



REVIEW ARTICLE

Alpha-terthienyl (A novel marigold derivative): An overview on nematicidal potential

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Abstract

Alpha-terthienyl (α -T), a plant-derived compound primarily found in the root exudates and leaves of marigold (*Tagetes spp.*), has shown strong potential as a sustainable alternative to chemical nematicides for managing plant-parasitic nematodes (PPNs) such as *Meloidogyne*, *Heterodera* and *Pratylenchus species*. Acting as a phototoxic agent, α -T generates reactive oxygen species (ROS) under ultraviolet (UV) light, leading to nematode mortality through oxidative stress. Its broad-spectrum efficacy, persistence in the rhizosphere and low toxicity to non-target organisms make it a promising candidate for integrated pest management. Studies indicate that α -T induces high mortality in *Caenorhabditis elegans* and *Meloidogyne incognita*, disrupts key metabolic enzymes like cholinesterase and glucose-6-phosphate dehydrogenase and exhibits strong photo-larvicidal effects in *Aedes aegypti* (LC₅₀: 0.002 ppm under UV light), as well as insecticidal activity against Lepidoptera and fire ants. Future research should aim to improve its formulation for field stability, explore synergistic effects with other biopesticides, investigate resistance mechanisms and assess its broader applicability in pest and vector control.

Keywords: α -terthienyl; marigold; nano formulation; photo activation; root knot nematode

Introduction

In soil habitats, nematodes are tiny roundworms that fall into two categories: plant-parasitic nematodes (PPNs) that harm lawns and crops and free-living nematodes that eat microorganisms. Their habitats are plant-based and subterranean, which make them pests and challenging to manage. They are not easily accessible to home gardeners, despite being used in commercial agriculture. In India alone, PPNS cause an estimated 21.3 % annual yield reduction, amounting to economic losses of INR 1.58 Crores (1). PPNS are broadly classified into ectoparasitic nematodes (which live and feed on the external surface of their host) and endoparasitic nematodes which live and feed within the host tissue). *Xiphinema sp.*, *Longidorus sp.* and *Trichodorus sp.* are some of the ectoparasitic nematodes that act as vectors of various viral diseases in plants. Endoparasitic nematodes like *Meloidogyne incognita*, *Globodera rostochiensis*, *Heterodera sp.* that causes disruption of vascular systems and nutrient transport systems in plants. The affected plants are more susceptible to secondary bacterial or fungal diseases. Marigold (*Tagetes erecta*) is a widely cultivated commercial flower of the Asteraceae family, valued for its ornamental

appeal, ease of cultivation and pest management properties (2, 3). In India, it is extensively grown across major floriculture states like Tamil Nadu, Karnataka and West Bengal with approximately 47.68 thousand hectares yielding around 501.87 thousand metric tons (4). Additionally, *Tagetes* species produce thiophenes, which exhibit nematicidal properties, making them effective in managing soil-borne pests. *Tagetes* species exhibits biopesticidal properties, acting as an insecticide, mosquitocide, nematicide, bactericide and fungicide (5). α -T a potent bioactive compound in marigold, effectively suppresses plant-parasitic nematodes like *Meloidogyne spp.* through allelopathy and root exudates (6, 7). Given its strong nematicidal properties, α -T offers a sustainable alternative for managing nematode-induced crop damage, responsible for 60% yield loss (8-10).

α -T demonstrates a distinct advantage over other nematicidal compounds due to its phototoxicity and multi-target action. Unlike Azadirachtin is primarily extracted from the neem tree (*Azadirachta indica* A. Juss) which disrupts molting by inhibiting ecdysone signaling in insects and nematodes, α -T acts via an oxygen-dependent phototoxicity, generating singlet oxygen and superoxide radicals that

induce oxidative stress and disrupt cellular antioxidant defences in nematodes (11, 12). Thymol, which is primarily extracted from thyme (*Thymus vulgaris*) acts mainly through membrane destabilization and is more lethal to nematodes since it lacks α -T's oxidative stress induction (13). Compared to synthetic nematicides like carbofuran and fenamiphos, which inhibit acetylcholinesterase and persist in soil for months (14), α -T degrades rapidly, minimizing environmental accumulation while maintaining comparable efficacy. These properties make α -T a more potent and environmentally sustainable alternative in nematode and pathogen management. Thus, exploring its insecticidal and nematicidal effects offers a sustainable alternative to synthetic pesticides and nematicides, which are often associated with environmental and health concerns. Additionally, this review can contribute to the development of eco-friendly pest and nematode management strategies, reducing dependency on chemical inputs and supporting sustainable agriculture.

Species of marigold in nematode suppression

Marigold exhibits antagonistic properties that helps in reducing nematode populations in tomato crops (15). Intercropping tomatoes with marigold significantly lower nematode infestation, with a 3:1 marigold root extract achieving 82.33 % mortality. This practice also enhances tomato growth and fruit quality. Additionally, endophytic bacteria isolated from *Tagetes* species have shown bio control potential against *Meloidogyne* spp., causing up to 93 % mortality *in vitro* and reducing nematode populations by 50-74 % in greenhouse trials (16). These results highlight marigold's potential in sustainable management of root-knot nematodes. Flor de Muerto and Polynema reduced J2 populations in soil and roots by 85-92 %. Single Gold and Scarlet Sophie showed selective suppression, reducing *M. incognita* and *M. javanica* by up to 88 %. Tangerine Gem was effective against *M. hapla* (80 %) but less so against other species (<70 %). In contrast, *T. erecta* showed limited control over *M. hapla* (17). *Tagetes minuta* and *T. patula* have antagonistic effects on

Pratylenchulus penetrans, indicating their potential to lower its population. Cultivars like *T. patula* 'Single Gold', *T. hybrid* 'Polynema' and *T. erecta* 'Cracker Jack' effectively suppressed four root-knot nematode species in tomatoes. While 'Polynema' and 'Cracker Jack' support reniform nematodes, *T. patula* 'Boy-O-Boy' reduced their numbers (18). Cold aqueous extracts (20 % w/v) of *T. erecta* significantly lowered *Meloidogyne incognita* infestations and root galling in tomato plants, with 40-day-old plant extracts proving more effective than those from older plants-comparable to carbofuran (19). These results suggest *T. erecta* extracts as a natural alternative to chemical nematicides. The susceptibility of various *Tagetes* species and varieties to different plant-parasitic nematodes is summarized in Table 1.

Biological properties of marigold

a. Marigold act as a host plant

Research indicates that certain plant-parasitic nematodes, such as ring nematode (*Criconea* sp.) stubby-root nematode (*Trichodorus obtusus*), sheath nematode (*Hemicycliophora arenaria*), reniform nematode (*Rotylenchulus reniformis*) and pin nematode (*Paratylenchus* spp.) are not adversely affected by marigolds and may even use them as host plants. Unknown species of *Tagetes* to be a suitable host for these nematodes (20). Here are a few ways marigolds function as host plants.

b. Attracting beneficial insects

French marigold (*Tagetes patula*) has been selected as an insectary plant for sweet corn (*Zea mays* L. subsp. *mays*) due to effectiveness as a companion or border plant. Research has shown that nectar from marigold flowers significantly boosts the longevity of the parasitic wasp *Trissolcus basal*, highlighting its value in enhancing biological control efforts (21). Studies have demonstrated the pest-repellent and predator-attracting benefits of marigolds when used alongside snap beans (*Phaseolus vulgaris* L.) and lima beans (*Phaseolus lunatus* L.). For instance, fewer Mexican bean beetles (*Epilachna varivestis*) on snap beans bordered by

Table 1. Susceptibility of several marigold species/varieties to some commonly occurring plant-parasitic nematodes

Marigold species/variety	<i>Dolichodorus</i> sp.	<i>Radopholus similis</i>	<i>Hoplotaimus</i> sp.	<i>Pratylenchus</i> sp.	<i>Rotylenchulus reniformis</i>	<i>Meloidogyne incognita</i>	<i>Belonolaimus longicaudatus</i>	<i>Trichodorus</i> sp.	References
<i>Tagetes erecta</i>	-	R	-	-	R	-	-	-	(19)
<i>Tagetes erecta</i> 'cracker Jack'	-	-	-	R	S	R	-	-	(19)
<i>Tagetes minuta</i>	-	-	-	-	-	R	-	-	(92)
<i>Tagetes patula</i>	S	-	I	R	R	-	S	S	(31), (18), (93)
<i>Tagetes patula</i> 'Single Gold'	-	-	-	-	-	R	-	-	(94)
<i>Tagetes patula</i> 'Boy-O-Boy'	-	-	-	-	R	-	-	-	(19)
<i>Tagetes hybrid</i> 'Polynema'	-	-	-	-	S	R	-	-	(19)
<i>Tagetes signata</i>	-	-	-	-	-	R	-	-	(31)

S: Susceptible, R: Resistant, I: Intermediate

marigolds compared to non-bordered controls (22). Previous study has shown that higher populations of chewing and piercing-sucking predators in the interior rows of lima beans interplanted with marigolds and bordered by snap beans, compared to monoculture lima bean plantings (23). These findings highlight marigolds' role in enhancing pest management as well as promoting natural predators in cropping systems.

c. Nematicidal potential of marigolds in crop rotation

Crop rotation with marigolds has demonstrated significant efficacy in suppressing PPNs, often proving superior to non-host crops and chemical fumigants. Growing the marigold cultivar 'Single Gold' provided better control of lesion nematodes (*Pratylenchus penetrans*) in strawberries than fumigation with 750 L/hect of metam sodium (18). This highlights marigolds as a natural and sustainable alternative for nematode management. The effectiveness of chemical fumigants can decline due to microbial degradation, reducing their long-term impact. The use of *T. patula* and *T. erecta* as rotation crops to the conventional practice of growing rye (*Secale cereale*) with chemical fumigation before transplanting flue-cured tobacco (*Nicotiana tabacum*). Marigold rotation effectively reduced *P. penetrans* populations below economic thresholds for three consecutive years, leading to an increased tobacco yield of 197 Kg/hect compared to the rye-plus-fumigation method (24). These findings highlight marigolds' long-term nematicidal effects, reinforcing their role in sustainable nematode management and improved crop productivity.

d. Allelopathic effect

Allelochemicals are plant metabolites or their by-products released into the environment, where they can exhibit toxic effects on other organisms (25). Marigolds produce biologically active compounds, including essential oils, which have demonstrated potential allelopathic properties. These compounds have been found to be effective against nematodes and plant pathogens, such as *Alternaria solani* (26, 27). The specific allelopathic compounds in marigolds responsible for nematode suppression remain only partially identified. However, previous studies proposed that α -T is a key component (28). The discovery of such compounds dates back to the mid-20th century, when a study identified bithienyl compounds in the aerial parts of *Tagetes* (29). Subsequent studies isolated α -terthienyl and another bithienyl compound from marigold roots, highlighting their potential role in nematode management (30). More recently, three nematicidal compounds-5-(ent-1-ol)-2,2-bithienyl, sigma-4,22-dien-3-beta-ol and 5-(4-acetoxy-1-butenyl)-2,2-bithienyl were isolated from the chloroform extracts of *T. erecta*, *T. patula* and *T. minuta* (31). Their study found that *T. erecta* extracts were more effective against nematodes than those from *T. patula* and *T. minuta*. Furthermore, the whole extract of *T. erecta* exhibited greater potency than the individual compounds, suggesting synergistic effects among the components.

e. Nematocidal activity of marigolds in intercropping

Intercropping with marigolds has demonstrated strong nematicidal effects, effectively suppressing *Meloidogyne*

incognita populations in various cropping systems. *Tagetes erecta* did not develop root galls and significantly reduced gall formation on intercropped water spinach (*Ipomoea reptans*) (32). Similarly, a study reported that *M. incognita* failed to induce galls on *T. erecta* and *T. patula*, leading to reduced root galling in soybean (*Glycine max*) when intercropped with these marigolds (33). Intercropping *T. patula* at varying spacings between mulberry (*Morus alba*) rows significantly decreased root galling and egg mass numbers, reinforcing its nematode-suppressive potential (34). However, research indicates that intercropping cucurbits with *T. patula* or alfalfa (*Medicago sativa*) did not reduce nematode populations, suggesting that the effectiveness of marigolds in nematode suppression may depend on host crops and specific agro ecosystem conditions (35).

f. Nematicidal potential of marigold as a soil amendment

Incorporating *Tagetes* species into the soil effectively suppresses plant-parasitic nematodes through the release of toxic compounds during decomposition. *T. patula* residues have been shown to reduce *Meloidogyne incognita* populations, though the suppression is more pronounced when marigolds are grown as cover crops rather than solely used as amendments (36). The decomposition of marigold residues releases bioactive compounds that directly inhibit nematodes, while also altering soil microbial communities to favour nematode-antagonistic organisms such as microbivores and nematode-trapping fungi (37). Additionally, the breakdown of organic matter can generate acidic by-products that are lethal to certain nematodes. *T. patula*'s amendment effects contribute significantly to *Pratylenchus penetrans* suppression. However, distinguishing the nematicidal effects of marigold residues from crop rotation benefits requires controlled experimental designs, such as those demonstrated in studies using sun hemp amendments (38).

Application of marigold as a bio insecticide

Tagetes species are known for their insecticidal, larvicidal and mosquitocidal properties, making them suitable for eco-friendly pest control (39). Interest in plant-based insecticides is growing due to their safety and low environmental impact (40). Essential oils from *T. erecta* and *T. minuta*, containing compounds like limonene, β -ocimene, eugenol and piperitone, are effective against pests such as mosquitoes, Lepidoptera and beetles (41). These properties support their use in integrated pest management (IPM) strategies. Research also underscores the pest control potential of *T. lucida* and *T. erecta*. Essential oils from *T. lucida* repel *Sitophilus zeamais* and reduce aphid populations on cabbage, mainly due to oxygenated monoterpenes and phenolics, though repeated application is needed as effectiveness declines over time. *T. erecta* oil, particularly from roots and flowers, shows strong larvicidal activity against *Aedes aegypti*, linked to piperitone and thiophenes. Its flower extracts are also effective against *Culex quinquefasciatus*, with the chloroform fraction showing the highest toxicity. Similarly, *T. minuta* oil is effective against *Anopheles gambiae* and *Aedes aegypti* (40).

T. erecta essential oil shows strong larvicidal activity against *Aedes aegypti*, mainly due to piperitone, D-limonene and piperitenone from its thiophene-rich roots and flowers

(42). Its flower extracts are also effective against *Culex quinquefasciatus*, with the chloroform fraction being the most potent. Likewise, *T. minuta* oil targets *Anopheles gambiae* and *Aedes aegypti* (43). In addition to oils, powdered *T. erecta* provides a cost-effective option, cutting pest control costs by 75 % without negative effects. Intercropping *T. patula* with tomatoes has successfully controlled whiteflies and its aqueous and methanolic extracts show insecticidal activity against *Bemisia tabaci* (44). *T. erecta* root extracts have shown higher toxicity against *Rhyzopertha dominica* and *Tribolium castaneum* compared to malathion (45). Marigold leaf powder (5 %) also exhibited greater residual toxicity (57.09 %) than *Azadirachta indica* (50.06 %) and *Cynodon dactylon* (43.28 %), indicating its potential as a natural alternative to synthetic insecticides for stored grain protection (46). Additionally, dichloromethane and methanol extracts of *T. erecta* are effective against *Sitophilus oryzae* and its flower extracts further support its use in pest control (45). A comprehensive overview of the effect of marigold cultivars on plant parasitic nematodes and crop yield is shown in Table 2.

Applications of marigold as bactericide and fungicide

Essential oils from *Tagetes* species, rich in terpenoids such as dihydrotagetones, tagetones and ocimenones, possess strong biopesticidal, antibacterial and antifungal properties, as confirmed by GC-MS analysis (42, 47). *T. minuta* shows significant antifungal activity against plant pathogens, while *T. lucida* further supports the genus's antimicrobial potential. A compound from *T. patula* roots, 2,5-dicyclopentenyl cyclopentanone, also demonstrates strong antifungal effects. An emulsion formulation of *Tagetes* oil, designed for better water solubility, effectively targets *Fusarium* species, with SEM imaging revealing hyphal damage (40, 48). Species like *T. minuta* also inhibit pathogens such as *E. coli* and *Rhizoctonia solani*, due to secondary metabolites like thiophenes, flavonoids and terpenoids-especially α -terthienyl, which disrupts microbial membranes and produces ROS under UV light. These light-activated effects enhance their potential for sustainable disease management in agriculture and medicine. Their efficacy against soilborne pathogens like *Fusarium verticillioides* further supports their use in integrated pest management (49). Future studies should

Table 2. Effect of marigold cultivars on plant parasitic nematodes and crop yield

Crop studied	Target nematode species	Cultivars	Tactics	Findings	Country	References
Carrot	<i>Meloidogyne javanica</i>	<i>Tagetes patula</i> 'Happy Days'	Grown in rotation	Numbers and weight of carrots with galls following marigold were low <i>M. javanica</i> number in field soil following marigold were lower and yield higher than carrot following corn and okra	Brazil	(84)
Soyabean	<i>Meloidogyne incognita</i>	<i>Tagetes erecta</i> & <i>Tagetes patula</i> mix	Co-cropped in pots	Reduced the number of nematodes and galls on Soyabean and in soil and also increase in Soyabean plant growth parameters	Egypt	(33)
Strawberry	<i>Pratylenchus penetrans</i>	Single Gold	Grown in rotation	Reduced the population density of Marigold. Total yield and quality better than Marigold	Netherlands	(18)
Cabbage & cauliflower	<i>Tylenchorynchus brassicae</i>	<i>Tagetes tenuifolia</i> , <i>Tagetes lucida</i>	In corporation in pot <i>T. minuta</i> mix	Inhibited population build up	India	(92)
Pineapple	<i>Rotylenchulus reniformis</i>	<i>Tagetes patula</i> , <i>Boy-o Boy</i>	Grow in rotation	Decrease of reniform nematode numbers but magnitude of drop similar to clean fallow	USA , Hawaii	(95)
Mulberry	<i>Meloidogyne incognita</i>	<i>Tagetes patula</i> cv.unknown	Intercropping	Reduced root galls on Mulberry	India	(96)
Potato	<i>Pratylenchus penetrans</i>	<i>Tagetes tenuifolia</i> 'Nemakill and Nemanon', <i>Tagetes patula</i> spp. and <i>Tagetes erecta</i> 'Cracker jack' <i>Tagetes erecta</i> X <i>Tagetes patula</i> 'Polynema' etc	Rotation	Marigold lowered density of <i>Pratylenchus penetrans</i> . Tuber yields higher in subsequent potato crop	Canada	(97)
Tomato	<i>Meloidogyne incognita</i>	<i>Tagetes patula</i> Dwarf French 'Lemon drop'	Intercrop pot experiment	Root population of both species on tomato grown with Marigold less than on tomato grown solo	USA	(98)
Snap beans	<i>Hoplolaimus galeatus</i> , <i>Paratrichodorus christiei</i> , <i>Belanolaimus longicaudatus</i>	<i>Tagetes patula</i> , Rusty red	Rotation	All three nematodes increased following Marigold plantings. Application of phenamiphos as a suplot treatment increased snap bean yields 57 % in marigold treatment plots	USA	(99)
Tomato	<i>Meloidogyne javanica</i>	<i>Tagetes erecta</i>	Intercrop	Root galls less on tomato plants grown with <i>Tagetes erecta</i> than tomato alone. Number of fruits and Weight of fruits also greater but root weight and shoot length slightly reduced in intercropping	Pakistan	(54)
Tobacco	<i>Pratylenchus penetrans</i>	<i>Tagetes patula</i> , dwarf double French	Rotation	Tobacco growth parameters improved and <i>Pratylenchus penetrans</i> and <i>Tagetes claytoni</i> population reduced for 3 and 1 st year respectively	USA	(100)

explore advanced delivery methods including nanotechnology, to boost the stability and effectiveness of *Tagetes*-based applications. These findings, along with the broader antibacterial and antifungal activities observed in *Tagetes* species, as summarized in Table 3., highlight its potential as a natural antimicrobial agent.

Applications as bio pesticides

Powdered leaves, stems and flowers of *T. erecta* have shown insecticidal activity, with incense sticks made from leaf powder providing effective mosquito repellence at 75 % lower cost than commercial products and without side effects (50). Intercropping French marigolds with tomatoes also effectively reduced whitefly (*Trialeurodes vaporariorum*) populations and limonene dispensers enhanced this control (51). Aqueous extracts of *T. patula* managed *Lygus hesperus*, while methanolic extracts achieved high mortality even at low concentrations. Both extract types showed dose-dependent toxicity against *Bemisia tabaci* (5). Further research is needed to isolate the active compounds and understand their mechanisms for commercial use of marigold-based biopesticides.

Methanolic extracts from the leaves, stems and roots of *T. patula* showed strong nematocidal effects, with higher concentrations increasing efficacy (52). Another study on five *Tagetes* varieties found that extracts from leaves, roots and flowers reduced egg hatching and increased juvenile mortality (53). *Tagetes patula* and *T. erecta* were especially effective in lowering root gall formation and nematode populations in crops like tomato and egg plant (54).

The dual role of marigolds in nematode management

Marigolds (*Tagetes spp.*) have a dual role in nematode interactions, serving as hosts for some species like *Rotylenchulus reniformis* and *Criconea sp.* while also acting as effective nematocidal agents (20). Beyond nematode management, marigolds enhance pest control by attracting beneficial insects such as *Trissolcus basalis* and by supporting natural pest predators through intercropping (21-23). Their nematocidal properties are achieved through crop rotation, soil amendment and allelopathy. Rotation with marigolds has been shown to reduce nematode populations more

effectively than some chemical fumigants (19). Bioactive compounds like α -terthienyl and bithienyl derivatives disrupt nematode metabolism and reproduction (29, 55). Soil incorporation of marigold residues also suppresses *Meloidogyne incognita* while fostering beneficial microbes (35, 36). Although intercropping results can vary by agro ecosystem (35), studies consistently show reduced root galling in crops like soybean and mulberry (56). These results highlight marigolds as a sustainable strategy for integrated nematode and pest management.

Phytochemicals present in marigold

Plants produce a diverse array of secondary metabolites, including phenols, flavonoids, terpenoids, quinones, tannins, alkaloids, saponins, coumarins and sterols. These compounds play a crucial role in plant defence, helping protect crops from pests and pathogens. The essential oil derived from the aerial flowering parts of *Tagetes* primarily contains monoterpene hydrocarbons such as ocimenes, limonene and terpinene, along with acyclic monoterpene ketones like tagetones, dihydro-tagetone and tagetenone as shown in Fig. 1. It also includes smaller amounts of sesquiterpenes and oxygenated compounds (57).

a. Extraction methods for phyto-chemicals present in marigold

Marigold (*Tagetes spp.*) flower extracts are rich in antioxidants, mainly phenolics like gallic acid and quercetin and carotenoids, with lutein being the dominant pigment (58-60). In *Tagetes* species, flavonoids and carotenoids are the primary pigments, with lutein esters being most abundant (61, 49). Flavonoids are valued for their strong antioxidant and metal-chelating properties, offering significant health benefits (62). The extraction method and solvent choice greatly influence the yield and biological activity of these bioactive compounds, requiring careful consideration of efficiency, stability, cost and availability (63). Studies show that dried marigold petals extracted with 95 % ethanol under controlled conditions yielded potent extracts. For *T. patula*, micropilot extraction using butylene glycol and ethyl alcohol achieved a high recovery of thiophenes, especially α -T, with a 90 % yield. Component analysis was performed using GC-MS and SIM techniques for accurate profiling (50).

Table 3. Antibacterial and antifungal activities of *Tagetes spp.*

Plant species	Targeted bacterial / fungal species	References
Wild marigold (<i>Tagetes minuta</i>)	<i>Rhizoctonia solani</i> <i>Sclerotium rolfii</i> <i>Salmonella typhi</i> <i>Escherichia coli</i>	(59)
Wild marigold (<i>Tagetes minuta</i>)	<i>Bacillus subtilis</i> <i>Aspergillus niger</i> <i>Candida albicans</i>	(62)
Wild marigold (<i>Tagetes minuta</i>)	<i>Fusarium verticillioides</i> <i>Fusarium proliferatum</i>	(63)
French marigold (<i>Tagetes patula</i>)	<i>Botrytis cinerea</i>	(101)
Irish -lace marigold (<i>Tagetes filifolia</i>)	<i>Sclerotium epicorium</i> <i>Colletotrichum cocodes</i> , <i>Alternaria solani</i> <i>Rhizoctonia solani</i> <i>Salmonella spp.</i>	(26)
Mexican marigold (<i>Tagetes lucida</i>)	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Trichophyton mentagrophyte</i>	(102)

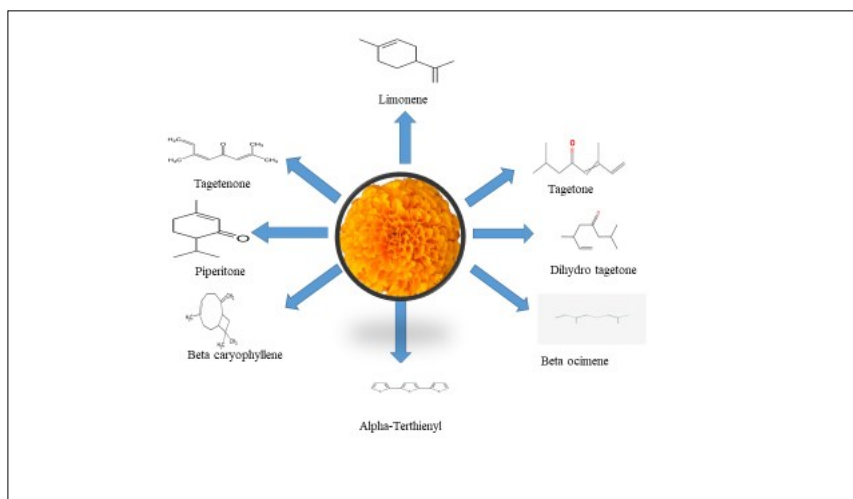


Fig. 1. Phytoconstituents present in marigold species.

Alpha terthienyl - a root metabolite of marigold

α -Terthienyl ($C_{12}H_8S_3$, MW 248.376), a blue-fluorescing compound initially isolated from marigolds and characterized by a group of researchers (29), is a key bioactive substance known for its nematicidal, insecticidal, fungicidal, antiviral and cytotoxic properties (64). It is considered the primary agent responsible for marigold's nematicidal effects and holds potential as an environmentally friendly pesticide with minimal residues (12, 64). Structurally, α -terthienyl consists of three conjugated thiophene rings linked by single carbon-carbon bonds, forming an extensive π -electron system that enhances its reactivity (12) (Fig. 2). Under UV light, it generates reactive oxygen species, contributing to its pesticidal and antimicrobial efficacy. It is sparingly soluble in water but dissolves well in organic solvents like ethanol, chloroform and dichloromethane. Its melting point ranges between 88–90 °C (65).

a. Mode of action of Alpha-terthienyl

α -T exhibits strong nematicidal and larvicidal properties, targeting phytoparasitic nematodes and mosquito larvae. It inhibits key enzymes like cholinesterase, glucose-6-phosphate dehydrogenase and malate dehydrogenase in *Ditylenchus dipsaci*, causing oxidative stress—a mechanism confirmed by the reduction of toxicity with singlet oxygen scavengers like NaN_3 , methionine and histidine (65). As an allelopathic agent in marigolds, α -T suppresses nematode populations without needing photoactivation, comparable to synthetic nematicides (66). Its ability to penetrate the hypodermis and induce oxidative damage boosts its effectiveness.

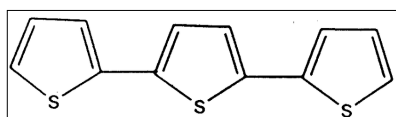


Fig. 2. Structure of Alpha-terthienyl.

In *Caenorhabditis elegans*, α -T is more effective against dauer larvae than adults due to its cuticle-penetrating and oxidative stress-inducing abilities (67). This mechanism aligns with oxidative responses in aerobic organisms, involving enzymes like superoxide dismutase (SOD) and catalase to mitigate oxidative damage (68). Additionally, α -T shows strong phototoxicity against mosquito larvae, with UV exposure at 366 nm causing complete larval mortality (69). These findings highlight α -T's dual role in integrated pest management, effectively controlling nematodes and mosquitoes.

b. Nematicidal activity of Alpha-terthienyl

Research indicates that α -terthienyl exhibits strong nematicidal activity against *Caenorhabditis elegans* and *Meloidogyne incognita*, with its potency significantly increasing under light exposure. This photoactivation enhances toxicity by generating reactive intermediates, making it highly effective against various developmental stages (50). In *C. elegans*, young adults and dauer larvae are affected, though the latter show greater resistance due to their protective adaptations (52). However, prolonged exposure and higher doses improve efficacy. In *M. incognita*, the J2 stage is particularly susceptible, highlighting α -terthienyl's potential for agricultural nematode control under controlled environmental conditions (54). The specific mortality percentages and LC_{50} values, which provide a clearer comparison of its efficacy across different nematode stages, are summarized in Table 4 for reference.

The nematicidal action of α -T is linked to oxidative stress, activating detoxification pathways in *C. elegans*. Key enzymes such as Superoxide Dismutases (SODs), Glutathione Peroxidases (GPXs), Catalases (CTLs) and Peroxiredoxins (PRDXs) neutralize reactive oxygen species (ROS) generated by the compound (70). Phase II detoxification enzymes, including Glutathione S-Transferases (GSTs) and UDP-Glucuronosyl transferases (UGTs), help eliminate oxidative by

Table 4. Lethal concentrations (LC_{50}) and mortality percentages of nematodes under photoactivation and non-photoactivation conditions

Nematode stage	Findings (Mortality % / LC_{50} Values)	References
<i>C. elegans</i> Young Adults	$LC_{50} = 22 \pm 1.1 \mu M$ (No Photoactivation)	(67)
<i>C. elegans</i> Young Adults	$LC_{50} = 1.93 \pm 0.03 \mu M$ (Photoactivation)	(67)
<i>C. elegans</i> Dauer Larvae	74 % (1 μM), 93 % (2.5 μM), 97 % (5 μM) (No Photoactivation)	(67)
<i>C. elegans</i> Dauer Larvae	99 % (1 μM), 100 % (2.5 & 5 μM) (Photoactivation)	(67)
<i>M. incognita</i> J2 Stage	58 % (1 μM), ~100 % (2.5 & 5 μM) (No Photoactivation)	(67)
<i>M. incognita</i> J2 Stage	$LC_{50} = 1.87 \pm 0.12 \mu M$ (Photoactivation)	(81)

-products (71). GST-4 is upregulated in response to α -T, indicating its involvement in the nematode stress response (72). The ROS mechanism is depicted in Fig. 3, which shows how α -terthienyl, upon UV activation, generates singlet oxygen and superoxide anion radicals, leading to Superoxide Dismutase (SOD) inhibition and acetylcholinesterase disruption. These effects impair the nematode nervous system, reducing their ability to locate and invade host roots, ultimately affecting their interaction with plant hosts.

Transcriptional regulation studies show that the SKN-1/WDR-23 pathway mediates detoxification responses. SKN-1 regulates GST-4 and SOD-1, while WDR-23 modulates SKN-1 (69). RNAi knockdown of SKN-1 reduces GST-4 and SOD-1 expression, diminishing the nematodes' oxidative stress response, whereas WDR-23 knockdown upregulates GST-4, suggesting a negative regulatory role (73). The compound's effects are primarily driven by SKN-1 activation rather than WDR-23 modulation (71). In experiments with *Meloidogyne* juveniles, nematodes moved freely within a gel matrix toward plant roots, such as tomato and *Arabidopsis*, with observed differences in movement speed toward the roots (67). Comparison of α -T with commercial nematicides and other biopesticides shown in Table 5.

c. Insecticidal activity of Alpha-terthienyl

c.1 Effect of Alpha-terthienyl on *Aedes aegypti* (caused by Zica virus): α -Terthienyl (α -T), a secondary metabolite from plants

in the *Compositae* and *Calendula* families, is a potent larvicide against mosquito species such as *Aedes aegypti* and *Anopheles gambiae* (74, 75). It exhibits toxicity to *A. aegypti* larvae even in darkness, but its effectiveness significantly increases under UV light. In first-instar larvae, exposure to α -T under UV light resulted in an LC_{50} of approximately 0.002 ppm, with nearly all surviving larvae developing into adults within 24 hrs. Fourth-instar larvae were also highly sensitive, with an LC_{50} of 0.45 ppm under photochemical treatment. Additionally, α -T demonstrated unexpected phototoxicity in 1–2-day-old pupae, with an LC_{50} of 0.06 ppm, marking the first instance of a photo-insecticide active against pupal stages. However, it was ineffective against pupae nearing adulthood and did not affect egg hatching (76).

Upon absorption through water, mosquito larvae store α -T in the gastric cecum or excrete it via the Malpighian tubules. Under light exposure, α -T disrupts larval physiology by inactivating superoxide dismutase at the anal fistula and inducing lipid peroxidation in the midgut and Malpighian tubules (77). Furthermore, α -T increases oxidized glutathione (GSSG) levels and generates reactive oxygen species (ROS), which can arrest the S phase of the cell cycle in both insect and human oocytes (78). These mechanisms highlight α -T's potential as an effective photochemical larvicide for mosquito control strategies as shown in Fig. 4.

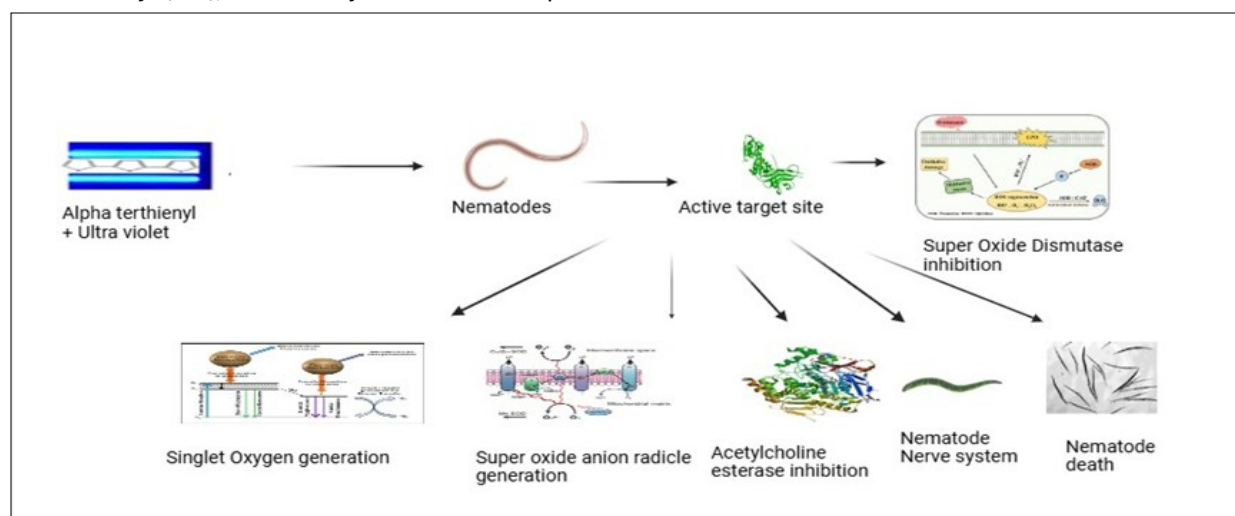


Fig. 3. Mechanism of action of α -terthienyl against nematodes.

Table 5. Comparison of α -T with commercial nematicides and other biopesticides

Compound/Formulation	Target nematode(s)	Concentration	Exposure time	Effectiveness	References
α -Terthienyl (α -T) (natural form)	<i>Nacobbus aberrans</i> (J2)	100 μ g/mL (0.01 %)	24 hrs	83.2 \pm 5.2 % immobility	(103)
Commercial α -Terthienyl	<i>Nacobbus aberrans</i> (J2)	100 μ g/mL (0.01 %)	24 hrs	71.74 \pm 8.8 % immobility	(103)
Commercial α -Terthienyl formulation	<i>Heterodera zae</i> larvae	0.125 %	24 hrs	100 % mortality	(104)
1-Phenylhepta-1,3,5-triyn	<i>Bursaphelenchus xylophilus</i>	2 mM	Not specified	Effective	(103)
5-Phenyl-2-(1'-propynyl)-thiophene	<i>Bursaphelenchus xylophilus</i>	2 mM	Not specified	Effective	(103)
β -Sitosterol	<i>Meloidogyne incognita</i>	1 %	12 hrs	60 % mortality	(104)
Stigmasterol + β -Sitosterol	<i>Meloidogyne incognita</i>	5 μ g/mL	Not specified	74.4 % mortality	(104)
Stigmasterol + β -Sitosterol	<i>Heterodera glycines</i>	5 μ g/mL	Not specified	55.3 % mortality	(104)
23a-Homostigmast-5-en-3 β -ol + Nonacosan-10-ol (mixture)	<i>Meloidogyne incognita</i>	50 μ g/mL	24 hrs	93.7 % mortality	(104)
23a-Homostigmast-5-en-3 β -ol (individual)	<i>Meloidogyne incognita</i>	100 μ g/mL	24 hrs	50 % mortality	(104)
Nonacosan-10-ol (individual)	<i>Meloidogyne incognita</i>	100 μ g/mL	24 hrs	50 % mortality	(104)

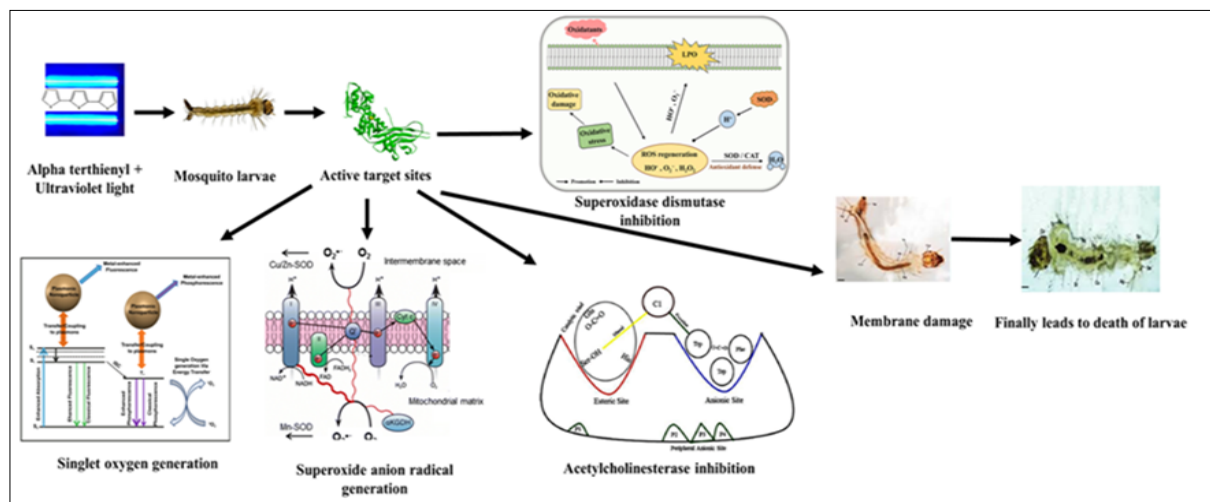


Fig. 4. Schematic representation of mechanism of action of alpha terthienyl in mosquito larvae.

c.2. Effect of Alpha-terthienyl on Lepidoptera : α -T, a natural compound from the Asteraceae family, exhibits phototoxic effects on insect larvae, particularly under ultraviolet-A (UV-A) light exposure. In *Manduca sexta* (tobacco hornworm) fifth-instar larvae, α -T was administered through an artificial diet and topical application. Toxicity was observed only when larvae were exposed simultaneously to α -T and UV-A light (320–400 nm); neither treatment alone caused noticeable adverse effects. A single ingested dose (50 μ g/g of larval weight) followed by 4 hrs of UV-A irradiation resulted in delayed, abnormal pupal development, with no adult emergence. Similarly, topical application of α -T (50 μ g/g) combined with UV-A exposure led to tissue necrosis, disrupting sclerotization and melanization of the pupal case during later developmental stages (79).

Beyond its larvicidal action, α -T induces oxidative damage in *Spodoptera litura* (SL) cells. Comparative toxicity studies using the MTT assay revealed an IC_{50} of 0.21 μ g/mL for α -T, significantly lower than rotenone (12.25 μ g/mL), indicating higher toxicity. Malondialdehyde (MDA) levels increased with α -T concentration, while glutathione (GSH) levels declined, suggesting oxidative stress. Transmission electron microscopy (TEM) analysis showed swelling of the cell membrane and nuclear envelopes, membrane

disintegration, leakage of intracellular contents and mitochondrial expansion, confirming severe structural damage. The oxidative stress induced by α -T disrupted antioxidant defenses, leading to significant cellular deterioration (80).

c.3 Effect of Alpha-terthienyl on red ant : α -T exhibits potent phototoxicity against the red imported fire ant (*Solenopsis invicta*), with knock-down rates of 93.08 %, 98.29 % and 100 % after 90, 120 and 180 min of UVA exposure, respectively, compared to only 7.41 %, 18.52 % and 25.93 % in darkness. Mortality progressively increases from 6.25 % to 52.08 % when ants are initially exposed to UVA for 30 min and then kept in darkness for up to 180 min. Behavioral impairments, including loss of aggregation, clinging and walking abilities, worsen over time, with walking and clinging completely ceasing after 180 min. Scanning electron microscopy reveals structural damage, indicating neural and sensory disruption. These findings underscore α -T's potential as a UVA-activated bioinsecticide for fire ant control (56).

Studies have demonstrated its efficacy against mosquito larvae, agricultural pests and parasitic nematodes, making it a promising candidate for pest control. The following Table 6. summarizes the documented effects of α -T on various nematodes and insects, highlighting its potential as a bioinsecticide and nematicide.

Table 6. Effect of α -Terthienyl on nematodes and insects

Organism	Effect of α -Terthienyl	References
Nematodes		
<i>Caenorhabditis elegans</i>	Mortality was concentration-dependent: 74 % at 1 μ M, 93 % at 2.5 μ M and 97 % at 5 μ M. Photoactivation increased mortality to 99 % at 1 μ M and 100 % at 2.5 and 5 μ M.	(67)
<i>Meloidogyne incognita</i> J2s	α -T nanofiber formulation (8 ppm) reduced nematode populations and enhanced tomato plant growth. Marigold-derived α -T inhibited <i>M. incognita</i> .	(105),(81)
<i>Ditylenchus dipsaci</i>	Inhibited cholinesterase, glucose-6-phosphate dehydrogenase and malate dehydrogenase, inducing oxidative stress and toxicity.	(106)
Insects		
<i>Aedes aegypti</i>	Larvae absorbed α -T through water, storing it in the gastric cecum or excreting it via Malpighian tubules. Under light, α -T disrupted larval physiology by inactivating superoxide dismutase and inducing lipid peroxidation.	(77)
<i>Manduca sexta</i> (Tobacco hornworm)	Toxicity observed only with UV-A exposure (320–400 nm). Ingestion (50 μ g/g) followed by 4 hrs of UV-A led to abnormal pupal development with no adult emergence.	(79)
<i>Spodoptera litura</i>	Induced oxidative damage in SL cells; toxicity (IC_{50} : 0.21 μ g/mL) was significantly higher than rotenone (IC_{50} : 12.25 μ g/mL). Caused membrane disintegration and mitochondrial damage.	(80)
<i>Solenopsis invicta</i> (Red imported fire ant)	Lethal under UV light exposure.	(56)

Challenges and limitations

α -terthienyl (α -T) shows strong promise as a natural nematicide, effectively suppressing egg hatching and juvenile survival of *Meloidogyne incognita* in laboratory and greenhouse trials (81). It also reduces nematode populations in soil and roots while promoting plant growth, especially at higher concentrations. However, practical field use faces challenges such as resistance development, environmental persistence, phototoxicity, inconsistent efficacy and risks to non-target organisms. Resistance is a key concern, as prolonged α -T exposure may trigger adaptive responses in nematodes. Species like *M. incognita* and *Heterodera zea* possess antioxidant defenses, including SKN-1-regulated detoxification enzymes, which help them withstand oxidative stress (82). Additionally, dauer larvae show reduced sensitivity due to lower expression of enzymes like GST-4 and SOD-1, potentially limiting long-term effectiveness and underscoring the need for integrated or rotational strategies (83).

Another limitation is α -T's reliance on UV light for activation. Its nematicidal action depends on the generation of reactive oxygen species (ROS) under UV exposure (84, 12). However, in natural soil conditions, especially shaded areas or deeper layers limited UV penetration can reduce its effectiveness. Its phototoxicity also raises environmental concerns, as UV exposure triggers production of reactive species like singlet oxygen, which may harm aquatic organisms through runoff (85). Field use is further complicated by factors such as soil composition, moisture, UV availability and climate, all of which influence α -T's stability and performance. High volatility, rapid breakdown in oxygen-rich soils and poor water solubility hinder its residual activity and bioavailability, making consistent application and dosage across varied agricultural settings difficult (86).

Future directions

α -T, a natural pesticide from marigold (*Tagetes* spp.), degrades quickly in sunlight with a half-life of about 4 hrs, reducing environmental residue (12). Its selective toxicity poses minimal risk to beneficial insects like bees and silkworms under controlled conditions. However, its UV-activated photo toxicity can harm aquatic life, as the ROS it generates such as singlet oxygen and superoxide radicals cause oxidative stress and damage to cells in fish and amphibians (85, 86). This highlights the need for cautious application to avoid ecological harm. Significant research gaps remain regarding the long-term environmental effects of α -terthienyl and its interactions with other biocontrol agents (70). Investigating its impact on nematode oxidative stress pathways, such as SKN-1/WDR-23, could improve its efficacy while reducing ecological risks. Additionally, combining α -T with other plant-based or microbial nematicides may enhance performance and lower the potential for resistance (87).

For sustainable use, thorough evaluations of α -T's ecological and economic impacts are essential. Long term studies should assess its persistence in soil, effects on soil microbiota and influence on non-target species across

diverse climatic conditions (88). Innovative delivery systems like nano carriers responsive to pH or moisture could improve stability and precision, reducing environmental exposure (89). Encapsulation methods may also extend its soil activity without relying on UV activation, enhancing field applicability (86). Future strategies could include integrating α -T with biofumigant crops such as *Tagetes* spp. to combine chemical and agronomic control methods (90). Molecular docking studies, supported by nematode genomics, may identify new targets like detoxification enzymes, aiding the development of synergistic, low-dose formulations. Ultimately, extensive field trials across varied environments are crucial to confirm its safety, effectiveness and economic viability for sustainable nematode control (91).

Conclusion

Marigold has shown promise as a natural nematicide, though its success as an intercrop varies based on planting density and cultivation methods. Its roots release α -T, a compound with strong nematicidal and insecticidal activity, believed to work through singlet oxygen generation under sunlight, which damages pests. Its low persistence and environmental safety make it a potential alternative to synthetic nematicides, which often pose ecological and health risks. However, scaling α -T for widespread agricultural use requires further evaluation. While it supports sustainable farming by reducing chemical use, limitations such as UV dependency, inconsistent field results and possible effects on non-target organisms remain. Advances in nanotechnology and controlled-release systems may enhance their stability and application. Future studies should focus on its long-term environmental effects, interactions with soil microbes and role in integrated pest management. Collaboration among researchers, policymakers and industry will be key to ensuring that α -T is both effective and sustainable.

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Authors' contributions

AHK and PGK were responsible for designing the study, conducting the statistical analysis, developing the protocol, drafting the initial manuscript and remaining all authors were contributed and revised the manuscript.

Compliance with ethical standards

Conflict of interest: There is no conflict of interest between the authors.

Ethical issues: None

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