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Research Article

Assessment of arbuscular mycorrhizal fungi (*Glomus* spp.) as potential biocontrol agents against damping-off disease *Rhizoctonia solani* on cucumber

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Abstract: Damping-off disease, caused by the fungus *Rhizoctonia solani*, is one of the most important diseases of cucumber plant and causes significant yield losses. *R. solani* possess some characters, such as wide host range and unlimited survival in soil, that make it as pathogen one of the most difficult agents to control. Therefore, the research for finding a biocontrol agent against this disease will be valuable. Two species of mycorrhizal fungi *Glomus mosseae* and *Glomus clarum* were evaluated against *R. solani* on cucumber plants. Mycorrhiza inoculated plants with both species showed a significant reduction in disease severity (DS), 21% and 25%, respectively, whereas the disease severity was 65% for non-inoculated plants. Furthermore, the effects of mycorrhizal fungi were evaluated on 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 noninoculated plants. It is concluded that both mycorrhiza species could be an important tool to control some soil-borne pathogens, increase plant nutrients absorption and increase resistance to abiotic stresses.

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

Introduction

Rhizoctonia solani Kühn, the causative agent of damping-off disease in a variety of crop plants such as cucumber, is an economically important soil-borne pathogen (Bartz *et al.*, 2010; Saberi *et al.*, 2013). *R. solani* is considered as a pathogen difficult to control due to characteristics such as the great variability in the population, a wide host range, and long-term survival in soil (Thakur *et al.*, 2018). Therefore, some cultural practices including the crop rotation, sanitation, and soil solarization are not sufficiently effective means of control. The application of chemical pesticides, mainly, methyl bromide fumigation, has been a reliable method to control *R. solani in the past*; however it causes serious risks to human health and pollutes the environment (Vinale *et al.*, 2008; Manganiello *et al.*, 2018). Therefore, the biological control method becomes an important component of the disease management to improve crop production and food safety (Justyna *et al.*, 2017).

The biological control has become an important target of many researchers in the fields of biological and agricultural sciences (Manganiello *et al.*, 2018). Bio-control agents use different mechanisms of action against fungal pathogens such as production of antimicrobial compounds, mycoparasitism or hyperparasitism, cell wall-lytic

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enzymes activity, and the induction of systemic resistance (ISR) activity (Vinale *et al.*, 2006). In addition, some bio-control agents are capable of improving some aspects of plant growth such as the germination rate, shoot and root weight, nutrients uptake, and yield (Liu *et al.*, 2018).

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 (Smith et al., 2003; Jacott et al., 2017). In return, the AM fungi are completely dependent on the nutrients exuded from living root system (Jacott *et al*., 2017). In addition, AM fungi have been known to increase host resistance to a wide range of fungal and bacterial pathogens, especially pathogens causing rots (Hoeksema *et al.*, 2010). The aim of this study was to examine the influence of two species of arbuscular mycorrhizal (AM) fungi (*Glomus* spp.) to promote systemic resistance against the agent of damping- off disease *R. solani* on cucumber *Cucumis sativus* L.

Materials and Methods

Infected samples of cucumber plants with wilting, yellowing, and dwarfing symptoms were collected 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 from the root 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 fungi grown in culture plates were diagnosed using classification keys (Domsch *et al.*, 1980).

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 chloramphenicol (Jaiswal *et al.*, 2014). Sterilized soils were separated on each pot (3 kg) and inoculated with 1 ml of pathogen broth culture, 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 planted in each pot. Germinated, not germinated seeds, and collapsed plants were recorded 7 and 10 days after planting, and disease intensity was calculated as recommended (Khan et al., 2006) 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; 4 = plant is dead. DS was calculated from disease grades 0-3, using the following formula (Abawi and Widmer, 2000):

$$DS = \frac{\sum (f \times v)}{N \times x} \times 100$$

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

Seeds of cucumber *Cucumis sativus* L. 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 laboratory. This mixture consisted of propagated units of *Glomus clarum* (Nicol. Schenck) and *Glomus mosseae* (Nicol. Gerd) in a suspension of (1×10^6 unit $^{-1}$ concentration). 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). For mycorrhizal inoculum, each pot was inoculated with the dilution of 5 ml of suspension of mycelial fragments of either *Glomus clarum* or *G. mosseae*/L^{-1} watered twice directly after the cultivation and after 14 days. Negative controls pots were not inoculated with either AM or the pathogen, positive controls received 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 under outdoor conditions. The diseases severity for each treatment was monitored and estimated as mentioned above (Al-Askar and Rashad, 2010).

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 in a hot air oven at 60 °C for 48h until constant weight (Manila and Nelson, 2017).

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 literature (Sharma *et al.*, 2005; Guleria *et al.*, 2007). Among five isolated pathogens, *R. solani* showed the highest diseases severity (DS) on cucumber plants, which was about 63% while *Penicillium* spp. showed the lowest diseases severity (DS), about 8% (Fig. 1). Therefore, *R. solani* was the most aggressive pathogen due to the suitable environmental conditions and the availability of susceptible hosts and was used for all subsequent studies.

Arbuscular mycorrhizal (AM) fungi effects *R. solani* pathogen on cucumber plant, inoculation of cucumber plants with the AM, *G. mosseae* + *G. clarum*, showed a significant reduction in disease severity of damping-off compared with control (Fig. 2). Diseases 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* (Fig. 2).

Arbuscular mycorrhizal effects on growth parameters of cucumber plants, both growth parameters shoot dry weight and root dry weight in arbuscular mycorrhizal (AM) fungi colonized plants were significantly increased compared with non-mycorrhizal plants (Table 1). Cucumber plants, colonized with AM *G. mosseae*, showed slightly greater increase in all growth parameters compared with plant colonized with AM *G. clarum*, which matches with our results on DS experiment (Table 1 and Figure 2).

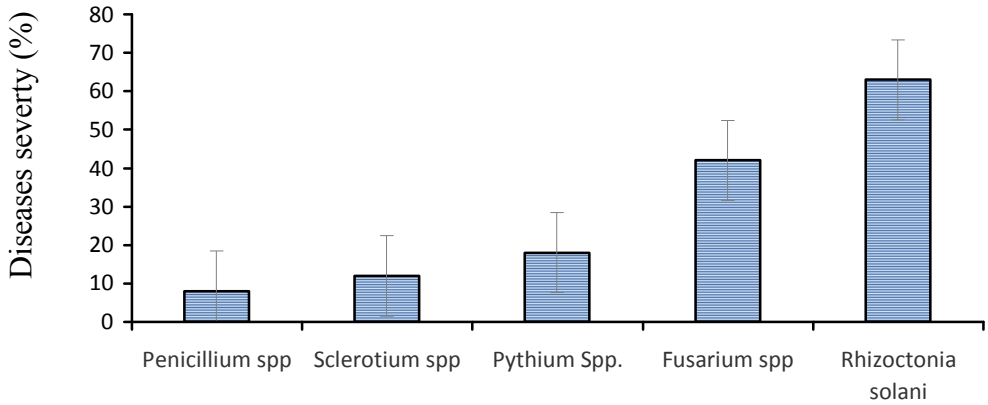

Figure 1 The pathogenicity test for isolated pathogens from infected area with damping-off diseases. Each column represents the mean of 5 replicates. Bars on the pillars represent standard error and LSD = 5.73 (P = 0.01).

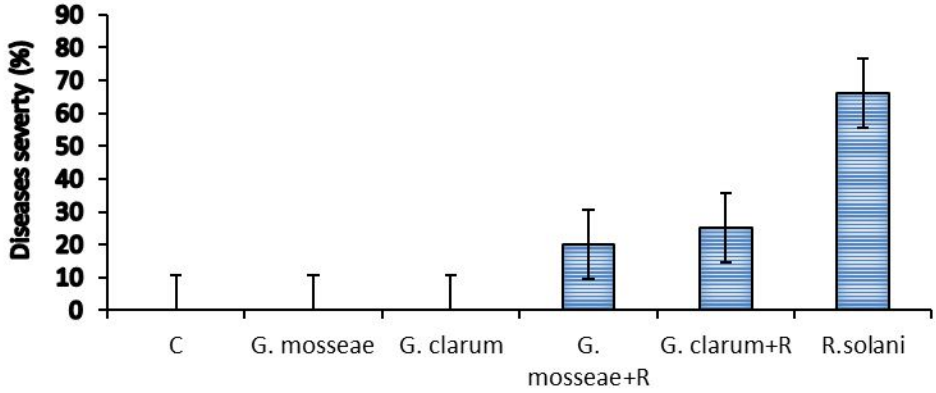

Figure 2 Evaluation of arbuscular mycorrhizal (AM) fungi on the severity of damping-off diseases on cucumber. Each column represents the mean of 4 replicates. Bars on the pillars represent standard error and LSD (P = 0.01).

Table 1 Evaluation of arbuscular mycorrhizal (AM) fungi on the growth parameters of cucumber plants.

Treatments	Shoot dry weight (g/plant)					Root dry weight (g/plant)				
	5 d	10 d	15 d	20 d	Mean	5 d	10 d	15 d	20 d	Mean
Control	0.50	0.80	0.90	1.10	0.83	0.20	0.40	0.70	0.90	0.55
Control + *R. solani*	0.10	0.30	0.40	0.50	0.33	0.08	0.10	0.20	0.30	0.17
Glomus clarum	0.40	0.60	0.70	1.20	0.73	0.15	0.30	0.60	0.80	0.46
G. mosseae	0.60	0.70	0.80	1.10	0.80	0.20	0.40	0.70	0.90	0.55
G. clarum + *R. solani*	0.30	0.50	0.60	0.90	0.58	0.15	0.20	0.50	0.60	0.36
G. mosseae + *R. solani*	0.40	0.60	0.80	1.00	0.70	0.20	0.30	0.60	0.70	0.45
Mean	0.38	0.58	0.70	0.97		0.16	0.28	0.55	0.70	

Mycorrhizal fungi are considered as ideal bio-control agents due to their ability to form mutualistic symbiosis relationship with roots of most vascular plant species (Song *et al*., 2015). Moreover, plant-mycorrhiza relationship benefit plants not only against soil-borne pathogens, but also enhance plant resistance to various abiotic stresses and increase nutrients absorption (Smith and Read, 2010).

In the present study, inoculated plants with mycorrhizal fungi reduced significantly the disease severity of R. solani pathogen, which may be attributed to increased nutrients status in the rhizophere, reduced direct competition for root space and resources with pathogen, induce plant immunity to involve certain systemic mechanisms such as systemic acquired resistance (SAR) and cell wall defenses, and enhance production of defense-compounds such as phenolics, -1,3-glucanase and chitinolytic enzymes (Jacott *et al.*, 2017). Additionally, inoculated plants with mycorrhizal fungus *G. mosseae* showed a lower disease severity than the other species *G. clarum*, which may lead to a potential active control tool. Furthermore, the inoculation with mycorrhizal fungi increases both root dry weight and shoot dry weight, which may work as extra fertilizer for fields that have nutrition deficiency.

Mycorrhizal fungi play main part in plant defense against pathogens and form a mutual relationship with plants. In summary, both mycorrhizal species could be an important tool to control some soil-borne pathogens, increase plant nutrients absorption, and increase tolerance to

abiotic stresses. In future research, specific systemic mechanisms that render mycorrhizal fungi good biocontrol agents should be investigated more.

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ارزیابی قارچ‌های میکروریز آربوسکولار (گونه‌های گلوموس) به‌عنوان عوامل احتمالی زیست کنترل در برابر بیماری بوته میری *Rhizoctonia solani* در خیار

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چکیده: بیماری بوته میری، ناشی از قارچ *Rhizoctonia solani*، یکی از مهم‌ترین بیماری‌های گیاه خیار است که باعث کاهش تلفات قابل‌توجهی می‌شود. *R. solani* با دارا بودن طیف وسیعی از میزبان و بقای نامحدود در خاک، به‌عنوان پاتوژن یکی از دشوارترین عوامل از جنبه کنترل محسوب می‌شود. بنابراین، تحقیقات برای یافتن یک عامل کنترل‌کننده زیستی در برابر این بیماری بسیار ارزشمند خواهد بود. دو گونه قارچ میکوریز *Glomus mosseae* و *Glomus clarum* در مقابل *R. solani* در گیاهان خیار ارزیابی شدند. گیاهان تلقیح شده میکوریزا با هر دو گونه کاهش معنی‌داری در شدت بیماری (DS)، به‌ترتیب ۲۱ و ۲۵ درصد نشان داد، درحالی‌که شدت بیماری در گیاهان تلقیح نشده ۶۵ درصد بود. علاوه بر این، اثرات قارچ میکرویز بر پارامترهای رشد گیاهان خیار بررسی شد. گیاهان تلقیح شده با هر دو گونه قارچ میکوریز در مقایسه با گیاهان تلقیح نشده از نظر وزن خشک اندام هوایی و وزن خشک ریشه تفاوت معنی‌داری نشان دادند. نتیجه‌گیری می‌شود که هر دو گونه میکوریز می‌توانند یک ابزار مهم برای کنترل برخی عوامل بیماریزای منتسب به خاک، افزایش جذب مواد مغذی گیاهان و افزایش مقاومت در برابر تنش‌های غیرزیستی باشند.

واژگان کلیدی: کنترل بیولوژیک، *Rhizoctonia solani*، میکوریز آربوسکولار، خیار، بیماری بوته میری