



Bio-nematicidal effect of botanicals against root-knot nematode (*Meloidogyne incognita*) in tomato (*Solanum lycopersicum*)

NEERAJ¹, ANIL KUMAR², VINOD KUMAR³, MANISH KUMAR⁴, AJAY SINGH SINDHU⁵,
SEWAK RAM GOEL⁶ and GURPREET SINGH⁷

CCS Haryana Agricultural University, Hisar, Haryana 125 004, India

Received: 17 May 2019; Accepted: 30 May 2019

ABSTRACT

Nematicidal efficacy of five botanicals, viz. turmeric (*Curcuma longa*), marwatulsi (*Origanum majorana*), mentha (*Mentha arvensis*), aonla (*Phyllanthus emblica*) and jatropa (*Jatropha curcas*) was evaluated under *in vitro* and *in vivo* conditions for their larvicidal properties against the root-knot nematode, *Meloidogyne incognita* in tomato during 2014–16 at Department of Nematology, CCSHAU, Hisar. Methanolic and hexane extracts of turmeric (Rhizome) showed varying degrees of lethality to *M. incognita* at different concentrations under *in vitro* conditions. Per cent larval mortality and inhibition of egg hatching were found to be directly proportional to the concentration of extracts and exposure period. In *in vivo* experiments, growth parameters and nematode reproduction factors (number of galls/plant, number of egg masses/plant and final nematode population in soil) were evaluated post-treatment with chopped leaves of botanicals. The study showed that the treatment improved plant growth and reduced pathogen indices. The study suggests the representative role of botanicals in managing *M. incognita* in tomato plants.

Key words: *Meloidogyne incognita*, Plant extract, Root-knot nematode, Tomato, Larvicide

Plant parasitic nematodes (PPNs) are the major biotic constraints to global food security. The economic damage caused by PPNs has been estimated at over 173 billion dollars worldwide on annual basis (Elling *et al.* 2013). Root-knot nematode (*Meloidogyne incognita*) ranks first among the top 10 most significant damaging PPNs due to its worldwide distribution and parasitizing ability in almost all plants. Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown throughout the world for consumption in various forms. Several viral, bacterial, fungal and nematode pathogens infest tomato and cause diseases of economic consequences. Among different yield-limiting factors, parasitic plant nematodes, particularly root-knot nematode *Meloidogyne* spp. is a serious threat to tomato cultivation in many countries including India. *M. incognita* causes severe yield losses to the tune of 27.21% in tomato in India (Jain *et al.* 2007). Different nematode management strategies, viz. cultural, physical, biological, host plant resistance and chemical methods have been

used. Among all the techniques, chemical methods are considered to be most reliable for minimizing the losses and nematode population density but there are several issues related to use of nematicides such as groundwater contamination, residual toxicity and carcinogenic effects. Management through chemicals although very effective causes toxicological and environmental problems. Use of botanical extracts could be a new alternative for the future. These nematicidal plant extracts are usually considered safe in comparison to the active synthetic chemical compounds isolated from plants and microbes. Botanical pesticides are readily available in many places, often cheaper than their synthetic counterparts and their crude extracts are easy to prepare even by farmers. In the present study, stem and leaves of turmeric, marwatulsi, mentha, aonla and jatropa that are traditionally used for pest management have been tested for their bio-nematicidal effect.

MATERIALS AND METHODS

Plant material and culture maintenance: Seeds of tomato cultivar Pusa Ruby were surface sterilized with 70% ethanol, solution of 0.1% SDS + 0.1% HgCl₂, and subsequently washed four-times with water. Ten pots of 15 cm diameter were filled with pot mixtures and used for seed sowing. Twenty-days old plants were inoculated with surface sterilized 500 J2s/plant. Egg-masses were harvested 60 days post inoculation and placed over modified Baermann funnel for hatching.

^{1,4,5}Ph D Scholar (Neeraj17051992@gmail.com, mkverma9608@gmail.com, ajaysinghsindhu@gmail.com), Division of Nematology, ICAR-Indian Agriculture Research Institute, New Delhi; ³Assistant Professor (vinodnagal09@gmail.com), ⁶Professor (sewakramgoel@gmail.com), ^{2,7}Ph D Scholar (anilvats1409@gmail.com, ppecef@gmail.com), Department of Nematology, CCS Haryana Agricultural University, Haryana.

Isolation of crude extracts: The rhizome of turmeric was collected, washed in tap water to remove dust and other foreign material. It was shade-dried for 6 to 7 days, after complete drying the dried material was powdered and filled into the Soxhlet extractor (dry weight of the material was taken before filling). Extraction was carried out by using solvents of different polarities (i.e. methanol and hexane). Extraction was done for 72 h in each solvent system. Solvent was removed using rotary evaporator to get a pure crude extract, the weight of the crude extract was recorded. All the three solvent extracts were stored at 4–8°C in refrigerator separately in conical flasks until analysis and nematocidal bioassay.

Preparation of formulations and test samples: The formulation (1000 ppm) of different crude extracts was prepared in distilled water containing Exalin (4%) by mixing ingredients for half an hour using magnetic stirrer followed by serial dilution to obtain 500, 250, 125, 62.5 concentrations for nematocidal activity.

In vitro hatching assay: Hatching assay was done according to the procedure suggested by Saravanapriya *et al.* (2004). Fresh egg masses of *M. incognita* were collected from infected tomato roots and repeatedly washed four-times through sterile distilled water. Egg-masses of same sizes were collected and placed in Petri dish at four egg-masses/plate. Nematode eggs were poured in 24 wells culture plate and 1 ml of different concentrations (ppm) of test solution of turmeric crude extracts (Methanolic & Hexane) was added and mixed. Exalin (4.0%) in distilled water was taken as control. Observations on egg hatching were recorded at 3, 6, 8, 9 & 12 days of exposure in each treatment. Hatching per cent was calculated by counting the number of hatched and unhatched eggs. Data, thus, obtained were subjected to angular transformation and analyzed by applying CRD factorial design.

In vitro testing for larval mortality: Mortality assay of turmeric extracts against *M. incognita* juveniles was performed by the method given by Prasad *et al.* 2005. The nematode suspension of 100J2s/ml was poured into each well assigned with different treatments including control in 24 wells culture plate and 1 ml of different concentrations of test solution of extracts was added and mixed. Exalin (4.0%) in distilled water was taken as control and kept in ambient temperature at 25–30°C in BOD incubator. Observations were recorded after 24, 48, 72 and 96 h of exposure in each treatment; all dead and alive 2nd stage juveniles were counted under a stereomicroscope. Mortality of nematodes was judged by keeping immobile nematodes in fresh distilled water for 24 h and observed under a stereomicroscope. The ratio of dead nematodes/number of total nematodes expressed the percentage mortality. Data obtained was subjected to angular transformation and analyzed by applying CRD factorial design.

Soil sterilization: Field soil was mixed with sand in the 3:1 ratio. The soil-sand mixture was steam-sterilized at 1.0546 kg/cm³ pressure for 4 h and stored in polythene bags. It was then exposed to open sunlight by spreading

over the polythene sheets for aeration. Before filling the soil into the earthen pots, they were surface sterilized with 4% formalin solution.

Nematode inoculation: Nematode inoculation was done carefully by removing the soil around the root zone of rice seedlings to ensure direct and easy approach of juveniles to root zone. The juvenile inoculum was vigorously bubbled and poured in to the vicinity of exposed roots with a pipette.

Greenhouse pot studies: The fresh leaves and delicate stem of different plants, viz. turmeric, marwatulsi, mentha, aonla and jatropha were chopped and mixed properly in 15 cm earthen pots filled with steam sterilized sandy loam soil at 20 g and 30 g per pot. These pots were left for 10 days for proper decomposition of plant materials. Two seedlings of 4-week-old tomato cv. Sel-7 were transplanted in each pot and after three days of transplanting one healthy seedling in each pot was maintained and others were uprooted carefully. Sets of pots, i.e. inoculated with nematodes, uninoculated and treated with carbofuran at 0.1 g/pot were maintained for comparison. Seedlings of all the treatments were inoculated with 2000 juveniles three days after transplanting. Each treatment was replicated four times. In pot experiments, plant growth characteristics such as shoot length, fresh shoot weight, root length, fresh root weight and nematode reproduction parameters such as number of galls per seedling and reproduction factors were measured.

Final nematode population: For recording nematode reproduction factor, 200 cc of soil from each pot was taken separately with respect to each treatment and replications and final soil population was analyzed by Cobb's Sieving and Decanting method and nematodes were extracted by modified Baermann's Funnel technique. The extracted nematodes per ml nematode suspension were counted under a stereoscopic binocular microscope with the help of counting dish, and finally, the soil population per 200cc soil was calculated.

Statistical Analysis: Data obtained from *in vitro* and pot experiments were analyzed by applying factorial CRD using OPSTAT software and are given in means of three and four replicates respectively.

RESULTS AND DISCUSSION

Effect of methanolic and hexane extract of turmeric on egg hatching: Effect of methanolic extract and hexane on hatching of egg masses is presented in Table 1. All extracts at different concentrations were found toxic in inhibition egg hatching. The hatching rates showed an inverse relation to the concentration of extract and duration of exposure. Minimum rate of hatching of eggs was observed in 1000 ppm while maximum hatching was observed at 62.5 concentration in all tested dilutions. In the hatching assay, it was found that three-factors including extracts, concentrations, and duration of exposure significantly played major role in hatching as individual as well in combination (Table 1). Based on the research finding, among methanol and hexane extracts, the methanolic extract obtained from turmeric showed most inhibitory effect followed by hexane at 1000 ppm.

Table 1 Effect of Methanolic and Hexane extracts of turmeric on egg hatching of *Meloidogyne incognita* (Average of three replicates)

Conc. (ppm)	Hatching (%)							
	Methanol extract				Hexane extract			
	3 DAI	6 DAI	9 DAI	12 DAI	3 DAI	6 DAI	9 DAI	12 DAI
1000	0.000 (0.000)	2.500 (7.753)	4.500 (12.148)	6.750 (14.969)	1.500 (5.958)	6.500 (14.712)	9.500 (17.868)	11.500 (19.759)
500	131.0 (10.5)	67.6 (9.4)	50.3 (6.9)	19.3 (4.6)	111.3 (13.1)	119.0 (10.9)	65.6 (8.1)	37.0 (6.2)
250	145.0 (11.9)	78.6 (9.6)	57.6 (7.1)	21.6 (4.7)	151.3 (14.6)	148.3 (12.2)	84.6 (9.2)	62.3 (8.3)
125	155.6 (10.3)	88.0 (8.3)	78.6 (5.6)	17.6 (4.4)	165.0 (12.6)	82.6 (9.1)	52.3 (7.30)	29 (5.4)
62.5	288.6 (16.9)	185.3 (13.7)	112.3 (10.6)	80.6 (9.0)	311.6 (20.4)	298.3 (17.7)	157.6 (12.5)	100 (10.)
Exalin (4%)	374.750 (45.692)	305.750 (51.780)	272.250 (58.221)	199.750 (59.828)				
Carbofuran	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)				

CD at 5%, exposure period(A)=(0.412), extracts(B)=(0.483) conc.(C)=(1.211), A×B=(1.145), A×C=(2.001), B×C=(2.181) A×B×C=(3.84). Figures in parenthesis are angular transformed value.

Minimum hatching (0.0 larvae) was recorded with mentholic extract at 1000 ppm while minimum egg hatching (0.0 larvae) was recorded with carbofuran as chemical control. The present investigation is in conformity with the finding of Murugeswari *et al.* (2016) that evaluated the effect of different concentrations (5, 10, 15 ppm) of the root extract of *Aerva lanata* against different inoculum levels (5, 10 and 15 egg masses) of root-knot nematode, *M. incognita* on biochemical constituents of Bengal gram, *Cicer arietinum* and found that the biochemical constituents of Bengal gram such a, protein, amino acid, and chlorophyll content were

decreased with increase in level of inoculum, number of egg masses and increased with increasing concentrations of root extract treatment except phenol content. Neeraj *et al.* (2017) tested the effect of ethanolic extracts of various plants against root-knot nematode at different dilutions, i.e. 1:5, 1:10, 1:20, 1:40 and 1:80. And found that ethanolic extracts of all plants showed nematocidal effect against *M. incognita* at varying degree.

Effect of methanolic and hexane extract of turmeric on nematode mortality: The data in Table 2 revealed that methanolic extract was sublethal to juveniles of *M.*

Table 2 Effect of Methanolic and Hexane extracts of turmeric on larval mortality of *Meloidogyne incognita* (Average of three replicates)

Conc. (ppm)	J2 mortality (%)							
	Methane				Hexane			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1000	90.000 (73.225)	94.000 (74.375)	96.250 (77.525)	98.250 (83.629)	73.300 (61.08)	80.150 (64.866)	86.150 (69.543)	96.000 (77.849)
500	81.000 (67.866)	84.750 (69.560)	89.000 (70.847)	96.250 (78.525)	55.000 (45.413)	65.011 (54.774)	74.500 (60.045)	85.250 (61.170)
250	73.500 (59.004)	78.750 (62.547)	80.500 (63.886)	85.750 (67.857)	40.000 (39.225)	51.500 (48.723)	68.000 (54.781)	79.12 (62.267)
125	49.000 (40.982)	64.750 (52.413)	71.250 (54.863)	81.000 (64.144)	32.250 (33.143)	42.500 (40.663)	62.000 (52.343)	69.000 (57.622)
62.5	28.000 (31.172)	59.250 (51.899)	69.350 (54.934)	78.000 (62.658)	23.250 (28.458)	30.500 (33.500)	45.500 (44.695)	52.000 (49.018)
Exalin (4%)	0.000 (2.211)	0.000 (2.211)	0.000 (2.211)	0.000 (2.211)				
Carbofuran	76.000 (55.940)	80.750 (65.575)	91.750 (75.191)	100.000 (88.227)				

CD at 5%, exposure period(A)=(0.496), extracts(B)=(0.375) conc.(C)=(0.676), A×B=(0.992), A×C=(1.789), B×C=(1.352) A×B×C=(1.578). Figures in parenthesis are angular transformed value.

incognita. The highest mortality of juveniles was observed at 1000 ppm (0.00) of methanol and hexane extract of turmeric while the lowest mortality was observed at the lowest concentration, i.e. 62.5 ppm. Per cent mortality was recorded to be zero in carbofuran as control. The methanol extract was found consistent in terms of larval mortality as it was effective up to 62.5 ppm. The present investigation is in conformity with the finding of Prasad *et al.* (2005) that tested four concentrations of root and leaf extracts of *Calotropis procera* and *Parthenium hysterophorus* (0.5, 1.0, 2.0 and 4.0% at different exposure periods of 24, 48 and 72 h) against reniform nematode and reported higher mortality of pre-adults of *R. reniformis* race-A infesting sunflower cv. Morden as compared to the control. Joyamati *et al.* (2013) tested the essential oil extracts of medicinal plants, viz. *Parkia javanica*, *Jatropha curcas*, *Vitex negundo*, and *Adathoda vasica* as seed soaking against *M. graminicola* on the common rice variety Dharam. The plants treated with essential oil extract of *Jatropha* showed improvement in plant growth parameters and reduction in disease incidence when compared with other treated plants. Nayak *et al.* (2013) evaluated the nematicidal property of methanolic extract of neem against second stage juveniles of *M. incognita*

and revealed that methanolic extract at 5 ml concentration exhibited maximum mortality (81%) after 24 h.

Phytotherapeutic effect of botanicals against root-knot nematode infesting tomato under screen house condition: Results revealed that chopped leaves of the all the plant species at both doses, i.e. 20 and 30 g/kg soil were significantly effective in improving the plant growth parameters (shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight) and reducing the nematode reproduction factors (number of galls per plant, number of egg masses per plant, final nematode population in soil) in comparison to untreated control (Table 3, Fig 1). Plant growth parameters were found maximum in case of mentha leaves at 30 g/kg soil applied in soil followed by the turmeric leaves at the same dose. This might be because plant leaves act as organic amendments and attribute to the available nutrients from leaf after decomposition and thus, promote the growth of the plants. The nematode reproduction factors were significantly reduced in case of mentha (30 g/kg soil) followed by turmeric leaves at same dose and carbofuran (0.1 g/kg soil); this is because plant leaves are also known to promote growth of predaceous soil biota that is unfavourable

Table 3 Effect of chopped leaves of various plants on growth parameters of tomato (cv. Sel-7) infested with *M. incognita* under screen house conditions (Average of four replicates)

Treatment	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)	No. of galls/ plant	No. of egg masses/ plant	Final nematode population in 200cc soil
T ₁ Turmeric @20 g	43.62	23.50	25.05	11.77	5.5	1.21	99.25 (10.01)	28 (5.40)	573.5 (23.97)
T ₂ Turmeric @30 g	45.02	26.00	28.02	12.92	6.0	1.41	80 (8.99)	19 (4.49)	487 (22.09)
T ₃ Marwa @20 g	41.42	23.27	23.05	11.42	4.9	0.96	136.5 (11.72)	38 (6.20)	657.5 (25.66)
T ₄ Marwa @30 g	43.95	25.87	25.60	12.72	5.3	1.24	97.5 (9.92)	30 (5.54)	600.5 (24.52)
T ₅ Mentha @20 g	45.30	27.60	27.00	11.62	5.9	1.40	56.75 (7.44)	19 (4.44)	421 (20.54)
T ₆ Mentha @30 g	48.12	28.77	28.47	12.97	6.0	1.49	54.75 (7.46)	14 (3.78)	360.8 (18.99)
T ₇ Aonla @20 g	38.85	22.07	23.22	10.78	4.9	1.08	166.5 (12.93)	48 (7.70)	880.5 (29.66)
T ₈ Aonla @30 g	40.97	24.60	24.65	11.73	5.3	1.14	116.3 (10.82)	26 (5.18)	784.5 (28.02)
T ₉ Jatropha @20 g	40.77	22.25	23.97	10.55	4.8	1.16	148 (12.20)	37 (6.16)	842.5 (29.04)
T ₁₀ Jatropha @30 g	41.35	24.60	25.97	11.20	5.3	1.18	102 (10.41)	29 (5.47)	813 (28.53)
T ₁₁ Carbofuran @0.1 g	43.25	25.47	26.70	11.50	5.7	1.18	60.75 (7.85)	16 (4.14)	434 (20.85)
T ₁₂ Untreated inoculated	37.82	20.50	20.07	8.50	3.7	0.88	249.3 (15.81)	59 (7.71)	1043.3 (32.31)
CD @ 5%	1.72	1.78	1.67	0.94	0.57	0.15	(0.39)	(0.38)	(0.56)

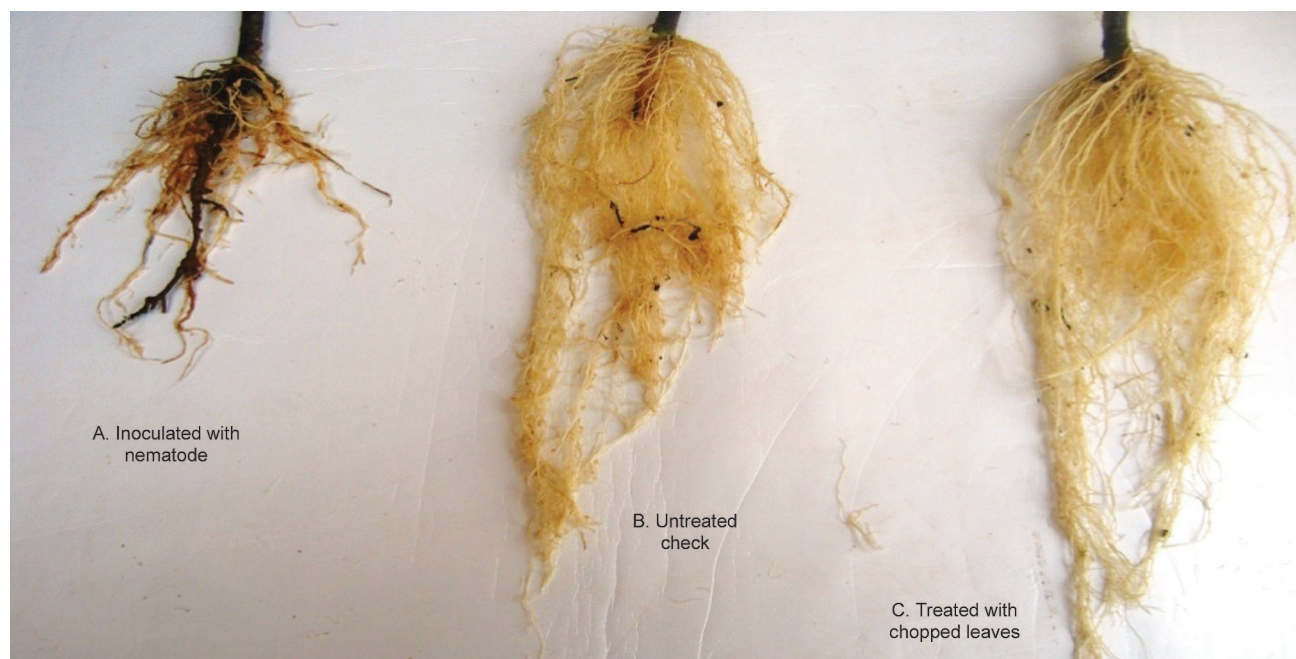


Fig 1 Effect of Chopped Leaves on root growth of tomato infesting by *Meloidogyne incognita*.

to nematodes either by direct parasitism or production of toxic metabolites. The present investigation is in conformity with the finding of Bawa *et al.* (2014) who conducted a pot experiment and evaluated nematicidal effect of ethanolic extracts from four plant species, viz. *Azadirachta indica* leaf, *Capsicum annuum* fruit, *Zingiber officinale* rhizome and *Parkia biglobosa* seeds applied at four levels of 250, 500, 750 and 1,000 ppm concentrations, applying 5 ml per tomato stand. At 1,000 ppm concentration, the *Azadirachta indica* extracts completely (100%) prevented root-knot juveniles from invading plants and significantly increased the growth of tomato plants over the control treatments. Neeraj *et al.* (2016) applied crude aqueous extracts of turmeric, marwatulsi, mentha, aonla and jatropa and a synthetic nematicide (Carbofuran) at the rate 50 ml and 75 ml per pot. Data revealed that aqueous extract of mentha at 75 ml/pot followed by an extract of turmeric at 75 ml/pot was effective in enhancing plant growth parameters and reducing nematode reproduction factors. Among different plant species, leaves of *Jatropha pandurifolia* at 50 g leaves/kg soil were found most effective in reducing the reproductive potential of *M. incognita* (Asif *et al.* 2013). Abbas *et al.* (2009) found that application of neem seed kernel at 0.2 and 0.4% w/w to soil significantly reduced the root galling and decline in nematode population in soil caused by *M. incognita* infesting tomato.

Use of botanicals is now emerging an important means for protection of crop and environment from pesticidal pollution. Therefore, it is necessary to investigate the efficacy, toxicity and potential of new botanicals as an alternative source for minimizing nematode population. Identified potential nematostatic or nematicidal plants can be used to search potent compounds involved in killing nematodes which further can be suggested for use as

nematicides itself, or can be useful for the synthesis of novel nematicides with least environmental and non-targeted impacts. The antimicrobial properties of essential oils and plant extracts have been recognized for many years. The members of the family Zingiberaceae were also reported as natural antimicrobial agents, especially their essential oils (Chen. *et al.* 2008). The tested antagonistic plants in this study could be an excellent source for screening compound through chemical profiling in the future.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received to Dr Anil Kumar (Assistant Nematologist, CCSHAU) and thank our Guide and Mentor Dr Sewak Ram (Principal Scientist, CCSHAU).

REFERENCES

- Abbas S, Gaur H S and Kamra A 2010. Comparative efficacy of neem seed and kernel granular formulations against root-knot nematodes *Meloidogyne incognita*. *Pakistan Journal of Botany* **39**: 196.
- Asif M and Siddiqui M A. 2013. Phytotherapeutic approach for the management of *Meloidogyne incognita* affecting *Abelmoschus esculentus*. *Phytopathology* **109**: 1210–5.
- Bawa J A, Mohammed I, Liadi S and Anglairs A E. 2014. Nematicidal effect of some plants extracts on root-knot nematodes (*Meloidogyne incognita*) of Tomato (*Lycopersicon esculentum*). *World Journal of Life Science and Medical Research* **3**: 120–4
- Chen I N, Chang C C, Ng C C, Wang C Y, Shyu Y T and Chang T L. 2008. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. *Plant Foods for Human Nutrition* **63**:15–20.
- Elling A A. 2013. Major emerging problems with minor *Meloidogyne* species. *Phytopathology* **103**: 1092–1102.
- Jain R K, Dabur K R and Gupta D C. 2007. Assessment of avoidable losses in yield due to root-knot nematode (*Meloidogyne*

- incognita*) in a few vegetable crops. *Indian Journal of Nematology* **20**: 236.
- Joyamati L, Zenith N G, Ronibala K H and Purnima P. 2013. Efficacy of essential oil extracts of medicinal plants against rice root-knot nematode *Meloidogyne graminicola* in pots. *Indian Journal of Nematology* **43**: 86–9.
- Murugeswari P, Murugan C A and Rajan M K. 2015. Nematicidal activity of root extracts of *Aerva lanata* and *Aerva javanica* against the root-knot nematode, *Meloidogyne incognita*. *World Journal of Agricultural Sciences* **12**: 78–83
- Nayak N and Sharma J L. 2016. Effect of methanol neem extracts on mortality of *Meloidogyne incognita* juveniles. *Indian Journal of Nematology* **39**:88–90
- Neeraj, Kumar A, Singh G. 2016. *In vitro* studies of the effectiveness of medicinal plants extract compared to carbofuran in controlling the root-Knot nematode *Meloidogyne incognita* in Tomato. *Environment & Ecology* **35** (3B): 2100–02
- Neeraj, Singh G, Kumar A, Ram Sewak and Kumar V. 2017. Evaluation of Nematicidal Activity of Ethanolic Extracts of Medicinal Plants to *Meloidogyne incognita* (Kofoid and White) Chitwood under Lab Conditions. *International Journal of Pure and Applied Sciences*. **5**(1): 827–31
- Prasad and Suverna N. 2005. Nematicidal toxicity of *Calotropis procera* and *Parthenium hysterophorus* extracts on pre-adult of *Rotylenchulus reniformis*. *Annals of Plant Protection Science* **13**(2): 445–9.
- Saravanpriya B, M Shivakumar, G Rajendran and S Kuttalam 2004. Effect of different plant products on the hatching of *Meloidogyne Incognita* eggs. *Indian Journal of Nematology* **34**: 690–7