Research Article

Nematoxic Effect of (Polar and Non-Polar) Chemical Constituents of Cumin (*Cyminum* Spp.) Against *Meloidogyne javanica*

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Abstract | The nematoxic effect was determined for the extracts of *Cyminum* spp. (*Cuminum cyminum* and *Nigella sativa*) in methanol, chloroform, ether, butanol, and hexane on egg hatching and mortality of the juveniles of root-knot nematode *Meloidogyne javanica*. After 72 hours, *C. cyminum* (MeOH) demonstrated the maximum nematicidal activity in egg hatching (15%±2.10) of *M. javanica*, followed by *N. sativa* (Ether), 22.33±6.86%, at the lowest administered dose of 0.25 % concentration. After 72 hours of treatment, *C. cyminum* (MeOH) and *N. sativa* (BeOH) had a non-significant difference in nematotoxic impact (90.0±10.3, 88.0±7.2 mortality %, respectively) in 1% concentration. According to decreasing order, MeOH>BuOH>Chloroform>ether >Hexane, all fractionates showed positive reactivity and potential.

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Introduction

Root-knot nematodes (RNK) belongs to the genus *Meloidogyne* and are important crop pests because they infect a wide range of plant types. It is an endo-parasite that resides in the root system's secondary roots. This pest is distinguished by its formation of enormous cells, often known as gall cells. RNK has the potential to develop over 2,000 different plant species (Sasser and Freckman, 1987). They are responsible for half of the total damage to plants caused by all types of nematodes (Sasser, 1980). Several extracts of spices, including *Eucalyptus* spp., *Aegiceras corniculatum*, *Avicennia marina*, *Ceriopstagal*, and *Rhizophora mucronata* have been reported to kill these nematodes (Mehdi and Dawar, 2008; Samreen *et al*., 2009).

Spices are dried fruits, seeds, bark, roots, and other vegetative material that are used as a food additive in nutritionally insignificant amounts to enhance the flavour of food by eradicating or inhibiting the spread of dangerous bacteria (Burkill, 1985). Cumin seeds come from the herb *Cuminum cyminum*, a member of the Apiaceae family that includes parsley and is native to the South Asia and East Mediterranean. Cumin seeds are yellow–grey in colour and oblong in shape. Black seed (also known as black cumin;

Nigella sativa) is from Ranunculaceae family, annual flowering plant native to Southern Europe, North Africa, and Southwest Asia. Cumin seeds are nutrient-dense, containing a high amount of fat (especially monounsaturated fat), dietary fibre, and protein. Cumin seeds are abundant in B and E vitamins, as well as a range of other nutrients, including iron. Cuminaldehyde, cymene, and terpenoids are the primary volatile components of cumin (Bettaieb *et al*., 2011). Commercial insecticides were found less efficient than essential oil from cumin and it was proposed in innovative green formulations in crop protection against *E. fetida* and *H. axyridis* (Dawar *et al*., 2007). *Cumin cyminum* is used to keep harmful bacteria at bay by either inhibiting their growth or killing them (Burkill, 1985). Pmentha-1, 4-dien-7-al, gamma-terpinene, cumin aldehyde, and beta-pinene are all found in *C. cyminum* essential oil (Lacobellis *et al*., 2005). Cumin's biological activities have been related to the active components, such as terpenes, phenols, and flavonoids, and their concentration and activity (Burkill, 1985; Bettaieb *et al*., 2011).

Materials and Methods

Collection of plants material

Cumin seeds were purchased from open market of Quetta city. Collected seeds were washed with distilled water and shade dried for two week. The step was followed by crushing and grinding of sample to convert into fine powderand stored in airtight bottles.

Preparation of extracts

To make the ethanol extract, 100 ml of methanol was used to extract 10g of dried powder on a rotary shaker at 190-220 rpm for 24 hours. The methanol extracts were fractionated on the basis of polarity of various solvents such as Methanol, Butanol, Chloroform, Hexane, and, Ether and then concentrated in a rotary evaporator and dried in a vacuum oven at 45 °C, using 200 g of seeds per extract.

It was then passed through five layers of muslin fabric and centrifuged at 5000 g for 15 minutes. The solvent was evaporated, and the supernatant was collected, resulting in a final volume that was one-fourth that of the original (Parekh *et al*., 2005). For future investigation, it was maintained in sealed vials at 6ºC. To make 1 percent, 0.50 percent, and 0.25 percent concentrations, a suitable amount of extract was dissolved in DMSO (Faizi et al., 2011).

Nematode culture preparation

Roots of infected root-knot nematodes plants were obtained in Balochistan's Quetta/Jaffarabad city. The perenialpattern that Taylor and Netscher (1974) described was used to identify root-knot nematodes. The isolated root-knot nematodes were treated to single egg-mass culture preparation. Using a 3 %NaOCl solution, RKN eggs were extracted from infected plants. With minor modifications, the acquired egg samples were preserved according to the procedure given in McClure *et al*. (1973). The eggs suspension was placed onto cotton-wool filter paper and kept at 28±2°C for 48 hours to yield freshly hatched juveniles (J_2).

Egg hatching test

Two ml of cumin seed extract was poured into glass cavity blocks (diameter 2.5 cm) after which two egg-masses of *M. javanica* were placed to evaluate the influence of different cumin seed extracts for egg hatching activity of *M. javanica*. The control group consisted of egg-masses kept in distilled water. Each treatment was put thrice to the bioassay. A stereomicroscope at (4X) magnification was used to count the number of hatched juveniles after 72 hours of exposure. The treatments were done in triplicate on the glass cavity blocks, which were randomized at room temperature (28ºC). The toxicity of cumin seeds extract was determined using the average percentage of hatching eggs.

Mortality test

To measure nematicidal activity, different extracts of cumin seeds were made at the concentrations of 1%, 0.50% and 0.25%, then placed on 2.5 cm diam. glass slides, and permitted to evaporate the organic solvent for 48 hours. To determine juvenile mortality after 24 hours, 48 and 72 hours, 2ml of the juvenile suspension (100 juveniles/ml) was put to each glass slide and kept at room temperature (28º C). Each treatment was tested three times. The negative control was only water whereas carbofuran served as positive control. The number of deceased juveniles was counted under a low power stereomicroscope after 72 hours of exposure. Toxicity of different extracts of cumin spp. was determined by calculating the average proportion of dead nematodes. If nematodes did not move when probed with a fine needle, they were declared dead.

Statistical analysis

Data were subjected to analysis of variance (ANOVA)

for which Least Significance Difference (LSD) and Duncan's Multiple Range Test (DMRT) (Sokal and Rohlf, 1995) were carried out.

Reversible effects were not expected. Mortality percent was calculated by the following formula (Cayrol *et al.*, 1989).

$$\text{Mortality\%} = \frac{\text{Number of dead } J_2 \text{ in a treatment}}{\text{Number of total tested } J_2 \text{ in the same treatment}} \times 100$$

Calculating lethal concentrations and toxicity index for *M. javanica* J_2, the following formula to calculate the toxicity index of each plant extract was used (Sun, 1950).

$$\text{Toxicity index} = \frac{LC_{50} \text{of the highest effective extract}}{LC_{50} \text{ of each extract}}$$

Inhibition rates were calculated according to the following formula:

$$\text{Egg hatching inhibition\%} = \frac{\text{No of hatched } J_2 \text{ in control} - \text{No: of hatched } J_2 \text{ in treatment}}{\text{No of hatched } J_2 \text{ in control}} \times 100$$

Graphs were plotted from the reading of egg hatching inhibition % using Microsoft excel.

Result and Discussion

Cumin seeds have potential source of phytochemicals as nematoxins (Samreen *et al.*, 2009). This information has been re-examining by using two species of cumin against root-knot nematodes. Their fractionates of varied concentrations in polar and non-polar solvents at different time intervals, were tested against root-knot nematode in this study.

Data obtained from the experiment had shown lowest egg hatching and also capable of causing significant mortality of *M. javanica* juveniles by *C. cyminum*as as compared to *Nigella sativa*. Among all the examined fractionates of both cumin spp., *C.cyminum* (MeOH) showed maximum nematicidal activity by obtaining (85.00%±12.10) egg hatching inhibition% of *M. javanica* eggs followed by *N. sativa* (Ether), 78.3%±12.23 at lowest applied dose i.e 0.25% concentrations after 72 hours (Table 1). Similarly mortality test was also significantly high for *C. cyminum* (MeOH) as compared to *Nigella sativa* (MeOH). Aqueous extract of *C. cyminum* had the best nematicidal action against *M. javanica* eggs, entirely reduced eggs at 100% w/v (Samreen *et al.*, 2009). Other spices, such as black pepper, were found to control *M. javanica* hatching and mortality. Parmer et al. (1997), identified Pipernonaline, a piperolein alkaloid. The presence of 18 components in the acetone extract of pepper contributed for 75.59 % of the total amount. The main chemicals were piperine (33.53 %), piperolein B (13.73 %), piperamide (3.43 %), and guineensine (3.23 %). The volatile oil in black seed (*Nigella sativa*), oil contains about 0.5-1.5 percent nigellone and thymochinone, which have anti-histamine, anti-oxidant, and anti-infective properties. Present observation showed that *C.cyminum* (MeOH) and *N. sativa* (BuOH) have non-significant difference and maximum nematoxic effect (90.0±10.3, 88.0±7.2 mortality percentage, respectively) in 1% concentration after 72 hours of treatment. Damasius *et al.* (2007) calculated the antioxidant activity of ethanolic and aqueous cumin extracts and it was shown that the aqueous extract had comparatively higher DPPH activity than the ethanolic extract which is consistent with the findings of our investigation. Previous researches have shown similar results, indicating that cumin's phenolic components play a key role in its nematicidal capability.

M. javanica was likewise killed by ethyl acetate and hexane fractions at varied concentrations, according to Siddiqui *et al.* (2000). Similarly, Mehdi *et al.* (2001) on *Avicennia marina* and *R. mucronata* and Tariq *et al.* (2007) on *Rhizophora mucronata* found that water, methanol, and chloroform extracts killed *M. javanica* significantly.

Aqueous extracts of *Z. officinale* and *C. carvi* displayed the most nematicidal activity against *M. javanica* eggs, while an ethanol extract of *F. vulgare* at 1000 ppm killed the most juveniles. At 1000 l/liter concentrations, the essential oils of *F. vulgare*, *C. carvi* and menthe rout displayed the highest nematicidal activity, according to Oka *et al.* (2000). Phellandrene and 78 % eugenol are found in this essential oil, which are similar to a cinnamon leaf. Spices with nematicidal activity showed promise in the control of root-knot nematode (*M. javanica*). The interesting finding of the present study was that the extracts in ethanol, chloroform, hexane, butanol and ether had different levels of nematoxic effect (Figure 1) Least activity was observed in hexane fraction of both type of seed *C.cyminum* and *N. sativa*at 1% after 72 hours

Table 1:.*Effect of cumin seeds Cuminumcyminum and Nigella sativa using polar and non-polar extracts on egg hatching and mortality of Meloidogyne javanica at different time intervals.*

S. No.	Sample	Conc. (%) [b]	Egg hatching %			R2	Mortality %[c]			LC_{50} [a] at 72 hrs	Toxicity index	R2
			24h[d]	48h	72h		24h[d]	48h	72h			
1.	*C.cyminum* (MeOH)	1	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.965	39.87±5.95Aa	49.00±5.83Ab	90.00±10.97Ac	0.57	0.00	0.873
		0.5	10.01±2.15 Ba	11.00±3.15Aa	13.33±2.36Aa	0.964	34.66±3.64Aa	34.33±5.84Ab	85.33±7.50Ac	1.04	0.548	0.853
		0.25	10.00±0.00Ba	12.32±1.98Aa	15.00±2.10 Aa[d]	0.934	29.33±5.90Aa	24.00±8.23Ab	85.33±5.42Ac	1.14	0.501	0.756
2.	*C.cyminum* (Chloro-form)	1	20.5±6.21Aa	43.33±3.45Ab	53.33±4.56Ab	0.912	26.66±5.34Ba	37.00±5.32Aa	69.66±6.97Ac	3.37	0.169	0.995
		0.5	20.1±2.34Aa	43.33±6.32Ab	60.00±3.25Ab	0.911	19.33±7.37Ba	33.33±4.65Aa	58.33±6.98Ab	4.91	0.116	0.994
		0.25	24.3±3.75Aa	29.66±4.75Ba	71.33±6.34Ab	0.834	17.33±4.64Ba	28.66±5.98Aa	58.66±6.99Ab	4.16	0.137	0.853
3.	*C.cyminum* (Ether)	1	15.33±5.36Aa	35.33±5.34Ab	50.66±10.78Ac	0.864	15.7±5.42Aa	35.66±6.32Bb	65.33±7.97Ac	3.04	0.187	0.775
		0.5	20.00±5.26Aa	41.66±8.86Ab	56.00±7.39Bb	0.930	14.0±5.83Aa	33.33±6.34Bb	49.00±4.45Ab	5.30	0.107	0.980
		0.25	21.66±4.36Aa	42.00±7.35Ab	70.33±6.38Bc	0.945	12.3±5.95Aa	26.66±4.95Bb	47.30±6.49Ab	5.48	0.104	0.912
4.	*C.cyminum* (BuOH)	1	45.33±5.43Ba	73.33±6.02Bb	86.33±9.24Ab	0.977	31.0±6.23Aa	50.32±7.32Ab	70.33±9.76Ab	2.65	0.215	0.936
		0.5	45.33±5.32Ba	75.66±4.86Bb	88.33±6.54Ab	0.999	27.7±4.95Aa	59.01±6.92Ab	69.66±7.97Ab	3.65	0.157	0.985
		0.25	45.33±5.32Ba	80.33±5.44Bb	89.00±7.98Ab	0.994	22.0±5.17Ba	38.33±6.12Ab	55.00±6.26Ab	3.72	0.153	0.990
5.	*C.cyminum* (Hexane)	1	50.66±4.36Ba	72.33±6.92Ab	85.00±7.45Ab	0.953	49.0±5.23Ba	54.33±6.91Ab	55.00±5.98Ac	3.16	0.180	0.930
		0.5	48.66±5.36Ba	78.00±6.12Ab	88.00±8.62Ab	0.040*	45.57±4.97Ba	54.33±5.12Aa	55.30±5.64Ab	4.53	0.125	0.898
		0.25	48.33±4.89Ba	78.33±6.60Aa	89.66±6.48Ab	0.065*	41.33±4.34Ba	53.66±4.69Aa	54.66±6.86Ab	4.36	0.130	0.977
6.	*N. sativa* (MeOH)	1	39.30±5.29Ba	72.33±6.86Bb	82.33±7.74Ab	0.077*	14.34±1.87Aa	56.33±5.56Ab	76.66±8.64Bb	3.99	0.142	0.876
		0.5	45.33±5.32Ba	76.66±6.94Bb	85.33±7.23Ab	0.056*	12.33±2.36Aa	52.33±3.76Ab	73.33±6.12Bb	3.85	0.148	0.959
		0.25	45.33±5.32Ba	80.66±5.44Bb	89.00±9.24Ab	0.853	11.33±3.25Aa	50.66±4.98Ab	60.00±6.64Ab	4.67	0.122	0.981
7.	*N. sativa* (Chloro-form)	1	42.00±3.12Ba	67.33±5.23Bb	77.00±7.48Ac	0.756	50.33±6.33Ba	55.33±5.76Bb	69.00±7.45Ab	3.65	0.166	0.836
		0.5	44.00±1.12Ba	68.33±4.21Bb	79.00±5.52Ac	0.995	41.66±5.45Ba	50.33±6.98Bb	585.3±8.56Ab	4.84	0.117	0.885
		0.25	45.66±6.32Ba	70.33±8.23Bb	82.33±6.43Ab	0.994	27.33±2.59Ba	38.00±3.43Bb	44.33±3.26Ab	5.023	0.113	0.990
8.	*N. sativa* (Ether)	1	17.66±4.31Aa	18.66±4.72Ba	19.00±6.23Ab	0.853	29.88±4.35Ba	46.00±5.87Bb	60.66±9.75Ab	4.99	0.114	0.876
		0.5	19.00±2.45Aa	20.33±6.32Ab	21.00±3.25Ab	0.775	23.30±5.98Ba	46.33±6.32Bb	58.33±8.98Ab	4.85	0.117	0.959
		0.25	19.33±4.87Aa	22.33±3.45Ab	22.33±6.86Ab	0.980	18.7±6.25Ba	32.66±7.86Bb	47.33±6.86Ab	5.672	0.100	0.981
9.	*N. sativa* (BuOH)	1	9.33±2.24Aa	33.33±4.78Bb	70.00±5.64A	0.812	45.33±4.44Ba	73.66±5.42Bb	88.66±7.37Ab	5.96	0.095	0.930
		0.5	10.00±324Aa	40.33±5.20Aa	75.33±8.74Ab	0.836	42.33±5.63Ba	68.33±5.86Bb	85.00±5.43Ab	2.36	0.241	0.898
		0.25	20.00±3.56Aa	53.33±4.38Aa	80.00±7.54Ab	0.885	13.03±5.34Ba	35.30±6.32Bb	41.00±7.35Ab	2.53	0.220	0.977
10.	*N. sativa* (Hexane)	1	19.66±5.73Aa	39.66±6.38Ab	69.33±6.39Ab	0.890	29.33±5.34Ba	43.33±6.86Bb	45.00±9.96Ac	4.37	0.130	0.999
		0.5	21.33±2.75Aa	46.33±4.97Ab	70.66±5.85Ab	0.830	27.33±6.25Ba	31.69±8.56Bb	42.33±7.34Ac	4.511	0.126	0.994
		0.25	24.33±3.86Aa	53.65±6.23Ab	74.33±6.54Ab	0.898	24.66±2.96Ba	29.33±4.74Bb	41.66±5.84Ac	5.0612	0.112	0.953
11.	Carbofuran	1	0.00±0.00Da	0.00±0.00Da	0.00±0.00Da	NA	100±0.00C	Dead	Dead	0.0065	NA	0.040*
		0.5	0.00±0.00Da	0.00±0.00Da	0.00±0.00Da	NA	100±0.00C	Dead	Dead	0.0065	NA	0.065*
		0.25	0.00±0.00Da	0.00±0.00Da	0.00±0.00Da	NA	100±0.00C	Dead	Dead	0.0065	NA	0.077*
12.	Water	1	100.00±0.00Da	100.00±0.00Da	100.00±0.00Da	NA	0.00±0.00Da	0.00±0.00Da	Dead	0.000	NA	0.000
		0.5	100.00±0.00Da	100.00±0.00Da	100.00±0.00Da	NA	0.00±0.00Da	0.00±0.00Da	0.00±0.00Da	0.000	NA	0.000
		0.25	100.00±0.00Da	100.00±0.00Da	100.00±0.00Da	NA	0.00±0.00Da	0.00±0.00Da	0.00±0.00Da	0.000	NA	0.000

a= values are in ppm.

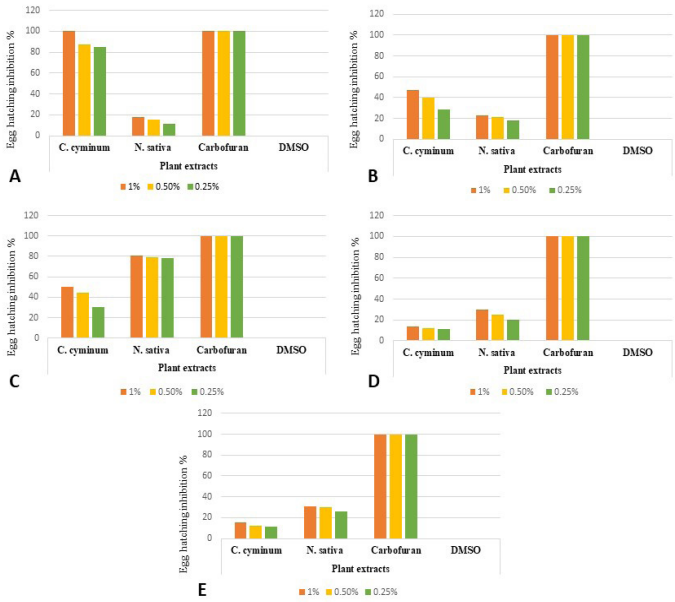

Figure 1: *Comparative graph of C.cyminum and N. sativa fractionates in different solvents, (A: MeOH, B: chloroform, C: ether, D: BuOH, E:hexane) for their performance in egg hatching inhibition% of Meloidogyne javanica after 72 hours of treatmnet.*

(55.0±5.98, 45±9.96 mortality %), respectively. The essential oil of *C. cyminum* included various quantities of monoterpenes and terpenes. The oil contained 23.6 percent hydrocarbons and 75.6 percent oxygenated monoterpenes (HMs, OMs), respectively (Johri, 2011). In comparison to the polar chemical elements of cumin spp., these non-polar molecules exhibited less promise. All other fraction also showed positive response but significance potential was in extract obtained from methanol. The positive potential according to decreasing order is mentioned below MeOH>BuOH> ether ≥Chloroform> Hexane.

Plants have a variety of secondary metabolites that might improve their efficiency. Phenolic molecules are one of them. Terpenes, monoterpenes, and sesquiterpenes are some of the other phytochemical elements (Skendi *et al.*, 2017). Compounds containing sugars per aglycone are found in a range of point conjugated forms, resulting in a wide variety of phenolic forms (Martinez *et al.*, 2015; Pimpao *et al.*, 2013). Phenolic compounds such as carvacrol, thymolhave been reported in many studies as being responsible for antimicrobial properties (Tajkarimia *et al.*, 2010; Burt, 2004). Such as cinnamic acid, catechol, caffeic acid, quinones have potential antimicrobial effects (Cowan, 1999). Flavonoids reported to have antimicrobial activity, form complexes with cell wall proteins (Cowan, 1999). Cumin polar fraction has been reported flavonoids, saponin, glucosinolatesand thiosulfinates showed antimicrobial effect. Numerous investigations on the antioxidant activity (Burits and Bucar, 2000), antibacterial activity (Hanafy and Hatem, 1991), antihyperglycemic impact, anticancer effect (Salomi *et al.*, 1992), and antinociceptive action of the essential oil of black cumin and thymoquinone have been undertaken (Abdel-Fattah and Kinzo, 2000; Shivakumar *et al.*, 2010). Cumin is a powerful antioxidant that suppresses radical-mediated lipid peroxidation by scavenging peroxy, hydroxy, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Manjree *et al.*, 2001). Cumin aldehyde, cuminal, -pinene, -terpinene, and safranal, which are found in essential oils, are responsible for antioxidant action (Surveswaran *et al.*, 2007). Pinocarveol, identified in the volatile cumin oil by El-Ghorab *et al.* (2010), may have antioxidant properties. Bettaieb *et al.* (2011) discovered terpinene, terpinene, and bornyl acetate as antioxidant components in cumin oils from stems, roots, leaves, and flowers, respectively. The nematode control potential of *C. cyminum* and *N. sativa* were found to be promising in the control of root-knot nematode (*M. javanica*). The results showed that plant has significant potential and thus can be a milestone in pesticide synthesis to protect plant crop.

Conclusions and Recommendations

It has been determined that the extracts of Cyminum spp. (*Cuminum cyminum* and *Nigella sativa*) in different polar and non-polar solvents such as methanol, chloroform, ether, butanol, and hexane has shown promising results to inhibit egg hatching and mortality of the juveniles of root-knot nematode *Meloidogyne javanica*. The findings demonstrated that plants have tremendous potential and can thus mark a turning point in the development of pesticides to safeguard plant crops.

Novelty Statement

The present research is the first time investigation of polar and non-polar chemical constituents of Cumin spp. against *Meloidogyne javanica*.

Author's Contribution

S.M. did the extraction of compounds and drafted the manuscript, A.S. did statistical analysis and reviewed the manuscript; E.I. did egg hatching test and

mortality test.

Conflict of interest

The authors have declared no conflict of interest.

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