BIOIOGICAL CONTROL OF DAMPING-OFF, ROOT-ROT/WILT DISEASES OF ALFALFA IN EGYPT

A.M. HASSANEIN[1], EBTEHAG EL-BAROUGY[1], A.M ELGARHY[1], PAIVI PARIKKA [2] AND T.A. EL-SHARKAWY[1]

1. *Plant Protection Res Inst. Agric Res Center, Cairo, Egypt.*
2. *Plant Pathology, Agric. Res. Center, Jokioinen, Finland.*

(Manuscript received 24 Nov., 1998)

Abstract

Alfalfa (Medicago sativa L.) is subject to stand injury and yield loss from several diseaes, such as damping-off, wilt and root-rot. *Rhizoctonia solani* and *Fusarium oxysporum* are the main pathogens of these diseases. There are great efforts to reduce environmental pollution by reducing the usage of agrochemicals to control pests. Biological control of many diseases is used as a mean to increase forage production. Some bioagents [*Trichodermaharzianum, T.viride, Bacillus subtilis* and Mycostop (*Streptomyces grisioviridis*)] significantly inhibited mycelial growth of *Rhizoctonia solani)]* and *Fusarium oxysporum in vitro*. *Trichoderma harzianum* and *T.viride* overlapped with the pathogens and suppressed the growth by 53 and 48% for *R.solani*, and 66 and 60.6 for *F.oxysporum*. Also, *B.subitilis* and Mycostop expressed inhibitory zone, and inhibited pothogen's by 36.1 and 59.4% for *R.solani* and 46.3 and 72.8% for *F.oxysporum*. Under greenhouse conditions, all tested biocontrol agents significantly decreased damping-off and root-rot/wdt diseases in alfalfa. *T.harzianum, Gliocladium virens* and Mycostop were more effective in controlling damping-off and root-rot/wilt diseases caused by *R.solani* and *F.oxysporum* in alfalfa.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a very important forage crop in many countries and it is one of the most promising forage crops in the newly reclaimed areas in Egypt. (Mohamed *et al.*, 1990). Damping off, root-rot/wilt diseases are the most important diseases affecting the production of legume forage crop in Egypt, causing considerable damge and losses in green matter and seeds. *Rhizoctonia solani and Fusarium oxysporum* are the main pathogens involved in these diseases (Abd El-Aziz, 1970l; Seif El Nasr and Leath, 1983 and El-Gantiry *et al.* 1994).

Many researchers used microorganisms as antagonists to *Fusarium* spp. and *Rhizoctonia solani* as an alternative control method to fungicides (Mitchell, 1973; Kommedahl, 1974; Yehia *et al.* 1982; Tahvonen, 1982 a,b and Tahvonen and Lahdenpera, 1988). The organisms described are mostly bacteria and fungi.

Fungi have been applied to seeds of alfalfa (Gregory *et al.*, 1952). The antagonistic activity of *Trichoderma harzianum* against several fungi, including *R.solani* and *Fusarium* spp was reported by Dennis and Webster, (1971 a,bc,) Sayed *et al.* (1992), El-Gantiry et al. (1994) and Hassanein *et al.* (1996). Elad *et al.* (1982) showed that the ability of *T.harzianum* isolates to control damping-off caused by *Pythium aphanadermatum* was corrolated to the level of hydrolytic enzymes production in soil. Tahvonen (1982a) found that *Streptomyces* spp. and *T.viride* effectively inhibited the growth of a number of soil and seed-borne fungi on a nutrient medium. Treating the peat or seeds with *T.viride* and *Streptomyces* spp isolates inhibited or reduced damping off caused by *R.solani* in cauliflower growing in the peat substrate. Also, *Gliocladium virens*, *T.harzianum* and *Paecillomyces lilacinus* significantly reduced root-rot infection of lentil caused by *Macrophomina phaseolina* and *Fusarium* spp. (Sayed *et al.*, 1992). This work was aimed to study the efficacy of some bioagents in controlling root-rot/wilt diseases of alfalfa *in vitro* and *in vivo*.

MATERIALS AND METHODS

In Vitro

Trichoderma harzianum, T.viride, Bacillus subtilis and Mycostop (Streptomyces griseoviridis) were tested, using PDA medium, as antagonists to *R.solani* and *F.oxysporum*.

Agar disks (5 mm) of antagonistic and pathogenic fungi were cut from the periphery of 7 days old cultures or streak for bacteria and *Streptomyces* were placed opposite to each other, (5cm apart) and incubated at 25±2°C for 7 days (Yeh and Sinclair, 1980). Five Petri dishes were used for each antagonist and the same number was kept as control with the pathogen alone plated on one side of Petri dish.

Inhibition was recorded according to Vincent (1967) and Iqbal *et al.* (1995) and treatments were compared by LSD at the 5% level probability.

In vivo

R.solani, F.oxysporum and *G.virens* were grown on sorghum sand medium (25:75 W/W) for 15 days at 25°C. *T.harzianum* and *T.viride* were grown on wheat bran medium for 7 days at 30°C (Hadar *et al.* 1979), while *B.subtilis* was grown on nutrient broth medium for 48 hr at 25°C. Field soil was sterilized with 5% formalin solution and left to aerate. Sterilized soils were infested with *R.solani* or *F.oxysporum* at the rate of 3% (w/w). *T.harzianum, T.viride* and *G.virens* were applied at the rate of 10 g/kg soil, *B.subtilis* at the rate of 50 ml/pot, and Mycostop

at the rate 50 ml/pot (2g/l). (Hadar *et al*, 1979). Twenty seeds of Cv. Sewa were sown in each pot (30 cm) 7 days after infestation. Each treatment was replicated four times. Pots containing infested soil with only the pathogen and also unifested soil were used as control treatments. Data were recorded after 30,60 and 90 days from planting.

RESULTS AND DISCUSSION

Data in Table (1) show that all tested biocontrol agents significantly reduced the mycelial radial growth of *R.olani* and *F.oxysporum*. The inhibition of growth was relativey more in *F.oxysporum* than in *R.solani*. *T.harzianum* and *T.viride* overlapped with the pathogen and inhibited its growth by 53 and 48% for *R.solani* and 66 and 60.6% for *F.oxysporum*, respectively.

Table 1. *In vitro* effect of antagonistic microorganisms on mycelial radial growth of *R.solani* and *F.oxysporum* on PDA medium.

Treatments (Antagonistic agent)	Mean linear growth (mm)		Reducion (%)	
	R.solani	*F.oxysporum*	*R.solani*	*F.oxysporum*
Trichoderma harzianum	42.3	30.6	53	66
Trichoderma viride	46.8	35.5	48	60.6
Bacillus subtilis	57.5	48.3	361	46.3
Mycostop	36.5	24.5	59.4	72.8
Control	90	90	0.0	0.0
L.S.D. at 1%	0.06	0.88		
5%	1.2	1.2		

Bacillus subtilis and Mycostop (*Streptomyces griseoviridis*) retarded colony growth of the pathogens at a distance by producing a clear inhibitory zone against the pathogens and inhibited their growth by 36.1 and 59.4% for *R.solani* and 46.3 and 72.8% for *F.oxysporum*, respectively.

Generally, Mycostop was the most effective antagonist to radial growth of the two pathogens, followed by *T.harzianum*, *T.viride* and *B.subtilis*, in a descending order.

This antagonistic effect occurred when the antagonist interacted with the pathogenic fungi and suppressed their growth on PDA medium. The antagonistic mi-

croorganisms which caused growth reduction by growing over the pathogen did so by fast growing and better saprophytic activity, with which the pathogen could not compete, resulting in reduced growth of the pathogen (Iqbal and Akhtar, 1987). Micoorganisms which caused reduction in colony growth of the pathogen by producting a clear inhibitory zone may have released toxic metabolites into the medium. (Wright, 1954, 1956 and Akhtar, 1982). Also, Abd El-Moity *et al.* (1992) proved that linear growth of *F.oxysporum* and *R.solani* was greatly affected in the presence of the antagonistic fungus *T.viride*, *T.spiralis* or *B.subtilits.* Trichodermin and peptide antibiotics are reported to be produced by *Trichoderma* spp. These antibioties can inhibit the growth of several fungi (Dennis and Webster, 1971a). Some *Trichoderma* spp. were able to produce acetaldehyde or other acidic volatiles (Dennis and Webester, 1971b). T.harzianum produced volatile inhibitory substances which inhibited the growth of *R.solani* (Wu,1980). Culture filtrate of *T.harzianum* suppressed the mycelial growth of *Fusarium solani* and *F.oxysporum* f.sp *glycine* (Khalifa, 1993).

Trichoderma harzianum, Trichoderma viride, Gliocladium virens and Mycostop (*Streptomyces grisiviridis*) were added singly into soil to study their effect on alfalfa damping-off and root-rot/wilt diseases caused by *R.solani* and *F.oxysporum* under greenhouse conditions. Data in Table (2) show that all biocontrol agents significantly decreased pre and post emergence dampng-off and increased surviving plants compared with the control treatment. Data also indicate that the two pathogens differed in their reaction to the same antagonist. In case of *R.solani, T.harzianum* and Mycostop were the most effective in reducing damping-off of alfalfa, while *B.subtilis* was the least effective. In case of *F.oxysporum, T.harzianum* and *G.virens* were the most effective in controlling disease infection and development and increased percentage of survivals with no significant difference followed by Mycostop. *B.subtilis* was the least effective biocontrol agent. This benificial effect may be due to the fact that *T.harzianum* is able to parasitize many soil-borne pahogens (Dennis and Webster, 1971c and Upadhyay and Mukhopadhyay, 1986). Moreover, *T.harzianum* is able to produce inhibitory substances retarding the growth of patho genic fungi (Dennis and Webster, 1971a and b and Wu, 1980). Essmat *et al.* (1995) reported that *T.harzianum, B.subtilis* (two isolates) and *Streptomyces gresies* significantly reduced pathogenicities of *S.rolfsii* and *R.solani* on Senna plants, with *T.harzianum* being more effective compared with *B.subtilis* and *S.gresies.*

It remains to evaluate the behavior of biocontrol agents tested in the present work under conditions of natural soils in the field with its complexity as far as the

inhabiting microorganisms.

It is expected that the efficacy would vary according to the ability of biocontrol agent to compete; the higher their competitive ability the more effieient they would be expected in providing protection against infection.

Data reported herein, however, indicate the possibility of employing certain biocontrol agents in controlling certain important soil-borne fungi inflicting great damage to alfalfa plants. Therefore, a safer alternative to agricultural chemicals may be made available in an attempt to preseve the ecosystem from chemical pollution and help restore the natural balance in the enviroment.

Table 2. Effect of some biological agents on damping off in alfalfa under greenhouse condition.

Bioagent	*R.solani*			*F.oxysporum*		
	damping off %		Survival Plants %	damping off %		Survival Plants %
	Pre-	Post		Pre-	Post	
T.harzianum	17.5	5.0	77.5	12.5	5.0	82.5
T.viride	20.0	6.2	73.6	17.5	7.5	75.0
G.virens	22.5	8.8	71.3	10.0	3.8	86.3
B.subtilis	29.0	7.5	63.5	22.5	10	67.5
Mycostop	18.5	7.5	74.0	15.2	8.3	76.5
Control	52.5	12.5	35.0	37.5	15.0	47.5
L.S.D at 5%	2.3	4.1	5.4	4.6	2.5	5.0

REFERENCES

1. Abd-El-Aziz, Ferial M.R. 1970. Studies on damping off and root-rot diseases of soybean and lucerne in U.A.R., M.Sc. Thesis, Fac. Agric., Alexandria Univ. 105 pp.

2. Abd-El-Moity, T.H.A.A. Khaled and S.A. Omar 1992. Biological treatments for control root-rot/wilt disease of faba bean in Egypt. J.Appl. Sci., 7 (11): 13-21.

3. Akhtar, C.M.1982. Investigation of the biological control of *Rhizoctonia solani* and *Fusarium oxysporum f.lini* causing root-rot and wilt diseases in cotton and linseed crops, respectively. Final report of linseed project No. PKARS-83, AARI/UA. Faislabad.

4. Brown, M.E. 1974. Seed and root bacterization. Annu. Rev. Phytopathol. 12: 181-197.

5. Dennis, L. and J. Webster. 1971a. Antagonistic properties of specific groups of Trichoderma. 1-production of non-volatile antibioties. Trans. Brit. Mycol. Soc., 57: 25-39.

6. Dennis, L. and J. Webster. 1971b. Antagonistic properties of specific groups of Trichoderma. II production of volatile antibiotics. Trans. Brit. Mycol. Soc., 57: 41-48.

7. Dennis, L. and J. Webster. 1971c. Antagonistic properties of specific groups of Trichoderma. III hyphal interaction. Trans. Brit. Mycol. Soc., 57: 363-369.

8. Elad, Y., I. Chet, and Y. Henis. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can. J. Microbiol., 28: 719-725.

9. El-Gantiry, S.M., R.M. Youssef, M.S. Agha, A.M. Hassanein and A.A. Mahmoud. 1994. Biocontrol of damping-off and wilt diseases of alfalfa. Egypt. J. Appl. Sci., 9 (7): 620-629.

10. Essmat, E.A., A.M. Abo-El-Ela and F.A.F. Mahmoud. 1995. Biological and chemical control of Senna root rot. Egypt. J. Phytophathol., 23 (2): 69-77.

11. Gregory, Y.K.F., O.N. Allen, A.J. Riker and W.H. Peterson. 1952. Antibiotics and antagonistic microorganisms as control agents against damping-off of alfalfa. Phytopathology, 42 : 613-622.

12. Hadar, Y.I. Chet and Y. Henis. 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopathology, 69: 64-68.

13. Hassanein, A.M., G.A. El-Morsy and N.A. Abou-Zeid. 1996. Biological control of damping-off, root rot and wilt diseases of lentil. Proc. Symposium Regional Sur les Maladies des Cereal et des Legumineuess Alimentaires 11-14 Nov., Rabat, Maroc.

14. Iqbal, S.M. and C.M. Akhtar. 1987. Biological control of sugarcane red rot (Colletotrichum falcatum Went.) J. Agric. Res., 25 (2): 195-202.

15. Iqbal, S.M., A. Bakhsh, S. Hussain and B.A. Malik. 1995. Microbial antagonism against *Slerotium rolfsii*, the cause of collar rot of lentil. LENS Newsletter, 22 (1/2): 48-49.

16. Khalifa, E.Z. 1993. Integrated biocontrol of Fusarium spp. on soybean. 6th International congress of Plant Pathology, Montreal Canada, July, 28-Aug., 6, 1993.

17. Kommedahl, T. 1974. Utilization of biological agents other than host resistance for control of plant pathogens. Pages 248-257 In F.G. Maxwell and F.A. Harris, eds. Proc. Summer Inst. Biological control of Plant Insects and Diseases June, 1972. Univ. Press Mississippi. Jackson. Miss. 647 p.

18. Mitchell, J.E. 1973. The mechanisms of biological control of plant diseases. Soil Biol. Biochem., 5: 721-728.

19. Mohamed, N.A., M.K. Ahmed and M.E. Mousa. 1992. Effect of indole acetic acid on the morphology and chemical compostion of alfalfa. Egypt. J.Agric Res., 70 (4): 1151-1175.

20. Sayed, E., R.Y. Hashmi and Abd El-Gaffar. 1992. Biological control of root rot disease of lentil LENS Newsletters, 19 (2): 43-45.

21. Seif El-Nasr, H.I. and Leath. 1983. Crown and root fungal diseases of alfalfa in Egypt. Plant Dis., 65: 509-511.

22. Tahvonen, R. 1982a. The suppresiveness of Finnish light coloured sphagnum peat. J. Sci. Agric. Soc. Fin. 54: 345-356.

23. Tahvonen, R. 1982b. Preliminary experiments into the use of Streptomyces spp. isolated from peat in biological control of soil and seed-borne diseases in peat culture. J. Sci. Agric. Soc. Finl. 54: 357-365.

24. Tahvonen, R. and M.L. Lahdenpera. 1988. Biological control *Botrytis cinerea* and *Rhizoctonia solani* in lettuce by *Streptomyces* sp. Annales Agric. Fenniae, 27: 107-116.

25. Upadhyay, J.P. and A.N. Mukhopadhyay. 1996. Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum* in sugar beet. Tropical Pest Mangment, 32: 215-220.

26. Vincent, J.M. 1967. Distribution of fungal hyphae in the presence of certain inhibtors. Nature, 159.

27. Wright, J.M. 1954. The production of antibiotics in soil. I. Production of gliotoxin by *T.viride*. An. Appl. Biol., 41: 280-289.

28. Wright, J.M. 1956. The production of antibiotic in soil. III Production of gliotoxin in wheat straw buried in soil. An. Appl. Biol., 44: 461-466.

29. Wu, W.S. 1980. Biological and chemical control seed treatment of soybeans. Memoirs of the college of Agriculture, National Taiwan Univ. 20: 1-16.

30. Yeh, C.C. and J.B. Sinclair. 1980. Effect of *Chaetomium cupreum* on seed germination and antagonism to other seed borne fungi of soybean. Plant Dis., 648-470.

31. Yehia, A.H., S.A. El-Hassan and A.H. El-Bahadli. 1982. Biological seed treatment to control Fusarium root rot of broad bean. Egypt. J. Phytophathol., 14 (1-2): 59-66.

المقاومة الحيوية لأمراض موت البادرات وعفن الجذور والذبول في البرسيم الحجازي في مصر

أحمد محمد حسنين[1]، إبتهاج الباروجي[1]، عبد الله الجارحي[1]،
بايفي باريكا[2]، طه أحمد الشرقاوي[1]

١ معهد بحوث أمراض النبات – مركز البحوث الزرعية – الجيزة – مصر.
٢ معهد بحوث وقاية النبات – مركز البحوث الزراعية ـ فنلندا.

يصاب البرسيم الحجازي بالعديد من الأمراض انتي تؤثر علي النباتات القائمة وتسبب خسارة في المحصول، مثل موت البادرات والذبول وعفن الجذور، والتي يسببها الفطران *رايزوكتونيا سولاني*، *فوزاريوم أكسي سبورم*. وهناك الكثير من المحاولات لتقليل تلوث البيئة بواسطة تخفيض إستعمال الكيماويات الزراعية لمقاومة الآفات.

تستخدم المقاومة الحيوية للعديد من الأمراض لزيادة إنتاج الأعلاف. وقد أدي إستخدام بعض العوامل الحيوية مثل *ترايكودرما هارزيانم*، *ترايكودرما فردي وباسيلس ساتليس* والمبيد الحيوي مايكوستوب إلي وقف نمو الميسليوم للفطرين *رايزوكتونيا سولاني وفيوزاريوم اكسي سبورم* علي بيئة أجار البطاطس دكستروز.

تحت ظروف العدوي الصناعية بالصوبة. وجد أن كل العوامل الحيوية المختبرة قللت معنويا موت البادرات وعفن الجدور والدبول في البرسيم الحجازي. وكان الفطر ترايكودرما هارزيانم والفطر جليوكلاديوم فير نز والمبيد الحيوي ميكو استوب أكثر تأثيرا في مقاومة موت البادرات وعفن الجدور والدبول المتسبب عن الفطرين *رايزكتونيا سولاني وفيوزاريوم أكسوسبوريم*.