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Application of Commercial Composts and/Or Trichoderma Harzianum for Controlling Lupine Root Rot Disease under Field Conditions

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Abstract- Three types of compost i.e. animal, plant and mixture and Trichoderma harzianum, were applied alone or in combination to study their effect on root rot incidence and yield of lupine plants under field conditions. Results indicate that all applied treatments either single or combined treatments significantly reduced the disease incidence. The most effective treatment is the combined treatments between mixture of animal plus plant compost and T. harzianum followed by the mixture of animal plus plant compost as single treatment. They recorded in respective order 7.0, 10.0% and 10.0, 14.0% 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 treatments of the other compost application either individually or combined with T. harzianum. It was also observed that the applied combined treatments of different compost types plus T. harzianum showed more reduction in disease incidence comparing with application of composts alone. Similar trend was observed concerning lupine yield.

Index Terms-**bioagent, disease control, compost, lupine, root rot, T. harzianum.**

I. INTRODUCTION

Lupine (*Lupineus termis* Forsk) is grown in Egypt for food, medical and industrial purposes. The total cultivated area and its average yield were decreased during the last decades. These decrements may partially be responsible for unexpected failure of lupine in Egypt in recent years. This coincides with unprecedented reports about considerable root-rot incidence due to the attack *Rhizoctonia solani* and *Fusarium solani* [1,2,3,4,5,6]. This disease appears during the growing season at the seedling stage of plant growth. Many soil borne fungi attack earlier at the pre-emergence stage, thus forcing the farmer to replant the missed hills or dead plants. Due to the economic importance of lupine, the farmers plant repeatedly on the same land. This practice leads to a high build-up of pathogens, causing serious losses that could reach up to 12% [7]. Thus, an effective method to control soil borne pathogens is needed. The management strategy followed by the farmers included an unwise, intensive use of fungicides. This strategy was not a satisfactory solution for controlling root rot disease. An investigation of root rot disease of lupine is considered particularly important due to its wide prevalence in Egypt, particularly in sandy soils. Thus far, because of scientific and practical difficulties, there is no economic way to control root rot disease in many crops. The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries [8]. However, this is not an easy method, and it is costly to apply. It is possible to use biological controls as the best control measure under greenhouse conditions. It was also reported that [9] *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 soilborne fungi [10,11,12]. Moreover, [13,14,15] observed that the application of wheat bran colonized by *T. harzianum* to soil infested with *R. solani* and *S. rolfsii*, reduced the incidence of root diseases caused by these pathogens in beans. On the other hand, there have been constant searches for alternative and efficient compounds for the control of plant pathogens. A number of investigations have demonstrated the effectiveness of composts of various origins in suppressing soil-borne plant pathogens [16,17,18,19,20,21], and their application to soil has been proposed to control many different diseases. Amendment of compost with *Trichoderma harzianum* also was reported to accelerate agricultural wastes composting and improved its diseases suppressive effect [22,23,24,25,26]. In this regards, several researchers have been recorded that bio compost application as soil amendment could suppress diseases caused by *R. solani* and Fusarium spp. on many economic crops [26,27,28]. The increasing interest in pesticide alternatives was due to the toxicity implication of pesticides for humans. Therefore, there will be an increasingly driven demand motivated by different priorities such as health benefits, cost, ecological benefits, ethical issues, food safety and sustainability of supply. Against this background, and the demand for natural products as raw material for new antifungal agents, the objective of the present work was aimed to determine the efficacy of applying three types of compost and *Trichoderma harzianum* alone or in combination as a soil treatment, was evaluated against root-rot incidence of lupine under field conditions.

II. MATERIALS AND METHODS

The field experiment was designed to investigate the effectiveness of compost and biological means to control root rot disease of lupine. Soil drench of compost alone or

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in combination with the bio agent *Trichoderma harzianum* were applied under field conditions. For achieving this target the following procedures were carried out for two successive growing seasons 2013 and 2014.

Source of compost and the bio-agent T. harzianum

Three types of compost *i.e.* animal, plant and mixture of animal plus plant were purchased from El- Nile Company, Giza, Egypt. 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.

Field experiment

A field study was carried out at the Researches and Experimental station of National Research Centre (NRC) in Nubaryia region, Beheira Governorate, Egypt for two successive growing seasons. The influence of different types of compost 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 two cultivation seasons. 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, compost types introduced to the soil at the rate of 20m^3/feddan (4200m^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 [9]. 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 for the two growing seasons.

Statistical analysis

Tukey test for multiple comparisons among means was utilized [29].

II. RESULTS AND DISCUSSION

The activity of integrated treatments of compost 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 mixture of animal plus plant compost and *T. harzianum* followed by the mixture of animal plus plant compost as single treatment. They recorded in respective order 7.0, 10.0% and 10.0, 14.0% 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 treatments of the other compost application either individually or combined with *T. harzianum*. It was also observed that the applied combined treatments of different compost types plus *T. harzianum* showed more reduction in disease incidence comparing with application of composts alone.

Table (1) Lupine root rot incidence in response to soil drench with different types of composts and/or *T. harzianum* under field conditions

Treatment	Lupine root rot incidence %	
	Pre-emergence	Post-emergence
Single treatments		
Plant compost	14.0 b	23.0 b
Animal compost	14.0 b	22.0 bc
Mixture of animal plus plant compost	10.0 d	14.0 d
T. harzianum	12.0 cd	20.0 c
Combined treatments		
Plant compost + *T. harzianum*	11.0 cd	18.0 c
Animal compost + *T. harzianum*	10.0 d	19.0 c
Mixture of animal plus plant compost + *T. harzianum*	7.0 e	10.0 e
Fungicide (Rizolex-T)	8.0 e	11.0 e
Control	35.0 a	42.0 a

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

Illustrated data in Fig. (1) showed that at pre-emergence stage the root rot disease incidence reduced by 80.0 and 71.4% at applied combined treatment of mixture of animal plus plant compost and *T. harzianum* and individual treatment of the mixture of animal plus plant compost, respectively. At post-emergence plant growth the same two treatments could reduce disease incidence by 76.2 and 66.7% over the control treatment, respectively. Data also showed that the fungicide treatment has more effect on the reduction of disease incidence than the other compost treatment either alone or combined with *T. harzianum* that they reduce root rot incidence by 77.1 and 73.8% over control treatment comparing with plant or animal compost treatments when applied individually or combined with *T. harzianum*. These later treatments reduced disease incidence by (60%; 68.6-71.4) and (45.2-47.6%; 54.8-

57.1) in respective order at pre-, and post-emergence stages of plant growth.

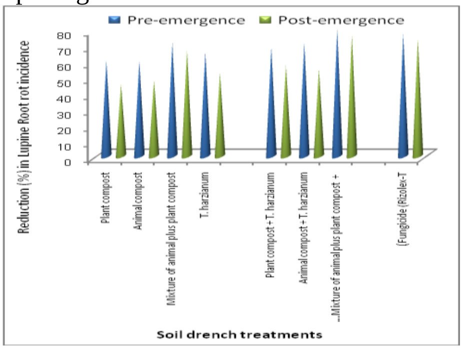

Fig. (1) Reduction in root rot incidence of lupine in response to application of composts and *T. harzianum* alone or in combination under field conditions

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 treatments of mixture of animal plus plant compost and *T. harzianum* showed higher yield production than the other applied using compost alone or combined with *T. harzianum* as well as fungicide treatment. Highly effective treatments which reflect in the obtained lupine yield and its increase were the mixture of animal plus plant compost combined with *T. harzianum* which recorded yield of 6.0 Kg/m^2 with increase of 57.8% over the control treatment, followed by individual treatment which recorded yield ranged between 5.0-5.2 Kg/m^2 with increase between 31.6-36.8%. Meanwhile combined treatments recorded yield as 5.5-5.5Kg/m^2 with increase of 42.1-44.7%. Seeds coated with the fungicide Rizolex-T, caused a yield increase estimated as 31.6% over the check control treatment.

Table (2) Lupine yield in response to soil drench with different types of composts and/or *T. harzianum* under field conditions

Treatment	Lupine yield (Kg/m^2)
Single treatments	
Plant compost	5.0 c
Animal compost	5.0 c
Mixture of animal plus plant compost	5.2 c
T. harzianum	5.0 c
Combined treatments	
Plant compost + *T. harzianum*	5.4 b
Animal compost + *T. harzianum*	5.5 b
Mixture of animal plus plant compost+ *T. harzianum*	6.0 a
Fungicide (Rizolex-T)	5.0 c
Control	3.8 d

Fig with the same letter are not significantly different ($P \leq 0.05$)

The present investigation has demonstrated the anti-fungal activity of all treatments tested. This work proves that some compost types have potential and could be use-ful when integrated with the bioagent *T. harzianum* against lupine root rot fungal pathogens. From the earlier reports [16,30] it is evident that some of the plant or animal wastes have antifungal compounds which do have the capacity to inhibit the fungal pathogens.

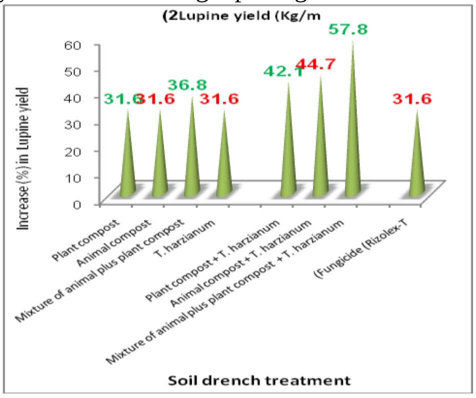

Table (2) Increase in Lupine yield in response to soil drench with different types of composts and/or *T. harzianum* under field conditions

Therefore, there is increasing interest in obtaining alternative antimicrobial agents for use in plant disease control systems. One of the main procedures used in the research of biologically active substances is a systematic screening for the interaction between microorganisms and plant products. This procedure has been a source of useful agents to control the microbial survival [31]. In the present study the introduction of the bioagent *T. harzianum* to the soil increased the efficacy of different compost types against root rot incidence under field conditions. Similar results were reported by [32]. 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 [8,33,34,35,36]. Moreover, a number of investigations have demonstrated the effectiveness of composts of various origins in suppressing soil-borne plant pathogens [8,18,19,20,30], and their application to soil has been proposed to control many different diseases. However, not all composts have been shown to exert beneficial effects on plant growth and health. Moreover, organic amendments play an important role as environmentally friendly and sustainable alternative approach to protect plants against soil borne pathogens. Soil amendments, using composted agricultural wastes fortified with bio control agents could be acceptable approaches in this regard. The use of organic agricultural wastes in this respect can be an advantageous both in soil fertility, recycling of agricultural residues and could provide a powerful tool for management of plant diseases. It has been reported that several composts and/or composts fortified with bio control agent used as soil amendments reduced pathogens propagules density and protected plants from soil borne plant pathogens [37,38,39]. Using agricultural wastes, domestic food wastes or some grains as substrates for *T. harzianum* growth formulation and directly delivery in soil for controlling soil borne pathogens on some crops were recorded [27,40,41]. Amendment of compost with *Trichoderma harzianum* also was reported to accelerate agricultural wastes composting and improved its diseases suppressive effect [22,23, 25,42,43,44]. 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 [35]. 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. Maintaining equilibrium between the pathogen population and its hosts by constant supply of a biocontrol agent that will keep disease to a minimum. One of the main procedures used in the research of biologically active substances is using compost for controlling soilborne plant pathogens and increasing the soil fertility as well. The suppressive activity of different compost types toward several plant pathogens is well documented in the recent review by [45]. Further, the investigation on the biological activity of these materials on antagonistic soil-borne fungi is of great interest, in that their contribution to biological control should be safeguarded. Very few information is reported in the literature on the suppressive effects of soil native humic substances (HS) and HS-like fractions on phytopathogenic fungi and also no information is reported on antagonistic soil-borne fungi. Although some information is available on the mechanisms responsible for the suppressive action of compost and compost extracts on plant pathogenic fungi [45] very limited information exists on the relationship between chemical properties of HS and HS-like fractions and fungal suppressive capacity. In this regards, several researchers have been recorded that bio compost application as soil amendment could suppress diseases caused by *R. solani* and *Fusarium* spp. on many economic crops [24,27,40]. Using agricultural wastes, domestic food wastes or some grains as substrates for *T. harzianum* growth formulation and directly delivery in soil for controlling soil borne pathogens on some crops were also recorded [24,27,40]. 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 [37,38,46,47,48]. On the light of the obtained results in the present work it may be concluded that soil application of compost combined with the bioagent *T. harzianum* is considered an applicable, safe and cost-effective method for controlling such soilborne diseases.

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