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Nematicidal Property of Phytochemical Alpha - Terthienyl against Root Knot Nematode, *Meloidogyne incognita*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Documentation of the nematicidal effect of Alpha-terthienyl on *Meloidogyne incognita.*
Study Design: Complete randomized design (CRD).
Place and Duration of Study: Department of Nematology, Tamil Nadu Agricultural University, Coimbatore between 2021-2023.
Methodology: This study used a purified synthetic compound of Alpha-terthienyl (α-T).it is a plant-derived phytochemical from Marigold (*Tagetes* sp.). This study examines the effect of alpha-

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terthienyl on egg hatching and juvenile mortality under laboratory conditions. Four different concentrations of alpha-terthienyl were tested for their influence on *in vitro* studies. A uniform size of single egg mass of *M. incognita* was incubated. Observations on hatching were recorded. Juveniles of *M. incognita* were incubated in alpha-terthienyl were examined.
Results: The result showed 100% percent inhibition at 2 ppm concentration. A similar trend was observed in Juvenile (J2) mortality also showed 100% mortality at 2 ppm. A check was maintained in tap water for comparison.
Conclusion: According to the findings of this study, Alpha-terthienyl was effective against *M. incognita* under in vitro conditions. Hence it could be used for the management of *M. incognita* in vegetable crops which also increase yield.

Keywords: Alpha-terthienyl; Meloidogyne incognita; nematicidal effect; egg hatching; juvenile mortality.

1. INTRODUCTION

Root-knot nematode, *Meloidogyne incognita* is the most common plant parasitic nematode [1] is highly polyphagous and causes damage to a wide range of economically important crops worldwide. *M. incognita* causes significant yield and economic losses in agricultural crops and vegetable crops [2]. According to the report, the yearly output losses caused by plant-parasitic nematodes will be close to $173 billion. It has a diverse host range [3]. Depending on the tomato variety, the root-knot nematode has a 25 to 100% yield loss potential, which leads to an estimated annual agricultural loss of USD 80 billion [4]. Plant parasitic nematode causes an estimated Rs.102,039.79 million (1.58 billion USD) which amounts to 21.3% yield loss in crops [5].

For several decades, the use of chemical nematicide was one of the primary means of control for root-knot nematodes [6]. Nowadays, chemical nematicide are losing their popularity among farmers for protecting their crops from nematode infestations because of their harmful effects and environmental pollution that led to an urgent need for safe and more effective options [7], Jonathan et al. 2012). Plant extracts or phytochemicals are non-harmful to the environment. The majority of the bioactive compounds present in the plant genera are alkaloids, terpenes, tannins and flavonoids that possess antioxidant and insecticidal properties [8,9] .

Alpha-terthienyl, a naturally occurring secondary plant metabolite is found in abundance in the roots of *Tagetes species* (Family: Asteraceae). The phytochemical of marigolds alpha-terthienyl, is known for its nematicidal, insecticidal, fungicidal, antiviral, and cytotoxic properties [10]. It is a phototoxic compound, which has great potential as a pest control agent is a potential insecticide/larvicide [11]. Alpha -terthienyl is phototoxic against several organisms such as nematodes, insects such as *Manducta* sexta, *Piaria rapae, Musca domestica.* It produces oxygen radical species which inhibit several enzymes like both *in vivo* and *in vitro* [12,9]. The susceptibility of nematodes to alpha-terthienyl modifies the expression of GST and SOD . It affects respiratory, digestive and nervous systems of larvae resulting in 100% mortality. This makes Alpha-terthienyl an effective Phyto nematicide. With this background, a laboratory study was conducted to test the effect of alpha-terthienyl against *M. incognita.*

2. MATERIALS AND METHODS

2.1 Preparation of Alpha-Terthienyl Stock Solution

Alpha-terthienyl (99.9% purity) synthetic chemical was purchased from TCI chemicals. It was insoluble in water, so it was dissolved in the solvent 2% DMSO (Sigma- Aldrich) in distilled water. The standard solution was prepared at a concentration of 1000 ppm. Further dilutions were prepared from the stock solution as a working standard for the experiments.

2.2 Maintenance of Pure Culture

M. incognita culture was obtained from the Department of Nematology, TNAU, Coimbatore. The species of the nematode was determined to be *M. incognita* based on morphological characters of perineal pattern present in the posterior region of the female body [13]. It was maintained in PKM-1 tomato variety grown in the pots containing a sterile pot mixture. The egg masses and juveniles from the pure culture is used for further experiments.

2.3 Egg Hatching Study

Different concentrations *viz*., 0.5, 0.75, 1, 2 ppm of alpha-terthienyl were prepared by diluting the stock solution. With the addition of distilled water various concentrations were transferred to a 5cm diameter Petri plate. Single egg masses of *M. incognita* having uniform size were inoculated to each Petri plate. A treatment with blank (tap water) was maintained as a check (Fig 3). These Petri plates were incubated at room temperature (28 ± 2℃). A number of hatched second-stage juveniles (J2) was observed at 24 h intervals upto 96hr.A number of unhatched eggs was counted and the percent egg hatch inhibition was calculated by using Abbot's formula . After 4 days of incubation the treated eggs were transferred to the tap water, to confirm the effect.

Hatching inhibition of eggs (%) = (Total number of eggs – Hatched number of eggs) / Total no.of eggs in treatment × 100

2.4 Juvenile Mortality Study

An in vitro test was carried out to study the impact of alpha-terthienyl on the mortality of second stage juvenile of *M. incognita*. The second-stage J2 was obtained from a pure culture maintained under glasshouse conditions. The egg masses of *M. incognita* were incubated at (28±2℃) for obtaining uniform stages of hatched J2. Four separate doses of the solutions at 0.5ppm, 0.75ppm, 1ppm and 2ppm, were prepared from stock solution with three replicate treatments. A treatment with blank was maintained as a check. A number of dead juveniles was counted at intervals of 24h,48h and 72h. juvenile mortality was calculated by N.G. Ravichandra [14]. After 72hrs the treated juveniles were transferred to tap water (Fig 9).

Mortality (%) = (Number of dead juveniles in treatment / Total number of juveniles in the treatment) ×100

2.5 Statistical Analysis

The data from egg hatching and juvenile mortality were subjected to Complete Randomized Design and DMRT [15] test analyzed using IBM SPSS Statistics (Version 27) .

3. RESULTS AND DISCUSSION

3.1 Effect of Alpha Terthienyl on Egg Hatching Study

All the concentrations *viz*.,0.5,0.75,1, 2 ppm showed an inhibitory effect on the hatching of *M. incognita* eggs when the overall effect was analyzed. The proportion of egg hatching was directly proportional to the exposure period and inversely proportional to the concentration of Alpha terthienyl. The highest rate of complete inhibition at 24h, 48h and 72h was noted at 2 ppm (100%) followed by 1 ppm (18.91%) at 48h and 72h. Egg damage is seen in (Figs. 4-7). After 72 hours of incubation, the eggs were transferred to tap water to study the nature of alpha-terthienyl as Nematostatic or nematotoxic. Nematicidal activity of alpha-terthienyl against *M. incognita* egg masses were evaluated are presented in Table 1.

3.2 Effect of Alpha Terthienyl on Juvenile Mortality Study

Similarly, the highest mean mortality for juveniles was observed at 2 ppm (100%) at 24h, 48h and 72h followed by 1 ppm (98.43%) at 72 h and the least mean mortality was observed in 0.5 ppm (35.1%) at 24 h. The percentage of death among juveniles (J2) was higher when the exposure time was increased. The nematotoxic effect of alpha-terthienyl was confirmed by transferring the treated juveniles to tap water (Figs 9). The J2 was unable to revive even after transferring them into normal tap water. The effect of alpha-terthienyl on juveniles was evaluated and the results of mortality of nematode as function of time are presented in Table 2.

4. DISCUSSION

Current investigation showed that the alpha-terthienyl compound present in Marigold has the inhibitory effects of the root- knot nematode *M. incognita.* [16] study confirmed that α-terthienyl has capacity of exerting toxic effects to nematodes even in the absence of light and applied as a plant extract similar to nematicides. Zhang *et al,* 2019 compared crude extract of alpha-terthienyl and commercial product of alpha-terthienyl evaluated. Synthetic compound of alpha-terthienyl showed toxicity in concentration of (33 ppb) causes 100% larval death with 55 min of exposure to alpha-terthienyl and ultraviolet light (366 nm) on *Aedes aegypti.*

Methanol extracts of the leaves, stem, and roots of *T. spatula* exhibited strong inhibitory effect against *M. incognita* egg hatching [17]. it is in accordance with the above experiment showing egg hatching inhibition. Five different varieties of *Tagetes*, including *Tagetes patula, T. erecta c.v. Atlantis orange, T. erecta c.v. single orange, T. erecta c.v. Indian Yellow, and Tagetes minuta*, were tested for their ability to inhibit egg hatching and promote second-stage juvenile mortality using acetone extracts from their leaves, flowers, roots, and stems [18]. Similar results reported in *H. zeae* when treated with commercially available α-terthienyl at concentrations of 0.125% (≈5 mM) for 24 h showed 100% juvenile mortality. This result coincides with the above findings of 100% mortality at a 2ppm concentration. Takahiro 2019 also α-terthienyl is an oxidative stress-inducing chemical that effectively penetrates the nematode hypodermis and exerts nematicidal activity. Expression induction of two major enzymes, glutathione S-transferase (GST) and superoxide dismutase (SOD), was restricted in *C. elegans.*

Table 1. Effect of alpha-terthienyl on egg hatching

TREATMENTS (Concentration)	% egg hatching			
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
0.5 ppm	43.30 (41.14) [d]	53.43 (46.96) [c]	67.4 (55.18) [d]	74.24 (59.49) [d]
0.75 ppm	33.5 (35.36) [c]	43.42 (41.21) [b]	52.27 (46.30) [c]	62.38 (52.16) [c]
1 ppm	10.59 (18.99) [b]	43.42 (41.21) [b]	18.91 (25.77) [b]	18.91 (25.77) [b]
2 ppm	0.00 (4.05) [a]	0 (4.05) [a]	0 (4.05) [a]	0 (4.05) [a]
Blank (DMSO)	79.21 (62.87) [e]	96.49 (79.20) [d]	98.85 (83.34) [e]	98.85 (83.34) [e]
Control	81.10 (64.23) [f]	97.81 (81.48) [e]	98.95 (84.11) [f]	98.95 (84.11) [f]
SE(d)	0.216	0.032	0.093	0.057
C.D.	0.476	0.07	0.204	0.126

(Figures in parentheses are arc sine transformed values. The column followed by alphabet are significantly different from each other at 1% level by DMRT)

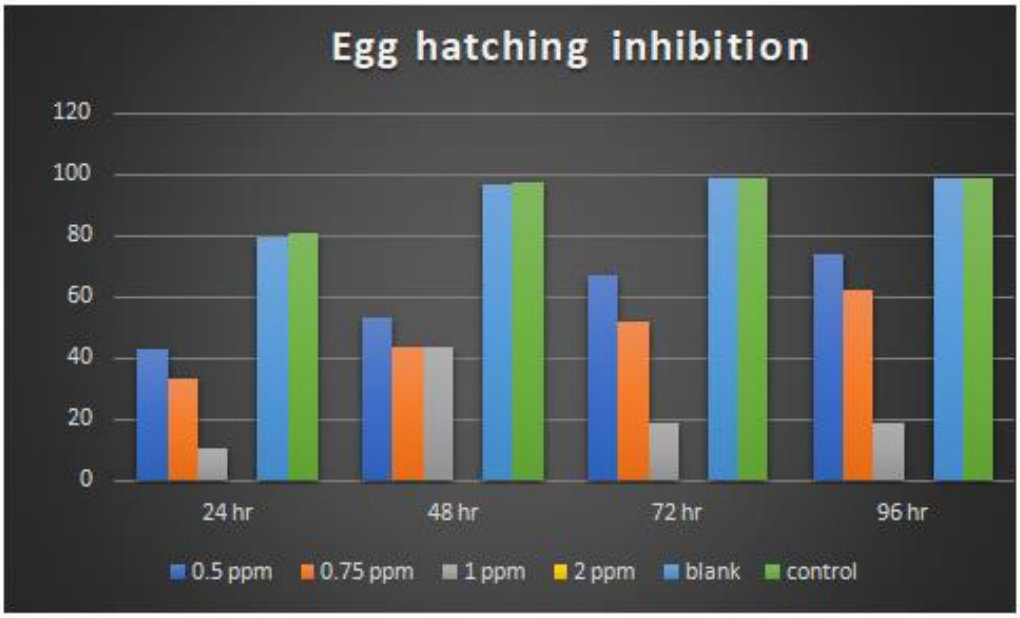

Fig. 1. Graph depicting the Egg-hatching inhibition

Table 2. Effect of alpha-terthienyl on juvenile mortality of *M. incognita*

TREATMENTS (Concentrations)	24h	48h	72h
0.5 ppm	35.10 (36.33) [d]	44.80 (42.01) [d]	53.13 (46.79) [d]
0.75 ppm	47.88 (43.78) [c]	62.19 (52.05) [c]	75.95 (60.63) [c]
1 ppm	88.02 (69.74) [b]	95.83 (78.21) [b]	98.43 (76.85) [b]
2 ppm	100 (90) [a]	100 (90) [a]	100 (90) [a]
Blank (DMSO)	15.78 (23.40) [e]	28.09 (32) [e]	32.02 (34.46) [e]
Control	7.32 (15.69) [f]	8.85 (17.30) [f]	12.85 (21) [f]
SE(d)	0.117	0.08	0.22
C.D.	0.25	0.18	0.49

(Figures in parentheses are arc sine transformed values. The column followed by alphabet are significantly different from each other at 1% level by DMRT)

Fig. 2. Graph indicating the Juvenile mortality test

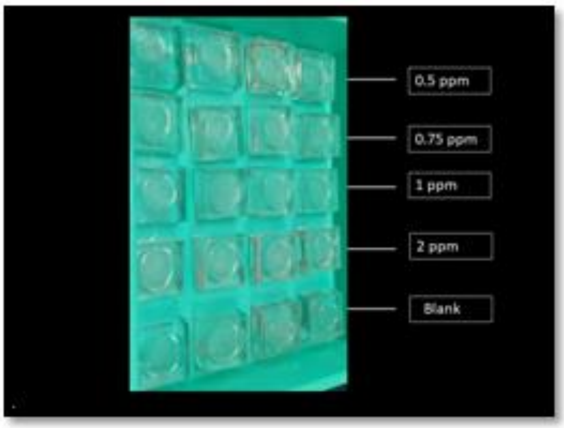

Fig. 3. Bioassay

Fig. 4. Healthy egg

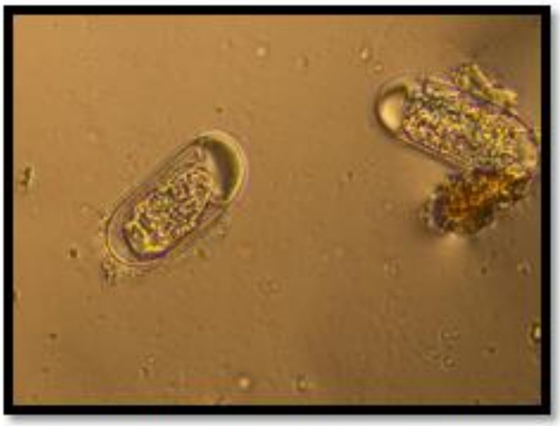

Fig 5 Protein deformation initiates in the corner of the egg masses

Fig 6 Protein deformation in higher magnification (40x)

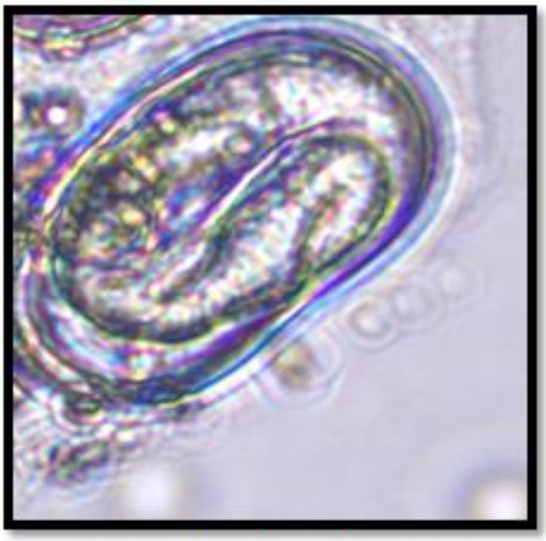

Fig 7 Malformation of egg along with juveniles

Fig 8 Healthy juvenile

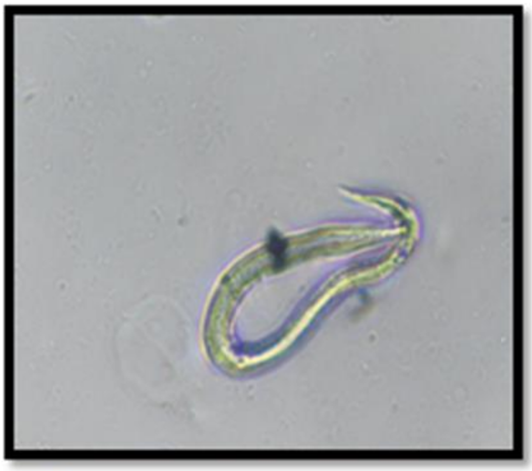

Fig 9 Damaged juvenile

5. CONCLUSION

The present investigation confirmed that the alpha-terthienyl can inhibit nematode egg hatching and causes nematode mortality. However, the mode of action of alpha-terthienyl is not well understood by these results. It may be considered as an alternative to synthetic nematicides since it is derived from plants. The development of suitable formulations that improve solubility and bioavailability is essential to develop a Phyto nematicide.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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