

EFFECT OF ROOT EXUDATES OF *TAGETES* SP. ON EGG HATCHING BEHAVIOR OF *MELOIDOGYNE INCOGNITA*

Iruthaya Kalaiselvam and Aruna Devaraj*

Department of Zoology, JA College for Women, (Autonomous) Periyakulam-625601, Theni District, Tamilnadu, India

Article Received on: 14/08/11 Revised on: 16/09/11 Approved for publication: 06/10/11

*Email: snakearuna@yahoo.com

ABSTRACT

The effect root exudates of pre-planted marigold intercropped with tomato in regulating the hatching behavior of root-knot nematode - *Meloidogyne incognita* eggs were investigated. Marigold cultivars *Tagetes patula*, *T. minuta*, *T. erecta*, *T. erecta* (var. Orange), *T. erecta* (var. Yellow) significantly reduced the numbers of second-stage juveniles (J2s) in subsequent tomato compared to the tomato-tomato control. Four different concentrations (25, 50, 75 and 100 %) of water soluble extract from the selected varieties of Marigold cultivars were filtered and added to the petri dish and infested with the eggs of *M. incognita*. Data indicate that egg hatching was significantly affected by root exudates of *Tagetes* sp. however, nematicidal activity was species dependent. Root exudates of *T. erecta* were lethal to J2 of *M. incognita* and were inhibitory to the hatch of eggs at the concentration of 75 % or higher.

Keywords: *Meloidogyne incognita*, Root-knot nematodes, *Tagetes erecta*, *Tagetes patula*, *Tagetes minuta*

INTRODUCTION

Plant diseases caused by parasitic nematodes are widespread¹. The mere presence of plant-parasitic nematodes in soil does not guarantee crop damage or yield loss, since a nematode population may remain below the damage threshold level for a specific field². Factors such as environmental conditions, soil type, previous cropping history, the specific nematode species and or race present, pathotype distribution, prevailing nematode distribution pattern, nematode multiplication rate, and plant cultivar grown will have a bearing on whether crop damage and yield reduction will be inflicted^{1,2,3}. Root-knot nematodes (*Meloidogyne* spp.) are obligate endoparasites of a wide range of plant species and globally cause large crop yield losses³. The infective second stage juvenile (J2), hatches from an egg in the soil and must find a host and establish a feeding site in order to survive and complete its life cycle. Nematode population dynamics are density dependent and are influenced by host growth, reproductive potential and environmental factors^{3,4}. However, little is known about the influence of environmental parameters on the egg hatching behavior and survival of the infective juvenile^{3,5,6}.

The root knot nematode, *M. incognita*, is an obligate parasite that causes significant damage to a broad range of host plants belonging to the families Solanaceae, Cucurbitaceae, Leguminosae, Liliaceae, Chenopodiaceae, Compositae, Umbelliferae, Cruciferae and Malvaceae. *M. incognita* is also highly pathogenic to some staple crops such as cereals, including rice, maize, potato, soybean, banana, plantain, sweet potato and yam; industrial crops such as tobacco, coffee, sugar cane, sugar beet, cotton and black pepper. *M. incognita* can infect 1,700 plant species^{3,6}. In particular, *M. incognita* is a serious problem on a number of economically important agricultural crops including grape (*Vitis vinifera*), papaya (*Carica papaya*), guava (*Psidium guajava*), tobacco (*Nicotiana tabacum*), watermelon (*Citrullus vulgaris*) and tomato (*Lycopersicon esculentum*).

M. incognita has been reported to increase the severity of bacterial wilt disease in plants⁷. Moreover, farms infested with *M. incognita* frequently experience a decline in the yield of subsequent crops. Current practice of management of *M. incognita* in the field condition primarily relies on application of nematicides⁸. However, because chemicals are of potential risk to the environment and human health, eco-friendly substitute to chemical nematicides are desirable.

Several plants have been shown to be effective in controlling nematodes on agricultural crops when grown in rotation, inter-

planted with susceptible crops, or used as a soil amendment. Among these plants, marigold (*Tagetes* sp.) is used for its nematicidal properties against plant-parasitic nematodes. Marigold can suppress 14 genera of plant-parasitic nematodes, with lesion nematodes (*Pratylenchus* sp.) and root-knot nematodes (*Meloidogyne* spp.) the most affected⁹. The suppression of *Meloidogyne* spp. by Marigold have been reported^{10,11}. African (*T. erecta*) and French (*T. palula*) marigolds are the most commonly used species. Each of the Marigold species consists of several varieties that differ in characteristics such as bloom size, shape, and color, as well as plant size and leaf shape. Although, marigolds had an overall suppressive effect on nematodes, French marigold cultivars (*T. palula*) was found to be most effective against wide range of nematodes^{10,11}.

The potential use of marigolds for the control of *Meloidogyne* species have been studied by several workers both in laboratory and in the field. Hackney and Dickerson¹², 1975 attributed suppression of *M. incognita* and *M. javanica* to early non preference of the nematodes for marigold roots, but Suatmadji⁹ 1969, reported that equal numbers of *M. hapla* invaded roots of tomato and marigold. When intercropped most cultivars of African marigold (*T. erecta*) and French marigold (*T. palula*) are effective in reducing the populations of *M. incognita*. However, selection of specific marigold species or cultivars to control targeted nematode pests seems to be the most important parameter for the effective control of the nematodes in the soil.

Alternatively, root-knot nematodes can be effectively managed without chemical pesticides⁹. Some plant species are known to be highly resistant to nematodes. The best documented of these include marigolds (*Tagetes* sp.), rattlebox (*Crotalaria spectabilis*), chrysanthemums (*Chrysanthemum* sp.), castor bean (*Ricinus communis*), margosa (*Azadiracta indica*), and many members of the family Asteraceae¹². The allelopathic effect of marigold root exudates is responsible for nematicidal activity and suppression of the hatching of nematode eggs is attributed to α -terthienyl¹³. Besides, the exudate inhibits the growth of disease promoting organisms such as fungi, bacteria, insects, and viruses. Further, nematicidal activity of marigold was detected in the root exudates but not in the homogenized extracts of roots and leaves⁸. Planting of marigolds for 65 to 105 days in fields highly infested with root-knot^{12,14} or root-lesion nematodes¹¹ resulted in significant decrease of nematode populations and increase in yields of subsequent crop up to 98 %.

Studies have shown that pre-planting resistant hosts significantly reduce nematode populations in the soil^{8,14,15}. Therefore, efficacy of *Tagetes* sp. as potential biocontrol agent in the management of *M. incognita* was evaluated both in the field and soil amendment experiments. In addition, plant extracts and root exudates from selected *Tagetes* sp. were tested to determine the mechanism of nematode suppression by affecting the hatching behavior of the eggs.

Recently, it has been reported that use of marigold as nematode biocontrol agent significantly improves the nematode-suppressive effect¹⁶. We hypothesize that marigolds suppress plant parasitic nematodes more effectively if it is planted right after a nematode-susceptible host, while targeted nematodes are still in their active yet vulnerable stage. This is because nematodes could undergo a survival stage and avoid coming into contact with allelopathic compounds released from marigold.

MATERIALS AND METHODS

A hail-net-cage experiment was followed by a microplot experiment during a consecutive season. Local *M. incognita* and tomato plants were used in the study. Hail-net-cage experiment: Black plastic bags (25,000 cm³ capacity) were filled with a methyl bromide-fumigated and steam-pasteurized, sandy-loam soil (3.9 % clay, 1.9 % silt, 93.6 % sand, 0.6 % organic material); pH of the soil was adjusted to 6.55. Plant nutrients were added according to a soil nutrient analysis as per the requirement. One seed was planted per bag. Inoculation was performed at planting by pipetting desired nematode inoculum density around each seed. The range of Pi used for each cultivar was approximately 100, 500, 1000, 5000, 10000 and 20000 *M. incognita* J2 per seed. The untreated control treatment was not inoculated (Pi = 0).

Microplot experiment: field plots used in this study consisted of 1m x 1m plots in the study area, tomato saplings were planted in alternative rows to *Tagetes* plants, tomato - tomato were used as control. Single plants were treated as replicates. Plants in both trials were watered by means of micro-irrigation three times a week, except when rainfall occurred. In such cases irrigation was rescheduled to prevent water logging in the plots.

Effect of root extract on egg hatching

A small drop of eggs suspended in distilled water was placed in Petri dishes and eggs were counted under a stereo microscope. Ten milliliter of undiluted root extract of each plant as added. Petri dishes containing distilled water served as controls. Each treatment was replicated four times. Petri dishes were incubated at room temperature. Hatching was observed after 7 days and percentage inhibition calculated.

RESULTS AND DISCUSSION

Previous studies have shown that pre-planting resistant hosts may effectively reduce nematode populations in the soil^{15,16}. Tomato has been reported as a better host for *Meloidogyne* species than any of the tested marigolds. The effect root exudates of pre-planted marigold intercropped with tomato in regulating the hatching behavior of root-knot nematode *M. incognita* eggs were investigated. So far, only *T. erecta* was reported effective in the management to decrease the number of *M. incognita* when it was grown in infested soil^{15,16}.

The relationships between number of egg masses and percentage yield loss in the hail-net-cage (Fig. 1a-f) and microplot (Table 1) experiment were strong and highly significant. Root exudates of all selected *Tagetes* sp. delayed the egg hatching of *M. incognita* (Table 1) when compared to the control in a concentration and time dependent manner. The effect of different concentration levels and exposure timings on the egg hatching behavior of *M. incognita*

varied significantly with the plant species in the field and the exposure time (0 to 72 h) (Fig. 1a – 1f).

It was observed that in the control, initiation of hatching started just after 1h whereas for rest of the treatments the hatching was delayed by at least 3h. Marigold cultivars *T. patula*, *T. minuta*, *T. erecta*, *T. erecta* (var. Orange), *T. erecta* (var. Yellow) significantly reduced the numbers of second-stage juveniles (J2s) in subsequent tomato compared to the tomato-tomato control. Four different concentrations (25, 50, 75 and 100 %) of water soluble extract from the selected varieties of Marigold cultivars were filtered and added to the petri dish and infested with the eggs of *M. incognita*, the percentage hatch of the eggs increased 10.38 % to 48.66 % from 12 to 72h in the control however, with significant variations among the selected cultivars of *Tagetes* sp.

Data indicate that two of the *Tagetes* sp. (*T. erecta* and *T. erecta* (var. Yellow)) were effective in the management of hatching of the nematode eggs and thus reduction in the population of the nematodes in the field (micro plot experiment). However, the other selected *Tagetes* sp. had no significant effect on nematode management (Fig. 1a-f).

Results of the present study suggest that *Tagetes* sp are an ideal antagonistic plant for use in the management of *M. incognita*. The roots of *Tagetes* exuded substances that were lethal to the J2 of *M. incognita* and inhibitory to egg hatch⁸. Results indicate that *Tagetes* sp. could be used to manage *M. incognita* as a rotation crop, a co-planted crop, or a soil amendment for control of root-knot nematode. The relative value of nematode resistance in susceptible host plants in the presence of variable nematode population levels, however, has been demonstrated clearly in this study. Moreover, root exudates were selectively toxic to the plant-parasitic nematodes but not to free-living organisms hence, Marigolds are a source of cheap and effective nematocides of root knot nematodes.

REFERENCES

1. Tsay TT. Chemical control of plant-parasitic nematodes. Plant Pathol Bull 1999; 8:41-50.
2. Khan MR. Plant Nematodes Methodology, Morphology, Systematics, Biology and Ecology. 2008; Science Publishers, New Jersey, USA
3. Williamson VM, Gleason CA. Plant nematode interactions. Curr Opin Plant Biol 2003; 6: 327-333.
4. Trudgill DL. Resistance to and tolerance of plant-parasitic nematodes in plants. Ann Rev Phytopathol 1992; 29:167-192.
5. Robinson AF. Movement of five nematode species through sand subjected to natural temperature gradient fluctuations. J Nematol 1994; 26: 46-58.
6. Sasser JN, Eisenback JD, Carter CC, Triantaphyllou AC. The International *Meloidogyne* Project - Its Goals And Accomplishments. Ann Rev Phytopathol 1983; 21:271-288.
7. Yen JH, Huang JW, Lin CY, Chen DY, Tsay TT. Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* fsp niveum in watermelon roots. Plant Pathol Bull 1998; 7:201-204.
8. Tsay TT, Wu St, Lin YY. Evaluation of Asteraceae Plants for Control of *Meloidogyne incognita*. J Nematol 2004; 36(1):36-41.
9. Suatmadji RW. Studies on the effect of *Tagetes* species on plant-parasitic nematodes. 1969; Wageningen, The Netherlands
10. Steiner G. Nematodes parasitic on and associated with roots of marigolds (*Tagetes* hybrids) Proceedings of the Biological Society of Washington 1941; 54:31-34
11. Pudasaini MP, Viaene N, Moens M. Effect of marigold (*Tagetes patula*) on population dynamics of *Pratylenchus penetrans* in a field. Nematol 2006; 8(4):477-484
12. Hackney RW, Dickerson OJ. Marigold, castor bean, and chrysanthemum as controls of *Meloidogyne incognita* and *Pratylenchus alleni*. J Nematol 1975; 7:84-90
13. Siddiqi MA, Alam MM. Control of plant-parasitic nematodes by *Tagetes tenuifolia*. Revue de Nematol 1988; 11:369-370.
14. Ploeg AT. Greenhouse studies on the effect of marigolds (*Tagetes* sp) on four *Meloidogyne* species J Nematol 1999; 31:62-69.
15. Hu SS, Huang MC. Effects of French marigold (*Tagetes patula* L) on the control of soil nematode National Chung Hsing University Horticulture 1978; 3:31-36
16. Prasad D, Nagla DK, Kumar S, Saini ML. Marigold plants for management of nematode populations in fields. Curr Nematol 1992; 3:15-18.

Table 1 Effect of Root Exudates of *Tagetes* sp. on egg hatching of *M. incognita*

Time (h)	Conc. (%)	Control	<i>T. palula</i>	<i>T. minula</i>	<i>T. erecta</i>	<i>T. erecta</i> (Orange)	<i>T. erecta</i> (Yellow)
12	25	10.38 ±0.33	4.32 ±0.12	6.32 ±0.37	20.73 ±0.37	5.12 ±0.42	18.64 ±0.14
	50	10.40 ±0.27	5.87 ±0.14	10.23 ±0.38	38.92 ±0.29	7.76 ±0.28	32.70 ±0.81
	75	10.30 ±0.36	9.23 ±0.17	13.68 ±0.31	44.65 ±0.29	10.79 ±0.75	42.85 ±0.41
	100	10.29 ±0.15	13.12 ±0.21	17.34 ±0.46	52.67 ±0.36	13.87 ±0.89	50.65 ±0.24
24	25	18.74 ±0.29	8.32 ±0.26	12.23 ±0.45	28.66 ±0.44	10.23 ±0.47	25.76 ±0.20
	50	18.75 ±0.30	11.89 ±0.30	14.68 ±0.42	45.64 ±0.33	14.32 ±0.74	43.73 ±0.23
	75	18.81 ±0.38	14.80 ±0.32	20.87 ±0.57	56.76 ±0.32	18.30 ±0.87	52.80 ±0.37
	100	18.77 ±0.45	19.53 ±0.26	23.32 ±0.62	62.87 ±0.49	23.00 ±0.72	59.88 ±0.38
36	25	23.66 ±0.33	12.32 ±0.30	14.32 ±0.64	32.72 ±0.48	14.12 ±0.51	31.72 ±0.11
	50	23.67 ±0.40	15.23 ±0.23	18.56 ±0.56	68.95 ±0.56	16.54 ±0.69	65.74 ±0.25
	75	23.65 ±0.42	20.87 ±0.35	24.54 ±0.70	75.64 ±0.36	26.22 ±0.89	74.73 ±0.39
	100	23.68 ±0.51	29.37 ±0.72	39.12 ±0.70	75.44 ±0.33	31.54 ±0.99	74.35 ±0.48
48	25	30.64 ±0.34	23.67 ±0.29	15.87 ±0.34	45.76 ±0.54	15.89 ±0.59	42.86 ±0.17
	50	30.85 ±0.74	24.23 ±0.29	22.32 ±0.69	72.85 ±0.42	23.88 ±0.52	70.64 ±0.18
	75	30.83 ±0.34	23.99 ±0.22	34.87 ±0.39	82.65 ±0.31	26.79 ±0.52	79.88 ±0.16
	100	30.15 ±0.98	35.34 ±0.27	44.87 ±0.23	87.75 ±0.35	36.45 ±0.71	85.75 ±0.27
60	25	37.44 ±0.37	32.77 ±0.26	23.56 ±0.35	51.75 ±0.43	28.66 ±0.58	49.75 ±0.16
	50	37.54 ±0.37	30.80 ±0.22	26.76 ±0.46	81.86 ±0.22	30.60 ±0.62	49.70 ±0.36
	75	37.46 ±0.37	27.46 ±0.32	38.76 ±0.46	95.83 ±0.23	31.87 ±0.74	93.64 ±0.27
	100	37.47 ±0.37	41.56 ±0.39	50.87 ±0.76	98.38 ±0.49	42.89 ±0.71	96.39 ±0.27
72	25	43.66 ±0.53	38.62 ±0.10	30.76 ±0.37	60.67 ±0.39	35.23 ±0.59	58.63 ±0.31
	50	43.67 ±0.45	35.23 ±0.24	42.75 ±0.61	92.77 ±0.23	40.32 ±0.84	90.97 ±0.38
	75	43.68 ±0.46	34.77 ±0.34	45.23 ±0.47	100.00 ±0.37	37.54 ±0.87	100.00 ±0.21
	100	43.70 ±0.43	49.00 ±0.51	58.87 ±0.75	100.00 ±0.43	50.80 ±0.97	100.00 ±0.29

Data represent mean value; n = 3

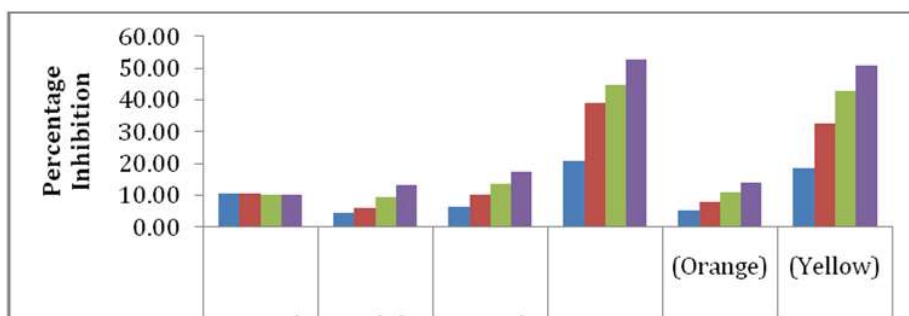


Fig. 1a Percentage inhibition of *Meloidogyne incognita* population by the root exudates of selected cultivars of *Tagetes* sp. after 12h of treatment



Fig. 1b Percentage inhibition of *Meloidogyne incognita* population by the root exudates of selected cultivars of *Tagetes* sp. after 24 h of treatment

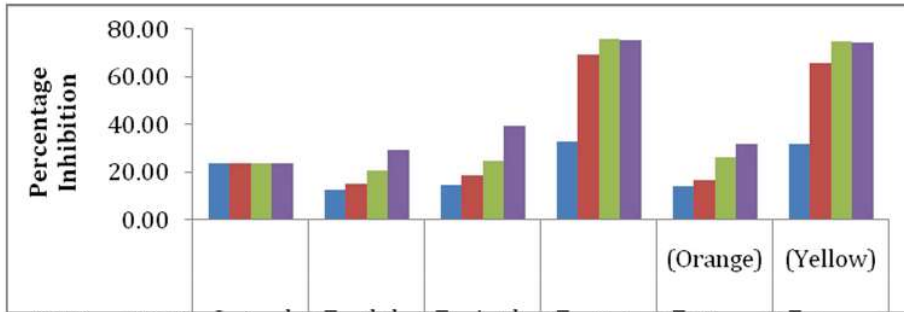


Fig. 1c Percentage inhibition of *Meloidogyne incognita* population by the root exudates of selected cultivars of *Tagetes* sp. after 36h of treatment

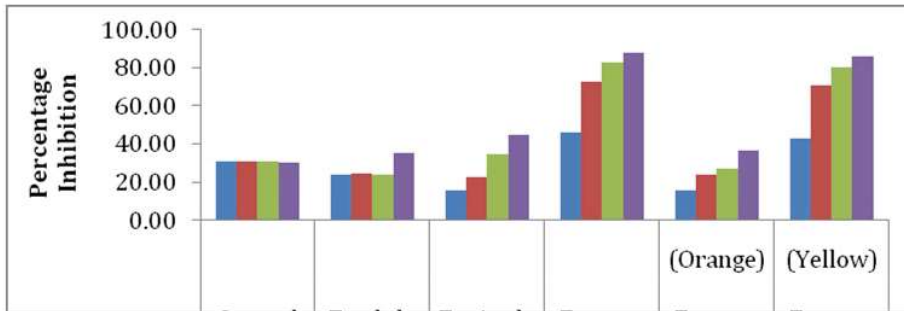


Fig. 1d Percentage inhibition of *Meloidogyne incognita* population by the root exudates of selected cultivars of *Tagetes* sp. after 48h of treatment

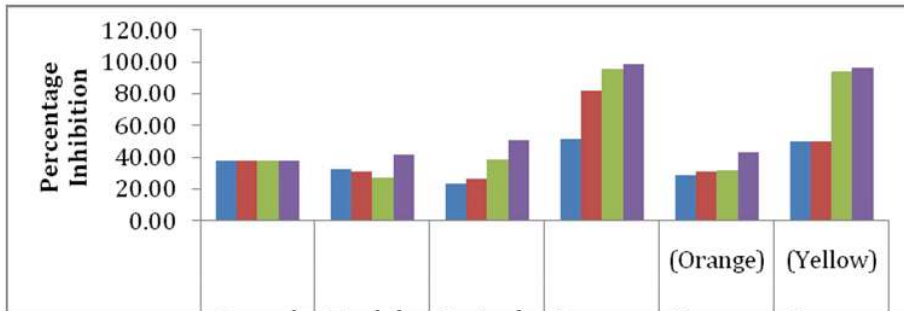


Fig. 1e Percentage inhibition of *Meloidogyne incognita* population by the root exudates of selected cultivars of *Tagetes* sp. after 60 h of treatment

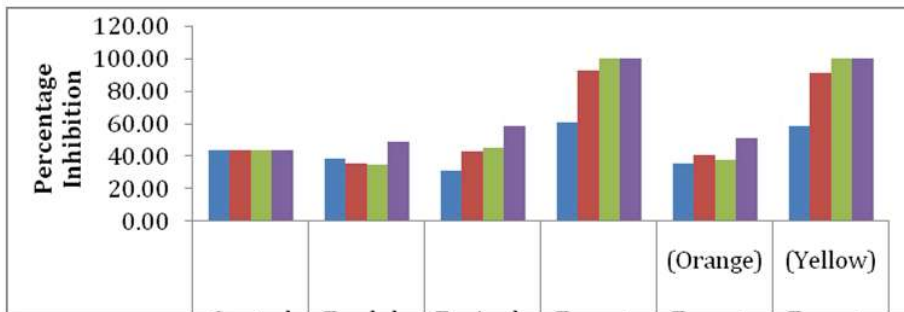


Fig. 1f Percentage inhibition of *Meloidogyne incognita* population by the root exudates of selected cultivars of *Tagetes* sp. after 72 h of treatment

Source of support: Nil, Conflict of interest: None Declared