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Integration of Urea Fertilizer and Trichoderma harzianum for Controlling Rhizoctonia Root Rot Disease of Lupine under Field Conditions

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Abstract- - Efficacy of different Urea fertilizers alone or in combination with Trichoderma harzianum in the control of root rot diseases of lupine was examined. All urea fertilizers alone or combined with the bioagent showed significant suppression of root infecting fungi on lupine comparing with control. Significant suppression of Rhizoctonia solani root rot incidence at pre- and post-emergence stages of plant growth was observed when urea fertilization plus T. harzianum was applied as soil drench. The most effective treatment is the combined treatments between Urea 400g/m^2 plus T. harzianum followed by Urea 200g/m^2 plus T. harzianum and Urea 100g/m^2 plus T. harzianum treatments, respectively. Data also revealed that seed dressing with the fungicide Rizolex-T showed superior effect on the root rot incidence followed by single applied treatments of the other Urea fertilization and T. harzianum. The harvested lupine yield in all treatments followed the same trend. The obtained results in the present work may be lead to conclude that soil application with urea fertilization combined with the bioagent T. harzianum is considered an applicable, safe and cost-effective method for controlling such soil borne diseases.

***Index Terms-* Disease control, lupine root rot, Rhizoctonia solani, Trichoderma harzianum, urea fertilization.**

I. INTRODUCTION

Lupine (*Lupinus termis* Forsk) is one of the easier and lower cost pulse crops to grow, but they are a feed priced commodity. They tend to be grown before cereals in a rotation, sometimes after a pasture phase. On infertile sands they are grown as a cash crop preceding pasture renovation.

In Egypt lupine plants is an important crop for food, medical and industrial purposes. The total lupine cultivated area and its average yield per Feddan (4200m^2) were decreased during the last decades. These decrements may partially be responsible for unexpected failure of lupine in Egypt during the recent years especially in sandy and new reclaimed soils. This coincides with unprecedented reports about considerable root rot incidence due to the attack of *Rhizoctonia solani* and *Fusarium solani* [1,2].

Soilborne fungal diseases are among the most important factors limiting the yield production of grain legumes in many countries, resulting in serious economic losses. Lupine plants are attacked by several soil borne fungi causing serious diseases.

Rizoctonia root rot disease caused by *Rizoctonia solani* is a soil borne infection and it has a wide host range, the fungus can survives as fungal fragments in soil and plant debris as well as dormant structures in form of sclerotia [3].

An investigation of root rot disease of lupine is considered particularly important due to its wide prevalence in Egypt, particularly in sandy soils. Treating seed with fungicides protect seedlings against root diseases. Seed dressings just before sowing reduce the incidence of seed-borne infection but cannot fully control root rot, especially under severe conditions. Seed dressing is desirable in close lupine rotations. Although some chemicals are effective in controlling these diseases but they are expensive and not environmentally friendly.

Therefore, alternative control methods are needed for managing these pathogens. The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries [4]. It is possible to use biological controls as the best control measure under greenhouse conditions. It was also reported that [5] *Trichoderma harzianum* introduced to the soil, was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T. In recent years, several attempts have been made to overcome this obstacle by applying antagonistic microorganisms. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by soil borne fungi [6,7,8].

On the other hand, there are few studies documenting the potential for mineral amendments to suppress plant root diseases in the field. Although a standard recommendation for controlling common root rot includes ensuring “adequate fertility”, there is little information in the literature on the actual impact of specific nutrients on pathogen survival, germination (inoculum potential) and subsequent disease development.

Common root rot was not affected by increasing soil pH through liming, or by the addition of either phosphate or ammonium nitrate–phosphate fertilizers [9]. Also, [10] found that soil amendments of urea (break down to NH^{4+}) resulted in less root rot than those involving ammonium nitrate (break down to NH^4+and NO^{3-}).

In this regard, Urea is an inexpensive form of nitrogen fertilizer with an NPK (nitrogen-phosphorus-potassium) ratio of 46-0-0. Urea is a low cost nitrogen fertilizer form. This is because of its high nitrogen composition and consequent low transport and storage costs.

Urea may be the fertilizer of choice when only nitrogen is needed in a soil fertility program.

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The objective of the present work was aimed to determine the efficacy of applying different doses of Urea fertilization and/or the bioagent *Trichoderma harzianum* as a soil drench against root-rot incidence of lupine under field conditions.

II. MATERIALS AND METHODS

The field experiment was designed to investigate the effectiveness of different Urea fertilizers alone or in combination with Trichoderma harzianum in the control of root rot diseases of lupine. Soil drench of Urea fertilizers alone or in combination with the bio agent Trichoderma harzianum were applied under field conditions. For achieving this target the following procedures were carried out for the growing season 2013 / 2014.

One isolates of *Trichoderma harzianum* obtained from the Plant Pathology Department of the National Research Centre, Giza, Egypt was used in the present study. This microorganism was isolated from the rhizosphere of various healthy and root rot infected leguminous crops, grown in the Delta and Middle Egypt regions, and proved its high antagonistic ability during previous work at the same department. *Trichoderma harzianum* isolate was grown in conical flasks containing 250 ml potato dextrose broth PDA medium at 28+1°C for 8 days. Then, the mycelial mats were harvested and the culture filtrates were collected and multiplied by growing on a substrate consisting of 2:1 (w:w) sterilized barley powder with a small quantity of malt extract in plastic bags for 14 days at 28±1°C.

A field study was carried out at the Researches and Experimental station of National Research Centre (NRC) in Nubaryia region, Beheira Governorate, Egypt. The influence of different levels of Urea fertilization applied alone or in combination with the bio agent, *T. harzianum* as soil drench treatments for controlling root rot disease of lupine was evaluated.

Field experiments consisted of plots (4x8 m) each comprised of 8 rows and 32 holes / row was conducted in a Complete Randomized Block design with three replicates (plots) for each particular treatment as well as untreated check control plots. The fungicide Rizolex-T 50WP applied as seed dressing at the rate of 3g/Kg seeds was used as comparison treatment. Traditional agricultural practices were followed throughout the growing season.

The bio-agent *T. harzianum* introduced to the soil in the form of fungal inoculum grown on barley powder medium, as mentioned above, at the rate of 250 g/m^2. Meanwhile, Urea fertilization introduced to the soil at the rate of 100,200 and 300g/m^2. All the above mentioned treatments were incorporated into the same cultivated row site on the top of 20 cm of the soil surface considering relevant treatments [11]. The experimental field was irrigated after treatments application two weeks before sowing.

All plots were cultivated with Lupine seeds *cv*. Giza 3. The percentage of disease infection were recorded after 15 and 30 days of germination date as pre-, and post-emergence root rot of each treatment as well as check treatment (control) for each growing season. The average percentages of disease incidence as well as the obtained yield were calculated.

Statistical analysis

Tukey test for multiple comparisons among means was utilized [12].

III. RESULTS AND DISCUSSION

The efficacy of integrated treatments of urea fertilization and/or bio-agent T. harzianum against root rot incidence of lupine was evaluated under field conditions. Results presented in Table (1) indicate that all applied treatments either single or in combination significantly reduced the disease incidence compared with control treatment.

The most effective treatment is the combined treatments between Urea 400g/m^2 plus *T. harzianum* followed by Urea 200g/m^2 plus *T. harzianum* and Urea 100g/m^2 plus *T. harzianum* treatments, respectively. They recorded in respective order 6.0, 9.0, 9.0% and 9.0, 16.0, 24.0% root rot incidence at pre- and post-emergence stages of plant growth.

Moreover, data also revealed that seed dressing with the fungicide Rizolex-T showed superior effect on the root rot incidence followed by single applied treatments of the other Urea fertilization and *T. harzianum*. It was also observed that the applied combined treatments of different Urea fertilization plus *T. harzianum* showed more reduction in disease incidence comparing with application of Urea fertilization and *T. harzianum* alone.

Table 1. Effect of different concentrations of urea and/or *Trichoderma harzianum* on root rot disease incidence of lupine plants under field conditions

Treatment	Root rot incidence %	
	Pre-emergence	Post-emergence
Single treatment		
Urea 100 g/m^2	16.0 b	29.0 b
Urea 200 g/m^2	15.0 b	22.0 c
Urea 400 g/m^2	13.0 c	14.0 e
T. harzianum	12.0 d	18.0 d
Combined treatment		
Urea 100 g/m^2 + *T. harzianum*	9.0 e	24.0 c
Urea 200 g/m^2 + *T. harzianum*	9.0 e	16.0 de
Urea 400 g/m^2 + *T. harzianum*	6.0 g	9.0 f
Rizolex-T (3g/Kg seeds)	8.0 f	12.0 f
Control	18.0 a	32.0 a

Figures with the same letter in each column are not significantly different ($P \leq 0.05$)

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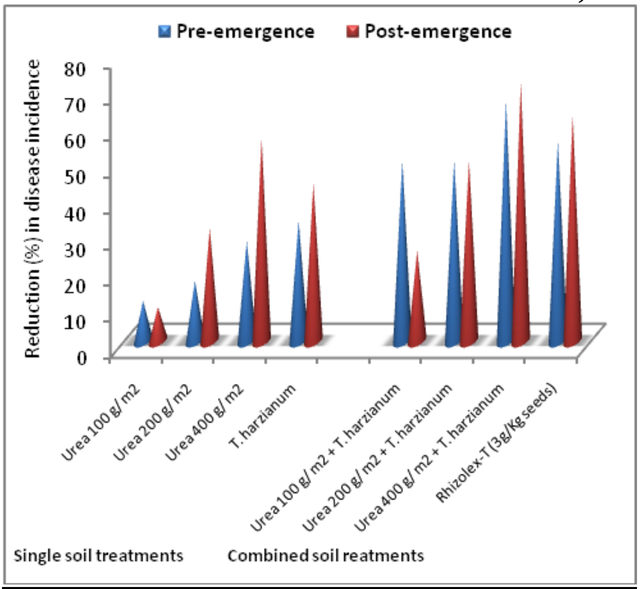

Fig. 1. Reduction in root rot disease incidence of lupine plants in response to different concentrations of urea and/or *T. harzianum* under field conditions

Illustrated data in Fig. (1) showed that at pre-emergence stage the root rot disease incidence reduced by 66.6, 50.0 and 50.0% at applied treatments of Urea fertilization at the rate of 400, 200, 100g/m^2 combined with *T. harzianum*, respectively. At post-emergence plant growth the same treatments could reduce disease incidence by 71.8, 50.0 and 25.0% over the control treatment, respectively.

Data also showed that the fungicide treatment has more effect on the reduction of disease incidence than the other single treatments that it reduce root rot incidence by 55.5 and 62.5 over control at pre-, and post-emergence of plant growth stages. Furthermore, the individual treatments of *T. harzianum* and Urea fertilization with 400, 200, 100g/m^2 reduced disease incidence by 33.3-34.7%; 27.7-56.2%; 16.6-31.2% AND 11.1-9.3% in respective order at pre-, and post-emergence stages of plant growth.

The harvested lupine yield in all treatments was significantly higher than that in the control treatment.

Data in Table (2) and Fig. (2) reveal that the combined treatments of Urea fertilization plus *T. harzianum* showed higher yield production than the other applied using Urea fertilization or *T. harzianum* as well as the fungicide treatment.

Highly effective treatments which reflect in the obtained lupine yield and its increase were the Urea fertilization at the rate of 400 g/ m^2 combined with *T. harzianum* which recorded yield of 7.3 Kg/m^2 with increase of 52.0% over the control treatment, followed by Urea fertilization at the rate of 200 and 100 g/ m^2 combined with *T. harzianum* treatments which recorded yield increase as 41.6%. Meanwhile single treatments of *T. harzianum* and Urea fertilization with 400, 200, 100g/m^2 recorded yield as 6.1, 6.5, 6.3 and 6.2Kg/m^2 with increase of 27.0, 35.4, 31.2 and 29.1%. Seeds coated with the fungicide Rizolex-T, caused a yield increase estimated as 16.6% over the check control treatment.

Table 2. Effect of different concentrations of urea and/or Trichoderma harzianum on yield of lupine plants under field conditions

Treatment	Lupine yield (Kg/m^2)
Single treatments	
Urea 100 g/ m^2	6.2
Urea 200 g/ m^2	6.3
Urea 400 g/ m^2	6.5
T. harzianum	6.1
Combined treatments	
Urea 100 g/ m^2 + *T. harzianum*	6.8
Urea 200 g/ m^2 + *T. harzianum*	6.8
Urea 400 g/ m^2 + *T. harzianum*	7.3
Rhizolex-T (3g/Kg seeds)	5.6
Control	4.8

Figures with the same letter are not significantly different (P≤ 0.05)

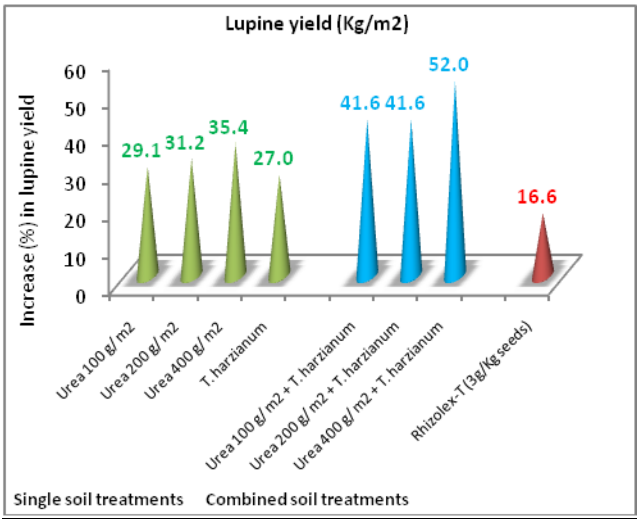

Fig. 2. Increase in lupine yield in response to different concentrations of urea and/or *T. harzianum* under field conditions

In the present study the introduction of the bioagent *T. harzianum* to the soil decreased the root rot disease incidence under field conditions. Similar results were reported by [13]. He stated that *T. harzianum* introduced to the soil was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T. Moreover, the application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries [4, 14,15,16,17]. Furthermore, antagonistic microorganisms have been suggested as one of several possible means for controlling plant pathogens without any damage to the host plant. Antagonists considered as a potential cost-effective means for reducing population of plant pathogens in soil [16]. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment.

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Moreover, in the present work it was clearly observed more reduction in lupine root rot incidence when the bioagent combined with Urea fertilization. In this regards, the use of fertilizers in the control of soil borne root rot diseases of crop plants is a common practice. Nitrogen present in the fertilizer is absorbed by the plant which is utilized in protein synthesis and seed production where as potassium is involved in many cellular functions including photosynthesis, phosphorylation, water maintenance, reduction of nitrates and reproduction. Potassium is also known to reduce *F. oxysporum* infection on tomato [18] and *R. solani* infection on hemp [19]. Urea also inhibits soil borne root-infecting fungi on mung bean [20].

Also, [21] observed that control of root infecting fungi with the use of mineral fertilizers could presumably be due to the increase in tolerance with the development of thicker cuticle and cell wall or more schlerenchyma tissue with different nutrient regimes which has been correlated with the difficulty in penetration of pathogen. Complete suppression of *M. phaseolina* was obtained when urea and DAP were used in combination with *A. marina* leaves powder on okra [22]. Similarly, [20] reported that urea showed significant reduction in *M. phaseolina* infection on mung bean. Similar results were observed by [23] that root rot diseases in mung bean caused by root infecting fungi viz., *Fusarium* spp., *M. phaseolina* and *R. solani* also reduced by the addition of urea and potash. Toxicity of ammonia ion released during degradation of urea exerted an adverse effect on soil borne pathogen [24]. Furthermore, root rot diseases caused by *F. oxysporum* and *R. solani* were reduced by the addition of mineral fertilizers [19].

In general, the greatest benefit to the plant is provided when full nutrient sufficiency is provided; however, the response to a particular nutrient may be different when going from deficiency to sufficiency than from sufficiency to excess. Since each nutrient functions as part of a delicately balanced interdependent system with the plants genetics and the environment, it is important to establish a nutrient balance for optimum crop response. Through an understanding of the disease interactions with each specific nutrient, the effects on the plant, pathogen, and environment can be effectively modified to improve disease control, enhance production efficiency, and increase crop quality.

A particular element may decrease the severity of some diseases, but increase others, and some have an opposite effect in different environments. Some forms of biological disease control and 'suppressive soils' are manifestations of microbial activity that influence nutrient availability [25,26]. A standard recommendation to maintain optimum N fertilization to decrease take-all of cereals derives from early observations that N deficiency predisposed plants to take-all. It is now known that a deficiency of most essential nutrients will increase severity of this disease. Barnyard manure high in zinc from animal rations that is applied prior to planting winter wheat greatly reduces the severity of spring blight caused by *Rhizoctonia cerealis* similar to providing pre-plant Zn. Copper deficiency causes male sterility in gramineous crops, and wheat deficient in Cu is predisposed to ergot as the florets open for cross-pollination. Providing a sufficiency of Cu for wheat nutrition greatly reduces ergot severity and increases wheat yield. Resistance of wheat and flax to rust, and maize to Stewart's wilt, may be lost under K-deficient conditions [27].

IV. CONCLUSION

Nutrient management through amendment, improved genetic efficiency, and modification of the environment is an important cultural control for plant disease and an integral component of efficient production agriculture. Disease resistance is genetically controlled but mediated through physiological and biochemical processes interrelated with the nutritional status of the plant or pathogen. The nutritional status of a plant determines its histological or morphological structure and properties, and the function of tissues to hasten or slow penetration and pathogenesis. Pathogen virulence and their ability to survive are also conditioned by various nutrients; however, most nutrients influence disease potential more than inoculum potential. The intricate relationship of the plant's nutritional status with plant pathogens, the a biotic environment and organisms in the environment is dynamic and the severity of most diseases can be greatly decreased by proper nutrient management. Knowledge of the relationship of plant nutrition to disease provides a basis for reducing disease severity in intense as well as integrated crop production systems. Such means comprise elimination of pathogens density in the soil and maintaining soil condition, favorable for root development and enhancement the competitive ability of bio agents against pathogens. Therefore, these methods introduced efficient disease control and increasing yield of many crops [28,29].

On the light of the obtained results in the present work it may be concluded that soil application of compost com-bined with the bioagent *T. harzianum* is considered an applicable, safe and cost-effective method for controlling such soil borne diseases.

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