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Phytofungitoxic Properties in the Aqueous Extracts of Some Plants

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Abstract: The effects of aqueous extracts of twenty plants on spore germination and vegetative growth of two pathogenic, terrestrial (*Alternaria solani)* and zoosporic (*Saprolegnia parasitica*) fungal species was examined. The extract of *Eugenia aromatica* completely inhibited the spore germination of *A.solani* and exerted a highly significant depressive effect on the mycelial growth of the two tested species. Also, the extracts of *Allium cepa* (cloves),*Eucalyptus rostrata* (leaves) and *Capsicum frutescens* (fruits) exhibited remarkable inhibitory effects against these two fungi. Some of the tested plant extracts showed a promotive effect either on spore germination or mycelial dry weight. Zoosporic fungus was relatively more sensitive than the terrestrial one.

Key words: Phytofungitoxic, *S. parasitica, A. soloni*

Introduction

Now, quick and effective management of plant diseases and microbial contamination in several agricultural commodities is generally achieved by the use of synthetic pesticides (Agriose,1997). Many pathogenic micro-organisms and insect pests have developed resistance against these chemical pesticides (Williams and Heymann,1998;Witte,1998). The incessant application of chemical pesticides have harmful effects on soil biosphere and create health hazards in human and animals due to their residual toxicity (Anon,1998). Plants have been known for their medicinal and antimicrobial properties since ancient times. For this reason attention has been diverted to an alternative, safe and cheap method for the management of pathogenic micro-organisms (Rice *et al.*,1998). Some plant extracts are hatching inhibitors, alter sporulation and vegetative growth. This investigation was initiated to elucidate the effects of aqueous extracts of various parts of common plants on spore germination and vegetative growth of two pathogenic fungal species: *Alternaria solani* and *Saprolegnia parasitica*.

Materials and Methods

Organisms

***Alternaria solani* (terrestrial fungus causing early blight of tomato):** Diseased tomato fruits were collected in clean plastic bags. Three infected fruits were selected and superficially sterilized using 75% ethyl alcohol. Under aseptic conditions, the sterilized Petri-dishes containing sterilized PDA medium (about 20 ml for each plate) with 100 μ g ml^{-1} streptomycin were inoculated from the diseased spots on the fruits. The plates were incubated at 25 $\pm$ 2°C. The developing colonies were identified (on the basis of morphological and microscopic characeristics) as *Alternaria solani* Sorauer (Ellis, 1971).

***Saprolegnia parasitica* (zoosporic fungus causing Saprolegniasis to fish):** Five fish were suspected to be naturally infected with saprolegniasis were collected. This disease is characterized by the presence of cotton- like, white to gray growth on the skin, fins, gills or eyes of fish (Bohm and Fuhrmann,1984; Singhal *et al.*,1987). Using sesame-seeds water culture and baiting technique (khallil,1990; Ahmed and khallil,1991), the growing fungus was isolated and identified as *Saprolegnia parasitica* Coker (Seymour, 1970).

Plant extracts: Twenty plants were selected (Table 1) to investigate the effect of aqueous extracts of different parts (e.g. leaves, storage roots,fruits, seeds and flower buds) on either spore germination or mycelial growth of the two pathogenic fungal species. The plant parts were thoroughly washed with 2% aqueous sodium hypochlorite and sterile distilled water. Aqueous extracts were prepared by blending 25 g of air–dried plant part in 100 ml sterile distilled water. The filtrate was used as the test extract and kept in refrigerator until used.

Effect of different extracts on spore germination: Spore suspension of *Alternaria solani* was prepared from 7- days old culture growing on PDA medium. Five ml of extractive stocks were added to 20 ml of 1% soil extract agar (1% sterilized soil extract solution and 15 g agar in 1 L distilled water). Soil extract agar without extractives was used as control. Soil extract agar was used in order to present the fungal spores with minimum of added nutrients (Abd El- Hady and Ouf,1993). Three drops of spore suspension were placed on each plate (three replicate plates were set up). The plates were incubated at 25 $\pm$ 2°C for 24 hours after which the percentage germination of spores was recorded.

Effect of some extracts on the mycelial growth: After preliminary screening on spore germination, ten plant extracts were finally selected to study their effects on growth of *Alternaria solani* and *Saprolegnia parasitica*. An aliquot (5 ml) of the tested plant extract was added to 20 ml sterile Sabauroud (for *A. solani*) and glucose- peptone (for *S. parasitica*) liquid media, contained in 100 ml Erlenmeyer flasks. In a control set, 5 ml sterile distilled water was mixed with the same amount of appropriate medium. Flasks were inoculated with mycelial agar disc (0.5 Cm in diameter) prepared from 7- days old culture, and incubated at 28 $\pm$ 2°C (*A. solani*) and 20 $\pm$ 2°C (*S. parasitica*) for ten days. The cultures were shaken at regular intervals. Three replicates were prepared for each treatment. Cultures were filtered and the mycelial dry weights were determined after drying to a constant weight at 60°C.

Results and Discussion

Of the twenty plant extracts tested, *Eugenia aromatica* completely inhibited conidial germination of *A. solani* (Table 1). The extracts of *Allium sativum* (cloves), *Eucalyptus rostrata* (leaves), *Capsicum frutescens* (leaves and fruits), *Ammi visnaga* (seeds), *Capsicum annum* (fruits) and *Allium cepa* (bulb) were also highly effective against the germination of conidia (germination percentage ranged between 6.47 - 44.10%). The remaining tested extracts were either less effective or exerted stimulative effects compared with the control treatment (Table 1). Similarly several reports dealt with the either inhibitory or stimulative effects of some plant

Table 1: Pecentage (%) of conidial germination and mycelial dry weight (mg/ 25 ml media) of *Alternaria solani* and *Saprolegnia parasitica* as affected by aqueous extracts of some plants.

Plant species	Common name	Part	% of spore Germination (*A. solani*)	Mycelial dry weight	
				A. solani	*S. parasitica*
Control			87.5 ± 2.3	1005.7±21.8	714.7±15.2
Allium cepa	Onion	Bulb	55.6± 7.1**	727.3± 25.7**	165.3±13.8**
A. sativum	Garlic	Cloves	6.5± 0.6**	184.3± 17.3**	36.3± 26.6**
Ammi visnaga	Tooth pick	seeds	35.3± 4.5**	604.0± 75.9**	134.3± 16.1**
Brassica rapa	Common turnip	roots	96.8 ± 3.6*	–	–
Cassia fistula	Indian laburnum	Leaves	89.9 ± 4.1		
Capicum annum	Hot peppers	Fruits	23.57 ± 4.2**	615.0±16.6**	74.0± 6.9**
		Leaves	82.9 ± 9.6		
Capsicum frutescens	Bird piper	Fruits	15.2 ± 2.6**	387.99**	82.7.0± 14.5**
		Leaves	44.1 ± 5.4**	–	–
Coriandrum sativum	Coriander	Leaves	84.1 ± 2.1	–	–
Datura stramonium	Thorn-apple	Leaves	69.1± 6.5**		
Daucus carota	Parsnip (Carrot)	Leaves	91.5 ± 1.4	1304.0± 34.2**	846.3±57.8**
		Roots	95.3 ±3.5*		
Eucalyptus rostrata	Eucalyptus	Leaves	10.5 ± 1.2**	346.0± 41.9**	144.7±39.27**
Eugenia aromatica	Clove	Flower bud	0.0**	65.0± 15.4**	19.0± 2.0**
Mentha piperita	Peppermint	Leaves	90.2± 4.6		
Nerium oleander	Oleander	Leaves	71.6 ± 1.4**	728.0± 75.7**	339.0± 36.3**
Petroselinum sativum			90.0 ± 5.9	–	–
Pimpinella anisum	Aniseed	Seeds	62.1 ± 6.3**	–	–
Raphinus sativum	Radish	Roots	93.9 ± 3.7	–	–
Riccinus communis	Castor bean	Leaves	84.8 ± 3.0		
Thymus capitatus	Thyme	Leaves	79.3 ± 5.1*	611.0± 19.16**	196.3±19.3**
L.S.D. at 5%			7.5	66.22	49.10
L.S.D. at 1%			10.1	90.0	66.73

* Significant difference compared with the control ** Highly significant difference compared with the control. - Unexamined

extracts on fungal spore germination . In this respect, Yoshida *et al*. (1987) and Singh *et al*. (1990) reported that garlic extracts and some of its derived compounds (ajoene and allicin) inhibited spore germination of some fungi (e.g. *Alternaria alternata, Colletotrichum sp., Curvularia sp., Candida albicans, Trichophyton mentagrophytes, Microsporum canis, M. gypseum* and *Epidermatophyton floccossum*). On the contrary, Singh *et al*. (1986) reported that exudate and extract of both young and mature leaves of Spinach stimulated conidial germination of *Alternaria alternata, Cladosporium cladosporioides, C. herbarum, Curvularia lunata (Cochliobolus lunatus), Drechslera australiensis (Pseudocochliobolus)* and *Fusarium oxysporum* but in case of *Cercospora beticola* , germination and growth were inhibited by exudates of young leaves. The petroleum ether and other extractives of *Solenostemma argel* are ineffective as a fungitoxicant to spore germination of *Aspergillus candidus* or *Penicillium lanosum* whereas the low concentrations of the other extractives (including water extract) were stimulative to *A. candidus* and suppressive to *P. lanosum* (Edwards and Ayres, 1981). It was reported that accumulation of fungitoxic substances in plant tissues is partly responsible for resistance of such tissues to infection.

Also,it was found that the aqueous extracts of selected plants (except those of Carrot storage roots) showed inhibitory action against the mycelial growth, produced by both tested fungal species when added to the growth medium. The most effective extracts were those obtained from *Eugenia aromitica* (dried flower buds) and *Allium sativum* (cloves). The growth of *A. solani* was suppressed by 93.54 % and 81.67% of the control when treated with two extracts ,respectively (Table 1). *Saprolegnia parasitica* was more sensitive and the mycelial dry weight was reduced by 97.34% and 94.92% of the control, respectively. The extracts of *Eucalyptus rostrata* leaves , *Capsicum frutescens* fruits, *Ammi visnaga* seeds and *Thymus capitatus* leaves were relatively less effective on the mycelial growth of both *A. solani* (inhibition by 39.24% - 65.60% of the control treatment) and *S. parasitica* (inhibition by 72.53% - 88.43% of the control). The mycelial dry weight of *A. solani* was suppressed by 19.76% - 38.85% of the control when treated with the extracts of *Allium cepa* bulb, *Capsicum annum* fruits, *Capsicum fruitescens* leaves and *Nerium oleander* leaves. These extracts exhibited higher inhibitory effects (by 52.57% - 89.65% 0f the control) on *S. parasitica* (Table 1). On the contrary, the storage root extract of *Daucus carota* exerted a promotive effect on the mycelial dry weight of either *A. solani* (129.63% of the control) or *S. parasitica* (118% of the control). Laboratory screening of plant extracts has given encouraging results, indicating their potential use in the management of diseases caused by several fungi. The high inhibitory effect of some tested extracts is correlated with the previous data. Garlic clove extract showed antifungal activity against the growth of *A. niger* and *A. flavus* (Tansey and Appleton,1975; Yoshida *et al.*, 1987; Yin and Cheng, 1998) and *Penicillium spp.* and *Candida albicans* (Manning and Moore, 1977). Several other studies have reported that garlic bulb extract can inhibit the growth of bacteria, fungi, viruses in culture media and food systems and it has been shown to possess insecticidal antiparasitic and antitumour properties (Ghanaoum, 1988; Pai and Platt, 1995; Kumar and Berwal, 1998; Satish *et al.*, 1999). The inhibitory action of garlic juice was suggested to be due to the presence of volatile sulphur compounds garlicin, phytoncides1`, allicin which acts as an inhibitor of respiratory - SH group enzymes, ajoene which destroys the integrity of cell wall (Yoshida *et al.*,1987; Fock *et al.*, 1990), phytocidin (Kovacs,1964) and thiosulfinate (Weber *et al.*,1991). Also, it was reported that *Eucalyptus* oil has established antiseptic, prophylactic and anthelmintic properties (Ansari and Shrivastava,1991). Similarly, the aqueous extracts of *Ammi visnaga* seeds and *Inula viscosa* leaves exerted an inhibitory effect against the dermatophytes (*Microsporum canis* and *Trichophyton rubrum*) and some bacterial species (Maoz and Neeman,1998). Recent findings have demonstrated that the aqueous extracts of some higher plants (e.g. *Agremone mexicana, Cyperus rotunds, Euphorbia hirta , Solanum nigrum, Capsicum annum,*

Melaleuca alternifolia , Mentha piperita, Salvia officinalis, Ricinus communis, Acacia arabica, Oxalis corniculata and Prosopis juliflora) contain antimicrobial substances (Chen *et al.*,1985; Masood and Ranjan, 1991; Hasan and Abdel-Mallek,1994; Shapiro *et al.*, 1994; Satish *et al.*,1999).

These inhibitory effects are interesting in connection with the prevention of either fungal or bacterial contamination in many foods, some diseases and they could be used instead of synthetic antimicrobial products.

References

Abd El-Hady, F.K. and S.A. Ouf, 1993. Fungitoxic effect of different substances from *Solenostemma argel* (Del) Hayne on some shoot surface fungi. Zentralbl. Microbiol., 148: 595-607.

Agriose, G.N., 1997. Control of plant diseases. In plant pathology, 4th ed. N. pp. 200 216. California: Academic Press.

Ahmed, Sh.M. and A.M. Khallil, 1991. Some investigations on Saprolegniasis in *Tilapia* species at Assiut, Egypt. Assiut Vet. Medical J., 25: 125 131.

Ansari, A.A. and A.K. Shrivastava, 1991. The effect of eucalyptus oil on growth and Aflatoxin production by *Aspergillus flavus*.Letters of Applied Microbiology, 13: 7577.

Anonymous, 1998. Pesticide incidents up for 1996/97 compared with previous year. International Pest Control, 40: 1 8.

Bohm, K.H. and H. Fuhrman, 1984. A Mycological survey of diseased freshwater fish. Bull. Eur. Ass. Fish Pathol., 4: 26 27.

Chen, H.C., M.D. Chang and T.T. Chang, 1985. Antibacterial properties of some spice plants befor and after heat treatment. Chinese J. Microbiol. Immunol., 18: 190-195.

Edward, M.C. and P.G. Ayres, 1981. Cell death and cell wall papillae in the resistance of Oak species to powdery mildews disease. New Phytologist, 89: 411-418.

Ellis, M.B. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. The Cambrian News Ltd. Aberystwyth, 608 p.

Focke, M.A., A. Feld and H.K. Lichtenthaler, 1990. Allicin, a naturally occurring antibiotic from garlic, specifically inhibits acetyl COA synthetase. FEBS Lett., 261: 106-108.

Ghannoun, M.A., 1988. Studies on antibacterial mode of action of *Allium sativum*. J. of general Microbiology, 134: 2917- 2924.

Hassan, H.A. and A.Y. Abdel Mallek, 1994. Inhibitory effect of aqueopus leaf extract of some plants on growth and Aflatoxin production by *Asperegillus flavus*. Dirasat, 21B: 215-219.

Khallil, A.M., 1990. Mycoflora associated with some freshwater plants collected from Delta region (Egypt). J. Basic Microbiol., 30: 663-674.

Kovacs, G., 1964. Studies on antibiotic substances from higher plants, with special reference to their plant physiological importance. K. Vet. Landhojsh. Kobenhagen, 47: 92.

Kumar, M. and V.D. Berwall, 1998. Sensitivity of food pathogens to garlic (*Allium sativum)*. J. Applied Microbiol., 84: 213-215.

Manning, W.J. and G.C. Moore, 1977. Effects of an aqueous garlic extract on several plant pathogenic fungi. Proc. Am. Phytopathol. Soc., 4: 192.

Maoz, M. and I. Neeman, 1998. Antimicrobial effects of aqueous plant extracts on the fungi *Microsporum canis* and *Trichophyton rubrum* and on three bacterial species. Letters in App. Microbiology, 26: 61-63.

Masood, A. and K.S. Ranjan, 1991. The effect of aqueous plant extracts on growth and aflatoxin production by *Aspergillus flavus*. Letters in Appl. Microbiology, 13: 32 34.

Pai, S.T. and M.W. Platt, 1995. Antifungal effects of *Allium sativum* (garlic) extract against the *Aspergillus* species involved in Otomycosis. Letters In App. Microbiology, 20: 14-18.

Rice, M.J., M. Legg and K.A. Powell 1998. Natural products in agriculture a view from the industry. Pesticides Science, 51: 227-234.

Satish, S., K.A. Raveesha and Janardhana, 1999. Antibacterial activity of plant extracts of phytopathogenic *Xanthomonas campestris* pathovars. Letters in App. Microbiology, 28: 145-147.

Seymour, R.L., 1970. The genus *Saprolegnia* .Verlag. Von. J. Cramer, Germany, pp: 124.

Shapiro,S., A. Meir, and B. Guggenheim, 1994. The antimicrobial activity of essential oils and essential oil components towards oral bacteria . Oral Microbiology Immunology, 9: 202-208.

Singh, P.N., I.R. Sindhu, and K. Gupta, 1986. Effect of leaf exudate and extract of spinash on some phylloplane fungi. Acta Botanica. Indica, 14: 104-110.

Singh, S.M., S. Sapana and P.K. Chatterjee, 1990. Clinical and experimental mycotic keratitis caused by *Aspergillus terreus* and the effect of subconjunctival oxiconazole treatment in the animal model. Mycopathologia, 112:127-137.

Singhal, R.N., S. Jeet, and R.W. Davis, 1987. Experimental transmission of *Saprolegnia* and *Achlya* to fish. Aquaculture, 64: 1-7.

Tansey, M.R. and J.A. Appleton, 1975. Inhibition of fungal growth by garlic extract. Mycologia, 67: 409-413.

Weber, N.D., A.D. Douglas, J.A. North, B.K. Murray, L.D. Lawson and B.G. Hughes, 1991. In Vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Medica, 5: 417-423.

Williams, R.J. and D.L. Heymann, 1998. Contamination of antibiotic resistance. Science, 279: 1153-1154 .

Witte, W., 1998. Medical consequences of antibiotic use in agriculture. Science, 279: 996-997.

Yin, M.C. and W.S. Cheng, 1998. Inhibition of *Aspergillus niger* and *A. flavus* by some herbs and spices. J. of food protection, 61: 123-125.

Yoshida, S.S., S. Kasuga, N. Hayashi, T. Ushiroguchi, H. Matsuura and S. Nakagawaa, 1987. Antifungal activitry of ajoene derived from garlic. App. Environ. Microbiol., 56: 615-617.