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Antagonistic Effect of Rhizobium, Bacillus, Pseudomonas, Trichoderma on Fusarium and Rhizocotonia Compared with Moncut *In Vitro*.

El-Sebaay, H. H.[1] and A. B. El-Sayed[2]
[1]Botany Department (Microbiology) Faculty of Agriculture Al-Azhar University Cairo.
[2]Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

ABSTRACT

This investigation was carried out in 2018 to study activity *Rhizobium leguminosarum* biovar*phaseoli*, *Bacillus subtilis*, *Pseudomonas fluorescens,Trichoderma album* and *T. hamatum* against of some soil pathogenic plant fungi; *Fusarium oxysporum* and *Rhizoctonia solani* causative agents root rot disease of common bean was evaluated on culture medium. The bio-agent Rhizobium was checked as culture filtrate, different concentrations; 25, 50,100,150,200 and 250%, also by their cells as opposite to test fungi to observe effect on radial growth and inhibition zone percent by dual method compared with control. In addition bio agent effect was compared with effect of Moncut fungicide; different concentrations; 1, 10, 25, 50 and 100 ppm by agar well diffusion method.The obtained results revealed that bio - agents gave significance effect and maximum inhibition growth of *Fusarium oxysporium* and *Rhizoctonia solani* compared with control as well as Moncut fungicide. Overall rhizobia nitrogen fixing, growth regulators production, also gave inhibition growth of test fungi reached 89.50 , 90.29% and in cultural filtrate conc.100% manner , but cells treatment were,59.96% and 38.86% in the previous order.

Keywords: Antagonism, Rhizobia, Bacillus, Pseudomonas,Trichoderma on Fusarium , Rhizoctonia , radial growth , inhibition percent, Moncut fungicide.

INTRODUCTION

Due to great harms caused by chemiecal pesticied control of some soil pathogenic plant , there is effect on enviorenment and public health. The researches towerds to bio-agent control by non-pathogenic and save microorganisms , adabted and alternative of chemical pesticide. Elbatanony *et al*. 2007; Mazen *et al.* 2008 ,Shanker and Shyam 2014).

A number of fungi and bacteria are known to be very effective to bio-agent against soil-borne plant pathogenic fungi (Shoda 2000).Bacillus-based biological control agents have great potential in integrated disease management (IDM) options,together with cultural control, resistant cultivars, fungicides or others biological control agents (Jacobsen *et al*., 2004). Genus Bacillus comprises a heterogeneous groups can be survive in many diverse environments, often with extreme variations in temperature, nutrient, and other stresses (Driks, 2004). These properties are associated with the ability to produce peptide antibiotics and contribute to the utilization of *Bacillus* spp. to manage several root and foliar diseases (Kloepper *et al*., 1999; Driks, 2004). In addition found that Actinomycetes, *Bacillus licheneforms* and fungi their effect by antimicrobial production (Abd-Elkhaleik *et al*., 2018). Enzymes, for example, chitinase that can lyses cell walls plant pathogenic fungi.(El-Mehalawy 2004), or plant growth enhancement through IAA production (Deshwal, *et al.*, 2003; Shoukry *et al.*,2018).

The inhibitory effect of cultural filtrate of some wild rhizobial isolates (M.L, L.C , 4 T.S and *R. leguminosarum* ICARDA 441 strain) against some fungi causing root rot disease of faba bean (*R. solani, Fusarium* spp. and *F. solani*) *in vitro* and their antimicrobial synergetic effect when combined with Arbuscular mycorrhiza (AM) fungi, were investigated.(El-Batanony *et al*. 2007).The bio-control agents have different mechanisms or combinations of mechanisms which may be involved in the suppression of different plant disease; for example, inhibition of pathogen by antimicrobial substances (antibiosis) (El-Mehalawy 2004); or production of diverse microbial metabolites like siderophore, rhizobiotoxin (Deshwal, *et al.* 2003); competition for nutrients supplied by seeds and roots and colonization sites; induction of plant resistant mechanisms; inactivation of pathogen germination factors present in seed and root exudates and degradation of pathogenicity factors of the pathogen such as toxins;parasitism that may involve production of extracellular cell wall-degrading.

The present study was evaluated the effect of *Rhizobium leguminsarum bv. phaseoli* ; *Bacillus subtilius; Pseudomonas fluorescens; T. album; T. hamatum* against *Rhizoctonia solani* and Fusarium oxysporium. In vitro the bioagent was checked as clture filtrate and measure. Agrowth and inhibition percent of *Fusarium* oxysporium and *Rhizoctonia solani*. In addition bio- effect of *Bacillus subtillus, Pseudomonas fluoresence, T. hamatum* and *T. album* on some soil fungi compared with moncut fungicide on medium.

MATERIALS AND METHODS

Materials: Antagonistic bio-agents bacteria *Rhizobium leguminsarum bv. phaseoli* (pea group) and *Bacillus subtilis* were taken from performer work and reconfermed to purified and identified characteristics. But *Pseudomonas flurescnce* , *Trichoderma album* and *T. hamatum* as well as test fungi *(Fusarium oxysporium ,Rhizoctonia solani*) as casative root rot plant pathogenic ; also fungicide moncot as. Each were carried out by plant pathology Institute , Agric. Res. Center. Minstry of Agiculture.

Methods: Antgonistic action was applied by agar well diffusion method on the solid medium as follow: Preparation of tested fungi casative root rot plant pathogenic fungi; *Fusarium oxysporium* and *Rhizoctonia solani* was prepared separately by inoculation on petri dishs of potato dixtrose agar (PDA) medium under asceptic condition and inocubation at $30C^0$ for 7 days.

Preparation of antagonistic microoganims: The antagonistic bacteria of *Rhizobium leguminsarum bv. phaseoli* inoculum produced by growing in (YEM) broth medium containing; Manitol 10g/l, Yeast extract 1g/l,K2HPO4 0.5g/l,MgSO4 0.2g/l,NaCl 0.1g/l, agar 20g/l,Distilled water 1000ml, Congored 10ml,pH 7.2. After sterilization, medium inoculated and inocubated at $30C^0$ for 48h. To *Bacillus subtilis* and *Pseudomonas flurscncnce* inoculum production by growing separatly on nutreint broth medium and inocubation at $30C^0$ for 48h.

The inoculum of antagonistic fungi; *Trhicoderma album* and *T. hamatum* production separatly by inoculation on (PDA) petri dishes medium and inocubation at $30C^0$ for 7 days.

Antagonistic application: All antagonistic tests were done on 9cm petri dishes three replicates to calculate the clear zones have been seen at 7days of incubation compared with control.

Antagonistic action between Rhizobium theirfore bring organism or their culture filtrate with test fungi; *Fusarium oxysporium* and *Rhizoctonia solani* was checked invidiualy. Rhizobial cells was streaked on oppsite side equal distance 5cm from test fungi, but rhizobial culture filtrate was experminted by filtration 5ml of broth rhizobial growing medium by using millibor filter syring(0·45µm) under aceptic condition. The cluture filtrat was injected as 1ml in agar well oppsite 5cm side distance from dsic test fungi. After inocubation resultes were observed and recorded of radial growth and inhibition zone compared with control test fungi alone,both experimental and control Petri plates were arranged in a completely randomized design with three replicates per treatment. Petri plates were incubated at 28 ± 2 °C for 7 days. The percentage fungal radial growth inhibition was calculated by following formula:

For antagonistic by *Bacillus subtilus* and *Pseudomonas flourescence* were examined *Fusarium* oxysporum and *Rhizoctonia solani* by growing in nurient broth medium and inocubation at 30^0C for 48h . The test organism fungi was inoculated as disc on PDA medium and antagonistic organism was straked on opsite side of petri dish as three plats replicates of each fungi indvidiually treatment. After inocubation period for 4days the radial growth of fungi and inhibition zone percent were observed, measured and recorded compared with pathogenic fungi alone as control.

For antagonistic root rot fungi by *Trichoderma album* and *T.hamatum* as bio-agent was carried by inoculation of test and antagonistic fungi as a disc separeatly each on one plate by use PDA medium. After that incubation at 30 ^{0}C for7 days, then under aceptic condition by cork porer get one disc of each put on one side opsite at equal distance 5cm between two fungi. Inoculated plates were inocubated at 30^0C for 4-6 days the results were observed , recorded and culculated compared with control (Titiya *et al.,* 2007).

All bio-agent antagonistic treatment was compared with Moncut fungicide by different concentrations ; 1 ,10 , 25 , 50 and 100 ppm/l. After inoculation pathogenic fungi as disc in PDA medium petri dish , agar well was done by cork porer, one ml of each concenration put in well of each test fungi.

Data analysis: The means were compared using the least significant difference test (LSD) between the antagonistic treatments at 0.05 and 0.01 significances (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Antagonistic of bio-agents on *Fusarium oxysporium* : The results in Table (1) revealed that effect of bio-agents on mycelial expansion(linear growth (cm) , efficacy (%) and inhibition growth (cm) by dual medium method as inoculum or disc opposite disc of test fungi. Antagonistic bacteria of *R. leguminsarum biovar. Phaseoli* was; 3.7cm , 59.96% and 1.86cm . It was pointed out by Chao that the *Rhizobium leguminosarum biovar phaseoli* had an effect on the inhibition of the *Fusarium* and *Rhizoctonia* species.Also *Bacillus subtilis* found to be 4.10cm, 53.01% and 2.00cm.While the effect of *P. fluorescence* was; 4.16cm, 53.01.% In addition the effect of *T. album* and *T. hamatum* were; 2.33cm, 2.30cm, 72.97% and 72.93% compared with control respectively.The researches towerds bio-agent control by non-pathogenic and save microorganisms , also adabted and alternative of chemical pesticide.Etheshaul- Haque & Ghaffar 1993; Deshwal *et al.* 2003; Sharif *et al.*2003; Elbatanony *et al.* 2007; Mazen *et al.* 2008 , Shanker and Shyam 2014.

Table 1. Antagonistic effect of bio-agents on *Fusarium oxysporium*.

Bio- agents		Liner growth (cm).				Efficacy %.				Inhibition zone (cm).			
Isolates		**R1**	**R2**	**R3**	**M.**	**R1**	**R2**	**R3**	**M.**	**R1**	**R2**	**R3**	**M.**
R.leguminsarum bv.phaseoli		3.50	3.60	4.00	3.7	66.25	59.09	54.55	59.96	1.90	2.00	1.70	1.86
B. subtilus		4.00	4.00	4.30	4.10	54.55	55.05	51.64	53.01	2.00	2.00	2.00	2.00
P. florescence		4.00	4.00	4.50	4.16	54.55	55.05	49.43	53.01	2.40	2.20	2.00	2.20
T. album		2.30	2.20	2.00	2.33	73.86	73.16	71.91	72.97	-	-	-	-
T. hamatum		2.0	2.40	2.50	2.30	73.86	73.03	71.91	72.93	-	-	-	-
Control		8.80	6.80	8.90	8.16	00.00	00.00	00.00	00.00	-	-	-	-
L.S.D.	0.05		0.94				5.12				0.18		
At	0.01		1.32				7.18				0.26		

***Values are mean of three replications. Significantly at p 0.05 and 0.01 levels.**

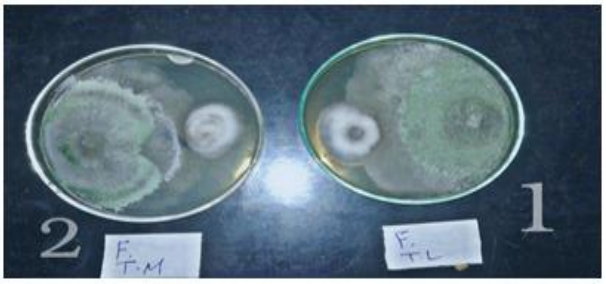

Photo 1. Antagonistic interaction between *F. oxysporium* + 1(*T. album*) , 2 (*T. hamatum).* on dual media.

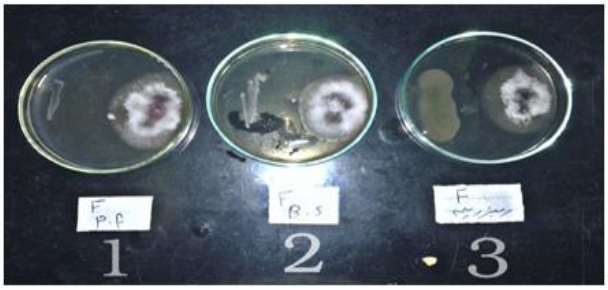

Photo 2. Antagonistic interaction between *F. oxysporium* with (1) *P. flourescnce* , (2) *B. subtilius* and (3) *R. leguminsarum*.

There were significance at 0.05 were; 0.94, 5.12 and 0.18, but at 0.01 were; 1.32, 7.18 and 0.26 of liner growth, efficacy and inhibition zone respectively. The results were agreement with investigators as;

Antagonistic of bio-agents microbes on *Rhizoctonia solani*: Data in Table(2) Improved that posative effect of bio-agents microorganisms on the radial growth (cm) and reduction growth percent of *Rhizoctonia solani* by dual medium method.The mean results were; 4.93 and 38.86% against by *R.leguminsarumbiovar.phaesoli*, 5.53 and29.91% . Moreover, some works explained the antagonistic properties of *Rhizobium leguminosarum* against *Fusarium oxysporum* f.sp. *lentis* due to excretion of antibiotics that have fungicidal action on condia of *F. oxysporum* Essalmani and Lahlou (2002). *Rhizobium* was reported to produce toxic metabolites which have inhibitory effect against soil borne plant pathogens Estevez *et al*.(2002). Defago *et al*.(1990) have also demonstrated by mutational analysis and complementation that production of HCN by *Pseudomonas fluorescens* strain, CHAO accounted for about 60% of the biocontrol activity.*B. subtilus* were;5.80 and 27.79%, while *P. florescence* were 3.10 and 64.76%Kishore *et al*.(2005) demonstrated that *Pseudomonas aeruginosa* which produced protease had significant inhibition (> 32%) against *Sclerotium rolfsii.* This is an indication that the enzyme protease has responsible effect on the phytophatogens. The *T. album* were , 3.43 and 61.12% in the other hand *T. hamatum* effect on the radial growth (cm) and reduction growth % compared with control respectively.Shoda, 2000 noteced that a number of fungi and bacteria are known to be very effective bio-agent against soil-borne plant pathogenic fungi.Jacobsen *et al*., 2004 showed that Bacillus-based biological control agents have great potential in integrated disease management (IDM) options,Together with cultural control, resistant cultivars, fngicides or others biological control agents.Driks, 2004 and Ahmed (2017) They found that Genus Bacillius comprises a heterogeneous groups can be survive in many diverse environments, often with extreme variations in temperature, nutrient, and other stresses.

Table 2. Antagonistic effect of bio-agents isolates on *Rhizoctonia solani*.

Bio-agents Isolates	Liner growth (cm)				Reduction of radial growth %			
	R1	R2	R3	M.	R1	R2	R3	M.
R.leuminsarum bv.phaesoli	5.50	5.30	4.00	4.93	37.50	39.77	39.32	38.86
B. subtilis	6.oo	6.30	4.30	5.53	31.81	28.40	29.54	29.91
P. florescence	6.50	6.40	4.50	5.80	26.13	27.72	29.54	27.79
T. album	3.00	3.10	3.20	3.10	65.90	64.77	63.63	64.76
T. hamatum	3.50	3.50	3.30	3.43	60.22	60.22	62.92	61.12
Control	8.80	8.80	8.90	8.83	00.00	00.00	00.00	00.00
L.S.D. 0.05	2.55				2.42			
At 0.01	3.57				3.39			

***Values are mean of three replications. Significantly at p 0.05 and 0.01 levels.**

Photo 3. antagonistic interaction between *R. solani* + (1) *P. fluorescnce*, (2) *B. subtilius* and (3) *R. leguminsarum*.

Effect of Moncut fungicide on Fusarium and Rhizoctonia growth on medium: The results in Table (3) showed that effect of moncut fungicide different concentrations on growth of *Fusarium oxysporum* and *Rhizoctonia solani* on medium. The concentrations 1,10,25,50 and 100ppm from fungicide were checked on the inhibition growth % of tested

Fungi. The heist mean percent effect on *R. solani* was found that at 50ppm ; 86.06% , also p. at 0.05 found 10.20 and 14.51 at 0.01.

On the other hand effect on *F. oxysporium* was86.03% at 50 ppm and P at 0.05 was 6.64 and 9.45 at P 0.01 these results were the same trend by Ghada *et al*; (2013), Amany *et al;* (2016) Ibrahim *et al* (2016) and Karima *et al*, (2012).

Table 3. Effect of Moncut fungicide different concentrations on *Fusarium oxysporum* and *Rhizoctonia solani* growth.

Test organism Replicates		Moncut concentrations (ppm)				
		1	10	25	50	100
		Inhibition (%)				
R. solani	R1	88.78	70.77	78.77	84.26	100
	R2	88.88	80.88	82.02	88.39	100
	R3	70.88	82.82	82.02	85.56	100
	Mean	82.84	78.15	80.93	86.07	100
LSD	0.05.			10.20		
At	0.01			14.51		
F. oxysporium	R1	60.22	79.58	77.27	78.40	100
	R2	61.36	70.78	76.40	89.88	100
	R3	62.80	73.03	77.52	89.83	100
	Mean	61.46	74.46	77.06	86.03	100
LSD	0.05			6.64		
At	0.01			9.45		

***Values are mean of three replications. Significantly at P 0.05 and 0.01 levels.**

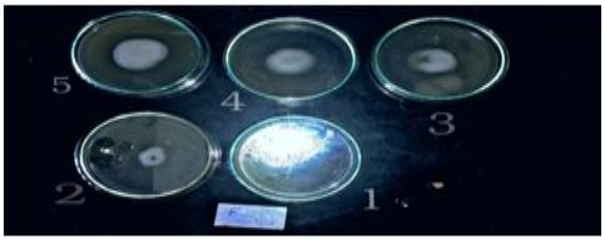

Photo 4. effect of moncut fungicide difrent concentration on *F. oxysporum* on the medium.

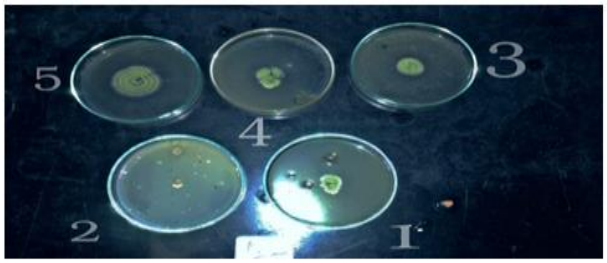

Photo 5. effect of moncut fungicide difrent concentration on *R. solani* on the medium.

Effect of Rhizobium culture filtrate on growth *Rhizoctonia solani:* Theresults in Table(4) showed that different concentration of rhizobium culturefiltrate effect on linear growth test fungi. Mean linear growth by (cm) was 3.23, 1.60 and0.86 (cm) at 25, 50 up to 100% concentration, from results obviously decreasing in expanded of *Rhizoctonia solani* mycelium. The results were confirmed that by increasing in efficacy percent which were; 67.92,81.78 and90.29 at 25, 50 up to 100% concentration. Also P at 0.05 was 0.43and3.47, but P at 0.01 was 0.60and4.82 of linear growth (cm) and efficacy % respectively. These results confirmed by *Mazen et al*, 2008 and El-Batanony *et al*. 2007 studied that the inhibitory effect of cultural filtrate of some wild rhizobial isolates against some fungi causing root rot disease of faba (*R. solani*, *Fusarium* spp. and *F. solani*) in vitro and their antimicrobial synergetic effect when combined with Arbuscular mycorrhiza (AM) fungi, also evaluate the bioactivity of *Rhizobium* spp.isolates and strain to determine the probable mechanisms of the bio-protection.

Table 4. Effect of *Rhizobium leguminsarum bv. Phaesoli* culture filtrate concentrations on the growth of *Rhizoctonia solani.*

Rhizobium culture filtrate conc. (%)	liner growth (cm)			Mean	Efficacy (%)			Mean
	R1	R2	R3		R1	R2	R3	
25%	2.50	3.70	3.50	3.23	70.78	62.22	70.78	67.92
50%	1.50	1.60	1.70	1.60	83.14	82.22	80.00	81.78
100%	0.90	0.80	0.90	0.86	89.88	91.11	89.88	90.29
150%	0.00	0.00	0.00	0.00	100	100	100	100
200%	0.00	0.00	0.00	0.00	100	100	100	100
250%	0.00	0.00	0.00	0.00	100	100	100	100
Control	8.90	9.00	8.90	8.93	0.00	0.00	0.00	0.00
L.S.D 0.05			0.43				3.47	
At 0.01			0.60				4.82	

***Values are mean of three replications.Significantly atP 0.05 and 0.01**

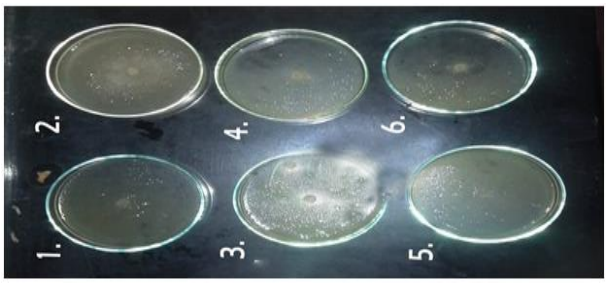

Photo 6. Effect of rhizobium culture filtrate difrent concentration on the growth of *R. solani* on the medium.

Effect of Rhizobium culture filtrate on growth *Fusarium oxysporum:* Data in Table(5) reviled that effect of culture filtrate of growth *Rhizobium liguminsarum bv. Phaseioli* cells on the linear growth of test fungi of *Fusarium oxysporium* . The mean results of different concentrations ; 25, 50 and 100% was;1.46, 1.00 and 0.93. Also results was confirmed with efficacy percent which was;87.70,88.71 up to reached91.04 of concentrations 25, 50 up to 250% respectively. The results were confirmed by calculate P at 0.05 was; 0.27 and1.19, but P at 0.01 was;0.38 and 1.65 of linear growth and efficacy percent respectively. These results was agreement by *Mazen et al*, 2008 and El-Batanony *et al*. 2007 .

Table 5. Effect of *Rhizobium leguminsarum bv. Phaesoli* culture filtrate on growth of *Fusarium oxysporium* on medium.

Rhizobium-filtrate conc. (%)	liner growth (cm)			Mean	Efficacy (%)			Mean
	R1	R2	R3		R1	R2	R3	
25%	1.90	1.30	1.20	1.46	88.22	88.39	86.51	87.70
50%	1.00	1.00	1.00	1.00	88.63	88.76	88.76	88.71
100%	1.00	0.90	0.90	0.93	88.76	89.88	89.88	89.50
150%	0.90	1.00	0.90	0.93	89.77	88.76	91.01	89.84
200%	0.90	0.80	0.80	0.83	88.76	89.88	89.88	89.50
250%	0.80	0.80	0.80	0.80	90.90	91.12	91.12	91.04
Control	8.70	8.90	8.90	8.83	00.00	00.00	00.00	00.00
L.S.D 0.05			0.27				1.19	
At 0.01			0.38				1.65	

***Values are mean of three replications. Significantly at p 0.05 and 0.01 levels.**

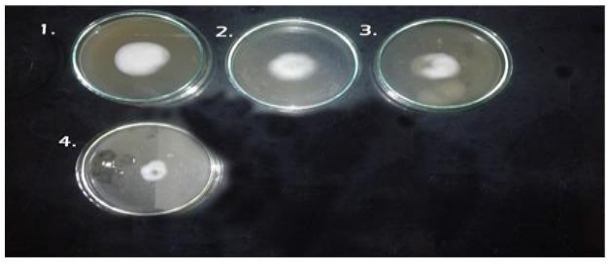

Photo 6. Effect of rhizobium culture filtrate difrent concentration on the growth of *F. oxysporum* on the medium.

CONCLUSION

This research was conducted to investigation the effect of antagonistic some microorganisms are; *Rhizobium leguminsarum biov. Phaseioli* as organism oppsite organism or culture filtrate different concentrations, *Bacillus subtilus , Pseudomonas fluoresense*, *Trichoderma album* and *T. hamatum*. On the test organism *Fusarium oxysporum* and *Rhizoctonia solani* on the medium all antagonistic organisms gave dcreasing in growth linear and inhibition percent by different degres. But striking Rhizobium theirfore bring organism or culture filtrate treatment which gave efficacy percent reached to 91.04% on fusarium at 250% in the effect up to 90.29 with *Rhizoctonia solani* at 100% filtrate concentration compared with control and moncut fungicide. These results support application bioagents as biocontrol to environmental integrity, public health and save food production.

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التأثير التضادى للريزوبيوم والباسيلس والسيدومونس والتريكوديرما على الفيوزاريوم والريزوكتونيا بالمقارنة بالمبيد الفطري مونكت معملياً

حسن هدايه السباعى[1] و عبدالناصر بدوى السيد[2]

[1]جامعة الأزهر- كلية الزراعة بالقاهرة- قسم النبات الزراعى فرع الميكروبيولوجى

[2]وزارة الزراعة- مركز البحوث الزراعية- معهد بحوث أمراض النبات

اجريت اختبارات هذا البحث فى عام 2018م لدراسة التأثير التضادى ببكتيريا ريزوبيوم فاصولاى وباسيلس ستيلس وسيدوموناس فلوريسنس وفطرتريكوديرما ألبم و تريكوديرما هماتم على فطرى فيوزاريوم أوكسيسبوريوم وريزوكتونيا سولانى بالمقارنة بالمعاملة بالمبيد الفطرى مونكت تركيزاتppm1;10;25;50;100على البيئة فى اطباق بترى كان متوسط التثبيط لفطر ريزوكتونيا سولانى100و86.07و80.93و78.15و82.84% على التوالى. فى حين كانت النتائج مع فطر الفيوزاريوم أوكسيسبوريوم كالتالى: 100و86.03و77.06و74.46و61.46% على التوالى. - أظهرت عزلات ميكروبات التضاد درجات مختلفة من التضاد مع الفطريات المختبرة . - كان أعلى متوسط درجات تضاد بين فطر ريزوكتونيا سولانى و*P.fluresense* 5.80cm تلاها*B.subtilus* 5.53cm ثم 4.93cm لبكتيريا الريزوبيوم ,3.11cm, 3.43cm لفطري تريكودرما هماتم وألبم على التوالى. - اعطى راشح مزرعة بكتيريا الريزوبيوم متوسط نسبة كفاءة مع فطر الريزوكتونيا كالتالى: 67.92%،81.78 %، 90.29%بتركيزات50,25 ,100% على التوالى. - فى حين كان متوسط نسبة الكفاءة مع فطر الفيوزاريوم،87.70 , 89.8488.71 ،89.50 ،91.04 % لتركيزات 250,200,150,100,50,25% على التوالى مقارنة بالكنترول والمعاملة بالمبيد الفطرى مونكت بتركيزات مختلفة، اضافة لتثبيت نتروجين الهواء الجوى وانتاج منظمات نمو النبات وزيادة خصوبة التربة.