Chapter

Assessment of Biocontrol Potential of Arbuscular Mycorrhizal (*Glomus* spp.) against Damping-off Disease (*Rhizoctonia solani*) on Cucumber

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Abstract

Rhizoctonia solani is one of the most important causative agents of damping-off diseases on cucumber plants and significantly reduces their yield. *R. solani* possesses some characteristics, such as wide host range and unlimited survival in soil, which made it most difficult to control. Therefore, the research for a biocontrol agent will be valuable to control this disease. Two species of mycorrhizal fungi (*Glomus mosseae* and *Glomus clarum*) that were evaluated against the agent *R. solani* reduced the damping-off disease on the cucumber plant. Mycorrhizal-inoculated plants with both species showed a significant reduction in disease severity (*DS*), which were 21 and 25%, respectively, whereas the disease severity was 65% for non-inoculated plants. Furthermore, the effects of mycorrhizal fungi were evaluated against the growth parameters of cucumber plants. Plants inoculated with both species of mycorrhizal fungi showed a significant increase in both shoot dry weight and root dry weight compared with uninoculated plants. In conclusion, both mycorrhiza species could be an important tool to control soil-borne pathogens, increase plant's nutrients' absorption, and increase resistance to abiotic stresses.

Keywords: biological control, *Rhizoctonia solani*, arbuscular mycorrhiza, cucumber, damping-off diseases

1. Introduction

Rhizoctonia solani Kühn, the causative agent of damping-off disease in a variety of crop plants such as cucumber, is an economical important soil-borne pathogen [1, 2]. *R. solani* fungus is considered as a difficult pathogen to control due to several characters such as the great variability in the pathogen population, a wide host range, and long-term survival in soil [3]. Further, some cultural practices including the crop rotation, sanitation, and soil solarization with *R. solani* are not sufficiently effective because the pathogen is able to survive for many years in soil. The application of chemical pesticides, mainly methyl bromide, is the most reliable method to

control *R. solani*; however, it causes serious risks including polluting the air, damaging the environment, building fungicides' resistance of pathogen, and harming the human health [4, 5]. Therefore, the biological control method becomes an important component of the disease management to increase crop production and food safety [6].

The biological control becomes an important target of many researchers in the field of biological and agricultural sciences [5]. Biocontrol agents use different mechanisms of action against fungal pathogens, such as antimicrobial compound production activity, mycoparasitism or hyperparasitism, cell wall-lytic enzyme activity, and the application of systemic resistance (ISR) activity [7]. In addition, some biocontrol agents are capable of improving some aspects of plant growth, such as the germination rate, shoot and root weight, nutrients' uptake, and yield [8].

Arbuscular mycorrhizal (AM) fungi have been known to form a symbiotic relationship with around 80% of vascular plants. The symbiotic relationship can provide the plant with many benefits, including enhancement of plant growth and germination rates, increasing supplement of water and nutrients [9, 10]. In return, the AM fungi are completely dependable on the nutrients that are coming from the living root system [9]. In addition, AM fungi have been known to increase the host's resistance to a wide range of fungal and bacteria pathogens, especially rot pathogens [11]. The aim of this study was to examine the influence of different species of arbuscular mycorrhizal (AM) fungi (*Glomus* spp.) to promote systemic resistance against the disease agent of damping-off disease (*R. solani* Kühn) on cucumber (*Cucumis sativus* L.).

2. Materials and methods

Infected samples were brought from cucumber plants with wilting, yellowing, and dwarfing symptoms from a field related to the College of agriculture, University of Al-Qadisiyah. The plants were washed with sterilized water to remove soil residues and were cut to small pieces. Then, the samples were sterilized with sodium hypochlorite (NaCIO) 1% for 2 min, washed with sterilized water twice, and dried with filter papers. Nine petri dishes of potato dextrose agar (PDA) were inoculated with five pieces of the infected plants and incubated for 3 days at 25°C. Soil samples were diluted for pathogen isolation and the petri dishes were incubated at 27°C. Both plant and soil samples were kept in a refrigerator at 4°C and diagnosed using classification keys [12].

Isolated pathogens were stored at 4°C prior to analysis and incubated at 25°C for 3 days. From the colony edge, four populated agar disks (7 mm) were cut and mixed in a 250 ml flask containing 100 ml of potato dextrose broth and 25 mg of chloramphenicol [13]. Sterilized soils were separated on each pot (3 kg) and inoculated with 1 ml from pathogen broth culture, and sterilized water was used for the control. Then, all pots were irrigated and covered for 3 days. Cucumber seeds were disinfected with sodium hypochlorite (NaCIO) 1% for 4 min and were planted in each pot. Germinated, not germinated seeds, and collapsed plants were recorded after 7 and 10 days for planting, and disease intensity was calculated as recommended [14]: 0 = no symptoms; 1 = seed rot, not germinated; 2 = brown rot on the stem base, plant is still standing; 3 = plant is wilted, laying on the ground; and 4 = plant is dead. *DS* was calculated from disease grades 0–3 using the following formula [15]:

$$DS = \frac{\sum(f * v)}{N * X} \times 100 \quad (1)$$

where *DS* = disease severity, *f* = infection class frequencies, *v* = number of plants of each class, *N* = total of observed plants, and *X* = highest value of the evaluation scale.

Cucumber seeds were surface-sterilized using 0.2% NaCIO for 2 min and rinsed several times with distilled water. Arbuscular mycorrhizal (AM) fungi were obtained from the Iraqi Ministry of Sciences and Technology's laboratory. This mixture consists of propagated units of *Glomus clarum* (Nicol. Schenck) and *Glomus mosseae* (Nicol. Gerd) in a suspension form (1×10^6 unit L^{-1} concentration). *Glomus* spp. were identified and separated in two tubes by the experts at Iraqi Ministry of Sciences and Technology's laboratory. Six healthy seeds of cucumber were planted in each pot (25 cm in diameter), which contained 3 kg of sterilized soil (clay:sand, 2:1, v/v) into each pot. For mycorrhizal inoculum, each pot was inoculated with dilution of 5 ml of either *Glomus clarum* or *G. mosseae*/L^{-1} water twice at the beginning of cultivation and after 14 days. As controls, the pots were provided with no AM + no pathogen, AM only, and pathogen only. For the pathogen inoculum, 5 ml of spore suspension (*R. solani*) was added at the beginning of cultivation. Six treatments were conducted as the following: *Glomus clarum*, *G. mosseae*, *G. clarum* + *R. solani*, *G. mosseae* + *R. solani*, control, and control + *R. solani*. Four replicates were made for each treatment. In this study, all plants did not receive any fertilizer and were watered when necessary at outdoor conditions. The disease severity for each treatment was monitored and estimated as mentioned above [16].

When the plants emerged above the soil surface, five plants were harvested from each treatment after 5, 10, 15, and 20 days. The plants were washed with tap water to clean off soil particles. Fresh and dry weights were evaluated and recorded after drying the samples by a hot air oven at 60°C for 48 h until gaining constant weight [17].

3. Results and discussion

Five pathogens were isolated form the infected plants and soil. The fungal identification was performed according to the morphological characteristic as previously reported in literatures [18, 19]. Among five isolated pathogens, *R. solani* showed the highest disease severity (*DS*) on cucumber plants, which was about 63%, while *Penicillium* spp. showed the lowest disease severity (*DS*), which was about 8% (**Figure 1**). Therefore, *R. solani* was the most aggressive pathogen due to the suitable environment condition, and the availability of susceptible hosts and was used for all subsequent studies.

The effect of AM fungi against *R. solani* on cucumber plants was studied by the inoculation of cucumber plants with the AM, *G. mosseae* + *G. clarum*, which showed a significant reduction in the disease severity of damping-off compared with control (**Figure 2**). Disease severity (*DS*) of mycorrhizal plants was reduced by 46% and 41%, respectively. Furthermore, inoculated plants with mycorrhiza showed fewer symptoms compared with non-mycorrhizal plants. Disease severity in AM-inoculated plants with *G. mosseae* was about 20%, which was slightly less than AM-inoculated plants with *G. clarum* (**Figure 2**).

The effect of AM fungi on the growth parameters of cucumber plants was assessed by shoot dry weight and root dry weight. AM fungi-colonized plants had significantly increased shoot and root dry weights when compared with the non-mycorrhizal plants (**Table 1**). Cucumber plants, colonized with AM (*G. mosseae*), showed a slight increase in all growth parameters compared with the plant colonized with AM (*G. clarum*), which matches with our results on the *DS* experiment (**Table 1**).

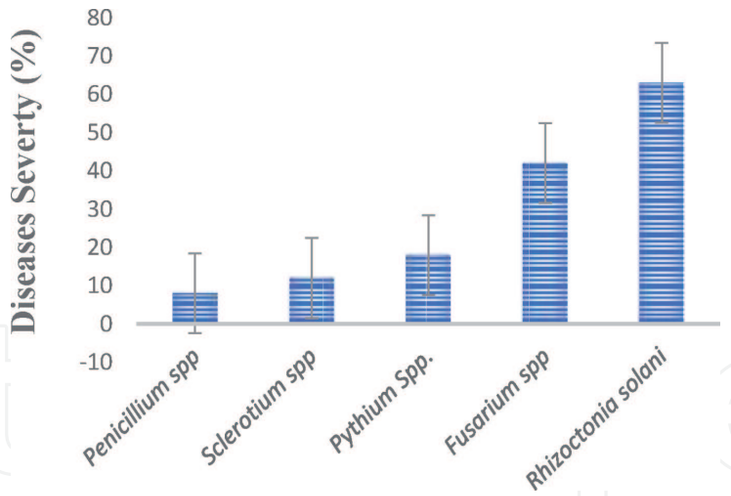

Figure 1.
Pathogenicity test for isolated pathogens against damping-off diseases on cucumber. Each column represents the mean of five replicates. Bars on the pillars represent standard error and LSD = 5.73 (P = 0.01).

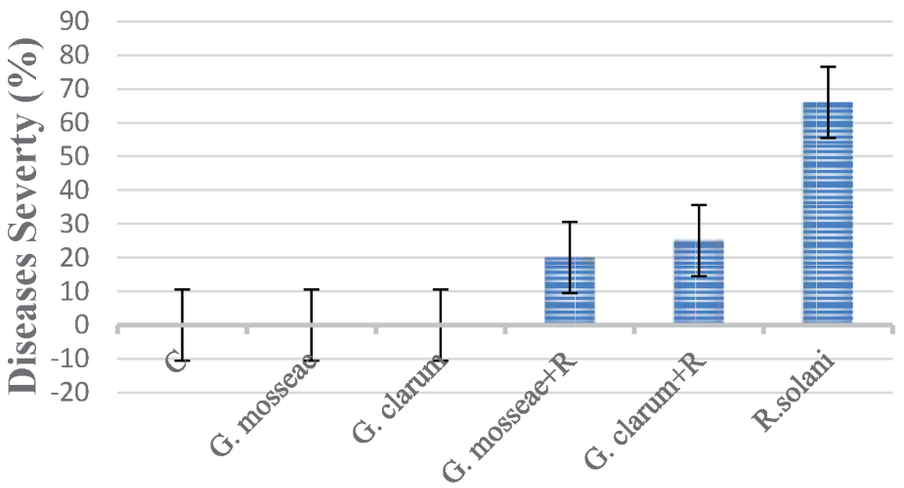

Figure 2.
Evaluation of arbuscular mycorrhizal (AM) fungi on the disease severity of damping-off diseases on cucumber. Each column represents the mean of four replicates. Bars on the pillars represent standard error and LSD (P = 0.01).

Mycorrhizal fungi are considered as ideal biocontrol agents due to some characteristics such as the ability to form a mutualistic symbiosis relationship with the roots of most vascular plant species [20]. Moreover, the plant-mycorrhiza relationship benefits the plant not only to control soil-borne pathogens but also to enhance the plant's resistance to various abiotic stresses and increases the nutrients' absorption [21].

In the present study, inoculated plant with mycorrhizal fungi reduces significantly the disease severity of *R. solani* pathogen, which may be attributed to increase the nutrients' status, reduce the direct competition for root space and resources with the pathogen, induce the plant's immunity to involve certain systemic mechanisms such as the systemic acquired resistance (SAR) and cell wall defenses, and enhance the production of defense compounds such as phenolics, -1,3-glucanase, and chitinolytic enzymes [9]. Additionally, inoculated plants with mycorrhizal fungi (*G. mosseae*) showed a lower disease severity than *G. clarum*, which may lead to a potential active control tool. Furthermore, the inoculation with mycorrhizal fungi increases both the root dry weight and shoot dry weight, which supports our hypothesis.

Mycorrhizal fungi play a main part in plant defense against pathogens and form a mutual relationship with plants. In summary, both mycorrhiza species could be

Treatment	Shoot dry weight (g/plant)				Root dry weight (g/plant)			
	5 days	10 days	15 days	20 days	5 days	10 days	15 days	20 days
Control	0.5	0.8	0.9	1.1	0.2	0.4	0.7	0.9
Control + *R. solani*	0.1	0.3	0.4	0.5	0.08	0.1	0.2	0.3
Glomus clarum	0.4	0.6	0.7	1.2	0.15	0.3	0.6	0.8
G. mosseae	0.6	0.7	0.8	1.1	0.2	0.4	0.7	0.9
G. clarum + *R. solani*	0.3	0.5	0.6	0.9	0.15	0.2	0.5	0.6
G. mosseae + *R. solani*	0.4	0.6	0.8	1	0.2	0.3	0.6	0.7

Table 1.
Evaluation of AM fungi on the growth parameters of cucumber plants.

an important tool to control soil-borne pathogens, increase plant nutrient absorption, and increase resistance to abiotic stresses. In future research, specific systemic mechanisms of mycorrhiza fungi against pathogens should be investigated more.

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