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Antagonistic Activity of Some Fungi and Cyanobacteria Species against *Rhizoctonia solani*

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ABSTRACT

This study was conducted to investigate the suppression effect of some antagonistic fungi and cyanobacterial species against *Rhizoctonia solani* as the causal agent of soybean root rot. Growth of *Rhizoctonia solani* as the causal agent of root rot of soybeans was inhibited (*in vitro* and greenhouse conditions) in the presence of some antagonistic fungi (*Gliocladium deliquescens, G. virens, Trichoderma hamatum* and *T. harzianum*) and cyanobacterial species (*Nostoc entophytum* and *N. muscurum*). The results show that *Trichoderma harzianum* was the best antagonistic fungi whereas *Nostoc entophytum* as cyanobacteria showed antifungal activity higher than *Nostoc muscurum*, the inhibitory effect was dependant on the type of the bioagent. In experiments carried out in greenhouse, the growth parameters (length, weight, carbohydrate, protein and nitrogen) of the infected soybean plants showed different responses to the tested biological agents as compared to untreated infected plant. It could be concluded from the obtained data the fruitful use of the tested biotic factors for controlling rot root of soybean induced by *Rhizoctonia solani*.

Key words: Antifungal activity, *Gliocladium* spp., *Nostoc* spp., soybean, *Trichoderma* spp.

INTRODUCTION

Plant diseases play direct role in the destruction of natural resources in agriculture. In particular, soil borne pathogens cause important losses, fungi being the most aggressive. The distribution of several phytopathogenic fungi, such as *Pythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia* and *Fusarium*, has spread during the last few years due to changes introduced in farming with detrimental effects on crops of economic importance. In addition, not only growing crops but also stored fruits are prey to fungal infections (Chet *et al*., 1997). Rhizoctonia root rot and hypocotyl rot caused by *Rhizoctonia solani*, is a common disease of soybean which is the most important commercial crops playing key role in economical and social affairs in Egypt and also an important nitrogen-fixing leguminous crop cultivated for food and feed (Bradley *et al*., 2002).

Rhizoctonia solani is common soil-inhabiting fungus with a wide host range that includes field crops, vegetables, fruits and ornamentals (Bohlooli *et al*., 2005). Rhizoctonia Foliar Blight (RFB) of soybean occurs in many tropical and subtropical regions, causing yield reductions of up to 70% and in Brazil, up to 60% (Meyera *et al*., 2006). *Fusarium oxysporum*, *Rhizoctonia solani*,

Macrophomina phaseolina and *Sclerotium rolfsii* are common fungal pathogens to soybean causing damping off, root rot and wilt diseases resulting in serious economic losses (Fayzalla *et al*., 2009).

Most soil-borne pathogens are difficult to control by conventional control measures such as the use of resistant cultivars and synthetic fungicides (Weller *et al*., 2002). Rhizoctonia diseases are difficult because this pathogen survives for many years as sclerotia in soil or as mycelium in organic matter under numerous environmental conditions and has an extremely wide host range (Grosch *et al*., 2003). Moreover, the use of fungicides, besides being expensive and involving risks to the environment associated with the application of chemicals, is not totally affective and may lead to the appearance of new, resistant strains of pathogens (Soylu *et al*., 2005). As a consequence, there is an increased emphasis on ways to minimize the use of fungicides.

Interest in biological control has increased recently, fuelled by public concerns over the use of chemicals in the environment (Whipps, 2001). Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts (Brimmer and Boland, 2003) and which reduce the disease and are perceived as less harmful than conventional fungicides (Washington *et al*., 1999). It has long been recognized that the biological control became recently an effective strategy for fighting plant pathogens (Kabeil *et al*., 2008).

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents against soil-borne pathogens, since the rhizosphere provides the front line of defense for roots against attack by pathogens (Lozovaya *et al*., 2004). Several antagonistic bacteria and fungi to soil borne pathogens were reported as biocontrol agents of many pathogens induced root rot and wilt diseases (Haggag, 1998). Fungal biological control agents have several mechanisms of action that allow them to control pathogens, including mycoparasitism, production of antibiotics or enzymes, competition for nutrients and the induction of plant host defenses (Brimmer and Boland, 2003). A broad spectrum of fungal antagonists was evaluated as potential Biocontrol Agents (BCAs) against the soil-borne pathogen *R. solani* (Grosch *et al*., 2006). Antagonists of phytopathogenic fungi have been used to control plant diseases and 90% of such applications have been carried out with different strains of the fungus *Trichoderma* (Hermosa *et al*., 2000). Furthermore, *Trichoderma* strains are effective in controlling plant diseases and the action of fungal hydrolytic enzymes is considered as the main mechanism involved in the antagonistic process (Szekeres *et al*., 2004). Gachomo and Kotchoni (2008) revealed the production of volatiles by *Trichoderma* species against the pathogenic microorganisms. *T. harzianum* is a well known biological controlling agent against several soil borne phytopathogens (Yadav *et al*., 2011).

Algae are one of the chief biological agents that have been studied for the control of plant pathogens (Hewedy *et al*., 2000). Cyanobacteria were found to be a rich source for various products of commercial, pharmaceutical or toxicological interest: primary metabolites, such as proteins, fatty acids, vitamins or pigments (Borowitzka, 1995).

Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity (Noaman *et al*., 2004). They have received little attention as potential biocontrol agents of plant diseases. Kulik (1995) stated that for a number of reasons, cyanobacteria and algae are suitable candidates for exploitation as biocontrol agents of plant pathogenic bacteria and fungi: Cyanobacteria and algae produce a large number of antibacterial and antifungal products, many can grow in quantity in mass culture and they are not a threat to the environment (except for the production of toxic blooms in freshwater and marine habitats and slimy areas on turf by a relatively small number of cyanobacteria).

The aim of the present study is focused on detection the ability of some antagonistic fungi and some cyanobacteria in suppressing root rot diseases of *Glycine max* L. caused by *R. solani in vitro* and *in vivo* and determine their effects on some growth parameters of *Glycine max* L.

MATERIALS AND METHODS

Biological agents

Fungi: The antagonistic fungi (*Gliocladium deliquescens, G. virens, Trichoderma hamatum* and *T. harzianum*) and Pathogenic fungus (*Rhizoctonia solani*) were procured from Plant Pathology Department, El-Gemmeiza Agricultural Research Station, (El-Gharbia Governorate, Egypt) and incubated on Potato Dextrose Agar (PDA) slants and plates at 28±1°C to establish growth then stored at 5°C in refrigerator.

Blue green algae (Cyanobacteria): *Nostoc entophytum* and *N. muscurum* were obtained from Botany Department, Faculty of Science, Tanta University. The identified cyanobacteria inoculated on BG_{11} (Rippka *et al.*, 1979) nutrient agar slants and left in a diffused light at room temperature (28±2°C) to grow for 12 days thereafter, they were kept in a refrigerator at 4°C.

Plant: Soybean (*Glycine max* (L.) Merrill) seeds, cultivar (Giza 111) were kindly supplied by the Legumes Department, El-Gemmeiza Agricultural Research Station, Agricultural Research Center, Egypt. All experiments were carried out in Mycology laboratory and greenhouses of Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.

Preparation of antifungal extracts: Cyanobacteria mass from an axenic culture growing in BG_{11} were separated from the culture medium by centrifugation after 12 days of incubation at 30°C under continuous illumination (30 $\mu E/m^2/S$). The pellets were dried at 60°C for 24 h (Khan *et al.*, 1988; Vlachos *et al.*, 1996) and their extract (acetone, chloroform, methanol and water) prepared according to the methods of Katircioglu *et al.* (2006).

Antifungal assay by the agar disc diffusion method: Petri dishes (9 cm in diameter) contains 15 mL of PDA medium were divided into two equal halves, the first half was inoculated with a disk (0.5 cm in diameter) of *Rhizoctonia solani* and the second half was inoculated with a disk (0.5 cm in diameter) of cyanobacteria extracts or a disk of the tested antagonistic fungi (Bauer *et al.*, 1966; Nair *et al.*, 2005). The percentage of inhibition (I%) was calculated after 4 days of incubation at 28±1°C according to Topps and Wain (1957) equation.

Determination of the total phenolic contents of cyanobacteria: The total phenolic contents of cyanobacteria were determined as described by Jindal and Singh (1975).

Determination of the polysaccharides of cyanobacteria: Polysaccharides of tested cyanobacteria (Intracellular (IPS) and Extracellular Polysaccharides (EPS)) were extracted and determined as the method described by Shi *et al.* (2007).

Biological control experiment (Preparation of pathogen and biological control inoculation under greenhouse conditions): Pathogencity test is primary test for determination of the suitable concentration of *R. solani* which the casual agent of rot root under greenhouse

conditions in early May 2009. The inoculum was prepared by dispensing 100 g of mixture wheat bran and sand (2:1) in bottles, then moistured with water. Contents of bottles were autoclaved for 20 min at 1.5 atm., then inoculated with *R. solani* which had been grown on PDA for one week and incubated at 28±1°C for 14 days. Autoclaved soil was placed in greenhouse and infested with inocula of *R. solani* one week before sowing at the rates of 10, 30, 50, 70 and 90 g kg^{-1} soil. Pre emergence damping-off was recorded using the following equation after 15 days of sowing as percentage of infected plants.

$$\text{Damping-off \%} = \frac{\text{No. of infected plant}}{\text{Total plant No.}} \times 100$$

Starter cultures of both pathogen and antagonistic fungi were cultured in sterilized mixture wheat bran and sand (2:1) in bottles, then moistured with water and incubated at 28±1°C for 14 days. The bottles were shaken daily to mix and spread the fungal inoculum well on the growth substrate. During the season (late May to June 2009), sterilized soil was placed into 25 cm diameter plastic pots, each pot contained 3 Kg soil. Soil infestation was carried out one week before sowing at the rate of 30 g kg^{-1} *R. solani* inoculum and the fungal inoculum was mixed with the sterilized soil one week before sowing at the rate of 3% (w/w) while the fresh cyanobacterial inoclume was added at 0.3% (w/w) and kept moist. Twenty sterilized soybean seeds were sown in each plastic pot and replicated five times for each particular treatment. Post-emergence damping off was recorded after 45 days of sowing for each treatment as mention above.

Plant analysis: Measurement of soybeans growth included post-emergence damping-off, surviving seedlings, plant height, fresh and dry weights of shoot and roots, carbohydrate content (Nelson, 1944; Naguib, 1964), protein (Bradford, 1976), nitrogen (Naguib, 1969) and phosphorous content (Allen *et al.*, 1974) after 45 days of sowing.

Statistical analysis: The presented results are the Means±SD (standard deviation) of at least five readings. One way Analysis of Variance (ANOVA) was done using the SAS (1996) program version 6.12. The objective of statistical analysis was to determine any significant different between treatments.

RESULTS

Antifungal activity of the tested antagonistic fungi *in vitro*: The results presented in Fig. 1 and show that all tested antagonistic fungi (*G. deliquescens*, *G. virens*, *T. hamatum* and *T. harzianum*) exhibited antifungal activity against *Rhizoctonia solani in vitro* after 4 days of incubation. The antimicrobial activities of the tested fungi could be arranged in the following sequence? *T. harzianum* (63%)> *G. virens* (55%)> *T. hamatum* (49.8%)> *G. deliquescens* (46.4%) at p = 5%.

Antifungal activity of the some extracts of the tested cyanobacteria *in vitro*: The antifungal activity of two cyanobacterial sp. (*N. entophytum* and *N. muscurum*) as acetone, chloroform, methanol and water extracts is represented in Fig. 2a and b. The results show that extracts exhibited antifungal activity except water extract of *N. muscurum* which showed no antifungal activity. However, the strongest antifungal activity was observed in water extract of *N. entophytum* (44.4%).

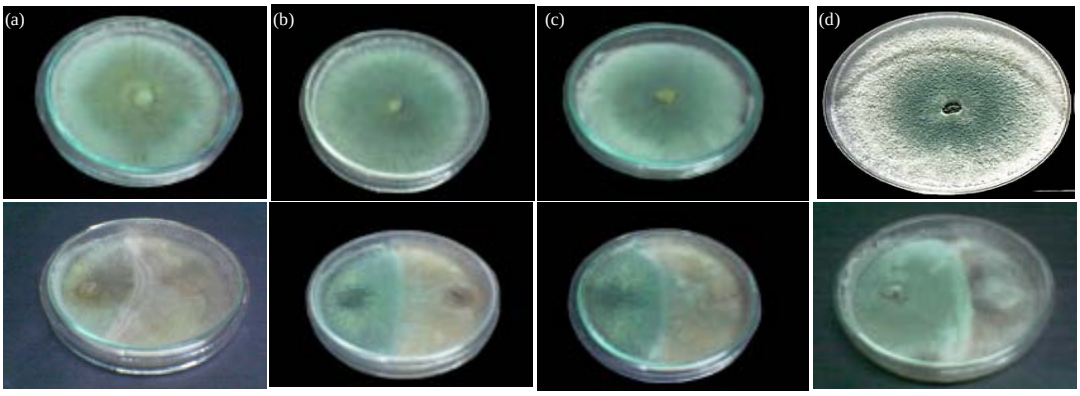

Fig. 1(a-d): Antagonistic effect between *Rhizoctonia solani* and some antagonistic fungi on PDA medium (four days old). (d) *T. harzianum* (b) *G. virens* (c) *T. hamatum* (a) *G. deliquescens*

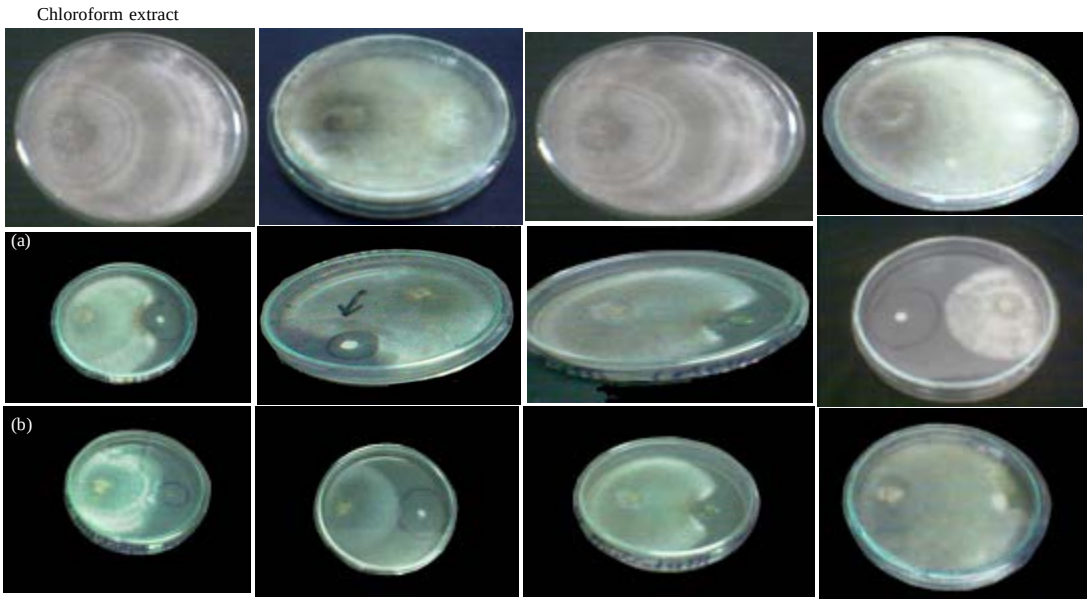

Fig. 2(a-b): Antifungal activity of some extracts of (a) *Nostoc entophytum* and (b) *Nostoc muscurum* against *Rhizoctonia solani* on PDA medium (four days old)

Role of phenol and polysaccharides content as antifungal agents: The relationship between the antifungal activity of the tested cyanobacteria and their polysaccharides and phenol contents were determined by estimating their contents in the tested cyanobacterial species. The results show that as the phenol contents of the tested cyanobacterial species were increased, their antifungal activity was increased (Table 1).

The content of polysaccharides content of *N. entophytum* was higher than *N. muscurum* and showed higher antifungal activity than *N. muscurum*.

Table 1: Total phenol contents and polysaccharides of blue green algae

Biological agents	Total phenol contents (mg g^{-1} DW)	Polysaccharides content (mg/g^{-1} DW)	
		IPS (mg g^{-1} DW)	EPS (mg mL^{-1})
Blue green algae			
Nostoc entophytum	0.91±0.02	2.8±0.3	156.3±4.3
Nostoc muscorum	0.88±0.01	2.2±0.02	135.3±2.3

IPS: Intracellular polysaccharides EPS: Extracellular polysaccharides. Values represent Mean±SD (n = 5)

Antifungal activity of the tested organisms under greenhouse conditions: This trial was conducted from May to June 2009. As a general trend, *R. solani* caused a highly significant reduction in the measured soybean growth parameter such as survival ratio by 77.22% and caused a highly significant increase in the post-emergence damping off by 83.3% after 45 days of sowing at $p<0.001$(Fig. 3a).

The root depth and shoot length of infected soybeans with *R. solani* exhibited progressive decreases throughout the cultivation period up to 45 days by about 31 and 48.3%, respectively at $p<0.001$ (Fig. 3b).

The infection of soybean with inoculum of *R. solani* was found to cause a highly significant decrease soybean fresh and dry weights amounted by 48.3 and 51.1%, respectively below the healthy control at $p<0.001$ (Fig. 1b, 3c, 3d).

Under greenhouse conditions, addition of (3 g kg^{-1}) *T. harzianum* induced highly significant increase in the soybean survival rates by 219.2% at $p<0.001$ (Fig. 3a). *T. harzianum* was the more effective than *G. virens* in increasing soybean root depth and shoot length by 53.8 and 46.4 % as compared to infected plant (Fig. 3b). Addition of 3% *T. harzianum* or *G. virens,* separately induced highly significant increase in the total fresh weight above the infected control level by 53.8 and 45.4% (Fig. 3c) and caused a highly significant increase in the total dry weight by 64.5 and 59.8%, respectively at $p<0.001$ (Fig. 3d).

However, treatment the infected soil with 0.3% *N. entophytum* or *N. muscurum* caused highly significant increase in the total fresh weight by 39.5 and 34.4% (Fig. 3c) and led to increase the total dry weight by 57.9 and 51.9%, respectively at $p<0.001$ above the control value after 45 days of sowing (Fig. 3d).

Compared to control culture, 3% (w/w) *R. solani* infected soybean had highly significant reduction in carbohydrate contents (DRV, TRV and sucrose) of soybean shoot system amounted by 24.8, 45.8 and 66.1%, respectively (Fig. 3e). The same treatments caused also decrease in root system DRV, TRV and sucrose by 57.8, 26.8 and 14.6% below the control value under greenhouse conditions at $p<0.001$(Fig. 3f). On the other hand, the carbohydrate content of soybeans shoot and root system cultivated in soil contained 0.3% (w/w) *T. harzianum* or *G. virens* had significant increase as compared with infected control after 45 days of sowing under greenhouse condition.

Protein content of the infected soybeans shoot and root showed significant increase by 75.2, 58% as compared with uninfected plant Hence nitrogen contents showed highly significant increase by 58, 42.6%, respectively after 45 days of sowing under greenhouse conditions (Fig. 3g, h). On the other hand, the protein and nitrogen contents of infected soybean treated with tested antagonistic fungi were lower than untreated infected plant but were higher than the uninfected plant (Fig. 3g, h).

The algal treatments e.g., *N. entophytum* and *N. muscurum* caused significant reduction in the protein (Fig. 3g) and nitrogen content below the infected untreated soybeans after 45 days of

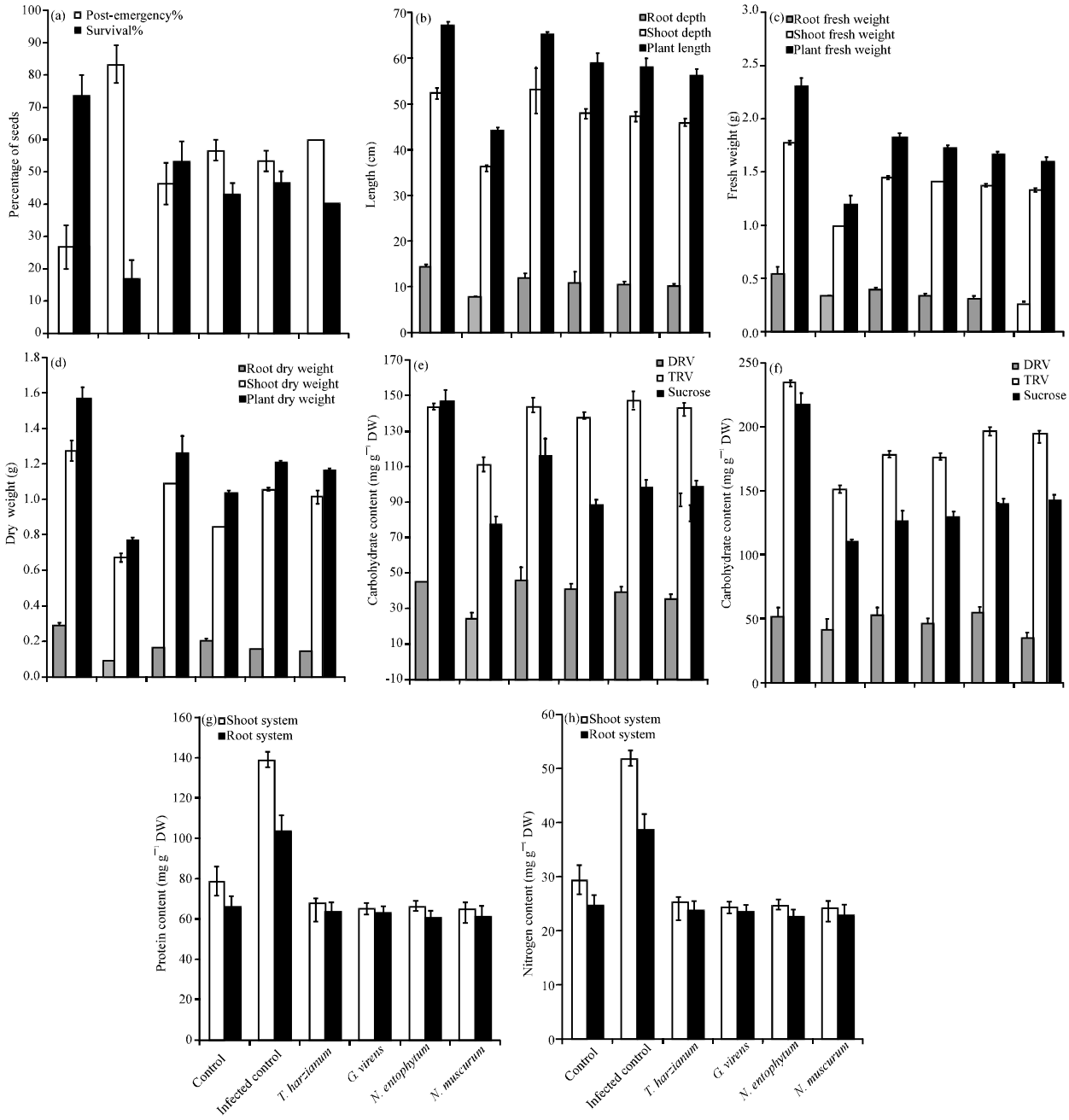

Fig. 3(a-h): Effect of the tested bioagents on (a) post damping off and survival percentage (I%) (b) length, (c) fresh, (d) dry weight, (e, h) carbohydrate, (g) protein and (h) nitrogen contents of infected *Glysine max* L. with *Rhizoctonia solani* after 45 days of sowing under greenhouse conditions

sowing but these contents of infected soybean treated with tested algae were higher than uninfected control (Fig. 3h). The results indicate that the antifungal activity of *N. entophytum* under greenhouses conditions were also higher than that of *N. muscurum*.

DISCUSSION

Antifungal activity of the tested organisms *in vitro*: The results show that all of the tested organisms exhibited inhibitory effect on *R. solani in vitro*. The inhibitory effects as measured by inhibition ratio were extremely variable as measured by the diameter of the inhibition zone according to the species of the tested organism. With respect to antagonistic fungi, *T. harzianum* (Fig. 1d) showed the strongest antagonistic effect followed by *G. virens* (Fig. 1b), *T. hamatum* (Fig. 1c) then *G. deliquescens* (Fig. 1a). These results are in accordance with the data obtained by El-Kader (1997) who found that *T. harzianum* (as a biocide) decreased *R. solani* growth which the causal organism of bean root rot disease by 69-74% *in vitro*. Singh and Chand (2006) recorded that *T. harzianum* gave maximum inhibition of the *R. solani* (75.55%) followed by *G. virens* which exhibited 57.77% mycelial growth inhibition under laboratory conditions. Kalai *et al.* (2008) stated that *Trichoderma* species are known to have strong antifungal effect partly as a result of their production of extracellular protease and chitinase enzymes which hydrolyse the main constituent of the fungal cell wall.

The obtained results showed that water extract of *N. entophytum* exhibited high antifungal activity while no activity was observed in water extract of *N. muscurum* against *R. solani*. On the other hand, chloroform extract showed marked antifungal activity in case of *N. muscurum* whereas the chloroform extract of *N. entophytum* showed lower activity. More or less similar results were reported by Piccardi *et al.* (2000) recorded that the bioactivity of *Nostoc* spp. was equally distributed between lipophilic and hydrophilic extracts and was mostly directed against *Penicillium expansum* and *R. solani*. El-Sheekh *et al.* (2006) stated that chloroform was the best solvent for extracting the active material of *N. muscurum*.

The antifungal activity of cyanobacteria could be attributed to their phenol content and/or polysaccharides content (Table 1). This interpretation based on the results concerning the content of these substances in the tested cyanobacteria where their antifungal effect were increased as their polysaccharides and/or phenol content increased. In agreement with our explanation, there are a number of reports by authors on the antifungal activity of phenolic substance e.g., De Cano *et al.* (1990) found that phenolic compounds in extracts from cells of *N. muscurum* significantly inhibited the growth of *Candida albicans* and *Staphylococcus aureus*. Furthermore, Samapundo *et al.* (2007) observed that the phenolic compounds e.g., vanillic and caffeic acid treatments caused reduction in *F. verticillioides* and *F. proliferatum* growth. Sekine *et al.* (2009) detected that phenolic hydroxyl compounds have antifungal activity against white- and brown-rot fungi.

With respect to polysaccharides which play important role as defense mechanism for cyanobacteria and reflect the antifungal activity of the tested cyanobacteria as demonstrated Table 1. This observation has been emphasized by Potin *et al.* (1999) who found that oligosaccharides from marine algae were used to protect from infections by pathogens. Cuero (1999) revealed that the antimicrobial activity of chitosan is well observed on a wide variety of microorganisms including fungi, algae and some bacteria.

Antifungal activity of the tested organisms under greenhouse conditions: The results show that *R. solani* caused soybean damping off and reduced the plant length, weight and carbohydrate contents (Fig. 3). Present results support the results obtained by Ismail and Ahmed (2000) who reported that *R. solani* was the most pathogenic fungus, it caused significant effects in all tested variables (pre, post-emergency damping off, survival plants and

plant height) of cotton seedlings. Heydari *et al.* (2007) observed that R. solani induced damping off symptoms on all emerged and non emerged cotton seedling. Haikal (2008) who showed that filtrates of *A. niger*, *F. culmorium*, *Penicillium* sp. and *R. solani* inhibited seed germination and seedling development of soybean due to their toxic metabolites in the media in which they were grown. Hwang *et al.* (2009) recorded that the height, shoot vigour and shoot dry mass of *Rhodiola rose* were significantly reduced by *R. solani* infection. Abdullah (2008) stated that *R. solani* decreased total carbohydrate content of wheat and barley. El-Daly and Haikal (2006) shown that the soil infection with 3% (w/w) *R. solani* drastically lowered the total carbohydrates of *Zea mays*.

It could be deduced from the previous mentioned data that *T. harzianum* and *G. virens* were the most effective antagonistic fungi to control by *Rhizoctonia solani* under laboratory conditions (Table 1) so we used the tested species under greenhouse conditions while 3% (w/w) of *T. harzianum* or *G. virens* reduced the post-emergency damping-off caused by *R. solani* and increased the survival rates of seedling. Present results are in agreement with Bazgir and Okhovat, (1996) who reported that the inoculation of *T. harzianum* to the soil one month before sowing reduced the level of *R. solani* on *Phaseolus vulgaris* beans. *Trichoderma* spp. or *G. virens* grew on the bran suppressed the spread of *R. solani* and significantly reduced its inoculum potential (Lewis *et al.*, 1998). *Trichoderma* spp., *Gliocladium* spp. and actinomycetes were plays a key role in the sustainability of agriculture systems and indicates the level of health of soil (Gil *et al.*, 2009).

Trichoderma harzianum was more effective than *G. virens* in controlling the pathogenic effect of *Rhizoctonia solani in vitro* and under greenhouse conditions. Our results are in conformity with those of Hanson and Howell (2002) who explained that *G. virens* have good biocontrol activity against *Rhizoctonia solani* on cotton but lack some of the commercially desirable characteristics found in *Trichoderma* species. *T. harzianum* gave maximum protection of the disease (72.72%) while *G. virens* and *Aspergillus* sp. were found to be the least effective in controlling root rot of mungbean Under greenhouse conditions (Singh and Chand, 2006).

The results present in Fig. 3 show that the application of antagonistic fungi to infected soybeans at 3% (w/w) increased the length, weight and carbohydrate accumulation of infected soybeans. It could be deduced from the obtained data that *G. virens* and *T. harzianum* act as stimulator for infected soybean elongation and weight as compared to untreated infected control. The stimulation effect of the tested fungi differs according to fungal species which correlated with antagonistic ability which confirmed previously *in vitro*. Our results are in agreement with Chen *et al.* (1996) who reported that the increased of carbohydrate content might be correlated to increase in growth rate due to the effect of stimulatory effect of the antagonistic fungi. De Paula Junior (2002) stated that *T. harzianum* increased bean growth in the presence of *R. solani*. Grosch *et al.*, 2006) stated that *Trichoderma* sp. either partly or completely controlled the dry mass loss of lettuce caused by *R. solani*. Biological control agents *T. harzianum* or *B. subtilis* or both initiated the increase of carbohydrate content of Z. mays infected with 3% (w/w) *R. solani* (El-Daly and Haikal, 2006). El-Mohamedy and El-Baky (2008) detected that *T. harzianum* stimulated carbohydrate accumulation in the infected pea with *R. solani*. *Trichoderma* have a strong aggressiveness against phytopathogens and produce trichotoxins that could inhibit plant pathogen and promote plant growth (Gachomo and Kotchoni, 2008).

Data in (Fig. 3g, h) show that the protein and nitrogen contents of infected soybeans were decreased by addition of 3% *T. harzianum* or *G. virens* to the soil as compared with infected control under greenhouse conditions. These results are more or less similar to that reported by

Naseby *et al.* (2000) who stated that *Trichoderma* strains reduced the activity of C and N cycle enzymes in pea. Inbar *et al.* (1994) reported that *T. harzianum* caused non significant changes in N content of cucumber and pepper.

The obtained data showed that the experimental cyanobacteria were able to inhibit the post-damping off effect of *Rhizoctonia solani* and increase the survival rate of soybean seedling under greenhouse conditions (Fig. 3a), hence *N. entophytum* and *N. muscurum* increased the number of survival seedling as compared with infested control. This positive effect may be due to their antifungal activity as demonstrated previously *in vitro*. In this context, Kulik (1995) mentioned that filtrates or cell extracts from cyanobacteria applied to seeds as protectants against damping-off fungi such as *Fusarium* sp., *Pythium* sp. and *R. solani*. De Caire *et al.* (1990) reported that extracellular products from *N. muscurum* are promising as a biological control of soybean seedlings damping off. Moore (1996) showed that *Nostoc* sp. (GSV 224) has potent fungicidal activity and may have use in the treatment of resistant fungal-induced diseases of domestic plants and agricultural crops.

Length, weights and carbohydrate contents of infected soybeans treated with of *Nostoc entophytum* and *N. muscurum* (Fig. 3b-f) showed an increase than untreated infected soybeans under greenhouse conditions. This increase may result from the effect of antifungal activity of cyanobacteria which suppressed the toxic effect of *Rhizoctonia solani* as demonstrated previously under laboratory conditions (Table 1). Very little data have been published on the effect of cyanobacteria on growth parameter of infected plant with *Rhizoctonia solani*. These results are in agreement with those obtained by Tiedemann *et al.* (1980) who found that plant biomass yield which inoculated with blue green algae were significantly greater than with the control treatment. Ordog (1999) found that the extract of cyanobacteria contain a special set of biologically active compounds including plant growth regulators which increased root and shoot development. Maqubela *et al.* (2009) stated that *Nostoc* spp. inoculation increased maize dry matter. The carbohydrate content of tomato (*Lycopersicon esculentum* L.) was increased by *Nostoc* spp. (Al-Khiat, 2009). The above mentioned stimulations in carbohydrate content of the different plant by cyanobacteria could be attributed to the stimulation of photosynthetic process by some factors present in such organisms.

With regard to the effect of tested cyanobacteria on protein and nitrogen contents of infected soybeans under greenhouse conditions, the obtained results show that the nitrogen content of infected soybeans was reduced by application of *N. entophytum* and *N. muscurum* (Fig. 3h), although cyanobacteria can fix N in soil (Metting, 1981). These results are in conformity with Adam (1999) who stated that *N. muscurum* improved the growth and nitrogen contents of noninfected wheat, sorghum, maize and lentil. Al-Khiat (2009) recorded that the tomato (*Lycopersicon esculentum* L.) protein content increased by *Nostoc* sp. It could be deduced from the above mentioned data that the infected plant failed to uptake nitrogen from the soil.

CONCLUSION

This work is endeavor for utilization of some antagonistic fungi and some cyanobacterial species as antifungal agent against *Rhizoctonia solani* which the causal agent of soybeans rot root. Our results indicated that the efficiency of the tested biological treatments (antagonistic fungi and cyanobacteria) for controlling *Rhizoctonia solani* under laboratory and greenhouse conditions, the degree of efficiency is different according to the types of biological treatments. All tested biological treatments are effective for decreasing the post-emergency damping off of soybeans caused by

Rhizoctonia solani and increased some growth parameter e.g., soybean severity, length, weights and carbohydrate content. On the other hand, they have negative effect on protein and nitrogen contents of infected soybeans as compared with untreated infected control under greenhouse conditions. The antifungal activity induced by such biotic factors could be attributed to phenol and polysaccharides content.

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