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Efficacy of *Tagetes erecta* (L.) Aqueous Leaf Extract against the Root Knot Nematode *Meloidogyne incognita*: In vitro

Manimegalai.T[1], and S. Iruthaya Kalai Selvam[1*]

PG and Research Centre of Zoology, Jayaraj Annapackiam College for Women (Autonomous), Periyakulam, Theni - 625 601 (Affiliated to Mother Teresa Women's University), Kodaikanal, India.

*Corresponding author - S. Iruthaya Kalai Selvam,

Email: iruthayakalaiselvam@gmail.com

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ABSTRACT

Root knot nematodes (*Meloidogyne incognita*) are among the most destructive soil borne pathogens of vegetable crops, causing severe yield losses worldwide. In the present study, *Meloidogyne incognita* was identified based on microscopic examination of mature females, which exhibited diagnostic features including a pear shaped body, distinct stylet, thick cuticle, and a squarish dorsal arch with wavy forked striae in the perineal pattern. In vitro assays revealed that aqueous leaf extract of *Tagetes erecta* (L.) exhibited significant nematicidal activity against second stage juveniles (J_2) of *M. incognita*. Juvenile mortality increased with extract concentration, reaching 100% at 1000 ppm, followed by 89%, 72%, 61.5%, 51.5%, and 18.5% at 800, 600, 400, 200, and 100 ppm, respectively. Statistical analysis (one-way ANOVA) confirmed significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) among treatments compared to the control. Probit analysis further revealed a progressive decline in LC_{50} values with increasing exposure time, from 220.85 ppm at 24 h to 120.45 ppm at 96 h, indicating enhanced nematicidal efficacy over time. These findings demonstrate that *T. erecta* (L.) leaf extract shows significant nematicidal potential and could serve as an effective botanical alternative for managing root knot nematodes.

INTRODUCTION

Globally, root knot nematodes (*Meloidogyne* spp.) are considered some of the most harmful sedentary endoparasitic nematodes (Kantor *et al.*, 2022; Shahid *et al.*, 2024). Root knot nematodes (*Meloidogyne* spp.) are among the most damaging plant parasitic nematodes, causing approximately 12.6% loss in global crop production. *Meloidogyne incognita* is the nematode species most commonly found in vegetables (Marquez *et al.* 2019). Plants infected with root knot nematodes develop root galls, which disrupt normal root function and reduce water and nutrient uptake from the soil (Rao *et al.*, 2017; Saeed & Mukhtar, 2024). The second stage juvenile (J2) is the only stage that can infect plants. Once inside the roots, J2s grow into the third (J3) and fourth (J4) stages before becoming adults. Adult females form feeding sites that cause root galls (Kumar & Yadav, 2020).

Effective management of root knot nematodes (RKN) often involves combining multiple approaches, including resistant or trap crops, organic amendments, microbial agents, and chemical nematicides. Although synthetic nematicides are highly effective and widely used, their application is increasingly limited due to potential risks to human health and the environment (Trambadiya *et al.*, 2023). The use of botanical extracts to manage plant parasitic nematodes is gaining attention due to the environmental concerns associated with persistent chemical pesticides. Despite restrictions on certain nematicides, their continued use poses risks, highlighting the need for safer, ecofriendly alternatives. In this context, aqueous plant extracts have been explored for their potential to effectively suppress nematode populations while minimizing environmental impact (Abbassy *et al.,* 2017).

Marigold (*Tagetes erecta* L.) is a widely cultivated plant, valued globally for its ornamental, medicinal, and cosmetic uses (Fonseca *et al.*, 2016). *Tagetes erecta* (L.) widely valued as an ornamental plant with medicinal properties, produces considerable plant waste during commercial flower cultivation. The leaves have important economic value and can be used for environmentally friendly purposes, not just for decoration. Their application in sustainable agriculture, pharmaceutical industries, and environmental management underscores their multifaceted importance (Mejia-resendiz *et al.*, 2025). Marigold leaves greatly lowered nematode numbers in the soil (Radwan *et al.*, 2007). Plant derived secondary metabolites have gained attention as effective, environmentally friendly, and sustainable alternatives for controlling plant parasitic nematodes. Among the most investigated compounds with nematicidal activity are alkaloids, terpenoids, saponins, flavonoids, coumarins, thiophenes, and other phytochemicals have all been linked to the nematicidal activity (Prabhu & Poorniammal, 2023; Ibrahim *et al.*, 2024). The objective of the present study was to evaluate the nematicidal efficacy of aqueous leaf extract of *Tagetes erecta* (L.) as an alternative to chemical nematicides and to investigate its effects against *Meloidogyne incognita* under in vitro conditions.

Materials and Methods

Collection of Root Knot Nematode

Tomato plants infected with root knot nematode (*Meloidogyne incognita*) were identified in the farmer's field. Tomato plants (*S. lycopersicum* L.) showing symptoms like stunted growth, yellowing, and leaf curling were collected from Kandamannur, Theni District, Tamil Nadu. Roots infected with *M. incognita* and bearing visible galls were gently washed. The roots were examined for egg masses and females, and protruding females were carefully selected for study.

Identification of Root Knot Nematode

Female *Meloidogyne incognita* obtained from root knots of infected tomato plants (*S. lycopersicum* L.) were morphologically identified by studying the arrangement of the perineal pattern. Mature females were carefully dissected from the infested roots using forceps and a dissecting needle. An incision was made with a scalpel in the middle of the female, cutting through the cuticle along the equator. The posterior region containing the perineal pattern was carefully removed, trimmed with a fine pointed needle, and the attached debris was gently brushed off. The trimmed perineal pattern was mounted on a slide with a drop of glycerine, keeping the anterior side facing the glass and the anal region positioned upward. A coverslip was then carefully placed over the specimen, and the pattern was examined under a microscope Kaur, H., & Attri, R. (2013).

Preparation of Plant Extracts

Fresh leaves of *Tagetes erecta* (L.) were collected from the campus of Jayaraj Annapackiam College for Women, Periyakulam, Theni District, Tamil Nadu, India. The leaves were washed thoroughly and oven dried at 58 ± 2 °C for 48 h. The dried material was ground into a fine powder using an electric grinder. For stock preparation, 10 g of leaf powder was dissolved in 1000 ml of double distilled water and kept for 24 h. The extracts were filtered through Whatman No. 1 filter paper and centrifuged at 5000 rpm for 10 min to remove debris. The resulting supernatant was used as the stock solution, from which working concentrations of 100, 200, 400, 600, 800, and 1000 ppm were prepared using sterile distilled water (Khan *et al.*, 2019).

Maintenance of inoculum

A pure population of *Meloidogyne incognita* was maintained on tomato plants (*S. lycopersicum* L.) (Var. PKM-1) in 5 kg capacity earthen pots under controlled conditions at the Research Centre, Jayaraj Annapackiam College for Women, Periyakulam, India. Twenty one day old seedlings were transplanted into sterilized pot mixture containing red soil, farmyard manure, and sand in a 2:1:1 ratio. After seedling establishment, second stage juveniles (J2) were inoculated at the rate @ 2 J2 per gram of soil in each pot. 45 days after inoculation, the plants were carefully uprooted and washed with water. Egg masses were collected and incubated in water to allow hatching. The hatched juveniles were used as inoculum for subsequent experiments (Taylor and Sasser, 1978).

Juvenile Mortality Assay

For the mortality test, 1.0 ml of double distilled water containing 100 freshly hatched second stage juveniles (J2) of *Meloidogyne incognita* was transferred into Petri dishes containing 9.0 ml of *Tagetes erecta* (L.) leaf extract at different concentrations (100, 200, 400, 600, 800, and 1000 ppm). Double distilled water served as the control. Each treatment was replicated four times. The Petri dishes were incubated at 28 °C, and observations were recorded after 24, 48, 72, and 96 h using a stereoscopic microscope. Nematodes exhibiting mobility or winding body shapes were considered alive, while those showing no movement and appearing straight were considered dead (Rokiek *et al.*, 2011). The number of live and dead nematodes was recorded, and mortality data were subjected to probit analysis for the calculation of LC_{50} values (Behreus *et al.*, 1953; Sakuma, 1998).

Mortality (%) = Control – Treatment / Control × 100

Statistical analysis

Mortality data of juveniles were recorded from four replicates (n = 4). Probit analysis was used to calculate the lethal concentration (LC50) at different time intervals. Statistical analyses were conducted using SPSS software, version 20 (SPSS Inc., Chicago, IL, USA). One way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to determine significant differences among treatments. Results are expressed as mean ± standard deviation, and differences were considered significant at $P < 0.05$.

Results

The present study evaluated the morphological identification of *Meloidogyne incognita* and assessed the nematicidal efficacy of *Tagetes erecta* (L.) leaf extract against second stage juveniles. Microscopic examination of mature females of *Meloidogyne incognita* revealed a characteristic pear shaped body with a distinct anterior stylet, well developed muscles, and a thick cuticle. The posterior region showed a visible vulva. Perineal pattern analysis further exhibited an oval to rounded shape with distinct lateral lines. A squarish dorsal arch with wavy striations and forked lateral lines characterized the posterior cuticular pattern (PCP). These features together confirm that the nematode species is *M. incognita*. (Plates 1(a), and Plate 2 (b)).

The results showed that (Figures 1 and 2) *Tagetes erecta* (L.) leaf extract caused mortality of juveniles at all the concentrations compared to the control. The juvenile mortality was high at 1000 ppm (100%), followed by 800 ppm (89%), 600 ppm (72%), 400 ppm (61.5%), 200 ppm (51.5%), and 100 ppm (18.5%), respectively. The study demonstrated that the *Tagetes erecta* (L.) leaf extract 1000 ppm had a more pronounced effect on juvenile mortality compared to other treatments. Statistical analysis using one way ANOVA identified significant differences among the treatment groups. A symbol above each bar in the figures indicates significant differences compared to the control group. Notably, the *Tagetes erecta* (L.) leaf extract significantly reduced ($p < 0.05$), ($p < 0.01$), ($p < 0.001$) the juvenile mortality at all concentrations.

The nematicidal activity of *Tagetes erecta* (L.) leaf extract against juveniles of *Meloidogyne incognita* is presented in Table 1. The LC_{50} values decreased progressively with increasing exposure time, indicating enhanced mortality of juveniles over time. At 24 hours, the LC_{50} was 220.85 ppm, which reduced to 178.30 ppm at 48 hours and 150.67 ppm at 72 hours. The lowest LC_{50} value (120.45 ppm) was recorded at 96 hours. These findings demonstrate that prolonged exposure to *T. erecta* leaf extract increases its nematicidal efficacy against *M. incognita* juveniles. Overall, the results indicate that extended exposure to *T. erecta* (L.) leaf extract substantially enhances its nematicidal activity, as evidenced by the progressive reduction in LC50 values over time.

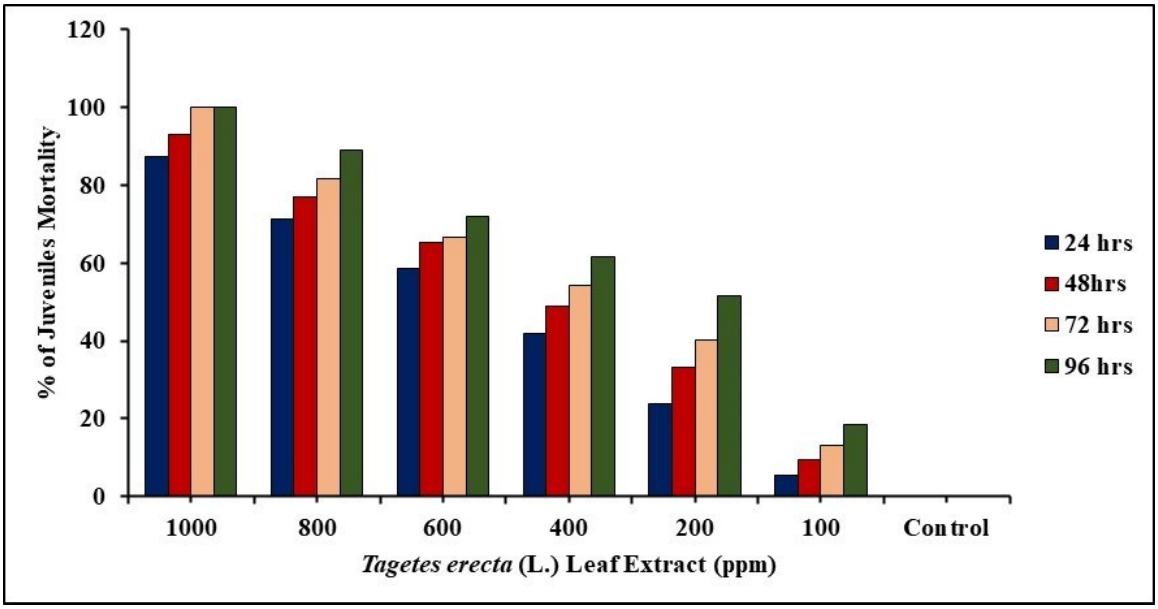

Figure 1: Effect of *Tagetes erecta* (L.) leaf extract on the juvenile mortality of *Meloidogyne incognita* in vitro. *Data represent mean (n = 4). Control-Distilled water; ppm- Parts per million.

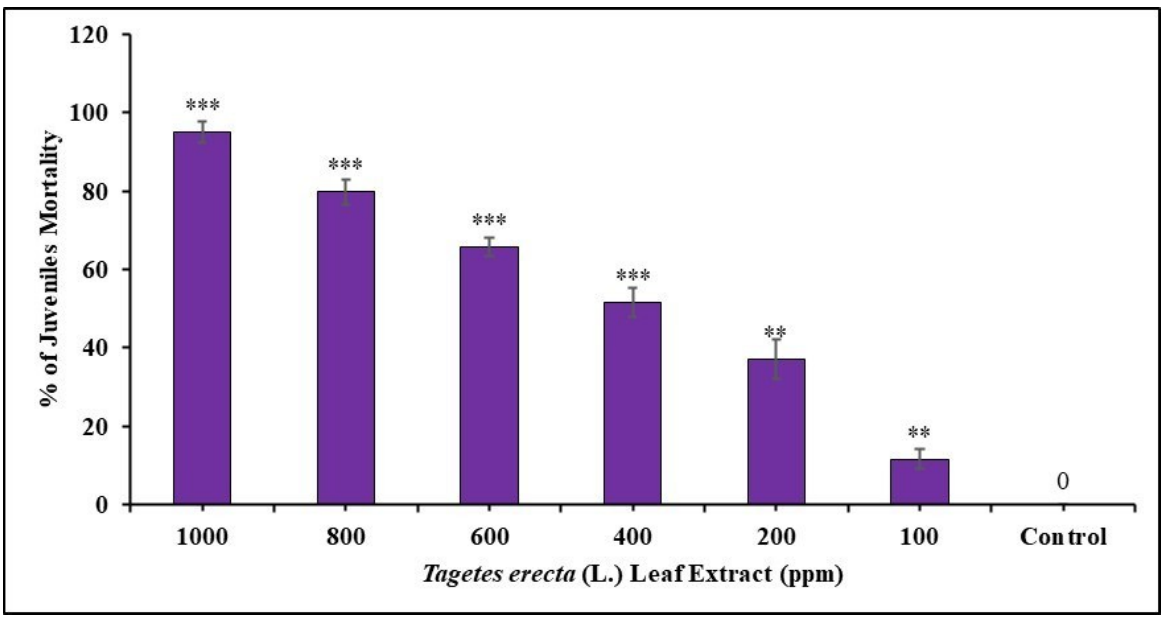

Figure 2. Antagonistic activity of *Tagetes erecta* (L.) leaf extract on juvenile mortality of *Meloidogyne incognita* at different intervals. Values are expressed as mean ± standard error of 4 independent replicates. Paired sample t-test was used to analyze the significant differences between the groups. The symbol (*), (**), and (***) above the bar represents the significant difference ($p < 0.05$), ($p < 0.01$), ($p < 0.001$) from the control.

Table 1 Nematicidal activity of *Tagetes erecta* (L.) leaves extract against juveniles of *Meloidogyne incognita*.

Treatments	Time Interval (hours)	LC50 value in ppm (95% CL)
Tagetes erecta (L.) leaf extract	24	220.85
	48	178.30
	72	150.67
	96	120.45

Note*: LC50– The lethal concentration required to cause 50% mortality at 24, 48, 72, and 96 hours, calculated with 95% confidence limits. CL-Confidence limits.

Plate 1: (a) Morphological Microscopic Image of Pear Shaped Mature Female *M. incognita*

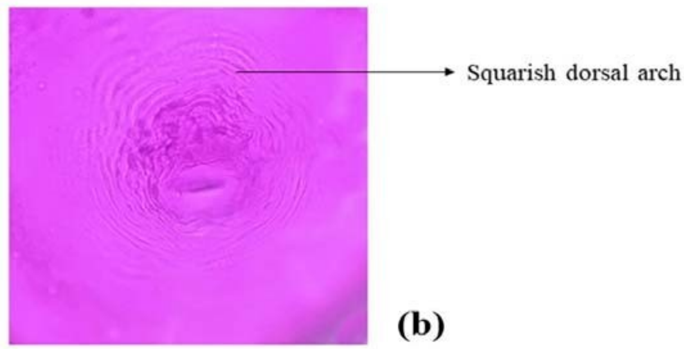

Plate 2: (b) Microscopic Image of *M.incognita* Pear Shaped Mature Female Perineal Pattern

DISCUSSION

The present findings on the nematicidal potential of *Tagetes erecta* (L.) leaf extract against *Meloidogyne incognita* are supported by previous studies, which collectively highlight the role of bioactive compounds and morphological confirmations in nematode identification and in vitro juvenile mortality assays. According to Deshwal *et al*. (2024), species confirmation of *M. incognita* was achieved through analysis of female perineal pattern morphology. Root knot nematode (*Meloidogyne incognita*) infecting passion fruit (*Passiflora edulis*) in Mizoram was identified by Khan *et al*. (2017) through morphological observations of its various life stages. The examination of perineal patterns in mature females isolated from infected root samples revealed a prominent, squarish dorsal arch accompanied by distinctwavy, forked striae. These morphological characteristics diagnose *Meloidogyne incognita*, thereby confirming its association as the predominant root knot nematode species in the surveyed samples (Kaur *et al*., 2022). In an in vitro study, distilled water leaf extracts of Neem (*Azadirachta indica*) and Bael (*Aegle marmelos*) caused 86.6% and 11.9% mortality, respectively, of *Meloidogyne javanica* second stage juveniles at 100% concentration (Shakya & Yadav, 2020). Similarly, Ali *et al*. (2018) observed that the mortality of second stage juveniles (J2) increased progressively with time, reaching the maximum value of 95.60% after 10 days of exposure in Petri dishes treated with 10% (w/v) marigold extract. Shah *et al*. (2018) observed that *Tagetes erecta* (L.) leaf extracts were more effective at higher concentrations, showing the greatest suppression of *M.incognita* population. (Degroote *et al*., 2024) showed that the in vitro evaluation of an innovative bio nematicidal compound mixture against *Meloidogyne incognita* showed significant efficacy, causing average mortalities of 70.0% and 82.3% after 1 and 2 days of exposure, respectively ($p < 0.001$).

Tariq *et al*. (2022) analyzed the nematicidal activity of various aqueous plant extracts against *M. incognita* J2. Among them, Parthenium hysterophorus was the most effective, with LC50 values of 1098.8, 853.4, and 664.9 µg/mL at 12, 24, and 48 h, respectively. *Tinospora cardifolia* showed the least activity, with much higher LC_{50} values of 2686.4, 2418.8, and 1419.0 µg/mL across the same exposure times. Moderate efficacy was observed for *Cymbopogon citratus* (1390.3, 1063.3, 769.3 µg/mL), *Eichhornia crassipes* (1736.7, 1349.3, 950.2 µg/mL), and *Monstera deliciosa* (2089.7, 1598.5, 1151.8 µg/mL). These results demonstrate that the toxicity of plant extracts varies with species, and the progressive decline in LC50 values over time reflects increased mortality with longer exposure. According to Aminu-Taiwo *et al*. (2015), *Tagetes erecta* extract was the most effective among the tested botanicals, causing complete juvenile mortality within 24 hours of exposure, with an LC50 value of 0.31 mg/ml. Overall, these observations reinforce that *T. erecta* leaf extract is a promising plant based agent for the effective control of *M. incognita*, with its efficacy strongly influenced by concentration and exposure duration.

CONCLUSION

The present study confirmed the identity of *Meloidogyne incognita* through morphological and perineal pattern analyses and demonstrated the strong nematicidal potential of *Tagetes erecta* (L.) leaf extract against its second stage juveniles. The extract induced significant mortality across all tested concentrations, with maximum effectiveness observed at 1000 ppm. The progressive decline in LC50 values with increasing exposure time further highlighted the importance of duration in enhancing nematicidal efficacy. These findings suggest that *T. erecta* (L.) leaf extract considerable promise as a natural alternative for nematode control, highlighting the need for further exploration through formulation studies and field evaluations.

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