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IN VITRO FUNGICIDAL ACTIVITY OF SPICES AGAINST ROOT INFECTING FUNGI

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Abstract

Fungicidal activity of 16 spices were tested *In vitro* against root rot fungi viz., *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* using paper disc and well methods. *R. solani* was inhibited by *Curcuma longa* and *Myristica fragrans* (flower) whereas *M. phaseolina* and *Fusarium solani* were inhibited by *Piper nigrum*, *Capsicum annum*, *Cuminum cyminum*, *Carum carvi*, *Secale cereale*, *C. longa*, *Allium sativum*, *Zingiber officinale* and *Coriandrum sativum.* Ethanol extract of spices was more effective in the control of root rot pathogens as compared to aqueous extract whereas 100% w/v aqueous extracts were more effective than 50% w/v aqueous extract. Paper disc and well methods were equally effective in the inhibition of test fungi.

Introduction

Spices have been traditionally used since ancient time for the preservation of food product as they have been reported to have antiseptic and disinfectant properties. A preliminary screening for antimicrobial activity of 35 spices has been carried out. Extract of different polarity from leaves and seed of coriander (*Coriandrum sativum*) and coriander oil were investigated for antioxidant activity (Murray, 2000). The main component of *Cuminum cyminum* oil was p-mentha-1, 4-dien 7-al, cuminaldehyde, gamma-terpine, and beta-pinene. *Carum carvi* has carvone, limonen, germacrene D, and trans-dihydrocarvone (Tacobellius *et al*., 2005). Turmeric oil and Curcumin isolated from *Curcuma longa* were studied against 15 isolated dermatophytes, 4 isolates of pathogenic molds and 6 isolates of yeast (Apisariyakul *et al.,* 1995). In *Myristica fragrans* seed, GC/MS, a major compound present were identified as alpha-pinene and terpine 4-ol 4.4%. The gingerol compound is present in ginger. Garlic extracts have a strong antifungal effect inhibiting the formation of mycotoxin like aflatoxin by *Asperjillus parasiticus* (Lawson, 1996). *Eugenia caryophyllus* has the main constituent of the essential oil, phenylpropanoids such as carvacol, thymol, eugenol and cinnamaldehyde. The synthetic fungicides viz., chlorothalorial dichlofuanid and mancoze and four commercially available compounds has been derived from *Piper longum* (Parmer *et al*., 1997). In *Foeniculum vulgare* seed, the main constituents of the oil were (E)-anethole (72.27%-74.18%), fenchone (11.32%-16.35%) and methyl chavicol (3.78%-5.29) which have an antifungal property (Dukic *et al.,* 2003). The soil borne pathogens plays important role in the development of root rot and root knot diseases complex of crop plants. Of soil borne root infecting fungi, *M. phaseolina* (Tassi) Goid, is known to produced charcoal rot, seedling blight, root rot, stem rot, pod rot on more than 500 species of plants (Dhingra & Sinclair, 1978; Sinclair, 1982) and about 72 hosts are reported from Pakistan (Mirza & Qureshi, 1978). *Rhizoctonia solani* Kühn exist active mycelium in soil which is known to produce seed rot, damping off of seedling, wilt and root rot on over 2000 species of plants (Parmeter, 1970), of which at least 63 hosts have been reported from Pakistan (Ghaffar, 1988), similarly *Fusarium solani* and *Fusarium oxysporum* which are very common in agriculture fields of Pakistan are known to cause

root rot, stem rot and wilt disease on a wide range of plants (Ghaffar, 1992). Various plants extracts like *Eucalyptus* sp., (Dawar *et al.,* 2007), *Avicennia marina* (Forsk.) Vierh, *Rhizophora mucronata* Lamk., *Ceriops tagal* (Perr.) C.B.Robinson and *Aegiceras corniculatum* (L.) Blanco aqueous and ethanol extracts were used for the inhibition of root rot fungi (Mehdi & Dawar, 2007). Experiments were therefore carried out to study the antimicrobial activity of spices in the inhibition of root rot fungi viz., *F. solani*, *R. solani* and *M. phaseolina*.

Materials and Methods

Common spices viz., Cyenne pepper or chilies (*Capsicum annum* L.), cumin seed (*Cuminum cyminum*/*Carum carvi* L.), coriander (*Coriandrum sativum* L.), turmeric (*Curcuma longa* L.), nutmeg and maco (*Myristica fragrans* Houtt.), cloves (*Myrtus caryophyllus* L.), black cumin (*Nigella sativa* L.), black pepper (*Piper nigrum* L.), garlic (*Allium sativum* L.), ginger (*Zingiber officinale* Rose.), wild rye (*Secale cereale* L.), fennel (*Foeniculum vulgare* Mill.), poppy (*Papaver somniferum* L.) and taiez pat (*Cinnamomum tamala* T.Nees & Eberm.) were collected from Karachi market and ground in an electric grinder.

For the aqueous extraction of spices, 200g powdered spices were soaked in 500 ml distilled water for 24 hrs, filtered with Whatman filter paper and stored at 6˚C which gives 100% extract. Half quantity of 100 % extract was diluted in distilled water which gave 50% extract. Likewise, 200g powdered spices were soaked in 500 ml ethanol for two weeks, filtered twice and filtrate was concentrated under a rotary vacuum evaporator. An appropriate amount of extract was dissolved in ethanol to make 1000, 500 and 250 ppm concentrations.

Paper disc and well methods were used to study the inhibition of root rot fungi with aqueous and ethanol extracts of different spices. The cultures of *R. solani*, *M. phaseolina* and *F. solani* were isolated from soil and grown on potato dextrose agar containing penicillin @ 20,000 unit/liter and streptomycin @ 200 mg/liter. A 5 mm disc of root infecting fungi viz., *R. solani, M. phaseolina* and *F. solani* was placed in the center and 5 mm surface sterilized paper discs soaked in aqueous extract of spices @ 0.1, 1 and 5% w/v and in other experiment paper disc soaked in ethanol extract of spices @ 1000 ppm, 500 ppm and 250 ppm w/v were placed on three corners of Petri dish. The disc soaked in sterilized water was placed at the fourth corner in both (aqueous and ethanol) which served as control. Each treatment was replicated three times for each root infecting fungus. Inoculated Petri dishes were incubated for 5-6 days and zone of inhibition was measured.

Results and Discussion

Aqueous extract: Results of paper disc method showed that 50% w/v aqueous extracts of *C. annum, C. cyminum, C. longa, M. fragrans* (flower), *P. nigrum*, and *A. sativum*, significantly inhibited the growth of *F. solani* whereas *C. longa* and *M. fragrans* (flower) were effective for the inhibition of *R. solani. M. caryophyllus*, *Curcuma longa* and *M. fragrans* (flower), inhibited the growth of *M. phaseolina* (Table 1). In case of 100% w/v aqueous extract of spices used @ of 0.1, 1 and 5% *P. nigrum*, *M. fragrans* (flower and fruit), *C. carvi*, *C. annum*, *C. cyminum* and *C. longa* were found to be effective for the inhibition of *F. solani* whereas *M. fragrans* (flower), *M. fragrans* (fruit) and *C. longa* were more effective for the inhibition of *R. solani. P. nigrum*, *A. sativum*, *M. fragrans* (flower), *C. carvi*, *M. caryophyllus*, *F. vulgare*, *C. annum*, *C. cyminum* and *C. longa* more effectively inhibited the growth of *M. phaseolina* (Table 2).

Table 1. Growth inhibition of root rot fungi by 100% aqueous extract of spices.

Treatments	Zone of inhibition (mm)											
	Paper disc method											
	F. solani (mm)				*R. solani* (mm)				*M. phasoelina* (mm)			
	C	0.1%	1%	5%	C	0.1%	1%	5%	C	0.1%	1%	5%
Allium sativum	17	18	21	24	5	6	16	20	20	22	27	30
Capsicum annum	20	22	24	28	8	10	14	18	20	22	26	27
Carum carvi	21	23	26	31	16	17	19	23	19	20	25	30
Cinnamomum tamala	14	15	21	25	F	5	10	20	15	19	21	28
Coriandrum sativum	20	22	23	25	15	20	24	25	15	16	21	24
Cuminum cyminum	15	22	25	32	F	F	6	13	16	18	22	30
Curcuma longa	18	22	23	24	18	22	24	28	12	16	27	29
Foeniculum vulgare	19	20	25	27	15	17	23	24	20	21	25	23
Myristica fragrans (f)	21	23	25	29	20	22	23	29	20	21	23	24
M. fragrans (fr)	23	26	29	35	22	26	28	35	15	20	28	33
Myrtus caryophyllus	18	18	19	21	F	F	F	F	20	22	26	29
Nigella sativa	16	20	22	24	7	11	15	19	17	19	20	24
Papaver somniferum	13	13	16	20	15	15	21	24	15	15	19	22
Piper nigrum	25	26	27	30	F	2	3	5	5	17	22	28
Secale cereale	18	19	21	25	15	16	19	23	20	21	24	28
Zingiber officinale	20	20	24	26	20	21	23	25	17	18	23	25
	Well method											
Allium sativum	17	20	24	30	5	6	16	20	20	23	24	28
Capsicum annum	22	22	26	28	23	24	26	27	16	17	21	25
Carum carvi	23	23	28	30	17	18	21	23	19	21	25	28
Cinnamomum tamala	13	13	20	23	5	6	12	22	11	15	21	27
Coriandrum sativum	20	22	23	24	15	18	24	28	17	18	22	25
Cuminum cyminum	16	22	26	30	F	3	6	15	16	18	23	30
Curcuma longa	13	15	21	25	15	18	21	25	18	20	23	28
Foeniculum vulgare	20	21	24	28	16	20	24	25	20	22	25	29
Myristica fragrans (f)	21	25	25	29	20	22	24	28	18	20	22	25
M. fragrans (fr)	20	25	29	33	20	25	28	35	15	20	25	35
Myrtus caryophyllus	11	14	20	24	F	F	F	F	23	24	27	28
Nigella sativa	16	19	23	25	10	15	17	18	10	15	18	19
Papaver somniferum	18	18	19	25	15	15	21	24	18	29	21	25
Piper nigrum	24	26	28	31	F	F	4	5	15	17	21	30
Secale cereale	17	20	21	26	12	12	16	18	21	22	25	28
Zingiber officinale	17	20	22	29	10	12	16	18	12	16	22	25

C = Control
F = Full growth

In well method, 50% aqueous extracts of spices viz., *P. nigrum*, *A. sativum*, *C. carvi*, *M. fragrans* (flower), *C. longa*, *P. somniferum* and *C. cyminum* used @ of 0.1, 1 and 5% w/v inhibited the growth of *F. solani* whereas *C. longa* and *M. fragrans* (flower), were effective for the inhibition of *R. solani* while *M. caryophyllus*, *C. longa* and *M. fragrans* (flower) inhibited the growth of *M. phaseolina* (Table 1). In case of aqueous extract of spices used @ 100% w/v *P. nigrum*, *A. sativum*, *C. carvi*, *M. fragrans* (flower), *C. annum*, *C. cyminum* and *M. fragrans* (fruit) were more effective for the inhibition of the growth of *F. solani*, *M. fragrans* (flower), *C. annum*, *C. cyminum*, *M. fragrans* (fruit) and *C. longa* more effectively inhibited the growth of *R. solani* while *P. nigrum*, *A. sativum*, *M. fragrans* (flower), *C. carvi*, *M. caryophyllus*, *F. vulgare*, *C. annum*, *C. cyminum* and *C. longa* were more effective in inhibiting the growth of *M. phaseolina* (Table 2).

Table 2. Growth inhibition of root rot fungi by ethanol extract of spices.

Treatments	Zone of inhibition (mm)											
	Paper disc method											
	F. solani (mm)				*R. solani* (mm)				*M. phasoelina* (mm)			
	C	250	500	1000	C	250	500	1000	C	250	500	1000
Allium sativum	23	26	25	24	19	24	22	20	22	28	25	24
Capsicum annum	21	26	25	23	20	28	24	22	25	30	27	26
Carum carvi	22	30	27	22	16	24	22	18	19	30	25	22
Cinnamomum tamala	16	25	22	17	8	18	15	12	12	19	16	14
Coriandrum sativum	21	27	24	22	20	25	24	22	20	28	23	20
Cuminum cyminum	15	30	23	22	2	10	5	4	17	25	21	18
Curcuma longa	30	36	33	32	20	30	25	23	25	31	31	28
Foeniculum vulgare	19	28	25	21	16	25	21	16	20	28	26	21
Myristica fragrans (f)	19	27	21	19	10	20	16	15	15	25	20	16
M. fragrans (fr)	19	30	25	22	18	28	22	21	21	29	26	23
Myrtus caryophyllus	18	26	22	19	4	10	6	4	23	29	26	24
Nigella sativa	17	25	22	20	10	25	20	15	18	25	22	19
Papaver somniferum	20	27	23	22	15	22	19	17	15	26	20	17
Piper nigrum	20	32	25	22	3	10	6	4	17	30	25	20
Secale cereale	19	25	21	20	15	23	19	16	20	28	25	22
Zingiber officinale	17	29	22	20	14	22	20	15	22	34	28	24
	Well method											
Allium sativum	19	28	25	21	5	10	7	6	17	23	24	20
Capsicum annum	18	26	22	19	15	24	20	19	22	30	28	24
Carum carvi	19	25	23	20	20	27	23	21	24	35	28	25
Cinnamomum tamala	21	30	27	22	18	25	21	19	19	30	25	21
Coriandrum sativum	12	20	16	14	13	21	18	14	13	19	17	14
Cuminum cyminum	19	26	24	22	20	25	23	21	20	32	27	23
Curcuma longa	19	30	23	21	6	14	10	6	18	24	21	19
Foeniculum vulgare	23	31	29	27	21	30	29	27	21	32	31	27
Myristica fragrans (f)	19	27	25	21	16	24	21	17	19	28	25	22
M. fragrans (fr)	15	22	20	16	21	26	22	22	15	21	18	16
Myrtus caryophyllus	20	30	25	22	21	32	25	22	20	28	23	21
Nigella sativa	19	26	23	19	5	10	7	6	23	29	26	23
Papaver somniferum	18	24	22	20	10	25	20	15	17	24	20	18
Piper nigrum	18	25	23	20	15	20	18	16	16	27	21	17
Secale cereale	19	28	25	21	5	10	7	6	17	23	24	20
Zingiber officinale	19	25	21	19	12	18	16	12	21	29	25	22

C = Control

Ethanol extract: In paper disc method ethanol extracts of spices were used @ of 250, 500 and 1000 ppm for the inhibition of root rot fungi like *F. solani*, *R. solani* and *M. phaseolina*. Inhibition of test fungi increased with the increase in concentration. Ethanol extracts of spices of *P. nigrum*, *C. longa*, *C. carvi*, *M. fragrans* (flower), *F. vulgare* and *C. cyminum* effectively inhibited the growth of *F. solani* whereas *M. fragrans* (flower), *C. annum* and *C. longa* were found to be highly effective in the inhibition of *R. solani* while *P. nigrum*, *C. sativum*, *M. fragrans* (flower), *C. annum*, *C. carvi*, *C. longa*, *F. vulgare*, *Z. officinale*, *S. cereale*, *M. caryophyllus* and *A. sativum* were effective for the inhibition of growth of *M. phaseolina* (Table 2).

In well method *P. nigrum*, *C. longa*, *C. carvi*, *M. fragrans* (flower) and *C. cyminum* were highly effective in the inhibition of growth of *F. solani* whereas *M. fragrans* (flower), *C. annum* and *C. longa* significantly inhibited the growth of *R. solani* whereas

C. sativum, M. fragrans (flower), *C. annum, C. carvi, C. longa, F. vulgare, Z. officinale, S. cereale, M. caryophyllus* and *A. sativum* were most effective for the inhibition of the growth of *M. phaseolina* (Table 2). Ethanol extracts of spices were more effective in the control of *F. solani, R. solani* and *M. phaseolina* as compared to aqueous extracts. Of the different concentrations of aqueous extracts used, 100% was effective than 50%. Paper disc and well methods were equally effective for the inhibition of test fungi (Tables 1,2). Paper disc and well methods showed inhibition in growth of test fungi viz., *F. solani*, *R. solani* and *M. phasoelina* by all concentration. Maximum inhibitions of test fungi was observed by 5% w/v followed by 1 and 0.1% w/v.

Present investigation showed that out of 16 most effective spices, *C. longa* and *M. fragrans* (flower) were highly effective in the inhibition of *F. solani*, *R. solani* and *M. phaseolina*. Shelef (1983) reported that Cinnamon and Clove have strong effect. All spices, Caraway, Corriander, Cumin and Oregano saye showed medium effect whereas Black pepper, red chilies and ginger showed weak antimicrobial effect. *C. longa* and *M. fragrans* (flower) were strongly effective against the test fungi. Similarly *C. longa* rhizome derived material was effective against *Botrytis cineria, Erysiphe graminis, Phytophothora infestans, Puccinia recondita, Pyricularia oryzae* and *Rhizoctonia solani* (Apisariyakul *et al.,* 1995). Jungjong *et al.,* (2007) found that plant extracts were potent *In vivo* antifungal activity against various plant diseases They also found that treatment with methanol extract of *M. fragrans* seed reduced the development of various plant diseases. *Curcuma* species with antifungal, antibacterial and anti-inflammatory activity has been reported (Apisariyakul *et al.,* 1995). Present result showed that aqueous and ethanol extracts of *Allium sativum* were effective against *F. solani* and *M. phaseolina.* Similarly Hughes & Lawson (1991) reported that ethanol and aqueous extracts of garlic have antifungal property. Allicin was the major compound responsible for the inhibition of fungal growth. Plants extract and essential oil showed antifungal activity against wide range of fungi. Ark & Thompson (1959) showed that garlic extract contain potent fungicide which were effectively protecting peaches against brown rot *Macrophomina fruticola. C. annum* extract have been found to have activity against the cercria of *Schistoma mansoin.* It is not possible that activity was related to capsaicininoid, or other compounds since the toxicity was assayed with the crude fruit extract (Friscкhkorn *et al.,* 1978). Black pepper were found effective for the inhibition of *Fusarium* sp. Hariselstran *et al*., (1954) have reported the powerful fungistatic action of black pepper metabolites, piperine, apopiperine, Beta-cinnamenyle, acrolyl hydrazide, Beta-cinnamenyl acrolyl on *Aspergillus versicolor*.

Present results showed that paper disc and well methods were found significant in the inhibition of growth of *F. solani*, *R. solani* and *M. phaseolina* when aqueous and ethanol extracts of spices were used. Similar results were observed by Tariq *et al.,* (2007) where well and paper disc methods showed significant inhibition of *F. solani*, *R. solani* and *M. phaseolina.* Spices were effective in the inhibition of test fungi when used @ 5% w/v. Such similar results were obtained by Dawar *et al.,* (2007) where aqueous extracts of *Eucalyptus* sp., plant part was more effective when used @ 5% w/v in the control of *F. solani*, *R. solani* and *M. phaseolina.* Of the different spices used, *C. longa* and *M. fragrans* (flower) showed highest antimicrobial activity against the test fungi viz., *F. solani*, *R. solani* and *M. phaseolina*. There is therefore need to carry out work *In vivo* for the control of root rot pathogens.

References

Apisariyakul, A., N. vaniltanakoun and D. Buddhasukh. 1995. Antifungal activity of termeric oil from *Curcuma longa* (Zingiberaceae). *Journal of Enthnopharmacology*, 49:(3): 163-169.

Ark, P.A. and J.P. Thompson. 1959. Control of certain disease of plants with antibiotic from garlic (*Allium sativum.* L). *Plant Dis. Rep*., 43: 276-282.

Dawar, S., S.M. Younus, M. Tariq and M.J. Zaki. 2007. Use of *Eucalyptus* sp., in the control of root infecting fungi on mung bean and chick-pea. *Pak. J. Bot.*, 39(3): 975-979.

Dhingra, O.D. and J.B. Sinclair. 1978. *Biology and pathology of M. phaseolina* Imprensa Universidade Federal Dvicosa-Minas Gerais-Brazil, pp.166.

Dukic, N.M., S. Kujundzic, M. Sokovic and M. Couladis. 2003. Essential oil composition and antifungal activity of *Foeniculum vulgare* Mill obtained by different distillation conditions. *Phytotherapy Research*, 17(4): 368-371.

Frischkorn, H.E. and E. Carrazzoni. 1978. Cercaricidal activity of some essential oils of plant from Brazil. *Naturwissenschaften* 65: 480-483 Full text via cross Ref view record in Scopus cited by in Scopus (9).

Ghaffar, A. 1988. Soil borne disease research center. *Final research report.* Dept. of Botany, University of Karachi-75270, Pakistan, pp.111.

Ghaffar, A. 1992. Use microorganism in the biological control of soil borne root infecting fungi. NSRBD project *Final research report.* Dept. of Botany, University of Karachi, Karachi-75950, Pakistan pp 95.

Hairselstran, T., G.P. Dateo, H.S. Levinson, W.H. Stahl, E.J. Hewth and K.S. Kenigshascher. 1954. Antioxidant activity and antagonist to microbial growth, piperine, isonicotynyl hydrazide and related compounds. *Fd. Res*., 19(4):373.

Hughes, B.G. and L.D. Lawson. 1991. Antimicrobial effect of *Allium sativum* L (garlic) *Allium ampeloprasum* (elephant garlic) and *Allium cepa* L. (onion) garlic compound and commercial garlic supplement products. *Phytother Res.*, 5: 154-158.

Jun-joung, C., G.J. Choi, S. Wanson, K. Soojang, H.K. Lim, S.O. Lee, N.D. Sung, K. Cho and J.C. Kim. 2007. Isolation and antifungal activity of lignans from *Myristica fragrans* against various plant pathogenic fungi. *Pest Mangment Science*, 63(9): 935-940.

Lawson, L.D. 1996. The composition and chemistry of garlic, colves and processed garlic. *In: The science and therapeutic application of Allium sativum* L., (Eds.): H.P Kocuh and L.D Lawson William and Wilkin, Baltimore, pp. 37-108.

Mehdi, F.S and S. Dawar. 2007. Use of mangrove of Indus Delta in the control of root rot disease and growth. *Promotion of crop plant. 2nd Technical Report*, pp. 94.

Mirza, J.H. and M.S.A. Qureshi. 1978. *Fungi of Pakistan*. Dept. of Plant Pathology, University of Agric, Faisalabad, pp. 311.

Murray, B.I. 2000. Herbal drug and phytopharaceuticals, medpharmGm b/f scientificpublisher, stuttgart. *Crop protection*, 9(8-10): 603-608.

Parmeter, J.R. 1970. *Rhizoctonia solani, Biology and pathology.* University of California press, Barkley. Los Angles and London, pp. 225.

Parmer, V.S., S.C. Jains, K.S. Bisht, R. Jain, P. Taneja, Jha., O.D. Tyagi, A.K. Prasad, J. Wengel, C.E. Oisen and P.M. Boll. 1997. Phytochemistry of the genus Piper. *Phytochem*., 46: 597-673.

Shelef, L.A. 1983. Antimicrobial effects of spices. *J. Food Safety*, pp 629-644.

Sinclair, J.B. 1982. *Compendium of soy bean disease.2nd ed.* American Phytopatholgical Society pp 104.

Tariq, M., S. Dawar, F.S. Mehdi and M.J. Zaki. 2006. Use of *Avicennia marina* in the control of root infecting fungi on okra and mash bean. *Pak. J. Bot.*, 38(3): 811- 815.

Tacobellus, N.S., D. Locantore, F. Capasso and F. Senatore. 2005. Antimicrobial activity of *Cuminum cyminum* and *Carum carvi* essential oil. *J. Agric Food Chem*., 53(1): 57-61.

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