

## **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 *Aspergillus 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											
	<i>F. solani</i> (mm)				<i>R. solani</i> (mm)				<i>M. phaseolina</i> (mm)			
	C	0.1%	1%	5%	C	0.1%	1%	5%	C	0.1%	1%	5%
<i>Allium sativum</i>	17	18	21	24	5	6	16	20	20	22	27	30
<i>Capsicum annum</i>	20	22	24	28	8	10	14	18	20	22	26	27
<i>Carum carvi</i>	21	23	26	31	16	17	19	23	19	20	25	30
<i>Cinnamomum tamala</i>	14	15	21	25	F	5	10	20	15	19	21	28
<i>Coriandrum sativum</i>	20	22	23	25	15	20	24	25	15	16	21	24
<i>Cuminum cyminum</i>	15	22	25	32	F	F	6	13	16	18	22	30
<i>Curcuma longa</i>	18	22	23	24	18	22	24	28	12	16	27	29
<i>Foeniculum vulgare</i>	19	20	25	27	15	17	23	24	20	21	25	23
<i>Myristica fragrans</i> (f)	21	23	25	29	20	22	23	29	20	21	23	24
<i>M. fragrans</i> (fr)	23	26	29	35	22	26	28	35	15	20	28	33
<i>Myrtus caryophyllus</i>	18	18	19	21	F	F	F	F	20	22	26	29
<i>Nigella sativa</i>	16	20	22	24	7	11	15	19	17	19	20	24
<i>Papaver somniferum</i>	13	13	16	20	15	15	21	24	15	15	19	22
<i>Piper nigrum</i>	25	26	27	30	F	2	3	5	5	17	22	28
<i>Secale cereale</i>	18	19	21	25	15	16	19	23	20	21	24	28
<i>Zingiber officinale</i>	20	20	24	26	20	21	23	25	17	18	23	25
Well method												
<i>Allium sativum</i>	17	20	24	30	5	6	16	20	20	23	24	28
<i>Capsicum annum</i>	22	22	26	28	23	24	26	27	16	17	21	25
<i>Carum carvi</i>	23	23	28	30	17	18	21	23	19	21	25	28
<i>Cinnamomum tamala</i>	13	13	20	23	5	6	12	22	11	15	21	27
<i>Coriandrum sativum</i>	20	22	23	24	15	18	24	28	17	18	22	25
<i>Cuminum cyminum</i>	16	22	26	30	F	3	6	15	16	18	23	30
<i>Curcuma longa</i>	13	15	21	25	15	18	21	25	18	20	23	28
<i>Foeniculum vulgare</i>	20	21	24	28	16	20	24	25	20	22	25	29
<i>Myristica fragrans</i> (f)	21	25	25	29	20	22	24	28	18	20	22	25
<i>M. fragrans</i> (fr)	20	25	29	33	20	25	28	35	15	20	25	35
<i>Myrtus caryophyllus</i>	11	14	20	24	F	F	F	F	23	24	27	28
<i>Nigella sativa</i>	16	19	23	25	10	15	17	18	10	15	18	19
<i>Papaver somniferum</i>	18	18	19	25	15	15	21	24	18	29	21	25
<i>Piper nigrum</i>	24	26	28	31	F	F	4	5	15	17	21	30
<i>Secale cereale</i>	17	20	21	26	12	12	16	18	21	22	25	28
<i>Zingiber officinale</i>	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											
	<i>F. solani</i> (mm)				<i>R. solani</i> (mm)				<i>M. phaseolina</i> (mm)			
	C	250	500	1000	C	250	500	1000	C	250	500	1000
<i>Allium sativum</i>	23	26	25	24	19	24	22	20	22	28	25	24
<i>Capsicum annum</i>	21	26	25	23	20	28	24	22	25	30	27	26
<i>Carum carvi</i>	22	30	27	22	16	24	22	18	19	30	25	22
<i>Cinnamomum tamala</i>	16	25	22	17	8	18	15	12	12	19	16	14
<i>Coriandrum sativum</i>	21	27	24	22	20	25	24	22	20	28	23	20
<i>Cuminum cyminum</i>	15	30	23	22	2	10	5	4	17	25	21	18
<i>Curcuma longa</i>	30	36	33	32	20	30	25	23	25	31	31	28
<i>Foeniculum vulgare</i>	19	28	25	21	16	25	21	16	20	28	26	21
<i>Myristica fragrans</i> (f)	19	27	21	19	10	20	16	15	15	25	20	16
<i>M. fragrans</i> (fr)	19	30	25	22	18	28	22	21	21	29	26	23
<i>Myrtus caryophyllus</i>	18	26	22	19	4	10	6	4	23	29	26	24
<i>Nigella sativa</i>	17	25	22	20	10	25	20	15	18	25	22	19
<i>Papaver somniferum</i>	20	27	23	22	15	22	19	17	15	26	20	17
<i>Piper nigrum</i>	20	32	25	22	3	10	6	4	17	30	25	20
<i>Secale cereale</i>	19	25	21	20	15	23	19	16	20	28	25	22
<i>Zingiber officinale</i>	17	29	22	20	14	22	20	15	22	34	28	24
Well method												
<i>Allium sativum</i>	19	28	25	21	5	10	7	6	17	23	24	20
<i>Capsicum annum</i>	18	26	22	19	15	24	20	19	22	30	28	24
<i>Carum carvi</i>	19	25	23	20	20	27	23	21	24	35	28	25
<i>Cinnamomum tamala</i>	21	30	27	22	18	25	21	19	19	30	25	21
<i>Coriandrum sativum</i>	12	20	16	14	13	21	18	14	13	19	17	14
<i>Cuminum cyminum</i>	19	26	24	22	20	25	23	21	20	32	27	23
<i>Curcuma longa</i>	19	30	23	21	6	14	10	6	18	24	21	19
<i>Foeniculum vulgare</i>	23	31	29	27	21	30	29	27	21	32	31	27
<i>Myristica fragrans</i> (f)	19	27	25	21	16	24	21	17	19	28	25	22
<i>M. fragrans</i> (fr)	15	22	20	16	21	26	22	22	15	21	18	16
<i>Myrtus caryophyllus</i>	20	30	25	22	21	32	25	22	20	28	23	21
<i>Nigella sativa</i>	19	26	23	19	5	10	7	6	23	29	26	23
<i>Papaver somniferum</i>	18	24	22	20	10	25	20	15	17	24	20	18
<i>Piper nigrum</i>	18	25	23	20	15	20	18	16	16	27	21	17
<i>Secale cereale</i>	19	28	25	21	5	10	7	6	17	23	24	20
<i>Zingiber officinale</i>	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. phaseolina* 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*, *Phytophthora 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 cercaria of *Schistoma mansoin*. It is not possible that activity was related to capsaicinoid, or other compounds since the toxicity was assayed with the crude fruit extract (Frisckhkorn *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.

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