ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 2, August 2014

Some Control Measures against Lupine Wilt Disease Incidence under Field Conditions

Abdel-Kader, M.M., El-Mougy, N.S., Nadia Gad

Abstract- Abstract- Different control measures, i.e. plant compost and seed treatment with Trichoderma harzianm or cobalt sulphate were applied to study their effect on wilt incidence and yield of lupine plants under field conditions. Results indicate that all applied treatments significantly reduced the disease incidence. The most effective treatment is the seed coated with T. harzianum followed by seed soaked with cobalt sulphate. They recorded in respective order 19.2, 18.0% and 20.7, 19.3% wilt incidence at the first and second growth seasons. Moreover, data also revealed that seed dressing with the fungicide Topsin-M70% showed superior effect on wilt incidence followed by treatment of compost application as soil drench. Similar trend was observed concerning lupine yield.

***Index Terms*- bioagent, cobalt, disease control, compost, lupine, T. harzianum, Topsin-M70%, wilt disease.**

I. INTRODUCTION

One of the appropriate ways of intensifying lupine production is to protect this crop against diseases and pests. Causative agents of lupine diseases are fungi, bacteria, viruses and nematodes, which cause different types of injuries: withering, decay, maculation, blotches, pustules, deformations, chloroses, etc. Losses in yield from diseases quite often reach 25-75% [1]. Protection of plants against numerous diseases and pests by means of agronomic and chemical techniques is sometimes either not efficient enough, or economically unsuitable. When studying disease resistance or selecting methods of analysis of breeding sources for resistance, it is necessary to know and to take into account biological and pathological features of pathogens. The worst damage to lupine is caused by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Lupinei* [2]. The infection first becomes apparent in the vegetative stage as leaves begin to darken. Obvious symptoms begin at budding or flowering time when leaves wilt, desiccate and rapidly defoliate. At this stage, infected roots are almost symptomless except for a brown zone under the epidermis. Brown streaking of the vascular tissue is sometimes visible on the upper stem and, under wet conditions; pink spore masses develop from the vascular streaks. Infected plants eventually die [3]. The existence of races of *F. oxysporum* f. *lupinei* specific to different lupine species emerged from the surveys of [4].

A Ukrainian researcher [5] considers *Aphanomyces euteiches* Drechsler, various fungi of the *Fusarium* genus, and *Verticillium alboatrum* the main agents of lupinee wilt. Fusarium wilt is a limiting factor that reduces lupine yield. This disease is conventionally controlled by chemical fungicides, but as these crops are used for human and animal consumption, chemical fungicides represent hazardous choice for disease control. Instead, other fungicide alternatives, *e.g.* plant resistance inducers, biological control offers a safer alternative way for disease control.

The aim of the present study was designed to evaluate the ability of some control measures against lupine wilt disease incidence under field conditions. Cobalt as seed soaking, the bio-agent *Thrichoderma harzianum* and compost (plant source) as soil drench were used in this experiment.

II. MATERIALS AND METHODS

Source of tested materials and lupine seed treatment

Plant compost was purchased from El- Nile Company, Giza, Egypt. Cobalt sulphate (CoSO4) was purchased from El-Nasr Chemical Company, Cairo, Egypt. In addition 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.

To evaluate the bio-control capability of the candidate organism, the seed coating technique [6] was adopted since this technique was found to be the most suitable means of application. The candidate organisms were grown on PDA medium for an appropriate period of time, then the spores of *T. harzianum* was harvested by flooding the culture with sterile distilled water (SDW). The fungal spore suspension was separately mixed with sterilized Carboxymethyl cellulose (CMC). The mixtures were intermingled with lupine seeds, and left to dry under aseptic conditions at room temperature 23-26ºC.

To evaluate Cobalt as disease resistance inducers, Cobalt (CoSO4.5H2O) was tested as seed soaking at concentration of 1.0 ppm. The lupine seeds were soaked for 12 hours and left to dry then sowed.

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. Evaluation of different types of control measures against wilt disease incidence was carried out for two successive growing seasons. The influence of cobalt (seed soaking), *T. harzianum* (seed dressing) and compost (soil drench) on control of wilt disease of lupine was evaluated. Compost

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 2, August 2014

introduced to the soil at the rate of $20m^3$/feddan ($4200m^2$). The later mentioned treatment was incorporated into the same cultivated row site on the top of 20 cm of the soil surface. 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 Topsin-M70% 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.

All plots were cultivated with Lupine seeds *cv*. Giza 3. The percentage of disease infection was recorded after 30 and 60 days of germination date as wilt incidence of each treatment as well as check treatment (control). The average percentages of disease incidence throughout two growing seasons as well as the obtained yield were calculated.

Statistical analysis

Tukey test for multiple comparisons among means was utilized [7].

III. RESULTS AND DISCUSSION

The activity of soil drench with compost, seed treatment with T. harzianum or cobalt against wilt incidence of lupine was evaluated under field conditions. Results presented in Table (1) indicate that all applied treatments significantly reduced the disease incidence compared with control treatment.

The most effective treatment is seed coating with *T. harzianum* followed by seed soaking with cobalt. They recorded in respective order 19.2, 18.0% and 20.7, 19.3% wilt incidence at the first and second growth seasons. Moreover, data also revealed that seed dressing with the fungicide Topsin-M70% showed superior effect on wilt incidence followed by treatment of compost application as soil drench. They recorded in average throughout the two growing seasons 21.8 and 22.8% disease incidence.

Table 1. Percentage of lupine wilt disease incidence in response to different control measures under field conditions

Treatment	Lupine wilt incidence %		
	First season	Second season	Average
Plant compost (soil drench)	23.1 b	22.4 b	22.8
Cobalt (seed soaking)	20.7 d	19.3 d	20.0
T. harzianum (seed coating)	19.2 e	18.0 e	18.6
Topsin-M 70% (seed dressing)	22.6 c	21.1 c	21.8
Control	32.7 a	30.6 a	31.6

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

Illustrated data in Fig. (1) showed that wilt disease incidence reduced by 41.1 and 36.7% at applied seed coating with *T. harzianum* and seed soaking with cobalt treatments. Also, 31.0 and 27.8% disease reduction was recorded at treatments of Topsin-M70% and plant compost application as seed dressing and soil drench, respectively.

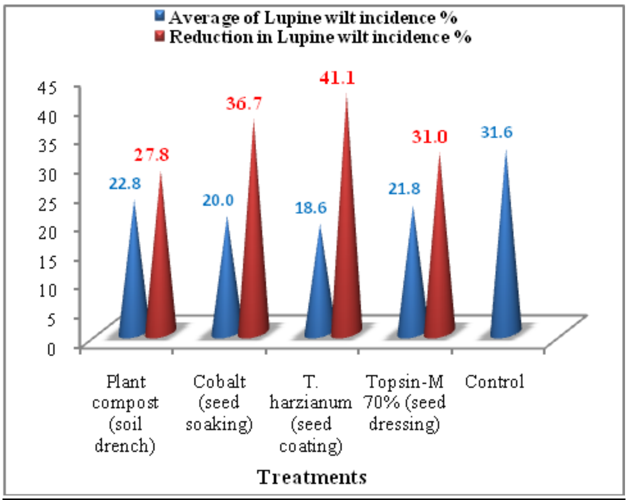

Fig. 1. Average lupine wilt disease incidence and its reduction in response to different control measures under field conditions

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 seed treatment with either T. harzianum and cobalt as well as soil drench with plant compost showed higher yield production than fungicide seed treatment. Highly effective treatments which reflect in the obtained lupine yield and its increase were seed coated with T. harzianum which recorded yield of 6.3 Kg/m2 with increase of 65.7% over the control treatment, followed by treatment of seed soaking with cobalt and soil drench with plant compost which recorded yield of 5.9 and 5.7 Kg/m2 with increase as 55.2 and 50.0%, respectively. Meanwhile, seed dressing with the fungicide Topsin-M70%, caused a yield increase estimated as 23.7% over the check control treatment.

Table 2. lupine yield in response to different control measures under field conditions

Treatment	Lupine yield Kg/plot		
	First season	Second season	Average
Plant compost (soil drench)	5.6 c	5.8 c	5.7
Cobalt (seed soaking)	5.8 c	6.0 b	5.9
T. harzianum (seed coating)	6.2 b	6.4 b	6.3
Topsin-M 70% (seed dressing)	4.6 d	4.8 d	4.7
Control	3.7 a	3.9 a	3.8

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

Since the use of pesticides is one of the environmental pollution factors, therefore there is an increasing interest in obtaining alternative antimicrobial agents for use in plant disease control systems. The present work proves that the evaluated control measures have potential and could be useful against lupine wilt fungal pathogen. From the earlier reports [8,9] it is evident that some of the plant or animal wastes have antifungal compounds which do have the capacity to inhibit the fungal pathogens. Moreover, a number of investigations have demonstrated the effectiveness of composts of various origins in suppressing soil-borne plant pathogens [10,11,12], and their application to soil has been proposed to control many different diseases.

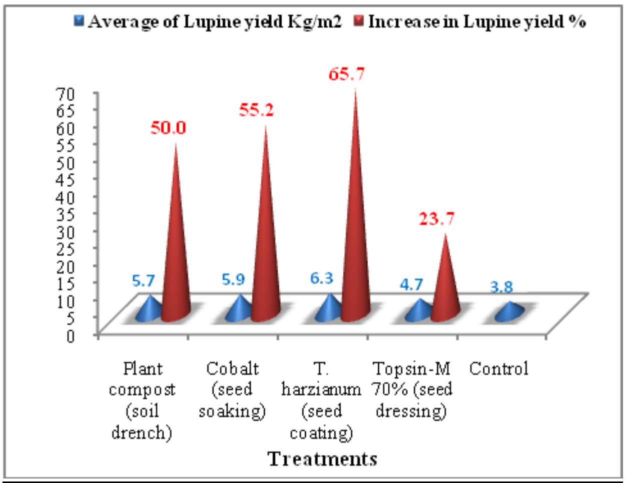

Fig. 2. Lupine yield in response to different control measures under field conditions

Also, 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 [13,14,15]. 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. In the present study the introduction of the bioagent *T. harzianum* as seed coating increased the efficacy of against wilt incidence under field conditions. Similar results were reported by [16]. 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 [17,18,19,20,21].

Microelements might play a positive role for stimulating natural defense mechanisms in plants. This phenomenon was observed in the present work when lupine seeds were soaked in cobalt sulphate which reflected in reduction in wilt disease incidence. Many investigators have explained the role of cobalt in enhancing the inducer resistance in plant [22,23]. They stated that, cobalt can be activated a new proteins, as chitinase and/or other pathogenesis-related proteins and activated many of enzymes which have a role in disease resistance. Moreover, cobalt is known to promote many processes of plant growth including leaf expansion, stem and. root elongation [24]. Using cobalt chloride ($CoCl_2$) at 1&2mg/l and calcium chloride ($CaCl_2$) at 8mM were the most superior treatments for minimizing incidence of charcoal rot (%) of sesame plants caused by *Macrophomina phaseolina* under greenhouse and field trials [25]. They added that cobalt chloride ($CoCl_2$) at 1&2mg/l, calcium chloride ($CaCl_2$) at 8mM and ferrous sulphate ($FeSO_4$) at 8mM were the most effective mineral salts for stimulating natural defense in sesame plants. Also, it was reported that three concentrations of cobalt Co++ 0.25, 0.5 and 1.0 ppm as inducer chemical were used as soaking treatment against wilt disease of watermelon. The most effective concentration was 0.5 ppm [26,27]. The role of Co++ as an inducer is due to inhibiting the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. This conclusion was supported by the observation that when Co++ was administered to IAA- treated tissue, endogenous ACC accumulated while ethylene production declined [28]. Also, treatment with Co++ led to an increase in enzymatic activity of peroxidase, polyphenol oxidase and β 1-3 glucanase. The increase of enzyme activity was correlated with increased formation of papillae in epidermal cells [26, 27,29,30]. Furthermore, in field experiment [31] studied the effect of Molybdenum (Mo) or Cobalt (Co) on lentil pathogens (*Rhizoctonia solani* and *Fusarium oxysporum*). They found that Mo and Co treatments showed significant decrease in pre and post-emergence damping off as well as dead plants (resulted from root rot and wilt). They suggested that soaking lentil seeds in Mo or Co element at 2 ppm is recommended to be incorporated into the production program of lentil to decrease root rot and wilt diseases as well as improve growth and productivity.

Such applied means in the present study 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 such crops. On the light of the obtained results in the present work it may be concluded that soil application of compost or seed dressing with the bioagent *T. harzianum* or cobalt considered an applicable, safe and cost-effective methods for controlling such soil borne diseases.

REFERENCES

[1] Allen, O.N. and Allen, E. K. 1981, The Leguminosae: A Source Book of Characteristics, Uses and Nodulation. Macmillan, London, 250p.

[2] Zian, A.H. 2005, Studies on lupinee root rot and wilt disease. M.Sc. Thesis, Faculty of Agriculture, Suez Canal University, Egypt.

[3] Kurlovich, B.S.; Rep'ev, S.I.; Shchelko, L.G.; Budanova, V.I.; Petrova, M.V.; Buravtseva, T.V.; Stankevich, A.K.; Kartuzova, L.T.; Alexandrova, T.G.; Teplyakova and T.E. and Malysh, L.K. 1995, Theoretical basis of plant breeding. Vol.111. The gene bank and breeding of grain legumes (lupinee, vetch, soya and bean), St. Petersburg, VIR, 438p.

[4] Richter, H. 1941, Lupinee fusariosen. Review of Applied Mycology 23. P.110.Ride, J.P. 1992, Recognition signals and initiation of host responses controlling basis incompatibility between fungi and plants. Society for Experimental Biology Seminar Series 48: Perspectives in Plant Cell Recognition. J.A. Collow and J.R. Green (Eds.), Cambridge University Press, pp. 213-37.

[5] Arafa, M.K.M.; Hassan, M.H.A.; Botro, S.A. and El-Gantiry, S.M.M. 2002, fungal diseases of lupine – species of pathogenic fungi, their occurrence and control recommendations. Assiut Journal of Agricultural Sciences, 33(4): 67–79.

[6] Taylor A.G.; Harman, G.E. and Nielson, P.A. 1994, Biological seed treatments using Trichoderma harzianum for horticultural crops. Horticulture Technology, 4:105–108.

[7] Neler, J.; Wassermann, W. and Kutner, M.H. 1985, Applied linear statistical models. Regression, analysis of variance and experimental design: 2nd ed. Richard, D. Irwin Inc. Homewood, Illinois.

[8] Litterick, A.; Harrier, M.; Wallace, P.; Watson, C.A. and Wood, M., 2004, The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production—a review. Crit. Rev. Plant Sci., 23: 453–479.

[9] Pascual, J.A.; Garcia, C.; Hernandez, T.; Lerma, S. and Lynch, J.M. 2002, Effectiveness of municipal waste compost and its humic fraction in suppressing Pythium ultimum. Microbial. Ecol., 44: 59–68.

[10] Hoitink, H.A.J. and Fahy, P.C. 1986, Basis for the control of soil-borne plant pathogens with composts. Annu. Rev. Phytopathol. 24: 93–114.

[11] Serra-Wittling, C.; Houot, S. and Alabouvette, C. 1996, Increased soil suppressiveness to fusarium wilt of flax after addition of municipal solid waste compost. Soil Biol. Biochem., 28: 1207–1214.

[12] El-Marsy, M.H.; Khalil, A.I.; Hassouna, M.S. and Ibrahim, H.A.H. 2002, In situ and in vitro suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. World J. Microbiol. Biotechnol., 18: 551–558.

[13] Huang, A. 1991, Control of soil borne crop diseases by soil amendments. Plant Protection Bulten Taipel 33: 113-123.

[14] Yogen, A.; Raviv, M.; Hadar, Y.; Cohen, R. and Katan, J. 2006, Plant waste based composts suppressive to diseases caused by pathogenic Fusarium oxysporum. Eur. J. Plant Pathology, 116:267-276.

[15] Khalil, I. and El-Mghrabia, K. 2010, Biological control of Fusarium dry rot and other potato tuber diseases using Pseudomonanas Fluorescens and Enterobacter cloacae. Biological Control, 53(3): 280-284.

[16] Abdel-Kader, M.M. 1997, Field application of Trichoderma harzianum as biocide for control of bean root rot disease. Egypt. J. Phytopathol., 25: 19–25.

[17] Sivan, A. 1987, Biological control of Fusarium crown rot of tomato by Trichoderma harzianum under field conditions. Plant Dis., 71: 587–592

[18] Chao, W.L.; Nelson, E.B.; Harman, G.E. and Hoch, H.C. 1986, Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology, 76: 60–65.

[19] El-Mougy, N.S. 2001, Field application of certain biological and chemical approaches on controlling Bean wilt disease. Egypt. J. Phytopathol., 29: 69–78.

[20] Wright, B.; Rowse, H.R. and Whipps J.M. 2003. Application of beneficial microorganisms to seeds during drum priming. Biocontrol Sci. Technol., 13: 599–614.

[21] El-Mougy, N.S. and Abdel-Kader, M.M. 2008, Long term activity of bio-priming seed treatment for biological control of faba bean root rot pathogens. Aust. Plant Pathol., 37 (5): 464–471.

[22] Zaky, W.H.; El-Sherbieny, S.N. and Mosa, A.A. 2002, Induced resistance of spearmint plant against rust disease caused by Puccinia menthae. Ann. Agric. Sci., Ain Shams Univ., Cairo (Egypt), 47 (1): 417-429.

[23] Morsy, K.M.M. 2005, Induced resistance against damping-off root rot and wilt diseases of lentil. Egypt. J. Phytopathol., 33 (2): 53-63.

[24] Atta, U.A.; Shehata, N.G. and Kobbia, T.U. 1991, Effect of cobalt on tomato plant growth and mineral content. Ann. Agric. Sci. Ain Shams Univ., Cairo, Egypt, 36: 617-624.

[25] Abdel-Aal1, A.E.; Abd-El-Kader, D.A; Khedr, M.A. and Khalifa, M.M. 2012, Induction of resistance in sesame plants against charcoal rot disease by some chemical inducers. Zagazig J. Agric. Res., Vol. 39 No. (2): 189-202.

[26] Abd-El-Kareem, F.; Ashour, W.E.; Diab, M.M. and Aly, M.M. 1993, Induction of resistance in watermelon plants against Fusarium wilt using biotic and chemical inducers. 5th Nat. Conf. of Pests and Dis. of Veg. and Fruits, Ismailia, Egypt, pp. 447-455.

[27] Mazen, M.M. 2004, Resistance induction against diseases of faba bean crop. Ph.D. Thesis, Fac. Agric., Suez Canal Univ., pp.196.

[28] Yeong, B.Y. and Yang, S.F. 1979, Auxin-induced ethylene production and its inhibition by aminoethoxyvinyl-glycine and cobalt ion. Plant Physiol., 64: 1074-1077.

[29] Aly, M.M; Ashour, W.E; Diab, M.M. and Abd-El-Kareem, F. 1993, Induced resistance to Fusarium wilt of watermelon under field conditions. 5th Nat. Conf. of Pests and Dis. of Veg. and Fruits, Ismailia, Egypt, pp. 41-445.

[30] Schneider, S. and Vllrich, W.R. 1994, Differential induction of resistance and enhanced enzymes activities in cucumber and tobacco caused by treatment with various a biotic inducers. Physiol. and Mol. Plant Pathol., 45: 291-304.

[31] El-Hersh, M.S.; Abd El-Hai, K.M. and Ghanem, K.M. 2011, Efficiency of Molybdenum and Cobalt Elements on the Lentil Pathogens and Nitrogen Fixation. Asian Journal of Plant Pathology, 5: 102-114.

AUTHOR BIOGRAPHY

First Author: Department of Plant Pathology, National Research Centre – Egypt, Email: mokh_nrc@yahoo.com.

Second Author: Department of Plant Pathology, National Research Centre – Egypt, Email: nehal_nrc@yahoo.com

Third Author: Department of Plant Nutrition and Soils & Water use, National Research Centre – Egypt, Email: drnadiagad@yahoo.com