

Integrated Control Management of Root Rot Disease in Lupine Plants by Using Some Bio-Agents, Chemical Inducers and Fungicides

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Abstract

Fusarium solani, *Rhizoctonia solani*, and *Macrophomina phaseolina* were found to be associated with root-rot symptoms of lupine plants. Bio-agents (Rhizo-N, Bio-Arc and Plant Guard), chemical inducers (salicylic acid, K_2HPO_4 and hydrogen peroxide), fungicides (Rhizolext-T, Vitavax-200 and Topsin-M70) were examined for controlling root-rot and growth promoting of lupine plants *in vitro* and *in vivo*. All treatments either individually inhibited growth of the tested pathogenic fungi under greenhouse and field conditions, all treatments indicate that is a significantly reduced root-rot or increased of survival plants. Also, under field conditions, all these treatments significantly increased growth parameters (plant height and number of branches per plant) and yield components (number of pods and seeds per plant, weight of 100 seeds and total yield per feddan) and the percentages of lupine seeds protein, oil and ash.

Keywords: Lupine; Bio-agents; Chemical inducers; Fungicides; Root-rots

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Citation: El-Sayed SA, Abdel-Monaim MF. Integrated Control Management of Root Rot Disease in Lupine Plants by Using Some Bio-Agents, Chemical Inducers and Fungicides. J Plant Sci Agric Res. 2017, 1:1.

Received: November 07, 2016; **Accepted:** January 23, 2017; **Published:** January 30, 2017

Introduction

Lupine (*Lupinus albus* L.) is cultivated around the Mediterranean and along the Nile valley where it is used for human consumption also for medical and industrial purposes. It can be considered as a friendly crop to the environment related to its efficient nitrogen fixation system, in addition to its improvement to the traditional cereal rotation and protein supply in low input farming system [1]. While lupine is subjected to attack by many pathogenic organisms under Egyptian conditions root-rot disease is considered the most serious disease of lupine in Egypt, causing a considerable loss in seed germination, plant stand and seed yield [2,3]. Scientists revealed that several soil borne fungi attack the roots of legume crops (faba bean, lentil, chickpea and lupine) [4-6]. They found that *Fusarium oxysporum* and *Rhizoctonia solani* were the most destructive fungi of such plants. Zain surveyed diseases of white lupine in Egypt and the list included Fusarium wilt (*F. oxysporum* f. sp. lupine) and root-rot diseases, including damping-off, *Fusarium* spp., *Rhizoctonia* spp. and *Macrophomina phaseolina*. Researchers isolated *R. solani* and *Fusarium solani* from diseased lupine plants in some areas of Dakahlia (Egypt) [7]. These fungi were the most dominant isolates. Scientists found that the frequency of the isolated fungi from infected lupine plants which were collected from different locations of five governorates

can be ranked in descending order as follows *F. oxysporum*, *Fusarium* spp. and *Alternaria* spp., contents in healthy resistant mutant were higher than that found in susceptible cultivar [8]. Researchers tested three strains of plant growth promoting Rhizobacteria (*Pseudomonas fluorescens*, *Bacillus subtilis* and *Rhizobium* sp.) against *Fusarium oxysporum* f. sp. Ciceris [9]. They found that all strains inhibited the linear growth of *F. oxysporum* and were effective in reducing wilt incidence. Few scientists proved that plant growth *Bacillus megaterium* against root-rot caused by *M. phaseolina*. In recent years the *B. megaterium* and *Trichoderma viride* have been extensively used for plant growth promotion and disease control [10]. Researchers indicate that *T. harzianum* caused the highest increase in total seed yield than fungicide treatment. Amendment with certain abiotic factors (inducers) appears to stimulate the disease resistance by indirectly stimulating indigenous populations of microorganism that are beneficial to plant growth and antagonistic to pathogens [11]. For example chitin amendment of soil has been found to stimulate the growth and antagonistic to microorganisms [12] increase the bio-control activity and stimulate the expression of plant defense proteins [13]. All these effects may culminate in enhancing plant protection. Similarly, SA and H_2O_2 amendment was tested in combination with biocontrol agents. Scientists tested the efficiency of *P. fluorescens* with tested in without

SA amendment in chickpea against *Fusarium* wilt infection [14]. Few researchers tested some plant resistance elicitors: Salicylic acid (SA) and Hydroquinone (HQ) in comparing to the fungicide Rhizolex-T as seed treatments for controlling lupine root-rot [7]. Few found that the effect of mustard and canola seed meal compared the fungicides Topsin-M70 on the control of lupine *Fusarium* wilt incidence under greenhouse conditions was studied [8]. Researchers also revealed that seed dressing with the fungicide Topsin-M70% showed superior effect on wilt incidence [15]. This work aimed to study the effect of some bio-agent, chemical inducers and fungicides for controlling of root-rot of lupine.

Materials and methods

Source of the pathogen

Pathogenic fungi of *Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina* were selected from the Leguminous Diseases Department, Plant Pathology Research Institute, Agriculture Research Center. The isolates were tested to confirm virulence.

Effect of biocides, chemical inducers and fungicides on linear growth of pathogen

Biocides (Table 1) were mixed with potato dextrose agar (PDA) medium. The plates centre were inoculated with *F. solani*, *R. solani*, and *M. phaseolina* discs (5 mm diameter) of PDA culture and incubated at 25°C for 5 days. Plates only inoculated with pathogen fungi at one side 5 mm from the plate edge kept as control. Three replicates were used for each treatment. Average radial growth was recorded when one plate was covered with the fungal growth.

Effect of selected chemical inducers on pathogenic fungi growth

in vitro: Salicylic acid (SA), potassium phosphate (K_2HPO_4) and hydrogen peroxide (H_2O_2) at different concentrations were tested to study their effect on the mycelia linear growth of pathogenic fungi, *in vitro*. Three concentrations of salicylic acid (SA) (2.5, 5 and 8 μ m), potassium phosphate at (5, 10 and 15 μ m) and hydrogen peroxide (2.5, 5 and 8 μ m) were prepared and added separately to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized petri palates (9 cm diameter). Plates were individually inoculated at the center with equal disks (5 mm) of 7 days old culture of pathogenic fungi and incubated at 25°C for 5 days. The average of the fungal linear growth was than recorded.

Table 1 L Biocides used as bio-agents against pathogenic fungi.

Biocide (trade name)	Producer name	Bio-agents (density/ml)	Dose	
			Per 1L. medium	Perkg/seed
Bio-ARC		<i>Bacillus subtilis</i> 3×10^7 cfu/ml	4.0 g	4.0 g
Bio-Zeid	El-Nasr Co.	<i>Bacillus megaterium</i> , 2.5×10^7 cfu/ml	2.5 g	2.5 g
Clean root		<i>Trichoderma harzianum</i> , 3×10^7 cfu/ml	4.0 ml	4.0 ml

Effect of tested fungicides on growth of pathogenic fungi, *in vitro*: Three fungicides, Rhizolex-T (Tolclophosemethul+Thiram), Vitavax-200 (Carboxin+Thiram) and Topsin-M70% (Thiophanate methyl 7%) at different concentration (0.10, 30, 50, 100 and 200 ppm) were used. The required concentrations of the fungicides were adjusted and added to autoclaved PDA medium, then poured to sterilized petri dishes (9 cm in diam.) and rotated gently. Plates were then inoculated at the center by equal discs (5 mm in diam.) taken from 7 days old cultures of the pathogenic fungi *F. solani*, *R. solani*, and *M. phaseolina*. Three replicate were used for each concentration. Plates were incubated at 25°C. Linear growth of each tested fungi was measured when the pathogenic fungi completely covered control treatment by taking two perpendicular diameters (in mm) and averaged.

Greenhouse experiment

Effect of bio-agents on lupine root-rot incidence under greenhouse conditions: Three commercial bio-products, i.e. Rhizo-N (*Bacillus subtilis*), Bio-Arc (*B. megaterium*) and plant guard (*T. harzianum*) were used for controlling lupine root-rots. Plant Guard were (4.0 g/1 water), (2.5 g/1 water) and (4.0 ml/L), respectively, for 2 hours before planting (Hussein, 2005). This experiment was carried out in sterilized pots (25 cm diam.) containing sterilized clay soil in the greenhouse. Both pots and soil were sterilized with 5% formalin solution. Soil was infested with *F. solani*, *R. solani*, and *M. phaseolina* were prepared by growing them separately on sand-barley medium for 15 days. Pathogenic fungi were mixed thoroughly, ten days before sowing. Control treatment was inoculated only with pathogens. Ten seeds (Giza 2) were sown per post and three replicates were used for each treatment percentages of pre-pot and three replicates were used for each treatment percentages of pre- and post-emergence damping-off as well as healthy survival plants in each treatment were determined 15 and 30 days after sowing, respectively using the next formula [16]. The disease severity of root-rot disease was determined after 45 days from sowing [17].

Effect of chemical inducers on the disease incidence under greenhouse conditions

This experiment was carried out in sterilized pots (25 cm diameter) containing sterilized clay soil in the greenhouse. Both pots and soil were sterilized with 5% formalin solution. Soil was infested with *F. solani*, *R. solani* and *M. phaseolina*. As mentioned before. Lupine seeds (cv. Giza 2) were soaked, before sowing in solution of three chemical inducers, salicylic acid (SA) at 2.5, 5.0 and 8 μ m, K_2HPO_4 at 5, 10 and 15 μ m and H_2O_2 at 2.5, 5.0 and 8 μ m for 2 h. Seeds were soaked in tap water, they using as control. Ten seeds were sown per pot, and three replicates were used for each treatment. Percentage of pre- and post-emergence damping-off, root-rot, and healthy survival plants were recorded after 15 and 30 days respectively.

Effect of using fungicides on lupine damping-off and root-rot incidence under greenhouse conditions: This experiment was carried out in sterilized pots (25 cm diameter) containing sterilized clay soil in the greenhouse. Both pots and soil were sterilized with 5% formalin solution. Soil was infested with *F.*

solani, *R. solani*, and *M. phaseolina*. As mentioned before. Lupine seeds (Giza 2) were treated with the tested fungicides, Rhizolex-T at 2 g/kg seed, Vitavax-200 at 3 g/kg seed and Topsin-M70 at 3g/kg seed by shaking them gently in glass container containing glue suspension (supper film 70) as a sticker material/kg seed. The same aforementioned methods were used without fungicides as control. Ten seeds were sown per pot, and three replicates were used for each treatment. Percentage of pre- and post-emergence damping-off, root-rot and healthy survival were recorded.

Field experiments

The efficacy of the promising bio-agents, chemical inducers and fungicides for controlling lupine seed rot was assessed under field conditions. The experiment was carried out at Sers El-Lyain Research Station, during 2013/2014-2014/2015 growing seasons. The experimental plot was divided to equal plots each consisting of 3 rows (3.5 cm long and 60 cm in width). The recommended agricultural practices i.e., preparation of the land, irrigation fertilization ... etc., were applied as usual. Lupine seeds (cv. Giza 2) were treated with the treatment (bio-agents, chemical inducers and fungicides) as mentioned before. The same previous mentioned methods were used without treatment as control. Three replicates were used for each treatment. Percentage of pre- and post-emergence damping-off, 15 and 30 days after sowing, respectively. Root-rot incidence was recorded after 75 day of sowing. Plant growth characters as plant height, number of branches, number of pods per plant, seed index as well as the average weight of seeds/feddan were recorded at harvest.

Also, the percentage of protein and oil contents of each crop seeds were determined [18,19].

Statistical analysis

Data were statistically analyzed using the Fisher's least significant difference (L.S.D.) [20].

Results

Effect of bio-agents on linear growth of fungi

Data presented in (Table 2) indicate that all bio-agents tested were significantly decreased the linear growth pathogenic fungi

Table 2 Effect of some bio-agents on linear growth (mm) *in vitro*.

Tested biocides	Tested pathogen						
	/ L./medium Rate	<i>F. solani</i>	Reduction %	<i>R. solani</i>	Reduction %	<i>M. phaseolina</i>	Reduction %
Rhizo-N	4.0 g	20.00	77.77	25.00	72.22	18.00	80.00
Bio-Arc	2.5 g	40.00	55.55	45.00	50.00	35.00	61.11
Plant Guard	4.0 ml	30.00	66.66	30.00	66.66	25.00	75.55
Control	-	90.00	0.00	90.00	0.00	90.00	0.000
L.S.D. at 5%	-	3.52	-	4.12	-	3.18	-

on PDA medium compared with the control. Data showed that Rhizo-N (*Bacillus subtilis*) inhibited the growth of *F. solani*, *R. solani* and *M. phaseolina* by 77.77, 72.22, 66.66 and 88.88% reduction, respectively. Rhizo-N gave the highest inhibition followed by Plant Guard. On the other hand, the least inhibition of the fungal growth was recorded on Bio-Arc. Generally, Rhizo-N was the most effective bio-agent followed by Plant Guard and Bio-Arc.

Effect of different concentrations of chemical inducers on the linear growth of fungi:

The effect of the tested chemical on mycelia growth as fungi, the cause of root-rot in lupine are shown in (Table 3). Data indicate that low concentrations of salicylic acid, potassium phosphate and hydrogen peroxide slightly reduced the linear growth of pathogenic fungi (*F. solani*, *R. solani* and *M. phaseolina*). The highest reduction in linear growth was achieved when salicylic acid at 8 μ m was used to present 88.88, 88.88, and 100.00% reduction. Generally, salicylic acid was the most effective chemical inducers followed by hydrogen peroxide and potassium phosphate.

Effect of different concentration of fungicides on linear growth of fungi:

Data in (Table 4) show that *F. solani* was completely inhibited by Topsin-M70 at rate 10 and 200 ppm, respectively, also *R. solani* was completely inhibited by Vitavax-200 and Topsin-M70, respectively. While, *M. phaseolina* was completely inhibited by Topsin-M70 at rate 30 ppm was completely inhibited by Topsin-M70 and Vitavax-200 at rate 30 ppm. All fungicides significantly reduced growth of fungi than the control. Vitavax-200 was the most effective followed by Topsin-M70 and Rhizolex-T. In general, effect of the fungicides on the mycelia growth was increased by increasing concentration of the fungicides.

Effect of bio-agent, chemical inducers and fungicides on lupine root-rot under greenhouse conditions

Data in (Table 5) show that there is a significantly the tested bio-agents in decreased percentage of pre- and post-emergence damping-off and increased percentage of healthy survival plants compared to check (control). However, all bio-agents significantly reduced the disease severity of root-rot symptoms caused by pathogenic fungi. Generally, Rhizo-N was the most

Table 3 Effect of three chemical inducers with three different concentrations on linear growth of four fungi *in vitro*.

Chemical inducers	Concentration	Tested pathogen					
		<i>F. solani</i>	Reduction%	<i>R. solani</i>	Reduction%	<i>M. phaseolina</i>	Reduction%
Salicylic acid (SA)	2.5 µm	55.00	38.88	50.00	44.44	33.00	63.33
	5.0 µm	30.00	66.66	20.00	77.77	15.00	83.33
	8.0 µm	10.00	88.88	10.00	88.88	0.00	100.0
Potassium phosphate (K ₂ HPO ₄)	5 µm	65.00	27.77	60.00	33.33	40.00	55.55
	10 µm	40.00	55.55	30.00	66.66	25.00	72.22
	15 µm	28.00	68.88	20.00	77.77	20.00	77.77
Hydrogen peroxide (H ₂ O ₂)	2.5 µm	60.00	33.33	55.00	38.88	35.00	61.11
	5.0 µm	35.00	61.00	25.00	72.22	20.00	77.77
	8.0 µm	23.00	74.44	20.00	77.77	10.00	88.88
Control	-	90.00	-	90.00	-	90.00	-
L.S.D. at 5%	-	4.32	-	5.07	-	4.90	-

effective biocides followed by plant guard and Bio-Arc. Also, data presented in (Table 5) show that there is significant difference between the tested chemical inducers a significant difference between the tested inducers in decreased percentage of pre- and post-emergence damping-off and increased percentage of healthy survival lupine plants and decreased percentage of disease severity. Salicylic acid was the most effective inducers with all fungi followed by H₂O₂ and K₂HPO₄. Data in (Table 5) show that there is a significant difference between the tested fungicides in decreased percentage of pre- and post-emergence damping-off increased percentage of healthy survival plants and decreased percentage disease severity %. Vitavax-200 was the most effective fungicide with the infests fungi followed by Topsin-M70 and Rhizolex-T.

Efficiency of the treatment bio-agents, chemical inducers and fungicides on reducing the pre- and post-emergence root rot lupine under field conditions

Effect on root-rot infection: Data in (Table 6) clearly show the influence of the tested bio-agents, chemical inducers and fungicides on root-rot disease of lupine under field conditions during two successive seasons. The results revealed that the treatment reduced the pre- and post-emergence damping-off compared to check (control). However there were visual variations among these treatments. Vitavax-200 gave the best results in decreasing the percentage of pre- and post-emergence root-rot during the two seasons of 2013/2014-2014/2015. Bio-Arc was the least effective treatments controlling pre- and post-infection. All treatments gave the highest reduction in the disease severity under field conditions.

Effect on yield component: As for the effect of the tested treatments on yield component of lupine under field conditions, the obtained in (Table 7) show a marked difference among all treatments and varieties on yield components as well as on root-rot diseases of lupine including plant height, number of branches, number of pods, 100 seed weight and weight of seeds/ feddan (kg/feddan). Data in (Table 8) show that the percentage of seeds protein, oil and ash were increased due to soaking seeds in treatments compared with untreated seeds. Vitavax-200 treatment had the first followed by Rhizo-N and salicylic acid treatment.

Discussion

White lupine (*Lupinus albus* L.) has many benefits for human and animal consumption, also for medical and industrial purposes. It could be considered as a friendly crop to the environment related its efficient nitrogen fixation system, in addition and protein supply in a low input farming system. Damping-off, root rot, char cod rot and anthracnose the most important diseases of this crop. The present work showed that damping-off, root-rot and char cool rot diseases is incited by a group of soil borne fungi including *R. solani*, *F. solani*, *F. oxysporum* and *M. phaseolina*. This is in agreement with results obtained by [2-4]. *In vitro*, *in vivo* and field conditions results obtained indicated that all the tested bio-agents effectively in habited the mycelia growth of *F. solani*, *R. solani*, and *M. phaseolina* on PDA. Rhizo-N (*Bacillus subtilis*) followed by plant guard (*Trichoderma harzianum*) and Bio-Arc (*B. megaterium*). In pots and under field condition, the obtained results showed that seed treatment with biocides decreased percentage pre- and post-emergence damping-off and root-

Table 4 Effect of three fungicide six different concentrations of linear growth three fungi *in vitro*.

Fungicides	Concentration (ppm)	Tested pathogen					
		<i>F. solani</i>	Reduction%	<i>R. solani</i>	Reduction%	<i>M. phaseolina</i>	Reduction%
Rhizolex-T	0	90.00	0.00	90.00	0.00	90.00	0.00
	10	30.00	66.66	35.00	61.11	41.00	54.44
	30	25.00	72.22	30.00	66.66	35.00	61.11
	50	20.00	77.77	22.00	75.55	20.00	77.77
	100	10.00	88.77	15.00	83.33	10.00	88.88
	200	0.00	100.0	0.00	100.0	0.00	100.0
Vitavax-200	0	90.00	0.00	90.00	0.00	90.00	0.00
	10	35.00	61.11	30.00	66.66	30.00	66.66
	30	20.00	77.77	0.00	100.0	20.00	77.77
	50	10.00	88.88	0.00	100.0	10.00	88.88
	100	0.00	100.0	0.00	100.0	0.00	100.0
	200	0.00	100.0	0.00	100.0	0.00	100.0
Topsin-M70	0	90.00	0.00	90.00	0.00	90.00	0.00
	10	20.00	77.77	35.00	61.11	24.00	73.33
	30	10.00	88.88	10.00	88.88	0.00	100.0
	50	0.00	100.0	0.00	100.0	0.00	100.0
	100	0.00	100.0	0.00	100.0	0.00	100.0
	200	0.00	100.0	0.00	100.0	0.00	100.0
L.S.D. at 5%	-	3.43	-	2.91	-	2.30	-

rot and increased percentage of healthy survival plants. Some byproducts of microorganisms (*T. harzianum* and *B. subtilis*) stimulated plant growth and at the same time reduced population density of plant pathogens [5]. Also they found that used *B. subtilis* as seed coating and early-sowing data against *R. solani* and *Fusarium* spp. decreased chick pea pre- and post-emergence damping-off seed treatment with biocides resulted in most cases increase in crop parameters compared with the control in the two growing seasons 2013/2014 – 2014/2015. Rhizo-N this result is in agreement with those obtained [5]. *In vitro* tested chemical inducers effectively inhibited the mycelia growth of pathogenic fungi. Our study under greenhouse and field condition proved lupine seed treatment of chemical inducers reduced pre- and post-emergence damping-off and root-rot and increase healthy survival plants SA at 8 am was the most effective chemical inducers with all fungi were infested. Seed treatment with chemical inducers recorded the highest increase in crop parameters. In conclusion, application of the inducers tested as seed treatment

caused by increase in crop parameters as compared with control plants [12-14]. The effect of the fungicides on the mycelia growth was increased by increasing concentrations of the fungicides. Our study under greenhouse and field conditions proved lupine seed treatment of fungicides showed a significant decreased percentage of pre- and post-emergence damping-off and root rot and increased percentage of healthy survival plants [18-20]. Seed treatment resulted in most cases increase in crop parameters. Vitavax-200 recorded the highest increase in crop parameters followed by Topsin-M70 and Rhizolex-T. This is in agreement with results [8,15]. The obtained results in the present study indicate that the application of some bio-agents, chemical inducers and fungicides alternatives could be used as an effective and safe technique for controlling soil borne plant pathogens in addition to avoid environmental pollution and significantly increased yield, protein and oil [21,22].

Table 5 Effect of bio-agents, chemical inducers, fungicides on lupine root-rot incidence under greenhouse conditions.

Treatments	Pathogenic fungi											
	<i>F. solani</i>				<i>R. solani</i>				<i>M. phaseolina</i>			
	Pre-emergence %	Post-emergence %	Survival %	Disease severity %	Pre-emergence %	Post-emergence %	Survival %	Disease severity %	Pre-emergence %	Post-emergence %	Survival %	Disease severity %
Rhizo-N	3.33	6.67	90.00	11.11	6.67	6.67	86.66	13.33	3.33	0.00	96.67	10.00
Bio-Arc	6.67	10.00	83.33	14.44	10.00	10.00	80.00	14.44	3.33	6.67	90.00	11.11
Plant Guard	6.67	6.67	86.66	12.22	6.67	10.00	83.33	14.44	3.33	3.33	93.34	12.22
Salicylic acid	3.33	6.67	90.00	9.99	3.33	6.67	90.00	10.00	0.00	0.00	100.0	11.11
K ₂ HPO ₄	13.33	10.00	76.67	14.41	13.33	13.33	73.34	15.55	10.00	6.67	83.33	13.33
H ₂ O ₂	6.67	6.67	86.66	11.11	6.67	10.00	83.33	10.00	3.33	3.33	93.34	12.22
Rhizolex-T	3.33	3.33	93.34	8.88	0.00	0.00	100.0	6.66	0.00	0.00	100.0	3.33
Vitavax-200	3.33	0.00	96.67	6.66	0.00	0.00	100.0	3.33	0.00	0.00	100.0	0.00
Topsin-M70	3.33	6.67	90.00	9.99	6.67	6.67	86.66	8.88	0.00	0.00	100.0	9.99
Control	30.00	25.00	45.00	38.88	33.00	34.00	33.00	40.00	20.00	22.00	58.00	40.00
L.S.D. at 5% :	Fungi=5.28			Treatments=4.46				Fungi × Treatments=10.54				

Table 6 Effect of treating lupine seeds with bio-agents, chemical inducers and fungicides on the incidence root-rot during two seasons under field conditions.

Treatments	Season 2013/2014				Season 2014/2015			
	Pre-emergence %	Post-emergence %	Survival %	Disease severity %	Pre-emergence %	Post-emergence %	Survival %	Disease severity %
Rhizo-N	3.33	3.33	93.34	11.11	6.67	3.33	90.00	10.00
Bio-Arc	6.67	6.67	86.66	14.44	6.67	10.00	83.33	15.55
Plant guard	6.67	3.33	90.00	13.33	6.67	10.00	83.33	13.33
Salicylic acid	3.33	0.00	96.67	9.99	3.33	6.67	90.00	10.00
K ₂ HPO ₄	6.67	6.67	86.66	13.33	6.67	10.00	83.33	14.44
H ₂ O ₂	3.33	6.67	90.00	15.55	6.67	16.67	76.73	16.66
Rhizolex-T	0.00	3.33	96.67	8.88	3.33	3.33	93.34	8.88
Vitavax-200	0.00	0.00	100.0	2.22	3.33	0.00	96.67	3.33
Topsin-M70	3.33	3.33	93.34	5.55	3.33	6.67	90.00	5.55
Control	16.67	20.00	63.33	26.66	20.00	20.00	60.00	30.00
L.S.D. at 5%	6.32	7.21	10.00	9.35	7.18	8.34	11.00	9.72

Table 7 Effect of treating lupine seeds with bio-agents, chemical inducers and fungicides on some yield component under field conditions.

Treatments	Season 2013/2014					Season 2014/2015				
	Plant height(cm)	Number of branches/plant	No. of pods / plant	Weight of 100 seeds (gm)	Weight of seed (kg) feddan	Plant height(cm)	Number of branches/plant	No. of pods / plant	Weight of 100 seeds (gm)	Weight of seed (kg) feddan
Rhizo-N	98.15	7.25	15.00	26.00	1350	95.31	6.30	13.00	23.55	1300
Plant guard	96.12	6.80	13.71	24.30	1300	94.22	5.90	12.80	22.75	1230
Salicylic acid	97.22	7.11	14.50	25.00	1450	94.75	6.70	13.90	23.00	1400
H ₂ O ₂	93.32	6.91	13.00	24.60	1420	94.45	6.10	13.20	20.63	1380
Rhizolex-T	98.02	7.13	15.00	25.91	1540	96.18	6.75	14.00	23.70	1470
Vitavax-200	98.72	7.23	15.27	26.23	1600	97.32	6.90	14.85	24.00	1530
Control	87.00	5.32	7.34	17.21	720	83.18	4.80	7.00	16.30	680
L.S.D. at 5%	1.73	0.90	1.82	1.67	-	1.32	0.73	1.90	1.55	-

Table 8 Effect of bio-agents, chemical inducers and fungicides on some chemical components of lupine seeds at Sers El-Layin during seasons 2013/2014 -2014/2015.

Treatments	Season 2013/2014			Season 2014/2015		
	Protein %	Oil %	Ash %	Protein %	Oil %	Ash %
Rhizo-N	23.18	1.03	3.58	22.92	1.01	3.50
Plant guard	23.11	1.01	3.50	22.85	1.00	3.45
Salicylic acid	23.15	1.02	3.55	22.90	1.01	3.50
H ₂ O ₂	23.10	1.01	3.52	22.88	1.01	3.48
Rhizolex-T	23.29	1.02	3.55	23.18	1.02	3.52
Vitavax-200	24.18	1.04	3.60	23.90	1.03	3.57
Control	17.06	0.69	3.20	16.72	0.61	3.12

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