Research Article

Syzygium cumini Leaf Extracts as a Nematicide against Root Knot Nematode *Meloidogyne javanica* in Tomato

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Abstract | Root-knot nematode (*Meloidogyne* spp.) is a pest of tomato *Solanum lycopersicum* (L.), causing reduction in yield and quality. The use of natural plant products as biopesticides in the prevention and control of pests and diseases has been increasing. Nematicidal activity of "Black plum *Syzygium cumini*, leaf aqueous, ethanol and methanol extracts were investigated for managing the population of *Meloidogyne javanica*, in tomato. In laboratory conditions second-stage juveniles (J2) mortality was recorded after 24, 48 and 72 h of incubation in concentrations of 0.25, 0.5 or 1%. Maximum mortality was achieved with ethanol extracts followed by aqueous and methanol extracts. In the greenhouse, leaf extracts at 60 days after inoculation produced reduction in root galling, egg masses, and nematode populations in plants in treated containers compared to untreated plants (control) with values increasing as time and concentration increased. During sixty days of treatment, no significant effect was recorded in plant height and shoot weight for extracts and concentration. Extracts of *S. cumini* may have potential for controlling *M. javanica* on tomato.

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Introduction

Root-knot nematodes (*Meloidogyne* spp.) are soil-borne pathogens, their infective second stage juveniles (J2) invade and penetrate tomato (*Solanum lycopersicum* L) roots, establish a permanent feeding site, and form knots in root tissues affecting the uptake of nutrients and water (Eloh *et al.*, 2015). The population of plant parasitic nematodes in agricultural fields can be minimized through proper sanitation, use of nematode free planting stock, natural enemies, resistant cultivars, cultural practices, and pesticides (Halbrend and LaMondia, 2004; Greco and Esmenjaud, 2004). The expenditure and registration of new, and listing, a potential new synthetic nematicide are obstacles. There is a need for new field control measures for this pathogen on tomato (Nikoletta and Pierluigi, 2012). Effective, safe, and eco-friendly pesticide, could possibly provide a viable solution. Thus, the development and production of environment-friendly botanical pesticides and their water-based formulations, to replace the highly toxic agro-chemicals and unsafe formulations, has gained significant importance towards developing appropriate strategies for crop protection (Ismail *et al.*, 2020).

Compounds derived from plants have novel target points of action, the chance of developing resistance is low (Nguyen *et al.*, 2015), and there is no residual

effect (Ujvary, 2001). *Syzygium cumini* L. is reported to have antibacterial and anti-fungal activity (Shafi *et al*., 2002; Oliveira *et al*., 2007; Tewari *et al*., 2017). Studies on the nematicidal activity of *S. cumini* have received little attention and hence, the present investigation was undertaken to evaluate nematicidal activity of extracts of *S. cumini* leaves against *Meloidogyne javanica* (Treub, 1885; Chitwood, 1949) in tomato.

Materials and Methods

Plant material

Leaves of *S. cumini* were collected in July 2018 from healthy trees located at the National Nematological Research Center, University of Karachi (NRRC), Karachi, Pakistan.

Nematode culture

Meloidogyne javanica were obtained from a pure culture maintained at the greenhouse of NNRC by single egg masses on tomato *Solanum lycopersicum*. Perennial pattern analysis was adopted for further confirmation about pure population of root knot nematodes Infested plants, with roots galls, were washed free from soil to pick egg masses with fine forceps. Fifty uniform size egg masses were hatched in spring water at 28±2˚C. Freshly hatched J2 were used for these studies.

Chemicals

For extraction of products from the leaves pure reagent grade ethanol (99.9%) and methanol (99.9%) (Sigma Aldrich, St. Louis, MO) were used.

Nematicidal activity

Fifty uniform sized fresh, healthy leaves of *S. cumini* were washed with water, air dried and crushed by mortar and pestle. Leaf powder was separately soaked in 200 mL of each solvent, or distilled water, for 24 h to extract soluble ingredients. The extracts were filtered through two layers of Whatman filter paper. Collected solvents were evaporated using a rotary evaporator at 50˚C. Crude extracts (50 mg·mL $^{-1}$) were re-dissolved in 5% dimethyl sulfoxide (DMSO), for nematicidal activity a measured amount of stock solution was added to attain 0.25, 0.5 or 1% Concentrations. One hundred root-knot J2 were hand-picked for each dose, placed in Petri dishes (5 cm dia) and kept at 25±2˚C, all treatments were repeated thrice. Observations were made after 24, 48 or 72 h with a stereoscopic microscope to determine percent nematode immobility; 5% DMSO was used as a control. Nematodes were considered deceased if they failed to respond to touch. Regained mobility was confirmed by transferring them to spring water.

"Seeds" of tomato, cv. Money Maker, were sown in an Earthen pots (15 cm dia) disinfected with 0.4% formalin containing sterilized sandy clay loam soil (sand, silt and clay 1:1, v:v). Pots were irrigated with approximately 200 mL at sowing and then at a 2 day intervals. At 15 days after germination, plants were thinned to 1/pot and watered daily. Seedlings were inoculated with approx. 2000 newly emerged J2 in the rhizosphere of each plant by making 3-4 holes in the soil.

Aqueous, ethanol and methanol crude extracts were dissolved in 5% DMSO. The stock solution (50 mg·mL^{-1}) of each was used for preparation of 0.25, 0.5 or 1% in 50 mL of water. There were four replicates of each treatment and an uninoculated one for control. Pots were arranged in a completely randomized block design on a greenhouse bench, at NNRC. The experiment was completed at 60 days post-inoculation. Plants were carefully removed from pots and roots washed with water to remove adhering soil particles. Plant height, fresh root weight, fresh shoot weight, numbers of egg masses and galls per root system were determined. Nematode populations in 250 g of soil were determined with a Baermann's funnel technique (Baermann, 1917).

Data analysis

Data were subjected to ANOVA multifactor analysis of variance in SAS (ver. 9.1, SAS Institute, Cary, NC). If the interaction was significant it was used to explain the results. In an interaction the data are analyzed with Least Squares Means and the means separated with Tukey's test. Line graphs, regression analysis and regression equation were developed with MS Excel (Microsoft, Redmond, WA USA).

Results and Discussion

All the treatments showed nematicidal potential towards *S. cumini* against root-knot nematode *M. javanica*. After 24 hours of treatment, ethanol extract caused maximum percentage J2 mortality (60) at its 1 % concentration (Figure 1). Aqueous and methanol caused (50, 30) J2 mortality respectively at same concentration (Figures 2, 3). J2s mortality lowered

with the decrease in concentration from 1% to 0.25 %, so treatments effect varied with concentrations. Juvenile mortality percentage was maximum in ethanol extract followed by aqueous and methanol extract at their all concentrations after 72 hours. After 72 h of ethanol, aqueous and methanol extracts treatment percentage mortality of J2 was (90, 80, 78) in 1 % concentration while at minimum 0.25 % concentration percentage mortality was (70, 50, 40) respectively. Effect of concentration on mortality percentage showed significant effect with the increase in time interval. A linear relationship was found in mortality and concentrations and regression equations were also drawn after time duration.

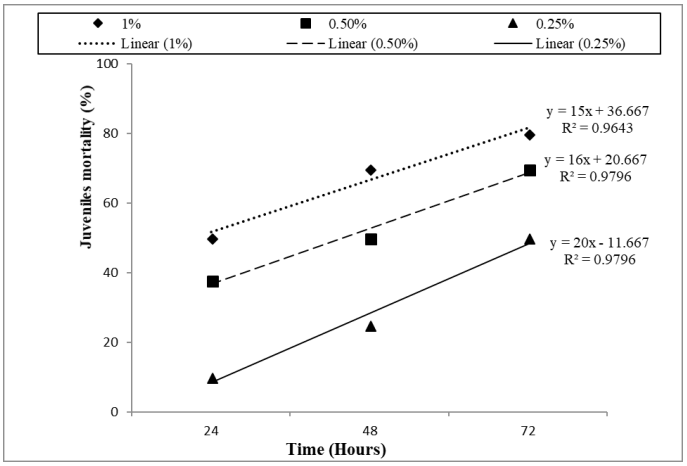

Figure 1: *Relationship between % Meloidogyne javanica (Treub, 1885) Chitwood, 1949 juvenile mortality and time (hours) for concentrations of aqueous extract from Syzygium cumini (L.) leaves.*

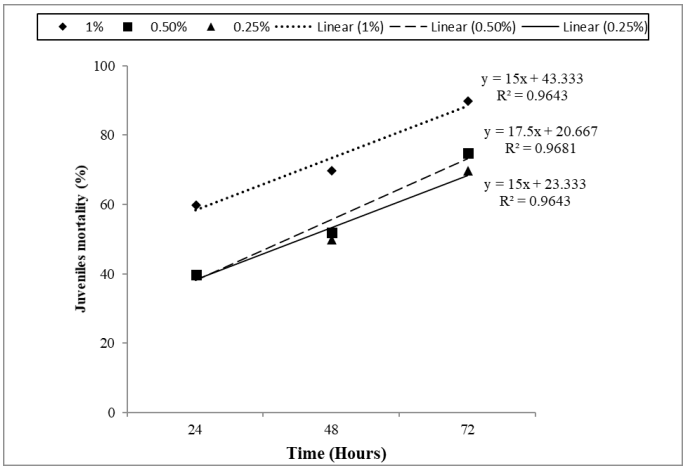

Figure 2: *Relationship between % Meloidogyne javanica (Treub, 1885) Chitwood, 1949 juvenile mortality and time (hours) for concentrations of ethanol extract from Syzygium cumini (L.) leaves.*

All three extracts and its concentrations caused a significant reduction in the number of galls. A significantly lower number of galls were observed in ethanol extract at 1% concentration (32.05) as compared to control treatment (130.10). The minimum number of egg masses were recorded in ethanol extract followed by aqueous or methanol extract at all concentrations. A significant improvement in treated plants height was recorded as compared to infected plants (control). Analysis of variance response to extract and concentration and their interaction on infection, plant development, and nematodes in the rhizosphere are showed that significant difference were found galls/root system, egg mass/root system and nematodes population in 250 g soil and non-significant difference found in plant height (cm) and root and shoot weight (gm). Galls root per system, egg masses, root weight and nematode population significantly reduced at all treatment as compared to control (Table 1) During 60 days time period effect of concentrations and treatment showed non-significant effect in plant height and shoot weight of tomato.

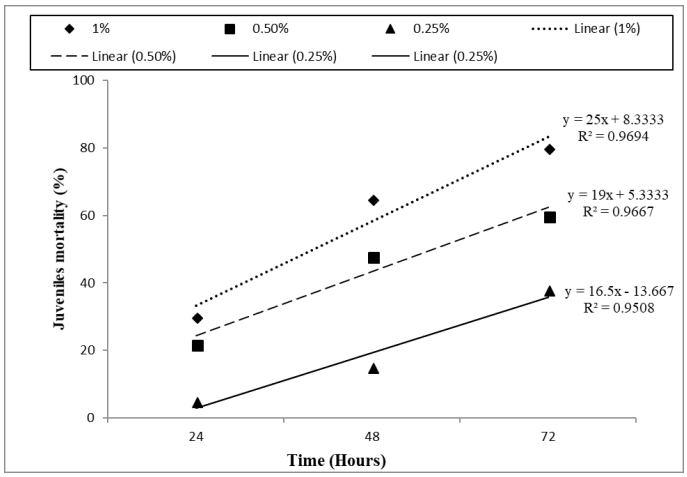

Figure 3: *Relationship between % Meloidogyne javanica juvenile (Treub, 1885) Chitwood, 1949mortality and time (hours) for concentrations of methanol extract from Syzygium cumini (L). leaves.*

Bioactive natural products are safer to the environment and are reported to possess broad spectrum activity against diverse insect pests and pathogens and also showed great potential to reduce nematode density (Chitwood, 2002). A large number of plant origin compounds have been analyzed for nematocidal activity. Plants defends itself against invaders by exuding and producing secondary metabolites. Investigations and new findings of phytochemicals involving the chemical-mediated interactions between a plant and other organisms in its environment have its roots in allelochemistry (Nikoletta and Pierluigi, 2012). These findings contribute important information

Table 1: *Interaction[a] effect of different extracts concentration of Syzygium cumini leaves in vivo against Meloidogyne javanica on plant growth parameters of Solanum lycopersicum.*

Extract	Concentration (%)	Galls/ root system	Egg masses/ root system	Plant height (cm)	Shoot weight (g)	Root weight (g)	Nematode population in 250 g soil
Aqueous	0.25	92.05 a	62.05 a	28.2 c	35.20 c	27.30 a	1588 a
	0.5	80.12 b	57.07 b	30.5 b	39.90 b	25.40b	1324 b
	1	72.08 c	40.25 c	38.40 a	42.50 a	23.10 c	1010 c
LSD		3.23	2.29	2.32	3.26	2.76	5.43
Ethanol	0.25	78.06 a	59.08 a	32.02 c	43.20 c	20.22 a	1250 a
	0.5	65.15 b	39.2 b	38.04 b	48.90 b	18.16 b	1012 b
	1	32.05 c	20.4 c	42.09 a	50.50 a	15.07 c	854 c
LSD		2.93	2.28	2.21	2.39	2.18	5.98
Methanol	0.25	85.07 a	52 a	28.02 c	38.25 c	24.02 a	1340 a
	0.5	66.14 b	45 b	30.08 b	42.15 b	20.08 b	1199 b
	1	42.05 c	28 c	35.07 a	45.29 a	18.12 c	920 c
LSD		2.72	3.43	2.43	2.73	1.83	5.20
Control		130.10	77.1	18.10	32.10	28.0	3500

[a] means separated with Tukey's test; [b] values in columns followed by the same letter are not significantly different, P>0.05.

that extracts of *S. cumini* reduced the penetration of infective J2. The effectiveness of phytochemical as a management strategy is economical, which revolves the utility of the alternative chemicals and nonchemical methods for nematode control practices accessible to a specific grower (Chitwood, 2002). Bio based pesticides are commonly used as alternatives to synthetic compounds in organic agriculture. In conclusion *Syzygium cumini* metabolites showed nematicidal potential, researchers also reported its antibacterial, anti-inflammatory and antioxidant effects (Salim *et al*., 2009). Current research is focused on the potential use of *S. cumini* leaf extracts for the improvement of new management strategies. Pests attacking agricultural commodities are major threat to crops. These pests are primarily controlled through chemical pesticides, which have caused harmful effects on the environment, contaminated ground water, causing poisonous residues on food chain which are threatening human and animal health. The present study reveals that *S. cumini* have some bioactive natural products which are safer to the environment (Chagas *et al*., 2015) and can be used as a nematicide for the control of destructive pests, root-knot nematodes.

Novelty Statement

This study revealed that leaf extracts of *Syzygium cumini* can be utilized for management of RKN and this method is environment friendly and free from hazards.

Author's Contribution

Salma Javed: Finalized the manuscript, performed data analysis.
Daniyal Siddiq: Carried out experimental work.

Conflict of interest
The authors have declared no conflict of interest.

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