# BIOLOGICAL CONTROL OF LUPIN (*LUPINUS TERMIS* L.) AND CHICKPEA (*CICER ARIETINUM* L.) DAMPNG OFF DISEASE

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## ABSTRACT

Six biocontrol agents namely *Trichoderma harzianum, T. Koningii, T.viride,* two isolates of *Bacillus subtilis* and one isolate of *Erwinia herbicola* were tested for their antagonistic action against five pathogenic fungi causing lupin and chickpea damping-off,.i.e. *Macrophomina phaseolina, Fusarium solani, Rhizoctonia solani* and two isolates of *F. semitectum*. For measuring the bioagent activity the fungicide vitavax captan was used as standard. *T. Koningii, T. harzianum* and *B. Subtilis* isolate no (1) caused the highest reduction in linear growth of all the tested pathogenic fungi. All tested bioagents significantly decreased damping-off disease caused by *M. phaseolina* and *F. solani* in lupin seedlings and two isolates of *F. semitectum* in chickpea seedlings. The tested bioagents gave variable results against *R. solani* in lupin and chickpea seedlings.

**Keywords**: Biological control, damping-off disease, *Trichoderm* sp., *Bacillus subtilis, Erwinia herbicola.*

## المقاومة الحيوية لمرض موت البادرات فى كل من الترمس والحمص

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تم اختبار التأثير التضادى لستة من العوامل الحيوية هى عزلتين من باسيلس ساتلس وعزله من ارونياهيربيكولا وثلاث عزلات من الفطر تريكودرما هي تريكودرما هارزيانم، تريكودرما قيردى وتريكودرما كوننجي ضد خمسه من الفطريات المسببه لمرض موت البادرات فى الترمس والحمص وهي ماكروفومينا فاسيولينا، فيوزاريوم سولانى فى الترمس وعزلتين من فيوزاريوم سميتيكتم فى الحمص. كما اختبر الفطر ريزوكتوينا سولانى فى كل من الترمس والحمص. واستخدم المبيد الفطرى فيتافاكس كابتان للمقارنه.

ففى الدراسة المعملية وجد ان تريكودرما هارزيانم وتريكودرما كوننجي وعزلة باسيلس ساتلس رقم (١) اعطت اعلى نسبه مئوية للنقص فى النمو الطولى لجميع الفطريات المختبرة.

اما الدراسة فى الاصص فقد اثبتت ان جميع العوامل الحيوية المختبرة قد قللت من موت البادرات فى الترمس نتيجة للاصابة بفطرى فيوزاريوم سولانى وماكروفومينا فاسيولينا وكذلك فى الحمص نتيجة للاصابة بعزلتين من فيوزاريوم سميتيكتم. اما الفطر ريزوكتونيا سولانى فقد كانت استجابته متباينة فى كل من الترمس والحمص . اما المبيد فيتافاكس كابتان فقد اعطى نتائج جيدة فى تقليل الاصابة بموت البادرات فى كلا المحصولين.

## INTRODUCTION

Legumes are the most important crops in Egypt. Lupin (*Lupinus termis* L.) is used as fodder crops or as green manure for sandy and poor soils. It is also used for medical and industrial purposes (Osman *et al.,* 1986). The chickpea (*Cicer arietinum* L.) is mainly used as fodder crop. Infection of roots with root rot and damping- off fungi cause serious economic losses for the yield (Khalifa, 1997). These diseases are chemically controlled by fungicides. Such chemical's pollute the environment, cause harmful effects of living organisms and reduce the population of the useful microorganisms in soil (Sivan and Chet 1986). There are many investigations about the efficacy of using biocontrol agents to control *Fusarium* sp., *M. phaseolina, R. solani* and other root rot and damping-off fungi (Abdel-Moity and Shatla, 1981 and Okhovvate and Karampour, 1996).

Osman *et al.* (1986) and EL-Safwani (1991) found that *T. harzianum* and *T. viride* reduced the Fusarium wilt of lupin crop. *T. harzianum* was used as seed treatment and soil application to control root rot and damping-off in many crops ( Ashour 1996 and Abdel-Kader 1997).

Sabet *et al.* (1998) used four biocontrol agents, *B. subtilis, Chaetomium atroporum, T. harzianum* and *Pseudomonas fluorescens* against *M. phaseolina, R. solani, Sclerotinia sclerotiorum* and *Sclerotium rolfsii. T. harzianum* highly decreased linear growth *in vitro* while ***B. subtilis*** gave best results in controlling broad bean damping-off disease *in vivo*. Several plant pathogenic fungi were controlled by using *B. subtilis* as seed treatment. The antagonistic mechanism of it can be attributed to antibiotic production (Leoffler *et al.,* 1986).

The present work was carried out to study the effect of six different biocontrol agents *T. viride, T. Koningii T. harzianum*, Two isolates of *B. subtilis* and *Erwinia herbicola* in comparison with the fungicide vitavax captan against damping-off disease in lupin and chickpea seedlings *in vitro* and under green house conditions.

## MATERIALS AND METHODS

**1. Isolation and identification of the causal fungi:**

Samples of naturally infested legume plants (lupin, chickpea and soybean), showing different degrees of root-rot and damping-off symptoms were collected from the Research Station of Fculty of Agriculture, Alexandria University and Noubria Research farm at Alexandria Governorate. Diseased roots were cut into small fragments. Roots pieces were surface sterilized by immersing them in 1% sodium hypochlorite for 2 minutes and then washed several times with sterilized distilled water. Root fragments were dried between two sterilized filter papers, then transferred into potato dextrose agar (PDA) medium and plates were incubated for 5 days at $25^0$C. Developed fungi were transferred to new PDA plates, purified and identified following Gilman (1957) and Barnett and Hunter (1972).

**2. Fungal and bacterial bioagents:**

Three species of *Trichoderma*, i.e. *T. viride, T. koningii* and *T. harzianum* were obtained from Plant Pathology Institute (ARC), two isolates of *Bacillus subtilis* and one isolate of *Erwinia herbicolal* were obtained from the stock culture collection of the Department of Plant Pathology, Faculty of Agriculture, Alexandria University.

**3. Pathoginicity test:**

The pathogenic potential of five fungi, i.e. two isolates of *Fusarium semitectum* Berk from chickpea plants, one isolate of *F. solani* Mart and *Macrophomina phaseolina* (Tassi) Goid from lupin plants and one isolate of *Rhizoctone solani* from soybean plants were tested.

One disc (5mm in diameter) 7 days old of each tested fungus was used to inoculate a glass bottle (500 ml) containing autoclaved cornmeal medium (50 gm corn and 40 ml water) then incubated for 2 weeks at $25^0$C. Autoclaved sandy clay soil (1:1, w/w) was thoroughly mixed with the inoculum of the tested fungi which grown in cornmeal medium, at the rate of 40 gm/kg soil. Plastic pots (20 cm in diameter) were filled with inoculated soil at the rate of one kg/pot. In check pots, soil was mixed only with uninoculated cornmeal at the same ratio. The infested soil was left for seven days to secure establishment of the inoculated fungi. Lupin (cv Giz 2) and chickpea (cv Gize 195) seeds were surface sterilized with sodium hypochlorite1% for 2 minutes and washed for three times with sterilized water. Ten seeds were sown in each pot. All pots were kept under greenhouse conditions and disease assessment was recorded 30 days after sowing as percentage of pre and post-emergence damping-off.

**3.1. In vitro tests:**

**3.1.1. Antagonistic effect of fungal bioagents on pathogenic fungi:**

Antagonistic effect of *Trichoderma* speceis (*T. viride T. Koningii* and *T. harzianum*) on the linear growth of the five tested fungi were carried out in petri dishes containing PDA medium. Each plate was divided into equal halves; one half was inoculated with disc (5 mm in diameter) of a Trichoderma sp. of 7 days old, the opposite half was inoculated with disc 7 days old culture of any of the tested pathogenic fungi. Four plates were used for each treatment. Plates were then incubated at $25^0$C for 7 days. Percentage of reduction in the linear growth of the tested fungi was determined using the formula stated by Topps and Wain (1957).

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

Where R= Percentage of growth reduction.
C= Diameter of the control hyphal growth
T= Diameter of the treated hyphal growth

**3.1.2. Antagonistic effect of bacterial bioagents on pathogenic fungi:**

In order to study the antagonistic effect of bacteria on the growth of the five tested pathogenic fungi, each bacterial bioagent was streaked at one side of PDA medium plates. Plates were incubated for 24 hours at $25^0$C, then one disc (5 mm in diameter) bearing 7 day-old growth of each tested fungus was placed in the opposite side of the plate. Plates were incubated for 7 days at $25^0$C, and four replicates were

used. Percentage of reduction occurred in linear growth of the tested fungi were determined.

**3.2. Pot experiments:**

Pots (20 cm in diameter) were filled with soil pre infested with any of the five tested fungi by the same method which mentioned under pathoginicity test. At the same time surface sterilized lupin (cv Giza 2) and chickpea (cv Giza 195) were immersed for one hour in one of the *Trichoderma* sp. spore suspension at concentration of $5x10^8$ spore /ml prepared from 10 day old cultures grown on PDA. On the other hand, surface sterilized seeds were soaked for one hour before sowing in 3 day- old liquid cultures of either one isolate of *B. subtilis* or *E. herbicola*. Concentration of bacterial cells was adjusted to be $10^8$-$10^9$ colony-forming unites (CFU) per ml ( Baker 1962). Ten seeds were sown in each pot and four replicates were used for each treatment. Untreated seeds were served as a control. The disease assessment of damping-off has been carried out as mentioned before.

Obtained data were statistically analyzed using Complete Randomized Block Designs suggested by Snedecor and Cochran (1967). Average were compared at 5% level of probability using Least Significant Difference (L.S.D.).

## RESULTS AND DISCUSSION

Pathogenicity test revealed that the five tested fungi were pathogenic to lupin and chickpea seedlings. All the tested fungi caused high percentage of pre-emergence damping-off and decreased the number of survived plants (Tables 2 and 3).

***In vitro* experiments:**

The effect of six antagonistic microorganisms on the reduction of mycelial growth of the tested fungi are presented in (Table 1). Obtained data showed that *T. Koningii T. harzianum* and *B. subtilis* isolate no (1) exhibited good results in reducing the mycelial growth of all tested fungi. Moreover *B. Subtilis* isolate no (2) and *E. herbicola* gave slight reduction in the mycelial growth of *R. solani* and a moderate reduction in the mycelial growth of the other four fungi. *T. viride* exhibited slight reduction on *R. soloni* and good results in reducing the mycelial growth of the other four fungi. These results are similar to these reported by Abdel-kader (1999) who found that *T. harzianum* (T-3) has high inhibitory effect on the mycelial growth of *F. oxysporum f.* sp. *capsici* isolates which cause wilt disease of hot pepper at the rate of 65.4-73.8%. Also Hwang and Chakravarty (1992) reported that in paired culture, *B. subtilis* inhibited mycelial growth of 2 isolates of *R. solani* isolated from rotted pea seedlings.

**Table (1): Reduction in the mycelial growth of the tested pathogenic fungi as a results of treatment with six antagonistic microorganism.**

| Tested bioagents | Reduction in linear growth of the tested pathogen % | | | | |
|---|---|---|---|---|---|
| | ***M. phaseolina*** | ***F. solani*** | ***F. semitectum (1)*** | ***F. semitectum (2)*** | ***R. solani*** |
| *T. viride* | 57.00 | 57.36 | 64.39 | 62.16 | 26.19 |
| *T. Koningii* | 70.67 | 64.39 | 67.00 | 69.56 | 49.58 |
| *T. harzianum* | 69.59 | 65.87 | 70.67 | 67.73 | 54.37 |

| | | | | | |
|---|---|---|---|---|---|
| *B. subtilis* (1) | 61.38 | 56.63 | 72.56 | 58.46 | 46.26 |
| *B. subtilis* (2) | 54.37 | 51.06 | 50.68 | 53.33 | 31.06 |
| *E. herbicola* | 54.37 | 52.19 | 52.16 | 57.36 | 10.71 |
| L. S.D. 5% | 4.84 | 8.59 | 9.07 | 9.85 | 12.31 |

**Greenhouse experiments:**

Evaluation of biological control of lupin damping-off caused by *M. phaseolina, F. solani* and *R. solani* by using six bioagents i.e. *T. viride, T. koningii, T. harzianum*, two isolates of *B. subtilis and E. herbicola* are presented in (Table 2). Obtained data showed that all the tested bioagents significantly decreased the pre-emergence damping-off incited by any of the three fungi in comparison with infested control treatment except *E. herbicola* against *R. solani. Data in Table 2 showed that T. viride, T. Koningii* and *E. herbicola* were nearly as effective as the fungicide vitavax captan in reducing the pre-emergence damping-off caused by *M. phaseolina* 15.0, 18.33, 6.66 and 10%, respectively while the pre-emergence damping-off in infested control was 75%. Meanwhile *T. koningii, T. harzianum, B. subtilis* isolate no (1) *E .herbicola* and the fungicide vitavax captan decreased the pre-emergence damping-off caused by F. solani 26.66, 6.66, 26.66, 18.33 and 18.33%, respectively. *T. viride, T. koningii* and *B. subtilis* isolate no (1) significantly reduced the pre-emergence damping-off caused by *R. solani* from 73.4 for infested control treatment to 18.33, 10.0, 3.33% respectively.

**Table (2): Effect of six biocontrol agents as seed treatment on lupin damping-off disease caused by three fungi.**

| Tested bioagents | Pre and Post-emergence damping –off (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *M. phaseolina* | | | *F. solani* | | | *R. solani* | | |
| | % Pre | % Post | % survivors | % Pre | % Post | % survivors | % Pre | % Post | % survivors |
| *T. viride* | 15.00 | 3.33 | 81.67 | 45.00 | 6.66 | 48.34 | 18.33 | 6.66 | 75.01 |
| *T. koningii* | 18.33 | 6.66 | 75.01 | 26.66 | 3.33 | 70.01 | 10.0 | 3.33 | 86.67 |
| *T. harzianum* | 28.33 | 3.33 | 68.34 | 6.66 | 6.66 | 86.68 | 26.66 | 3.33 | 70.01 |
| *B. subtilis* (1) | 35.00 | 3.33 | 61.67 | 26.66 | 0.0 | 73.34 | 3.33 | 3.33 | 93.34 |
| *B. subtilis* (2) | 35.00 | 3.33 | 61.67 | 35.00 | 0.0 | 65.00 | 45.0 | 0.0 | 55.0 |
| *E. herbicola* | 6.66 | 13.33 | 80.01 | 18.33 | 3.33 | 78.34 | 65.0 | 0.0 | 35.0 |
| Vitavax captan | 10.00 | 6.66 | 83.34 | 18.33 | 3.33 | 78.34 | 18.33 | 3.33 | 78.34 |
| Infested control | 75.00 | 0.0 | 25.00 | 65.00 | 10.0 | 25.0 | 73.39 | 0.0 | 26.61 |
| Uninfested control | 0.0 | 0.0 | 100 | 3.33 | 0.0 | 96.67 | 6.66 | 0.0 | 93.3 |
| L.S.D. 5% | 19.35 | N.S | 18.66 | 15.26 | N.S | 15.97 | 18.93 | N.S | 20.71 |

There were no significant differences between infested control and all treatments in case of post-emergence damping- off caused by any of the tested fungi. The above mentioned results revealed that the tested bioagents are very effective to control the pre-emergence damping-off and consequently the number of survived plants is significantly increased. Similar results had bean reported by Yehia *et al.* (1982) who used *T. viride, Streptomyces griseus* and *B. subtilis* as seed treatment to control broad bean root rot caused by *F. solani.* They found that seed coating with *T. viride* and *B. subtilis* could be considered as an effective biocontrol for Fusarium root rot. Meanwhile Khalifa (1997) found that both soil and seed treatments with the biocontrol agent *T. harzianum* were nearly as effective as the seed treatment with fungicide benlate for controlling damping -off and root-rot diseases of faba bean caused by *F. solani.*

Evaluation of biological control of chickpea damping- off caused by two isolates of *F. semitectum* and *R. solani* by using six bioagents are presented in (Table 3). Obtained data showed that all tested bioagents significantly decreased the pre-emergence damping-off incited by the two isolates of *F. semitectum.* Data in table 3 revealed that *T. koningii, B. subtilis* isolate no (2) and *E. herbicola* were nearly as effective as the fungicide vitavax captan in reducing the pre-emergence damping-off caused by *F. semitectum* isolate no (1) 18.33, 10, 6.66 and 6.66%, respectively while the pre-emergence damping-off in infested control was 65%. It is also clear that *T. koningii,* the two isolates of *B. subtilis* and the fungicide vitavax captan exhibited good results in reducing the pre-emergence damping-off caused by isolate no (2) of *F. semitectum* 3.33, 11.7, 15.0, 6.66 and 6.66%, respectively while in the infested control was 74%. The above mentioned results revealed that the tested bioagents are very effective to control the pre-emergence damping-off and consequently the number of survived plants is significantly increased.

**Table (3): Effect of six biocontrol agents as seed treatment on chickpea damping-off disease caused by three fungi.**

| Tested bioagents | Pre and Post-emergence damping –off (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***F. semitectum* (1)** | | | ***F. semitectum* (2)** | | | ***R. solani*** | | |
| | % Pre | % Post | % survivors | % Pre | % Post | % survivors | % Pre | % Post | % survivors |
| *T. viride* | 23.33 | 0.0 | 76.67 | 26.66 | 6.66 | 66.68 | 55.0 | 0.0 | 45.0 |
| *T. koningii* | 18.33 | 0.0 | 81.67 | 3.33 | 3.33 | 93.34 | 55.0 | 0.0 | 45.0 |
| *T. harzianum* | 35.0 | 0.0 | 65.0 | 28.33 | 3.33 | 68.34 | 35.0 | 0.0 | 65.0 |
| *B. subtilis* (1) | 23.33 | 0.0 | 76.67 | 11.70 | 3.33 | 84.97 | 65.0 | 0.0 | 35.0 |
| *B. subtilis* (2) | 10.0 | 6.66 | 83.34 | 15.0 | 0.0 | 85.0 | 55.0 | 0.0 | 41.67 |
| *E. herbicola* | 6.66 | 3.33 | 90.01 | 45.0 | 0.0 | 55.0 | 55.0 | 6.66 | 38.34 |
| Vitavax captan | 6.66 | 6.66 | 86.68 | 6.66 | 6.66 | 86.68 | 6.66 | 3.33 | 90.01 |
| Infested control | 65.0 | 6.66 | 28.34 | 74.0 | 3.33 | 20.67 | 75 | 0.0 | 25.0 |
| Uninfested control | 3.33 | 0.0 | 96.67 | 0.0 | 0.0 | 100 | 6.66 | 0.0 | 93.34 |
| L.S.D. 5% | 16.22 | N.S | 21.25 | 20.46 | N.S | 24.71 | 15.42 | N.S | 11.08 |

Five bioagent gave slight reduction in the pre-emergence damping-off caused by *R. solani* in chickpea seedlings. *T. harzianum* was the best bioagent against *R. solani* in chickpea seedlings (35%) while the infested control was 75%. There were no significant differences between infested control and all treatment in post-emergence damping-off caused by any of the tested fungi. These results are in agreement with these reported by Okhovvat and Karampour (1996). They used the antagonistic fungi *including T. harzianum, T. viride* and *T. koningii* to control chickpea root rot caused by *F. solani.* The results showed that the antagonistic fungi decreased root rot by 40, 56, 69% respectively. Similar results were obtained by other researchers. Hervas *et al.,* (1998) reported that chickpea cultivars were treated with either *B. subtilis*, Bacillus sp., non-pathogenic *F. oxysporum* or *T. harzianum* prior to inoculation with *F. oxysporum f.* sp. *ciceris.* They found that protection from Fusarium wilt varied among the chickpea cultivars. Also Landa *et al.* (1997) determined the antagonistic activity of 74 bacterial isolates obtained from the rhizosphere of chickpea plants against *F. oxysporum f.* sp. *ceceris.* The antagonistic Bacillus sp. isolates suppressed disease caused by the highly virulent strain of *F. oxysporum f.* sp. *ceceris*. Cook (1993) reported that there are three strategies in considering biological control with introduced microorganism: (a), reduction of the

population of the pathogen (b) prevent the pathogen from infesting the plant and (c) limit disease development after infection.

Therefor the prime purpose of biological control of damping -off disease was not only to raise the plant growth and yield, but also to reduce density of soil-borne pathogens and find the ways to make biocontrol effective and competitive with chemical seed treatment.

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## المقاومة الحيوية لمرض موت البادرات فى كل من الترمس والحمص

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**مركز البحوث الزراعية - معهد بحوث امراض النباتات- الصبحية- الاسكندرية**

تم اختبار التأثير التضادى لستة من العوامل الحيوية هى عزلتين من باسيلس ساتلس وعزله من ارونياهيربيكولا وثلاث عزلات من الفطر تريكودرما هي تريكودرما هارزيانم، تريكودرما قيردى وتريكودرما كوننجي ضد خمسه من الفطريات المسببه لمرض موت البادرات فى الترمس والحمص وهي ماكروفومينا فاسيولينا، فيوزاريوم سولانى فى الترمس وعزلتين من فيوزاريوم سميتيكتم فى الحمص. كما اختبر الفطر ريزوكتوينا سولانى فى كل من الترمس والحمص. واستخدم المبيد الفطرى فيتافاكس كابتان للمقارنه.

ففى الدراسة المعملية وجد ان تريكودرما هارزيانم وتريكودرما كوننجي وعزلة باسيلس ساتلس رقم (١) اعطت اعلى نسبه مئوية للنقص فى النمو الطولى لجميع الفطريات المختبرة.

اما الدراسة فى الاصص فقد اثبتت ان جميع العوامل الحيوية المختبرة قد قللت من موت البادرات فى الترمس نتيجة للاصابة بفطرى فيوزاريوم سولانى وماكروفومينا فاسيولينا وكذلك فى الحمص نتيجة للاصابة بعزلتين من فيوزاريوم سميتيكتم. اما الفطر ريزوكتونيا سولانى فقد كانت استجابته متباينة فى كل من الترمس والحمص . اما المبيد فيتافاكس كابتان فقد اعطى نتائج جيدة فى تقليل الاصابة بموت البادرات فى كلا المحصولين.