
Effect of bacterial inocula on *Fusarium oxysporum* f sp *sesami* and their pathological potential on sesame

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El Sayed H. Ziedan., Mostafa H Mostafa and Ibrahim S. Elewa (2012) Effect of bacterial inocula on *Fusarium oxysporum* f sp *sesami* and their pathological potential on sesame. Journal of Agricultural Technology 8(2): 699-709.

Fusarium oxysporum f.sp. *sesami* (Zap) Cast. causing wilt disease of sesame plants causing high losses in quality and quantity of sesame seed yield. Bacterial isolates of *Bacillus megatherium* and *Azospirillum brasilense* were the main biological component respectively of commercial biofertilizers *i.e.*, phosphorene and cerialin respectively in Egypt. These bacteria were reduced linear growth and conidiospores production of *Fusarium oxysporum* f.sp. *sesami* their effects were increased in combination with fungicide (Topsin) which increased by increasing fungicide concentration. Furthermore, several morphological changes of fungal hyphae by *Bacillus megatherium* stress. In laboratory, plate culture of *Fusarium oxysporum* f.sp. *sesami* was used for evaluate ability of *i.e.*, cerialin, phosphorene, rhizobacterin and blue green algae as well as fungicide (Topsin M-70 %) for protection sesame seedlings from *Fusarium* blight. All different commercial biofertilizers and Topsin were significantly protection sesame seedling from infection by *Fusarium oxysporum* f.sp. *sesami*. than the control. Phosphorene as a single treatment was the best effective in this respect. Meanwhile, combined treatments were significantly than single for reducing wilt disease of sesame seedlings. Cerialin + phosphorene and cerialin + phosphorene + Topsin were the most effective treatments reduced wilt of sesame seedlings. Under natural field infestation by wilt pathogen, root dipping of sesame transplantings before cultivation of biofertilizers suspension *i.e.*, cerialin, phosphorene as single and combined treatments as well Topsin at (1000 ppm) were reduced wilt disease incidence and significantly increase yield than the untreated transplanting and sesame plant obtained from seed coated by V/captan. Topsin M 50% as combined treatments with cerialin or phosphorene gave a good effect than single treatments of each components mentioned before. Furthermore, combination of cerialin + phosphorene + Topsin (1000 ppm) was the best treatment significantly reduced wilt disease incidence and significantly increased morphological characters of sesame plant and seed yield.

Key words: Sesame, wilt, *Fusarium oxysporum* f.sp. *sesami*, biofertilizer

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Introduction

Wilt disease of sesame (*Sesamum indicum* L.) caused by *Fusarium oxysporum* f.sp. *sesami* is the most serious diseases causing losses in seed yield in Egypt (Elewa *et al.*, 1994; Khalifa , 1997; Sahab *et al.*, 2001 ,Mostafa *et al.*, 2003 ; El-Fiki , *et al.*, 2004 ; El-Bramawy *et al.*, 2008 , Sahab *et al.*, 2008, Elewa *et al.*, 2011 and Ziedan *et al.*, 2011) . Application of a biotic and biotic agents with sesame transplantings as root dipping before cultivation were protect sesame plant from wilt disease for a long time and significantly increased sesame yield than with classical sowing by seeds (Elewa *et al.*, 1994; Sahab *et al.*, 2001 ; Mostafa *et al.*, 2003 ; Elewa *et al.*, 2011 and Ziedan *et al.*, 2011). Several biocontrol agents were able to control root diseases of sesame in field using *Trichoderma* spp. (Khalifa , 1997; Sahab *et al.*, 2001 ; Abou Sereih (Neven *et al.*, 2007) ; Elewa *et al.*, 2011. *B. subtilis* (Sahab *et al.*, 2001 ; Jacobsen *et al.*, 2004 and Leclere *et al.*, 2005) . VAMycorrhizae (*Glomus* spp.), (Khalifa , 1997 , Sahab *et al.*,2001; Elewa *et al.*, 2011 and Ziedan *et al.*, 2011). Plant growth promoting rhizobacteria (PGPR) are a group of free-living bacteria that colonize the rhizosphere. This group of rhizobacteria had the potential to improve the plant growth and can contribute to the biological control of plant pathogens *i.e.*, root-rot of wheat caused by *R.solani* (Zarrin(Fatima) *et al.*, 2009). Fungal disease suppression in tomato (Hariprasad and Niranjana, 2009). This work aimed to study the application of biofertilizers for control sesame wilt diseases and their effect on yield production.

Materials and methods

Pathogen

Fusarium oxysporum f.sp. *sesami* (Zap) Cast was obtained from Plant Pathology Department, National Research Centre , Dokki, Cairo , Egypt

Biofertilizers

Commerical biofertilizers *ie.*, cerialin, phosphoren,rhizobacterin and blue green algae were obtained from Agriculture Research Centre. Ministry of Agriculture , Egypt.

Fungicides

Topsin M- 70% w.p (Thiophanate) Diethyl 4-4 (O-phenylene) bis 3-thiolllophanate ,manufacture by Nippa Soda, Japan .Vitavax/captan w.p

(carboxin+captan) Vitavax: 5,6 dihydro-2-methyl-1,4-oxathin 3-carboxinlide (37.5%) + captan: N- ((Trichloromethyl) thio) -4-cyclohexene -1,2-dicarboximide, manufacture by Uniroyal Chemical ,USA.

Antagonistic of biofertilizers agents on pathogen

Antagonistic potential of the main biological components of cerialin and phosphoren are *Azospirillum brasilense* and *Bacillus megatherium* respectively were assayed against *F. oxysporum* f.sp. *sesami* in dual culture on potato dextrose agar medium. Each bacterial isolates were streaked at two margins of plate supplemented by different rate of Topsin M-70% *i.e.*, 0,1,2,5,10 and 20 ppm. Each plate was inoculated in the center by disk 4-mm of fungal growth 7 days old. Five Peti dishes (9cm) were used for each treatment and five plates free were served as a control. Plates were incubated at 28 C for 5-7 days. Linear growth of fungal and conidiospores were calculated 7 days after incubation as well as morphological changes of fungal hyphae was observed 21 days after inocubation using light microscopy.

Biofertilizers inocula and inoculation

Fresh suspension of each biofertilizers (1%) was prepared and supplemented with 0.1% Arabic gum as a stiker before each treatment. Sesame seeds and root of transplantings (25-30 days old) were soaked of each suspension containing one or more than agents for 15 minutes before sowing .

Screening of biofertilizers on Fusarium disease on sesame (in vitro)

Preliminary evaluation of biofertilizers *i.e.*, cerialin, phosphoren, rhizobacterin and blue green algae was carried out on germinated seed of sesame . Sesame seeds Cv. local Kafr El -Sheickh were germinated on wetted filter paper in Petri dishes for two days then soaked of each biofertilizer suspension for 15 minutes and fungicide (Topsin M-70%) at 50 ppm and set on fungal mycelium growth 7 days old. Plates were incubated at 27 C for 5 days . Ten plates were used as replicates and ten plates were served as a control . Disease incidence was determined 7 days after incubation according (Ziedan, 1998).

Treatments

Control

Topsin M-70%

Blue green algae

Rhizobacteren

Cerialin
Phosphoren
Cerialin +Topsin
Phosphoren +Topsin
Cerialin + Phosphoren
10-Cerialin + Phosphoren + Topsin

Field application

Field experiment was carried out at Kafer El Sheickh Governorate Egypt . Sesame seed Cv. local Kafr El-Sheickh was coated by V./captan by rate 3g/kg of seed. Transplantings (30 days old) were soaked of each biofertilizer as mentioned before for 15 days before sowing in the field. Each treatment was replicated three times (replicate = 1/400 feddan). Each plot included 3 rows (1/2 x 7 m) .Replicates were distribution in a complete randomize block design . Transplantings free treatment was served as a control.

Treatments

(A) Seed cultivation

1 - Seed coated by V./captan (3g/kg seed)

(B) Transplanting cultivation

1- Control

2-Topsin

3-Cerialin

4-Phosphoren

5-Cerialin +Topsin

6-Phosphoren +Topsin

Cerialin + Phosphoren

Cerialin + Phosphoren + Topsin

Wilt disease incidence

Percentage of diseased plants and disease severity were recorded 70 days after transplanting. Disease severity was determined of sesame plant shoot on linear scale from 0 to 5 according to (Ziedan , 1993) as follows : 0 = healthy plant 1= chlorosis 2=1/3 plant wilted 3=2/3 plant wilted 4=whole plant wilted 5= plant dead.

Morphological characters and seed yield

At the end of experiment ,plant morphological characters i.e., plant height, number of branches, number of pods per plant and seed yield were determined according to (Ziedan, 1998).

Statistical analysis

Data obtained was statistical analysis using Duncan's multiple range test according to (Snedecor and Cochran, 1980).

Results

Effect of biofertilizers agent and fungicide on fungal pathogen

Data in Table (1) and Fig (1) indicated that *Azospirillum brasilense* and *Bacillus megatherium* are the main biological components of cerialin and phosphoren respectively as well as Topsin M-50% significantly reduced linear growth and condiospores of *Fusarium oxysporum* f.sp. *sesami* than the control. Increasing Topsin M-70% concentrations was increased reduction of fungal growth and condiospore production. Topsin at 20 ppm highly reduced fungal linear growth and completely suppress conidiospores production. In this regard *Bacillus megatherium* showed pronounced effect than *Azospirillum brasilense*.The combination between bacterial isolates and fungicide significantly reduced pathoen linear growth and conidiospors on potato dextrose agar (PDA) agar medium which increased by increasing fungicide concentration. In addition the pattern of *Fusarium oxysporum* f.sp. *sesami* was affected by *A. brasilense* and *B. megatherium* . Hyphae of the fungus malformed ,less thickend ,vaculation and swelling of fungal mycelial cells were observed by light microscopy as shown in Fig (2).

Table 1. Effect of *A. brasilense* ,*B.megatherium* and agents on *F. oxysporum* f.sp.*sesami*

Treatment	Topsin M- 50% (ppm)									
	0		1		5		10		20	
	Linear growth	Conidia No/cm2	Linear growth	Conidia No/cm2	Linear growth	Conidia No/cm2	Linear growth	Conidia No/cm2	Linear growth	Conidia No/cm2
<i>A. brasilense</i>	7.6 b	6.3 b	6.9 a	3.6 b	5.3 a	4.0 b	4.6 a	3.0 a	1.2 a	0.0
<i>B.megatherium</i>	5.1 c	3.0 c	4.4 b	2.0 c	3.4 b	1.3 c	2.8 b	0.8 b	1.0 b	0.0
Topsin M-50%	8.1 b	9.3 a	7.2 a	6.0 a	5.8 a	5.0 a	4.6 b	3.0 a	1.3 a	0.0

Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple test



Fig. 1. Effect of *A. brasilense* , *B. megatherium* and Topsin on linear growth of *F.oxysporum* f. sp. *sesami* Above plates treated by Topsin.,Middle plates treated by Topsin + *A. brasilense* ,Downen plates treated by Topsin + *B. megatherium* , 0 = *F.oxysoprum* f.sp.*sesami* free stress ,S = *A. brasilense* ,P = *B.megatherium* ,) 1= 1ppm and 2 = 5 ppm of Topsin



Fig. 2. Morphological changes of *F.oxysporum* f. sp. *sesami* mycelia by *B. megatherium* stress

Screening of biofertilizers on wilt incidence of sesame

In vitro, the preliminary evaluation of commercial biofertilizers *i.e.*, cerialin, phosphoren, rhizobacterin and blue green algae. Data in Table (2) indicated that all treatments included biofertilizers as combination together or with fungicide (Topsin) significantly reduced sesame seedling infection by *F.oxysporum* f.sp. *sesami* than the control. Phosphoren was the best individual

biofertilizer reduced disease incidence of sesame seedlings followed by topsin. Combined treatments between topsin and each biofertilizer more effective than individual treatment of each components. Combined treatment of cerialin + phosphoren was the best and significant treatment in this respect.

Table 2. Effect of biofertilizers on wilt disease incidence of sesame

Treatment	Disease %	*D.severity
Control	85.0 a	3.4 a
Topsin	49.0 c	2.0 b
Blue green algae	49.0 c	2.0 b
Rhizobacteren	56.0 b	1.2 c
Cerialin	55.0 b	2.2 b
Phosphoren	38.0 d	1.5 bc
Cerialin + Topsin	25.0 e	1.0 c
Phosphoren + Topsin	44.0 c	1.8 b
Cerialin + phosphoren	09.0 f	0.4 d
Cerialin + phosphoren + Topsin	00.0 g	0.0 e

*Diseases severity was determined on linear scale from 0 to 5 according to (Ziedan, 1998) 0 = Healthy seedlings ,1= Root brownish and chlorosis of hypocotyl, 2 = Root brownish and yellowing of hypocotyl, 3= Root and stem brownish and fungal growth hypocotyl, 4= Germilings completely collapsed..

-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple test



Fig. 3. Linear scale from 0 to 4 of Fusarium blight on sesame seedlings
 0 = Healthy seedlings ,1= Root brownish and chlorosis of hypocotyl
 2 = Root brownish and yellowing of hypocotyl, 3= Root and stem brownish and fungal growth hypocotyl, 4= Germilings completely collapsed

Application of biofertilizers on wilt incidence of sesame

Data in Table (3) revealed that all sesame root dipping of biofertilizers and Topsin suspension as single or combined treatments as well sesame transplantings free treatment significantly increased survival plants percentage ,reduced wilt disease percentage and disease severity than seed soaked with V./captan. Cerialin was the best singl treatment recorded high percentage of sesame survival plant and reduced wilt disease percentage followed by Topsin then phosphoren treatments. In this respect, combined treatments between biofertilizers and fungicide as in combined treatments more effective than individual treatment of each. Cerialin + phosphoren + Topsin as combined treatment was the best treatment significantly increased plant survival of sesame, wilt disease incidence followed by Cerialin + phosphoren and Cerialin + Topsin.

Table 3. Effect of biofertilizers on wilt disease incidence of sesame

Treatment	Survival plant %	Wilt incidence	
		Infection %	D.severity
Seed cultivation			
Seed coated V./Captan	35.3 e	81.0 a	4.1 a
Transplanting cultivation			
Control	50.7 d	56.9 b	2.8 b
Topsin	63.7 c	45.6 c	2.3 b
Cerialin	67.9 c	36.0 d	1.8 c
Phosphoren	59.7 d	47.1 c	1.7 c
Cerialin + Topsin	74.8 b	29.8	1.5 c
Phosphoren + Topsin	70.0 b	33.6 d	1.3 cd
Cerialin + phosphoren	85.3 ab	19.1 e	1.0 d
Cerialin + phosphoren + Topsin	97.8 a	17.5 e	0.9 d

Diseases severity was determined on linear scale from 0 to 5 according to (Ziedan, 1998) 0 =Healthy plant, 1=chlorosis only, 2 =1/3 plant wilted , 3 =2/3 plant wilted , 4=whole plant wilted and 5=dead plant .

Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple test

Application of biofertilizers on yield components of sesame

Data in Table (4) revealed that all sesame root dipping of biofertilizers and Topsin suspension as single or combined treatments as well sesame transplantings free treatment significantly increased shoot heigh of plants no of branches,number of pods of each plant and seed yiela of each tratment than seed soaked with V./captan. In this respect, combined treatments between biofertilizers and fungicide as in combined treatments more effective than

individual treatment of each. Cerialin + phosphoren + Topsin as combined treatment was the best treatment significantly increased number of branches, pods and seed yield followed by Cerialin + phosphoren and phosphoren + Topsin.

Table 4. Effect of biofertilizers on morphological characters and yield components of sesame

Treatment	Shoot length (cm)	No branch	No pods	Seed yield aradeb/ feddan
Seed cultivation				
Seed coated V./Captan	125.0	2.3 e	33.3 f	2.7 c
Transplanting cultivation				
Control	133.3	4.0 cd	70.0 e	2.7 c
Topsin	131.0	4.6 c	105.0 d	3.0 b
Cerialin	130.0	4.6	108.3	3.4 b
Phosphoren	135.0	5.0 bc	105.0 d	3.4 b
Cerialin + Topsin	135.0	5.7 a	108.0	4.1 a
Phosphoren + Topsin	133.3	4.3 d	138.7 c	4.2
Cerialin + phosphoren	135.0	6.0	147.0	4.4 ab
Cerialin + phosphoren + Topsin	128.3	6.3 b	203.3 a	4.6 b

Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple test

Discussion

In Egypt, sesame (*Sesamum indicum* L.) are subject to be attacked by many soil borne pathogens. The most destructive diseases are Fusarium wilt caused by *F.oxysporum* f.sp. *sesami* at any growth stages and causing considerable losses of seed yield (Ziedan ,1993 & 1998 ; Elewa *et al.*, 1994 ,Khalifa , 1997 ; Sahab *et al.*, 2001 , Mostafa *et al.*, 2003 ; Abou Sereih (Neven) *et al.*,2007; El-Bramawy *et al.*, 2008 : Sahab *et al.*, 2008 ; Elewa *et al.*, 2011 and Ziedan *et al.*, 2011). Transplanting of sesame gave a good opportunity with biotic and chemical agents to protect plants for a long time to soil borne diseases by suitable agents than in case of seed treatment which may be effective for a short time after sowing (Elewa *et al.*, 1994; Ziedan , 1998 ; Sahab *et al.*, 2001 ; Mostafa *et al.*, 2003 ; Alasee (Najwa) 2006 and Elewa *et al.*, 2011 and Ziedan *et al.*, 2011). There have been many reports of successful uses of biological control agents to control soil borne pathogenic fungi by *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas flourescens* (Harman *et al*, 2004 ; Leclere *et al.*,2005 , Abou Sereih (Neven) *et al.*,2007 , Elewa *et al.*, 2011 and Ziedan *et al.*, 2011).The antagonistic potential

of *Bacillus megatherium* and *Azospirillum brasilense* against *F. oxysporum* f.sp. *sesami* was studied and showed high reduction of such pathogens growth, sporulation these observation were confirmed (Jacobsen *et al.*, 2004 and Leclere *et al.*, 2005 , Elewa *et al.*, 2011 and Ziedan *et al.*, 2011). Application of biofertilizers caused significant reduction wilt of sesame plants incidence and significantly increased yield components such as number of branches, number of pods per plant as well as seed yield . Similar results were obtained by (Khalifa , 1997 ; Ziedan , 1998; Sahab *et al.*, 2001 and ; El-Fiki , *et al.*, 2004, Elewa *et al.*, 2011 and Ziedan *et al.*, 2011) . PGPRs were inhibited root-rot of wheat caused by *R. solani* Zarrin (Fatima) *et al.*, 2009 and Fungal disease suppression in tomato (Hariprasad and Niranjana, 2009). The enhancement of plant growth characters and yield components may be due to ability of biofertilizers agents to provide plant by nutritional requirements and plant growth regulators and vitamins secret . Zarrin (Fatima) *et al.*, 2009 mentioned that three isolates of Azotobacter and Azospirillum produce IAA and possessed phosphorus solubilization capability of *R.solani* growth as well as production, ACC deaminase, siderophore, salicylic acid, hydrogen cyanide, cellulase, chitinase and α -1, 3-glucanase Hariprasad and Niranjana, Zarrin (Fatima) *et al.*, 2009). Application of mixture of two biofertilizers viz, creialin and phiosphoren has resulted in much more intensive plant growth promotion and diseases reduction compared to single biofertilizer treatment .It can be concluded that bifertilizers used successfully for biological control of soil borne plant pathogens.

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(Published in March 2012)