14	21.15	23.85	26.0	23.69
75	28.78	33.21	35.22	32.40	22.85	25.56	33.0	26.47
100	29.99	37.22	39.15	35.45	28.11	33.0	36.22	32.44
L.S.D (P≤0.05)	Con.=5.1 Per. = 4.2 Conc .× Per 9.0				Con.=5.2 Per. = 4.0 Conc .× Per 9.1			

Table 3: Effects of filtrate *of T. harzianum* concentrations on developmental period and fecundity of adult of *M. persicae*.

Concentration	Developmental period (days)	Productivity nymph / Female
0	6.6	28.33
50	8.0	22.00
75	8.6	19.00
100	9.0	17.00
L.S.D (P≤0.05)	0.2	0.4

where the average numbers of births adult female of *M. persicae* significantly reduced from 28.33 nymph in the control treatment to 17.00 nymph at 100% concentration.

Discussion

In our study, laboratory experiments show that filtrate of *T. harzianum* has role effective as a pesticide against green peach aphid *M. persicae* because of its contents of compounds and enzymes, which appeared clearly in an analysis gas chromatography-mass spectrometry. The effect of filtrate appeared to be dose and time-dependent and increased by increasing concentration and time after application. Our results are in harmony with Mohmed (2019a) who found that the crude filtrates of *Isaria fumosorosea* and *T.harzianum* were effective against the nymphs and adults of Aphis fabae. The result looks similar to those of Kim *et al.,* (2013) who reported that the filtrate of *Beauveria bassiana* Bb08 showed the highest mortality 78% against green peach aphid three days after treatments. These results were in agreement with the research obtained by Binod *et al.,* (2007) who showed that the culture filtrate *T. harzianum* exhibited high toxicity to control *Helicoverpa armigera* where was its role as a potent antifeedant as it reduced the feeding rate and bodyweight of the larvae It reduced the successful pupation and increased larval and pupal mortality. The increase in the growth period and the decrease in the productivity of adults may be due to the chemical compounds and enzymes contained in the filtrate of *T. harzianum* and that caused disruption in the physiology of the *M. persicae* and ability of the insects to digest food, as well as occurring disturbance of endocrine system which controlling growth and moulting. The mortality of nymphs was more than adults because they possess soft exoskeletons at this stage of development while being hard in adults, which may facilitate the penetration of fungal filtrate into the cuticle of the insect, as reported by Moussian (2010). The results of the study were consistent with Mohmed (2019b) observed that the effects of the conidial suspension of *I. fumosorosea* leads to an increase of the nymphal stage and reduced the number of offspring produced from *Aphis gossypii* . The results obtained from these studies have exposed the good potential for the use of filtrate of *T. harzianum* for the control and can be considered as a promising effective pesticide against green peach aphid *M. persicae*.

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