INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2007/09–3–459–461
http://www.fspublishers.org

Improving Biological Control of *Fusarium* Root-rot in Cucumber (*Cucumis sativus* L.) by Allelopathic Plant Extracts

NAHED Z. HAIKAL
Botany Department, Faculty of Science, Cairo University, Giza 12613, Egypt
Corresponding author's e-mail: tarekmoussa@yahoo.com

ABSTRACT

The use of allelopathic plant extracts to improve the effectiveness of biological control agent (*Trichoderma harzianum*) is being popularized in recent years. The present work was designed to evaluate the potential of aqueous extracts of three plants in Saudi Arabia (*Azadirachta indica; Ziziphus spina-christi & Zygophylum coccineum*) against the pathogenic fungus *Fusarium solanmi*, the causal agent of root-rot in cucumber seedling. The fungus was grown in 100 mL liquid sabouraud dextrose medium containing 0.0, 5.0, 10.0, 15.0, 20.0 and 25% leaves extracts of the three plants. Fungal growth was estimated after 4 and 8 days of incubation. A high response was exhibited by the test pathogen to employed extract treatment in all concentrations resulting in a pronounced decrease in fungal biomass production when the seeds of *C. sativus* soaked in the 15% extracts concentration and sown in soil artificially infested by *F. solani* alone or with *F. solani* and *T. harzianum* the incidence of root-rot disease in seedling decreased.

Key Words: Biological control; Root-rot; Plant extracts

INTRODUCTION

The soil borne pathogens, *Rhizoctonia solani*; *Pythuim ultimum* and *Fusarium* spp., can cause severe economic losses to field and greenhouse grown cucumber (Kaulizakis, 1997; Roberts *et al*., 2005). To avoid the implication of yield losses due to plant disease, variety of control methods are used. In addition to physical methods such as sunlight or UV radiation (Spadaro & Gullino, 2005) the use of chemical compounds toxic to fungi is the most commonly known means of controlling fungal disease in field and greenhouses (Washington & McGee, 2000; Fravel *et al*., 2005). Although this method has been very effective in controlling plant fungal disease, some major problems threaten to limit the continued use of fungicides. Firstly some fungi have developed resistance to chemicals; secondly some fungicides are not readily biodegradable and tend to persist for years in environment. This leads to a third problem, the detrimental effects of chemicals on organisms other than target fungi (Brady, 1984). Because of these associated problems, researchers are now trying to use environmentally safe alternative methods of fungal control as biological control or aqueous extract of many allelopathic plants. Allelochemicals reduce the germination of spores and mycelial growth of pathogenic fungi (Jayashree *et al*., 2001; Sarovenan & Marinuthu, 2003; Yulianti *et al*., 2006). Studies have been carried out to screen different plants for their antifungal and antimicrobial properties against different plant pathogens. Among these, Neem (*Azadirachta indica*) is important. It contains compound callerd limonoids, which possess antifungal activities (Loke, 1990; Kazmi *et al*., 1993). Bajwa *et al.* (2001) have observed that the aqueous extracts of *Dicanthium annulatum, Imperata cylindrical, Cenchrus pennistiformis* and *Desmostackya bipennata* have potential to control *Fusarium moniliforme* and *F. oxysporum*.

The objectives of the present study were to evaluate the potential of aqueous extract of aerial parts of *A. indica; Z. spina-christi* and *Z. coccineum* to control the pathogenic fungus *F. solani* and to use the extracts in combination with the bio-control agent *T. harzianum* to improve the efficacy of biological control of cucumber root-rot.

MATERIALS AND METHODS

Fresh healthy leaves of *A. indica, Z. spina-chrisli* and *Z. coccineum* grown in Jeddah were collected and washed thoroughly under running tap water, dried with blotting paper. A 100% (w/v) stock solutions was obtained by soaking the crushed leaves in sterilized water for 48 h at room temperature (Bajwa *et al*., 2003), filtered through Whatman filter paper and finally bacterial filter and stored at 5°C. Different concentrations were prepared by adding appropriate quantities of liquid media to stock solution.

Aqueous extract bioassays were carried out in liquid medium (Sabouraud dextrose) containing 10 g peptone and 40 g glucose per 1000 mL distilled water. Inoculum discs of 5 mm diameter, obtained from the belifery of six days old fungal culture of *F. solani* was transferred to flasks aseptically. The flasks were incubated at 25 $\pm$ 2°C for 8 days in which the dry weight of biomass yield was estimated at four days intervals. The mycelial biomases from triplicates samples for each treatment was collected on pre-weighed filter papers. The dry weight yield of mycelial

biomass was determined after 24 h oven drying at 70°C.

Pot experiments. Pathogen inoculant (3%) was mixed together with sterile vermiculite in each pot. Surface sterile cucumber seeds were sown in each pot and pots were wetted with 100 mL distilled water containing 25% leaf extract as a drench. There were 2 controls one of them contained only sterile seeds sown in sterile soil, and the other contained sterile seeds in soil drenched by 25% leaves extract. Another pot experiment was carried out in which biological agent *T. harzianum* was mixed in potting soil at 3% inoculum level with 3% pathogenic *F. solani* inoculum and the sterile seeds of cucumber were sown, some pots were irrigated by distilled water and the other were irrigated by 25% leaves extract. The number of diseased seedlings was counted after three weeks of planting, and length, fresh and dry weight of healthy seedlings were determined.

***F. solani* or *T. harzianum* inoculum.** The inoculum was produced according to the method of Sivan *et al*. (1984). A 25 g of crushed barley seeds was added to 75 g of vermiculite and wetted by about 10 mL distilled water in 500 mL conical flasks and autoclaved for 15 min at 121°C. The flasks were inoculated by 5 mm discs of *F. solani* or *T. harzianum* and incubated for 15 days at 25 ± 2°C. This preparation was added to the soil at the level 3.0% (w/w) inoculum before sowing 15 cucumber seeds in each pot.

RESULTS

All concentrations of the tested plants were found to be inhibitory to fungal growth and the rate of inhibition increased gradually by increasing the concentration (Table I). At 25% concentration, the percent inhibition was 82.28; 79.15 and 76.95% in the presence of extracts of *A. indica*, *Z. spina-christi* and *Z. coccineum*, respectively in comparison with the control treatment at the end of incubation period. Aqueous extract of *A. indica* was most effective than extracts of *Z. spina-christi* or *Z. coccineum*.

Table II shows the control of *Fusarium* root-rot in cucumber seedlings by the aqueous extracts or by *T. harzianum.* The use of the three aqueous extracts alone or in combination with 3.0% *T. harzianum* were effective in reducing the incidence of the disease especially in the case of *A. indica* with *T. harzianum*. The percentage of disease incidence in the presence of 25% aqueous extracts of *A. indica*; *Z. spina-christi* and *Z. occinum* were 14.63, 21.95 and 24.39%, respectively in comparison with 41.46% in the presence of 3.0% *R. solani* only. The disease incidence decreased when the bio-control agent. *T. harzianum* was used in combination with 25% extracts, which was in the order of *A. indica* < *Z. speina-christi* < *Z. coccineum* with *T. harzianum* (2.44, 16.35 & 19.51%, respectively).

The growth parameters of healthy cucumber seedlings were increased by the extracts of the three tested plants either alone on in combination with the antagonistic fungus *T. harzimuer*, increased growth parameters of healthy cucumber seedlings.

Table I. Effect of various concentrations of aqueous extracts of *A. indica, Z. spina-christi* and *Z. coccineum* on the dry weight gain by *F. solani* felts at 4-days intervals of growth on liquid media (mg Biomass; Means ± S.E)

Treatments	Concentration (%)	1st 4 days	2nd 4 days	Total 8 days
Control	0.0	48.4.7±44.3	196.3±15.0	681.0±7.4
	5.0	2080.7±22.2••	122.0±31.7•	330.7±6.8•
	10.0	177.7±11.5•	87.3±8.5•	265.0±29.8
A. indica	15.0	145.3±14.5••	62.3±10.9•	207.7±13.7••
	20.0	116.7±13.9•	49.7±5.2	166.3±23.8••
	25.0	82.0±6.3•	38.7±6.6	120.7±20.4••
	5.0	231.3±4.1•	143.7±25.7	375.0±27.1
Z. spira	10.0	200.3±21.5	106.3±9.3	306.7±27.1
Christi	15.0	165.0±10.7•	88.0±2.9	253.0±20.9•
	20.0	121.0±19.2••	69.3±2.6•	190.3±11.5••
	25.0	96.7±6.7•	45.3±13.9	142.0±21.6•
	5.0	249.7±7.3	168.0±4.3•	417.6±18.8
Z.	10.0	210.0±18.2	168.0±4.3•	417.6±18.8
coccineum	15.0	178.3±12.1•	89.3±7.5•	267.7±9.9•
	20.0	145.3±15.2	67.0±7.5•	212.3±12.7••
	25.0	105.7±9.3•	51.3±2.0	157.0 ± 22.7

• Significant at 5%; •• significant at 1%

DISCUSSION

The results obtained in the present study revealed that generally aqueous extracts of *A. indica, Z. spina-christi* and *Z. coccineum* reduced the pathogenic fungal biomass production in all the test concentration; many workers using other fungal species and different plants found similar effects. Hussain *et al*. (1992) reported marked suppression in growth of *Helminthosporium turcicum*, *H. maydis* and *H. carbounum* by the aqueous leaves extracts of *Anagalis arvensis*. According to Khan *et al*. (1998) aqueous extracts of *Allium cepa* exerted antifungal activity against *H. turzcicum* and *Atternaria radicina* Recently Devanath *et al*. (2002) and Pavlou and Vakalounakis (2005) reported similar inhibitory effect of the aqueous extracts of some medicinal plants. Gangwar *et al*. (2000), Jayashree *et al*. (2001) and Paul and Sharma (2002) reported that the aqueous extracts of *A. indica* inhibited vigorously the growth of soil borne pathogenic fungi. Data in Table II further revealed a decrease in disease incidence in cucumber seedlings by the aqueous extracts of the three tested plants added to the artificially infested soil by the pathogenic fungus *F. solani* in the presence or absence of the bio-control agent *T. harzianum.* Sarovenan and Marimuthu (2003) reported that *A. indica* has improved the biological control of *F. oxysporum* f. sp. *cubense*, the causal agent of wilt disease in banana seedlings, when mixed with the bio-control agents such as *Pseudomenas fluerescens* or *T. harzianum* and *T. viride*.

Table II. Effect of Plant extracts or *T. harzianum* or the incidence of root-rot disease in cucumber seedlings and on the growth parameters of seedlings (Means±SE)

Treatments	% of healthy seedlings			Length of seedlings cm			Fresh weight mg/seedling			Weight mg/seed		
	A. indica	*Z. spina-christa*	*Z. coccineun*	*A. indica*	*Z .spina-christa*	*Z. coccineun*	*A. indica*	*Z. spina-christa*	*Z. coccineun*	*A. indica*	*Z. spina-christa*	*Z. coccineun*
Control	91.1+2.2	997.3+6.9	997.3+6.9	20.4+0.4	20.1++0.4	20.1+0.4	997.3+6.9	997.3+6.9	997.3+9.6	98.0+2.2	98.0+2.2	98.0+2.2
3% *F. solani*	53.3+6.7••	53.3+6.7••	53.3+6.7••	14.3+0.4••	14.3+0.4••	14.3+0.4••	679.3+16.9••	679.3+16.9••	679.3+16.9••	71.2+5.5•	71.2+5.5•	71.2+5.5•
Strile soil + 25% extracts	94.1+0.7•	91.1+1.3•	88.2••	20.8+0.5••	20.4+0.3	20.4+0.4••	1063.0+32.1	1041.7+43.4•	1005.3+24.6•	101.2+3.1	99.2+4.5	97.7+3.7•
3% *F. solani* + 25% extract	77.8+5.9•	71.1+2.2•	68.9+2.2	18.2+0.4•	17.9+0.3••	18.0+0.6•	837.7+5.4.5	821.0+57.1•	807.7+29.6••	89.5+3.0••	84.3+2.7••	83.0+4.5•
3% *F. solani* + 3% *T. harzianum*	82.2+4.5••	80.2+4.5••	82.2+4.5••	19.7+0.4••	19.7+0.4	19.7+0.4	975.2+46.2	975.2+46.2	975.2+46.2	91.3+1.2	91.3+1.2	91.3+1.2
3% *F.solani* + 3% *T. harzianum* + 25% extract	88.9+2.2	76.2+6.5	73.3+3.9•	20.6+0.5	20.5+0.4•	20.5+0.3•	1032.2+35.0•	1001.3+27.1•	996.7+19.4•	100.8+10.0	96.2+4.5	93.0+6.3

• Significnt at 5%; •• Significant at 1%

Acknowledgements. The author thanks to Dr. Gashgary, Faculty of Girls Education, Jeddah, Saudi Arabia for help in identification the pathogenic fungus *F. solani*, isolated from infected cucumber seedling and the bio-control agent *T. harzianum* isolated from rhizosphere of cucumber seedlings.

REFERENCES

Bajwa, R., N. Akhtar and A. Javaid, 2001. Antifungal activity of allopathic plant extracts 1. Effect of allelopathic plant extracts of three alleopathic *Asteraceous* species on growth of Aspergillii. *Pakistan J. Biol. Sci.*, 4: 503–7

Bajwa, R., A. Khalid and T.S. Cheema, 2003. Antifungal activity of allelopathic plant extracts III: Growth response of some pathogenic fungi to aqueous extracts of *Parthenium hysterophorus. Pakistan J. Pl. Pathol.*, 2: 145–56

Brady, N.C., 1984. *The Nature and Properties of Soils*, P: 528. MacMillan Publishing Company, New York

Devanath, H.K., J.J. Pathank and L.C. Bora, 2002. *In vitro* sensitivity of *Ralstonia solanacearum*, causing bacterial wilt of ginger towards antagonists, plant extracts and chemicals. *J. Interacademicia*, 6: 250–3

Fravel, D.R., K.L. Deahl and J.R. Stommel, 2005. Compatibility of the bio-control fungus *Fusarium oxysporum* strain CS-20 with selected fungicides. *Biol. Control*, 34: 165–9

Gangwar, S.K., S.M. Qadri, M.D. Maji, P.M. Kumar and B. Saratchandra, 2000. Evaluation of fresh plant extracts for the control of mulberry powdery mildew. *Indian J. Sericult*., 39: 76–8

Hussain, I., M.A. Nasir and M.R. Haque, 1992. Effect of different plant extract on brown rust and yield of wheat. *J. Agric. Res.*, 30: 127–31

Jayashree, K., Rajeswari and G. Thiribhvanamala, 2001. Effect of plant extracts on the spore germination of rice blast pathogen. *Madras Agric. J.*, 87: 144–6

Kaulizakis, M., 1997. National Agricultural Foundation, Sub-tropical plant and olive trees. *Instt. China Lab. Pl. Pathol. Agrokipio Chinia Crete Greece*, 4: 383–6

Kazmi, A.R., J. Niaz and G. Jillani, 1993. Evaluation of some plant extracts for antifungal properties. *Pakistan J. Phytopathol*., 5: 93–7

Khan, T.Z., M.A. Gill, M.A. Nasir and S.A.A. Bukhan, 1998. Antifungal properties of some plant extracts. *Pakistan J. Phytopathol.*, 10: 62–5

Loke, J.C., 1990. Activity of extracted neem seed oil against fungal plant pathogens. *In*: *Neem Potential in Pest Management Programmes*, Pp: 132–6. Department Agriculture U.S. Agriculture Research Survey Maryland

Pavlou, G.C. and D.J. Vakalounakis, 2005. Biological control of root and stem rot of greenhouse cucumber caused by *Fusarium oxysponum* f. sp. *Radicis-cucumerinum* by lettuce soil amendments. *Crop Prot*., 24: 135–40

Paul, P.K. and P.D. Sharma, 2002. *Azadizachta indica* leaf extract induced resistance in barley against leaf strip disease. *Physiol. Molec. Pl. Pathol.*, 16: 3–13

Roberts, D.P., S.M. Lohrke, S.L.F. Meyer, J.S. Buyer and J.H. Bowers, 2005. Bio-control agents applied individually and in combination for suppression of soil borne diseases of cucumiber. *Crop Prot.*, 24: 141–55

Sarovenan, M.M. and T. Marimuthu, 2003. Development of integrated approach to manage the fusarial wilt of banana. *Crop Prot*., 22: 1117–23

Sivan, A., Y. Elad and I. Chet, 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium phanidermatum*. *J. Phytopathol*., 74: 498–501

Spadaro, D. and M.L. Gullino, 2005. Improving the efficacy of bio-control agents against soil-borne pathogens. *Crop Prot.*, 24: 601–13

Washington, W.S. and P. McGee, 2000. Dimethomorph soil and seed treatment of potted tomatoes for control of damping off and root rot caused by *Phytophthora nicotianae* var *micotianae*. *Australian Pl. Pathol.*, 29: 46–51

Yulianti, Y., K. Sivasithamparam and D.W. Turner, 2006. Saprophytic growth of *Rhizoctonia solani* kühn in soil amended with fresh green manure affects the severity of damping-off in canola. *Soil Biol. Biochem.*, 38: 923–30

(Received 07 October 2006; Accepted 10 March 2007)