APPLICATION OF MYCORRHIZAE FOR CONTROLLING ROOT DISEASES OF SESAME

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Abstract: Vesicular arbuscular mycorrhizae fungi (VAM) was evaluated as a biotic agent for controlling wilt and root-rot diseases of sesame caused by *Fusarium oxysporum* f. sp. *sesami* (Zap.) Cast and *Macrophomina phaseolina* (Moubl) Ashby pathogens can infect sesame plant at any growth stage causing considerable losses of seed yield. Spores of VA mycorrhizae fungi (*Glomus* spp.) were collected from the soil around the root systems of sesame plants then propagated on roots of Suddan grass (*Sorghum vulgare* var. *sudanese*). Under green house and field conditions, two hundreds sporocarps of *Glomus* spp. were added as a soil drench beside the sesame plant. *Glomus* spp. (VA mycorrhizae) significantly reduced wilt and root-rot incidence of sesame plants. *Lums* spp. (VA mycorrhizae) also significantly increased plant morphological characters such as plant height, number of branches and number of pods for each plant. Application of *Glomus* spp. to protect sesame plants by colonizing the root system, significantly reduced colonization of fungal pathogens in sesame rhizosphere as well as pathogenic activity of fungal pathogens increased lignin contents in the sesame root system were also observed. Furthermore, mycorrhizae treatment provided selective bacterial stimulation for colonization on sesame rhizosphere. These bacteria belonging the Bacillus group showed highly antagonistic potential to fungal pathogens. Application of mycorrhizae together with other biocontrol agent such as *Trichoderma viride* or *Bacillus subtilis* significantly effected than individual treatments for controlling these diseases incidences and increasing morphological characters and seed yield of sesame.

Key words: biocontrol, mycorrhizae, *Sesamum indicum,* wilt, root-rot

INTRODUCTION

Wilt and root-rot diseases of sesame (*Sesamum indicum* L.) caused by *Fusarium oxysporum* f. sp. *sesami* (Zap.) Cast and *Marophomina phaseolina* (Moubl) Ashby are 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; Abou Sereih (Neven) *et al.* 2007; El-Bramawy *et al.* 2008; Sahab *et al.* 2008; Elewa *et al*. 2011 and Ziedan *et al*. 2010]. Application of biotic agents such as *Trichoderma* spp. and VA mycorrhizae (*Glomus* spp.) protected sesame plant from wilt and root-rot disease and significantly increased seed yield. [Khalifa 1997; Sahab *et al.* 2001; Abou Sereih (Neven) *et al.* 2007], *B. subtilis* (Sahab *et al.* 2001; Jacobsen *et al.* 2004; Leclere *et al.* 2005). Sukhada *et al.* (2010) found that plant application of *Glomus mossease* + *Trichoderma harazianum* in field soil infested by *F. oxysporum* f. sp. *cubense* improved banana plant height and reduced the population of *Fusarium* in soil.

This work aimed to control wilt and root-rot diseases on seasame using VA mycorrhizae and rhizospheric biocontrol agents.

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MATERIALS AND METHODS

Pathogens and biocontrol agents

F. oxysporum f. sp. *sesami, M. phaseolina, Bacillus subtilis, T. viride* and VA mycorrhizae (*Glomus* spp.) were obtained from the Plant Pathology Department, National Research Centre , Dokki, Cairo , Egypt.

Chosen antagonistic isolates against pathogens

Antagonisntic potential of *B. subtilis* and *T. viride* isolates against both pathogenic fungi was assayed in dual culture on Potato Dextrose Agar medium (PDA). The Inhibition zone of fungal growth was recorded and the percentage of fungal growth reduction was calculated according Elewa *et al.* (2009).

Mycorrhizae colonization of sesame root

The percentage of sesame root colonization by Vesicular arbuscular mycorrhizae fungi (VAM) was determined (Phillips and Hayman 1970) at the flowering stage. The root system was washed with tap water several times to remove the adhering soil particles. Roots were cut into small segments and treated with 10% potassium hydroxide (KOH) in test tubes, then heated in water bath for 10 minutes at 80–90°C. Thereafter, root segments were washed with tap water followed by 10% HC1, then stained with a 0.5% trypan blue solution, and heated again at 80–90°C for 5 minutes. Root segments were placed on glass slides, then a few drops of lactic acid were added. Mycorrhizal infection was recorded in each segment to calculate the percentage of root infection.

Biocontrol agents inocula

T. viride suspension 1x10^8 CFU/ml was prepared from 14-days-old culture on PDA. One percent Arabic gum as a sticker was added to the suspension (Ziedan 1998). *B. subtilis* suspension at 1x10^8 was obtained from slants of nutrient agar medium from 2-days-old at 28°C. *Glomus* spp. spores were collected from rhizosphere of sesame regions then propagated by *Sorghum vulgare* var *sudanese*. VA mycorrhizae were isolated by the wet sieving and decanting technique described by (Gerdemann and Niclson 1963). A set of sieves with pores sizes of 400, 315, 250, 160, 71 and 63 mM were used and counted under 50x magnification of a binocular microscope. Spores were kept on wet Whatman No. 1 filter papers in Petri-dishes. Two hundred spores were added as a soil drench at the stem base of the sesame plant after transplanting. The following were the treatments:

1 – the control,
2 – *B. subtilis*,
3 – *T. viride*,
4 – *Glomus* spp. (VAM),
5 – VAM + *B. subtilis*,
6 – VAM + *T. viride*,
7 – VAM + *B. subtilis* + *T. viride*.

Pot experiments

The experiment was carried out at the National Research Centre, Plant Pathology Dept. in soil that was infested by *F. oxysporum* f. sp. *sesami* or *M. phaseolina*. Infested soil was prepared using the technique described by Ziedan 1998. Sesame transplants (30-days-old) of vv. Giza 25 had their roots dipped in each suspension of *B. subtilis* and *T. viride* for 15 minutes before sowing (Elewa *et al.* 1994). Meanwhile, *Glomus* spp. was added at the stem base of the plants after sowing at the rate of 200 spores/plant according to Ziedan (1998). Four pots were used for each treatment. Six transplants were set in each pot. A set of five uninfested pots (25 cm diameter) served as control.

Field application

The experiment was carried out in El-Fayoum Governorate, Egypt in the field which had a long history of wilt and root-rot diseases of sesame. Inoculation by *Glomus* spp. inoclum, was done as mentioned before. Six replicates for each treatment using a randomized block design were made. A replicate = 1/400 feddan (10.5 m^2).

Pathogens count in sesame rhizosphere

Fusarium spp. was estimated on peptone PCNB agar medium (Nash and Snyder 1962) and *M. phaseolinea* was estimated on PDA amended by PCNB and streptomycin sulfate as the selective medium (Alabouvette 1977) at a dilution of 10^{-3}. Fungal plates were incubated at 27±2°C. Colony growth was observed after 3–6 day.

Wilt and root-rot diseases incidence

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

Sesame plant growth

Morphological characters *i.e.*, length, diameter and fresh weight of shoot, root size, number of branches and pods, seed yield and oil percentage were determined according to Ziedan (1998).

Statistical analysis

Statistical analysis was done using Duncan's multiple range test at 5% significance according to Snedecor and Cocharn (1980).

RESULTS

Antagonistic potential against pathogens

B. subtilis and *T. viride* isolates exhibited obvious antagonism against *F. oxysporum* f. sp. *sesami* and *M. phaseolina*. *B. subtilis* exhibited better antagonism against both pathogens. Data in figures 1 and 2 show the observed clear zone of pathogens hyphal growth as well as *Glomus* spp. showed inter-and intracellular growth of cortex cell tissue.

F. oxysporum — *F. oxysporum* + *T. viride* — *F. oxysporum* + *B. subtilis*

Fig. 1. Antagonism of *T. viride* and *B. subtilis* on *F. oxysporum*

M. phaseolina | *M. phaseolina* + *T. viride* | *M. phaseolina* + *B. subtilis*

Fig. 1. Antagonism of *T. viride* and *B. subtilis* on *M. phaseolina*

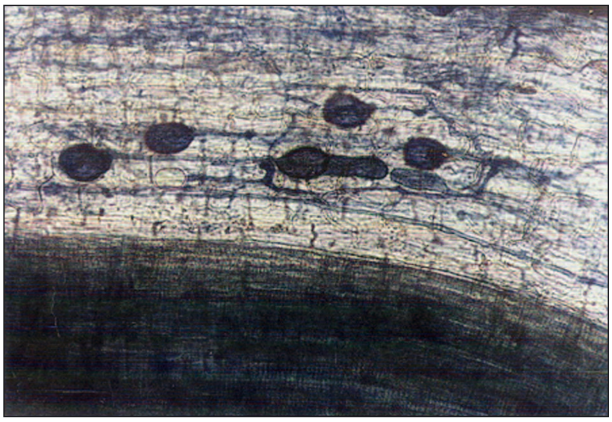

Fig. 2. Colonization of *Glomus* spp. sporocarps and mycelium hyphae on sesame root

Pot experiments

Effect of mycorrhizae on wilt disease and morphological characters of sesame

Data in table 1 indicate that all treatments significantly reduced wilt disease caused by *F. oxysporum* f. sp. *sesami*. *Glomus* spp. individually or in combinations with *B. subtilis* or *T. viride* significantly reduced wilt disease of sesame. *Glomus* spp. + *B. subtilis* followed by *Glomus* spp. + *B. subtilis* + *T. viride* were the best treatments. These treatments significantly increased shoot length and fresh weight as well as number of pods per plant.

Effect of mycorrhizae on root-rot disease and effect of mycorrhizae on the morphological characters of sesame

Data in table 2 indicate that all treatments significantly reduced root-rot disease caused by *M. phaseolina*. *Glomus* spp. individually or in combination with *B. subtilis* or *T. viride* significantly reduced root-rot disease of sesame and significantly increased shoot length, fresh weight of shoot system and number of pods per plant. No significant differences between VAM, *B. subtilis* and *T. viride* were noticed as treatments for reducing root-rot disease. The combined treatment *Glomus* spp. + *B. subtilis* + *T. viride* was the best treatment. It significantly increased shoot length and fresh weight of sesame.

Effect of mycorrhizae on pathogen count of sesame rhizosphere

Data in table 3 indicate that all biological treatments significantly reduced number of colony forming unites of two fungal pathogens. VA mycorrhizae suppressed the *M. phaseolina* count in sesame rhizosphere followed by *T. viride*.

Table 1. Effect of mycorrhizae on wilt disease and the effect of mycorrhizae on morphological characters of sesame

Treatments	Wilt disease incidence		Morphological characters/plant		
	% of diseased plants	disease severity	length [cm]	fresh weight [g]	No. of pods
Control	79.2 a	4.0 a	68.3 d	7.4 d	6.0 c
B. subtilis	66.7 ab	3.3 b	80.0 c	11.7 c	6.7 c
T. viride	50.0 b	2.5 c	103.8 ab	12.6 c	14.7 b
VAM	50.0 b	2.5 c	80.0 c	14.4 c	6.8 c
VAM + *B. subtilis*	29.2 d	1.5 d	115.6 a	18.7 b	19.0 a
VAM + *T. viride*	36.7 c	1.3 d	93.3 b	15.0 c	14.0 b
VAM + *B. subtilis* + *T. viride*	37.5 c	1.1 d	106.0 ab	25.3 a	20.0 a

VAM – Vesicular arbuscular mycorrhizae fungi

Diseases severity was determined on a linear scale from 0 to 5 according to Ziedan 1993:

0 – healthy plant, 1 – chlorosis only, 2 – 1/3 of plant wilted,

3 – 2/3 of plant wilted, 4 – whole plant wilted, 5 – dead plant

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

Table 2. Effect of mycorrhizae on root-rot disease and effect of the effect of mycorrhizae on morphological characters of sesame

Treatments	Root-rot incidence		Morphological characters /plant		
	% of diseased plants	disease severity	length [cm]	fresh weight [g]	No. of pods
Control	91.7 a	4.6 a	77.5 c	5.52 d	4.61 f
B. subtilis	50.0 c	2.5 c	101.9 a	19.4 a	12.6 b
T. viride	45.8 d	2.3 c	101.3 a	18.1 a	10.3 c
VAM	45.8 d	2.5 c	80.0 b	8.1 c	6.0 e
VAM + *B. subtilis*	45.8 d	2.4 c	104.4 a	18.2 a	9.8 d
VAM + *T. viride*	43.7 b	3.3 b	104.2 a	17.7 b	9.5 d
VAM + *B. subtilis* + *T. viride*	41.7 c	2.1 d	103.8 a	19.3 a	13.0 a

VAM – Vesicular arbuscular mycorrhizae fungi
Diseases severity was determined on a linear scale from 0 to 5 according to Ziedan 1993:
0 – healthy plant, 1 – chlorosis only, 2 – 1/3 of plant wilted, 3 – 2/3 of plant wilted, 4 – whole plant wilted, 5 – dead plant
Values followed by the same letter are not significantly different at $p \geq 0.05$ according to Duncan's multiple range

Table 3. Effect of mycorrhizae on pathogen count of sesame rhizosphere

Treatments	Pathogen count x 1,000/g soil	
	F. oxysporium	*M. phaseolina*
Control	233.0 a	30.0 a
B. subtilis	46.6 c	9.6 b
T. viride	76.9 b	3.5 c
VAM	29.9 d	0.0 d

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

Field application

Application of mycorrhizae for wilt and root-rot diseases of sesame

Data in table 4 reveale that all treatments significantly increased plant survival and reduced the number of wilt and root-rot diseased sesame plants and that treatments reduced disease severity. *T. viride* was the best individual treatment which increased plant survival and reduced disease in sesame plants caused by wilt and root-rot. The second best individual treatment was VAM. All combined treatments included VAM + *T. viride* or *B. subtilis* were better than the individual treatments for reducing wilt and root-rot incidence of plants. VA mycorrhizae + *T. viride* was the best treatment recorded high survival percentage of sesame plants and significantly reduced wilt and root-rot incidence. The combined treatment of *Glomus* spp. + *B. subtilis* + *T. viride* was next best.

Table 4. Effects of the application of mycorrhizae on wilt and root-rot of sesame

Treatments	Wilt and root-rot incidence		
	% of survival plants	% of diseased plants	disease severity
Control	51.0 d	55.9 a	2.8 a
B.subtilis	54.1 c	50.0 b	2.5 b
T.viride	67.5 b	39.2 c	1.9 c
VAM	56.7 c	48.4 bc	2.4 b
VAM + *B. subtilis*	66.6 b	34.2 cd	1.7 c
VAM + *T. viride*	79.3 a	23.3 e	1.2 e
VAM + *B. subtilis*+ *T. viride*	76.0 a	30.9 d	1.5 cd

VAM – Vesicular arbuscular mycorrhizae fungi
Diseases severity was determined on a linear scale from 0 to 5 according to (Ziedan 1993):
0 – healthy plants, 1 – chlorosis only, 2 – 1/3 of plant wilted, 3 – 2/3 of plant wilted, 4 – the whole plant wilted, 5 – dead plants
Values followed by the same letter are not significantly different at $p \geq 0.05$ according to Duncan's multiple range test

Effect of mycorrhizae on morphological characters and effect of mycorrhizae on yield components of sesame

Data in table 5 indicate that *B. subtilis, T. viride* and *Glomus* spp. as single or combined treatments significantly increased shoot length, diameter, root size. number of branches, pods and seed yield. In general, combined treatments were better than single treatments. The VAM + *B. subtilis* + *T. viride* mixture was the best treatment and it increased shoot length and diameter, root size, number of branches, pods and seed yield. No significant differences between all the treatments and the control as far as sesame seed oil concerned were noticed.

Effect of mycorrhizae on pathogens of sesame rhizosphere

Data in table 6 reveal that all treatments significantly reduced the total amount of Fusarium in sesame rhizosphere. VA mycorrhizae as individual treatment was the best in reducing the Fusarium count followed by *B. subtilis*. The best treatment of all which reduced Fusarium counts and Fusarium's pathogenic potential was the combined treatment of VA mycorrhizae + *T. viride.* On the other hand, a significant in the reduction pathogenic activity of Fusarium isolates obtained from sesame rhizosphere was obtained with the use of *Glomus* spp. + *B. subtilis* + *T. viride* followed by *Glomus* spp. + *T. viride* and then VA mycorrhizae as an individual treatment.

Effect of the application of mycorrhizae on lignin content in sesame root

Data in table 7 indicate that all treatments of *B. subtilis, T. viride* and VA mycorrhizae significantly increased lignin content in sesame roots. *T. viride* followed by VAM were the best treatments for increasing the lignin content in sesame root.

Table 5. Effects of the application of mycorrhizae on morphological characters, seed yield and % of oil in sesame seeds

Treatments	Shoot		Root size	Number/plant		Seed yield aradeb/ feddan	Oil [%]
	length [cm]	diameter [cm]		branches	pods		
Control	185.0 e	1.76 d	25.0 f	3.75 f	112.5 e	2.53 d	59.5
B. subtilis	196.3 c	1.99 b	50.0 b	5.3 e	197.5 c	4.55 c	56.9
T. viride	180.0 d	1.88 c	35.0 d	7.5 b	212.5 b	4.91 c	57.8
VAM	195.0 c	1.85 c	30.0 e	5.0 e	160.0 d	5.14 b	57.4
VAM + *B. subtilis*	210.0 a	1.77 d	35.0 c	6.75 c	196.3 c	4.95 c	57.1
VAM + *T. viride*	202.5 b	1.82 c	47.5 b	6.0 d	198.0 c	5.05 b	57.2
VAM + *B. subtilis* + *T. viride*	202.5 b	2.33 a	70.0 a	8.5 a	232.5 a	5.79 a	57.8

Values followed by the same letter are not significantly different at $p \geq 0.05$ according to Duncan's multiple range test
VAM – Vesicular arbuscular mycorrhizae fungi

Table 6. Effects of the application of mycorrhizae on the *Fusarium* spp. count in sesame rhizosphere

Treatment	*Fusarium* spp.		
	No./g of soil x10	count reduction [%]	pathogenic [%]
Control	36.25 a	0.00 e	36.3 b
B.subtilis	8.31 bc	77.08 c	76.7 a
T.viride	9.71 b	73.22 c	75.0 a
VAM	5.59 d	84.58 b	33.3 b
VAM + *B. subtilis*	9.93 b	69.68 d	–
VAM + *T. viride*	10.77 b	70.29 c	30.0 b
VAM + *B. subtilis* + *T. viride*	2.94 e	91.89 a	15.0 c

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

Table 7. Application of mycorrhizae on lignin content of sesame root

Treatment	Lignin content of sesame root [mg/g] dry root
Control	225 d
B. subtilis	265 c
T. viride	475 a
VAM	350 b

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

DISCUSSION

In Egypt, sesame is attacked by many soil borne pathogens. The most destructive of the soil borne pathogens are Fusarium wilt caused by *F. oxysporum* f. sp. *sesami* and *M. phaseolina.* Fusarium wilt caused by *F. oxysporum* f. sp. *sesami* and root-rot is incited by *M. phaseolina.* Both pathogens can infect plants at any growth stage and cause considerable losses in the 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; Ziedan *et al*. 2010].

The use of biological agents to control soil borne pathogenic fungi is an attractive possibility. There have been many reports of the successful uses of *T. harzianum*, *T. viride*, *B. subtilis* and *Pseudomonas flourescens* [Kang and Kim 1989; Deacon and Berry 1993; Hyun *et al*. 1999; Harman *et al.* 2004; Leclere *et al.* 2005; Abou Sereih (Neven) *et al*. 2007]. Transplanting of sesame provided a good opportunity for using biotic and chemical agents to protect plants for a long time against soil borne diseases in comparison to seed treatment which may only be effective for a short time after sowing [Deacon and Berry 1993; Elewa *et al.* 1994; Ziedan 1998; Sahab *et al.* 2001; Mostafa *et al.* 2003; Alasee (Najwa) 2006; Elewa *et al*. 2011].

The antagonistic interaction between *Trichoderma* spp. and *F. oxysporum* f. sp. *sesami* and *M. phaseolina* was extensively studied. *T. viride* attacks *F. oxysporum* f. sp *sesami* by coiling around the hyphae and penetrating into hyphae. (Chung and Choi 1992; Harman *et al*. 2004; Elewa *et al.* 2011). Also, the antagonistic potential of *B. subtilis* against both pathogens was studied. *B. subtilis* showed a high reduction of pathogen growth, sporulation and sclerotial formation (Shin *et al.* 1987; Jacobsen *et al*. 2004; Leclere *et al*. 2005; Elewa *et al*. 2011). Application of *Glomus* spp. caused significant reduction of wilt and root-rot of sesame. Similar results were obtained by Khalifa (1997), Ziedan (1998), Sahab *et al*. (2001), El-Fiki *et al*. 2004 and Ziedan *et al*. (2010). VA mycorrhizae was able to colonize sesame roots in soil infested by *F. oxysporum* f. sp. *sesami* than in the soil infested with *M. phaseolina*. (Khalifa 1997; Ziedan 1998; Sahab *et al*. 2001). In this respect, Sukhada *et al*. (2010) found that application of *G. mossease + T. harazianum* to banana field soil infested by *F. oxysporum* f. sp. *cubense* improved plant height and reduced population of *Fusarium*. VA mycorrhizal fungi protect plants by illuminating the pathogens in sesame rhizosphere and/or reducing the pathogenic activity and improving resistance due to the increase of antifungal chitinase enzymes in roots (Dehne *et al*. 1978). VA mycorrhizae fungi also increases lignin content in root system (Linderman 1992; Ziedan 1998; Mostafa *et al*. 2003; Ziedan *et al*. 2010). The use of mixed inocula of mycorrhizal symbionts and biocontrol agents can be more effective than the use of a single species.

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