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NEMATICIDAL ACTIVITY OF SPICES AGAINST *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD

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Abstract

Nematicidal activity of some spices against *Meloidogyne javanica* root knot nematode was examined. *In vitro* results showed that aqueous extract of *Cuminum cyminum* (100 % w/v) and ethanol extract of *Capsicum annum*, *Cinnamomum tamala* and *Curcuma longa* (1000 ppm) significantly inhibited egg hatching of *Meloidogyne javanica.* Aqueous extract of *C. longa, Nigella sativa* and *Piper nigrum* in 100% w/v whereas ethanol extract of *C. tamala* and *P. nigrum* in 1000 ppm caused appreciable mortality of second stage juveniles of *M. javanica.* Ethanol extract was found better as compared to aqueous extract. The concentration used @ 100% and 1000 ppm were found more effective and produced significant results as compared to 50%, 500 ppm and 250 ppm.

Introduction

Spices are dried seed, fruit, root, bark or vegetative substance used in nutritionally insignificant quantities as a food additive for the purpose of flavoring by killing or preventing the growth of harmful bacteria (Burkill, 1985). Many of these substances are also used for other purposes such as medicine, religious rituals, cosmetics, perfumery or eating as vegetables. There are reports that clove, cinnamon, bishop's weed, chilli, horse raddish, cumin, tamarind, black cumin, pomegranate seed, nutmeg, garlic, onion tejpat, cellary cambodge have potent antimicrobial activity against *Bacillus subtillis*, *Escherichia coli* and *Saccharomyces cerevisiae* (De *et al.,* 1999). Extract of different polarity from leaves and seed of coriander (*Coriandrum sativum*) and coriander oil was investigated for antioxidant activity (Murray, 2000).

Lacobellis *et al*., (2005) observed that the main component of *C. cyminum* oil was p-mentha-1, 4-dien-7-al, cumin aldehyde, gamma-terpinene and beta-pinene whereas those of *C. carvi* were carvone, limonen, germacrene D and trans-dihydrocarvone. Seed also contain essential oil up to 1%, linalool is the main component. In *Myristica fragrans* seed, GC/MS, a major compound is 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 have been derived from *Piper longum* (Parmer *et al*., 1997). Eugenol, piperine and piper longumine are main compound of *Piper longum*. In *Foeniculum vulgare* seed, main constituent of the oil were (E)-anethole (72.27%-74.18%), fenchone (11.32%-16.35%) and methyl chavicol (3.78%-5.29) (Mimica *et al.,* 2003). The common spices have a long history of use in eastern culture as food flavors. These are exotic spices and herb.

Root-knot nematodes (RKN) are one of the most important nematode pests of crop plants and have a diverse host range. RKN (*Meloidogyne* spp.) are sedentary root endoparasites and are involved in the development of specialized feeding structures known as giant cells. Root knot nematodes (*Meloidogyne* spp.) are capable of reproducing on over 2,000 species of plants (Sasser & Freckman, 1987) and are responsible for approximately 50% of overall nematode damage. The various species of *Meloidogyne* induce major morphological and physiological changes within roots, attack nearly every crop sown where not only yields are greatly affected but quality is also reduced (Sasser, 1980). Various plants extract like *Eucalyptus* sp., (Dawar *et al.*, 2007), *Avicennia marina*, *Rhizophora mucronata*, *Ceriops tagal* and *Aegiceras corniculatum* were reported against root knot nematode (Mehdi & Dawar, 2008). Present research was carried out to test activity of spices against *M. javanica* root knot nematode.

Materials and Methods

Collection of material: Fresh spices viz., *Allium sativum* L., *Capsicum annum* L., *Cinnamomum tamala* Nees & Eberm., *Coriandrum sativum* L., *Cuminum cyminum/Carum carvi* L., *Curcuma longa* L., *Foeniculum vulgare* Mill., *Myristica fragrans* Houtt., *Myrtus caryophyllus* L., *Nigella sativa* L., *Papaver somniferum* L., *Piper nigrum* L., *Secale cereale* L., and *Zingiber officinale* Rose., were collected from local market. Fresh materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extract preparation: Aqueous extract (10 % w/v) was prepared by soaking the powder for 6 hours in sterilized distilled water at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This process was repeated twice and after 6 h., the supernatant was concentrated to make the final volume one-fourth of the original volume (Parekh *et al*., 2005). It was autoclaved at 121˚C and 15 lbs pressure and then stored at 6˚C which gives 100% extract. Half quantity of 100% extract was diluted in distilled water which gave 50% extract.

For preparation of ethanol extract, 10 g of dried powder was extracted with 100 ml of ethanol kept on a rotary shaker at 190-220 rpm for 24 h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Parekh *et al.*, 2005). It was stored at 6˚C in airtight bottles for further studies. An appropriate amount of extract was dissolved in ethanol to make 1000, 500 and 250 ppm concentrations.

Culture preparation of root knot nematodes*:* Roots of plants infested with root-knot nematodes were collected from Karachi University garden. The root knot nematodes were identified with the help of perennial pattern as described by Taylor & Netscher (1974). The root-knot nematode *M. javanica* (Treub) Chitwood, was cultured on brinjal seedlings in a greenhouse from a single egg mass. Nematode eggs were extracted from infested roots using a 2% NaOCl solution and the eggs released from the roots were collected using the modified technique described by McClure *et al.*, (1973). The eggs suspension was poured on a cotton-wool filter paper and incubated at 28±2˚C to obtain freshly hatched juveniles (J_2). Juveniles collected within 48 h were used.

Table 1. Ethnobotanical information of some traditionally used species selected for nematicidal activity (Burkill, 1985).

Spices	Family	Common name	Therapeutic use
Allium sativum	Alliaceae	Garlic	Food, stomach troubles, anti-microbial.
Capsicum annum	Solanaceae	Chili, sweet pepper	Medicines, food, Agri-hotriculture
Carum carvi	Umbelliferae	Caraway	Roots edible, seeds used in breads, cakes.
Cinnamomum tamala	Lauraceae	Tezpat	Flavor in food, bark used as an inferior substitute of cassia.
Coriandrum sativum	Umbelliferae	Coriander	Agri-hotriculture, weeds, for stomach troubles, flavoring.
Cuminum cyminum	Umbelliferae	Cumin	Flavors, sauces, ornamental
Curcuma longa	Zingiberaceae	Turmeric	Anti-arthritic, anti-cancer
Foeniculum vulgare	Umbelliferae	Fennel	Anti-spasmodic, diuretic
Myristica fragrans (f)	Myristicaceae	Nutmeg	Medicine in paralysis, spasm.
M. fragrans (fr)	Myristicaceae	Nutmeg	Flavoring
Myrtus caryophyllus		Cloves	Topical anesthetic, anti-dyspeptic
Nigella sativa	Ranunculaceae	Black cumin	Antimicrobial
Papaver somniferum	Papaveraceae	Poppy	Anti-spasmodic, sedative
Piper nigrum	Piperaceae	Black pepper	Antimicrobial
Secale cereale	Gramineae	Wildrye	-
Zingiber officinale	Zingiberaceae	Ginger	Rhizome use as alcoholic drink

Egg hatching test: To determine the effect of aqueous (50 & 100%) and ethanol (250, 500 & 1000 ppm) extract of spices on egg hatching activity of *M. javanica*, 2 ml of the spices extract was transferred in watch glasses (diameter 2.5 cm) into which two medium size egg masses hand-picked from the knots of egg plant were placed. Egg masses kept in distilled water served as control. Each treatment was replicated thrice. After 72 h exposure, the number of juveniles hatched were counted with the aid of a stereomicroscope (X 6). Treatments were in triplicate and watch glasses were randomized at room temperature (28±2˚C). The toxicity of spices extract was assessed as the mean percentage of the hatched eggs.

Mortality test: To determine the nematicidal activity, dilutions of 250, 500 and 1000 ppm of ethanol extract were prepared, transferred to 2.5 cm diam., glass slides and left for 48 h to evaporate the organic solvent. Two ml of the juvenile suspension (40-50 juveniles/ml) were added to each glass slide to assess juvenile mortality after 24 h and incubated at room temperature (28±2˚C). Each treatment was replicated thrice. The glass cavity block without ethanol extract and aqueous of spices served as control. After 72 h exposure, the number of killed juveniles was counted under a low power stereomicroscope. The toxicity of spices was assessed as the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al.*, 1989).

Statistical analysis Data were analyzed and subjected to analysis of variance (ANOVA) including Least Significance Difference (LSD) and Duncan's Multiple Range Test (DMRT) (Sokal & Rohlf, 1995).

Results and Discussion

Species are nutritionally important food additive for the purpose of flavouring, medicinal, antimicrobial and antiarthritic (Burkill, 1985) (Table 1). The results showed that the aqueous and ethanol extracts of powdered spices inhibited egg hatching and is capable of causing appreciable mortality of *M. javanica* juveniles. Of the aqueous extracts of spices *C. carvi, Z. officinale* showed maximum nematicidal activity against *M. javanica* eggs followed by *A. sativum, C. tamala, C. longa, N. sativa* at 100% w/v. Aqueous extract of *C. cyminum* showed greatest nematicidal activity against *M. javanica* eggs as it completely reduced eggs at 100% w/v ($p<0.001$) (Table 2). Minimum nematicidal activity in aqueous extract at 100% w/v was shown by *M. fragrans* (flower) followed by *F. vulgare, P. nigrum, S. cereale, P. somniferum, M. caryophyllus* and *M. fragrans* (fruit). The influence of ethanolic extract of spices against *M. javanica* eggs were examined and results showed that *C. annum, C. tamala* and *C. longa* completely inhibited hatching of eggs at 1000 ppm compared to control whereas *C. longa* showed strong nematicidal activity against *M. javanica* eggs at 500 and 250 ppm after 72 hrs of exposure. Minimum inhibition of eggs of *M. javanica* was observed in *A. sativum* followed by *C. carvi* and *M. fragrans* (fruit).

The potential of spices in both aqueous and ethanol extract against *M. javanica* second stage juveniles were observed which showed that *C. longa, N. sativa* and *P. nigrum* caused maximum mortality of juveniles at 100% w/v of aqueous extract. Significant ($p<0.001$) mortality percentage of juveniles of *M. javanica* exerted maximum lethal effect at 100% w/v in aqueous and 1000 ppm of ethanol extract of spices as compared to control. Ethanol extract of *C. tamala* and *P. nigrum* at 1000 ppm showed maximum killing of juveniles followed by *M. fragrans* (flower and fruit), *C. sativum, N. sativa, M. caryophyllus, F. vulgare, C. longa, C. cyminum, C. carvi, A. sativum* and *C. annum* (Table 3). Of the different concentrations used, 1000 ppm showed more significant result in contrast to 500 and 250 ppm in ethanol extract whereas in aqueous extract, 100% w/v were found more effective. Ethanol extract showed more significant results as compared to aqueous extract.

Table 2. Effect of spices aqueous extract on hatching and mortality % of *Meloidogyne javanica* at different time intervals.

Treatments	Time (hrs)											
	Hatching %						Mortality %					
	50 %			100 %			50 %			100 %		
	24	48	72	24	48	72	24	48	72	24	48	72
Control	8	36	58	8	36	58	0	5	15	0	5	15
Allium sativum	2	6	12	0	2	4	8	16	27	13	23	33
Capsicum annum	0	7	12	0	0	10	6	19	25	14	21	31
Carum carvi	6	8	19	0	0	3	6	19	30	11	25	35
Cinnamomum tamala	0	0	7	0	2	4	8	13	24	9	19	28
Coriandrum sativum	2	7	10	0	3	11	7	14	21	11	24	31
Cuminum cyminum	2	6	17	0	0	0	7	18	27	12	24	30
Curcuma longa	0	0	5	0	0	5	9	20	30	14	25	36
Foeniculum vulgare	0	2	15	0	4	14	9	17	26	9	20	28
Myristica fragrans (f)	0	8	17	2	12	15	7	14	25	10	20	29
M. fragrans (fr)	5	9	14	4	4	12	6	20	29	15	23	33
Myrtus caryophyllus	4	6	25	2	6	12	8	18	27	13	20	30
Nigella sativa	0	10	15	0	0	5	9	20	32	13	27	36
Papaver somniferum	0	8	13	3	7	12	7	18	29	12	21	31
Piper nigrum	3	6	50	3	4	13	10	15	27	10	25	36
Secale cereale	5	9	14	0	0	12	6	14	23	10	22	26
Zingiber officinale	0	4	6	0	2	3	7	15	26	12	23	32
$LSD_{0.05}$Treatment		1.66			1.44			3.11			3.49	
$LSD_{0.05}$Time		0.70			0.60			1.30			1.46	

Present result showed that aqueous extract of *Z. officinale* was effective in reducing the hatching of eggs of *M. javanica.* Rhizome of Z. *officinale* when subjected to steam distillation yields ginger oil, which is the major constituent. These compound exhibited activity against *Spilosoma obliqua* and the fungus *R. solani* (Manjree *et al*., 2001). *Capsicum annum* extract has been found to show activity against the cercria of *Schistoma mansion*. Different *Capsicum* spp., tissue (fruit and leaves) and *Heliopsis longipes* root extract have previously been assayed for antimicrobial activity (Chewicz & Thorpc 1996; Gutierrez – leugo *et al*., 1996). Black pepper was found to be effective in the hatching and mortality of *M. javanica.* Parmer *et al*., (1997) isolated the compound piperolein alkaloid, pipernonaline. The acetone extract of pepper showed the presence of 18 component accounting for 75.59% of the total amount. Piperine (33.53%), piperolein B (13.73%), piperamide (3.43%) and guineensine (3.23%) were the major compounds. Present results showed that *Coriandrum sativum* was effective on *M. javanica.* Linalool is the main compound present in it and linalool (59.6-71.6%) has been reported as the main constituent of essential oil of *Coriandrum* fruit which are antimicrobial. The black seed (*Nigella sativa*) oil also contain about 0.5-1.5% volatile oil, including nigellone and thymochinone which are resposible for the anti-histamine, anti-oxidant and anti-infective effect. Present observation showed that *Cinnamomum tamala* reduced the hatching and increased the mortality rate of *M. javanica.* Essential oil resembles to cinnamon leaf and contains phellandrene and 78% eugenol. Nematicidal activity of spices showed promising results in the control of root knot nematode (*M. javanica*). Present observation showed that aqueous and ethanol extracts of spices were found to be effective in reducing the hatching and mortality of eggs of *M. javanica.* Siddiqui *et al*., (2000) also found that ethyl acetate and hexane fraction at different concentration showed mortality of *M. javanica*. Similarly Tariq *et al*., (2007) on *Rhizophora mucronata* and Mehdi *et al*., (2001) on *Avicennia marina* and *R. mucronata* observed the significant mortality of *M. javanica* by aqueous, methanol and chloroform extracts.

Table 3. Effect of spices ethanol extract on hatching and mortality % of *Meloidogyne javanica* at different time intervals.

Treatments	Time (hrs)																	
	Hatching %									Mortality %								
	250 ppm			500 ppm			1000 ppm			250 ppm			500 ppm			1000 ppm		
	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Control	8	36	45	8	36	45	8	36	45	0	5	15	0	5	15	0	5	15
Allium sativum	0	10	15	0	9	14	0	3	10	6	16	27	9	18	30	13	25	35
Capsicum annum	0	3	5	0	0	3	0	0	0	9	15	26	13	18	28	13	22	33
Carum carvi	0	10	13	2	10	12	1	7	9	6	16	29	11	20	33	15	25	35
Cinnamomum tamala	0	0	4	0	0	3	0	0	0	9	16	26	9	20	30	15	27	38
Coriandrum sativum	0	3	6	0	0	5	0	0	3	8	15	23	8	16	28	11	25	36
Cuminum cyminum	1	2	3	0	0	2	0	0	2	8	18	29	9	20	33	15	26	35
Curcuma longa	0	0	0	0	0	0	0	0	0	5	17	29	10	18	33	13	26	35
Foeniculum vulgare	0	3	9	0	2	7	0	0	4	8	12	19	8	18	25	14	24	35
Myristica fragrans (f)	0	4	12	0	3	7	0	0	6	6	16	25	11	20	31	14	26	37
M. fragrans (fr)	3	7	13	1	7	12	0	5	8	6	17	30	11	19	33	14	26	37
Myrtus caryophyllus	0	3	5	0	3	5	0	0	2	7	14	23	10	13	24	15	24	35
Nigella sativa	0	5	10	0	3	7	0	0	5	9	13	25	12	21	34	15	24	35
Papaver somniferum	1	11	12	6	1	10	1	3	7	7	13	23	10	14	29	14	25	33
Piper nigrum	0	3	6	0	0	3	0	0	3	11	15	29	8	15	33	9	22	38
Secale cereale	2	4	9	0	3	8	0	0	3	7	10	19	13	16	27	13	23	32
Zingiber officinale	0	2	6	0	2	4	0	2	2	10	16	27	9	19	29	14	23	33
$LSD_{0.05}$Treatment					1.55									15.22				
$LSD_{0.05}$Time					0.37									3.69				

The aqueous extracts of *C. carvi* and *Z. officinale* showed maximum nematicidal activity against *M. javanica* eggs whereas *F. vulgare* at 1000 ppm of ethanol extract showed maximum killing of juveniles. Oka *et al*., (2000) observed the essential oils of *C. carvi*, *F. vulgare*, menthe rout at 1,000 mul/liter concentration showed the highest nematicidal activity. Sukul *et al*., (1974) identified the nematicidal properties of ginger (*Z. officinale*), chilli pepper (*C. annum*) and garlic (*A. sativum*).

The results presented in this paper lead to the conclusion that spices exhibited some nematicidal compound which cause reduction in egg hatching and death of second stage juveniles of *M. javanica.* There is therefore need to find out toxic compounds released by spices and carry out experiments *In vivo* for the control of root knot disease by *M. javanica*.

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