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Control of Pythium Damping-off of Squash (*Cucurbita pepo*) by Seed Treatment with Crop Straw and Soil by the Biocontrol Agent *Trichodema harzianum*

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Abstract: Seed treatment by non-sterilized powdered straw from 4 crops was tested for Pythium damping-off of squash. The tested straws including wheat, faba bean, soybean and sorghum were effective in controlling the disease in soil artificially infested with *Pythium ultinum*. Sterilizing straws eliminated the efficacy of these straw, faba bean and soybean straws inhibit the mycelial growth of *Pythium* more than wheat and sorghum on potato dextrose agar when the straws were mixed with sterilized distilled water and left to ferment for 3 days. The soil treatment by the biocontrol agent *T. harzianum* control the damping-off disease in artificially infested soil and combination between seed treatment by straw powder and soil treatment by *T. harzianum* improve the efficacy of the biocontrol agent.

Key word: Squash (*Cucurbita pepo*), crop straw power, *Phythium* damping off

INTRODUCTION

Squash (*Cucurbita pepo*) is susceptible to the damping-off pathogen *Pythium* spp. *Pythium ultimum* is wide spread in soil and has a wide host range (Martin and Loper, 1999). Although fungicide seed treatment remains until nowadays still the main control method for the disease, increasing evidence suggests that seeds or soil treatment with biocontrol agents could be viable alternative. For example, coating seeds with bacterial agents such as *Pseudmonas fluorescens* or *Bacillus subtilis* was effective for the control of different seed and root diseases (Campo *et al*., 1994; Pizzinatto and Freitas, 1996; Bardin and Huang, 2003). Also the same results were obtained when the infested soil was treated by the antagonistic fungi such as *T. haezianum* and *Gliocladiune virens* (Inglis and Kawchuk, 2002; Ding *et al*., 2003; Roberts *et al*., 2005).

Plant residues have been used as amendment to increase soil organic matter and improve plant growth (Huang and Huang, 1993). Organic soil amendment may induce chemical and physical changes that also affect soil microflora (Gamliel *et al*., 2000; Pascual *et al*., 2000; Lazarovite, 2001; Panagouleas *et al*., 2003; Yulianti *et al*., 2006). In addition some organic matter can stimulate the production of lytic enzymes involved in the degradation of plant pathogens. Degradation of *Pythium ultimum* cell walls was accomplished by B-glucanases and cellulose enzymes (Thrane *et al*., 1997; Inglis and Kawchuk, 2002). The presence of organic substances rich in cellulose was also shown to stimulate growth of organisms with cellulose activity. Microbial degradation of plant residues cane also produce secondary products with antifungal activity, for example degradation of cruciferous plants resulted in the production of sulfur containing compounds with antifungal properties (Mayton *et al*., 1996; Ouf *et al*., 2004 and Yalienti *et al*., 2005). Also degradation of agonic residues containing nitrogen release volatile ammonia that reduce the survival of certain pathogenic fungi (Lazarovite, 2001 and Bardin *et al*., 2004). The aim of this study was to find out the crop straw powders effective for control (Pythium damping-off of squash when applied as seed treatment. Also the efficacy of soil treatment by the antagonistic fungus *T. harzianum* with the crop straw powders was also investigated.

MATERIALS AND METHODS

Control of damping-off of squash by seed treatment with crop straw powders: Plant straws from wheat (*Triticum aestivum*); faba bean (*Vicia faba*); soybean (*Glycine max*) and sorghum (*Sorghum vulgare*) were collected after harvest, dried at 50°C oven for one week and ground to powder. Squash seeds were coated with each straw powder by the following procedure; the seeds were soaked for 15 min in 1% methyl cellulose solution (Bardin and Huang, 2003) at a concentration of 3 mL per 10 g seeds. The seeds were then removed and placed in a plastic bag containing 2 g straw powder per 20 g seeds. The bag was inflated with air and shaken vigorously. This experiment was repeated again but with sterilized straw powder. Protection of squash seedlings from pythium damping off disease by straw powder was tested by planting the coated seeds in potting soil infested artificially with *P. ultimum* in wheat bran preparation. The pathogenic fungi was isolated from diseased squash seedlings (Al-Sarhance, 2003). After 3 weeks from planting the percentage of healthy seedlings were estimated. *T. harzianum* was kindly obtained from Faculty of Agriculture, King Saud University.

Control of damping-off disease in squash seedling by soil treatment with *T. harzianum* with on without seeds treatment with crop straw. A Similar experiment was conducted in which soil was artificially infested with 3% inoculum level of *T. harzianum* and 30% *P. ultimum* before planting by sterilized squash seeds or seeds treated by straw powders.

Effect of straw powders on the radial growth of *P. ultimum* and the total nitrogen content in straws: The powdered straws were tested for the production of volatile substances suppressive to the growth of *P. ultimum* in plate assay. The plate assay consisted in forming a paste of the non sterilized or sterilized straw powder. The paste was made by mixing 2.0 g of each straw powder with about 5 mL sterile distilled water. The paste of each straw was spread on the bottom of each *Petri* dish (9 cm diameter). A disc of 5 mm diameter of *P. ultimum* was taken from the periphery of 5 days old culture and placed at the center of the bottom of another plate containing Potato Dextrose Agar (PDA) medium the lids of the straw-containing and *Pythium*-inoculated plates were removed, the *Pythium* inoculated plate was inverted on the top of the straw containing plate and the two plates were sealed with parafilm. Mycelium growth of *P. ultinum* was measured after 3 days of incubation at 25°C±2 and was compared to the control which was the growth of *P. ultimum* on a PDA plate in the absence of a straw paste. The treatment of each experiment was replicated three times.

The total nitrogen content in each straw powder was determined according to Nagiub *et al*. (1983) .

RESULTS AND DISCUSSION

The data in Table 1 revealed that there was significant decrease in disease incidence by *P. ultimum* when seeds were treated by non-sterilized straw powders especially the straw powder of both soybean and faba bean than of wheat and sorghum. The data also showed that sterile straw powders have low ability to protect squash seedling from *Pythium* damping-off when coated on seeds. Also, when soil was treated by *T. harzianum* there was significant decrease in disease incidence. When seeds were treated with crop straw powder and the soil was treated with *T. harzianum*, this condition was more effective for the control of damping-off disease in squash, than, soil treatment with biocontrol agent alone.

Table 2 showed that the growth of *P. ultimum* on the solid media was reduced in the presence of non-sterilized straw powdered especially these of faba bean and soybean than wheat and sorghum straw. In the case of sterilized powders the rate of inhibition was very low in comparison with growth on sterilized powder. Still faba bean and soybean straw powders were more effective than wheat and sorghum straw.

The data in Table 3 revealed that the amount of total nitrogen in straw powders varied by the kind of the crop. Dicot crops especially leguminosae contains nitrogen more than monocot.

In this investigation the crop straws was used as seed coating material for the protection of squash seeds against *Pythium* damping-off disease the pots experiment revealed that straw powder of faba bean and soybean were effective for the control of damping-off of squash caused by *P. utimum* Previous studies (Huang and Huang, 1993; Gamliel *et al*., 2000) indicated that soil amendments with organic wastes for the control of plant disease often requires high amount of organic matter and thus is not cost-effective. However the present study

Table 1: Control of Pythium damping-off in squash seedling by seed treatments with sterile and non-sterile straw powders with or without the presence of *T. harzianum* as biocontrol agent (% of healthy seedling, Means±SE)

Treatments	Wheat	Faba bean	Soybean	Sorghum
Control (sterile soil±sterile seeds)	91.11+5.88	91.11+5.88	91.11+5.88	91.11+5.88
Sterilized seeds and soil infested with *P. ultimum*	44.45+2.22**	44.45+2.22**	44.45+2.22**	44.45+2.22**
Infested soil with *P. ultimum*+ seeds coated by sterile straw powder	42.22+2.22**	52.22+1.11*	55.55+2.22*	44.45+2.22*
Infested soil with *P. ultimum*+ seeds coated by non-sterile straw powder	71.11+4.44*	75.55+2.22	82.22+2.2	68.89+2.22
Sterile seeds +soil infested with *P. ultimum* and treated by *T. harzianum*	75.55+2.22	75.55+2.22	75.55+2.22	75.55+2.22
Seeds coated by non-sterile straw powders soil infested with *P. ultimum* and treated by *T. harzianum*	77.78+4.45	82.22+2.22	84.45+2.22	71.11+2.22*

*'**Significant at 5 and 1%, respectively

Table 2: Effects of straw powder paste on the radial growth and growth rate of *P. ultimum* (mm/disc, means±SE)

Treatments		Radial growth after 3 days	(%) of Radial growth
	Control	6.58+0.24	100.00
Wheat	Sterilized paste	6.50+0.15	98.38
	Non-sterilized paste	6.10+0.12*	92.71
Faba bean	Sterilized paste	6.2+0.11	94.23
	Non-sterilized paste	4.57+0.19**	69.45
Soybean	Sterilized paste	5.93+0.03*	90.12
	Non-sterilized paste	4.20+0.15*	63.83
Sorghum	Sterilized paste	6.23+0.07	94.68
	Non-sterilized paste	5.87+0.09*	89.21

*Significant at 5% *significant at 1% *'** Significant at 5 and 1%, respectively

Table 3: The total nitrogen content in straw powders of wheat, faba bean, soybean and sorghum as mg N/1 g dry straw (means±SE)

Crop straw	mg N/1 g dry straw
Wheat	87.26+0.91
Faba bean	184.45+4.25
Soybean	191.97+0.92
Sorghum	82.23+4.18

demonstrated that by coating certain types of straw into squash seeds, only very small amount of the straw was needed to protect the seeds from the disease, possibly by changing the chemical properties of the soil at the interface with the seed.

Faba bean and soybean straws were effective in controlling damping off disease in squash, more than coating wheat or sorghum straw powder on squash seeds (Table 1). This might be explained on the basis that both faba bean and soybean non-sterilized straw powders when fermented by the accompanied microorganisms which suppressed the incidence of disease and at the same time the sterilized straws were unable to suppress the disease. This was confirmed by the effects of straw powders paste on the radial growth of *P. ultimum* on PDA media (Table 2). Both faba bean and soybean straw powders contain a large amount of nitrogen than the nitrogen content of wheat and sorghum straw powders (Table 3). It also appears from the results in this study that the antagonistic fungus *T. harzianum* suppressed the damping-off disease in green houses experiments. Many researches suggested that *T. harzianum* can produce lytic enzymes which analyze mycelium of the pathogenic fungi (Viterbo *et al.*, 2004; Wen *et al.*, 2005). Also *T. harzianum* secreted some metabolites as mycotoxins and antibiotics which inhibited the pathogenic fungi found in the rhizrosphere of the plant (Benhamou and Chet, 1993; Pristchepa and Voitka, 1999; Jones, 2002; Harman *et al.*, 2004; Chung *et al.*, 2005). The combination between straw powders and *T. harzianum* improved the efficacy of biological control might be explained on the basis that straw produced volatile ammonia and *T. harzianum* also produced some secondary product which suppressed the growth of the pathogenic fungus *P. ultimum*.

REFERENCES

Al-Sarhance, A.I., 2003. The effect of the extracts of some wild plants from Al-Jouf region on the growth and metabolic activities of the pathogenic fungus that causes damping-off disease in squash (*Cucurbita pepo*) M.Sc. Thesis, Girls College-Jeddah Saudi Arabia.

Bardin, S.D. and H.C. Huang, 2003. Efficacy of stickers for seeds treatment with organic matter or microbial agents for the control of damping-off of sugar beet. Plant Pathol. Bull., 12: 19-26.

Bardin, S.D., H.C. Huang and J.R. Moyer, 2004. Control of pythium damping-off of sugar beet by seed treatment with crop straw powders and a biocontrol agent. Biol. Cont., 29: 453-460.

Benhamou, N. and I. Chet, 1997. Cellular and mechanisms involved in interaction between Trichoderma harzianum and Pythium ultimum. Applied Environ. Microbiol., 36: 2095-2099.

Campo, R., E. Shroder, R.E. Chave and M. Zapata, 1994. Biological control of *Macrophomina phaseolina* (Tassi) Goid on bean (*Phaseolus vulgaris* L.) by rhizobacteria. Fitopathologia Colombiana, 18: 36-41.

Chung, W.C., J.W. Huang and H.C. Huang, 2005. Formulation of soil biofungicide for control of Chinese cabbage (*Barassica chinensis*) caused by *Rhizoctonia solani*. Biological Control, 32: 287-294.

Ding, W.L., H.Z. Cheng and J. Chen, 2003. Presearch on preventing the medicinal plant disease with *Trichoderma harzianum* preparation. Zhongguo Zhong Yao Za Zhi, 28: 24-27.

Gamliel, A., M. Austerrveil and G. Kritzman, 2000. Non-chemical approach to soilborne pest management-organic amendments. Crop Prot., 19: 847-853.

Harman, G.E., C.R Howell, A. Viterbo, I. Chet and M. Lorito, 2004. *Trichoderma* species opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol., 2: 43-56.

Huang, H.C. and J.W. Huang, 1993. Prospects for control of soil borne plant pathogens by soil amendment. Curr. Top. Bot. Res., 1: 223-235.

Inglis, G.D. and L.M. Kawchuk, 2002. Comparative degradation of oomycete, ascomycete and basidiomycete cell walls mycoparasitic and biocontrol fungi. Can. J. Microbial., 48: 60-70.

Jones, R.W., 2002. Expression of an antifungal peptide in *Saccharomyces*: A new approach for biological control of the postharvest disease caused by *Colleotrichum coccodes*. Phytopathology, 29: 33-37.

Lazarovite, G., 2001. Management of soil-borne plant pathogen with organic soil amendments a disease control strategy salvaged from the past. Can. J. Plant Pathol., 23: 1-7.

Martin, F.N. and J.E. Loper, 1999. Soil borne plant diseases caused by *Pythium* spp. ecology, epidemiologyand prospects for biological control. Crit. Rev. Plant Sci., 18: 111-181.

Mayton, H.S., C. Olivier, S.F. Vaughn and R. Loria, 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology, 86: 267-271.

Nagiub, M.I., A.A. Hamed and N.Z. Haikal, 1983. Effect of tin on nitrogen metabolism of *Cunninghamella echinulata* and *Fusarium oxysporum* under various cultural conditions. Fac. Sci. Bull. Cairo Univ., 3: 113-128.

Ouf, S.A., N.S. Gewelly and N.A. Tharwat, 2004. Interaction of cruciferous amendments with bacteria antagonist on pathogenicity and some metabolic activities of *Pythium irregulare*. J. Arab. Biol., 13: 69-87.

Panagouleas, C., H. Skaltsa, D. Lazari, A.L. Skaltsow and M. Soklvic, 2003. Antifungal activity of secondary metabolities of *Centaurea raphanin* growing with in Greece. Pharm. Biol., 41: 266-270.

Pascual, J.A., T. Hermandez, C. Garcia, F.A.A.M. De Leij and J.M. Lynch, 2000. Long-term suppression of *Pythium ultimum* in arid soil using fresh and composted municipal wastes. Boil. Fertil. Soils, 30: 478-484.

Pizzinatto, M.A. and S.S. Freitas, 1996. Effect of *Fluorescent Pseudomonas* spp. on in seed health and germination and on cotton plant growth. Phytopathologia, 22: 9-14.

Pristchepa, L.I. and D.V. Voitka, 1999. Effectiveness of trichodermin in protection of tomato and cucumber against disease. Bull. Polish Acad. Sci. Biol. Sci., 47: 179-182.

Roberts, D.P., S.M. Lohrke, S.L.F. Meyer, J.S. Buyer and J.H. Bowers, 2005. Biocontrol agents applied individually and in combination for suppression of soilborne diseases of cucumber. Crop Prot., 24: 141-155.

Thrane, C., A. Tronsmo and D. Funk Jensen, 1997. Endo-1, 3-B-glucanase and cellulase from *Trichoderma harzianum* purification and partial characterization, induction of and biological activity against plant pathogenic *Pythium ultimum*. Phytopathology, 70: 730-733.

Viterbo, A., M. Harel and I. Chet, 2004. Isolation of two aspartyl proteases from *Trichoderma asperellum* expressed during colonization of cucumber roots. FEMS Microbiol. Lett., 238: 151-158.

Wen, Z., W. Liao and S. Chen, 2005. Production of cellulase by *Trichoderma reesei* from dairy manure. Bioresource Technol., 96: 491-499.

Yulianti, Y., K. Sivasithamparam and D.W. Turner, 2006. Saprophytic growth of *Rhizoctonia solani* Kühn in soil amended with fresh green manures affects the severity of damping-off in canola. Soil Biol. Biochem., 38: 923-930.