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Research Article

Nematicidal Effects of Various Fractions of *Curcuma longa* against *Meloidogyne incognita* (root knot nematodes)

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ABSTRACT

Parasitism is an important limiting factor responsible to cause damage to agricultural production. Plant parasitic root knot nematodes attack several economic crops in Pakistan. Plant-parasitic nematodes considerably add huge losses to economies in the top tomatoes crop producing countries throughout the world, instead of controlling the main pathogenic nematode species as usual; one of the innovative strategies to control plant-parasitic nematodes would be to manage diversity in communities in order to lead them to be less pathogenic. The plants and their materials are one of the potential remedies for nematodes management. Turmeric (*Curcuma longa*) along with its several biological applications may serve as a biopesticide against Meloidogyne incognita, a nematode species. A bioassay guided isolation of various fractions of turmeric was subjected to nematicidal activity in comparison with *Azadirachta indica* against *Meloidogyne incognita* larvae at the concentration of 0.25, 0.5, and 1% for 48 hours. Alongside, Larvae and eggs of nematodes were inoculated around the tomato seedlings in experiments with turmeric in a growth chamber. The control contains water instead of turmeric. Root gall severity and final nematode population were suppressed significantly. It was observed that the use of turmeric is very important for selected plant parasitic nematodes management.

RESEARCH ARTICLE

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INTRODUCTION

The nematodes (Root Knot Nematodes) are known worldwide as a plant parasitic. These are the roundworms, aquatic and small in size. Nematodes are found in plant roots and water occupied soil areas. Root and plant tissues are source of feeding for nematodes. They are major reason of decrease in crop production. Nematodes can destroy all fruits and vegetable crops. Nematodes are the utmost ample animals in the world (Van den

Hoogen *et al.*, 2019) as well as leading constituents in soil (Bardgett and Van der Putten, 2014). *Meloidogyne* spp. were first reported on tomato in 1957 in the Eastern region of Saudi Arabia. Since then, many other plant parasitic nematodes have been reported causing serious losses to many economically important agricultural crops (Al-Yahya, 2018). In form of low crop production, almost 358 $ billion loss per year as per available data of 2010-13, of the farmer's community by plant-parastic nematodes in 37 different crops (Abd-Elgawad and Askary, 2015). Nematicides is a very effective strategy for the control over root knot nematodes (Hajihassani *et al.*, 2009; Medina Canales *et al.*, 2019); while since last few years, due to health and environmental degradation as well as contamination/toxicity of resources, many toxic chemicals were removed from world markets such as methyl bromide, etc. (Kim *et al.*, 2018; Xiang *et al.*, 2018). Due to severely attack by root knot nematodes, ultimately cause of huge loss quantity of crop production (Stephen, 2020). The capacity of exactly illustrate and classify *Meloidogyne* varieties is a critical step if the menace by *Meloidogyne* varieties for the production of crop in affected fields.

Around 80% loss of crop yield due to hit by root knot nematodes (Kaşkavalcı, 2007). To control over crop loss by root-knot nematodes some chemicals were replaced and successfully captured the markets with effective results (Desaeger *et al.*, 2017); still new chemicals are regularly getting their share against root knot nematodes management. Xiao *et al.* (2018), during research used *B. cereus* strain Jdm1 to control over *Meloidogyne incognita* in tomato. Good efficiency was seen in the field study and control efficiency up to 50% for gall index 30 DPI. The rhizosphere was immediately recovered after some impact during the treatment. The effect of root-knot nematodes is almost negative on all types of crops throughout the world. Present approach to overcome on root knot nematodes is not impressive and adequate for the entirely tide over (Forghani and Hajihassani, 2020). Almost three important techniques are common and effective against the plant parasitic nematodes, such as biological control, cultural control, and chemical control (Lamber and Bekal, 2002). All of these; the biological control against nematodes has potential and very effective in controlled environment (Lamber and Bekal, 2002). Crop rotation is a useful technique for bound the growth of nematode's growing population and can lessen nematode stages under damage threshold throughout the year (Lamber and Bekal, 2002). The 11 vegetable crops yield losses by nematodes were observed in one province of Pakistan; dramatically the losses ratio was very high and observed around 25.6% average, and 40% in tomato, as compared to same crops 6.6 average and only 0.2% of tomato in the United States of America (McSorley *et al.*, 1987; Safdar and Mckenry, 2012). Among the commonly found root-knot species, *Meloidogyne incognita* is pre-dominant. *Curcuma longa* (turmeric) belongs to Zingiberaceae (ginger family) is a small fleshy rhizomatous perennial herb of bright yellow to orange color in its root system. It is originated from southeastern Asia. India is the main cultivator of this spice (Damalas, 2011). It is an incredible natural antiseptic, disinfectant, anti-inflammatory, and analgesic. Turmeric, also known as the queen of spices, has received very little input in terms of nematological research. The study was aimed to investigate nematoxic impacts of turmeric on hatching and survival of RKN (*M. incognita).*

MATERIALS AND METHODS

Plant Material

Turmeric is cultivated for its underground rhizome. Fresh *Curcuma longa* was collected from local markets in Karachi. The plenty of water was used for cleaning the samples, dried (only rhizomes dried for one month at room temperature) by air and then homogenized to good powder and airtight jars were used for store.

General Experimental Procedures

Nematode inoculums and plant material

Artificial inoculums experiment of healthy plants with *M. incognita* were performed to study the effect on nematode population. As root knot nematodes commonly infect tomato plants; Starting from a single egg of nematode, the inocula were increased on tomato plants (Nico *et al.*, 2004; Wesemael *et al.*, 2011). Inoculum consisted of second-stage juveniles (J2s) and eggs, which were extracted from tomato roots by the NaOCl procedure (Hussey and Barker 1973). The population density of *M. incognita* was determined by 10 replications of 1-ml aliquots of the inoculum's suspension. In initial stage 90 nematodes were taken and 90 larvae have been studied.

Nematode population analysis

After incubation period of two months, the roots of individual plants were washed make them free from soil. The root galling severity in the *Meloidogyne* infected tomato plants was assessed on a 0–5 rating scale according to the percentage of galled tissue, in which 0=0–10% of galled roots; 1=11–20%; 2=21–50%; 3=51–80%; 4=81–90%; and 5=91–100% (Neher and Campbell 1994; Hoeksema *et al.*, 2000). Nematodes from 100 cm^3 samples of infested potting mixtures and from 5 g samples of roots were extracted by centrifugation (Palomares Rius *et al.*, 2012), as described for inoculum preparation. Final population densities of nematode were estimated by using extracted nematodes. (Nematodes were considered dead when there is no movement upon checking with a fine needle physically).

Growth chamber experiment

The research work was carried out in the growth chamber with controlled environment at 30°C ± 5°C, 50-60% humidity in summer season.

Extraction and isolation

Roots of turmeric (5 kg dry weight) were air dried and extracted with ethanol (100 L) which was then concentrated to a gum (710 g). The gum was dissolved in distilled water and extracted thoroughly with petroleum ether (40 L) and then with hexane (60 L). After that hexane, soluble portion was dried (46.8 g). The remaining aqueous layer was acidified with acetic acid to pH 3 and then, extracted with CHCl3. The CHCl3 soluble portion was dried (158.0 g). Extraction of aqueous extract with ethyl acetate (5 L) yielded an impure mixture of ethyl acetate extract.

Nematicidal activity

The research work was carried out in a controlled environment at 28±2°C. For maintaining of stock culture, fresh eggs were used in root tissues of tomato for the hatching of egg. After 48 hours the larvae were seen from the sample of egg masses at

30°C incubation for larvae mortality studies. (We did experiments in sterilized petri dishes. 10 ml of each extract was used and 1 ml of larwae suspension was poured into the labeled dish. (1 ml volume was found to have ± 90 larwae). Assay was performed in triplicate. Water with nematodes larwae was taken as a control). Standard nematicide *Azadirachta indica* (0.05%) was taken for comparison and tap water taken as control.

RESULTS and DISCUSSION

Turmeric has a wide spectrum of biological activities. The nematicidal activity of extracts of *C. longa* was performed and the results of various fractions have been shown in Table 1. It was observed that antinematal activity of turmeric is almost insignificant against *M. incognita* as maximum mortality was achieved up to 20%.

The maximum antinematal activity was observed when 1% concentration of extract was used and the % mortality was found in the range of 15-24%. Similarly, the range of 8-14% was found with 0.5% extract solution, while 2-11% with 0.25% solution of all fractions. According to Figure 1, the comparison of all fractions shows that chloroform fraction has greater mortality followed by ethyl acetate and methanol fraction has the least mortality among the tested fractions. The order of antinematal activity of tested turmeric fractions was observed as follows.

Table 1. Nematicidal activity of different fractions of rhizome extract isolated from. *Curcuma longa* on the larval mortality of *M. incognita* (R). Percent mortality/concentration after 24 h.

Fractions (%)	24 h 1	48 h 1	24 h 0.5	48 h 0.5	24 h 0.25	48 h 0.25
Crude extract	15	20	10	12	7	9
Methanol	10	18	8	9	4	6
Chloroform	20	24	14	10	10	11
Ethyle acetate	16	20	12	8	8	9
n-Hexane	15	18	8	12	2	2

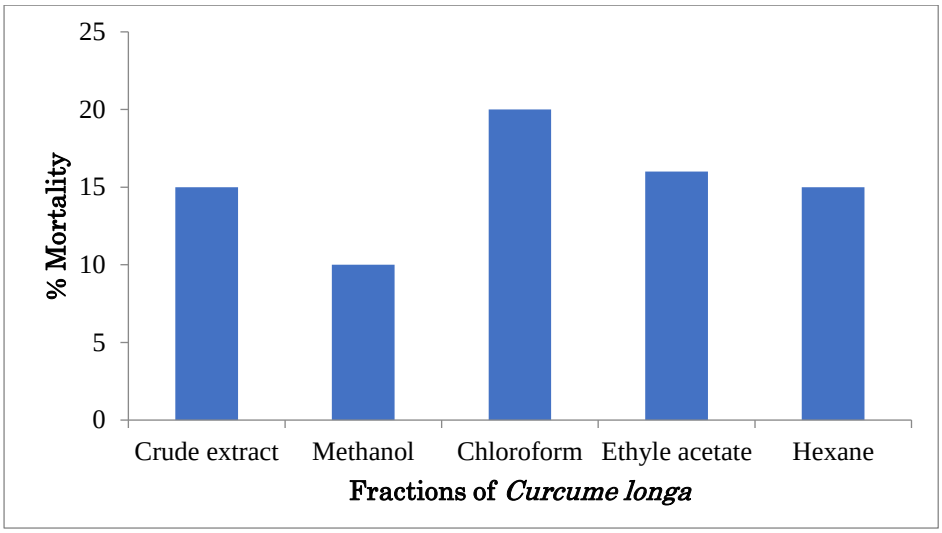

Figure 1. Comparative mortalities of different fractions of *C. longa* against *M. incognita* (mean ± S.E., n = 3) in 24 hours.

Chloroform> Ethyl acetate> Crude extract > n- Hexane > Methanol

Figure 2 shows the comparative viability of all fractions with respect to time and concentration against the selected nematode.1% concentration of all fractions showed significant mortality results. The chloroform extract showed maximum mortality in all concentrations i.e., for 1%, 0.5% and 0.25% concentration, the mortality rate is 20%, 14% and 10% for 24 hours and 25%, 10% and 11% for 48 hours respectively. Ethyl acetate fraction showed 16% and 20% mortality rate with 1% concentration and the lower concentrations showed moderate mortality. n-Hexane fraction showed moderate mortality results but methanol fraction has the least activity against nematodes.

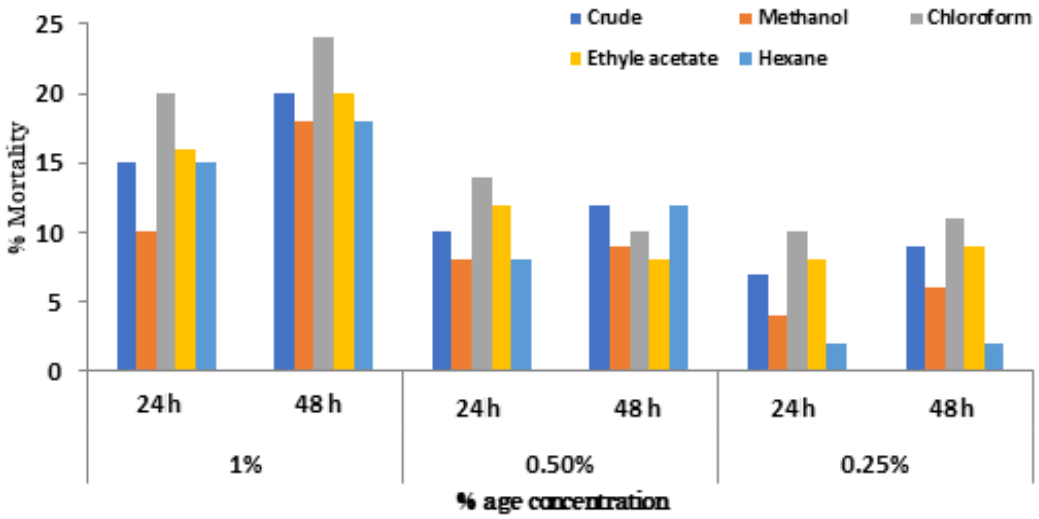

Figure 2. Comparison nematicidal activity of various fractions of turmeric on the larval mortality of *Meloidogyne incognita* (root knot nematodes) with respect to time. (mean ± S.E., n = 3).

The results reveal that the chloroform extract has more potent and active components against root knot nematodes. It may be deduced that the mortalities of all fractions at 24 hours have considerably increased at the concentration of 1%. Moreover, the effect of time on mortality was observed almost insignificant at the concentrations of 0.5 and 0.25%. Many essential oils and phytochemicals like monoterpenes and leaf extracts of *Lantana camara* for their nematicidal activity have been reported (Ahmad *et al.* 2010; Echeverrigaray *et al.* 2010). The active components would be isolated and analyzed in future which may be used as eco-friendly biopesticides against root knot nematodes. Being the important components in our diet, the turmeric components will not dangerous for man and environment. They will be helpful in controlling the harmful effects of nematodes in future.

CONCLUSION

The antinematicidal effect of turmeric against root knot nematodes was studied. Chloroform extract showed significant antinematicidal activity in all concentration range, which leads to the conclusion that the study may be helpful after isolation and analysis of active constituents from turmeric, these bioactive components, may be used as eco-friendly biopesticides against root knot nematodes from chloroform extract. It is suggested that nematode suppression in this research may rely on nematoxic compounds released from the composted material. Turmeric agro-industrial waste is sufficient for nematode management. Additionally, this approach may be suitable to integrate with other management tactics to lessen the production reduce in vegetable and woody crops caused by *Meloidogyne* spp.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no conflict of interest

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Uzma Rashid: All extracts preparations, data evaluation and manuscript writting.
Aijaz Panhwar: Compilation, literature review and corresponding of the article.
Aysha Farhan: Experimental work.
Musarrat Akhtar: Nematology expert.
Nusrat Jalbani: Technical reviewer.
Durdana Rais Hashmi: Literature survey, and reference writing.

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