BIOCONTROL OF *PHASEOLUS VULGARIS* ROOT ROT USING ARBUSCULAR MYCORRHIZAE

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Abstract

The arbuscular mycorrhizal fungi (AM fungi) as mixture of *Glomus* spp. was used to induce resistance in *Phaseolus vulgaris* against root rot disease caused by *Rhizoctonia solani.* Results showed that % disease incidence was significantly reduced in AM-inoculated plants as compared with infected and AM-free control. Production of hydrogen peroxide (H_2O_2) and level of lipid peroxidation increased in both roots and shoots bean plants with increasing the time of infection. AM fungi markedly alleviated oxidative stress in infected bean plants, thus, levels of H_2O_2 and lipid peroxidation was markedly reduced. Also, activity of antioxidant enzymes (catalase, ascorbate peroxidase and superoxide dismutase) significantly increased in bean tissues in response to both *R.solani* and/or AM fungus. The highest enzyme activity was recorded in shoots of 14 days old plants inoculated with AM fungi. Total phenolic compounds and various phenolic acids especially cinnamic and ferulic acids greatly increased in roots of bean plants infected with *R.solani* and/or inoculated with AM fungi. However, coumarin was found only in AM-treated plants. But quercetin markedly increased in bean roots infected by *R.solani* and decreased in AM-treated plants, as compared with non-infected control. Finally, activity of lignification enzymes (polyphenol oxidase, peroxidase and phenylalanine-ammonia lyase) increased in all infected plants. Our results indicate that AM fungi increased resistance in infected bean plants by inducing both antioxidant system and phenolic compounds pathway.

Key words: AM fungi – *Rhizoctonia solani* – *Phaseolus vulgaris* – antioxidant enzymes – phenolic compounds

INTRODUCTION

Traditional methods used to protect crops from diseases have been largely based on the use of chemical pesticides. Fungicides are available to manage damping off incidence, they will not be reliable as a long term solution because of the concerns about exposure risks, health and environmental hazards and residue. Moreover, frequent applications of fungicides lead to the development of tolerance in the target pathogens. In recent years, the focus has been towards biological control of diseases using biocontrol agents, which is safe and promising alternative to fungicides.

Biocontrol agents including plant growth promoting fungi (PGPF) belonging to the genera *Tichoderma, Penicillium , arbuscular mycorrhiza*, …etc., have been reported to be beneficial to several crop plants, not only by promoting their growth but also by protecting them from disease (Shoresh and Harman, 2008).

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that colonize about 80% of the higher plants in nature and increase plant growth under certain nutrient deficiency condition. They have been shown to play a different role, namely, improved plant nutrition, damage compensation, competition for colonization sites or photosynthates, changes in the root system, changes in rhizosphere microbial populations, and activation of plant defense mechanisms. Several mechanisms can be operative simultaneously, depending on environmental conditions, timing of the interaction, and partners involved.

Rhizoctonia solani is a soil-borne pathogen that affects many plant species and induces a deleterious effect to the susceptible hosts (Anderson, 1982). Although studies of plants infected separately with AM fungi or pathogen have been reported, recent analyses of plant-microbe interaction suggest that root infection by pathogenic fungi and AM fungi differ at the molecular level (Harrison, 2005). In most pathogenic interaction, plant resistance is associated with complex changes that include a hypersensitive response, accumulation of phytoalexins and other secondary metabolites, formation of structural defensive barriers such as lignin and hydroxyl proline-rich cell wall proteins and production of new enzymes, which often result in antifungal effect. However, the early stages of AM fungal colonization are accompanied by a weak but transient accumulation of phytoalyxins and by the expression of genes encoding plant defense processes.

Phaseolus vulgaris is infected by a number of diseases, among them is damping-off caused by *Rhizoctonia solani*. Root rots are the main diseases caused by *Rhizoctonia solani* and occur wherever common bean is cultivated (Beebe and Pastor-Corrales, 1991). They cause loss of plants, seriously limit establishment and can lead to reduced plant development.

The present work was carried out to evaluate the efficiency of mycorrhizal fungi (mixture of *Glomus* spp.) in improving bean defense mechanism against the damping-off pathogen, *Rhizoctonia solani* under greenhouse conditions. Thus, oxidative stress, antioxidant defense system and phenolic compounds and its related enzymes were investigated in bean tissues.

MATERIALS AND METHODS

Bean seeds (*Phaseolus vulgaris* cv. Proncho), obtained from the Agricultural Research center, Giza, Egypt, were surface sterilized for 1 min in 75% ethanol, immersed for 3 min in sterile distilled water, and then left to dry overnight. Sterilized seeds were sown in large pot (30 cm in diameter) containing non sterilized sandy loamy soil (2:1 w/w) under greenhouse conditions. The pots were divided into three groups (10 pots/group) and treated as the follows:

I. Soil of the 1st group were left without any treatments (non-infected control).

II. Soil of the 2nd group were infested with the pathogenic fungus *Rhizoctonia solani* (infected control).

III. Soil were treated with AM fungi (mixture of *Glomous* spp.) (250 spores/pot) + *R. solani*.

Preparation of pathogen inoculum

Inocula of *Rhizoctonia solani* isolates were prepared by growing the fungus on corn sand meal medium supplemented with 0.2% peptone solution. Flask containing the medium was inoculated with equal disks (0.5 cm) of three days old *R.solani* cultures. Inoculated flasks were then incubated at 20° C for 30 days.

Mycorrhizal inoculum

The mycorrhizal fungi were isolated locally. It was extracted from the rhizosphere of maize plants by the wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and was identified by the Biofertilizers Unit, Faculty of Agriculture, Ain Shams University. The inoculated mycorrhizal dosage was 2.5 ml of spore suspension per pot, containing approximately 250 spores. The inoculum was placed 3 cm under bean seeds at sowing time. The un-inoculated pots were supplied with filtered washing (2.5 ml/pot) to provide the associated microorganisms other than mycorrhizal propagules.

Soil infestation

Inocula of *R.solani* were added to soil at the rate of 3% of soil weight, one week before planting, watered resularly to ensure its distribution in the soil.

Plant Harvest and Analysis

Plants were harvested at two stages (14 and 28 day after planting). Shoots and roots were separated and immediately frozen in liquid nitrogen until using for various biochemical analyses**.**

The percentage of disease incidence (DI) was assessed using the following formula according to (Yehia et al., 2007):

$$DI = \frac{\text{No. of infected plants} \times 100}{\text{Total No. of seeds sown}}$$

Assay of hydrogen peroxide (H_2O_2) and Lipid Peroxidation

Hydrogen peroxide levels were determined according to Velikova et al. (2000). Half g fresh wt. of bean tissue was homogenized in 2 ml of 0.1% trichloroacetic acid (TCA) solution. After centrifugation at 12,000 g for 15 min, 0.5 ml of the supernatant was added to the reaction mixture containing 0.5 ml 10mM potassium phosphate buffer (pH 7.0) and 1 ml of 1M potassium iodide KI. Absorbance was determined at 390 nm. The blank was prepared in the same manner except that 1 ml of 10 mM potassium phosphate buffer (pH 7.0) instead of the sample. The amount of H_2O_2 was calculated from calibrated samples using (1, 5, 10 mM H_2O_2) standard solutions, one half ml of each standard solution was added to the reaction mixture containing 0.5 ml 10mM potassium phosphate buffer (pH 7.0) and 1 ml of 1M potassium iodide KI. Absorbance was determined at 390 nm.

Level of lipid peroxidation was measured as the amount of malondialdhyde (MDA), which react with thiobarbituric acid (TBA) to form TBA-MDA complex and measured at 532 and 600 nm. Total MDA equivalent were calculated according to the following equation:

$$\textbf{Total MDA (n mol/g FW)} = \frac{\textbf{Amount of extraction buffer (ml)} \times \textbf{amount of supernatant (ml) x [(Abs 532-Abs 600/ 155)] x10}^3}{\textbf{Amount of sample (g)}}$$

Extraction of oxidizing enzymes

Extraction was made as reported by Silvana et al. (2003). One g fresh weight of plant tissue homogenized in prechilled mortar and pestle at 4 ºC with 10 ml special mixture containing (50 mM phosphate buffer pH 7.4, 1 mM EDTA, 1 g polyvinylpyrrolidone (PVP) and 5% (v/v) Triton X-100) under ice cold condition. The homogenate was centrifuged at 10000 g for 20 min at 4 ºC and the supernatant was used for the assay of peroxidase (POX), catalase (CAT), ascorbate peroxidase(APOX) and superoxide dismutase (SOD).

Assay of Peroxidase Activity (POX)

Peroxidase activity was determined following the method of Jiang et al. (1984). POX activity was measured in a reaction mixture consisting of supernatant (1 ml) and guaiacol as a substrate. Three ml of reaction mixture consisting of (100 mM sodium phosphate buffer, pH 7.0 and 20 mM guaiacol). The increase in absorbance at 470 nm was measured spectrophotometrically after 20 µL H_2O_2 was added. Enzyme activity was defined as a change in the optical density/ g fresh wt./ min.

Assay of Catalase Activity (CAT)

The catalase activity was determined by recording the consumption of H_2O_2 at 240 nm for 30 sec in 3 ml of reaction mixture containing 100 mM phosphate buffer pH 7.0 and 20 µL of 30 % H_2O_2 and 30 µL enzyme extract (Aebi, 1995).

Assay of Superoxide dismutase (SOD)

The activity of total superoxide dimutase was determined according to Silvana et al. 2003). The activity was assayed by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT). In a test tube, 200µl of enzyme supernatant plus 300 µl buffer solution (50 m M K- phosphate buffer pH 7.8 and 0.1 m M EDTA) were added to 3.5 ml of O^{-2} generator mixture (14.3 mM methionine, 82.5 µM NBT and 2.2µM riboflavin). The test tube was shaken and placed 30 cm under the direct lamp for 10 min. Reading were carried out at wave length 560 nm. Blanks and controls were run in the same way but without illumination and enzyme, respectively. Superoxide dismutase activity unit defined as mg protein required to cause 50% inhibition of the reduction of Nitroblue tetrazolium (NBT) at 560 nm under the assay conditions.

Assay of Ascorbate Peroxidase (APOX)

Ascorbate peroxidase (APOX) activity was determined according to the method described by Rao et al. (1996). APOX activity was recorded by following the decrease in A290 for 3 min in 1 ml of reaction mixture containing 100mM phosphate buffer pH 7.5, 0.5 mM ascorbate and 0.2 H_2O_2 mM and 30µL enzyme extract. Enzyme activity was expressed as m mol oxidized ascorbate min^{-1} gm^{-1} F.wt.

Extraction of polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL):

Extraction of PPO and PAL was made as reported by Yingsanga et al. (2008). Powdered samples (0.5 g) were homogenized with buffer containing 20 ml of 100 mM sodium phosphate buffer (pH 7.0) and 0.5 g polyvinyl pyrrolidone (PVP) (Mol. wt 40,000) for the assay of the activities of PPO. PAL activity was measured in powder extracted with 50 mM sodium phosphate buffer (pH 8.8) containing 5 mM β-mercaptoethanol. The extracts were filtered through two layers of miracloth and the filtrates were centrifuged at 27,000 x g at 4°C for 30 min.

Assay of polyphenol oxidase (PPO)

PPO activity was assayed as described by Luh and Phithakpol (1972), where 1 ml of supernatant was mixed with 1 ml of sodium phosphate buffer (100mM, pH 7.0) and 1 ml pyrocatechol (50 mM). Change in absorbance at 410 nm was measured spectrophotometrically. PPO activity was expressed as the change in the optical density / gm fresh wt./ min.

Assay of Phenylalanine ammonia lyase (PAL)

PAL activity was measured following the method of Ross and Sederoff (1992). The assay mixture, containing 100 µl of plant extract,500 µl of 50 mM Tris HCI (pH 8.8), and 600 µl of 1 mM L- Phenylalanine, was incubated for 60 min at room temperature, and reaction was arrested by adding 2 N HCI. The assay mix was extracted with 1.5 ml of toluene by vortexing for 30 sec. Toluene was recovered after centrifugation at 1000 rpm (CRU-5000 centrifuge ITC) for 5 min. The absorbance of the toluene phase containing *trans*- cinnamic acid was measured at 290 nm against the blank of toluene. Enzyme activity was expressed as nmol *trans*- cinnamic acid released min^{-1} g^{-1} fresh weight

Extraction and analysis of phenolic compounds

Frozen tissues (1 g) were homogenized with 10 ml of 80% methanol and left for 24 h at room temperature before centrifugation at 15,000 g for 10 min**.** One ml of the methanolic extract was added to 5 ml of distilled water and 250µL of Folin-ciocalteau reagent, and the solution was kept at 25 ^{o}C for 30 min. then 1 ml of a saturated solution of Na_2 CO_3 and 1 ml of distilled water were added, and the mixture was incubated for 1 h at 25 ^{o}C. The absorption of the developed blue colour was measured using spectrophotometer at 725 nm. The total phenolic content was calculated by comparison with a standard curve obtained from a folin reaction with phenol. On the other hand, various phenolic acids in bean roots and shoots were determined using HPLC. Phenolic acids present in the samples were identified by comparing retention time (RT) of standard. Concentrations were calculated by comparing peak areas of compounds with those in the samples run under the same elution condition.

Statistical Analysis

The obtained data were analysed statistically using the one way analysis of variance as described by Snedecor and Cochran (1969). The means were compared by L.S.D. using Statistical Package for Social Sciences (SPSS) program version 12.

RESULTS AND DISCUSSION

Plants are attacked by a variety of pathogen and have evolved a wide range of mechanisms to defend themselves**,** including constitutive and induced defences. Many reports have shown that induced disease resistance in plants by biotic and abiotic elicitors is a very effective method for restricting the spread of fungal infection (Qin et al., 2003).

The use arbuscular mycorrhizae fungi (as bioagent) for controlling soil borne disease have been well-documented (El-Khallal, 2007). Results in Table (1) showed that % disease incidence is significantly increased in bean plants infected by R.*solani*

comparing with non-infected control. Low level of disease incidence in the control may be due to accidental low soil contamination (El-Khallal 2007). On the other hand, results showed that inoculation with AM fungi along with the pathogen resulted in a lower level of disease incidence than when the pathogen was used alone ,

Table 1. Induction of systemic resistance (as disease incidence %) in bean plants treated with bioagent (AM) against root rot caused by *Rhizoctonia solani* after 15 days from sowing.

Treatment	Disease incidence (%)
Control (non-treated)	$13.00^{c} \pm 2.3$
R.*solani.*	$44.82^{a} \pm 5.2$
R.*solani* + AM fungi	$26.32^{b} \pm 3.8$
LSD at 5 %	6.54

Means within the same column with the same letter are not significantly different ($P \leq 5\%$)

Assessment of disease showed striking differences in the rate and extent of fungal colonization, where disease incidence were reduced from 44.82% in pathogen–infested soil to 26.32 % in arbuscular mycorrhial treatments. Our results are in harmony with other reports (El-Khallal, 2007).

Arbuscular mycorrhizal fungi (AM) are known to improve the nutritional status of their host plants, and lead to alterations in the hosts physiology and exudation from roots. There is accumulating evidence that AMF can reduce disease incidence and promte propagation of several soil-borne pathogens inhibitive to *Meloidogyne incognita* (Li et al., 2006).

In addition, the efficiency of AM fungi for controlling and reducing disease incidence in bean plants might be related to the elevated level of jasmonic acid, upon mycorrhization and may enhance defence status in mycorrhized tissues (El-Khalal, 2007).

Oxidative status

H_2O_2 is an endogenous signaling molecule involved in plant responses to abiotic and biotic stresses. Infection by *Rhizoctonia solani* significantly increased levels of H_2O_2 in both roots and shoots of bean plants. Reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide during so-called"oxidative burst" are the earliest responses following successful pathogen recognition. ROS may be directly involved in pathogen killing and strengthening of plant cell walls, as well as triggering hypersensitive cell death (HR) and in production of systemic resistance signaling (Shetty et al., 2007).

Table 2. Levels of hydrogen peroxide (H2O2) and malondialdehyde, (MDA) in root and shoot of *Phaseolus vulgaris* plants infected with *Rhizoctonia solani* (R.solani) and grown in soil infested with AM fungi.

Treatment	H_2O_2 (µmol g^{-1}f.wt.)				MDA(n mol g^{-1}f.wt.)			
	Root		Shoot		Root		Shoot	
Days after planting	14	28	14	28	14	28	14	28
Control (uniafected)	0.22 [b]	0.35 [b]	0.42 [c]	0.56 [c]	8.22 [b]	4.46 [c]	11.91 [b]	12.70 [b]
R.solani	0.63[a]	0.81 [a]	0.83 [b]	1.05 [b]	10.19 [a]	10.73 [a]	14.73 [a]	15.85 [a]
R.solanis + AM fungi	0.21 [b]	0.19 [c]	1.70 [a]	1.83 [a]	9.88 [a]	8.93 [b]	10.24 [c]	13.36 [b]
LSD at 5%	0.12	0.14	0.20	0.32	0.54	1.06	1.22	1.28

Means within the same column with the same letter are not significantly different ($P \leq 5\%$)

Application of AM fungi markedly decreased levels of H_2O_2 in roots and increased it in shoots of bean plant through the experimental time, as compared with infected control (Table 2). Induction or suppression of H_2O_2 in roots and shoots of these treatments could be related to the activity of scavenging antioxidant enzymes. In addition, ROS was proposed to act synergistically in a signal amplification loop with salicylic acid (SA) to drive the HR and the establishment of systemic defense. AM fungi can elicit defense responses mediated by enhancing Jasmonic acid and SA signals in AM-treated plants**.**

Accumulation of H_2O_2 in stressed plants attacked by *R.solani* led to enhanced potential for the production of hydroxyl radicals, which lead to lipid peroxidation as shown in (Table 2). The measurement of malondialdehyde (MDA) levels is routinely used as an index of lipid peroxidation. MDA level significantly increased in roots and shoots of infected bean plants. These increments markedly decreased in response to AM fungi application (Table 2). It is well known that ROS-induced lipid peroxidation of membranes is a reflection of stress-induced damage at the cellular level. However, subsequent products of lipid peroxidation have been shown to posses antibacterial properties and signaling functions.

Changes in the Activity of Antioxidant Enzymes

Suppression of ROS scavenging enzymes plays a key role in the development of efficient defense responses in plants by enhancing pathogen-induced HR-mediated cell death. Results in Table (3) showed that roots and shoots of bean plants infected with *Rhizoctonia solani* showed significantly increased activity of SOD, CAT and APOX at 14 and 21 day of growth as compared with healthy control. Treatments with AM markedly

increased activity of H_2O_2 scavenging enzymes (POX, CAT and APOX) and greatly decreased SOD activity.

Superoxide dismutase SOD is an essential component of plants anti-oxidative defense system. It plays an important role in dismutation of free hydroxyl radicals by the formation of hydrogen peroxide. Our result showed that greater increase in the activity of SOD in infected plants might be effective in scavenging mechanism to remove excess accumulation H_2O_2 in roots and shoots. It has been extensively demonstrated that pathogens induce changes in the antioxidant status of plant cells. The amount of SOD enzyme increase in *Phaseolus vulgaris* and this induction led to the hypersensitive response of resistant leaves**.** On the other hand, accumulation of H_2O_2 in shoots of infected plant treated with AM fungi could be related to the increase in SOD, POX, CAT and APOX. AM fungi specific alterations in the pattern of anti oxidative enzymes, indicate that the corresponding genes might be expressed specifically during the colonization process**.** This activation corresponds to the occurrence of ROS in arbusculated root cortical cell.

Table 3. Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APOX) activities of shoots and roots in AM and non-AM Phaseolus vulgaris seedlings infected with Rhizoctonia solan.i

Treatment	SOD (U g^{-1}fresh wt.)				CAT (µ mol H_2O_2 min^{-1} g^{-1} fresh wt.)				APOX (µ mol ascorbate oxidized min^{-1} g^{-1} fresh wt.)			
	Root		Shoot		Root		Shoot		Root		Shoot	
Days after planting	14	28	14	28	14	28	14	28	14	28	14	28
control	13.44[b]	23.36[b]	6.30[c]	17.22[a]	30.81[c]	72.24[b]	108.08[c]	187.61[b]	45.8[c]	50.4[c]	48.3[b]	52.6[c]
R.solani	16.91[a]	27.44[a]	7.53[b]	17.92[a]	56.34[a]	102.2[a]	147.84[b]	193.86[a]	49.8[b]	73.6[a]	50.4[b]	77.8[b]
R.solanis + AM fungi	13.58[b]	20.86[c]	9.58[a]	15.82[b]	57.12[a]	103.88[a]	156.24[a]	186.76[a]	53.9[a]	57.3[b]	83.2[a]	84.7[a]
LSD at 5%	0.42	1.85	1.04	1.20	6.58	10.46	7.82	4.34	3.08	5.47	6.88	5.90

Means within the same column with the same letter are not significantly different ($P \leq 5\%$)

Changes in phenolic compounds and its related enzymes

It is known that several fungal products such as proteins, glucoproteins or oligosaccharides can trigger the defense mechanisms in plants. In the presence of pathogen, plants develop a vast array of metabolic defense responses sequentially activated by a complex multicomponent network that may be local and/or systemic**.**

Defense responses to pathogen infection include the production of several secondary metabolites such as phenolics.

In the present study, results showed that R. *solani* and the bioagent AM fungi significantly increased various phenolic compounds in roots of bean plants, as compared with non-infected control (Table 4). The highest total phenolics was recorded in AM fungi treated plants. Therefore, application of AM could be effective, because accumulation of phenolics may imply an increased resistance of plants to pathogen infection, through its role in lignification and suberization of the plant cell wall. However, levels of certain phenolic acids greatly changed in roots of bean plants in response to pathogen, with inoculation, AM fungi as compared with healthy control. Cinnamic, gallic, ferulic and quercetin acids markedly increased in infected bean plants with *Rhizoctonia solani* as compared with healthy plants. While AM applied having high levels of cinnamic and ferulic acid, low level of gallic, and quercetin acid as compared with normal control.

High level of cinnamic acids in infected roots especially those treated with AM fungi might be related to the activation of phenylpropanoid pathway through activation of PAL activity (Table 5). However, induction of ferulic acid in roots in response to pathogen infection indicate that this acid act as antifungal (Agrios 2005), supported its role in reducing disease through the formation of defense barrier and activation of defense responses.

Table 4. The contents of total phenolic acids and some phenolic acids (mg g-1 fwt) in roots of bean plants (15 days after pathogen inoculation) treated with AM as bioagent and grown in Rhizoctonia solani infested soil.

Treatment	No of peaks	Total phenolic acids	Known of phenolic acids				
			Cinnamic acid	Gallic acid	Ferulic acid	Quercetin	Coumarin
Non-infected Control	7	432.13	20..33	21.97	32.44	11.40	_
Infected control (*R.solani*)	10	325.62	28.94	11.25	33.86	26.39.	_
R.solani + AM fungi	13	365.21	30.22	7.33	35.66	3.83	29.00

Activation of PAL in *Asparagus densiflorus* could directly affect accumulation of secondary toxic compounds, such as phytoalexins, which might be released in root exudates and on root segment surfaces from the inoculated plants to inhibit fungal spore germination and growth (He et al., 2001). Rapid and transient increase in PAL activity was observed in sugar beet seedlings treated with cell wall protein fraction obtained from mycelial mat of *Pythium oligandrum* (Takenaka et al., 2003).

In parallel to the induction of various phenolic acids, results in Table (5), it appeared that activities of lignification enzymes PPO, POX and PAL significantly increased in both roots and shoots of infected bean plants treated or untreated with AM fungi. This induced increase increased with time. The highest activity of PPO and PAL was recorded in shoots of 28 day old bean plants inoculated with AM fungi. POX and PPO are important in the defense mechanism against pathogens, through its role in the oxidation of phenolic compounds to quinones, resulting in increased antimicrobial activity (El-Khallal, 2007). Thus, quinones play an important role in halting pathogen development by accelerating the cellular death at the infection site and by generating a toxic environment which will inhibit the growth of the pathogen inside the cells. Also, Cordier et al. (1998) reported that plant bioprotection by mycorrhizal fungi could be drawn from the accumulation of phenolic compounds in the plant cell wall reflecting increased lignification. Lignification, considered as an important mechanism for disease resistance, may contribute to reducing pathogen proliferation in mycorrhizal roots.

Increase polyphenol oxidase activity in host tissues in response to infection by the pathogen has been reported (El-Khallal, 2007). The phenol-oxidizing enzyme plays a vital role in tissue browning by way of its capacity to oxidize phenols to quinones. The toxic substances, quinones, which are more reactive and have more antimicrobial activity than the phenols already existing in plant, account for the increased host resistance against the invading pathogen.

Table 5. Activities of polyphenol oxidase (PPO), phenylalanineammonia lyase (PAL) and peroxidase (POX) in root and shoot of Phaseolus vulgaris plants infected with Rhizoctonia solani (R.solani) and treated with AM as bioagent.

Treatment	PPO(enzyme activity g-1 f wt. min-1)				PAL (mM t-cinnamic acid g-1 f wt. h-1)				POX(enzyme activity g-1 f wt. min-1)			
	Root		Shoot		Root		Shoot		Root		Shoot	
Days after planting	14	28	14	28	14	28	14	28	14	28	14	28
control	36.57^{c}	49.81^{c}	18.80^{c}	42.21^{b}	13.65^{c}	11.95^{c}	12.34^{c}	14.05^{c}	46.57^{c}	47.81^{b}	16.84^{c}	40.22^{c}
R.solani	51.32^{a}	55.03^{b}	24.88^{b}	56.83^{a}	17.07^{b}	20.06^{b}	14.08^{b}	18.35^{b}	50.31^{b}	50.92^{b}	24.61^{b}	51.83^{b}
R.solani + AM fungi	46.44^{b}	64.12^{a}	40.22^{a}	47.91^{a}	21.10^{a}	24.02^{a}	17.86^{a}	27.35^{a}	59.12^{a}	69.37^{a}	52.37^{a}	56.98^{a}
LSD at 5%	3.72	4.44	4.85	5.02	1.32	1.87	0.98	3.04	3.22	5.38	6.62	5.00

Means within the same column with the same letter are not significantly different ($P \leq 5\%$)

Finally, our results indicated that application of AM fungi greatly induced bean resistance against root rot disease caused by *Rhizoctonia solani*. Thus, induction of antioxidant enzymes activity and various phenolic compounds and its related enzymes played an important role in increasing resistance in AM treated plants against root rot disease.

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المقاومه الحيوية لمرض عفن الجذور في نبات الفاصوليا باستخدام فطريات الميكوريزا

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لقد تم تعميم استخدام المقاومة الحيوية للأمراض النباتية في الزراعة كمصدر طبيعي و اقتصادي. و في هذه الدراسة استخدمت فطريات الميكوريزا لاستحثاث مقاومة نباتات الفاصوليا لمرض العفن الجذري المتسبب عن فطر الرايزوكتونيا و قد توصلت الدراسة إلى النتائج التالية :

- انخفضت النسبة المئوية لحدوث مرض العفن الجذري انخفاضا معنويا في النباتات المعاملة بفطريات الميكوريزا مقارنة بالنباتات الغير معاملة و المحقونة بفطر الرايزوكتونيا الممرض فقط .
- زاد مستوى كلا من فوق أكسيد الهيدروجين و الليبيد بيروكسيديز في جذور و سيقان نباتات الفاصوليا المصابة بفطر الرايزوكتونيا الممرض بزيادة مدة الإصابة ، و لقد كان لفطريات الميكوريزا دور فعال في تخفيف الأثر الضار لمسببات الأكسدة من نواتج الأكسجين الحرة في النباتات المصابة وذلك بانخفاض محتوى النباتات من فوق أكسيد الهيدروجين و الليبيد بيروكسيديز.
- زاد نشاط الإنزيمات المضادة للأكسدة (الكتاليز – الأسكوربيت بيروكسيديز و السوبر أكسيد ديزميوتيز) زيادة معنوية في أنسجة نبات الفاصوليا المعاملة بفطر الرايزوكتونيا الممرض و فطريات الميكوريزا (معا أو كلا على حدة). و لقد تم تسجيل أعلى نشاط إنزيمي في سيقان النبات المعاملة بفطر الميكوريزا في النباتات النامية لمدة 14 يوم .
- زاد تركيز المركبات الفينولية الكلية و الأحماض الفينولية المختلفة خاصة حمض السينامك و حمض الفيروليك زيادة ملحوظة في جذور نباتات الفاصوليا المعاملة بفطر الرايزوكتونيا و فطريات الميكوريزا (معا أو كلا على حدة) ، و لقد وجد الكومارن في النباتات المعاملة بالميكوريزا فقط أما بالنسبة لحمض الكويرسيتنى فقد زاد تركيزه في النباتات المصابة بفطر الريزوكتونيا و قل في النباتات المعاملة بفطريات الميكوريزا بالمقارنة بالنباتات الغير مصابة.
- زاد نشاط إنزيمات البولي فينول اوكسيديز و البيروكسيديز و الفينل ألانين امونيا لاييززيادة كبيرة في جميع النباتات المصابة .
- و لقد توصلت هذه الدراسة إلى أن معاملة نباتات الفاصوليا المصابة بفطر الرايزكتونيا الممرض بفطريات الميكوريزا الداخلية كان لها الدور الفعال في زيادة مقاومة النباتات باستحثاث نشاط انزيمات الأكسدة و المركبات الفينولية.