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EVALUATION OF SOME FUNGI AND BACTERIA AS BIOLOGICAL AGENTS TO FUSARIUM WILT OF BANANA

Ziedan, E. H. [1]; Eman S. Farrag[2] and Moataza M. Saad[3]

1- Plant Pathology Dept. National Res. Center, Cairo, Egypt.

2- Agric. Botany Dept. Fac. of Agric. South Valley Univ. Quina, Egypt.

3- Microbial chem. Dept. National Res. Center Cairo, Egypt.

ABSTRACT

Fusarium oxysporum Schlechtend : Fr f.sp . *cubense* (FOC) (E F. Smith). Snyder & Hansen were the most causal organism causing wilt disease of banana plant at Beheira , Gharbea and Assuit Governorates, Egypt. Fusarium wilt disease of banana showed to be similar to those of other plants. Discoloration brown to dark of pseudostem and rhizomes was the most indicator of Fusarium wilt of banana plants.

In vitro four microbial isolates from the banana root and rhizosphere showed highly antagonistic activity to *F.oxysporum* f. sp. *cubense*. These isolates were identified as *Bacillus subtilis* (Ehrenberg) Cohn (No.1), *Pseudomonas fluorescens* Migula (No.3), *Trichoderma harzainum* Rifai (No.6) and *Laetisaria arvalis* Burds (4). *Bacillus subtilis* (Ehrenberg) Cohn (No.1); *Pseudomonas fluorescens* Migula (No.3) and *T. harzianum* isolates were the most antagonistic isolates inhibiting mycelial growth of *F. oxysporum* f.sp. *cubense*.These antagonistic strains were excreted antifungal lytic enzymes on liquid medium containing dead mycelium of *F. oxysporum* f. sp. *cubense i.e*. chitinase, β1-3,β1-4 glucanases and protease. Chitinase and protease enzymes were the most lytic produced by all antagonistic strains.while β1-3and β1-4 glucanases were produced at a moderate rate.

Bacillus subtilis (Ehrenberg) Cohn (No.1) was the most antagonistic strain producing chitinase and protease followed by *L. arvalis* and *T. harzianum*. On the other hand, *Bacillus subtilis* and *L. arvalis* were the most antagonistic producing difusible and volatile antibiotics followed by *P. fluorescens*. while, *T. harzianum* was the least antagonistic phase of antibiotics. Pot trails conducted during a summer season, revealed that dipping banana roots transplanting root in the suspension of *B.subtilis*, *P.fluorescens* Migula, *T.harzianum* Rifai and *Laetisaria arvalis* Burds $3x10^8$ (CFU/ ml) for one and two hours or soil drench with 100 and 200 ml / pot were effectively reduced wilt disease incidence of banana plants, respectively. Under field naturally infestation with *F.oxysporum* f.sp. *cubense* soil dranch of banana around rhizome region by suspension of *B.subtilis, P.fluorescens* Migula, *T.harzianum* Rifai, and fungicide (Topsin) reduced the wilt incidence than control . Soil drenched with 300 ml of *B. subtilis* significantly reduced the wilt incidence by 50% compared with control. Whereas the *P. fluorescens* Migula failed to control Fusarium wilt. *B. subtilis* was the most promising biocontrol agent against wilt disease of banana.

Keywords: Banana, *Fusarium oxysporum* f.sp. *cubense*, biological control, Fusarium wilt .

INTRODUCTION

Banana (*Musa* spp.) is one of the most important horticultural crops in tropical and subtropical countries. Banana disease (Fusarium wilt) is one of the most serious problems of banana plantation in the world. Fusarium wilt of banana caused by *F.oxysporum* f. sp. *cubense* (FOC) is considered to

have originated in Southeast Asia probably in association with wild species or early land races (Jones, 1994). Chemical control of Fusarium wilt is economically impracticable and its efficient control has been based on the use of tolerant varieties of banana (Stover and Simmonds, 1987 and Ploetz *et al.,* 1990). The breeding programmes to obation the disease resistant plants is difficult in banana because the most of the widely cultivated varieties are triploids and have poor seed production (Mastumoto *et al*., 1999). Currently, eight species of microorganisms are registered by the U.S.Environmental. Protection agencey of commerical use against soil borne plant pathogens in the Untited States.These include *Trichoderma harzianum* G-21, Gram negative bacteria(*Pseudomonas fluorescens* EG-1053) and Gram positive bacteria (*Bacillus subtilis* GB03, *B. subtilis* MBI 600 each of these microorganisms are intended to control damping- off diseases and improve stand establishment (Cook *et.al*., 1996). Biological control has been suggested an alternate method of controlling soil borne disease of banana by different methods(Sivamani & Ganamanickam (1988), and Ziedan *et.al*.,2005) . Dipping banana sucters in the suspension of antagonistic isolates of *P. fluorescens* (10^6 CFU / ml) or *T.viride* (10^6 CFU /ml) along with application of 500 g wheat bran , were effectively reduced Fusarium wilt incidence and produced the highest yield (Raguchander *et. al.,* 1997) . Also , Narendrappa and Gowda , 1995 in USA found that soil application of banana with *P. fluorescens* strain Pf10 reduced the wilt incidence 50% compared with the control.

Thus the current studies were designed to evaluate banana antagonistic microorganisms to controlling Fusarium wilt under greenhouse and field conditions .

MATERIALS AND METHODS

Isolation and identification of causal organisms

Twenty samples of diseased banana roots and rhizomes were collected from Beheira, Gharbeia and Assiut Governorates . The diseased tissues were disinfected in 1% sodium hypochlorite solution for 2 min, rinsed in tap water three times and placed on sterilized tissue paper at room temperature until dry . The sterile tissues were then plated on potato dextrose agar (PDA) , Czapek-s and peptone glucose agar media for 3-5 days at 25+2°C . Fungal isolates were purified using hyphal tip and single spore culture techniques. Identification was carried inconsaltation with information from Gilman, (1957) ;Nelson *et al*.,(1983) and Barnett and Hunter (1998).

Pathogenicity test

Pathogenicity test was carried out in Plant Pathology Dept. (NRC), plastic pots (30 cm- diameter) containing clay sandy soil were infested with each fungal inoculum prepared by growing each fungal isolates in sterilized corn meal and sand medium (75g corn meal grain + 25g clean pur sand +100 ml water) at 28 °C, for two weeks. Soil was infested with the rate of 10% (w/w). Pots were watered every two days for a week before planting one plant of banana Cv. Williams was cultivated in each pot. Five pots were

used for each isolate as replicates. Four months later, the percentage of wilt and disease severity were determined according to Woltz and Arthur, (1973), wheares:
0=healthy plant, 1=yellowish +1/3 plant wilted, 2= 2/3 plant wilted, 3= whole plant wilted and 4=plant dead

Host range of *F.oxysporum*

Lupni ,cotton , watermelon , cowpea ,sesame,tomato , eggplant and pepper, were used for studying the host specific of *F.oxysporum* isolate revealed to be able to induce wilt disease of banana .

Biological control studies

Isolation of rhizospheric microflora

A number of bacterial and fungal were isolated from rhizospheric soil samples collected from banana growing areas of Beheira, and Gharbeia using the method adopted by (louw and Webley, 1959). Dilutions were made up to $1x10^{-7}$, $1x10^{-4}$ for isolating bacteria and fungi respectively. Soil extract agar medium was used for bacterial isolation (Skinner *et al.,* 1952), peptone dextorse agar medium for fungal isolation (Martin , 1950) and one ml from the above dilutions was spreaded on the prepared media in a Petri-dish and four replicate were used. The bacterial and fungal were checked 2-5 days after incubation in dark at 28 ◌̊C.

Screening of antagonistic microorganisms to *F. oxysporum* f.sp *cubense*

The interaction between *F.oxysporum* f. sp. *cubense* and the rhizospheric microoganisms as well isolate of *Laetisaraia arvalis* which obtained during isolation traila of fungi associated banana infected plant by wilt disease were assaed *in-vitro* on (PDA) according to (Ziedan ,1993). Inhibition or reduction in the linear growth of the pathogenic fungi were recorded 5 days after incubation at 27 ± 2°C when mycelial growth covers the entire surface in control plates. The percentage of inhibition in mycelial growth of *F.oxysporum* f. sp. *cubense* was calculated using the formula as follows:

$$I= (C-T/C) \times 100$$

Whears: I = Percentage of inhibition of *F.oxysporum*
C=linear growth of control (without antagonistic agent) and T = linear growth of treatment.

Identification of antagonistic microorganisms:

Antagonistic bacterial isolates were identified according to morphological, physiological, and biochemical characters according to (Harrigan and McCane , 1976 and Sneath, 1986). Also, antagonistic fungal isolates were identified according to morphological characters (Gilman , 1957 and Barnett & Hunter, 1998).

Antibiosis activity of antagonistic microorganisms toward *F. oxysporum* f.sp. *cubense*

Propagation of pathogenic fungi

The fungal isolate was grown in Erlenmeyer flasks (250 ml) each containing 50 ml of Czapek·s Dox broth medium supplemented with glucose

as a sole source of carbon at a concentration of 1 % (w / v). Each flask was separately inoculated by one disk (4 mm- diameter) of a fungal growth and incubated shaken at 28± 2 °C for 5 days, then the mycelium was then collected by filtration and dried to a constant weight.

Production of difusible antibiotics

Plates containing dextrose agar medium (PDA)were covered with a cellophane membrane, inoculated in the center with 100µL of antagonistic isolate suspention (5×10^8 CFU ml^{-1}). After incubation for 72 hrs at 22°C, the membrane with the grown isolate was involved and the plate inoculated in the middle with 10 mm mycelium disk of *F. oxysporum* f.sp. *cubense* **.** Plates were futher incubated at 22°C for 48 hrs and the growth of the pathogen was measured.Control was run by sterile distilled water. Each experiment considering for each bacterial or fungal isolates. Treatments were run in triplicates and repeated at three times. Fungal growth of *F. oxysporum* f.sp *cubense* was determined of each antagonistic compare with in the control, according to (Press *et al*., 2001).

Production of volatile antibiotics

One hundred µL of antagonistic bacteria suspention (5×10^8 CFU ml^{-1}) were placed at the center of one half Petri dish containing King·s B medium. Disk 8mm (age 4 days) of pure culture *F. oxysporum* f.sp *cubense* was used for seeding placed at the center of another Petri dish containing PDA. Both half places were placed face to face perventing any physical contact between the pathogen and the antagonistic suspension and sealed to isolate the inside atmosphere and to prevent loss of volatiles formed. Plates were incubated at 22 °C for 48 hrs and the growth of the pathogen was measured in compared to the controls developed free antagonistic. Each experiment considering a single fungal or bacterial isolates were run in triplicate and repeated three times. Results are express as means of inhibition% ± S.D. of growth of *F. oxysporum* f.sp *cubense* according to (Press *et al*., 2001).

Secretion of antifungal enzymes

Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis and *L. arvails* were separately grown on a medium developed by Okon *et al*.(1973) which contained (g / L): $MgSO_4$.$7H_2O$,0.2 ; K_2HPO_4 , 0.9 ; KCl , 0.2 ; NH_4NO_3 , 1.0 ; $FeSO_4.7H_2O$, 0.002 ; $MnSO_4$. H_2O, 0.002 and $ZnSO_4.7H_2O$, 0.002. This medium (pH 6.5) was supplemented with dead fungal mycelium (*Fusarium oxysporum*) as inducers for enzyme productions at a concentration of 1.0 % and dispensed in Erlenmeyer flasks (250 ml) , each flask contained 50 ml of medium. The flasks were autoclaved and inoculated with 1.0 ml of precultured *Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis* and *L. arvails* The cultures were shaken incubated at 180 rpm. on a rotary shaker (New Brunswick Scientific Co.,USA) at 28± 2°C . Two flasks from each antagonistic isolate were analyzed daily for 5 days.

Enzymes assay

Chitinase activity was assayed following the release of N–acetylglucosamine according to the method of Monreal and Reese (1969). One ml of 1 % colloidal chitin in 0.1 M citrate phosphate buffer (pH 6.5) was incubated with 1.0 ml of culture filtrate at 37 °C for 2 hrs. One unit of enzyme

activity is defined as the amount of enzyme required to produce 0.5 µM / ml of N – acetylglucosamine per hour. Specific activity was expressed as units / mg protein.

β-1,4-glucanase activity was assayed following the release of free glucose from carboxy methyl cellulose (CMC) as a substrate . The activity was defined and measured according to the method of Mandels *et al*. (1976). One unit of enzyme was defined as the amount of enzyme that releases1.0 µM of glucose / min. Glucose equivalents (reducing sugar) generated during assay were estimated by using 3,5 dinitrosalicylic acid (DNS) method (Miller, 1959), with glucose as standard.

β-1,3-glucanase activity was assayed by incubating 1ml of 0.5% (w/v) laminarin in 0.1M sodium phosphate buffer , (pH 4.0) with 1ml of culture filtrate at 55°C for 5 min . The reaction was stopped in an ice bath, 3 ml of 3,5 dinitrosalicylic acid (DNS) reagent was then added and the mixture was heated in a boiling water- bath for 12 min. As a control, 1 ml laminarin solution was incubated and cooled, then 1ml of enzyme solution together with 3 ml of DNS reagent were added to correct for the reducing sugars in the substrate and the enzyme solution. Reducing sugar equivalents were measured in both the original and the control solutions according to the method of Miller (1959), with glucose as standard.

For determination of protease activity, a reaction mixture containing 1.0 ml of 1 % soluble casein in 0.05M-citrate phosphate buffer (pH 6.5) and 1.0 ml of culture filtrate was used. The reaction mixture was incubated for 1 h at 37°C and was stopped by adding 10 % trichloroacetic acid (TCA), kept for another 20 min at the same temperature and followed by centrifugation at 4000 rpm for 20 min. Samples of 75 µL were removed and tyrosine was determined according to Lowry *et al*. (1951). One unit of the enzyme activity was defined as the amount of enzyme required for the formation of 1.0 µM. of the product / min of the reaction under the standard assay conditions.

Biological control application

Pot experiment

This experiment was carried out at National Research Center (NRC), Plant Pathology Dept in plastic pots (30cm-diameter) infested with *F. oxysporium* f.sp. *cubense* as mentioned before . Banana plant (Cv Williams) tissue culture transplants were used in this study. Pots were divided into two groups. First group banana transplants dipped roots for one and two hours before sowing for each biocontrol agents suspension(3×10^8 CFU ml^{-1}). Second group, soil was drenched with 100 and 200ml of each agents suspension before sowing.One transplant was sown / pot. Four pots were used as replicates for each treatment and four plant were served as control according to Sivamani &Ganamanickam,(1988) and Ziedan *et. al*.,(2005).

Field application

This experiment was carried out at Gharbeia Governorate. Infected banana plants (Cv. Williams)were used in this study. Soil around rhizomes drenched with different biological agents suspension (300 ml/plant). Each biocontrol agent ($3x10^8$ CFU/ml) of *B.subtilis* , *P.fluorescens* , *T.harzianum* and *L*. *arvails*. Topsin 70% (fungicide)was used by the rate 10g suspended

of 200ml / plates. Eight banana plant were used for each treatment and eight banana plants were served as a control.

Prepartion of biocontrol agents inocula and inoculation method

Fungal inocula

Each isolate of *Trichoderma harzianum* and *L. arvails* were grown on PDA broth in Conical flask 250 ml for 7 days at 27 ◌̊C ±2 ◌̊C . Ten replicates were used for each isolate . Conidia spores and mycelial growth were harvested to obation fungal suspension $3x10^8$ CFU/ml ,according to Ziedan *et. al*.,(2005).

Bacterial inocula

Each isolate of *Bacillus subtilis* and *Pseudomonas fluorescens* were individually grown on nutrient broth medium (Beef 5 g , peptone 3g and distilled water 1000 ml) according to ATCC., 1984 , 100 ml in sterilized medium for each conical flask 250 ml . Flasks were incubated at 30 ◌̊C for 72hrs. Cell suspension of each test bacterium were centrifuged at 400 g / min for 15 minutes and adjusted to 3×10^8 cell / ml.

Wilt disease assessments

The precentage of banana wilted plants was determined, 3 months after sowing . Disease severity of shoot system was calculated as mentioned before . Also, disease severity of pseudostem basal and corms was determined according to percentage of discoloration area of cross section as follows:

0= No discoloration
1= 25% discoloration
2= 50% discoloration
3= 75 % discoloration
4=100 % discoloration

Statistical analysis:

The obtained data were statistically analyzed according to Snedecor and Cocharn (1980)

RESULTS AND DISCUSSION

Survey of Fusarium wilt disease of banana plants

The syndrome of Fusarium wilt of banana plants was commonly observed on the aerial plant parts showing yellowing, wilting, stunting and discoloration of pesudostem and rhizome of cross section at Gharbeia, Beheira and Assuit Governorates during 2002, 2003 and 2004.

Symptomatology of banana wilt disease

The expression of symptoms in wilt disease of banana caused by *F. oxysporum* sp. *cubense* showed in Fig.1 and Fig.2 .The first visible sing of the disease, then tern to wilted followed by collapse of the petiole in the middle, the inner most leaves remain green and erect. Pseudostem is sometimes surrounded by a skirt of dead leaves in Fig (1). Longitudinal section of rhizomes showing discolouration brown to dark colour. Cross section of pseudostem is discoloration with purple colour Fig (1). These symptoms are considered the most diagnostic due to *F.oxysporum* f. sp.

cubense (Stover and Simmonds, 1987, Jones, 1994, Ploetz *et al*., 1990 , Kung, 1995 and Moore *et al*., 1995). In this study disease severity of banana plants were grades in four group in Fig (2) as follows:

0= healthy ; 1=yellowish+1/3 plant leaves wilted ;
2= 2/3 plant leaves wilted 3= whole leaves wilted
4= plant dead

Fig (1) Fusarium wilt symptoms of banana plant (Cv. Williams)
A-External symptoms of banana leaves remain erect and yellowish
B-Internal symptoms of banana vascular discoloration in rhizome
C-Internal symptoms of banana discoloration of in pseudostem
0= Healths, 1 = 25% discoloration, 2 = 50 % discoloration, 3=100% discoloration

Isolation, identification and pathogenicity test of fungal isolates from banana

A number of fungal isolates from diseased banana with yellowing, wilting, stunting and discoloration of root system as shown in Figs 1 and 2 were prepared in pure culture. Isolates of *F. oxysporum, Aspergillus niger* and *Macrophomina phaseolina* from Beheira ,Gharbeia and Assuit were tested of banana (Cv. Williams one year – old).

Data in Table (1) show that *F. oxysporum* isolates from Beheria, Gharbeia and Assiut were the most and significantly pathogenic isolates induced wilt symptoms 66.5%, 33% and 66.5%, respectivilly. *Macrophomina phaseolina*

(Mauble)Ashby, isolated from Beheira induced root-rot syndrome (100%) and disease severity (4.0). *Laetisaria arvalis* was used as biocontrol agent to in this study . Simillar results were obtained before by several investigators of banana wilt disease(Stover and Simmonds 1987; Ploetz *et al*., 1990; Mastumoto *et al*., 1999 and Jones, 1994 ; Conway *et al*.,2000& Babba and Conway2003).

Fig(2) Disease severity of banana plant (Cv. Williams) infected by *F. oxysporium* f.sp. *cubense*

0 = healty plant **1 = 1/3 plant leaves wilted**
2 = 2/3 leaves wilted **3 = whole leaves wilted**

Host range of *F. oxysporum*

The studying of host range to the highly virulence *F. oxysporum* which able to induce wilt disease to banana plant and was failed to causing wilt symptoms, of the plants tested i.e. Tomato (*Lycopersicon esculentum*), Eggplant (*Solanum nigrum*),*Sesame* (*Sesamum indicum* L) Cv.Giza 32, Cotton (Gossypium spp.),Pepper (*Capsium annum*),Lupini (*Lupinus terms*)*Cowpea* (*Vigno sineces* Cv.) Black andWater melon (*Cucurbita pepo* Cv.) Eskandarmnni. This result confirmed that the compelety identifiction of wilt causal of banana is formae specially *F.oxysporum* f. sp. *cubense*.

Table (1) Pathogenicity test of fungal isolates

Location	Fungal name	Wilt and root-rot of banana		
		Infection %	D. severity	Symptoms
Beheira	*Aspergillus niger*	00.0d	0.0b	-----
(Nobareia)	*Aspergillus niger*	00.0d	0.0b	-----
	F. oxysporum	66.5b	3.0a	wilt
	M. phaseolina	100.0a	4.0a	root-rot
Gharbeia	*Laetisaria arvalis*	00.0d	0.0b	----
	F.oxysporum	33.0c	2.0a	wilt
Assiut	*F. oxysporum*	66.5b	3.0a	wilt
Check	Without	00.0d	0.0b	---

Numbers within columns followed by the same letter are not significantly different at ≥ 0.05.

Fusarium wilts are caused by pathogenic *Fusarium oxysporium*. There are a high level of specificity between susceptible host plants and pathogenic *F. oxysporium* strains. This specificity is characterized by formae specials and races to which *F. oxysporum* belongs (Armstrong and Armstrong, 1981)**Biological control studies**

Screening antagonistic isolates

Direct antagonism in dual culture between *F. oxysporium* and rhizospheric isolates was calculated in Table (2) show the isolated fungi and bacterial from banana rhizophere *i.e* bacterial isolates (*B.subtilis and one isolate of P. fluorescens*. Fungal isolates were including on *T. harzianum* and *T. viride*, 2 isolates of *A. niger*; one isolate of *Pencillium* sp. and one isolate of *L. arvalis*; *T.harzianum, P. fluorescens*. *Bacillus subtilis* (No. 1) showed a highly antagonistic effect against *F.oxysporum* f.sp. *cubense.* A high reduction of pathogenic fungal growth by *B.subtilis* (No1)*, P. fluorescens* , *T. harzianum* and *L. arvalis* .

Table(2) Antagonistic effect of banana rhizosphere microorganism to *F.oxysporum* f.sp. *cubense* .

Microorganism	***F.oxysporum f.sp.* cubense**	
	R. Growth %	**Zone of inhibition (cm)**
Bacillus subtilis (1)	72.00a	3.1a
Bacillus ssubtilis (2)	00.00d	0.0d
P. fluorescens (3)	27.00c	3.0a
L. arvalis (4)	50.8b	2.0b
Penicillium sp. (5)	66.7a	0.0d
T.harzianum (6)	72.2a	2.5b
T. viride (7)	50.0b	0.0d
Aspergillus niger (8)	45.0b	0.9c
Aspergillus niger (9)	50.0b	1.0c

The fungal growth of the tested fungal was severly inhibited in the presence of *B.subtilis* (No1) followed by *P. fluorescens.* No physical contact was observed between any of the antagonistic isolates and *F.oxysporum* . Moreover, an inhibitory hallo was observed suggesting the presence of fungistic metabolites secreted by the isolates. It has been described that all isolates can secrete several antifungal metabolites such antibiotics or lytic enzymes (Brian and McGowan, 1945, Broadbent *et. al*., 1971,Dennis and Webster, 1971, Pérez *et al*., 2002 and Ziedan *et. al*.,(2005).

Difusible antibiotics and volatile antibiotics :

All antagonistic isolates gave inhibitory effect on the growth of *F.oxysporum* but *Bacillus subtilis*, L. arvalis and *P. fluorescens* showed the best inhibitory effect on the growth of *F.oxysporum* (Table 3). Such results are in line with those obtained by (Reddy and Rahe,1994), indicated that an antibiotic complex containing bacilysin and fengmycin (Fengycin) produced by *Bacillus subtilis*, Brian and McGowan, 1945 were described a highly fungistatic antibiotic, viridin produced by *T.viride*.Dennis and Webster (1971) showed that *Trichoderma* spp. produce antibiotics such as trichodermin by *T.viride* and *T. polysporum* and other peptide antibiotic by *T. hamatum*.

Table (3) Effect of difsusible and volatile antibiotics secreted by antagonistic microorganisms on PDA at 22°C for 72hrs

Isolates	Inhibition of *F.oxysporum* %	
	difusible antibiotics	volatile antibiotics
Bacillus subtilis	33.94a	55.99a
P. fluorescens	32.99a	54.97a
T.harzianum	19.55b	21.22b
L. arvalis	33.8a	55.6a

Secretion of antifungal enzymes by antagonistic isolates

Bacillus subtilis , *P. fluorescens* ,*T.harzianum* and *L. arvalis*.were selected after the observation of clear zones around the growing isolates on medium containing only dead mycelium of *F.oxysporum* mentioned in table (2) suggesting an lytic activity of these strains against *F.oxysporum.*

In order to determine the production of lytic enzymes , 1% of fungal mycelium were used as a sole carbon source in the medium inoculated with *Bacillus subtilis* , *P. fluorescens T.harzianum* and *L. arvalis* respectively . Percentages of lysis and lytic enzymes activities were determined . Data in Table (4) showed that *Bacillus subtilis*, *P. fluorescens* ,*T.harzianum* and *L. arvalis* gave high chitinolytic activity (50.2, 43.5, 40.6 and 41.3 units/ ml respectively) and protease(61.3, 22.8, 56.4 and 66.2 units / ml respectively) enzyme activities and completely lysed fungal mycelium after 72 hrs for *Bacillus subtilis* and *P. fluorescens* or 69 hrs for *T.harzianum* and *L. arvalis* of incubation at 28±2°C. On the other hand, *β* 1,3 and *β* 1,4 glucanase gave low enzyme activities It may be concluded that they use these two latter mechanisms of biocontrol as opposite to some fungal biocontrol microorganisms that also use fungal cell wall hydrolyzing enzymes within their biocontrol mechanisms (Dennis and Webster 1971,Pérez *et al*., 2002 and Ziedan *et al*., 2005).

Table(4) Lytic enzymes produced by antagonistic microorganisms grown on dead mycelium of *F.oxysporum* f.sp. *cubense*

Organisms	Residual dead mycelium mg/50 ml medium	Lysis %	Lytic enzymes (units/ ml)			
			chitinase	β-1,3 glucanase	β 1,4 glucanase	protease
Check	1.0	0.0a	0.0a	0.0a	0.0a	0.0a
Bacillus subtilis	0.0	100b	50.2b	10.4b	15.2c	61.3c
P. fluorescens	0.0	100b	43.5b	18.9c	9.3b	22.8b
T.harzianum	0.0	100b	40.6b	12.1b	16.3c	56.4c
L. arvalis	0.0	100b	41.3b	11.3b	8.2b	66.2c

Numbers within columns followed by the same letter are not significantly different at ≥ 0.05.

Pot experiment

Data in Table (5) indicate that all antagonistic microorganisms to causal wilt disease of banana which isolated from rhizosphere significatly reduced wilt disease incidence of banana plant with different inoculation techniques than the control (untreated plants), increasing inoculum rate or

exposure period for each treatments increasing reduction of wilt disease percentage and disease severity .

Table (5) Effect of biological treatment on wilt disease of banana caused by *F.oxysporum* f.sp. *cubense*

Treatment	transplanting				Soil drench/pot			
	1h		2h		100 ml		200ml	
	Inf. %	D.S	Inf%	D.S	Inf%	D.S	Inf%	D.S
B.subtilis	00.0a	0.0a	0.0a	0.0a	50.0b	4.0b	0.0a	0.0a
P.fluorescens	75.0c	4.0b	25.0b	4.0b	25.0a	4.0b	25.0b	4.0b
T.harzianum	25.0b	4.0b	0.0a	0.0a	25.0a	3.0a	0.0a	0.0a
L. arvalis.	25.0b	4.0b	0.0a	0.0a	25.0a	4.0b	0.0a	0.0a
Chech	100d	4.0b	100c	4.0b	100c	4.0b	100c	4.0b

Numbers within columns followed by the same letter are not significantly different at ≥ 0.05.

Bacillus subtilis was the most antagonistic microbes significantly reduced wilt disease incidence of banana plant as root dipping and soil treatment with bacterial suspension followed by *T.harzianum* .

Meanwhile *P.fluorescens* was the least effective to suppress wilt disease of banana plants .

Field appplication

Data presented in Table (6) soil treatment around pseudostem with 300 ml suspension (3 x 10^8 /ml) , for each antagonstic microorganism *i.e., B.subtilis* and *T.harzianum* were the most effective for controlloing wilt disease of banana plant under natural infection than the *P.fluorescens* and in the untreated plant (control) .

B.subtilis suppress external wilt syndrome of banana plant to 50% than 100% in case of control untreated plant and plant treatment with *T.harzianum P.fluorescens* and fungicide (Topsin) . Also *B.subtilis* and *T.harzianum* were significally suppression internal wilt syndrome if corms and stem basal (discoloration percentage in cross section) 50% than the control followed by *P.fluorescens* 30% and Topsin 12.5%.Results are inagreement with those obtained by (Sivamani& Gnanamanickam, 1988,Mardis and Poetz,1990, Narendrappa and Gowda, 1995, Raguchander *et.al*.,1997,Nasir *et al*., 1999). *Bacillus* species, as a group, offer several advantages over *Fluorescent pseudomonas* and other Gram-negative bacteria as seed inoculants for protection against root pathogens, including longer shelf, because of their ability to form endosporess and the broad- spectrum activity of their antibiotics. One of the best known examples is *B. subtilis* A13, isolated more than 25 years ago in Australia (Broadbent *et al*., 1971). *Bacillus* species as biocontrol agents can be enhanced using a protocol designed to isolate root-associated, endospore- forming bacteria exclusively and then screening candidate strain against the species of the pathogens likely to cause root diseases on the same plants.

The use of biocontrol agent (biological control) as a new approach in plant protection showed promising results in controlling certain plant disease to avoid environmental hazards have proven great efficacy in biocontrol of many plant diseases .

Table (6) Effect of biological treatment on wilt disease of banana

Treatment	Wilt disease incidence			
	External		Internal	
	Inf %	D. Severity	Inf %	D. Severity
Check	100.0b	4.0b	100.0c	3.3b
P.fluorescens	100.0b	4.0b	70.0b	3.2b
T.harzianum	100.0b	4.0b	50.0a	4.0b
B.subtilis	50.0a	2.0a	50.0a	2.5a
Topsin	100.0b	3.9b	87.5b	3.3b

Numbers within columns followed by the same letter are not significantly different at ≥ 0.05.

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تقييم الفطريات و البكتريا التى تعمل على مقاومة اصابات الفيوزاريوم المرضية للموز

السيد حسين زيدان*, ايمان صالح فراج و معتزة محمود سعد*****

*** قسم امراض النبات- المركز القومى للبحوث-القاهرة-مصر**

**** قسم النبات الزراعى –كلية الزراعة - جامعة جنوب الوادى –قنا -مصر**

***** قسم كيمياء الكائنات الدقيقة-المركز القومى للبحوث- القاهرة-مصر**

يسبب *فطر F.oxysporium f.sp. cubense* مرض ذبول الموز فى محافظة البحيرة و الغربية وأسيوط. وتظهر الاعراض على المجموع الخضرى على هيئة أصفرار للاوراق الخارجية سرعان ما تجف ويظل متدلين بجوار الساق الكاذبة على حين يظهر تلون فى اسفل الساق

الكاذبة والرايزوم ويمتاز ذلك الفطر أنه متخصص لاصابة نباتات الموز دون غيرها من النباتات الاقتصادية الاخرى.

وفى محاولة للحد من انتشار المرض وبعيد عن استخدام المبيدات الكيماوية تم عزل العديد من العزلات الميكروبية من المنطقة المحيطة بجذور نباتات الموز كان منها العديد من العزلات الفطرية والميكروبية التى ثبت قوتها التضادية العالية للفطر المسبب لمرض ذبول الموز وهو *F.oxysporium* fsp. *cubense*. وقد عرفت العزلات المضادة بأنها عزلة من الفطر *Trichoderma harzianum* وعزلة الفطر *Laetisaria arvalis* وعزلتين من البكتيريا *Bacillus subtilis* و *Pseudomonas fluorescences* وبعمل بعض الدراسات المعملية على الميكروبات المعزولة وجد ان لها قدره عالية على انتاج الانزيمات المحللة لجدر خلايا الفطر مثل انزيمات الكيتينيز و البروتيز وكذلك انزيمات البيتا ١-٤ والبيتا ١-٣ جلوكانيز كذلك وجد ان هذه الميكروبات لها القدرة ايضا على انتاج المضادات الحيوية مما يزيد من كفاءة هذه الميكروبات فى ايقاف وتحلل الفطر المسبب للمرض.

وقد أثبت التطبيق على نطاق التربة المحتوية صناعيا على الفطر المسبب لمرض ذبول الموز بعدد من المعاملات المختلفة وذلك بنقع جذور وكورمات الموز ($3x10^8$ CFU/ ml) لمدة ساعة الى ساعتين أو معاملة التربة عند الزراعة بمعلق الميكروبات المضادة بمعدل ١٠٠مل وكذلك ٢٠٠ مل لكل اصيص بعد الزراعة حيث ادى الى خفض حدوث المرض وكانت المعاملة ب ٢٠٠ مل هى أفضل المعاملآت واكثرها فاعلية على وجه الاطلاق الى منع استيطان المسبب المرضى لذبول فى الموز. أما فى حالة التطبيق الحقلى: تم عمل مقارنة بين الكائنات المستخدمة وبين المبيد الفطرى توبسين وذلك باستخدام تركيزات مختلفة من معلق الميكروبات كلا على حده وجد ان تركيز ٣٠٠ مل كان افضل التركيزات المستخدمة وقد وجد ان سلالة *Bacillus subtilis* هى افضل السلالات المستخدمة على عكس السلالة *Pseudomonas fluorescences* التى فشلت فى ايقاف المرض . كذلك تشير الدراسة الى امكانية الاستفادة من ميكروبات الريزوسفير المضادة لمسببات الامراض كبديل حيوى آمن وذلك حفاظا على البيئة من التلوث بالمبيدات.