Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Induction of systemic resistance against damping-off and root-rot of white lupine (*Lupinus albus* L.) using some bioagents, chemical inducers and a mycorrhizal fungus

Sahar A. El-Sayed[1,3]*, Rania Z. El-Shennawy[1], Thuraya Ahmed Mohammed[2,3], Amany M.F. Attia[4]

1. *Plant Pathology Research Institute, Agriculture Research Centre, 12619, Giza, Egypt*
2. *El Nelain University, Faculty of Science and Technology, Khartoum, Sudan*
3. *College of Arts and Sciences in Baljurashi, Department of Biology, Al-Baha University, Saudi Arabia*
4. *Plant Pathology Department, Faculty of Agriculture, Cairo University, Egypt*

** Corresponding author's E-mail: Sahar_abbas2002@yahoo.com*

ABSTRACT

Damping-off and root-rot diseases of lupine are considered the main problem in lupine production in Egypt. In this study, the effect of two bioagents *i.e.*, *Trichoderma harzianum* and *Bacillus megaterium*, two chemical inducers *i.e.*, salicylic and citric acid, and mycorrhizal fungus compared to the fungicide Topsin-M70 were tested *in vitro*, greenhouse and field conditions against *Fusarium exysporium*, *F. Solani*, and *Rhizoctonia solani* are the causes of root rot and damping-off of lupine plants. All of the tests were done in vitro. The tested linear growth of bacteria was significantly slowed by bioagents of the three pathogenic fungi, *B. megaterium.* The most significant reduction was induced by *T. harzianum*, which was followed by *T. harzianum*. Sowing lupine in the seeds soil artificially inoculated with any of the three pathogenic fungi with a large increase in fresh and dry weight. The incidence of pre-and post-emergence damping-off was significantly reduced compared to the control treatment. *B.megaterium* and Topsin-M70 exhibited the highest percentages of survived plants. In general, in all the tested bio-agents, chemical inducers, the mycorrhizal fungus, and Topsin-M70 under field circumstances, the incidence of damping-off was dramatically reduced. The data obtained revealed all the tested treatments. Peroxidase, polyphenol oxidase, and chitinase enzymes, which play critical roles in plant metabolism, saw a significant increase inactivity, defense mechanisms that work against pathogens infection.

Keywords: Bioagents, Chemical inducers, Lupine, Mycorrhizal fungi, Plant defence-related enzymes, Topsin-M70.
Article type: Research Article.

INTRODUCTION

The leguminous white lupine (*Lupinus albus* L.) has been cultivated in Egypt for human and animal nourishment, as well as medical and industrial reasons. It can be regarded as an environmentally friendly crop because of its effective nitrogen fixation system, as well as its enhancement of traditional cereal rotation and protein supply in low-input farming systems (Julier *et al.* 1994, Shevchenko *et al.* 2021). Lupine-borne infections such as *Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani* damage the roots and stem base of lupine plants, and are one of the most critical factors restricting yield production (Zian 2011; El Sayed 2015; Maslienko *et al.* 2021). In addition, *T. harzianum* induces plant systemic resistance against soil-borne pathogens, while *B. subtilis* produces secondary metabolites which suppress the pathogens (Asaka & Shoda 1996). Many strategies are normally required to control soil-borne diseases, including soil heating and biological soil disinfestation, organic improvement implementation, yield management, and biological control agents (Katan 2004). Trichoderma species are excellent infection competitors, can modify the rhizosphere, are pesticide tolerant or resistant, can grow and survive in un-favourable conditions, are efficient in utilizing soil nutrients, have full

Caspian Journal of Environmental Sciences, Vol. 20 No. 3 pp. 571-583 Received: Jan. 02, 2022 Revised: March 23, 2022 Accepted: May 06, 2022
DOI: 10.22124/CJES.2022.5693

Publisher: University of Guilan, Iran

aggressiveness against phytopathogenic fungi, and promote plant growth (Vinale *et al.* 2006). Antioxidant-induced resistance is also a viable strategy for controlling diseases caused by soil-borne pathogens. The salicylic acid and oxalic acid-induced systemic resistance have been reported in soybean against root rot under controlled environments (El-Gendy *et al*. 2016). However, according to El-Mohamedy & Abd-All (2013) and El Mohamedy *et al.* (2015), using bio-priming seed treatment to prevent root rot soil-borne pathogens as a substitute for chemical fungicides is possible without posing any damage to humans, animals, or the environment. To alleviate the adverse effect of root rot caused by *Fusarium solani*, *Rhizoctonia solani* logically, arbuscular mycorrhizal fungi are one of the most effective biological techniques identified to treat root rot and wilt diseases, according to biologists (Al-Hmoud and Al Momany 2015). Mycorrhizal fungi are common and help plants grow and develop by improving nutrient uptake and soil health in the rhizosphere (Nahiyan & Matsubara 2012; Al- Hmoud & Al-Momany 2015). Resistance was induced by mycorrhizal fungus by increasing the defense system's accumulation (Alqarawi *et al.* 2014; Abd-Allah *et al.* 2015; Akhter *et al.* 2015). When *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., and *Serratia marcescens* were used as bioagents for chitinase, the accumulation of enzymes like chitinase, peroxidase, and polyphenol oxidase, which play an important role in plant defense mechanisms against pathogens infection, increased in treated bean plants more than in untreated ones (Abd El-Khair *et al.* 2011; Ahmed 2011). The objective of the present work is to investigate the effect of the biocontrol agent, antioxidants, and Mycorrhizal fungi in comparison with two fungicides on the growth of fungi responsible for causing damping-off and root rot of lupine. The work was expanded to control both dampening and root rot. Lupine infections in greenhouses and the treated plants, determine the activities of the enzymes peroxidase, polyphenol oxidase, and chitinase.

MATERIALS AND METHODS

The used materials

Plant material

White lupine seeds (*Lupinus albu*s L.), and cultivar Giza 2 were obtained from the Legume Research Department, Field Crops Research Institute, ARC, Giza, Egypt.

Source of the pathogens

Virulent isolates of *Fusarium oxysporium, F. solani*, and *Rhizoctonia solani*, previously isolated from lupine roots were obtained from Mycology and Plant Disease Survey Department, Plant Pathology Res. Institute, ARC, Giza, Egypt.

Source of the bio-agents

Two commercial bioproducts, *i.e.*, Plant Guard (*Trichoderma harzianum*, 3×10^7 CFU mL^{-1}) and Bio- ARC (*Bacillus megaterium*, 2.5×10^7 CFU mL^{-1}) were used. Loops from each product were streaked on PDA and nutrient agar media to obtain the bioagent of each product and maintained in slants containing PDA and nutrient agar media, respectively.

Mycorrhizal fungus

The vesicular arbuscular mycorrhizal (VAM) fungus (*Glomus* sp.) was obtained as a formulation from the Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, ARC, Giza, Egypt.

Chemical inducers

Citric and salicylic acids as plant systemic resistance inducers were obtained from El- Nasr Company, Egypt.

Laboratory experiments

Inhibitory effect of the tested bio-agents, chemical inducers, and Topsin-M70 on the linear growth of the three tested pathogenic fungi

T. harzianum was grown on a PDA medium, while *B. megaterium* on a nutrient agar medium. To test the antagonistic effect of *T. harzianum* in vitro on the linear growth of the tested pathogens of lupine damping-off and root-rot. Petri dishes (9 cm in diameter), each containing 20 mL PDA medium were inoculated with discs (5 mm in diameter) of any of the tested pathogens, taken from 7 day-old cultures. The discs were placed near the edge of each Petri-dish. At the same time, plates were inoculated with equal discs of *T. harzianum*. For each treatment, three plates were used as duplicates. The antagonistic effect of *B. megaterium* on the linear growth of the same pathogens was tested by streaking the growth *B. megaterium* on PDA plates containing PDA medium close to the edge of each Petri-dish, while the inoculation with the tested pathogen was done near the opposite near edge of each Petri- dish as mentioned before. Control plates were prepared without the bioagents. In the PDA medium, the effect of chemical inducers on the growth of pathogenic isolates was tested. Twenty mm of PDA medium containing either 10 mg of citric or salicylic acid were produced and infected with 5 mm discs of either of the three pathogenic fungi studied. Plates containing PDA medium amended with 500 ppm of the fungicide Topsin M-70 were prepared and inoculated with 5-mm discs of any of the three examined pathogenic fungi. Control plates were prepared without any of the bioagents, chemical induces and the fungicide Topsin M-70 were inoculated with 5mm discs of any of the three tested pathogenic fungi. All plates were incubated at 28 °C until the control treatment's growth reached the plate's edge. Colony diameter was measured and the percentages of growth inhibition for the pathogen were calculated and the reduction percentage of fungal growth was calculated according to the following formula:

Reduction (%) **= (C-T)/C × 100**

where:

C = the growth of the pathogen (check)

T = the growth of the pathogen with each treatment.

Scanning Electron Microscope (SEM) for the interaction between the bio-agent *T.harzianum* and the pathogen *F. solani*

A disc of 8 mm in diameter covered in *Trichoderma* and the pathogen hyphae was obtained for SEM investigation to show the interaction areas between *T. harzianum* and *F. solani*. The discs were soaked in 5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, rinsed in the same phosphate buffer, dehydrated in a graded aqueous ethyl alcohol series (10, 30, 50, 75 and 95%), and then placed in 100% ethanol at room temperature for a few minutes. They were then coated with gold-palladium using an anion sputtering device after being dried with a critical point drier unit attached on aluminum stubs with silver adhesive. Thereafter, the samples were inspected using a scanning electron microscope at Ain-Shams University's SEM unit (Manzali *et al.* 1993; El-Habbaa 1997; El Sayed 2006).

Effect of treating lupine seeds with bio-agents, chemical inducers, and a mycorrhizal fungus compared to Topsin-M70 *in vivo* on the incidence of damping-off

These trials were conducted in greenhouse conditions. Sterilized plastic pots (25-cm-diameter) filled with autoclaved sandy clay (1:2 w/w) soil were utilized to evaluate the efficiency of the bioagents *T. harzianum* (Plant Guard), *B. megaterium* (Bio-ARC), and VAM fungus (*Glomus* sp.) for controlling the infection by the three pathogenic fungi. The inoculum of *T. harzianum* was prepared by growing on autoclaved corn-meal sand medium (100 g corn-meal, 50 g sand, and 100 mL tap water in 500-mL bottles). The bottles were inoculated with 5 mm in diameter fungal disks taken from the margin of 7-day-old culture. The inoculated bottles were incubated at 28 ºC for 15 days. The inoculum of *T. harzianum* was added to the soil at the rate of 25g kg^{-1} soil before inoculating the pathogen (Abd El- Ghany 2007). While Bio-ARC (*B. megaterium*) was added to the soil as suspension (5 g L^{-1} water) at the rate of 100 mL pot^{-1}. Corn roots colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus (*Glomus* sp.) were added to the soil during the sowing of lupine seeds and before adding the pathogen at the rate of 10 g root segments as a layer of 3 cm under the surface-sterilized lupine seeds. In addition, soon before sowing, lupine seeds were soaked for 2.5 h (Shalaby1997) in a 10.0 mM concentration of citric or salicylic acid. The inoculum of any of the tested three pathogens was added to the soil, at the rate of 5% inoculum level. Pots were inoculated with the tested pathogens served as control treatment. The pots were irrigated before sowing one week before sowing to homogenous distribution and to stimulate the pathogens and bioagents growth. Finally 10 lupine seeds (cv. Giza 2), surface sterilized with 2% sodium hypochlorite were sown in each pot and three pots were used as treatment. All pots were kept under greenhouse conditions.

Disease assessment

Pre-emergence damping-off, post-emergence damping-off, and the proportion of survived plants were measured 15, 21, and 60 days after planting, respectively, to determine disease incidence. To avoid root damage, the entire plant was gently pulled up and rinsed under running tap water. Plants were then divided into roots and shoots, weighed, and placed in a 70 °C- oven for 72 h to estimate their dry weight. Damping-off was assessed using the following formula as follows:

$$\text{Pre-emergence damping-off (\%)} = \frac{\text{No. of non-germinated seeds after 15 days}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Post-emergence damping-off (\%)} = \frac{\text{No. of dead seedlings after 30 days}}{\text{Total No. of planted seeds}} \times 100$$

Survived seedlings =Total No. of planted seeds – (pre+ post emergence damping-off). Also, root–rot severity was assessed 60 days after sowing using the devised scale (0-5%) according to Salt (1982) as follows:

$$\text{Root – rot severity (\%)} = \frac{\text{Sum of (nxv)}}{5N} \times 100$$

where:
n= Number of roots in each category.
v= Numerical value of each category.
N= Total number of roots in the samples.
5= High numerical value.

Field experiments

Field experiments were carried out in fields have a back history of high infestation with the causal of lupine damping-off and root-rot diseases during growing season 2021/2022 on November 5 and 7, 2021 at two locations *i.e.*, Giza Agric. Res. Stat. at Giza and the farm of Fac. of Agric., Menoufia Univ., respectively, to evaluate the efficiency of the tested bioagents (*T. harzianu*m and *B. megaterium*), chemical inducers (citric and salicylic acids), and the mycorrhizal fungus (*Glomus* sp.) compared to the fungicide Topsin-M70 for controlling damping-off and their effect on some crop parameters. The experimental design was a complete randomized block with three replicates. The experimental unit area was 10.5 m^2 (3 m long × 3.5 m width) of 6 rows. Lupine seeds (cv. Giza 2) treated with the examined treatments as described before were sown in hills at the rate of 2 seeds/hill, 25 cm apart, on one side of the rows in both locations. Also, untreated seeds were used as control treatment in the same manner. The incidence of pre-and post-emergence damping-off was assessed 15 and 30 days after sowing as mentioned before. At harvest, plant height (cm), the number of branches / plant, the number of pods/ plant, and weight of seeds/plant and 100-seed weights were estimated and recorded.

The activity of oxidative and catalyzed enzymes

With the studied bioagents and chemical inducers, Tospin M-70 treatments, the activity of peroxidase (PO), polyphenol oxidase (PPO), and chitinase were evaluated in leaves from the treated lupine plants. Samples for enzyme testing were taken one month following the first treatment to the plants by the various treatments. For enzyme assays, one gram of lupine leaves was homogenized in an ice bath with 2 mL of 0.1 M sodium phosphate buffer (pH 7.0). The homogenates were then centrifuged for 10 minutes at 10.000 ppm. The activity of defense-related enzymes such as PO, PPO, and chitinase were measured in supernatants.

Activity of PO

50 L enzyme extract was combined with 2.85 mL of 0.1 M phosphate buffer (pH 7.0) and 0.05 mL of 20 mM guaiac reagent to determine peroxidase activity (PO: Fu & Huang 2001). To begin the reaction, 0.02 mL of 40 mM hydrogen peroxide was added to the mixture. Over a one-minute period, the rate of rising in absorbance at 470 nm was observed. A change in absorbance of 0.01 for 1 g of fresh enzyme activity was defined as one unit of enzyme activity.

The activity of PPO

Mayer *et al.* (1965) proposed a method for measuring the activity of polyphenol oxidase (PPO). 200 mL enzyme extract and 1.5 mL 0.01 M catechol were included in the reaction mixture. Changes in absorbance at 495 nm min^{-1} mg^{-1} of protein were used to calculate the activity.

Activity of chitinase

The activity of chitinase was determined using Boller and Mauch's technique (1988). Mm N-acetyl glucose amine equivalent released g fresh weight tissue 60 minutes was used to measure the enzyme's activity.

Statistical analyses

The gathered data were statistically analyzed using Snedecor and Cochran's techniques (ANOVA). The least significant difference test L.S.D was used to compare treatment means at a 5% level of probability.

RESULTS

Effect of some bioagents, chemical inducers, and the fungicide Topsin-M70 on the linear growth of the three pathogenic fungi

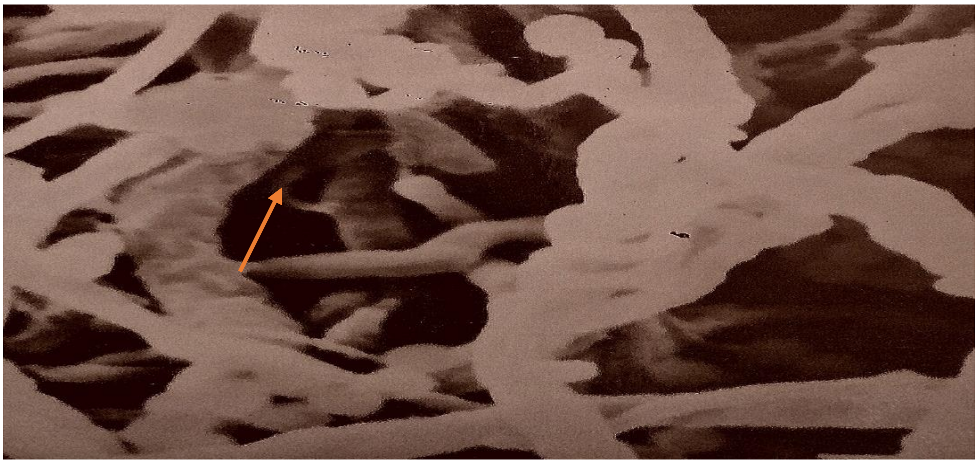
Data presented in Table 1 show the effect of the tested bioagents, chemical inducers, and the fungicide Topsin-M70 on linear growth of *F. oxysporum*, *F. solani* and *Rhizoctonia solani*, the causal of lupine damping-off and root-rot diseases. All treatments significantly reduced the linear growth of the three tested fungi. The average reduction in the linear growth of the tested fungi was recorded at 73.33, 72.22, and 63.44; 68.88; 66.67 and 66.11; 55.56, 50.0 and 44.44, and 60.0, 55.56 and 46.66 when *B. megaterium*, *T. harzianum*, citric acid, and salicylic acid were tested, respectively. However, the fungicide Topsin-M70 was the superior treatment in this regard, where the three tested fungi failed to grow.

Table 1. Effect of some biocontrol agents, and chemical inducers compared to the fungicide Topsin-M70 on the growth of the three pathogenic fungi.

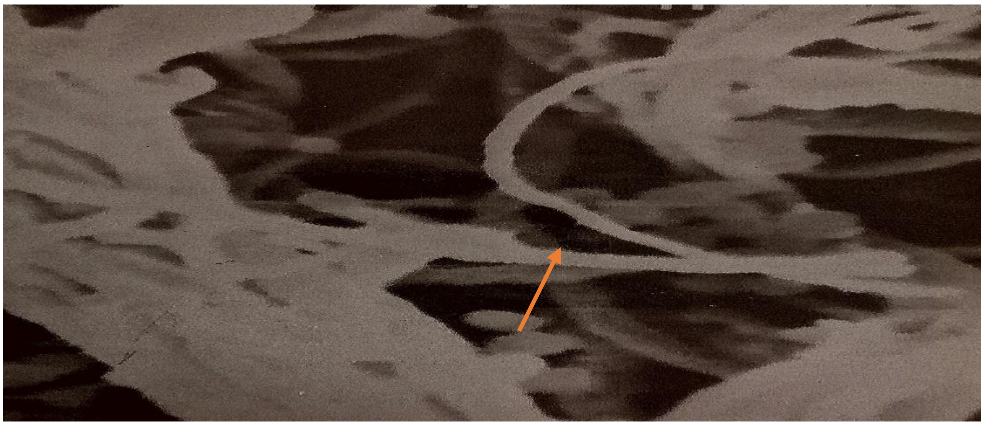
Treatments	*F.oxysporum*		*F. solani*		*R. solani*	
	Linear growth (mm)	**Reduction (%)**	**Linear growth (mm)**	**Reduction (%)**	**Linear growth (mm)**	**Reduction (%)**
B.megaterium	24	73.33	25	72.22	32	64.44
T. harzianum	28	68.88	30	66.67	35	61.11
Citric acid	40	55.56	45	50.0	50	44.44
Salicylic acid	36	60.00	40	55.56	48	46.66
Topsin-M70	0.0	100	0.0	100	0.0	100.0
Control	90	------	90	------	90	-------
L.S.D.	6.0	------	5.0	------	5.0	------

Scanning Electron Microscope (SEM) of the interaction between *T. harzianum* and *F. solani*

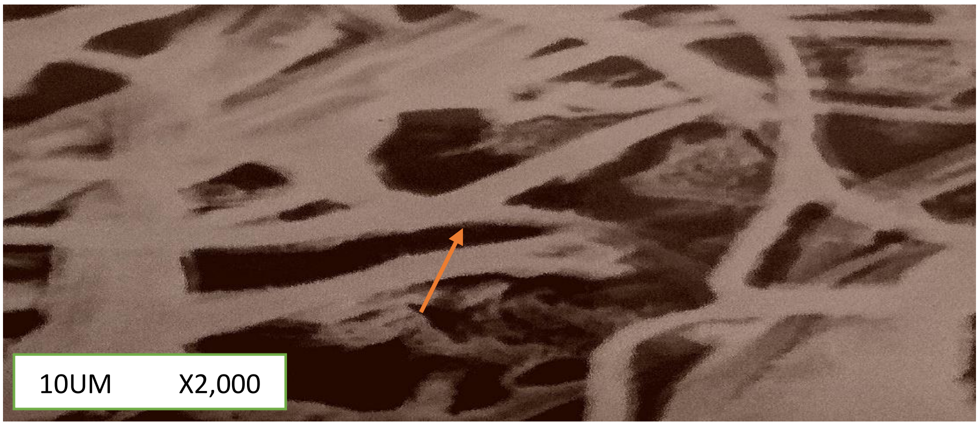
The illustrated results are in Figs. 1 (A, B and C) show that scanning electron microscope (SEM) illustrates the interaction sites between *T. harzianum* and the pathogenic fungus *F. solani*. In Fig. 1 (A) *Trichoderma* hyphae coiled around the host hyphae of *F. solani*. On the other hand, Figs. 1 (B and C) illustrate hook and pincer-shaped hyphal branches of *T. harzianum* and its penetration to the hyphae of *F. solani*.



A.



B.



C.

Fig.1. (A, B and C)**.** Scanning electron microscope (SEM) showing different types of *Trichoderma* parasitism on *F. solani* hyphae (2000X). A: Hooked parallel hyphae of *Trichoderma* which looking for penetration. B: Parallel hyphae which penetrate the mycelium and C: Adhesive hyphae of *Trichoderma* as well as its appressorium – bodies.

Effect of the tested bioagents and chemical inducers compared to Topsin-M70 on the incidence of damping-off under greenhouse conditions

Table 2. Effect of the tested bioagents and chemical inducers compared to Topsin-M70 of damping -off under greenhouse conditions.

Pathogens	Treatments	% Damping –off		%, Survived plants	%, Reduction in		% Increase in survive plants
		Pre-emergence	Post-emergence		Pre-emergence	Post-emergence	
F.oxysporum	*B.megaterium*	3.33	6.67	90.00	89.91	80.94	181.25
	T. harzianum	6.67	6.67	86.66	79.79	80.94	170.81
	Citric acid	10.00	10.0	80.00	69.70	71.43	150.00
	Salicylic acid	6.67	10.0	83.33	79.79	71.43	160.41
	Topsin-M70	0.00	0.00	100.00	100.00	100.00	212.50
	Control	33.00	35.00	32.00	0.00	0.00	0.00
F.solani	*B.megaterium*	5.00	5.00	90.00	85.71	83.33	157.14
	T. harzianum	10.00	5.00	85.00	71.43	83.33	142.86
	Citric acid	10.0	10.0	80.00	71.43	66.67	128.57
	Salicylic acid	5.00	6.25	88.75	85.71	79.17	153.57
	Topsin-M70	0.00	0.00	100.00	100.00	100.00	185.71
	Control	35.00	30.00	35.00	0.00	0.00	0.00
R.solani	*B.megaterium*	3.33	3.33	93.34	88.90	88.90	133.35
	T. harzianum	3.33	6.67	90.00	88.90	77.77	125.00

Citric acid	6.67	10.00	83.33	77.77	66.67	108.33
Salicylic acid	3.33	6.67	90.00	88.90	77.77	125.00
Topsin-M70	0.00	0.00	10.00	100.00	100.00	150.00
Control	30.00	30.00	40.00	0.00	0.00	0.00
L.S.D. at 0.05	8.54	7.39	9.85	-	-	-

The same trend was found in the case of the fungus *R. solani*, being 93.34, 90.0 and 90.0% survived plants were recorded, respectively. Meanwhile, in the case of *F. solani*, *B. megaterium* followed salicylic acid then *T. harzianum* were the best treatments in increasing the survived plants, being 90.0, 88.75, and 85.0%, respectively. Data presented in Table 3 indicate that the vascular arbuscular mycorrhizal fungus (*Glomus* sp.) was the most effective in reducing the severity of root-rot infection. In this respect, application of *F. oxysporum* with *Glomus* sp., led to decrease in the severity of root-rot from 33.3 to 25.6 %, while with *F. solani*, the percentage of reduction was from 27.8 to 11.1% and with *R. solani* was from 27.7 to 22.2%. No apparent symptoms of root-rot were observed on the control treatment.

Table 3. Effect of vascular arbuscular mycorrhizal fungus (*Glomus* sp.) on the severity of lupine root- rot under greenhouse conditions, 90 days after sowing.

Treatments	%, Root-rot severity	%,Reduction
F.oxysporum + VAM	25.6	42.5
F.solani + VAM	11.1	66.7
R.solani +VAM	22.2	33.3
F.oxysporium	33.3	-
F.solani	27.8	-
R.solani	27.8	-
Control with VAM only	0.00	-
Control	0.00	-
L.S.D at 0.05	4.02	

Data presented in Table 4 indicate that treated lupine seeds with the tested bioagents, and chemical inducers compared to fungicides significantly increased the fresh and dry weight of lupine shoots and roots under artificial inoculation with the three tested fungi under greenhouse conditions. The increases in shoot fresh and dry weight, were 120.47 and 64.65% in the case of inoculation with *R. solani*. It was 118.99 and 101.23%, in the case of inoculation with *F. solani*; 107.50 and 74.0%, in the case of inoculation with *F. oxysporum*, which have resulted from the treatment with salicylic acid. In the case of *B. megaterium*, 81.12 and 47.0% increases were recorded for *F. oxysorum*; 101.86 and 72.73% for *F. solani*; and 103.6 and 42.58 % for *R. solani* respectively. Meanwhile, the increases due to the treatment with citric acid were 64.67 and 38.6% for *F. oxysorum;* 83.80 and 52.83% for *F. solani;* and 76.08 and 22.85 % for *R. solani*, respectively. In addition, the increases due to the treatment with *T. harzianum* were 13.43 and 2.8 % for *F. oxysporum;* 35.84 and 26.04 % for *F. solani;* and 35.61 and 12.11% for *R. solani*, respectively. Also, the increases in root fresh and dry weight due to the treatment with salicylic acid were 41.25 and 28.62% for *F. oxysporum;* 46.32 and 31.23% for *F. solani;* and also 43.64 and 29.56% for *R. solani* respectively. In addition, the increases due to the treatment with the bacterium *B. megaterium* were 40.24 and 24.62% for *F. oxysporum;* 41.89 and 17.61 % for *F. solani;* and 44.69 and 24.21% for *R. solani* respectively. Moreover, the increase due to the treatment with citric acid was recorded at 23.74-18.15% for *F. oxysporum;* 23.58-28.57% for *F. solani;* and also 20.41 and 22.64 % for *R. solani* respectively. Whereas, the treatment with the fungicide Topsin-M70 recorded the lowest increase in root fresh and dry weight for the three tested fungi, being 18.31 and 14.15 % for *F. oxysporum;* 20.0 and 10.16% for *F. solani;* and 18.98 and 13.21% for *R. solani* respectively.

Effects of some bioagents, and chemical inducers, a mycorrhizal fungus, and the fungicide Topsin-M70 on incidence of damping – off under field conditions

Data shown in Table 5 indicate that, all tested bioagents, chemical inducers, a mycorrhizal fungus, and the fungicide Topsin-M70 were significantly effective in decreasing pre-and post-emergence damping-off and increasing the survived plants under field conditions at Agric. Res. Stat. and Fac. of Agric., Menoufia Univ. as for the disease control at the seedling stage in terms of rate (%) of survived seedlings. Topsin-M70 recorded the highest increase over control followed by *B. megaterium, T. harziaum* and salicylic acid, respectively.

Table 4. Effect of the tested bioagents and chemical inducers compared to the fungicide TopsinM-70 on shoot and root fresh and dry weight of lupine plants under greenhouse conditions.

Pathogens	Treatments	Shoot weight (g plant^{-1})		Root weight (g plant^{-1})		Increase (%)			
						Shoot weight(g plant^{-1})		Root weight (g plant^{-1})	
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
F.oxysporum	*B.megaterium*	31.17	7.35	6.97	4.05	81.12	47.0	40.24	24.62
	*T. harzianu*m	19.52	5.14	6.23	3.91	13.42	2.80	25.35	20.31
	Citric acid	28.34	6.93	6.15	3.84	64.67	38.6	23.74	18.15
	Salicylic acid	35.71	8.70	7.02	4.18	107.50	74.0	41.25	28.62
	Topsin-M70	24.15	6.23	5.88	3.71	40.33	24.6	18.31	14.15
	Control	17.21	5.00	4.97	3.25	-------	--------	--------	--------
F.solani	*B.megaterium*	8.16	7.03	6.74	3.54	101.86	72.73	41.89	17.61
	*T. harzianu*m	18.95	5.13	6.03	3.39	35.84	26.04	26.95	12.62
	Citric acid	25.64	6.22	5.87	3.87	83.80	52.83	23.58	28.57
	Salicylic acid	30.55	8.19	6.95	3.95	118.99	101.23	46.32	31.23
	Topsin-M70	22.79	5.95	5.70	3.33	63.37	46.19	20.00	10.63
	Control	13.95	4.07	4.75	3.o1	--------	--------	--------	--------
R.solani	*B.megaterium*	30.62	7.30	7.09	3.95	103.6	42.58	44.69	24.21
	*T. harzianu*m	20.41	5.74	6.71	3.81	35.61	12.11	36.94	19.81
	Citric acid	26.50	6.29	5.90	3.90	76.08	22.85	20.41	22.64
	Salicylic acid	33.18	8.43	7.04	4.12	120.47	64.65	43.67	29.56
	Topsin-M70	23.34	6.03	5.83	3.60	55.08	17.77	18.98	13.21
	Control	15.05	5.12	4.90	3.18	-------	--------	--------	--------
L.S.D at 0.05		1.34	0.95	0.82	0.53	-------	--------	--------	--------

Table 5. Effects of the examined bioagents, chemical inducers, a mycorrhizal fungus and the fungicide Topsin-M70 on the incidence of damping-off and the survived plants under field conditions during the 2021-2022 growing seasons.

Treatments	Agric. Res. Stat., Giza			Fac. of Agric., Menoufia Univ.		
	Damping -off (%)		Survived plants (%)	Damping-off (%)		Survived plants (%)
	Pre-emergence	Post-emergence		Pre-emergence	Post-emergence	
B.megaterium	6.66	3.33	90.01	6.66	6.66	86.68
*T. harzianu*m	10.00	6.66	38.34	12.18	10.00	77.82
Citric acid	10.00	14.20	75.8	12.18	16.53	71.29
Salicylic acid	10.0	10.0	80.00	12.25	10.0	77.75
Glomus sp.	10.12	13.00	76.88	13.00	15.04	71.96
Topsin-M70	3.33	3.33	93.34	3.33	6.66	90.01
Control	18.30	16.53	65.17	20.83	22.63	56.54
L.S. D at 0.05	1.59	1.74	1.92	1.09	1.93	2.17

Effects of the tested bioagents, chemical inducers, a mycorrhizal fungus, and Topsin-M70 on some crop components of lupine plants under field conditions

Data in Table 6 indicate that the tested bioagents, chemical inducers, the mycorrhizal fungus, and Topsin-M70 significantly increased the estimated crop parameters in both locations of the experiment compared to untreated ones. So that, Topsin-M70 was the superior treatment followed by the bioagents then salicylic acid. All the tested treatments showed also significant protection against the disease over the control.

Effect of the tested bioagents, and chemical inducers compared to the fungicide TopsinM-70 on the activity of peroxidase, polyphenol oxidase, and chitinase enzymes in lupine plants

Results presented in Table 7 indicate that treating the seeds of lupine with the tested bioagents, chemical inducers, a mycorrhizal fungus and Topsin-M70 resulted in a considerable increase in the activity of peroxidase, polyphenol oxidase, and chitinase enzymes compared to the untreated control. Generally, *B. megaterium, T.*

harziaum, and salicylic acid were superior for increasing the activity of the peroxidase enzyme. Meanwhile, citric acid and Topsin-M70 exhibited the lowest effects in this regard. On the other hand, *B. megaterium*, salicylic acid, and *T. harziaum* were the most effective treatments in increasing the activity of polyphenol oxidase, whereas, citric acid and Topsin-M70 exhibited the lowest effective ones. Moreover, all the examined bioagents, chemical inducers, and the mycorrhizal fungus increased chitinase activity higher than Topsin-M70. In all cases, control treatment recorded the lowest activity for the three enzymes.

Table 6. Effect of some bio-agents, chemical inducers, a mycorrhizal fungus, and the fungicide Topsin-M70 on some crop parameters under field conditions.

Treatments	Agric. Res. Stat., Giza				Fac. of Agric. Menoufia Univ.			
	Plant height (cm)	No. of branches/ plant	No. of pods/ plant	100 seed weight (g)	Plant height (cm)	No. of branches / plant	No. of pods/ plant	100 seed weight (g)
B.megaterium	125.27	5.60	31.00	38.60	127.31	5.80	32.00	41.00
*T. harzianu*m	115.35	4.60	26.70	32.33	121.00	5.00	28.00	33.15
Citric acid	106.21	3.85	22.33	27.18	109.22	3.90	25.00	28.00
Salicylic acid	118.31	4.75	25.00	31.00	120.35	5.18	27.00	30.15
Glomus sp.	115.33	4.18	25.75	30.15	120.37	4.25	26.80	32.00
Topsin-M70	129.18	5.00	32.00	35.00	133.70	5.20	34.00	37.00
Control	75.20	3.20	20.67	22.67	79.81	32.00	21.18	24.00
L.S.D. at 0.05	7.25	0.31	1.98	1.65	6.77	0.45	2.31	2.95

Table 7. Effect of the tested bioagents, chemical inducers, and the fungicide Topsin-M70 on the activity of peroxidase, polyphenol oxidase, and chitinase enzymes in lupine plants.

Pathogens	Treatments	Activity of		
		Peroxidase	Polyphenol oxidase	Chitinase
F. oxysporum	*B. megaterium*	0.982	0.181	2.355
	*T. harzianu*m	0.953	0.175	2.345
	Citric acid	0.914	0.153	1.340
	Salicylic acid	0.943	0.165	1.355
	Glomus sp.	0.570	0.115	1.824
	Topsin-M70	0.358	0. 088	0.925
	Control	0.210	0.097	1.661
F.solani	*B.megaterium*	0.875	0.177	2.213
	*T. harzianu*m	0.813	0.158	2.315
	Citric acid	0.745	0.145	1.423
	Salicylic acid	0.815	0.157	1.583
	Glomus sp.	0.493	0.119	2.120
	Topsin-M70	0.354	0.083	1.135
	Control	0.250	0.091	9.654
R.solani	*B.megaterium*	0.856	0.168	2.613
	*T. harzianu*m	0.817	0.165	2.435
	Citric acid	0.710	0.144	1.235
	Salicylic acid	0.790	0.150	1.436
	Glomus sp.	0.390	0.105	2.238
	Topsin-M70	0.215	0.087	0.995
	Control	0.195	0.099	1.225

*Expressed as absorption after 30 sec. at appropriate wave length.

DISCUSSION

White lupine (*Lupinus albus* L.) is one of the world's oldest crops, utilized not only as a protein source in fodder production but also to improve soil quality. Because of its high protein (35-45%) and oil content, a lupine seed is a good source of nourishment (10-15%). Many soil-borne fungi, including *Fusarium oxysporum, F. solani,* and *Rhizoctonia solani* are responsible for infecting the lupine plant causing damping-off and root-rot diseases (Zian 2011; El-Sayed 2015). Due to the limited cultivated area in Egypt, no crop rotation is applied, so plant-soil pathogens become threatened the cultivated plants. Recently, due to the great pollution with agrochemicals, which cause a hazardous effect on all alive organisms, biocontrol microorganisms and chemical inducers are safe and recommended to use as an alternative to chemical pesticides for the management of plant pests. *Bacillus* spp. produce a variety of chemicals that aid in the biological control of plant diseases and promote plant growth, making them a potential PGPR for a variety of agricultural and biotechnological uses. Bacilli also display antagonistic activity because they excrete extracellular metabolites such as cell wall hydrolases, antibiotics, and siderophores. *Bacillus* spp. also produces the induced systemic resistance, which improves plant response to pathogen attack. *Bacillus* spp. are also present phosphate solubilization, nitrogen fixation, and phytohormone synthesis, all help plants growth. *Bacillus* spp. antagonistic and plant growth-promoting strains could thus be beneficial in developing marketable treatments (Miljakovic *et al*. 2020). *Bacillus* spp. have been found as possible biocontrol agents and plant growth promoters in various studies of a wide range of plant species in recent years. When compared to the control treatment, the studied antagonists, *B. megaterium* and *T. harziaum*, and chemical inducers as well as citric and salicylic acid, induced a considerable reduction in the linear growth of the three pathogenic fungi in vitro. Greenhouse experiment revealed that soaking lupine seeds in the tested bioagents, chemical inducers, a mycorrhizal fungus and Topsin M-70 significantly reduced pre-and post-emergence damping-off under artificial inoculation with the three pathogenic fungi, exhibiting a significant elevation of the fresh and dry weight of lupine shoot and the roots of the plants. Topsin-M70 prevents lupine plants from infection by damping off. In all cases, *B. megaterum*, *T. harzianum* and salicylic acid displayed the highest percentages of survived plants, while citric acid displayed the lowest efficient. *Trichoderma* species are excellent infection competitors, can modify the rhizosphere, are tolerant or resistant to soil pesticides, can grow and survive in unfavourable conditions, are efficient in utilizing soil nutrients, are aggressive against phytopathogenic fungi, and promote plant growth (Vinale *et al.* 2006). *Trichoderma* spp. are also known to control a variety of plant pathogens, either indirectly by competing for space and nutrients, modifying environmental conditions, enhancing plant defensive mechanisms and antibiosis, and promoting plant growth, or directly by inhibiting pathogen growth and sporulation via mycoparasitism and enzyme production (Ragab *et al.* 2015). The induction of systemic acquired resistance by chemical inducers sensitizes the plant response rapidly after infection responses and may induce the accumulated phytoalexins and lignifications as well as inducing enhanced activities of chitinase and β-glucanase (Metranx & Boller 1986). Kessmann *et al.* (1994) mentioned that the mechanism of systemic acquired resistance is multifaceted, likely resulting in stable broad-spectrum disease management and they could be used preventatively to general plant health, resulting in long-term lasting protection. The vascular arbuscular mycorrhizal (VAM) fungi colonize the roots of many crop plants and are of great value in encouraging the uptake of phosphorus, minor elements, and water (Siddiqui *et al.* 2001). They also reduce the incidence and severity of the infection by several plant diseases (Demir & Akkopru 2007; Akhtar & Siddiqui 2008). These responses greatly may be due to the production of antibiotics, wall appositions, siderophores, and defence enzymes, which adversely affect the pathogens. The interaction sites between *T. harzianum* and the pathogenic fungus *F. solani* by the Scanning Electron Microscope (SEM) illustrated that *Trichoderma* hyphae coiled around the host hyphae of *F. solani*, then the hooked and pincer-shaped hyphae of *T. harzianum* penetrated to the hyphae of *F. solani*. This mode of interaction between *T. harzianum* and the pathogenic fungi hyphae was previously mentioned by many authors (Manzali *et al.* 1993; de Melol & Faull 2000; Noval *et al.* 2021). In comparison to the control, adding *Glomus* sp. to the pathogenic fungus studied resulted in a significant reduction in the severity of root-rot. Vinale *et al.* (2006), Akhtar & Siddiqui (2008), Abd-All (2013), El Gendy *et al.* (2016), and Aboelmagd *et al.* (2016) all reported similar results. The three enzymes, i.e. peroxidase, polyphenol oxidase, and chitinase were shown to be considerably elevated in the leaves of all treatments, compared to the control treatment. These enzymes are vital in the defensive mechanisms of plants against infections. The peroxidase enzyme oxidizes phenolic chemicals to more fungal hazardous molecules like quinines, which limit both fungal growth and spore germination, according to Morkunas *et al.* (2007). Peroxidase was also discovered to be involved in the lignin production process. Furthermore, Melo *et al.* (2007) pointed out that the active phenoloxidase system is required for the participation of an endogenous supply of phenolic chemicals in plant disease resistance. Abd El-Khair *et al.* 2011 found that using *Trichoderma* spp. as bioagents caused the increased enzymes such as chitinase, peroxidase, and polyphenol oxidase in treated lupine plants. As a result of treatments with various antioxidants, many oxidative enzymes such as peroxidase, catalase, ascorbate oxidase, and polyphenol oxidase were discovered

(Takahame & Oniki 1994; El- Khallal 2007; Abdel-Monaim 2008). Bioagents, chemical inducers, *Glomus* sp., and the fungicide Topsin M-70 were found to considerably reduce the incidence of pre-and post-emergence damping-off with a significant increase in estimated crop parameters under field conditions. Polyphenol oxidase is a copper-containing enzyme that converts phenols to highly poisonous quinines, as is widely known. This enzyme is also involved in the final oxidation of damaged plant tissue, and its involvement in disease resistance is attributed to this role (Kosuge 1996). The peroxidase enzyme, on the other hand, is involved in a variety of plant functions. Various compounds, such as salicylic acid and 2,6-dichloroisonicotinic acid (INA), as well as aminobutyric acid, have also been investigated for their ability to activate defence responses in plants (Kessmann *et al.* 1994).

CONCLUSION

In general, the results confirmed that antagonists, chemical inducers, the mycorrhizal fungus, and the fungicide Topsin-M70 reduced significantly pre-and post-emergence damping-off infections and elevated the survived lupine plants under greenhouse and field conditions. *B. megaterum* was the best treatment in this regard followed by *T. harzianum*. The combination between *Glomus* sp. and any of the bio-agents, i.e., *B. megaterum* and *T. harzianum*, resulted in a great reduction of root-rot severity. In addition, salicylic acid exhibited the best protection effect against damping-off followed by citric acid. The three enzymes, i.e., peroxidase, polyphenol oxidase and chitinase greatly increased in the leaves of all treatments, compared with the control. More detailed investigations on the interaction among the bioagents, the mycorrhizal fungi, and the host plant of various pathosystems are needed in future studies to further develop the efficacy of the biological control with the mycorrhizal fungi.

REFERENCES

Abd Allah, ES, Hashem, A, Algarawi, AA & Alwathnani, HA 2015, Alleviation of adverse impact of cadmium stress in sunflower (*Helianthus annvus* L.) by arbuscular mycorrhizal fungi. *Pakistan Journal of Botany*, 47: 785-795.

Abd El Khair, HR, Khalifa, KhM & Haggag, KHE 2011, Effect of *Trichderma* species on damping–off diseases incidence, some plant enzymes activity and nutritional status of bean plants. *Journal of American Science*, 7: 156-167.

Abd El Ghany, M 2007, Pathological studies on Apricot Root-rot in the New Reclaimed Lands in Egypt. PhD Dissertation, Faculty of Agriculture, Al-Azhar University, 82 p.

Aboelmagd, HE, Mohamed, FG, Eid, KhE & El Fiki, IAI 2021, Efficacy of some bio-agents, chemical inducers and fungicides in controlling tomato root-rot disease caused by *Rhizoctonia solani*. 5th International Conference on Biotechnology Applications in Agriculture, (ICBAA), Benha University, 8 April, Egypt (Conference Online), 197-210.

Abo Rehab, ME 1997, Studies on Root-rot of Persimmon and its Control in the New Reclaimed in Egypt. MSc. Dissertation, Faculty of Agriculture, Cairo University, 94 p.

Ahmed, GA 2011, Induction Resistance of Cucumber Plant (*Cucumber sativus* L.) against Fusarium Wilt Disease under Protected Houses conditions. PhD Dissertation, Kazakh National Agrarian University, 151 p.

Akhter, A, Hage Ahmed, K, Soja, G & Steinkellner, S 2015, Compost and biochar alter mycorrhization tomato root exudation and development of *Fusarium oxysporum* f. sp. *lycopersici*. *Frontiers in Plant Science*, 6: 529. http://doi.org/10.3389/fpls.2015.00529.

Algarawi, AA, Abd Allah, FF & Hashem, A 2014, Alteration of salt induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. *Journal of Plant Interactions*, 1: 802-810, http://www. tandfonline.com/ foi/tJpi20.

Al Hmoud, G & Al Momany, AJ 2015, Effect of four mycorrhizal products on Fusarium root- rot different vegetable crops. *Journal of Plant Pathology & Microbiology*, 6: 2 https://dio.org/10.417212157-7471.1000255.

Akhtar ME & Siddiqui, ZA 2008, *Glomus intraradices*, *Pseudomonas alcaligenes*, and *Bacillus pumilus*: effective agents for the control of root-rot disease complex of chickpea (*Cicer arietinum* L.). *Journal of General Plant Pathology*, 74:53–60. DOI: 10.1007/s10327-007-0062-4.

Asaka, O & Shoda, M 1996, Bio-control of *Rhizoctomia solani* damping–off of tomato with Bacillus subtillus RB14. *Applied and Environmental Microbiology*, 62: 4081- 4085.

Boller, T & Mauch, F 1988, Colorimetric assay for chitinnase. *Methods in Enzymology*, 161: 430-435.

de Melol, IS & Faull, JL 2000, Parasitism of *Rhizoctonia solani* by strains of *Trichoderma* spp. *Scientia Agricola*, 57: 1.

Demir, S & Akkopru, A 2007, Use of arbuscular mycorrhizal fungi for biocontrol of soil-borne fungal plant pathogens. In: SB Chincholkar, KG, Mukerji (eds.), Biological control of plant diseases. Howarth, New York, 17-37.

El Mohamedy, RS & Abdel Alla, MA 2013, Bio-primary seed treatment for biological control of soil borne fungi causing root-rot of green bean (*Phaseolus vulgaris* L.). *Journal of Agricultural Science and Technology*, 9: 589-599.

El Mohamedy, RS, Shafeek, MR & Rizk Fatma, A 2015, Management of root-rot diseases and improvement growth and yield of green bean plants using plant resistance inducers and biological seed treatments. *Journal of Agricultural Science and Technology*, 11: 1219-1234.

El Gendy Hala, M, El Sayed Sahar, A & Mosa, AM 2016, Efficacy of two natural extracts and fungicides in reducing soybean damping-off and Root –rot disease Egypt. *Journal of Phytopathology*, 44: 35-50.

El Sayed Sahar, A 2006, Use of Intercropping and other Treatments for Controlling Faba-bean Diseases. PhD Dissertation, Faculty of Agriculture, Benha University, Egypt.

El Sayed Sahar, A, El Gendy Hala, M & Abd El Motaleb, FA 2015, Efficiency of some micro-element root-rot disease yield characters and chemical composition of lupine. *Journal of Biological Chemistry and Environmental Sciences*, 10: (625-640).

Fu, J & Huang, B 2001, Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany*, 45: 105-114.

Julier, B, Wuyghe, C & Papineall, J 1994, Dry matter and nitrogen accumulation in determinate autumn-sown white lupine (*Lupinus albus*) c.v. lunoble. *European Journal of Agronomy*, 3: 153-160.

Katan, J 2004, Soil Disinfestation: One minute before methyl bromide, phase out. 4th International Symposium on Chemical and Non-chemical Soil and substrate Disinfestation, 698: 19 -26.

Kessmann, H, Sataub, T, Hofmann, C, Meatzke, T & Herzog, J 1994, Induction of systemic acquired disease resistance in plants by chemicals. *Annual Review of Phytopathology*, 32: 439-459.

Kosuge, T 1996, The role of phenolics in host response to infection to infection. *Annual Review of Phytopathology*, 7:195–222.

Manzali, D, Nipoti, P, Pisi, A, Filippini, G & D'Ercole, N 1993, Scanning electron microscopy study of *in vitro* antagonism of *Trichoderma* spp. strains against *Rhizoctonia solani* Kühn. *Phytopathologia Mediterranea*, 32: 1-6 .

Mayer, AM, Harel, E & Shaul, RB 1965, Assay of catechol oxidase a critical comparison of methods. *Phytochemistry*, 5:783-789.

Maslienko, LV, Voronkova, AK, Datsenko, LA & Efimtseva, EA 2021, Antagonistic effect of the promising fungal producer strain of microbiopreparation T-1 Trichoderma sp. on oil flax Fusarium blight. *Caspian Journal of Environmental Sciences*, 19: 883-890

Melo, GA, Shimizu, MM & Mazzafera, P 2006, Polyphenol oxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. *Phytochemistry*, 67: 277-285.

Metranx, JD & Boller, T 1986, Local and systemic induction of chitinase in cucumber plants in response to fungal, bacterial and viral infections. *Physiological and Molecular Plant Pathology*, 28: 161-169

Miljakovic, D, Marinkovic, J & Balesevic Tubic, S 2020, The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms*, 8: 1037. DOI: 10.3390/ microorganisms8071037.

Morkunas, I & Gemerek, J 2007, The possible involvement of peroxidase in defence of yellow lupine embryo axes against *Fusarium oxysporum*. *Journal of Plant Physiology*, 164: 497-506.

Nahiyan, ASM & Matsubara, Y 2012, Tolerance Fusarium root-rot and changes in antioxidative ability in mycorrhizal asparagus plants. *Horticultural Science*, 47: 356-360.

Noval, AM, Abd El Rahman, M, Abdelghany, TM & Abd El Mongy, M 2020, Mycoparasitic nature of Egyptian Trichoderma isolates and their impact on suppression Fusarium wilt of tomato. *Egyptian Journal of Biological Pest Control*, 31: 103.

Ragab, MMM, Abada, KA, Abd El Moneim, ML & Abo Shosha, YZ 2015, Effect of different mixtures of some bioagents and Rhizobium phaseoli on bean damping-off under field condition. *International Journal of Scientific and Engineering Research*, 6: 1009-1106.

Salt, GA 1982, Factors affecting resistance to root rot diseases (Eds. G, Hawtin, & C, Webb) faba bean improvement. IARDA, Aleppo, Syria, 260-270.

Shalaby, SIM 1997, Effect fungicidal treatment of sesame seeds on root-rot infection, plant growth and chemical components. *Bulletin of Faculty of Agriculture*, Cairo University, 48: 397-411.

Shaltout, AMA 2002, Use mycorrhizal fungi and some other bioagents to control root-rot and stalk-rot of corn. PhD Dissertation, Faculty of Agriculture, Cairo University, 127 pp.

Shevchenko, VA, Soloviev, AM, Popova, NP 2021 Eligibility criteria for joint ensilage of maize and yellow lupine on poorly productive lands of the Upper Volga region. *Caspian Journal of Environmental Sciences*, 19: 745-751

Siddiqui ZA, Iqbal, A & Mahmood, I 2001, Effects of Pseudomonas fluorescens and fertilizers on the reproduction of Meloidogyne incognita and growth of tomato. *Applied Soil Ecology*, 16: 179-185.

Snedecor, GW & Cochran, WG 1989, Statistical methods. Oxford and J. pH. Publishing Cam. 8th edition.

Virale, F, Marra, R, Scala, F, Ghisalberti, E, Lorito, M & Sivasitham Param, K 2006, Major secondary metabolites produced by two commercial Trichoderma strains active against different phytopathogens. *Letters in Applied Microbiology*, 43: 143-148.

Zian, AH 2011, Studies on Fusarium Wilt Disease of White lupine Plant in Egypt. PhD Dissertation, Faculty of Agriculture - Suez Canal University, Egypt, 167 p.

Bibliographic information of this paper for citing:

El-Sayed, S,A, El-Shennawy, R,Z, Ahmed Mohammed, T, Attia, A,M,F 2022, Induction of systemic resistance against damping-off and root-rot of white lupine (*Lupinus albus* L.) using some bioagents, chemical inducers and a mycorrhizal fungus. Caspian Journal of Environmental Sciences, 20: 571-583.