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EFFICACY OF CERTAIN FUNGICIDES AND ANTAGONISTIC MICROORGANISMS ON MYCELIAL GROWTH OF *Fusarium oxysporum* ISOLATED FROM DATE PALM IN NORTH SINAI

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ABSTRACT

In this study the efficacy of three chemical fungicides as well as five different biological control agents were tested *in vitro* against several isolates of *Fusarium oxysporum* from wilted date palm trees grown in North Sinai. The three commercial fungicides were: Tachigaren 30% SL, Rhizolex-T 50% WP and Dithane M-45 80% WP. Whereas the biological control agents included three fungal species namely: *Trichoderma album*, *T. harzianum* and *T. strigosum*. As well as the two bacteria: *Bacillus megaterium* and *B. subtilis*. Tachigaren was the most effective fungicide in reducing the fungal growth of all tested isolates of *F. oxysporum*. Rhizolex T-50 ranked second while Dithane M-45 was the least effective fungicide in reducing the mycelial growth of the tested isolates of *F. oxysporum in vitro*. *Trichoderma harzianum* was the most effective biocontrol agent in inhibiting the mycelial growth of all tested *F. oxysporum* isolates followed by *T. album* and then *T. strigosum*. On the other hand, *Bacillus megaterium* and *Bacillus subtilis* were generally non effective in reducing mycelial growth of the tested isolates of *F. oxysporum in vitro*.

Kew word: Certain Fungicides, antagonistic microorganisms, *Fusarium oxysporum* isolated, date palm, North Sinai.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is a major fruit crop in most Arab countries. It has historically been connected with sustaining human life and tradition of the people in the old world as a major agricultural crop. Arab countries possess 70% of the 120 million world's date palms and are responsible for 67% of the global date production **Loutfy (2010)**. Fusarium wilt diseases caused by *Fusarium oxysporum* are among the most severe plant diseases in the world. Fusarium wilts affect many plant species belonging to all the botanical families. *F. oxysporum* is one of the most common species among soil fungi in cultivated soil allover the world. It includes a large diversity of strains: saprophytic, parasitic and pathogenic.

Fusarium oxysporum (Fo) is a ubiquitous inhabitant of soils worldwide and causes diseases such as wilt, yellows and damping-off in different plant species **Ratul and Narendra (2010)**. The fungus can invade plant's roots with its conidial germ tube or mycelium. The roots can be infected directly through the root tips, wounds in the roots or at the formation point of lateral roots. Mycelium enters the xylem vessels branches and produces micro-conidia which are carried upward in the sap stream. Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves stomata to close, the leaves wilt and the plant eventually dies. Infection usually leads to chlorosis, leaf wilting and browning of the vascular system. Several procedures have been attempted for managing

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the fusarium wilt disease in the greenhouse and in field, **Ioannou (1999)**. Bayoud of date palm caused by the fungus *Fusarium oxysporum* f.sp. *albedinis* (Foa) is the most important disease of this crop. It is currently confined to Morocco and the western and central regions of Algeria, having destroyed approximately 15 million trees since it was first discovered, sometime before 1870, **Djerbi (1983)**.

In this research we studied the effects of some chemical fungicides as well as bio-fungicides, on the growth of several isolates of *Fusarium oxysporum* isolated from date palm grown in North Sinai.

MATERIALS AND METHODS

Isolation of Fungal Pathogens

Fusarium oxysporum cultures were isolated from infected roots of date palm trees showing wilt, drying, yellow leaves and progressive die back from different districts located in four different geographic regions in North Sinai. Root samples were washed carefully with tap water to remove the adhering soil. Diseased root samples were cut into pieces (1cm long). Surface sterilized with dipping in 0.5% sodium hypochlorite solution for 2 minutes, then washed thoroughly with several changes of sterile distilled water. The cuted pieces were dried between sterile filter papers. The surface sterilized pieces were transferred to Petri dished containing acidified potato dextrose agar medium (PDA) then incubated at 25 ± 2°C for two weeks then inspected for fungal growth, **Rashed (1991), El-Morsi (1999), Abdalla *et al.* (2001), El-Morsi (2004) and Baraka *et al.* (2011)**.

Fusarium oxysporum Control *In vitro*

Effect of chemical fungicides on the linear growth of *F. oxysporum*

Three fungicides: Hymexazol (Tachigaren 30% SL), Tolclofos- methyl (Rhizolex-T 50% WP) and Mancozeb (Dithane M-45 80% WP) were tested individually at different concentration (10, 50, 100, 500 and 1000 ppm). The effects of the fungicides were assayed by using the poisoned food technique according to the method described by **Abdalla *et al.* (1992)**. Fungicides were added to autoclaved PDA medium before dispensing medium in Petri plates, when temperature of the medium was about 50°C. Control treatment was PDA medium without fungicides additions.

Disks (5mm) in diameter from 7 days old cultures of the tested fungus were transferred to the center of Petri plates then of the plates were incubated at 27±2°C for 16 days. After 4, 8, 12 and 16 days of incubation the linear growth of the tested was assessed by measuring two perpendicular diameters in cm and the average was recorded.

Biological control

The *Trichoderma album* isolate was isolated from the commercial biological control product called "Bio zeid", *Trichoderma strigosum* and *Trichoderma harzianum* were isolated by one gram of dry soil from each sample was added to test tubes containing 9 ml sterile water and each test tube was shaken periodically for approximately 15 minutes. After shaking one ml of suspension was added to test tube containing 9 ml sterile water, and the process was repeated to make serial dilutions up to 1:10000 for each fungus isolation. Aliquots 1 ml of serial dilutions was spread on APDA plates. All plates were incubated at 25°C. After 2-7 days the isolated microorganisms, were identified according to their morphological characters, **Samuels and Hebbar (2015)**. Selected microorganisms were purified and transferred on to APDA slants; pure cultures were stored at 4°C for further studies. The antagonistic fungi were identified at Unit of Identification of Microorganisms, Plant Pathology Research Institute, Agricultural Research Center, using BIOLOG ™ system.

The *Bacillus megaterium* culture was isolated from the commercial biological control product called "Bio Arc" and the *Bacillus subtilis* culture was isolated from the commercial biological control product called "Clean Root".

The biological control agents were tested against *F. oxysporum* isolates *in vitro* using acidified potato dextrose agar (APDA) medium in 9cm diameter Petri plates. Five mm diameter disc of a four days old fungal growth of the tested *F. oxysporum* isolates were placed at about two cm from the edge of the plates.

On the opposite side of the Petri plates a five mm disc of the tested antagonistic fungi was placed. Plates were incubated at 25^{o}C and kept under daily observation and data were recorded one week later as linear growth of the *F. oxysporum* cultures as described by **Abd El-Moity *et al.* (1993)** and **Perveen and Bokhari (2012)**. The bacterium was maintained on PDA medium at 33±2^{o}C. The *Bacillus* sp. isolate was tested against *F. oxysporum* using PDA medium in 9 cm diameter Petri plates. 5 mm diameter discs of the tested fungus was placed two cm from the edge of the plate. On the opposite side of the Petri plates at about 2 cm from the edge a streak of the tested antagonistic bacterium was placed as suggested by **Agarwal *et al.* (2011)**. These experiments were repeated twice and data were recorded.

RESULTS AND DISCUSION

Fusarium oxysporum Control *In vitro*

Effect of chemical fungicides on linear growth of *F. oxysporum*

In vitro studies Table 1 indicate that Tachigaren 30 % SL was very effective in controlling radial growth of *F. oxysporum* at 1000 ppm on tested isolates (D_2, E_1 and F_7) 16 days after incubation, but it showed little effect on isolate B_2 16 days after incubation. Rhizolex-T 50% WP ranked second in controlling radial growth of *F. oxysporum* isolates (E_1 and F_7). It was less effective on linear growth of *F. oxysporum* isolate D_2, and showed no significant effect on isolate B_2 after 16 days of incubation. The fungicide Dithane M-45 80% WP was the least effective fungicide in controlling radial growth of *Fusarium oxysporum* isolates (B_2, D_2, E_1 and F_7) at all tested concentrations 16 days after incubation on APDA medium.

These results are in agreement with those obtained by **Haggag and El-Gamal (2012)** who found that Tachigaren was the most effective fungicide in reducing the mycelial growth of *Fusarium solani*. Similarly, **Song *et al.* (2004)** demonstrated that Prochloraz and Carbendzim were more efficient in reducing mycelial growth of *F. oxysporum* f.sp. *lycopersici* compared with Tachigaren and Dithane M-45.

In vitro antagonism of microorganisms on the pathogen *Fusarium oxysporum*

All tested isolates of *Trichoderma strigosum*, *T. harzianum* and *T. album* suppressed the growth of *F. oxysporum* isolates (B_2, D_2, E_1 and F_7), this inhibition occurred before any direct mycelial interaction took place (Table 2 and Plate 1).

Trichoderma strigosum isolate significantly suppressed the growth of *F. oxysporum* isolates and it subsequently mycoparasitised the pathogen expect in case of isolate B_2, *T. strigosum* did not overgrow it.

Trichoderma harzianum isolate also significant suppressed the growth of *F. oxysporum* isolates and it subsequently mycoparasitised the pathogen expect in case of isolate B_2, *T. harzianum* did not overgrow it.

Trichoderma album isolates was significantly inhibiting the growth of the pathogen *F. oxysporum* isolates and it subsequently mycoparasitised the pathogen, expect isolate B_2.

Table (1): Effect of five concentrations of three fungicides "Tachigaren, Rhizolex and Dithane M-45" on the linear growth (mm) of *F. oxysporum in vitro* isolate (B_2, D_2, E_1 and F_7) 4, 8, 12 and 16 days after incubation.

Treatments			Rate growth rate of *Fusarium oxysporum* isolates after incubation (day)			
			4 days	8 days	12 days	16 days
Fungal isolate "B_2" from Bear Al Abd	Tachigaren 30% SL	Control	30.00 a	50.00 fgh	90.00 a	90.00 a
		10 ppm	21.50 ghi	44.00 jklm	60.50 jkl	76.15 ij
		50 ppm	19.75 ijkl	42.87 lmn	56.50 mn	78.75 hi
		100 ppm	16.62 n	37.25 pq	50.25 op	82.00 fgh
		500 ppm	4.50 vw	13.50 DE	27.25 z	22.60 yz
		1000 ppm	3.62 w	11.75 E	16.75 B	18.87 A
	Rhizolex-T 50 % WP	Control	30.00 a	50.00 fgh	90.00 a	90.00 a
		10 ppm	23.00 defg	41.00 mno	71.00 hi	90.00 a
		50 ppm	17.00 mn	34.00 rs	61.50 jk	90.00 a
		100 ppm	12.25 q	25.50 wx	46.00 qrs	72.82 kl
		500 ppm	9.00 tu	20.50 zA	41.50 tu	62.75 p
		1000 ppm	5.75 v	14.50 CDE	32.75 xy	55.62 qr
	Dithane M-45 80 % WP	Control	30.00 a	50.00 fgh	90.00 a	90.00 a
		10 ppm	22.25 efgh	46.28 ij	69.25 hi	88.75 abc
		50 ppm	19.25 kl	40.56 nno	59.75 jklm	80.50 gh
		100 ppm	16.18 no	36.81 pqr	53.43 no	86.50 bcd
		500 ppm	14.31 p	31.93 stu	50.43 op	84.25 def
		1000 ppm	11.96 qrs	23.93 xy	46.50 qr	89.17 ab
Fungal isolate "D_2" from Rafah	Tachigaren 30% SL	Control	30.00 a	50.00 fgh	90.00 a	90.00 a
		10 ppm	14.50 op	30.50 tu	37.25 vw	53.10 rs
		50 ppm	12.06 qr	26.25 wx	38.25 uv	49.62 t
		100 ppm	10.21 st	24.50 wxy	34.25 wxy	47.82 tu
		500 ppm	0.00 x	0.00 F	0.00 D	2.50 B
		1000 ppm	0.00 x	0.00 F	0.00 D	0.00 B
	Rhizolex-T 50 % WP	Control	30.00 a	55.00 cd	82.00 c	90.00 a
		10 ppm	24.55 cd	54.25 de	80.25 cd	90.00 a
		50 ppm	10.28 rst	31.25 stu	49.00 pq	75.45 jk
		100 ppm	9.08 tu	25.57 wx	38.00 v	56.45 q
		500 ppm	0.00 x	16.00 BCD	18.75 B	42.82 v
		1000 ppm	0.00 x	0.00 F	13.00 C	21.77 zA
	Dithane M-45 80 % WP	Control	30.00 a	55.00 cd	82.00 c	90.00 a
		10 ppm	25.01 c	50.00 fgh	77.00 de	89.50 ab
		50 ppm	21.20 hij	42.50 lmn	69.50 hi	89.75 ab
		100 ppm	19.77 ijkl	38.25 op	59.50 jklm	85.25 def
		500 ppm	19.55 jkl	42.00 lmn	53.25 no	71.37 lm
		1000 ppm	14.65 op	32.75 st	44.50 rst	65.00 op

Table (1): Con. Effect of five concentrations of three fungicides "Tachigaren, Rhizolex and Dithane M-45" on the linear growth (mm) of *F. oxysporum in vitro* isolate (B_2, D_2, E_1 and F_7) 4, 8, 12 and 16 days after incubation.

Treatments			Growth rate of *Fusarium oxysporum* isolates after incubation (day)			
			4 days	8 days	12 days	16 days
Fungal isolate "E_1" from Al Shaikh Zewayed	Tachigaren 30% SL	Control	30.00 a	65.00 a	90.00 a	90.00 a
		10 ppm	22.50 efgh	48.50 ghi	68.00 i	85.75 cde
		50 ppm	18.50 lm	41.25 lmno	58.50 klm	82.95 efg
		100 ppm	19.00 l	34.25 qrs	53.00 o	75.45 jk
		500 ppm	3.42 w	14.25 DE	18.75 B	25.50 y
		1000 ppm	0.00 x	0.00 F	0.00 D	0.00 B
	Rhizolex-T 50% WP	Control	30.00 a	65.00 a	90.00 a	90.00 a
		10 ppm	20.90 hijk	47.50 hi	57.75 lm	72.50 kl
		50 ppm	11.42 qrs	29.50 uv	43.25 rst	69.00 mn
		100 ppm	8.87 tu	19.00 zAB	33.00 xy	44.67 uv
		500 ppm	0.00 x	14.50 CDE	22.37 A	35.40 w
		1000 ppm	0.00 x	0.00 F	0.00 D	20.90 zA
Fungal isolate "E_1"	Dithane M-45 80% WP	Control	30.00 a	65.00 a	90.00 a	90.00 a
		10 ppm	24.87 c	52.05 def	75.50 ef	90.00 a
		50 ppm	19.07 l	44.12 jkl	62.75 j	90.00 a
		100 ppm	21.42 ghi	43.00 klmn	61.50 jk	90.00 a
		500 ppm	22.20 fgh	47.25 hi	75.75 ef	90.00 a
		1000 ppm	23.05 defg	48.25 ghi	77.50 de	90.00 a
Fungal isolate "F_7" from Al Arish	Tachigaren 30% SL	Control	30.00 a	63.00 a	90.00 a	90.00 a
		10 ppm	10.50 qrst	27.02 vw	43.00 st	71.00 lm
		50 ppm	9.57 tu	21.50 yz	35.50 vwx	56.25 qr
		100 ppm	7.85 u	17.50 ABC	31.25 y	31.00 x
		500 ppm	0.00 x	0.00 F	0.00 D	0.00 B
		1000 ppm	0.00 x	0.00 F	0.00 D	0.00 B
	Rhizolex-T 50% WP	Control	29.00 a	63.00 a	90.00 a	90.00 a
		10 ppm	27.02 b	59.00 b	77.25 de	90.00 a
		50 ppm	15.22 nop	42.50 lmn	71.75 gh	90.00 a
		100 ppm	11.45 qrs	26.00 wx	44.00 rst	66.62 no
		500 ppm	0.00 x	12.85 E	27.50 z	50.00 st
		1000 ppm	0.00 x	0.00 F	0.00 D	21.10 zA
	Dithane M-45 80% WP	Control	29.00 a	63.00 a	90.00 a	90.00 a
		10 ppm	27.07 b	51.25 efg	88.50 ab	90.00 a
		50 ppm	23.00 defg	50.25 fgh	72.50 fgh	90.00 a
		100 ppm	23.50 cdef	46.00 ijk	74.75 efg	90.00 a
		500 ppm	24.00 cde	58.00 bc	86.25 b	90.00 a
		1000 ppm	27.00 b	65.25 a	90.00 a	90.00 a
	LSD 0.05		1.777	3.014	3.342	3.257

Table (2): The antagonistic effect of the bio-control agent: *T. strigosum*, *T. harzianum*, *T. album*, *Bacillus megaterium* and *B. subtilis* against *F. oxysporum* isolates growth (mm) *in vitro* 8 days after incubation.

Origin of fungal isolates	Treatment	Main linear growth of *F. oxysporum* (mm)		
		Microorganism against *F. oxysporum*	Control	* PGI (%)
Fungal isolate "B_2" from Bear Al Abd	***T. strigosum***	25.50 e	90	71.66
	T. harzianum	26.75 e		70.27
	T. album	19.72 fgh		78.17
	B. megaterium	50.00 a		44.44
	B. subtilis	45.00 b		50
Fungal isolate "D_2" from Rafah	***T. strigosum***	25.05 e	90	72.16
	T. harzianum	18.35 gh		79.61
	T. album	24.82 e		72.42
	B. megaterium	45.00 b		50
	B. subtilis	37.00 d		5.88
Fungal isolate "E_1" from Al Shaikh Zewayed	***T. strigosum***	26.00 e	90	71.11
	T. harzianum	18.72 gh		79.2 0
	T. album	20.87 fg		76.81
	B. megaterium	48.00 a		46.66
	B. subtilis	42.00 c		53.33
Fungal isolate "F_7" from Al Arish	***T. strigosum***	22.07 f	90	75.47
	T. harzianum	16.91 h		81.21
	T. album	17.97 gh		80.03
	B. megaterium	48.00 a		46.66
	B. subtilis	45.00 b		50
LSD 0.05				

***** PGI (Percent Growth Inhibition) = Colony growth in control plate – Colony growth in intersecting plate \ Colony growth in control plate × 100

- Each treatment represented the mean of six replicates for two experiment.

Similar results were obtained by **Perveen and Bokhari (2012)**. They studied the antagonistic potential of *T. harzianum* and *T. viride* against *F. oxysporum* isolated from date palm. They concluded that all tested isolates of *Trichoderma* showed appreciable inhibition for the mycelial growth of *F. oxysporum*.

Bacillus megaterium was non-significant in controlling radial growth of all tested *Fusarium oxysporum* isolates 8 days after incubation, no inhibition zones were observed with any of these isolates after incubation on PDA medium.

Bacillus subtilis was very effective in controlling radial growth of *F. oxysporum* isolates (B_2, D_2 and E_1) except isolate F_7 it was non-significant in controlling radial growth of the pathogen after incubation on PDA medium.

Several researchers have investigated bio-control strategies using various bacterial species to control *F. oxysporum* f.sp. *albedinis*

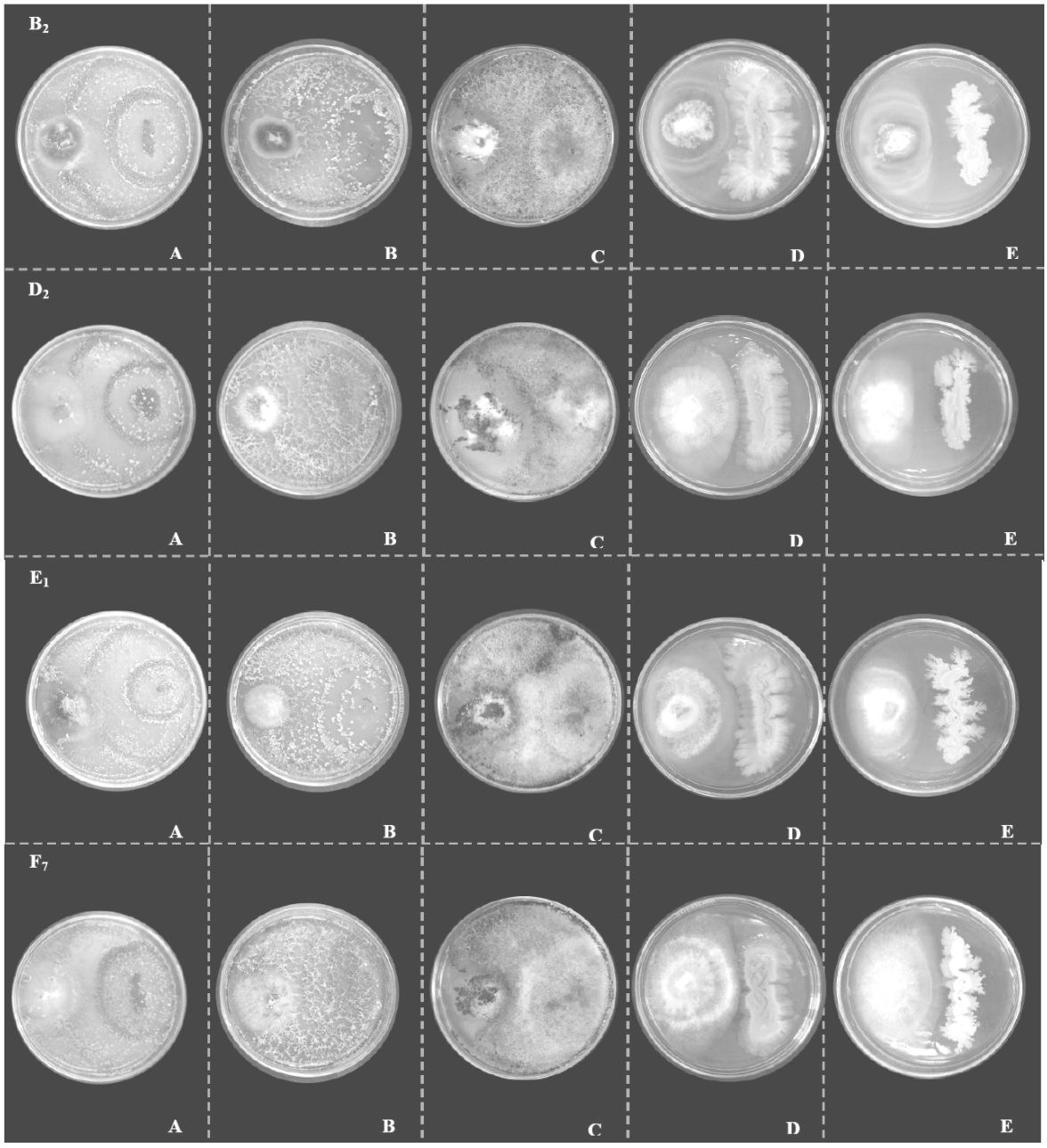

Plate (1): The antagonistic effect of *T. strigosum* (A), *T. harzianum* (B), *T. album* (C), *B.megaterium* (D) and *B. subtilis* (E) against *F. oxysporum* isolates growth *in vitro* after 8 days incubation

which cause the most destructive disease of date palm, called Bayoud disease. For instance, **Dihazi *et al.,* (2012)**, found that *Bacillus amyloliquefaciens* and *Burkholderia cepacia* significantly inhibited growth and sporulation of *F. oxysporum* f.sp. *albedinis*. Also, selected three bacterial species namely: *B. pumilus*, *Rahnella aquatilis*, *Bacillus oereus* for their high inhibition toward mycelial growth of *F. oxysporum* f.sp. *albedinis*.

These success in finding biological control agents against *F. oxysporum* are providing hope for establishing biocontrol strategy against *Fusarium* wilt of date palm.

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الملخص العربى

تأثير بعض المسببات الفطرية والكائنات البيولوجية المضادة علي نمو فطر الفيوزاريوم أوكسيسبورم المعزول من نخيل البلح في شمال سيناء

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تم في هذا البحث دراسة تأثير بعض المبيدات الكيميائية المتداولة وكذلك بعض الفطريات والبكتريا المستخدمين في مجال المكافحة الحيوية علي عدة عزلات من الفطر *Fusarium oxysporum* والتي تم عزلها من جذور أشجار نخيل مصابة معملياً في محافظة شمال سيناء، في هذا البحث تم استخدم ثلاث مبيدات فطرية تجارية هي التاتشجرين والرايزوليكس والدايثين م-٤٥ وذلك بخمس تركيزات هي (١٠-٥٠-١٠٠-٥٠٠-١٠٠٠ جزء في المليون) بالإضافة إلي الكائنات الحية الدقيقة المستخدمة في المقاومة الحيوية مثل فطر الترايكوديرما هارزيانم والترايكوديرما البوم والترايكوديرما ستراي‌جوزم وبكتريا الباسيليس ميجاتيريوم والباسيليس سيبتيليس، كان المبيد الفطري تاتشجرين هو أكثر تأثيراً في تثبيط النمو الميسليومي للمسبب المرضي الفيوزاريوم أوكسيسبورم في المعمل يليه الرايزوليكس ثم الدايثين م-٤٥ الذي أظهر أقل تأثير في تثبيط نمو الفطر. فطريات الترايكوديرما كانت الأعلي تأثيراً في تثبيط نمو الفيوزاريوم أوكسيسبورم حيث كان الفطر تراكوديرما هارزيانم هو أكثر الأجناس المضادة لنمو المسبب المرضي في المعمل يليه ترايكوديرما البوم ثم ترايكوديرما ستراي‌جوزم بينما بكتريا الباسيليس ميجاتيريوم لم تظهر أي تأثير يذكر في تثبيط نمو الفطر، أما البكتريا باسيليس سابتيليس كان لها تأثير واضح في تثبيط نمو الفطر عدا العزلة F_7 لم يكون لها أي تأثير مضاد مع المسبب المرضي.

الكلمات الاسترشادية: المسببات الفطرية، الكائنات البيولوجية المضادة، فطر الفيوزاريوم أوكسيسبورم، نخيل البلح، شمال سيناء.