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FIELD APPLICATION OF THE BIOCIDE *TRICHODERMA HARZIANUM* TO PROTECT BEAN ROOT

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ABSTRACT

Efficiency of *Trichoderma harzianum* to be used against the danger of bean rot disease on the pre-emergence stage of the bean seed is investigated . Soil drench with the *T. harzianum* showed more significant decrease in root rot incidence than seed treatment although all treatments decreased incidence significantly than the control.

INTRODUCTION

Certainly, legumes are of paramount importance in the Middle East table . Obviously, bean is one of the most important leguminous crops. High quality of green pods and seeds are used for fresh meal and food industries, while hay and low quality seeds are used for animal feed . Unfortunataly, bean is attacked by many of soilborne fungi which cause daming - off and root-rot diseases. *Rlizoctonia solani* reported as one of the main pathogens causing root- rot disease of Bean [1,2].

In many countries, the application of biological control using antagonistic microorganisms proved to be successful for controlling various plant diseases [3] It is still not easy and cost in application, however, it can serve as the best measure under greenhouse conditions. In addition, its application is safe , unhazardous for human, farm animals and avoid the environmental pollution [4].

The present study was carried out to investigate the efficiency of *T harzianum* as biocide to control bean root disease caused by *R. solani* in comparison with the prevaling chemical control method with Rhizolex under field conditions.

MATERIALS AND METHODS

Samples of bean plants showing root rot symptoms collected from three locations at Al-Aiat territory , Giza Governorate, Egypt were subjected to isolation of the causal organism. The isolated fungi were identified as three isolated of *Rhizoctonia solani* after cultural and microscopical examinations according to previous studies by several authors [5,6] .

The antagonistic effect of *T. harzianum* against *R. soloni* isolates was determined . *In vitro* test consisted of removing 5 -mm - diameter disks from the edge of expanding colonies of *T. harzianum* and *R. solani* isolates grown for 5 days on PDA medium in Petri dishes . The pair organisms were placed on opposite sides of Petri dishes containing PDA medium . Incubation period was 5 days at 25°C and five replicates were used for each tested isolate. Decrease of *R. solani* growth was determined using formula stated by **Ferreira et al.** [7] as follows :

$$R = \frac{A-B}{A} X\ 100$$

where :

R =percentage of growth decrease;
A : the distance of mycelial growth of pathogenic fungus away from the antagnositic fungus ;

B : the distance of mycelial growth of pathogenic fungus towards the antagonistic fungus .

The effect of fungicide Rhizolex on growth of *R. solani* was tested *in vitro* . Disks 5-mm diameter from 5 days grown culture of *R. solani* were placed on centre of Petri dishes containing PDA medium supplied with Rhizolex at different concenterations , i.e. 0, 50, 100, 200 and 400 ppm. Incubation period was 5 days at 25°C and five replicates were used for each concentration tested. Inhibitory effect of Rhizolex on *R. solani* was calculated as percentage of growth decrease using formula as :

$$R = \frac{C-T}{C} X\ 100$$

where :

R = Percentage of growth decrease;
C : growth diameter in control
T: growth daimeter in fungicide treatment.

Evaluation of *T. harzianum* as biocide under field conditions was carried out as seed treatment or soil drench in naturally heavily contaminated field with root rot pathogen at Al-Aiat , Giza Governorate. Inoculum of *T. harzianum* grown on sand - barley medium (1 : 1 , w: w and 40% water) was used for soil drench and seeds treatment . Biocide inoculum was mixed with soil

surface of planting row site to 20 cm depth at two rates 60 & 120 g/m^2, while bean seeds were immersed in *Trichoderma* spore suspension at concentration of 6 X 10^3 spore / ml for 1 hr. Rhizolex 50% as seed dressing fungicide at the recommended dose of 3g/kg seeds was used in this study as a reference for evaluation the bioagent fungus . Field consists of plots (7.5 x 10.5m) comprised for 12 rows and 30hill/row were used in this experiment . Three plots as replicates were used for each previous particular treatment as well as control . All treatment were sown with bean seeds cv. Giza, 3 at the rate of 3 seeds / hill. Bean plants received the usual agricultural practices . Average percent of pre- and post -emergence root rot incidence up to 60 days (the experimental period) was calculated.

RESULTS AND DISCUSSION

Laboratory test for biological antagonistic effect of *T. harzianum* against *R. solani* is a simple approach for understanding a small sector of biological systems in disease control as well as the influence of chemical fungicide on root rot pathogen for the same purpose.

Data in Table (1) reveal that , high growth decrease of tested *R. solani* isolates by 69.7; 72.6 and 74.4% was observed as a result of the anatgonistic effect of *T. harzionum*. **Bell et al.** [8] scored the degree of antagonism on a scale of classes, i.e. *Trichoderma* could completely overgrow the pathogen and cover the entire medium surface, or overgrow at least two thirds of the medium surface.

On the other hand, the pathogen growth was inhibited in ascending order by increasing Rhizolex concentrations in medium . Complete growth inhibition by 100% was recorded for all tested. *R. solani* isolates at 400 ppm. Many investigators recorded similar trend , where *R. solani* growth was completely inhibited within a range of 200 -400 ppm of Rhizolex [9,10]. Those results are confirmed by these obtained in present study.

Table (1) :Percentage growth decrease of *R. solani* affected by bio-fungicide *in vitro*.

Isolates of *R. solani*	Antagonistic tester *T. harzianum*	Rhizolex at different concentrations (ppm)			
		50	100	200	400
No. 1	74.4	35.0	55.0	79.0	100
No. 2	72.6	33.8	52.5	77.5	100
No. 3	69.7	28.8	50.0	73.7	100

Evaluation of biological control for Bean root rot using the biocide *T. harzianum* is presented in Table (2) . Data obtained show that at preemergence stage, all treatments significantly decrease root rot incidence in comparison with the control . No significant differences were observed between the two rates of soil drench and seed coating with biocide or Rhizolex . These results may be due to the almost equality of population density of *T. harzianum* in the seed bed in the two treatments.

Table (2) : Percentage of root rot incidence affecting by bio-fungicidal treatment in field conditions.

Treatments		Av. Percentage of root rot incidence	
		Pre-emergence	Post-emergence
Soil drench	60g/m^2	15.3*	9.1**
T. harzianum	120g/m^2	14.7	5.3
Seed coating	*T. harzianum* 6X10^3 spore/ml	16.4	12.3
	Rizolex 3g/kg seeds	14.8	14.8
Control		27.3	18.9
L.S.D. at 5%		8.5	4.1

* Based on emerged plants in relative to No. of sown seeds.

** Based on diseased plants in relative to No. of emerged seedlings.

At post- emergence stage, all treatments significantly affected root rot incidence than control . The high rate of soil drench (120g/m^2) with biocide *T. harzianum* showed the best treatment decreasing root rot incidence followed by the low rate (60g/m^2) Seed coating with either biocide or Rhizolex resulted in 12.3 and 14.8% root rotted plants and were less effective than the two levels of soil drench . These differnces could be due to the intial inoculum of *T. harzianum* introduced into the soil . The high fungal population density introduced through soil drench technique enables the fungus to adapt itself against environmental conditions [11] resulting in dominant high population of *T. harzianum* .

The biological equilibrium between the biocide *Trichoderma* and the other soil microflora in the favour of *Trichoderma* gives satisfactory antagonistic effect

against *R. solani* providing successful biocontrol. Rhizolex at recommended dose, 3 g/kg seeds decreased root rot incidence in a lower extent in comparison with biocide treatments . It gives less protection for seed germination and seedling grwoth when introduced into the soil and exposed to the environmental conditions which cause loss in its efficiency . On the other hand , the biocontrol agent established itself is soil and increase its population to at least constant density sufficient to antagonise plant pathogens.

However, biological control is still problem in application on a large scale under field conditions [12-14], the obtained results in the present study indicate that the usage of *T. harzianum* as biocide agent against *R. Solani* is promising technique for application to control various soilborne fungi causing root rot and damping -off diseases.

It can be concluded that the amount of *Trichoderma* preparation could be decreased by adjusting the mode of application to each crop according to the common agricultural practice. Studying fungal - fungal interaction as well as the ecology and survival of *Trichoderma* in the field may lead to the development of more efficient strains of *Trichdoerma* high antagonistic against soilborne plant pathogens and other competators of soil microflora.

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التطبيق الحقلى للمبيد الحيوى الترايكودرما هارزيانم لحماية جذور الفاصوليا

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تناول هذا البحث استخدام الترايكودرما هارزيانم للوصول إلى أفضل طريقة للحد من خطورة عفن الجذور على انبات بذور الفاصوليا. أظهرت النتائج المعملية قدرة عالية للترايكودرما هارزيانم على تقليل نمو العزلات المختبرة بنسب تتراوح فيما بين ٦٩-٧٤٪ .

أوضحت النتائج أنه فى حالة الاصابة بعفن الجذور فى مرحلة ماقبل الأنبات لم يكن هناك فروق معنوية بين كل المعاملات بالرغم من وجود معنوية بينها وبين الكنترول.