Improvement of antagonism and fungicides tolerance in *Trichoderma harzianum* and *T.viride* local isolates by Ultra-Violet irradiation

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Abstract
Among 22 Iraqi *Trichoderma harzianum* and *T.viride* isolates, T3,T5,D5,S1 and S6 related to *T. harzianum* showed a higher antagonistic activity against the plant pathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani*. These isolates were selected for improvement their biocontrol activity by usi
ng uv-irradiation. The optimum exposure time to ultraviolet irradiation is 30 min, in which, the percentage of kill was 98-99% .After UV-irradiation treatment (Quartz lamps 30W at 240-280 nm, peak 254nm, distance from the source was 20 cm), antagonistic capability against *R. solani* and *F. oxysporum* was improved in mutants MT3,MT5,MS1 and MS6, in which antagonistic reaction score were 3.6,3.9,3.2 and 3.6 against *F. oxysporum* and 3.7,4,3.4 and 4 against *R. solani* compared to 3.2, 2.8, 2.6 and 2.8 against *F. oxysporum* and 2.7, 2.9,3.1 and 3 against *R. solani* in the wild types T3, T5, S1 and S6,respectively. The growth of *T. harzianum* isolates (estimated as colony diameter) was significantly increased in all *T. harzianum* mutants except MT3 and MD5 mutants, CMCase activity was increased from 13.2, 16.2, 10 and 15.8 U/ml / mg protein in the wild isolates T3, T5, S1 and S6 to 18.6, 20.3, 17.7 and 22.5 U/ml / mg protein in the mutants MT3, MT5, MS1 and MS6, respectively. Chitinase activity was also increased in these mutants to 88.4,132.3,86.1 and 136.7 U/ml / mg protein, compared to 82.7, 93.2, 74.5 and 103.2 U/ml / mg protein in their wild, respectively**.** As comparison between wild types and mutants, the maximum growth was 5.2 and 4.8 cm (as colonies diameters) by MT5 in present of 25% of the recommended field rate of Topsin and Benomyl, compared to 3.3 and 2.2 cm in the wild type T5, respectively, followed by MS6,in which, the maximum growth in this mutant was 4.8 and 4.2 cm in the same concentration of these two fungicides, compared to 3.4 and 2.5 cm in the wild type S6, respectively. According to these results, the mutants MT5 and MS6 developed from UV-irradiation treatment, showed as promising *T. harzianum* mutants through their antagonistic activity (at mycoparasitism level) , fast growth, higher CMCase and chitinase activity and growth in 25% of the recommended field rate of Elsa , 25 and 50% of the recommended field rate of Diathen and Mizab and all concentrations of Topsin and Benomyl with complete inhibition of pathogenic fungi *F. oxysporum* and *R. solani*.

Key words: Biocontrol, Phytopathogenic fungi, fungicides reduction, *T. harzianum*, Ultra-Violet irradiation.

Introduction
Biological control of plant pathogens has attracted significant recent attention as an alternative disease management strategy due to its ability to provide environmental friendly disease control, particularly when included in an integrated pest management strategy (1,2). *Trichoderma harzianum* has been identified as a promising biocontrol agent of many plant diseases caused by the soil-borne pathogen (3).
Trichoderma species are known to suppress infection of root by soilborne pathogens like fungi such as *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* species and *Pythium* species on various crops (4,5,6) and root-knot nematodes (7).In addition ,species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (5,8).
Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds (9,10). In Iraq, many plant diseases have been identified in different fields that threaten rapeseed production every year and sometimes lead to the high economic losses (11). In the most cases, *Trichoderma* sp. required enough time for their activity against pathogens, and in sever plant diseases, *Trichoderma* sp. has a little or no effect against pathogens (12) in this situation chemical fungicides must be applied. The use of chemical biocides to control microbial, fungal and insect plant pests has long been a feature of conventional agricultural practice and their use has made it possible to increase crop yields and food production (13). However, many of these biocides have toxic effects on human, plants, animals and ecosystem as a whole, in addition, the toxic effects of chemical biocides are not confined to their target species, and their application can impact upon organisms that benefit the wider agroecosystem (ie. beneficial organisms), for this reasons there is a worldwide demand for a reduction in chemical use in agriculture and therefore a need to find economic, social and environmental alternatives.
The aims of the present study are (i) Production of *T. harzianum* mutants (by using uv- irradiation) via improvement of antagonistic capability of the local Iraqi *T. harzianum* isolates against the pathogenic fungi, *Fusarium. oxysporum* and *Rhizoctonia solani*.(ii) Improvement of capability of these isolates for tolerance to fungicides in order to determine their compatibility with fungicide applications as part of an integrated disease management programme (iii) Protect the environment and human health from chemical substances hazards by reduce the chemical

fungicides at the minimum concentrations with encourage of biocontrol agents applications.

Materials and methods

Isolation of *T. harzianum* and *T.viride* isolates

Fungi were isolated from samples collected from different locations of Salah-Aldin Governorate in Iraq (Table 1). The samples including soil, humus, and compost .Dilution plate method was used for isolation of fungi. *T. harzianum* and *T.viride* isolates (22 isolates) were selected from fungi population and identified according to(14) depend on visual observations on petri dishes (The mode of mycelia growth, colour, odour and changes of medium colour) and micro-morphological observations in slide culture(shape, size, arrangement and development of conidiophores or phialides),the identified cultures subsequently, they were transferred to Malt extract Agar (MEA) slants. Slants were incubated at 25°C for 5 days. The isolates were maintained on MEA at 4ºC.

Table 1. Regions , numbers and codes of . *T. harzianum* and *T.viride* isolates

Region	No. of isolates	*Trichoderma* species	Code of isolates
Tikrit	5	*T. harzianum*	T1,T3,T4, T5, T6
	3	*T.viride*	T2,T7, T8
Al-Dor	3	*T. harzianum*	D3, D4, D5
	2	*T.viride*	D1, D2
Bejy	1	*T. harzianum*	B1
	2	*T.viride*	B2, B3
Sammara	3	*T. harzianum*	S1,S5,S6
	3	*T.viride*	S2, S3, S4

Causal pathogens

Soilborne root infecting fungi including *Fusarium. oxysporum* and *Rhizoctonia solani* were obtained from Plant Protection Department – Agriculture College / Tikrit University. These fungi were maintained on Potato Dextrose Agar medium (PDA) at 4ºC.

Antagonistic capability of *T. harzianum* and *T.viride* isolates against *F. oxysporum* and *R. solani* in vitro:

Fifteen ml of MEA medium in 9 cm Petri-dishes were inoculated with two disks (7 mm in diameter) of five-day old antagonistic fungi (*T. harzianum* and *T.viride* isolates). Two disks of the tested pathogen obtained from four-day old cultures were then placed at the periphery of each plate at the same distance. The inoculated plates in addition to plates inoculated with the pathogen only (control treatment) were kept at 25°C. Three replicates were used for each treatment.

Antagonistic effect was evaluated by scoring the width of the inhibition zone (clear area) (15), where:

0 : no inhibition
1 :<10 mm (slight antagonism)
2 :10-20 mm (moderate antagonism)
3 : > 20 mm (high antagonism).
4 :over growth (mycoparasytism)

T. harzianum and *T.viride* isolates that showed a high antagonistic capability screened for subsequent studies.

Antagonistic capability of *Trichoderma* sp. mutants was also determined in the same procedure.

Effect of Ultra Violet irradiation time (min) on the percentage spores germination of selected *T. harzianum* isolates

Five ml of conidial suspension from 5-day-old grown culture of *Trichoderma* isolates that showed a high antagonistic capability (all these isolates were related to *T. harzianum*) was placed on Petri dishes (9cm) was then placed under Ultra Violet (UV) source for different periods including 0,10,20,30 and 40 min. The UV source was Quartz lamps 30W (240-280 nm, peak 254nm). The distance from the source of irradiation was 20 cm, after irradiation, Petri dishes were incubated at 25ºC in normal conditions. The percentage of irradiated spores germination was determined after 12, 24 and 48 h.The growth (as colony diameter) of the selected mutants of *T. harzianum* and their wild types was also determined.

Tolerance to fungicides

Tolerance to fungicides in selected *T. harzianum* isolates was evaluated using food poison method before and after UV- irradiation. Tested fungicides are listed in Table 2. Fungicides were added to PDA to get final concentration of 100, 50 and 25% of the recommended field rate by produced companies (table 2) in addition to PDA without fungicide as control. A 5mm inoculum disc of *T. harzianum* isolates was cut from the margin of actively growing colony and placed in centre of each Petri plate. Petri plates were incubated at 25±2ºC.Radial growth of *T. harzianum* isolates was observed daily, the colony diameter was measured after period of full growth *T. harzianum* in control.

Table 2. Types of fungicides

Fungicides (product name)	Active ingredient	recommended field rate by produced companies g /100Lit. ml/100Lit*	produced companies
Elsa	Carbendazin 50%	70	Dupont de Nemours/ France
Kopralin	Copper hydroxide 77%	200	Drexel Chem. Company/USA
Contaf	Hexaconazole 10%	100*	Rallis India LTD /India
Diathane M-45	Diathane	350	Dow Agrosciences(Rohm&Haas)
Mizeb	Mancozeb 80%	200	Agria / Bulgaia
Topsin	Thiophanate methyl 70%	200	Nippon-soda / Japan
Benomyl	Benomyl 50%	60	United Phosphorus (LTD)/India

Preparation of enzyme sources

Five-day-old mycelial discs (5 mm) of both wild and mutant types of *T.harzianum* grown individually on PDA medium was used for inoculating the Malt extract broth (basal medium) . After 14 days of incubation, the growth medium was filtered through Whatman No. 1 filter paper. The culture filtrates thus collected were preserved with sodium azide (0.02 % w/v) at 4 °C, which served as enzyme sources. The protein estimation was done following the method of Bradford (17) using Coomassie Brilliant Blue reagent (Fluka).

Carboxymethyl cellulase

Carboxymethyl cellulase (CMC-ase) activity was assayed by dinitrosalicylic acid (Sigma) following the method of Burns (17). The reaction mixture contained 0.5 ml of culture filtrate, 0.5 ml of sodium acetate buffer (0.05 mol/l at pH 5.0) and 1 ml of 1 % carboxymethyl cellulose (Sigma) dissolved in the same buffer (pH 5.0) at 50 °C for 30 min. The reaction was stopped by boiling and the amount of reducing sugar released was estimated. The enzyme activity was expressed as release of µmol glucose/ml of culture filtrate/min/mg protein.

Chitinase

Enzymatic hydrolysis of colloidal chitin was assayed following the release of free N-acetyl-glucosamine (NAGA) from colloidal chitin (18). The reaction mixture containing 1 ml of 0.5 % colloidal chitin, 2 ml of Mcllvaines's buffer (equal volume of 0.2 mol/l disodium hydrogen phosphate and 0.1 mol/l citric acid at pH 4.0) and 1 ml of culture filtrate was incubated for 20 min at 37 °C in a shaker bath and the reaction was stopped by boiling for 3 min. After centrifugation of this mixture at 2000 rpm for 30 min, 1.5 ml of supernatant fluid was mixed with 2 ml of potassium ferricyanide reagent (0.05 % potassium ferricyanide in 0.5 mol/l sodium carbonate) and heated in boiling water bath for 15 min. The amount of NAGA released was estimated from absorbance of reaction mixture at 420 nm. The enzyme activity was expressed in unit as release of 1 µmol N-acetyl glucosamine/ml of culture filtrate/min/mg protein.

Statistical analysis

Data Statistically analyzed by Statistic Analysis System, version 9 (SAS Institute, Cary,NC).Treatment means were compared by Least Significant Difference ($P<0.05$).

Results

Antagonistic capability of *T. harzianum* and *T.viride* isolates against *F. oxysporum* and *R. solani* in vitro

Among 22 Iraqi *Trichoderma harzianum* and *T.viride* isolates, antagonism screening test showed that the T3, T5, D5, S1 and S6 related to *T. harzianum* were the best isolates as wild types with a higher antagonistic reaction score (Fig. 1), the antagonistic capabilities of these isolates were 3.2, 2.8, 2.8, 2.6 and 2.8 against *F. oxysporum* and 2.7, 2.9, 3, 3.1 and 3 against *R. solani* in the wild types ,respectively.

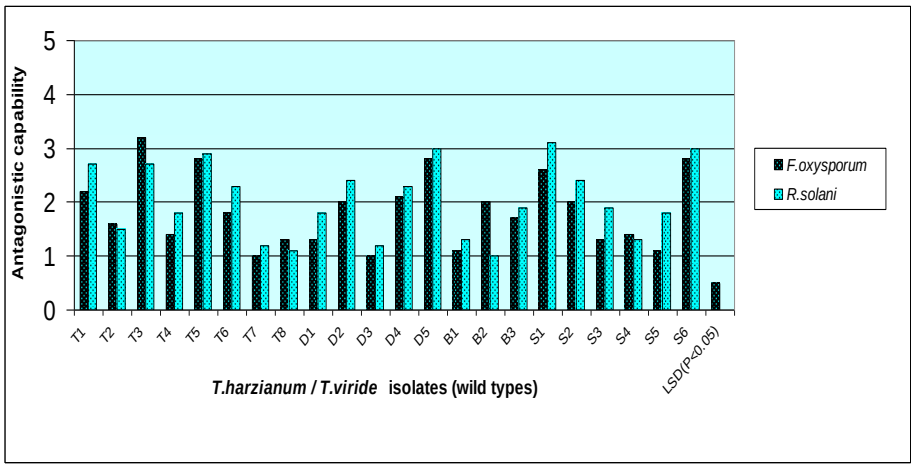

Fig.1. Antagonistic capability of the *T. harzianum* and *T.viride* isolates (wild type) against *F. oxysporum* and *R. solani*

Effect of Ultra Violet irradiation time (min) on the percentage spores germination of selected *T. harzianum* isolates

UV irradiation completely inhibited conidial germination of all selected *T. harzianum* isolates after 40 min, this inhibition increased with irradiation time (table 3), after 30 min of the UV irradiation, the lower percentage of conidial germination was recorded, there are only 2% of conidial germination in T3,T5 and S1, and 1% of conidial germination in D5 and S6, compared to 80,82,78,80 and 76% in non irradiated T3,T5,D5,S1 and S6, respectively. Recovery was observed after incubation for 24 and 48h in non-irradiation (normal) conditions, the percentage of conidial germination was 6-8% after 24 h, then increased after 48h to 14-16% (table 3).All the tested mutants (MT3,MT5,MD5,MS1 and MS6) were selected from the 30min uv-radiation exposure.

Table 3. Effect of Ultra Violet irradiation time (min) on the percentage spores germination of selected *T. harzianum* isolates.

Irradiation time (min)*	Percentage spores germination after														
	12 h.					24 h.					48 h.				
	T3	T5	D5	S1	S6	T3	T5	D5	S1	S6	T3	T5	D5	S1	S6
0	80	82	78	80	76	92	93	92	94	92	100	100	100	100	100
10	11	13	11	13	12	21	23	22	22	24	37	36	38	36	37
20	5	5	4	5	6	12	12	13	12	14	18	17	18	16	18
30	2	2	1	2	1	7	6	7	6	8	14	14	15	14	16
40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

***** UV source : Quartz lamps 30W (240-280 nm, peak 254nm)

Antagonistic capability of the selected *T. harzianum* mutants against *F. oxysporum* and *R. solani*

After irradiation, antagonistic capability against *R. solani* and *F. oxysporum* was improved in mutants MT3,MT5,MS1 and MS6, in which antagonistic reaction score were 3.6,3.9,3.2 and 3.6 against *F. oxysporum* and 3.7,4,3.4 and 4 against *R. solani* compared to 3.2, 2.8, 2.6 and 2.8 against *F. oxysporum* and 2.7, 2.9,3.1 and 3 against *R. solani* in the wild types T3, T5, S1 and S6, respectively. Although antagonistic capability of MD5 against *F. oxysporum* was improved but against *R. solani* was not.(Fig. 2 and 3).

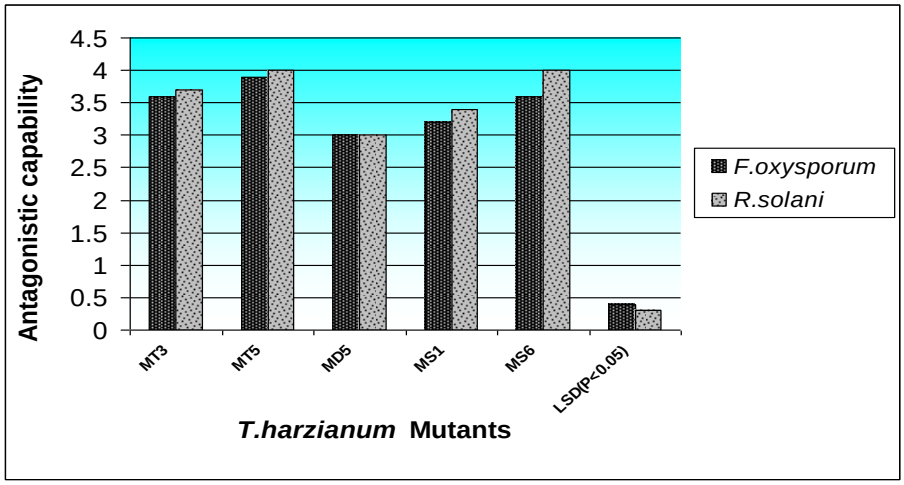

Fig.2. Antagonistic capability of the selected *T. harzianum* mutants against *F. oxysporum* and *R. solani* (MT3, MT5, MD5, MS1 and MS6 are mutants arise from the wild type T3, T5, D5,S1 and S6, respectively) .

Growth of *T. harzianum*

The growth of *T. harzianum* isolates (estimated as colony diameter) was significantly increased in all *T. harzianum* mutants except MT3 and MD5 mutants, the maximum colony diameter was 9 cm in the mutants MT5 and MS6 compared to 7.8-8.6 cm in wild isolates, but there is no effect of irradiation on the mutant MT3 growth and there is a decrease in MD5 growth compared to its origin wild type (Fig. 3)

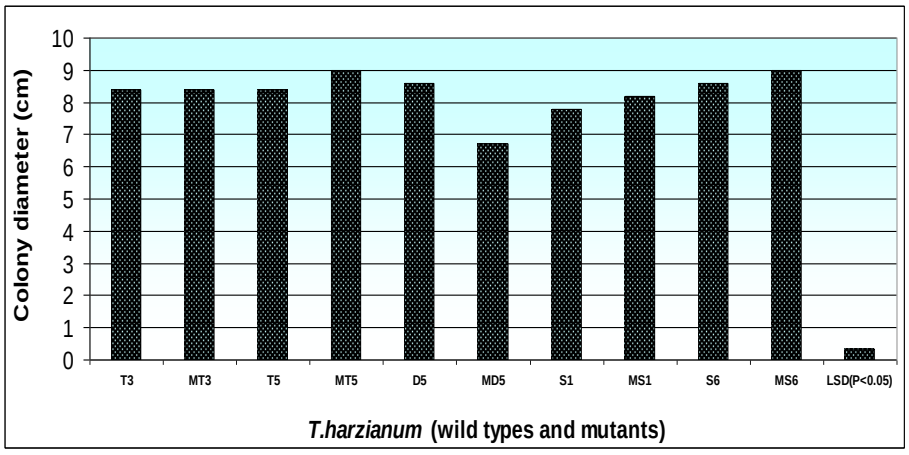

Fig. 3 Colony diameter (cm) of *T. harzianum* (wild types and mutants)
(MT3, MT5, MD5, MS1 and MS6 are mutants arise from the wild type T3, T5, D5,S1 and S6, respectively)

CMCase and Chitinase activities

CMCase activity was increased from 13.2, 16.2, 10 and 15.8 U/ml / mg protein in the wild isolates T3, T5, S1 and S6 to 18.6, 20.3, 17.7 and 22.5 U/ml / mg protein in the mutants MT3, MT5, MS1 and MS6, respectively. while CMCase activity was decreased from 14.4 U/ml / mg protein in the wild isolate D5 to 9.7 U/ml / mg protein in the mutant MD5(Fig.4).

Chitinase activity was also increased in *T. harzianum* mutants to 88.4,132.3,86.1 and 136.7 U/ml / mg protein in MT3, MT5, MS1 and MS6, respectively, compared to 82.7, 93.2, 74.5 and 103.2 U/ml / mg protein in the wild isolates T3, T5, S1 and S6, respectively, while the mutant MD5 showed decrease in Chitinase activity (88.2 U/ml / mg protein) compared to the wild isolate D5 (91 U/ml / mg protein) (Fig 5).

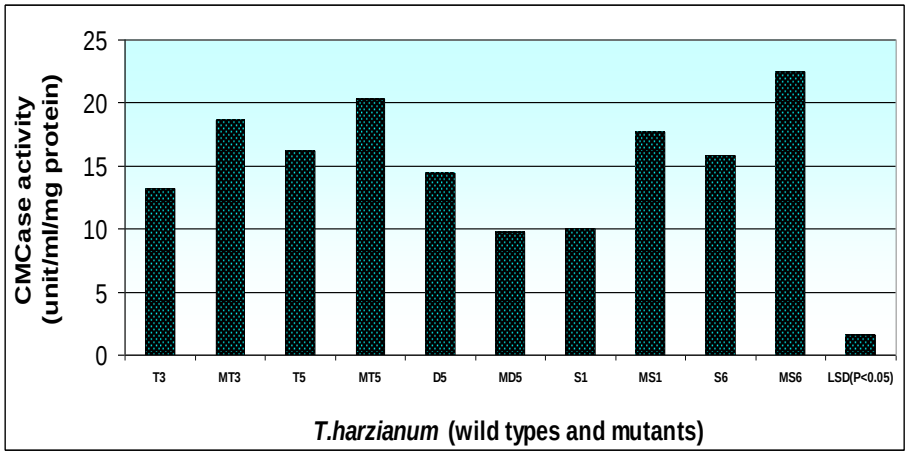

Fig. 4. CMCase activity of *T. harzianum* (wild types and mutants)
(MT3, MT5, MD5, MS1 and MS6 are mutants arise from the wild type T3, T5, D5,S1 and S6, respectively).

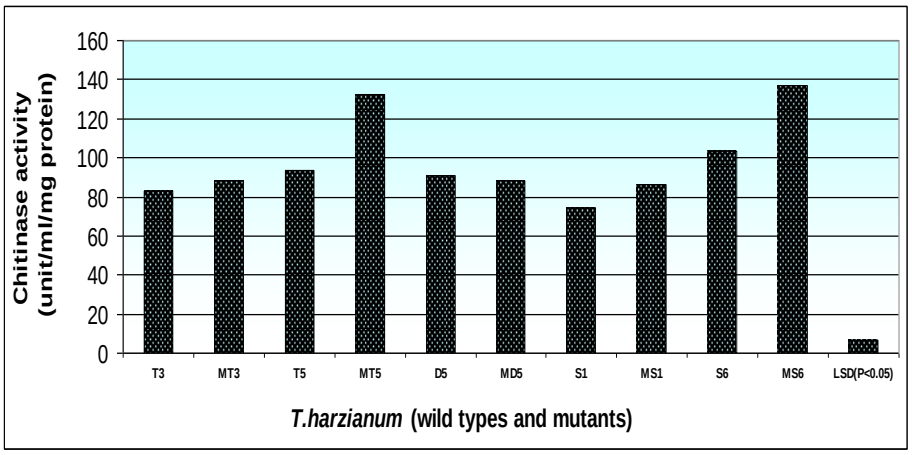

Fig. 5. Chitinase activity of *T. harzianum* (wild types and mutants)
(MT3, MT5, MD5, MS1 and MS6 are mutants arise from the wild type T3, T5, D5,S1 and S6, respectively) .

Tolerance to fungicides

Fungicides including Elsa and Cotaf at all tested concentrations completely inhibited the growth of all selected *T. harzianum* isolates (T3,T5,D5,S1 and S6) in addition to the pathogenic fungi *F. oxysporum* and *R. solani* (table4). These selected *T.harzianum* isolates showed growth on medium containing Diathen and Mizab at 25% of the recommended field rate but no growth was observed in other concentrations, in this minimum concentration (25%), the maximum colony diameter was 1.8 cm by T3 isolate in present of Diathen, while the maximum colony diameter was 2.4 cm by T3,S1 and S6 isolates in present of Mizab

Selected *T. harzianum* isolates in addition to pathogenic fungi *F. oxysporum* and *R. solani* showed a high resistance toward Kopralin at 25 and 50% of the recommended field rate, on the other hand, a negative correlation was recorded between concentration of Topsin and Benomyl and the growth of selected *T. harzianum* isolates,

Although there was no growth of *F. oxysporum* and *R. solani* in media contained all concentrations of Elsa , Cotaf and Diathen, the minimum concentration (25%) of the recommended field rate of Topsin , Mizab and Benomyl showed significant suppression in growth of these pathogenic fungi compared to selected *T.harzianum* isolates. The colonies diameters of *F. oxysporum* and *R. solani* were 2.3 and 1.8 in 25% of Topsin, respectively, while these diameters were 1.5 and 1.2 cm in 25% of both fungicides Mizab and Benomyl, compared to 3-3.7 and 2.2-2.4 and 2-2.5 cm of the selected *T. harzianum* isolates grown on media contained 25% of Topsin, Mizab and Benomyl, respectively.

Only T3 and S6 isolates were grown on the media contained Topsin even with the maximum concentration (100%) of the recommended field rate, in this concentration, colony diameter of both T3 and S6 isolates was 1.5 cm (table 4).

After irradiation the mutants MT5 and MS6 were the best mutants that grown in the present of Elsa at 25% of the recommended field rate, the colonies diameters were 2.4 and 2 cm, respectively. These two mutants also grown on the media contained 50% of the recommended field rate of Diathen and Mizab, the colonies diameters of both mutants were 2 cm compared to complete inhibition in the wild types T3 and S6 (table 4).

The results also showed that MT5 and MS6 can grow on all concentrations of Topsin and Benomyl, the colonies diameters in present of Topsin at 100,50 and 25% of the recommended field rate were 1.8,3.7 and 5.2 cm by MT5 and 2.5, 3.5 and 4.8 cm by MS6, respectively. While in the present of Benomyl at 100,50 and 25% of the recommended field rate, the colonies diameters were 2.3, 3.2 and 4.8 cm by MT5 and 1.6, 2.8 and 4.2 cm by MS6, respectively. As comparison between wild types and mutants, the maximum growth was 5.2 and 4.8 cm (as colonies diameters) by MT5 in present of 25% of the recommended field rate of Topsin and Benomyl, compared to 3.3 and 2.2 cm in the wild type T5, respectively, followed by MS6, the maximum growth in this mutant was 4.8 and 4.2 cm in the same concentration of these two fungicides, compared to 3.4 and 2.5 cm in the wild type S6, respectively.

Table 4. Colonies diameters (cm) of selected *T. harzianum* isolates (wild types), mutants and pathogenic fungi *F. oxysporum* and *R. solani* grown on various fungicides at 100,50 and 25% of the recommended field rate.

Fungi	Fungicides																	
	control	Elsa			Kopralin			Contaf			Diathen			Topsin			Mizab			Benomyl		
		100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25
T. harzianum wild isolates:																						
T3	9	0.0	0.0	0.0	8.2	9	9	0.0	0.0	0.0	0.0	1.8	1.5	3.1	3.7	0.0	0.0	2.4	0.0	1.0	2.5	
T5	9	0.0	0.0	0.0	7.4	9	9	0.0	0.0	0.0	0.0	1.4	0.0	2.5	3.3	0.0	0.0	2.2	0.0	0.0	2.2	
D5	9	0.0	0.0	0.0	7.6	9	9	0.0	0.0	0.0	0.0	1.6	0.0	2.5	3.0	0.0	0.0	2.2	0.0	0.0	2.2	
S1	9	0.0	0.0	0.0	8.5	9	9	0.0	0.0	0.0	0.0	1.6	0.0	2.5	3.0	0.0	0.0	2.4	0.0	1.0	2.0	
S6	9	0.0	0.0	0.0	8.5	9	9	0.0	0.0	0.0	0.0	1.6	1.5	2.7	3.4	0.0	0.0	2.4	0.0	1.0	2.5	
Mutants:																						
MT3	9	0.0	0.0	0.0	8.4	9	9	0.0	0.0	0.0	0.0	1.8	1.5	3.1	3.7	0.0	0.0	2.4	0.0	1.0	2.5	
MT5	9	0.0	0.0	2.4	7.7	9	9	0.0	0.0	0.0	2.0	3.6	1.8	3.7	5.2	0.0	2.0	3.2	2.3	3.2	4.8	
MD5	9	0.0	0.0	0.0	8.5	9	9	0.0	0.0	0.0	0.0	1.8	0.0	2.5	3.3	0.0	0.0	2.4	0.0	0.0	2.2	
MS1	9	0.0	0.0	0.0	8.5	9	9	0.0	0.0	0.0	0.0	1.6	0.0	2.7	3.2	0.0	0.0	2.4	0.0	1.0	2.2	
MS6	9	0.0	0.0	2.0	8.5	9	9	0.0	0.0	0.0	2.0	3.2	2.5	3.5	4.8	0.0	2.0	3.3	1.6	2.8	4.2	
Pathogens:																						
F. oxysporum	7.8	0.0	0.0	0.0	6.0	6.8	7.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	1.5	0.0	0.0	1.5	
R. solani	7.2	0.0	0.0	0.0	5.4	6.1	6.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	1.2	0.0	0.0	1.2	
LSD(P<0.05)	0.27	0.0	0.0	0.2	0.6	0.3	0.5	0.0	0.0	0.0	0.1	0.7	0.3	0.5	1.2	0.0	0.1	0.5	0.3	0.4	0.9	

(MT3, MT5, MD5, MS1 and MS6 are mutants arise from the wild type T3, T5, D5,S1 and S6, respectively)

Discussion

The increased exposure time to ultraviolet irradiation caused a decreased number of surviving conidia of *T. harzianum* isolates, may be due to a damage effect on the level of cell at all. The optimum exposure time to ultraviolet irradiation at the conditions of the present study is 30 min, in which, the percentage of kill was 98-99% in five selected *T. harzianum* isolates (T3,T5,D5,S1 and S6) after 12 h of the incubation in normal conditions, the percentage of kill decreased to 92-94 and 84-86% after 24 and 48h of the incubation in normal conditions, respectively, thus the mutants were selected from this treatment (exposure time to ultraviolet radiation =30 min) owing to maximum percentage of kill was recorded. The decrease of percentage of kill after incubation in normal conditions may be suggests that the *T. harzianum* isolates have different repair mechanisms, producing surviving colonies resistant to ultraviolet action. Similar results were obtained by (18) such results showed that the decrease of the survival of the primary conidia was proportional to the increase of the radiation doses. Stevenson and Weimer (19) observed a decrease up to 1000 times in the viability of conidia in suspension irradiated by 10 minutes, and five centimeters away from the ultraviolet radiation source.

The superior of antagonistic capability of *T. harzianum* mutants MT3,MT5,MS1 and MS6 against the pathogenic fungi, *R. solani* and *F. oxysporum* , in addition to significantly increases in colony diameters of *T. harzianum* mutants MT5 , MS1 and MS6 and significantly increases in CMCase and chitinase activities of all *T. harzianum* mutants (except in MD5), indicated that the genetic changes (mostly mutations) were carried out in these isolates by uv-irradiation. Besides, the increase of some *T. harzianum* mutants growth compared to the wild type refers to an increase in competition for space and nutrients, an additional effect for the biocotrol action.

Although the screening and / or breeding of local *T. harzianum* isolates has been reported to improve the efficiency of bio-control ((15) , and to obtain the fungicides tolerance isolates (20,21),ultraviolet and sometimes microwave radiation, due to their efficiency, simplicity and safety of use in a laboratory, are recommended to induce mutants ((18,22). In addition , exposure to ultraviolet radiation may be also useful in order to obtain other kinds of mutants.

Hydrolytic enzymes of *Trichoderma* have been described as the critical elements of its mycoparasitic action against fungal plant pathogens (23,24) as they play key roles in cell wall disintegration of target fungal pathogens resulting in mycoparasitism of *Trichoderma* sp. as a consequence. Genetic alteration of biocontrol fungi, local *T. harzianum* isolates by ultraviolet irradiation results in the improvement of their antagonistic potential as biopesticides, thus the most mutants exhibiting higher enzymes activities (including chitinase and CMCase) than their parental strains (wild types) indicative of their altered gene action that induces the enhanced activities of the enzymes, and the efficiency of mycoparasitic activity of the mutants through recognize the cell wall components of the host fungi, hydrolyze, solubilize and finally utilize them as substrates was higher than the wild types.

The action of the uv-irradiation is not the same in all *T. harzianum* isolates, the uv-irradiation has a negative effect with some mutants like MD5 through the decrease in colony diameter and enzymes activities compared to wild type D5, according to this result, mutations had positive and negative effects owing to their random effects on the DNA. The maintains of positive mutation normally achieved by selection.

The results showed that the various fungicides had a different effect on the growth of *T. harzianum* isolates in addition to the pathogeneic fungi *F. oxysporum* and *R. solani* and this may be attributed to difference due to biological variation of these fungi, this results agree with other studies (25)

Several studies (12,20,21) suggested that antagonistic activity of biocontrol agents might be effective if it is integrated with other control practice and may result in acceptable levels of disease control with reduce level of chemicals use. The results of the present study would help in the improvement of antagonistic capability of Iraqi local *T. harzianum* isolates by uv-irradiation , which can be used, with reduced dose of selected fungicides for the control of plant pathogenic fungi.

The results of the present study are agreement with (26),who refers to the combination of fungicide tolerant biological control agents with reduced levels of fungicide integrated control strategies would promote the degree of disease suppression similar to that achieved with full dosage of fungicides.

Compared to the biological control agents like *T. harzianum*, the chemical fungicides were the most effective treatment because biological control agents required more time (several days) for growing and became a competitive agent (Al-Kurtany *et al*., 2009), our results showed that the genetic changes of some *T. harzianum* isolates especially the mutants MT5 and MS6 by uv-irradiation allowed them to be relative tolerance to some fungicides in low concentrations (25-50% of the recommended field rate), in the same time, the low concentrations of fungicides had inhibitory effect against the pathogens *F. oxysporum* and *R. solani*.

Conclusion

As conclusion, the mutants MT5 and MS6 developed from UV-irradiation treatment, showed as promising *T. harzianum* mutants through their antagonistic activity (at mycoparasitism level), fast growth, higher chitinase activity and growth in 25% of the recommended field rate of Elsa and 25 and 50% of the recommended field rate of Diathen and Mizab and

all concentrations of Topsin and Benomyl with complete inhibition of pathogenic fungi *F. oxysporum* and *R. solani*. This lead to possibility to use a combination of fungicide tolerant *T. harzianum* mutants(MT5 and MS6) as very active biological control agents with reduced levels of fungicide in integrated control strategies. Further studies are required for application and evaluation these results on the field level.

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تحسين التضاد وتحمل المبيدات الكيمياوية الفطرية في عزلات الفطر *Trichoderma harzianum* و *T.viride* المحلية بوساطة التعرض للاشعة فوق البنفسجية

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الملخص

بين 22 عزلة محلية للفطرين *Trichoderma harzianum* و *T.viride* ، اظهرت العزلات T3 و T5 و D5 و S1 و S6 اعلى فعالية تضاد ضد الفطرين الممرضين للنبات *Fusarium oxysporum* و *Rhizoctonia solani* . انتخبت هذه العزلات لتحسين فعالية مقاومتها الاحيائية باستخدام الاشعة الفوق بنفسجية. كان وقت التعرض لهذه الاشعة الامثل 30 دقيقة ، في هذا الوقت بلغت نسبة القتل 98–99%. بعد المعاملة بالاشعة (مصدرها مصابيح كوارتز 30 واط عند طول موجي 240–280 نانوميتروبمسافة 20 سم) فقد تحسنت قابلية التضاد ضد *R. solani* و *F. oxysporum* في كل من الطافرات MT3 و MT5 و MS1 وMS6 والتي بلغ دليل التضاد فيها 3.6 و3.9 و 3.2 و 3.6 ضد الفطر *F. oxysporum* و 3.7 و4 و 3.4 و 4 ضد الفطر *R. solani* مقارنة بـ 3.2 و 2.8 و 2.6 و 2.8 ضد الفطر *F. oxysporum* و 2.7 و 2.9 و 3.1 و 3 ضد الفطر *R. solani* في الطرز البرية الناشئة منها T3 و T5 و S1 و S6 ، على التعاقب ، كما ارتفع معنويا معدل نمو جميع هذه الطافرات (مقدر بقطر المستعمرة) عدا MT3 وMD5. ارتفعت فعالية انزيم الكاربوكسي مثيل سليليز من 13.2 و16.2 و 10 و 15.8 وحدة/مل/ملغ بروتين في الطرز البرية T3 و T5 و S1 و S6 الى 18.6 و 20.3 و17.7 و 22.5 وحدة/مل/ملغ بروتين في الطافرات MT3 و MT5 و MS1 وMS6، على التعاقب. وارتفعت ايضا فعالية انزيم الكايتينيز في هذه الطافرات الى 88.4 و 132.3 و 86.1 و136.7 وحدة/مل/ملغ بروتين مقارنة بـ 82.7 و 93.2 و74.5 و 103.2 وحدة / مل/ملغ بروتين في طرزها البرية،على التعاقب. وعند مقارنة الطافرات مع طرزها البرية فان اعلى نمو بلغ 5.2 و 4.8 سم (قطر المستعمرة) من قبل الطافرة MT5 بوجود 25% من التركيز الحقلي الموصى به للمبيدين توبسين وبينوميل، مقارنة بـ 3.3 و 2.2 سم في طرازها البري T5 ،على التعاقب. تعقبها الطافرة MS6 والتي بلغ اقصى نمو لها 4.8 و 4.2 سم للتركيز نفسه لهذين المبيدين ، مقارنة بـ 3.4 و 2.5 سم في الطراز البري S6،على التعاقب.وحسب هذه النتائج فان الطافرتان MT5 و MS6 الناتجة من المعاملة بالاشعة فوق البنفسجية اظهرت كطافرات واعدة للفطر *T. harzianum* من خلال فاعليتها التضادية (الى مستوى التضاد الفطري) وسرعة نموها و اعلى فعالية لانزيمي الكاربوكسي مثيل سليليز والكاينينيز ونموهما بتركيز 25% من التركيز الحقلي للمبيد الساوتركيزي 25 و50% من التركيز الحقلي للمبيدين دياثين والميزاب وفي جميع تراكيز المبيدين توبسين وبينوميل مع تثبيط تام لنمو الفطريين الممرضين *F. oxysporum* و *R. solani* بهذه المبيدات.

الكلمات المفتاحية: المقاومة الاحيائية ، الفطريات الممرضة للنبات ، اختزال المبيدات الفطرية ، *T. harzianum* ، الاشعة فوق البنفسجية