APPLICATION OF MYCORRHIZAE FOR CONTROLLING ROOT DISEASES OF SESAME

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Received: December 7, 2010
Accepted: June 6, 2011

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 Sudan grass (Sorghum vulgare var. sudanense). 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 Macrophomina 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 mossea + Trichoderma harzianum 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 sesame using VA mycorrhizae and rhizospheric biocontrol agents.

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

Antagonistic 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% HCl, 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 1x10⁸ 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 1x10⁸ 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 Nicolson 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. A replicate = 1/400 feddan (10.5 m²).

**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. inoculum, was done as mentioned before. Six replicates for each treatment using a randomized block design were made. A replicate = 1/400 feddan (10.5 m²).

**Pathogens count in sesame rhizosphere**

*Fusarium* spp. was estimated on peptone PCNB agar medium (Nash and Snyder 1962) and *M. phaseolina* 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.
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 combination with *B. subtilis* or *T. viride* significantly reduced wilt disease 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 units 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Wilt disease incidence</th>
<th>Morphological characters/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of diseased plants</td>
<td>disease severity</td>
</tr>
<tr>
<td>Control</td>
<td>79.2 a</td>
<td>4.0 a</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>66.7 ab</td>
<td>3.3 b</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>50.0 b</td>
<td>2.5 c</td>
</tr>
<tr>
<td>VAM</td>
<td>50.0 b</td>
<td>2.5 c</td>
</tr>
<tr>
<td>VAM + <em>B. subtilis</em></td>
<td>29.2 d</td>
<td>1.5 d</td>
</tr>
<tr>
<td>VAM + <em>T. viride</em></td>
<td>36.7 c</td>
<td>1.3 d</td>
</tr>
<tr>
<td>VAM + <em>B. subtilis</em> + <em>T. viride</em></td>
<td>37.5 c</td>
<td>1.1 d</td>
</tr>
</tbody>
</table>

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 ≥ 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 reveals 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Wilt and root-rot incidence</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of survival plants</td>
<td>% of diseased plants</td>
</tr>
<tr>
<td>Control</td>
<td>51.0 d</td>
<td>55.9 a</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>54.1 c</td>
<td>50.0 b</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>67.5 b</td>
<td>39.2 c</td>
</tr>
<tr>
<td>VAM</td>
<td>56.7 c</td>
<td>48.4 bc</td>
</tr>
<tr>
<td>VAM + <em>B. subtilis</em></td>
<td>66.6 b</td>
<td>34.2 cd</td>
</tr>
<tr>
<td>VAM + <em>T. viride</em></td>
<td>79.3 a</td>
<td>23.3 e</td>
</tr>
<tr>
<td>VAM + <em>B. subtilis</em> + <em>T. viride</em></td>
<td>76.0 a</td>
<td>30.9 d</td>
</tr>
</tbody>
</table>

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 ≥ 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot length [cm]</th>
<th>Shoot diameter [cm]</th>
<th>Root size branching</th>
<th>Seed yield aradeb/ feddan</th>
<th>Oil [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>185.0 e</td>
<td>1.76 d</td>
<td>25.0 f</td>
<td>3.75 f</td>
<td>25.0 f</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>196.3 c</td>
<td>1.99 b</td>
<td>30.0 e</td>
<td>5.3 e</td>
<td>4.91 c</td>
</tr>
<tr>
<td>T. viride</td>
<td>180.0 d</td>
<td>1.88 c</td>
<td>35.0 d</td>
<td>7.5 b</td>
<td>4.91 c</td>
</tr>
<tr>
<td>VAM</td>
<td>195.0 c</td>
<td>1.85 c</td>
<td>30.0 e</td>
<td>5.0 e</td>
<td>5.14 b</td>
</tr>
<tr>
<td>VAM + B. subtilis</td>
<td>210.0 a</td>
<td>1.77 d</td>
<td>35.0 c</td>
<td>6.75 c</td>
<td>4.95 c</td>
</tr>
<tr>
<td>VAM + T. viride</td>
<td>202.5 b</td>
<td>1.82 c</td>
<td>47.5 b</td>
<td>6.0 d</td>
<td>5.05 b</td>
</tr>
<tr>
<td>VAM + B. subtilis + T. viride</td>
<td>202.5 b</td>
<td>2.33 a</td>
<td>70.0 a</td>
<td>8.5 a</td>
<td>5.79 a</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different at p ≥ 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fusarium spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./g of soil x10</td>
</tr>
<tr>
<td>Control</td>
<td>36.25 a</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>8.31 bc</td>
</tr>
<tr>
<td>T. viride</td>
<td>9.71 b</td>
</tr>
<tr>
<td>VAM</td>
<td>5.59 d</td>
</tr>
<tr>
<td>VAM + B. subtilis</td>
<td>9.93 b</td>
</tr>
<tr>
<td>VAM + T. viride</td>
<td>10.77 b</td>
</tr>
<tr>
<td>VAM + B. subtilis + T. viride</td>
<td>2.94 e</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different at p ≥ 0.05 according to Duncan’s multiple range test

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lignin content of sesame root [mg/g] dry root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>225 d</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>265 c</td>
</tr>
<tr>
<td>T. viride</td>
<td>475 a</td>
</tr>
<tr>
<td>VAM</td>
<td>350 b</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different at p ≥ 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.; 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 coating 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. mosseae* + *T. harzianum* 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.

REFERENCES


