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B Aswini
M.Sc. Scholar, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

B Anita
Professor, Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

A Shanthi
Professor and Head, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

D Jeya Sundara Sharmila
Assistant Professor, Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author
B Aswini
M.Sc. Scholar, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Nematicidal potential of Mexican sunflower, (*Tithonia diversifolia)* against the root-knot nematode, *Meloidogyne incognita*

B Aswini, B Anita, A Shanthi and D Jeya Sundara Sharmila

Abstract
Meloidogyne incognita, an endoparasitic root knot nematode, is mostly responsible for substantial yield loss in horticultural crops. Recently, increasing attention has been placed on creating environmentally friendly nematode management techniques for sustained crop production. Utilizing botanicals is one such eco-friendly method. The effectiveness of *Tithonia diversifolia*, a herbaceous blooming plant that has been widely used as an attractive plant and also possesses nematicidal qualities, was investigated through experiments. From plants kept in a glass house, *T. diversifolia* leaves were obtained. Using ethyl acetate, fresh leaf extract was prepared and centrifuged at 3000 rpm for 10 minutes. The extract was purified using Whatman no. 1 filter paper and utilised as a stock solution for further research. The extract was made in various dilutions, and its effectiveness against *M. incognita* was tested under *in vitro* conditions. It was observed how *T. diversifolia* leaf extract affected nematode egg hatching and juvenile mortality. When *Meloidogyne incognita* eggs were exposed to a 20 percent extract of *Tithonia diversifolia*, the eggs' capacity to hatch fell by 99 percent in under 24 hours. Similarly, when juvenile *Meloidogyne incognita* (J2) was exposed to a 20% extract of *Tithonia diversifolia*, 99 percent of the juveniles died within 72 hours.

Keywords: *M. incognita*, eco-friendly management, *Tithonia diversifolia*, nematicidal properties

Introduction
The most challenging crop pests to manage are phytoparasitic nematodes. Particularly in the tropical and subtropical climates, where environmental variables favour their survival and dissemination, they severely harm a wide variety of agricultural and horticultural crops, causing severe output loss. *Meloidogyne incognita* (Southern root-knot nematode), *M. arenaria* (peanut root-knot worm), *M. javanica* (Javanese root-knot nematode), *M. hapla* (Northern root-knot nematode), and the recently developing *M. enterolobii* (guava root-knot nematode) are the different species of root knot nematodes that affects most of the horticultural crops. *M. incognita* is the root-knot nematode that is most commonly found (Eisenberg *et al*., 1991).

One of the main methods of control in crops that are produced more intensively is the use of synthetic nematicides. However, with sustained usage, their effectiveness may diminish and they have a significant negative influence on the environment. Consequently, there is growing curiosity about finding nematicidal chemicals in plants (Olubunmi & Atolani *et al*., 2020) [2]. One of the most promising approaches to investigate the possibility of nematicidal or nematostatic qualities for the management of nematode problems is the use of plant products (Nikoletta *et al*., 2012)

One such plant is the Mexican sunflower (*Tithonia diversifolia*), a herbaceous flowering plant that has been widely introduced as an ornamental and has escaped from cultivation to become invasive, mostly in disturbed sites, along roadsides and in ruderal areas near cultivation (Jessica *et al*., 2021). *T. diversifolia* is a successful invader of new habitats through its tolerance to heat and drought, its rapid growth rates and its large production of lightweight seeds which are easily dispersed by wind, water and animals. Allelopathic activity has also been reported for this species (Sudeeptongma *et al*., 2017 and Maria *et al*., 2015) [7]. *Tithonia diversifolia* is recommended to be used as a green manure or as a major component of compost (Olayinka Samuel *et al*., 2007). Belay Feyisa *et al*., (2016), conducted a study to evaluate the effect of different locally available botanicals including Tithonia and an antagonistic fungus, *Trichoderma harzianum* to control root-knot nematode attacking tomato under *in vitro*

condition. Aqueous extracts of all the botanicals inhibited egg hatching of nematode and resulted in 100% mortality of the second stage juveniles of *M. incognita*. Hence the study was taken to assess the nematicidal property of *T. diversifolia* leaf extract against the root knot nematode, *Meloidogyne incognita.*

Materials and Methods

Maintenance of pure culture of *Meloidogyne incognita*

Pure culture of root knot nematode, *Meloidogyne incognita* was maintained in a glasshouse at the Department of Nematology (Fig 1). Fifteen days old tomato seedlings (cv. CO_3) were transplanted into 5kg pots (27 pots) containing pot mixture (1 part organic manure: 2 part red earth: 1 part sand). Egg masses were collected from the root knot nematode infected galled roots and kept in a beaker containing distilled water for hatching. After 3-5 days, freshly hatched juveniles (3000 J2) were inoculated near the root regions of 25 days old tomato seedlings. For laboratory studies egg masses were collected from *M. incognita* infected galled roots of tomato and sterilized with 0.5% sodium hypochlorite solution for 10 seconds. Infective juveniles were obtained from fully matured egg masses of *M. incognita.*

Fig 1: *M. incognita* affected galled roots of tomato

Preparation of *Tithonia diversifolia* leaf extract

Tithonia diversifolia leaves were collected from the glasshouse, Department of Nematology and near the temple, TNAU, Coimbatore. Cuttings of *Tithonia diversifolia* were planted and maintained for further research purpose (Fig 2).

10 g of *Tithonia diversifolia* leaves were taken. The leaves were grinded using mortar and pestle by adding 100 ml of ethyl acetate. The extracts were centrifuged at 3000 rpm for 10 minutes. They were filtered through Whatman no. 1 filter paper. The filtered extract served as the stock solution for preparing different dilutions.

Preparation of different concentrations of *Tithonia diversifolia* leaf extract

The stock solution (100% extract) was used to prepare different concentrations viz., 5%, 10%, 15%, 20% (Fig 3). These concentrations were evaluated for their efficacy on nematode hatching and mortality.

Fig 2: Mexican Sunflower (*Tithonia diversifolia*) plant

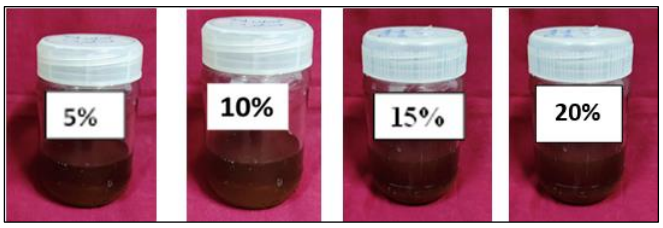


Fig 3: Preparation of different Concentrations of *Tithonia diversifolia*

Bioefficacy of *Tithonia diversifolia* leaf extracts on hatching

Different concentrations (5%, 10%, 15%, 20%) of *Tithonia diversifolia* leaf extracts were tested on sterilized egg masses of the root knot nematode, *M. incognita* under in-vitro conditions (Leonard *et al*., 2021) [1]. Distilled water was taken as control. From each concentration, 2ml was taken and tested against one nematode egg mass for each replication (Fig 4). Totally five treatments and four replications were maintained in a Completely Randomized Block Design. Total number of hatched juveniles from each egg mass was observed at 24hrs, 48hrs and 72 hrs time intervals.

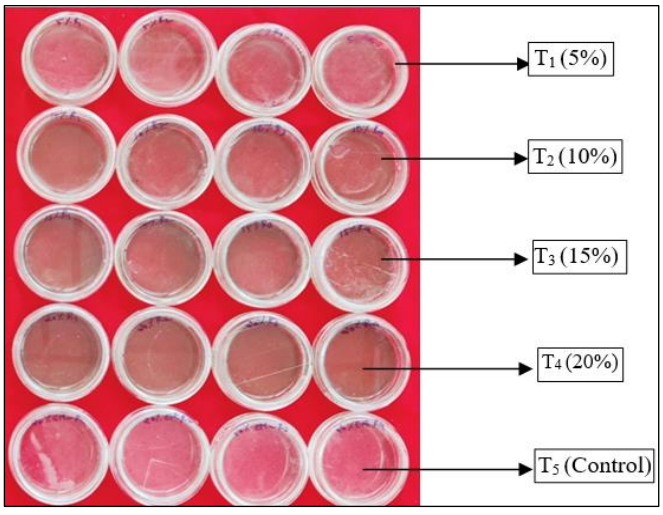


Fig 4: Egg Hatching Test

Bioefficacy of *Tithonia diversifolia* leaf extracts on juvenile mortality

One ml suspension of water containing 100 infective juveniles was added to different concentrations (5%, 10%, 15%, 20% and control) in Petri-plates (Fig 5). Totally five treatments and four replications were maintained in a Completely Randomized Block Design. Number of dead juveniles was observed at 24 hrs, 48 hrs and 72 hrs time intervals.

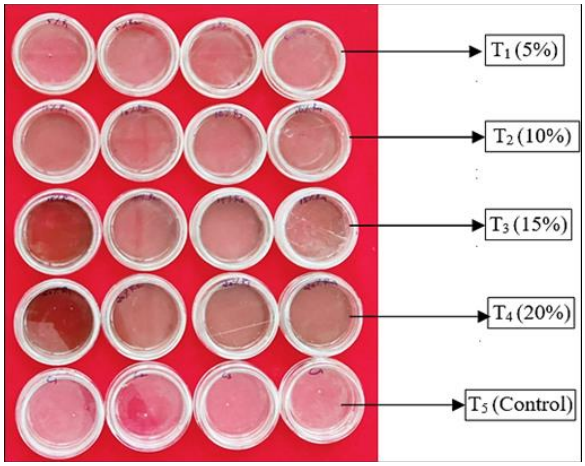


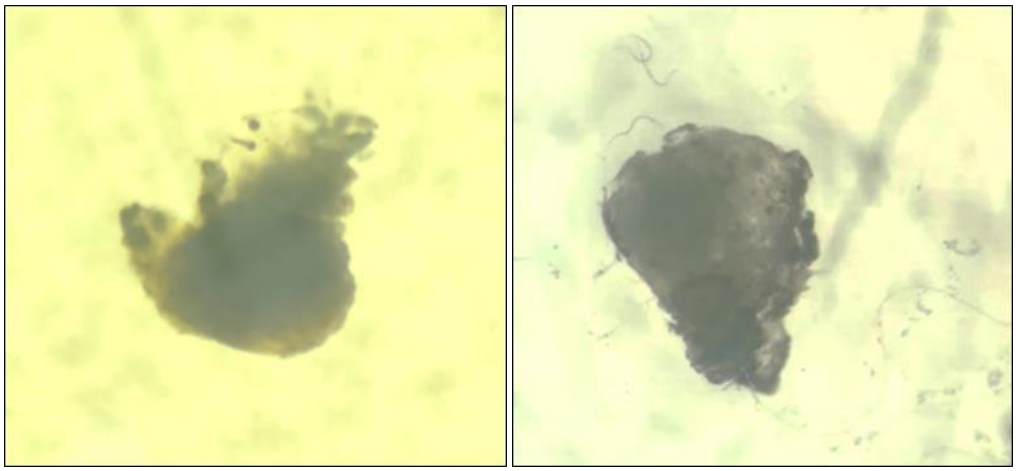
Fig 5: Juvenile Mortality test

Statistical analysis

The data obtained from the various experiments were analyzed statistically using ANOVA and Duncan's Multiple Range Test (DMRT) (Panse and Sukhatme, 1954) [13].

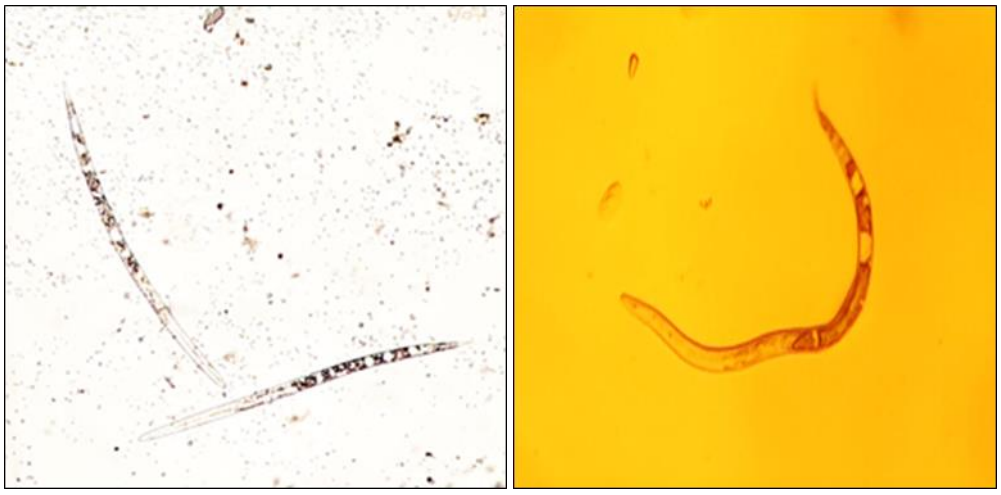
Results and Discussion

Using *Tithonia diversifolia*, leaf extracts were made in different concentrations and tested for nematode egg hatching and juvenile mortality. Among the six different concentrations evaluated, exposure of root knot nematode egg mass to 20% concentration of the leaf extract decreased the egg hatching of *M. incognita* significantly by 100% within 24 hours of exposure compared to control and other concentrations of leaf extract. (Table 1, Fig 6). *Tithonia diversifolia* leaf extract was found to degrade the gelatinous matrix of egg masses. Mortality of *M. incognita* infective juveniles was significantly high in 20% concentration compared to control. In- vitro results revealed that exposure of juveniles of *M. incognita* to *Tithonia diversifolia* leaf extract at 20%concentration caused 99% mortality within 24 to 72 hrs time interval period (Table 2, Fig 7) when compared to other treatments.



a) Treated eggmass showing unhatched juveniles b) Untreated eggmass showing hatched juveniles

Fig 6: Efficacy of 20% concentration of *Tithonia diversifolia* leaf extract on hatching of *M. incognita* eggs



a) Treated juveniles showing mortality b) Untreated juveniles showing active movement

Fig 7: Efficacy of 20% concentration of *Tithonia diversifolia* on nematode mortality of *M. incognita* juveniles

Table 1: Effect of *T. diversifolia* leaf extracts on hatching of *M. incognita* eggs under *in vitro* conditions

Treatments	Number of eggs hatched					
	24hr		48hr		72hr	
	Transformed values	Percent hatched (%)	Transformed values	Percent hatched (%)	Transformed values	Percent hatched (%)
T_1	13.00^{b} (3.61)	13	18.00^{b} (4.24)	18	19.00^{b} (4.36)	19
T_2	11.00^{b} (3.32)	11	13.00^{c} (3.61)	13	14.00^{c} (3.74)	14
T_3	3.00^{c} (1.73)	3	5.00^{d} (2.18)	5	6.00^{d} (2.45)	6
T_4	0.00^{d} (0.50)	0	0.00^{e} (0.50)	0	0.00^{e} (0.50)	0
T_5	53.00^{a} (7.28)	53	76.00^{a} (8.72)	76	95.00^{a} (9.75)	95
S.Ed.	1.1131		1.1699		1.1886	
CD(=0.01%)	3.2799		3.4473		3.5026	

*Figures in parentheses are square root transformed value. In a column, means followed by common alphabet are significantly different from each other at 1% level by DMRT.

Table 2: Effect of *T. diversifolia* leaf extracts on mortality of *M. incognita* juveniles under *in vitro* conditions

Treatments	Number of dead juveniles					
	24hr		48hr		72hr	
	Transformed values	Percent hatched (%)	Transformed values	Percent hatched (%)	Transformed values	Percent hatched (%)
T_1	22.00^{d} (4.69)	22	24.00^{d} (4.90)	24	25.00^{d} (5.00)	25
T_2	54.00^{c} (7.35)	54	55.00^{c} (7.42)	55	56.00^{c} (7.48)	56
T_3	81.00^{b} (9.00)	81	82.00^{b} (9.06)	82	84.00^{b} (9.17)	84
T_4	98.00^{a} (9.90)	98	99.00^{a} (9.95)	99	100.00^{a} (9.97)	100
T_5	0.00^{e} (0.50)	0	1.00^{e} (1.00)	1	1.00^{e} (1.00)	1
S.Ed.	1.3129		1.6105		1.5524	
CD(=0.01%)	3.8689		4.7459		4.5745	

*Figures in parentheses are square root transformed value. In a column, means followed by common alphabet are significantly different from each other at 1% level by DMRT.

Various reports have revealed the nematicidal activity of the botanical, *Tithonia diversifolia*. Studies of Odeyemi and Adewale, (2011) [10] revealed the phytotoxic properties and nematicidal potential of extract and residue of *Tithonia diversifolia* on *Meloidogyne incognita* infecting yam. Alkaloids and saponins were found to be present in the constituents of *Tithonia* ethanol extracts. They observed reduction in reproduction, number of eggs and juveniles and galling of *M. incognita* infecting yams, thus concluding *T. diversifolia* have potential for the control of root-knot nematodes. Similarly, Leonard *et al*., (2021) [1], conducted an experiment showing that the incorporation of sunn hemp and Mexican sunflower each at 10 and 20seedlings/plot significantly reduced the populations of *M. incognita*, *Rotylenchulus* and *Xiphinema* spp. and increased the yields of the vegetables.

Philippe *et al*., (2019), conducted an experiment to find the efficiency of *Tithonia diversifolia* on managing burrowing (*Radopholus similis)* and root-lesion (*Pratylenchus coffeae*) nematodes under greenhouse and banana plantation conditions. Thus, *Tithonia diversifolia* helps in the production of "organic banana" as an alternative to the use of nematicides.

Odeymi *et al*., (2014), conducted screen house studies to assess the efficacy of *Tithonia diversifolia* and *Chromolaena odorata* powder in comparison with the local commercial neem organic fertilizer in the management *of Meloidogyne incognita* infection on cowpea *(Vigna unguiculata var. Ife Brown*). These studies reveal that *Tithonia diversifolia* is having nematicidal properties and thus it can be used for managing the root knot nematodes.

Further extracts of *T. diversifolia* have been used traditionally for the treatment of diarrhoea, fever, hematomas, hepatitis, malaria and wounds in human beings (Jian-Qiao Gu *et al*., 2001). It is revealed that the *Tithonia diversifolia* extract is capable of inhibiting non-inflammatory reactions as well as

inflammatory pain (Victor *et al*., 2003) [16]. Similarly, aqueous and methanolic extracts of *T. diversifolia* leaf contain antimalarial substances with properties that showed both preventive and curative effects on malaria parasites (Oyewole *et al*., 2008) [12].

Conclusion

To conclude, the findings of current study revealed that the botanical, *Tithonia diversifolia* leaf extract has the ability to suppress *Meloidogyne incognita* showing high egg hatching inhibition and juvenile mortality percentage. As it is revealed that the botanical *Tithonia diversifolia* is having nematicidal properties it can be further explored for the management of root knot nematodes.

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Conflict of interest: None.

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