ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2025): 4.97
IJAFS 2025; 7(12): 790-793
www.agriculturaljournals.com
Received: 18-11-2025
Accepted: 20-12-2025

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Suppressive effect of marigold (*Tagetes erecta)* against root knot nematode, *Meloidogyne incognita*

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DOI: https://www.doi.org/10.33545/2664844X.2025.v7.i12i.1108

Abstract
This study was aimed to determine the nematicidal efficacy of marigold (*Tagetes erecta*) against root-knot nematode, *Meloidogyne incognita* which are most destructive nematode pest infecting almost all crops. In this study, aqueous extract of different parts of marigold *viz*., leaf, flower and root were tested at different concentration (10%, 20% and 30%). Each treatment was tested with three replications and the experiments were designed with the completely randomized block design. Results showed that highest inhibition of nematode egg hatching was recorded in 30 per cent concentration of marigold roots extracts (88.0 per cent) followed by 20 per cent of roots extracts (59.7) within 48 hr. Highest juvenile mortality was recorded in 30 per cent concentration of marigold roots extracts (88.7 per cent) followed by 20 per cent of roots extracts (86.7) on 7th day. On the other hand, no juvenile mortality was observed upto 4 days in untreated control. It can be concluded from the results using marigold may significantly reduce the damages caused by the root-knot nematodes.

Keywords: Root knot nematode, antagonistic plant, *Tagetes erecta*, leaves, flower, root

Introduction
Root-knot Nematodes belonging to the genus *Meloidogyne* are considered the most important group of plant-parasitic nematodes worldwide attacking nearly every crop. These nematodes inflict great losses to various crops and about 5 per cent of the total world crop yield is damaged due to root knot nematodes (Sasser, 1987) [13]. Chemical nematicide is one of the most rapid method to manage nematodes, but they are detrimental to both human and environment and are relatively unaffordable to small scale farmers (Washira *et al*., 2009) [15]. Hence, there is a need to develop alternative method that are cheap and ecofriendly and are economically viable to the farmers. Many botanical extracts have been found to contain phytochemical such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls (Chitwood, 2002) [5] which are effective against plant parasitic nematodes (Adegbite, 2003) [1]. Botanical pesticides are often readily available, cheaper than the synthetic nematicides and their crude extracts are easy to be prepared even by farmers. Thus, the present investigation was done to evaluate the nematicidal efficacy of different parts (leaf, flower and root) of marigold (*Tagetes erecta*) aqueous extracts on nematode egg hatching inhibition and juvenile mortality of root knot nematode, *M. incognita* under *in vitro* condition.

Materials and Methods
Preparation of aqueous extracts of marigold
The different parts of African marigold (*Tagetes erecta*) *viz*., leaves, flowers and roots (var. local yellow) were used for preparation of fresh aqueous extract. It was prepared by grinding 75g of leaves, flowers, and roots with 300ml of distilled water separately. To obtain a clear and transparent extract, the aqueous extracts were filtered through a muslin cloth and then centrifuged at 4000 rpm for 10 minutes. The supernatant solution was considered as stock solution and stored it in a refrigerator for laboratory studies.

Multiplication of Root knot nematode, *Meloidogyne incognita*
The root knot nematode, *Meloidogyne incognita* required for this study was maintained as pure culture on tomato cv. PKM 1 raised in pots with sterilized pot mixture (2:1:1 red soil,

sand and farmyard manure) at Dr.M.S.Swaminathan Agricultural College and Research Institute, Eachangkottai, Thanjavur.

Egg mass collection

Root-knot nematode infected tomato plant (cv. PKM 1) from the pure culture pot was up-rooted and washed gently under running tap water. Egg masses of *M. incognita* were picked up from the galled root using sterile dissecting needle and forceps. The collected egg masses were kept in distilled water at 10^0C in a refrigerator to prevent hatching before application of treatments.

***M. incognita* egg hatching inhibition assay**

The effect of different parts of marigold extract on *M. incognita* egg hatching inhibition was evaluated under *in vitro* by following the methodology suggested by Saravanapriya *et al.*, (2004) [12]. Leaves, flowers and roots extracts of each concentration (10, 20 and 30 percent) considered as separate treatment and 3 replications were maintained. Two uniform sized egg masses of *M. incognita* were transferred to each treatment, while egg masses in distilled water only served as control. The experiment was laid out in completely randomized design (CRD). The number of hatched second stage juveniles was counted after 24, 48, 72, 96, 120, 144 and 168 hours (up to 7 days). The suspension from each petri plate was transferred to nematode counting dish and retain the egg masses in petri plate. The number of juveniles was counted under stereoscopic microscope under 10x. Fresh extract/distilled water was added into respective petri plates and kept again at the laboratory bench for observation.

Root knot Nematode, *M. incognita* juvenile mortality test

Freshly hatched second stage juveniles of *M. incognita* were Transferred to different cavity blocks (10 juveniles/cavity block) containing 10, 20, 30 percent concentration of leaves, flower and root extracts (3ml / cavity block), while juveniles in distilled water only served as control. For each treatment three replications were maintained. Percent juvenile mortality was counted at 24 hours intervals upto 7 days. Dead juveniles were counted under stereoscopic microscope under 10x and the mortality was confirmed by touching the juvenile with a needle. The percentage of juvenile mortality was calculated by number of dead juveniles / total number of juveniles. The data were subjected to statistical analysis using excel.

Results

Efficacy of marigold extracts on hatching inhibition of *M. incognita* eggs

Nematicidal efficacy of marigold (*T. erecta*) extracts *viz*., leaf, flower and roots on hatching inhibition of *Meloidogyne incognita* eggs was made *under in vitro* condition. The efficacy of marigold extracts on hatching inhibition of *Meloidogyne incognita* eggs showed that all parts of marigold extracts at different concentration were found to inhibit the hatching of *M. incognita* eggs. On the other hand, enhanced egg hatching was found in all untreated control.

Highest nematode egg hatching inhibition was recorded in 30 per cent concentration of marigold root extracts (88.0 per cent) followed by 20 per cent of root extracts (59.7) within 48 hr. The effect of treatments namely, 20 percent conc. of root extracts was on par with 30 per cent conc. of leaf extracts, accounting for 59.7 and 55.3 per cent inhibition on nematode egg hatching compared to untreated control. This *in vitro* study showed that inhibition of *M. incognita* egg hatching increased with increasing concentration of marigold extracts (Table 1).

Table 1: Efficacy of different parts of marigold (*Tagetes erecta*) aqueous extracts on hatching inhibition of root knot nematode, *M. incognita* eggmass

Treatments	T	Percent hatching inhibition of egg mass						
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
10% Marigold leaf extracts	T_1	47.0	46.3	45.3	44.0	42.3	38.0	37.3
20% Marigold leaf extracts	T_2	54.3	52.3	51.0	50.3	46.7	42.3	39.3
30% Marigold leaf extracts	T_3	59.0	55.3	52.3	50.7	49.7	44.0	41.7
10% Marigold flower extracts	T_4	42.7	40.3	39.0	38.7	36.0	34.0	31.0
20% Marigold flower extracts	T_5	45.3	42.7	40.3	39.0	38.3	36.0	32.7
30% Marigold flower extracts	T_6	47.3	43.7	42.0	41.0	39.0	36.3	33.3
10% Marigold root extracts	T_7	61.3	58.7	53.7	53.0	51.7	46.7	43.7
20% Marigold root extracts	T_8	62.0	59.7	57.7	57.3	54.7	48.7	46.3
30% Marigold root extracts	T_9	76.7	88.0	80.0	78.3	74.7	73.7	72.3
Untreated Control	T_{10}	41.3	40.0	37.7	35.0	35.3	32.3	29.7
Mean		53.7	52.7	49.9	48.7	46.8	43.2	40.7
CD (P = 0.05)		1.60	1.58	1.00	1.23	1.73	1.51	1.65
SEd		3.33	3.31	2.09	2.56	3.60	3.14	3.45

Efficacy of marigold extracts on mortality of *M. incognita* juveniles

The efficacy of marigold extracts on juvenile mortality of *M. incognita* showed that all tested marigold plant extracts of different concentration were found to be enhance the mortality of *M. incognita* juveniles. On the other hand, juvenile mortality was not found upto 4 days in untreated control. Highest juvenile mortality was recorded in 30 per cent concentration of marigold root extracts (88.7 per cent) followed by 20 per cent root extracts (86.7) on 7th day. The effects of treatments namely, root extracts were on par with leaf extracts of marigold compared to untreated control respectively. The *in vitro* study showed that mortality of *M. incognita* juvenile increased with increasing concentration of marigold extracts and also increased with increased exposure time (Table 2).

Table 2: Efficacy of different parts of marigold (*Tagetes erecta*) aqueous extracts on juvenile mortality of root knot nematode, *M. incognita*

Treatments	T	Percent mortality of juveniles						
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
10% Marigold leaf extracts	T_1	67.0	69.0	72.0	74.3	77.7	79.0	81.0
20% Marigold leaf extracts	T_2	69.3	72.3	75.7	76.0	78.0	80.0	82.3
30% Marigold leaf extracts	T_3	73.0	75.7	77.0	79.0	81.0	82.0	83.7
10% Marigold flower extracts	T_4	52.0	55.3	56.0	58.0	60.7	62.3	63.0
20% Marigold flower extracts	T_5	53.7	56.3	57.3	58.7	62.0	62.7	63.7
30% Marigold flower extracts	T_6	55.0	56.7	58.3	59.0	63.3	64.0	65.7
10% Marigold root extracts	T_7	74.3	78.3	79.3	81.0	82.3	83.3	84.0
20% Marigold root extracts	T_8	77.3	80.0	80.3	82.7	84.0	85.0	86.7
30% Marigold root extracts	T_9	80.7	83.3	84.7	85.0	86.3	87.3	88.7
Untreated Control	T_{10}	0.0	0.0	0.0	0.0	5.7	7.7	11.0
Mean		60.2	62.7	64.1	65.4	68.1	69.3	71.0
CD (P = 0.05)		1.98	1.76	1.32	1.26	1.41	1.02	1.10
SEd		4.13	3.67	2.75	2.64	2.95	2.13	2.29

Discussion

Marigold (*Tagetes spp.*) may reduce the plant parasitic nematode populations by several means, including acting as a non-host or a poor host, producing allelopathic compounds that are toxic or inhibit plant parasitic nematode development, creating an environment that favors nematode antagonistic flora or fauna (Wang *et al*., 2007) [14]; or behaving as a trap crop (Pudasaini *et al*., 2008) [11]. These mechanisms may occur separately or in combination resulting in lower plant parasitic nematode population.

The effect of different parts of marigold extracts on egg hatching and juvenile mortality could be due to the presence of α-terthienyl and bithienyl compounds which have been reported to kill nematodes. Alpha-terthienyl is a heterocyclic, sulfur-containing compound, usually abundant in *Tagetes* tissue (Morallo-Rejesus and Decena, 1982) [9] and with reported nematicidal, insecticidal, antiviral, and cytotoxic activity (Arnason *et al*., 1989; Marles *et al*., 1992) [2, 7].

This *in vitro* study showed that inhibition of *M. incognita* egg hatching increased with increasing concentration of marigold extracts and exposure time. Highest inhibition on hatching of nematode eggs was recorded in 30 per cent concentration of marigold roots extracts within 48 hr. Highest juvenile mortality was recorded in 30 per cent concentration of marigold roots extracts on 7th day. This was in agree with Hasabo and Noweer (2005) [6] who found that the mortality effect of an extract on nematode is concentration dependent. The number of dead juveniles also increased with increase in exposure time. According to Bohlmann and Herbst (1962) [3] the inhibitory effect of marigold extracts might be due to bithienyls compounds present in the freshly ground roots of *T. erecta* and *T. patula* that possess nematicidal properties.

Neriman and Atilla, 2018 revealed that highest gall index was recorded from the marigold applications with only flowers and the lowest gall index was noticed from the marigold applications with green parts of the plants concluded that using of marigold in crop rotation may significantly reduce the damages caused by the root-knot nematodes.

Mali *et al*., 2019 [8] reported that French marigold *Tagetes patula* caused significant reduction in the number of root galls (8 / plant), root nematode population (29.80 / 8 galls) and soil nematode population (150.25 / 50 ml) with a remarkable growth of tomato plant (31.5 cm) as compared to other marigold cultivars and control.

Conclusion

The results of both egg hatching inhibition and juvenile mortality of root knot nematode, *M. incognita* under *in vitro* revealed that the marigold extracts are highly effective for suppressing *M. incognita*. Among the different parts of marigold extracts, root and leaf extracts proved to be most effective followed by flower extracts. These findings proved that different parts of marigold extract possess nematicidal activity and thus, root and leaf extracts of marigold can be used for the management of root knot nematode. It available easily inexpensive and eco-friendly.

Acknowledgments

The authors declare no conflicts of interest regarding this manuscript

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