



MARIGOLD BIOPESTICIDE AS AN ALTERNATIVE TO CONVENTIONAL CHEMICAL PESTICIDES

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ABSTRACT

Marigold (*Tagetes* spp) which is considered to be a traditional medicinal plant, has significant therapeutic value and is used in treatment of a number of ailments. This review mainly emphasizes on the role of *Tagetes* as biopesticides especially as insecticide, mosquitocide, nematocidal, bactericide and fungicide. The essential oils obtained from the aerial part of *Tagetes* mainly contain monoterpene hydrocarbons (ocimenes, limonene, terpinene etc) and acyclic monoterpene ketones (tagetones, dihydrotagetone and tagetenone) in addition to lower amount of sesquiterpene and oxygenated compounds. These components are mainly responsible for insecticidal, larvicidal and mosquitocidal property. On the other hand species of *Tagetes* also show strong nematocidal effect. Thus they can be used to suppress nematode species in the field by different techniques such as crop rotation or intercropping system or by exerting allelopathic effect. *Tagetes* can show a remarkable biochemical interaction known as allelopathy by producing a potentially bioactive component α -thienyl that is mainly responsible for nematocidal action. This sulphur containing compound has nematocidal, insecticidal, fungicidal, antiviral and cytotoxic effect. The essential oil of *Tagetes* also shows strong bactericidal and fungicidal effect against a number of plant pathogenic bacteria and fungi. This review not only discusses the role of marigold as biopesticides especially as insecticides, mosquitocides, nematocides, bactericides and fungicide but also elucidates its eco-friendly potential to be used in the agricultural field as an alternative to chemical synthetic pesticides.

Keywords: *Tagetes*, Essential oil, Biopesticide, Eco-friendly.

1. INTRODUCTION

Marigold, belonging to the genus *Tagetes*, is an annual herbaceous plant of Asteraceae family. *Tagetes* is native of Mexico and other warmer parts of America and are cultivated elsewhere in the tropics and subtropics. In India, this was introduced by the Portuguese [1]. The name marigold is however applied to several genera of Asteraceae with golden or yellow capitula inflorescence. There are about 33 species of the genus *Tagetes*, out of which, five species have been introduced into the Indian gardens viz. *Tagetes erecta* L. (Aztec or African Marigold), *Tagetes minuta* L. (*Tagetes glandulifera* Schrank), *Tagetes patula* L. (French Marigold), *Tagetes lucida* Cav. (sweet scented Marigold), *Tagetes tenuifolia* Cav. (Striped Marigold) [2] (Fig. 1). *Tagetes* is a plant of various uses having ornamental, ritual, medicinal, anthelmintic, insecticidal, colorant, food, and forage applications [3, 4]. Healing properties of *Tagetes* species have been

reported in folk medicine and alternative medicines through ages [5].

Bio pesticides are pesticides based on living microorganisms or natural products. They have been used widely for pest management worldwide [6]. Bio pesticides may be of microbial or of plant product in origin. Many phytochemical pesticides show broad spectrum of activity against pests and other diseases. They have long been considered as potent alternative to synthetic chemical pesticides as they are biodegradable, target specific, and produce little or no toxic effect to the environment or to human health. Besides, cost of production of biopesticides is significantly lower than the synthetic chemical pesticides [7]. Plants can produce a wide range of secondary metabolites such as phenol, flavonoids, terpenoids, quinones, tannins, alkaloids, saponins, coumarins and sterols which can play a very important role in plant defence and can protect large

number of crops from pest and pathogens. The major components of essential oil of the aerial flowering plant of *Tagetes* mainly constitute monoterpene hydrocarbons (ocimenes, limonene, terpinene etc), acyclic monoterpene ketones (tagetones, dihydro-tagetone and tagetenone) and lower amount of sesquiterpene and oxygenated compounds [1,8] (Fig. 2). It has been reported earlier that these compounds have potent pesticidal effects. In the present review an extensive study has been carried out to elucidate the role of marigold as biopesticide especially as insecticides, mosquitocides, nematocides and fungicide.

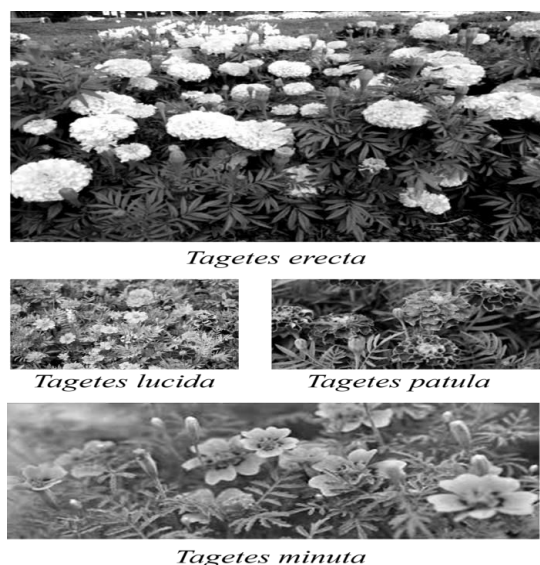


Fig. 1: Some *Tagetes* spp. found in India

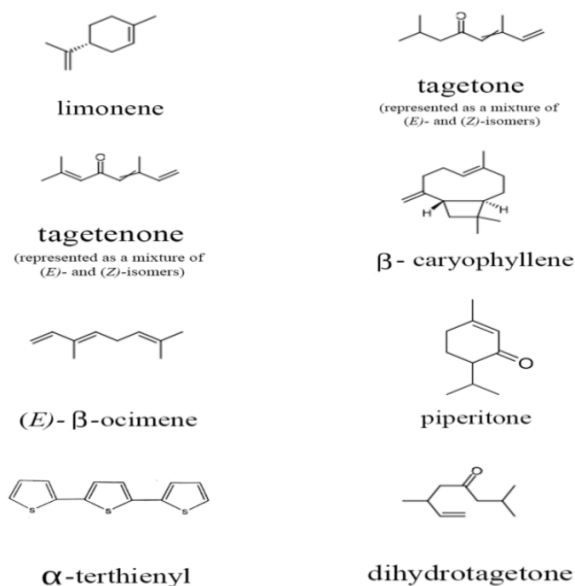


Fig. 2: Important chemical compound present in essential oils (EOs) of *Tagetes* spp.

2. BIO INSECTICIDES

Plant extracts are widely used as insecticides as they are eco-friendly and safe to the environment. Unlike chemical insecticides, plant extracts as alternative insecticides are non-toxic to human and animals and do not cause environmental contaminations [9]. There are earlier reports of *Tagetes* species which have insecticidal [10-13], larvicidal [14] and mosquitocidal effects [11, 15]. Several compounds obtained from essential oil from *T. erecta* plants are responsible for its insecticidal property. The major components in the essential oil of aerial parts, capitulum and leaves of *T. erecta* are limonene, (Z) β -ocimene, eugenol, linalol, β -caryophyllene, linalyl acetate, piperitone [16]. Ravikumar, 2010 evaluated the chemical composition of *T. erecta* and *T.patula* and elucidated its insecticidal property. The hexane extract of *T. erecta* flower showed better insecticidal property against *Acyrtosiphon gosypii* (1000ppm) and *Spodoptera frugiperda* (5000ppm) [17]. *T. erecta* showed insecticidal activity against species of *Tribolium* which are considered to be common pests of cereal silos, mills and warehouses. The quantity and quality of stored food is highly affected by the presence of these insect species [11]. Presence of insecticidal pyrethrin was found in the callus tissue of *T. erecta* maintained on revised Murashige and Skoog's medium (RT) as static cultures. The maximum pyrethrin content (1.68%) was observed in 6 weeks old tissue when grown in the medium supplemented singly with 1000 mg/l of exogenous ascorbic acid. When pyrethrin mixture was screened against *Tribolium* spp immediate 'knock down' effect was observed [10].

It was reported earlier that *T. erecta* and *T. patula* have phytotoxic compounds that can be utilized as a natural insecticide [18]. The hydroethanolic and ethanolic extracts of both the plant species of *Tagetes* showed strong antioxidant property and insecticidal activity against maize weevil *Sitophilus zeamais*. This is a cosmopolitan insect that can attack many hosts like wheat, corn, rice, barley and oats and can attack the seeds both in the field and also in the store house, thus causing serious economic loss. The effect of marigold plants as a resource plant to natural enemies in onion fields was investigated by Silveira *et al*, 2009 [19]. The experiment was set in a certified organic farm in Sao Paulo, Brazil using marigold rows at a center of an onion field. Samples were taken from marigold and the onion plants 5 m (near) and 30 m (far) from the flowering strips. Higher numbers of arthropod pests such as *Thrips*

tabaci and *Therioaphis trifolii* were observed in onion plants 30 m from the marigold strip, while higher numbers of predators and parasitoids especially *Stomatothrips angustipennis*, *Stomatothrips rotundus* and *Franklinothrips vespiformis*, which were twice as abundant in onion plants near the marigold strip at 5m distance. In this field study, marigold strips were used as an alternative to insecticide sprays for control of onion pests.

Several insecticidal compounds have been isolated from essential oil of *T. minuta* [20-23]. Insecticidal activity of *T. minuta* has been observed against Lepidoptera viz. fall army worm [24]; Anopheline [25] and other mosquitoes [21, 22] and Coleoptera [26] group of insects. Four major components viz. limonene, β -ocimene, dihydrotagetonone and tagetonone were identified in the essential oil of *T. minuta*. Weaver et al, 1994 investigated the insecticidal activity of floral, foliar and root extracts of *T. minuta* against adult Mexican bean weevil *Zabrotes subfasciatus*. Results indicated that floral and foliar extract showed similar insecticidal activity whereas root extract was required in less concentration but slower in action [12]. Essential oil extracted from seeds of *T. minuta* using n-hexane solvent, showed insecticidal activity against three different grain pests *Tribolium castaneum* (Red flour beetle), *Rhyzopertha dominica* (Lesser grain borer) and *Callosobruchus analis* (Pulse beetle) [27].

Essential oil of *T. lucida* was evaluated and its repellent activity against *Sitophilus zeamais* was observed [28]. Most oil components were oxygenated monoterpenoids or phenolic compounds. Ethanolic extracts of *T. lucida* was used to control aphid (*Aphis brassicae*) on cabbage plant. Remarkable reduction of aphid population was observed during the first six days after application. After nine days, however the pesticidal effect was lost and another application was needed to obtain long period of protection on cabbage against Aphid infestation [29].

Marques et al, 2011 [30] evaluated the activity of essential oil from *T. erecta* against III instars of *Aedes aegypti* and determined the amounts of larvicidal thiophenes in all plant tissues. The oil obtained by steam distillation and analyzed by gas chromatography/mass spectrometry showed 14 compounds. The main compounds were piperitone (45.72%), D-limonene (9.67%) and piperitenone (5.89%). The essential oil was active against larvae of *Aedes aegypti*, with LC₅₀ of 79.78 μ g/ml and LC₉₀ of 100.84 μ g/ml. The larvicidal thiophene contents were higher in the roots and flowers as demonstrated by high-performance liquid chromatography analysis. The

Mosquitocidal activity in Ethanolic, chloroform and petroleum ether extracts of *T. erecta* flower against different strains of *Culex quinquefasciatus* was studied [31]. Among the tested samples the chloroform soluble fraction showed the highest toxicity. An important photoactive component α -terthienyl of *T. minuta* was evaluated for mosquito control [32]. The essential oil of *T. minuta* also showed high larvicidal activity against mosquito *Anopheles gambiae* [33] and *Ades aegypti* [20].

3. BIO NEMATICIDES

Nematodes are unsegmented roundworms that are usually microscopic in size. There are many different kinds of nematodes which live in terrestrial habitat. Nematodes can be free-living that feed on fungi, bacteria, or other microscopic organisms. Nematodes that feed on plants are called plant-parasitic nematodes. Plant-parasitic nematodes can seriously damage or even kill crops, turf and ornamental plants. They generally feed on plant roots causing swelling or galls within the roots obstructing the flow of water, mineral salts and nutrients. Plant-parasitic nematodes are difficult to control because they live underground or inside of plants. African (*T. erecta*) and French marigolds (*T. patula*) are the most commonly used species which are well known for possessing nematicidal property. Marigold can suppress about 14 plant parasitic nematodes such as lesion nematode (*Pratylenchus* sp) and root knot nematode (*Meloidogyne* sp) [34]. Nematode suppression by *Tagetes* spp is influenced by crop plants, nematode species, and soil temperature [35]. There are a number of methods marigold species are found to adapt for nematode suppression such as by acting as trap crop [36, 37]; by exerting allelopathic effect [38]; by enhancing the nematode antagonistic microorganisms [39, 40] or by acting as host plant for nematode [40, 41]. The main method by which marigolds suppress plant-parasitic nematodes is through a biochemical interaction known allelopathy; a phenomenon where a plant releases compounds in the microenvironment and are toxic to other organisms [42]. Marigold plants produce a number of potentially bioactive compounds, among which α -terthienyl is recognized as one of the most toxic substance. This sulfur-containing compound is abundant in marigold tissues, including roots. It has nematicidal, insecticidal, fungicidal, antiviral, and cytotoxic activities, and it is believed to be the main compound responsible for the nematicidal activity of marigold [43, 44]. *T. patula* 'Single Gold', *Tagetes* hybrid Polynema and *T. erecta*

'Cracker Jack' effectively suppressed four root-knot nematode species: *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, and *M. Hapla*. The nematodes may be killed either by entering the root system of a marigold plant or contacting soil containing marigold's bioactive compounds [45]. The efficacy of *Tagetes* spp as biocontrol agent to control root knot nematode *Meloidogyne incognata* was evaluated both in the field and soil amendment experiment [46]. In this investigation when marigold species *T. erecta*, *T. patula* and *T. minuta* were pre-planted with tomato crops, reduction in the numbers of second stage juveniles (J2s) in subsequent tomato plantation was observed than in control. Four different concentrations of water soluble extract of marigold cultivars were filtered and added to the Petridish infested with the eggs of *M. incognita*. Root exudates of *T. erecta* were lethal to J2 of *M. incognita* and were inhibitory to egg hatching at concentration of 75 % or higher. *T. erecta* was also reported in effective management of *M. incognita* when it was grown in infested soil [47]. The bioactive compounds of different marigold species and cultivars may differ in composition, quality and quantity. Thus, certain species may be highly effective against one nematode species but have limited to no impact on or possibly increase populations of other plant-parasitic nematodes. Nematicidal activity of different parts (leaf, flower, seed, and root) of *T. lucida* was observed on reniform, lance (*Hoplolaimus indicus*) and spiral (*Helicotylenchus indicus*) nematodes, and it was reported that the flower extracts had the strongest nematicidal activity, followed by seed, leaf and root extracts [48]. Similar result was obtained by Hassan *et al*, 2003 [49] who reported leaf extract of *T. patula* was toxic to juveniles of *M. javanica* in petridish assay. These findings suggest that aerial parts of marigold is more toxic than roots and photoactivation is necessary for nematicidal activity of α -terthienyl [50-52]. However, it was also reported that nematicidal activity occurred even without photoactivation [53]. It was observed that, under dark conditions (without photoactivation), α -terthienyl was an oxidative stress-inducing chemical that effectively penetrated the nematode hypodermis and exerted nematicidal activity, suggesting high potential for its use as a practicable nematode control agent in agriculture [54]. Nematicidal activity of α -terthienyl against the model organism *Caenorhabditis elegans* and the root-knot nematode, *Meloidogyne incognita* was investigated. It was observed that induction of two major enzymes, glutathione S-transferase (GST) and superoxide

dismutase (SOD), was restricted in *C. elegans* hypodermis following treatment with α -terthienyl. The susceptibility of nematodes to α -terthienyl changed when the expression of GST and SOD was induced or suppressed.

4. BACTERICIDE AND FUNGICIDE

Essential oil (EO) of *Tagetes* sp. shows biopesticidal activity. It was documented that EO components especially terpenoids such as dihydrotagetonones, tagetonones and ocimenones are potent antimicrobial agents [55]. Various *Tagetes* oils analysed by GC/MS were shown to contain limonene, dihydrotagetenone and oscimenone which can inhibit gram-positive bacteria and fungi [56]. *T. minuta* is the most promising species among marigold which can be used as bactericide and fungicide. Essential oils and plant extracts of *T. minuta* have been reported to have antifungal activity against pathogenic fungi [57]. Essential oil from leaves [58] and thiophene rich extracts from root of *T. minuta* showed significant antifungal activity against soil borne and aerial fungal pathogens. The major component of the leaf extract of *T. minuta*, quercetagenin-7-arabinosyl-galactoside showed significant antibacterial activity [59]. In field experiment, floral extract of French marigold *T. patula* reduced canker disease of tomato (62.82%), early blight (61.53%), wilt (18.42%), Fruit spot (27.41%), blossom end rot (50.43%) and sun scald (26.44%) in comparison to control [60]. It was found that intercropping with *T. erecta* reduced early blight of Tomato caused by *Alternaria solani* [61]. Flower extracts of *T. patula* exhibited toxicity against soil borne fungus *Fusarium oxysporum* f.sp. *lycopersici* causing wilt disease in tomato plant [62]. *T. lucida* extracts also showed high antifungal and antimicrobial activity [63, 64]. Antifungal activity of the main ketone active component 2,5-dicyclopentenyl cyclopentanone was evaluated from the root extract of *T. patula*, (*Tagetes* fungicide) [65]. An emulsion of fungicide was developed to increase its water solubility for better application. *In vitro* study revealed that the fungicide exhibited strong antifungal activity against *Fusarium oxysporum* f.sp. *Niveum*, *Fusarium oxysporum* f.sp. *Capsicum* and *Fusarium graminearum*. Scanning electron microscopy analysis revealed that the fungicide had a significant role in modification of hyphal morphology. Some examples of antibacterial and antifungal activities of *Tagetes* spp are given in the table 1.

Table 1: Antibacterial and antifungal activities of *Tagetes* spp

Plant species	Targeted bacterial/fungal species	References
<i>T. minuta</i>	<i>Rhizoctina solani</i> , <i>Sclerotinia sclerotiorum</i> , <i>Sclerotium rolfsii</i>	[58]
<i>T. minuta</i>	<i>Salmonella typhi</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Aspergillus niger</i> , <i>Candida albicans</i>	[66]
<i>T. minuta</i>	<i>Fusarium verticillioides</i> and <i>Fusarium proliferatum</i>	[67]
<i>T. patula</i>	<i>Botrytis cinerea</i>	[68]
<i>T. minuta</i> and <i>T. filifolia</i>	<i>Sclerotium cepivorum</i> , <i>Colletotrichum cocodes</i> and <i>Alternaria solani</i> .	[69]
<i>T. lucida</i>	<i>Rhizoctonia solani</i> , <i>Trichophyton mentagrophyte</i> , <i>E.coli</i> , <i>Proteus mirabilis</i> , <i>Klebsiella pneumoniae</i> , and <i>Salmonella sp</i>	[70]

5. APPLICATIONS AS BIOPESTICIDE

Powdered material of different parts (leaf, stem and flower) of *T. erecta* were used to assess the insecticidal property [71]. Incense sticks were prepared from the powdered material and were burnt to test the mosquito repellent activity. The leaf powder showed effectiveness as mosquito repellent agent when compared with a commercial product. It was found to be advantageous as there were no side effects and the cost of production was quite less (75%) compared to commercial product. Significant control of whitefly (*Trialeurodes vaporariorum*) was achieved when french marigolds were intercropped amongst tomatoes from the beginning of the growth period. Application of limonene in the form of limonene dispenser also showed promising results in controlling white flies [72]. Similar kinds of results were obtained when aqueous foliar extracts of *T. patula* was applied in management of western tarnished bug *Lygus hesperus*. Highest mortality was observed with the lowest concentration of the methanolic extract [73]. Both aqueous and methanolic extracts of *T. patula* also exhibited dose dependent toxicity against *Bemisia tabaci*. However, further investigation is needed to identify specific toxic active components and their modes of action in order to commercially implement marigold as an alternative to conventional pesticides.

It has been mentioned earlier that *Tagetes* spp showed potent nematicidal property. Several cultural practices are practised in the field to control nematodes using marigold. Among different techniques crop rotation using marigold as cover crop is the most frequently used method to control nematodes. Marigold, which is a popular bedding plant, can be used as a cover crop. *T. erecta* produces more biomass than several cultivars of *T. patula* and thus establishes well in the field, making it ideal for use as a cover crop. However, *T. patula* 'SingleGold' can also generate a significant amount of biomass, similar to *T. erecta* 'Cracker Jack', and thus may be an ideal marigold cover crop [45]. Reynolds et

al. 2000 [74], compared the effects of *T. patula* and *T. erecta* as rotation crops with the traditional practice of growing rye. Rotation crop and chemical fumigation were implemented before transplanting tobacco (*Nicotiana tabacum*) in a field trial. Marigolds reduced *Pratylenchus penetrans* population densities below the economic threshold for the cash crop for 3 years and increased tobacco yield by 197 kg ha⁻¹ compared to rye and chemical fumigation. Similarly, it was found that rotating *T. erecta* with strawberry lowered nematodes to below detection levels [75]. Intercropping is another method of nematode management. Intercropping is the practice of cultivating two or more crops concurrently within the same field [76]. Tsay et al, 2004, in a greenhouse experiment observed that intercropping water spinach (*Ipomea reptans*) with *T. erecta* reduced root galls caused by root knot nematode *Meloidogyne incognita* [77]. Similarly, it was observed that *M. incognita* did not form galls on soybean plants when intercropped with marigold species *T. erecta* and *T. patula* [78]. Allelopathic plants may prove valuable under conditions where multiple nematode species are present since they have the ability to suppress multiple nematode pests. For example, banana plantings typically have mixed populations of nematode species with different feeding habits [79]. When *T. erecta* was intercropped with banana, populations of four important nematode pests, *Radopholus similis*, *Helicotylenchus multicintus*, *R. reniformis*, and *Hoplolaimus indicus* were suppressed [80]. Xie et al, 2007 investigated the efficiency of crop rotation and intercropping systems with *T. erecta* for controlling root knot nematodes (*Meloidogyne* sp) in angelica (*Angelica sinensis*) [81]. Crop rotation model showed higher nematode control efficiency than intercropping model. There are further reports that in the field marigold can be added to the soil as green manure [82] or plant extracts can be applied as nematicides [83, 84] for the purpose of nematode control.

6. CONCLUSION

From the extensive study in this review, it is revealed that apart from therapeutic uses, *Tagetes spp* can also be utilized as potential biopesticide. This approach is not only an eco- friendly biological control method but it is also cost effective. However before application of marigold as biopesticide in the field of agriculture, the active components responsible for the pesticidal effect should be completely analysed and their mode of action is also needed to be understood. Besides, assessment of residual traces of marigold phytochemicals are needed to be done to nullify their off target effects on beneficial arthropod community. The hazardous effects of these constituents, if any, on human health are also essential to be tested before commercially using marigold biopesticide as an alternative to conventional chemical pesticides.

Conflict of Interest

None declared

7. REFERENCES

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