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## Bioactive properties of *Tagetes minuta* L. (Asteraceae) essential oils: A review

**Martin Muthee Gakuubi, Wycliffe Wanzala, John M Wagacha and Saifuddin F Dossaji**

**Abstract**

Mexican marigold (*Tagetes minuta* L.) and its accruing products have a long worldwide history of human uses such as food, therapeutics and aromatherapy which are inherent in the plant's unique chemical composition and bioactivities. In the recent past, *T. minuta* essential oils (EOs) have received great attention in research, and their phytochemistry, bioactivities and uses remain the focus of considerable scientific studies. The interest in EOs is largely due to increased demand by consumers for natural-based products such as additives, drugs and pesticides, whose global acceptability and safety is highly regarded compared to synthetic products. The purpose of this review is to document the existing value addition and evidence-based multipurpose potential and considerations of *T. minuta* as a new generation crop as provided for by in-depth scientific studies of its EOs. Among the bioactivities and therapeutic properties attributed to *T. minuta* EOs include: antihelminthic, carminative, arthropod repellency, sedative, weedicidal, antiseptic, diaphoretic, spasmolytic, germicides, stomachic, antispasmodic, antiprotozoal, bactericidal, emmenagogue, nematocidal, insecticidal, fungicidal, antiviral and other microbicidal properties against a wide range of plant, human and animal pathogens, pests and parasites. Oil of *T. minuta* is therefore a potentially useful agent for protecting food crops on farm and in storage and livestock, thereby enhancing food security and improving human livelihoods. Nevertheless, increased value addition and the need for validation of traditionally claimed usages and applications of *T. minuta* EOs through in-depth scientific studies should be prioritized to globally position this plant as a new generation crop.

**Keywords:** Asteraceae; bioactivity; essential oil; new crop; *Tagetes minuta*.

**1. Introduction**

Plants produce a wide array of secondary metabolites during their growth and development [1]. Essential oils also known as ethereal or volatile oils are among the most important compounds of secondary metabolism of aromatic plants [2]. The term 'essential' indicates that the oil carries a distinctive scent or essence of the plant. Generally, essential oils consist of volatiles, complex mixtures of natural, aromatic oily liquid blends characterized by a strong fragrance. They are usually liquid at room temperature though some are solid or resinous and ranging in colour from pale yellow to emerald green and from blue to dark brownish depending on the plant species producing it [3].

The biosynthesis and storage of essential oils is not restricted to specialized plant parts but rather occur in various parts of the producing plants such as leaves, flowers, seeds, fruits, roots and barks. Essential oils are extracted using one of several techniques such as steam distillation, hydrodistillation, cold pressing and solvent extraction [4, 5]. Being secondary metabolites, essential oils are not vital for growth and development of the producing plant. Their role has been hypothesized to include protection against pathogens and pests by acting as antifeedants, antibacterials, antivirals, antifungals and insecticides [6]. In a number of plants, the essential oils suppress growth of neighbouring plants through allelopathic effects hence offering the producing plant a competitive advantage [7].

Chemically, essential oils (EOs) belong to two biosynthetically related groups namely; terpenes and aromatic compounds of low molecular weight of which monoterpenes form the major component [8, 9]. Essential oil of a single plant species can contain between 20 and 60 components or more at varying concentrations [10]. However, they are characterized by two or three major constituents, which are present at high concentrations (20-90%) and various minor components present in trace amounts [7]. While it has been suggested that major components of EOs are the primary determinants of their biological properties [4, 11], there is also evidence that

minor/trace components of EOs play an important role in determining the biological properties of the EOs such as antimicrobial activities through synergism [12, 13].

Biosynthesis of essential oils is restricted to approximately 3000 plant species from more than 60 families with about 300 plants being the basis for the production of most of the economically important essential oils in the world [4, 14]. However, most of these plant species are not fully being utilized at subsistence and commercial levels in order to realize their value to humanity. The aim of this review therefore is to present the existing value addition and evidence-based multipurpose potential and considerations of *T. minuta* plant as an important yet underutilized new generation crop based on in-depth scientific studies of its essential oils.

## 2. *Tagetes minuta* L.

### 2.1 Taxonomy and origin of *Tagetes minuta*

*Tagetes minuta* L. is an annual aromatic herb that belongs to the family Asteraceae and the genus *Tagetes*, which is one of the most abundant plant taxonomical groupings, comprising of about 1,000 genera and over 23,000 species [15]. *Tagetes minuta* is known by several common names such as Mexican marigold, wild marigold, stinkweed, stinking roger and khaki bush [16]. In East Africa, the plant is known by different local and indigenous ethnic-based names such as, Ol' bangi (Maa), Omosumo (Kisii), Kivangi (Kamba), Ang'we/Anyach (Luo), Mūbangi (Kikuyu), Etakanyi (Luhya), Chemiasoriet (Kalenjin), Mubangi (Meru), Mukazimurofa (Runyankole), Rwasesa (Rukonjo) and omukazimurofu (Rutooro) [17]. The plant is native to the temperate grasslands and montane regions of southern South

America, including countries such as Argentina, Chile, Bolivia, Peru [18, 19]. The plant has since been introduced to many parts of the world including Africa, Australia, New Zealand, United States, Europe and Asia where it grows wildly and as a weed although it has lately been grown commercially in many regions of the world [20].

### 2.2 Morphology and habit of *Tagetes minuta*

*Tagetes minuta* is a strongly scented annual herb reaching heights of 50-150 cm and consisting of erect and at times highly branched stem [21]. The leaves which are 3-30 cm long and 0.7-8 cm wide are mostly opposite but often alternate in upper parts of the plant. The leaves are dark-green to slightly glossy green, pungent, glabrous and pinnately compound with 9-17 leaflets. The leaf margins are acute and serrate while the leaflets have linear-lanceolate margins finely serrulate, with orange translucent glands and 2-4 cm long [19]. The undersurface of the leaves has small, multicellular and punctate glands, which are orangish in colour and when punctured produces a liquorice-like aroma [22]. The glands may be found on stem and involucre bracts too [15, 19]. The heads are numerous and usually supported by a flat-topped cymes yellowish green in colour and 10 to 15 mm long, 2-3 mm wide with each surrounded by four or five fused involucre bracts. There are usually 3 to 5 lemon-coloured ray florets, and 10 to 15 yellow-orange disk florets per capitula. The achenes are cylindrical, dark brown to black in colour, 10 to 12 mm long with pappus consisting of 1-4 tiny, unequal awn-like scale, 2-3 mm long, and 3-5 ovate to lanceolate scales 0.5-1 mm long (Authors' field observations) (Figure 1).



**Fig 1:** Habit and morphological characteristics of *Tagetes minuta* L. (A) Habit, (B) Leaf under surface, (C) Leaf upper surface, (D) Heads, and (E) Leaflet.

### 2.3 Essential oils of *Tagetes minuta*

Essential oils of *T. minuta* have been extensively studied both *in vivo* and *in vitro* for various bioactivity properties and have been reported to have antimicrobial, insecticidal, nematicidal, acaricidal and repellency action against a wide range of human, livestock and plant pests and pathogens [23]. The chemical composition of essential oils from *T. minuta* sampled from different regions of the world have been studied and compared [24, 25]. Significant differences in the chemical profiles of essential oils of *T. minuta* have been reported and these have been attributed to a wide range of human and environmental-based factors. These factors include: the harvesting method, geographical location of the target plant [25, 26], growth stage at harvest [24, 27], plant parts used [24, 26, 28] and the climatic conditions under which the plant grows [29]. The variations in the chemical composition of EOs from the same plant species result in chemotypes, which are the result of biological variations

caused by the effects of different soils, temperature, weather conditions and light among other factors [5]. This implies that it is possible for the chemical composition of plants that are botanically identical to vary. For example, a comparison of the chemical composition of *T. minuta* EOs from Madagascar showed that the (*Z*)- $\beta$ -ocimene and dihydrotagetone constituents were considerably lower than those of *T. minuta* EOs from India, Turkey, Rwanda and France [30].

Chemotypic variation of *T. minuta* essential oils has been reported in several studies. For instance, untargeted analysis of GC-MS data and hierarchical clustering analysis (HCA) of *T. minuta* EOs from South Africa revealed two major chemotypes [31]. Further orthogonal projections to latent structures-discriminant analysis (OPLS-DA) showed that (*E*)-tagetone, dihydrotagetone and (*Z*)-tagetone were the characteristic marker constituents for chemotype 1, while (*Z*)- $\beta$ -ocimene, (*E*)-ocimene and (*Z*)-ocimene were the characteristic marker

constituents for chemotype 2. In another study, the chemical profiles of essential oils from aerial parts of *T. minuta* grown in Egypt, South Africa and UK were evaluated [25]. The study identified two chemotypes; one characterized by higher content of tagetones in the UK sample, and another characterized by elevated proportion of ocimenes and ocimenones in the Egypt and South Africa samples.

### 3. Antibacterial activity of *Tagetes minuta* essential oils

Antibacterial activity is one of the most widely studied areas in essential oil research. Antibacterial activity of *T. minuta* EOs against a range of human, plant and animal pathogenic bacteria has been studied. A study was conducted to assess the antimicrobial and antioxidative activities of essential oils of three South African plants including *T. minuta* [32]. Agar diffusion assays were used to determine the antimicrobial activities of EOs against two Gram-positive bacteria - *Bacillus subtilis* and *Staphylococcus aureus* - and two Gram-negative bacteria - *Escherichia coli* and *Pseudomonas aeruginosa*. *Tagetes minuta* EOs whose major components were (Z)- $\beta$ -ocimene and dihydrotagetone exhibited a concentration-dependent antibacterial activity against the test bacteria, where the activity of the EOs increased with concentration. However, Gram-positive bacteria were more sensitive to the EOs than Gram-negative bacteria, a finding that concurred with previous studies, which reported that Gram-negative bacteria are generally more resistant to the effects of EOs than Gram-positive bacteria [33, 34]. In another study, the chemical composition, antioxidant, antimicrobial and cytotoxic activity of *T. minuta* EOs were evaluated. The antimicrobial effect of the essential oils was evaluated against two Gram-negative bacteria *Salmonella typhi* and *E. coli* and two Gram-positive bacteria *S. aureus* and *B. subtilis*. In addition, microdilution method was used to assess the minimum inhibition concentrations (MICs) of the EOs on the test pathogens. There was a concentration-dependent antibacterial activity of *T. minuta* EOs whose main components were dihydrotagetone, (E)- $\beta$ -ocimene, tagetone, (Z)- $\beta$ -ocimene, limonene and epoxyocimene. The MICs for *S. typhi*, *E. coli*, *S. aureus* and *B. subtilis* were  $150 \pm 8$ ,  $165 \pm 9$ ,  $67 \pm 8$ , and  $75 \pm 7$   $\mu\text{g/mL}$  of *T. minuta* EOs, respectively. Similar to the previous findings, the EOs had a significantly stronger antibacterial effect on the Gram-positive bacteria than on Gram-negative bacteria [35]. Antibacterial activity of limonene enantiomers ((+), (-) and the racemate) singularly and in combination (1:1) with 1,8-cineole has been reported against *S. aureus* and *P. aeruginosa* [36].

Antibacterial activity of *T. minuta* EOs obtained from different geographical regions and having different chemical profiles has also been studied. In one such study, the antibacterial activity of EOs extracted from aerial parts of *T. minuta* from Egypt, South Africa and the United Kingdom and having different percentage chemical compositions were tested against eight bacterial pathogens by determining the minimum inhibitory concentrations using broth dilution method. The EOs had great inhibitory activity against Gram-positive bacteria (*B. cereus*, *B. subtilis*, *S. aureus* and *S. faecalis*) than Gram-negative ones (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *E. coli*, and *S. typhi*) [25]. The study further showed variation in bioactivity of essential oils from the three geographical locations which was attributed to differences in the percentage compositions of the major and minor essential oil constituents with the EOs from the United Kingdom exhibiting a higher antibacterial activity compared to EOs from Egypt and South Africa.

Essential oils from a single plant may comprise of hundreds of

major and minor components resulting in a myriad of possible biological interactions and anticipated effects [37]. It has been reported in literature that generally, essential oils that contain phenolic structures such as carvacrol, eugenol and thymol produce the highest antimicrobial activity [38, 39]. However, more often than not, the level of antibacterial activity of EOs is usually as a result of unique complex synergistic and antagonistic biological interactions among different constituents of the EOs [37]. Systematic and comprehensive investigations such as subtraction bioassays should therefore be conducted to accurately determine the levels of biological interaction, mechanisms and combinations that are responsible for the antibacterial activity of active components of the EOs, which have been isolated by bioassay guided fractionations.

As previously stated, many studies have established that EOs are more efficacious towards Gram-positive than Gram-negative bacteria. The variation in sensitivity of Gram-positive and Gram-negative bacteria to EOs can be attributed to structural differences in cell walls of the two microbe groups. Roughly, 90% of the cell wall of Gram-positive bacterial is made up of peptidoglycan (murein), a mesh-like polymer of sugar and amino acids coupled with other molecules such as teichoic acid and proteins. This configuration of Gram-positive bacteria cell wall easily allows hydrophobic compounds to penetrate the cell and thus act on either the cell wall within the cytoplasm or both, thus making the Gram-positive bacteria more susceptible to antibacterial effects of EOs and other similar natural compounds [40]. On the contrary, Gram-negative bacteria possess a more complex cell wall that has a much thinner peptidoglycan surrounded by an outer membrane consisting of proteins and lipopolysaccharide (LPS). It has been suggested that the presence of LPS and more specifically its O-side chain is responsible for greater resistance of Gram-negative bacteria to hydrophobic EOs by restricting their diffusion into the cytoplasm [40, 41, 42].

### 4. Antifungal activity of *Tagetes minuta* essential oils

Essential oils and plant extracts in general have been reported to have antifungal activity against a broad range of pathogenic fungi [43]. High levels of activity against *Botrytis cinerea* were reported in 13 out of 345 plant extracts [44]. In the study, among the 49 essential oils analyzed, the most commonly occurring components in oils exhibiting high levels of antifungal activity were: limonene, 1,8-cineole,  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene and camphor, which have been identified as some of the common components of *T. minuta* essential oils. Limonene, 1,8-cineole,  $\alpha$ -pinene and camphor have been shown to have antifungal activity against a wide range of fungi such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Penicillium digitatum*, *Aspergillus niger*, *Verticillium fungicola* and *Trichoderma harzianum* [45, 46]. *Tagetes minuta* essential oils have been evaluated for their fungicidal effects against a broad range of fungal pathogens. Essential oils extracted from leaves of *T. minuta* had higher activity than oils extracted from flowers of the same plant against eight phytopathogenic fungi namely: *Rhizoctonia solani*, *Fusarium solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum pisi*, *Sclerotium rolfsii*, *Pyricularia grisea*, *Fusarium oxysporum lentis* and *Alternaria solani* [47]. The percent inhibition of EOs from flowers at  $1000 \mu\text{g mL}^{-1}$  were in the range of 8.9 to 35.1% with the highest activity reported against *F. oxysporum pisi* and the lowest against *P. grisea*. Leaf EOs on the other hand had the highest and least activity against *S. rolfsii* and *F. oxysporum lentis*, respectively. Concentration-dependent antifungal activity of EOs of *T. minuta* extracted from aerial parts was reported

against *Aspergillus niger* and *Candida albicans* with corresponding MIC values of  $135 \pm 15$  and  $115 \pm 8$   $\mu\text{g/mL}$ , respectively [35]. Similarly, a concentration-dependent antifungal activity of *T. minuta* EOs against the fungus *A. niger* and yeast *C. albicans* was reported with the activity increasing with increase in the concentration of essential oil [32]. However, in some studies, diluted oils have been shown to result in larger zones of inhibition than those of undiluted oils. These cases of deviation from the expected results may be explained by the hypothesis that diluted oils diffuse more easily in the media compared to undiluted oils [32, 48].

### 5. Insecticidal activity of *Tagetes minuta* essential oils

Chemical control of pests using insecticides remains one of the most effective strategies in pest management [49]. Indeed, synthetic insecticides have played a major role in the control and management of many economically important pests thus boosting crop yields and animal productivity and enhancing global food security [50]. However, increased public awareness of the negative effects associated with synthetic insecticides such as high persistence of residues, toxicity to non-target organisms, increased cases of development of resistance in some pests, health risk to humans and general damage to environment has generated interest in biopesticides with botanical-based ones experiencing the greatest revival. Most plant-insect interactions are chemically mediated by secondary metabolites. Generally, plants that show well-developed defense mechanism against pests are usually good sources of new insecticidal substances and comprise the main focus by Scientists seeking to develop plant-based insecticides [51]. Essential oils as candidate for novel insecticides have numerous advantages; they are easy to extract, are biodegradable and hence ecofriendly, have low persistence in soil and water, and require less stringent approval and regulatory mechanisms due to their long usage history [52, 53].

Insecticidal activity of *T. minuta* essential oil against head lice, *Pediculus humanus capitis*, a human ectoparasite that causes *Pediculosis capitis* has been studied [54]. In the study, a linear regression model reported a Lethal Time (LT<sub>50</sub>) value of  $16.4 \pm 1.62$  min with histopathological analysis of the treated adults showing pronounced disassembly of actin and myosin filaments. In another study, *T. minuta* EOs whose main constituents were limonene (13.0%), piperitenone (12.2%), and  $\alpha$ -terpineol (11%) produced a dose-dependent toxicity against the cabbage aphid *Brevicoryne brassicae* with high dose ( $125.8 \mu\text{L}^{-1}$  air) resulting in >90% insect mortality [55]. *Tagetes minuta* essential oils from fresh and dried plant materials have also been reported to be highly effective against the larvae of *Anopheles stephensi* with the LC<sub>50</sub> values of 1.0532 and 1.0315 mg/L, respectively [56]. In another study, significant insecticidal activity of the EOs from aerial parts of *T. minuta* was reported against *Aedes aegypti* larvae [57]. Essential oils of *Tagetes minuta* were also found to have repellent and antifeedant activity against diamond backmoth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) [58]. Essential oils from three genotypes of *T. minuta* were screened for their potential toxicity against three stored product beetle species: *Callosobruchus maculatus* (Fabricius), *Sitophilus oryzae* (Linnaeus) and *Tribolium castaneum* (Herbst) [59]. The EOs from genotype 1 of *T. minuta* (TM-1) induced 100% adult mortality in the three beetle species at dosages of 50,000 ppm and 500  $\mu\text{g/insect}$  in fumigant and contact toxicity tests, respectively. Furthermore, essential oils from TM-1 deterred oviposition and suppressed egg hatchability by 81% and 91%, respectively in *T. castaneum* at a dosage of 70, 000 ppm.

### 6. Acaricidal activity of *Tagetes minuta* essential oils

Plant essential oils have been studied extensively for their biocidal effect against a broad range of economically important arachnids especially ticks and mites revealing promising results. Control of tick species that infest livestock is especially of great importance in the livestock industry as it boosts productivity. Ticks cause extensive socio-economic losses to livestock industry by weakening the affected and infested animals through blood loss, reduction in the quality of the hides and more importantly, the ticks acting as vectors and reservoirs of a broad range of viral, rickettsial, bacterial and protozoan pathogens which are responsible for great economic losses globally [60, 61]. A study to evaluate the acaricidal activity of essential oils from leaves and stems of *T. minuta* against several tick species demonstrated that EOs of *T. minuta* had over 90% efficacy against four tick species at a concentration of 20%, an efficacy comparable to a number of referenced conventional acaricides [62]. In another study, EOs of *T. minuta* whose major constituents were dihydrotagetone (54.10%) limonene (6.96%), tagetone (6.73%) and (*E*)- $\beta$ -ocimene (5.11%) were shown to have significant activity against larvae, nymph and adult *R. microplus* [63].

Essential oils of *T. minuta* from different plant parts in varied growth stages were investigated for their biocidal activity against *Varroa destructor*, an ectoparasitic mite of the *Apis mellifera* [64]. The study findings revealed that the medium percentages of dead mites after six hours of treatment were 97.7, 98.3 and 100 for EOs from leaves of bloomed plants, leaves of non-bloomed plants and flowers, respectively. In a closely related study, *T. minuta* essential oils whose major components were (*E*)- $\beta$ -ocimene (62.8%), (*Z*)-ocimenone (10.2%), (*E*)-ocimenone (6.6%), limonene (5.8%) and dihydrotagetone (4.2%) were evaluated for their biological activity against *V. destructor* [65]. The percentage values of mite mortality sprayed with the EOs concentrations of 3, 4 and 5% were 64, 72 and 72%, respectively. In addition, significant acaricidal efficacy of *T. minuta* essential oil against *V. destructor* adult mite when administered through pulverization has been reported [66].

### 7. Repellent activity of *Tagetes minuta* essential oils

Repellents have been defined as substances that acting locally or at a distance, deter an arthropod from flying to, landing on or biting host human and/or animal skin or a surface in general [67]. Currently, considerable research efforts have been directed towards the development of alternative repellents to synthetic chemicals for control of insects and arthropods most of which are vectors to important diseases affecting humans and livestock. This is especially important because overreliance on synthetic chemicals is not without serious environmental and health concerns. Among the potential candidates in this regard are essential oils. Indeed, essential oils belonging to plants from numerous species have been studied extensively to assess their repellent properties with a number of them being the basis of some commercial repellent formulations [68]. *Tagetes minuta* essential oils have been studied for their repellent activities against a number of human and animal parasites such as ticks and mosquitoes [37].

#### 7.1 Repellency against ticks

The essential oils of *T. minuta* were investigated for their effect on the climbing response of adult *R. appendiculatus* using a dual-and no-choice assay arrangement [49]. Both assay methods used in this investigation independently showed a significant repellent effect of *T. minuta* essential oils against the test

organisms. The repellent effect was however less in no-choice assay compared to the dual-choice assay, especially for lower doses. Moreover, the study confirmed that *T. minuta* essential oils had effect on the adult *R. appendiculatus* both in the presence and absence of host-derived stimuli.

In a related study, the repellency of essential oils of *T. minuta* obtained from three agro-ecological zones in Kenya was evaluated against *R. appendiculatus* using a dual-choice tick climbing assay apparatus [69]. The study established a significant difference in chemical composition and dose-dependent repellency of *T. minuta* essential oils from the three agro-ecological zones. The higher activity of essential oil from one of the three regions was associated with the presence of relatively high amount of guaiacol, compared to oils from the other two regions, with synthetic blend assays showing that guaiacol and guaiacol-alloocimene blend had the highest repellency.

In addition to *R. appendiculatus*, the activity of essential oils of *T. minuta* has been studied against other types of ticks. Anti-tick properties of the essential oils of *T. minuta* obtained by hydrodistillation from a combination of flowers, leaves and soft stems were evaluated for anti-tick properties against *Hyalomma rufipes* [70]. *Tagetes minuta* EOs whose major components were (*Z*)- $\beta$ -ocimene (28.5%), (*E*)- $\beta$ -ocimene (16.83%) and 3-methyl-2-(2-methyl-2-butenyl)-furan (11.94%) had a dose-dependent repellent activity against *H. rufipes*. Furthermore, probit analysis revealed a repellent EC<sub>50</sub> of *T. minuta* EO to be 0.07 ml/mL and 0.07 ml/mL for male and female ticks, respectively. Additionally, the essential oils were found to induce delayed molting in 60% (s.e $\pm$ 4.7) of nymphs after 25 days in comparison with the control group.

### 7.2 Repellency against mosquitoes

The repellent activity of *T. minuta* essential oils against some mosquito species has been investigated. The chemical composition and repellent effect of *T. minuta* essential oil on the host-seeking female *Anopheles arabiensis* mosquitoes was investigated using the human-bait technique [37]. This study which was designed to simulate field situation was based on the proportion of host-seeking female *A. arabiensis* mosquitoes landing on the bait (human forearm) and biting it following its treatment with either the essential oils of *T. minuta* or Vaseline® (a non-perfumed petroleum jelly), which was used both as a diluent as well as a control. There was a significant dose-response effect of repellency with more test mosquitoes significantly landing and biting the control arm treated with pure petroleum jelly than the arm treated with the essential oil of *T. minuta* whose main constituents included (*Z*)- $\beta$ -ocimene, (*E*)- $\beta$ -ocimene, (*Z*)-tagetone, (*E*)-tagetone, dihydrotagetone, and piperitenone.

In another study, the repellency effect of forty-one essential oils that included those of *T. minuta* against three mosquito species namely: *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* was investigated [71]. The repellent test employed the human-bait technique with the testing period lasting up to 8 hours. Furthermore, since the test mosquitoes included both day and night biters, different testing periods were used i.e. 0800 to 1600 h for *A. aegypti* and 1600 and 2400 h for *A. stephensi* and *C. quinquefasciatus*. The protection time was calculated as the duration between the application of a repellent and the first two bites or two bites in successive observations. *Tagetes minuta* essential oils offered a protection of 60 minutes against *A. aegypti* and 480 minutes (the maximum protection time) for both *A. stephensi* and *C. quinquefasciatus*. In a closely related study, promising repellent activity of *T. minuta* essential oil

against the three aforementioned mosquito species was obtained with the test oils providing 86.4, 84.2 and 75% protection against *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti*, respectively after a study period of 6 hours [72]. In addition to mosquitoes, *T. minuta* EOs have been investigated for their repellent properties against other insects. *Tagetes minuta* EOs whose main constituents were (*Z*)-tagetone, (*E*)- $\beta$ -ocimene and dihydrotagetone were found to have good repellent activity against *Triatoma infestans* (the insect vector of Chagas disease). At a concentration of 0.5% (w/v), the essential oils of *T. minuta* produced an average repellency of 94.7% [73].

### 8. Nematicidal activity of *Tagetes minuta* essential oils

Plant-parasitic nematodes are important parasites that cause great damage to many agricultural crops resulting in losses estimated in the order of billions each year [74]. Botanicals have received much attention in the management of nematodes where they are used in four main ways: 1) used as part of the plant directly, 2) using extracts of parts of the botanicals, 3) using compounds of botanicals possessing nematicidal activity and 4) using plants as oil seed cakes, mature crop residue and organic amendments [75].

The toxicity of two compounds; (*Z*)- $\beta$ -ocimene and dihydrotagetone isolated from the EOs of *T. minuta* was investigated against the eggs and juveniles of *Meloidogyne incognita*, a plant-parasitic nematode [74]. *Tagetes minuta* essential oils at a concentration of 4%, 3%, 2% and 1% were strongly toxic to both the eggs and juveniles of the test nematode. Moreover, the egg-hatch inhibitions by the above oil concentrations were in the range of 72 to 79% in 14 days with the inhibitory activity being concentration-dependent. The study additionally showed that of the two compounds, dihydrotagetone was more toxic to the eggs of *M. incognita* and the compound killed the juveniles much faster (in 72 hours) compared to (*Z*)- $\beta$ -ocimene (in 96 hours).

In addition to plant-parasitic nematodes, *T. minuta* essential oils have been studied for their nematicidal activity against animal gastrointestinal nematodes such as *Haemonchus contortus*, an important pathogenic nematode of ruminants [76]. In the study, two bioassays: an egg hatch test (EHT) and larval development test (LDT) were used. *Tagetes minuta* essential oils displayed a dose-dependent effect in the EHT, inhibiting 98.1% of *H. contortus* larvae hatching at a concentration of 2.5 mg mL<sup>-1</sup>. Furthermore, the effective EOs concentration that suppressed 50% (EC<sub>50</sub>) of egg hatching was 0.53 mg mL<sup>-1</sup>. With regard to the larval development test, *T. minuta* EOs at a concentration of 10 mg mL<sup>-1</sup> inhibited 99.5% of *H. contortus* larval development with an EC<sub>50</sub> value of 1.67 mg mL<sup>-1</sup>.

### 9. Allelopathic effect of *Tagetes minuta* essential oils

Allelopathy, which is a biochemical interaction among higher plants in which certain plants release metabolites that have either stimulatory or inhibitory effects on the growth of the receptor plants has been recognized and studied for many years [77, 78, 79]. Allelopathy is generally recognized as an important ecological factor that determines the structure and composition of plant communities [80]. This phenomenon of certain plant species possessing the capability to affect surrounding plants has been widely documented and is currently being explored as a potential method of weed control and management [79, 81].

*Tagetes minuta* essential oils and their principal components in the vapour phase were studied for their allelopathic activity on roots of Maize (*Zea mays* L.) [80]. The volatile oils of *T. minuta* whose main components were limonene,  $\beta$ -pinene and  $\alpha$ -pinene

showed strong inhibitory and oxidant effect on the root of *Z. mays* seedlings. The phytotoxic action of *T. minuta* EOs against *Z. mays* was attributed to increased lipid peroxidation rates indicating an induction of ROS-generated oxidative stress. With regard to the terpenes studied, ocimene had the highest inhibitory effect on the root growth and the highest oxidative value. Thus, the high phytotoxicity action of *T. minuta* EOs was attributed to its high content of ocimene. In another study, the allelopathic potential of EOs of *T. minuta* against three invasive weeds: *Chenopodium murale* L., *Phalaris minor* Retz., and *Amaranthus viridis* L. was investigated [81]. The results of the study established a concentration-dependent response in which volatile oils of *T. minuta* reduced germination in the three test weeds with maximum reduction observed in *C. murale* followed by *P. minor* and least in *A. viridis*. Moreover, LC<sub>50</sub> values for *C. murale* and *P. minor* were lower (0.761 and 0.822 mg/petri dish, respectively) compared to those of *A. viridis* (2.745 mg/petri dish). Similarly, seedling length also followed the same pattern with maximum reduction observed in *C. murale*. Two compounds that have been commonly isolated from *T. minuta* EOs i.e.  $\alpha$ -pinene and its isomer  $\beta$ -pinene have been shown to have strong allelopathic effects, and their ecological role on the allelopathic interactions among plants is well documented [82].

The phytotoxic effect of aqueous extracts and essential oils of *T. minuta* on the callus growth and induction on four plant species; *Oryza sativa* (Dongjinbyeon), *Brassica campestris* subsp. *Napus* var. *pekinensis*, *Raphanus sativus* var. *acanthiformis* and *Sesamum indicum* (Ansanggae) have been studied [83]. Species-specific inhibitory trends from the essential oils and the aqueous extracts were observed. The percentage of total callus induction on *O. sativa* though slightly decreased in proportion to the concentration of the EOs was not significantly different to the control. For *B. campestris* var. *pekinensis*, however, the percentage of total callus induction was significantly inhibited by 20  $\mu$ l essential oil. With regard to the callus growth, when tested in 20  $\mu$ l essential oil, callus growth of the four receptor species decreased significantly compared to that of the control. The callus relative growth rate (RGR) in 20  $\mu$ l essential oil was repressed in the following order: *S. indicum*, *B. campestris*, *O. sativa* and *R. sativus*.

#### 10. Antioxidant activity of *Tagetes minuta* essential oils

An antioxidant is defined as any substance, which when present at low concentrations in combination with an oxidisable substrate, significantly delays or prevents oxidation of the substrate [84]. Oxygen though a vital component for living system, is a highly reactive atom that is capable of forming damaging molecule (free radicals). Free radical-mediated oxidation of various biological substances has been associated with numerous diseases such as cardiovascular disease, Alzheimer's disease, arthritis, inflammation, diabetes, Parkinson's disease and several types of cancers [4, 84]. To prevent oxidative damage, many diseases have for a long time been treated using antioxidants [85]. The use of synthetic antioxidants has raised health concerns especially after some such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were reported to be toxic and potentially carcinogenic, by inducing DNA damage [86]. As a result, there is increased need to find alternative naturally-based antioxidants especially from plants and plant metabolites. Indeed, numerous studies have been conducted on essential oils as potential sources of natural antioxidants [84, 87, 88].

The antioxidant activity of essential oils isolated from *T. minuta* flowers was studied with the hydrogen atom-or-electron

donating ability of the EOs assessed using the 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay [88]. The DPPH scavenging activity of the EOs was compared with those of the standard antioxidant, ascorbic acid. The DPPH free radical scavenging activity of the essential oil was lower (73.4%) at a concentration of 200  $\mu$ g/mL, in comparison to that of ascorbic acid (94.1%) at similar concentration. *Tagetes minuta* EOs at a 200  $\mu$ g/mL concentration reduced the concentration of DPPH free radical with an efficacy of about 75  $\mu$ g/mL concentration of the standard antioxidant. Moreover, *T. minuta* EOs achieved 50% reduction of the DPPH radical with EC<sub>50</sub> value of 86.35  $\mu$ g/mL. A study that similarly used the DPPH radical scavenging assay to evaluate the antioxidant activity of *T. minuta* essential oil whose main constituents included, trans-ocimene (51.7%), cis-tagetone (17.7%) and cis-ocimene (7.7%), reported an EC<sub>50</sub> value of 0.8mg/mL [89]. Another study similarly employed the DPPH radical scavenging assay to evaluate the antioxidant properties of both the essential oils and solvent extracts of *T. minuta* [84]. In the study, the active constituents were isolated by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The active fractions were later detected using gas chromatography/mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR), with the active components tested quantitatively for their radical scavenging activity by Ultraviolet-visible spectroscopy (UV/Vis). *Tagetes minuta* essential oils whose main chemical components included (*E*)- $\beta$ -ocimene (15.90%), L-verbenone (15%) and limonene (8.02%) were reported to have strong antioxidant activity with an LC<sub>50</sub> of 1.49 g/l<sup>-1</sup> after 30 minutes of evaluation.

#### 11. Anticancer activity of *Tagetes minuta* essential oils

Cancer is a leading cause of death worldwide, accounting for more than eight million deaths in 2012 [90]. It is estimated that roughly 60% of drugs currently used for cancer treatment have been isolated from natural products with plants being the most important sources [91]. Systematic screening of plants especially those that are prominently used in various types of ethnic medicine has led to the discovery of important drugs such as taxol, camptothecin and camptothecin [92]. Essential oils from a wide range of plants have been extensively studied for their anticancer activities including melanoma, leukemia, glioblastoma, and oral cancers, besides breast, cervix, colon, liver, pancreas, prostate and uterus cancers [93].

Anticancer activity of *T. minuta* and *Ocimum basilicum* EOs was conducted on two human promyelocytic leukemia cell lines (HL-60 and NB4) and an experimental animal model cancer cell line, Ehrlich ascites carcinoma cells (EACC) [88]. For *in vivo* studies, three treatments: initiation, pre-initiation and post-initiation were used to study the chemopreventive and/or chemotherapeutic efficacies of the two essential oils based on the survival of tumor (EACC) transplanted in female mice. In the case of the *in vitro* tests, *T. minuta* essential oils showed a higher anticancer activity against NB4 and EACC cell lines compared to *O. basilicum* EOs, while *O. basilicum* essential oils exhibited higher bioactivity than *T. minuta* EOs against HL-60 cell line. For *in vivo* studies, the pre-initiation treatments were more effective for both essential oils compared to initiation and post-initiation treatments on the tumor (EACC) transplanted female mice. In another closely related study, chemical composition, antioxidant, antimicrobial and cytotoxic bioactivities of the two aforementioned EOs was studied [35]. In the study however, the activity of the EOs against two human tumor cell lines namely, nasopharyngeal cancer cell line (KB)

and liver hepatocellular carcinoma cell line (HepG2) was studied using a modified MTT assay. At lower concentrations, (<50µg/mL), *T. minuta* EO had no significant effect on KB and HepG2 viability. At much higher concentrations (50-200µg/mL) however, *T. minuta* essential oils showed concentration-dependent effects on the cell viability with maximum reduction in cell viability observed at concentrations of >200µg/mL. Additionally, IC<sub>50</sub> values for KB and HepG2 were 75±5 and 70±4 µg/mL of *T. minuta* essential oils, respectively. In another study, *T. minuta* EOs whose main constituents were (*Z*)-ocimene (15.9%), (*E*)-ocimene (34.8%) and (*Z*)-β-ocimene (8.3%) showed potent cytotoxic activity against MCF-7 breast tumor cells, with an IC<sub>50</sub> of 54.7±6.2 µg/mL<sup>[94]</sup>. Furthermore, 1,8-cineole, a common occurring constituent in *T. minuta* EOs has been shown to induce apoptosis of human colon cancer cells<sup>[95]</sup>.

## 12. Other activities of *Tagetes minuta* essential oils

There have been numerous reports of bioactivities of *Tagetes minuta* essential oils and their components including: antidepressant activity via negative modulation on GABAergic function<sup>[96]</sup>, tranquilizing, hypotensive, bronchodilatory, spasmolytic and anti-inflammatory bioactivities<sup>[97, 98]</sup> and antifeedant activities<sup>[58]</sup>.

Essential oils have been considered relatively non-toxic to mammals and hence completely safe to use because they are 'natural'<sup>[99]</sup>. However, most EOs can be toxic at high concentrations, especially when taken orally<sup>[100]</sup>. Some EOs can be toxic when applied topically or orally even at very low concentration due to the presence of toxic components. *In vitro* and *in vivo* studies have shown that *T. minuta* EOs are phototoxic<sup>[101]</sup>. It is for this reason that the Scientific Committee on Consumer Products (SCCS) has recommended a maximum level of 0.01% *T. minuta* EOs in leave-on products (except sunscreen cosmetic products), provided that the terthiophene content in the EOs does not exceed 0.35%. Additionally, SCCS recommends that *T. minuta* extracts and oils should not be used as ingredients of sunscreen products<sup>[102]</sup>. With the exception of aromatherapy, there is little information in literature on safety considerations in using *T. minuta* EOs. Thus, comprehensive toxicity tests are required in order to ascertain the safety of using the EOs of *T. minuta* in pharmaceuticals, agrochemicals, food and beverages and in sociocultural ethnic practices, among other applications.

## 13. Conclusion

The biological activities of *Tagetes minuta* essential oils and their components are widely documented worldwide, ethnobotanically inherent in people's culture/taboo and well recognized in modern life styles. Validation of some of the useful folkloric claims about *T. minuta* through scientific evaluation of its essential oil has shown the plant to contain compounds and/or blends that have a wide range of bioactive, cosmetic, food and therapeutic properties, such as antihelminthic, carminative, repellency, sedative, weedkilling, antiseptic, diaphoretic, spasmolytic, germicides, stomachic, antispasmodic, antiprotozoal, bactericidal, emmenagogue, nematocidal, insecticidal, fungicidal, antiviral, and other microbicidal properties. Undertaking in-depth scientific studies to properly understand the underlying science of these properties of *T. minuta* essential oils and their components will indisputably enhance and facilitate a high level of acceptability and henceforth, value addition usage and applications of these oils in the pharmaceutical, agricultural, food and perfumery

industries. This review therefore presents *Tagetes minuta* as a promising multipurpose new crop of the world whose potential is yet to be optimally utilized at subsistence and commercial levels.

## 13.1 Conflict of interest

The authors declare that they have no conflict of interest.

## 14. References

- Ramakrishna A, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*. 2011; 6(11):1720-1731.
- Rehman R, Hanif MA, Mushtaq Z, Al-Sadi AM. Biosynthesis of essential oils in aromatic plants: A Review. *Food Reviews International* 2015; 32(2):117-160.
- Bassolé IH, Juliani HR. Essential Oils in Combination and Their Antimicrobial Properties. *Molecules*. 2012; 17(4):3989-4006.
- Shaaban AE, El-Ghorab AH, Shibamoto T. Bioactivity of essential oils and their volatile aroma components: Review. *Journal of Essential Oil Research*. 2012; 24(2):203-212.
- Rohloff J. Cultivation of Herbs and Medicinal Plants in Norway – Essential Oil Production and Quality Control. PhD. Thesis, Norwegian University of Science and Technology, Norway, 2003.
- Ibrahim MA, Kainulainen P, Aflatuni A, Tiilikkala K, Holopainen JK. Insecticidal, repellent, antimicrobial and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. *Agricultural and Food Science*. 2001; 10:243-259.
- Abad MJ, Bedoya LM, Apaza L, Bermejo P. The *Artemisia* L. Genus: A review of bioactive essential oils. *Molecules* 2012; 17:2542-2566.
- Seigler DS. *Plant secondary metabolism*. Kluwer Academic Publishers, New York, 1998, 325-367.
- Yap PX, Yip BC, Ping HC, Lim SE. Essential oils, a new horizon in combating bacterial antibiotic resistance. *The Open Microbiology Journal*. 2014; 8:6-14.
- Pandey AK, Singh P, Tripathi NN. Chemistry and bioactivities of essential oils of some *Ocimum* species: an overview. *Asian Pacific Journal of Tropical Biomedicine*. 2014; 4(9):682-694.
- Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - A review. *Food and Chemical Toxicology*. 2008; 46(2):446-475.
- Marino M, Bersani C, Comi G. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *International Journal of Food Microbiology*. 2001; 67:187-195.
- Burt S. Essential oils: their antimicrobial properties and potential application in foods - A review. *International Journal of Food Microbiology*. 2004; 94(3):223-253.
- Chi PT. Chemical composition, antioxidant and antimicrobial activities of essential oils extracted from citrus varieties in Vietnam. MSc Thesis, Vietnam National University, Vietnam, 2013.
- Sadia S, Khalid S, Qureshi R, Bajwa A. *Tagetes minuta* L. a useful underutilized plant of family Asteraceae: a review. *Pakistan Journal of Weed Sciences Research*. 2013; 19(2):179-189.
- Randall R, Kessal O. National list of naturalized invasive and potentially invasive garden plants. WWF Australia, Sydney, 2004, 98.
- Maundu PM, Ngugi GW, Kabuye CH. Traditional food

- plants of Kenya. English Press Limited, Nairobi, 1999, 220.
18. McVaugh R. Botanical collections of the La Plata expedition of 1853-1855. *Brittonia*. 1943; 5:64-79.
  19. Soule JA. *Tagetes minuta*: A potential new herb from South America. In: J Janick, Simon JE (eds.). *New crops*. Wiley: New York, 1993, 177.
  20. Naqinzhad A, Mehrvarz SS. Some new records for Iran and flora Iranica area collected from Boujagh National Park, N. Iranian Journal of Botany. 2007; 13(2):112-119.
  21. Shahzadi I. Chemical and molecular characterization of marigold (*Tagetes* species) found in Pakistan. PhD Thesis, COMSATS Institute of Information Technology, Abbottabad, Pakistan, 2012.
  22. Prakaso R, Syamasundar K, Gopinath C, Ramesh S. Agronomical and Chemical studies on *Tagetes minuta* grown in a red soil of a semiarid tropical region in India. *Journal of Essential Oil Research*. 1999; 11:259-261.
  23. Shahzadi I, Hassan A, Khan UW, Shah MM. Evaluating biological activities of the seed extracts from *Tagetes minuta* L. found in Northern Pakistan. *Journal of Medicinal Plant Research*. 2010; 4(20):2108-2112.
  24. Chalchat JC, Garry RP, Muhayimana A. Essential oil of *Tagetes* from Rwanda and France: chemical composition according to harvesting, location, growth stage and part of plant extracted. *Journal of Essential Oil Research*. 1995; 7(4):375-386.
  25. Senatore F, Napolitano F, Mohamed M, Harris PJ, Mnkeni PN, Henderson J. Antibacterial activity of *Tagetes minuta* L. (Asteraceae) essential oil with different chemical composition. *Flavour and Fragrance Journal*. 2004; 19:574-578.
  26. Chamorro ER, Ballerini G, Sequeira AF, Velasco GA, Zalazar MF. Chemical composition of essential oil from *Tagetes minuta* L. leaves and flowers. *Journal of Argentine Chemical Society*. 2008; 96:80-86.
  27. Moghaddam M, Omidbiagi R, Sefidkon F. Changes in content and chemical composition of *Tagetes minuta* oil at various harvest times. *Journal of Essential Oil Research*. 2007; 19:18-20.
  28. Weaver DK, Wells CD, Dankel FV, Bertsch W, Sing SE, Sirharan S. Insecticidal activity of floral, foliar and root extracts of *Tagetes minuta* (Asterales: Asteraceae) against adult Mexican bean weavils (Coleoptera: Bruchidae). *Journal of Economic Entomology*. 1994; 87:1718-1725.
  29. Mohamed M, Harris P, Henderson J, Senatore F, Napolitano F. Effect of drought stress on the yield and composition of volatile oils of drought-tolerant and non-drought-tolerant clones of *Tagetes minuta*. *Planta Medica*. 2002; 68:472-474.
  30. Ramarason-Raonizafinimanana B, Ramanoelina PA, Rasoarahona JR, Gaydou EM. Chemical compositions of aerial part of *Tagetes minuta* L. chemotype essential oils from Madagascar. *Journal of Essential Oil Research*. 2009; 21(5):390-392.
  31. Tankeu SY, Vermaak I, Viljoen AM, Sandasi M, Kamatou GP. Essential oil variation of *Tagetes minuta* in South Africa - A chemometric approach. *Biochemical Systematics and Ecology*. 2013; 51:320-327.
  32. Muyima ON, Nziweni S, Mabinya LV. Antimicrobial and antioxidative activities of *Tagetes minuta*, *Lippia javanica* and *Foeniculum vulgare* essential oils from the Eastern Cape Province of South Africa. *Journal of Essential Oil Bearing Plants*. 2013; 7(1):68-78.
  33. Smith-Plamer F, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils against five important food-borne pathogens. *Letters in Applied Microbiology*. 1998; 26:118-22.
  34. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C *et al.* Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy*. 2005; 49(6):2474-2478.
  35. Shirazi MT, Gholami H, Kavooosi G, Rowshan V, Tafsiry A. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. *Food Science & Nutrition*. 2014; 2(2):146-155.
  36. Van Vuuren SF, Viljoen AM. Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. *Flavour and Fragrance Journal*. 2007; 22(6):540-544.
  37. Wanzala W, Ogoma SB. Chemical composition and mosquito repellency of essential oil of *Tagetes minuta* from the Southern slopes of Mount Elgon in Western Kenya. *Journal of Essential Oil Bearing Plants*. 2013; 16(2):216-232.
  38. Cosentino S, Tuberoso CG, Pisano B, Satta M, Mascia V, Arzedi E, *et al.* *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus herba-barona* essential oils. *Letters in Applied Microbiology*. 1999; 29:130-135.
  39. Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*. 2001; 91(3):453-462.
  40. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. 2013; 6:1451-1474.
  41. Helander IM, Alakomi H, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, *et al.* Characterization of the action of selected essential oil components on gram-negative bacteria. *Journal of Agricultural and Food Chemistry*. 1998; 46:3590-3595.
  42. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*. 2008; 8:639-652.
  43. Grange M, Ahmed S. *Handbook of Plants with Pest-Control Properties*. John Wiley & Sons (Wiley-Interscience), Inc., New York, 1988, 470.
  44. Barkai-Golan R. *Postharvest Diseases of Fruits and Vegetables: Development and Control*. Elsevier B.V., Amsterdam, 2001, 125.
  45. Matasyoh JC, Kiplimo JJ, Karubiu NM, Hailstorks TP. Chemical composition and antimicrobial activity of essential oil of *Tarhonanthus camphorates*. *Food Chemistry*. 2007; 101:1183-1187.
  46. Marei GK, Rasoul MA, Abdelgaleil SA. Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. *Pesticide Biochemistry and Physiology*. 2012; 103(1):56-61.
  47. Saha S, Walia S, Kundu A, Kumar B, Joshi D. Antifungal acetylinicthiophenes from *Tagetes minuta*: potential biopesticide. *Journal of Applied Botany and Food Quality*. 2012; 85:207-21.
  48. Hood JR, Wilkinson JM, Cavanagh HM. Evaluation of common antibacterial screening methods utilized in essential oil research. *Journal of Essential Oil Research*. 2003; 15(6):428-433.
  49. Wanzala W. Ethnobotanicals for Management of the Brown Ear Tick, *Rhipicephalus appendiculatus* in Western Kenya.



- Ponsen & Looijen, Wageningen, 2009, 231.
50. Kupferschmied P, Maurhofer M, Keel C. Promise for plant pest control: root-associated *Pseudomonads* with insecticidal activities. *Frontiers in Plant Science*. 2013; 4(287):1-17.
  51. Prakash A, Rao J. Botanical pesticides in agriculture. CRC Lewis Publ. Boca Raton, 1997, 481.
  52. Zygadlo JA, Grosso NR. Comparative study of the antifungal activity of essential oils from aromatic plants growing wild in the central region of Argentina. *Flavour and Fragrance Journal*. 1995; 10:113-118.
  53. Isman MB. Botanical insecticide deterrents and repellents in modern agriculture and increasingly regulated world. *Annual Review of Entomology*. 2006; 51:45-66.
  54. Cestari MI, Sarti SJ, Waib CM, Branco AC. Evaluation of the potential insecticide activity of *Tagetes minuta* (Asteraceae) essential oil against the head lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae). *Neotropical Entomology*. 2004; 33(6):805-807.
  55. Motazedian N, Aleosfoor M, Davoodi A, Bandani AR. Insecticidal activity of five medicinal plant essential oils against the cabbage aphid, *Brevicoryne brassicae*. *Journal of Crop Protection*. 2014; 3(2):137-146.
  56. Hadjiakhoondi A, Vatandoost H, Abousaber M, Khanavi M, Abdi L. Chemical composition of the essential oil of *Tagetes minuta* L. and its effects on *Anopheles stephensi* larvae in Iran. *Journal of Medicinal Plants Research*. 2008; 26(7):33-39.
  57. Chantraine JM, Laurent D, Ballivian C, Saavedra G, Ibanez R, Vilaseca LA. Insecticidal activity of essential oils on *Aedes aegypti* larvae. *Phytotherapy Research*. 1998; 12:350-354.
  58. Reddy SE, Kirti DS, Koundal R, Singh B. Chemical composition and insecticidal activities of essential oils against diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). *Natural Product Research*. 2015; 1-5.
  59. Alok K, Veena P, Bhasneya S, Tripathia AK, Sushil K. Potential toxicity of new genotypes of *Tagetes* (Asteraceae) species against stored grain insect pests. *International Journal of Tropical Insect Science*. 2005; 25(2):122-128.
  60. Estrada-Peña A, Jongejan F. Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission. *Experimental & Applied Acarology*. 1999; 23(9): 685-715.
  61. Rajput ZI, Hu S, Chen W, Arijo AG, Xiao C. Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University Science B*. 2006; 7(11): 912-921.
  62. Garcia MV, Matias J, Barros JC, Lima DP, Lopes RS, Andreotti R. Chemical identification of *Tagetes minuta* Linnaeus (Asteraceae) essential oil and its acaricidal effect on ticks. *Revista Brasileira de Parasitologia Veterinária*. 2012; 21(4): 405-411.
  63. Andreotti R, Garcia MV, Cunha RC, Barros JC. Protective action of *Tagetes minuta* (Asteraceae) essential oil in the control of *Rhipicephalus microplus* (Canestrini, 1887) (Acari: Ixodidae) in a cattle pen trial. *Veterinary Parasitology*. 2013; 197(1): 341-345.
  64. Chamorro ER, Sequeira AF, Velasco GA, Zalazar MF, Ballerini GA. Evaluation of *Tagetes minuta* L. essential oils to control *Varroa destructor* (Acari: Varroidae). *Journal of Argentine Chemical Society*. 2011; 98: 39-47.
  65. Eguaras MJ, Fuselli S, Gende L, Fritz R, Ruffinengo SR, Clemente G, Gonzalez A, Bailac PN, Ponzi MI. An *in vitro* evaluation of *Tagetes minuta* essential oil for the control of the honeybee pathogens, *Paenibacillus larvae* and *Ascosphaera apis*, and the parasitic mite, *Varroa destructor*. *Journal of Essential Oil Research*. 2005; 17: 336-340.
  66. Ruffinengo S, Maggi M, Faverin C, García SB, Bailac P, Principal J, Eguaras M. Essential oils toxicity related to *Varroa destructor* and *Apis mellifera* under laboratory conditions. *Zootecnia Tropical*. 2007; 25(1): 63-69.
  67. Choochote W, Chaithong U, Kamsuk K, Jitpakdi A, Tippawangkosol P, Tuetun B, Champakaew D, Pitasawat B. Repellent activity of selected essential oils against *Aedes aegypti*. *Fitoterapia*. 2007; 78: 359-364.
  68. Nerio L S, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: a review. *Bioresource Technology*. 2010; 101(1): 372-378.
  69. Makang'a OB. Composition and repellency of essential oils of *Tagetes minuta* from different zones in Kenya against brown ear tick (*Rhipicephalus appendiculatus*). MSc. Thesis, Kenyatta University, Kenya, 2012.
  70. Nchu F, Magano SR, Eloff RN. *In vitro* anti-tick properties of the essential oil of *Tagetes minuta* L. (Asteraceae) on *Hyalomma rufipes* (Acari: Ixodidae). *Onderstepoort Journal of Veterinary Research*. 2012; 79(1):1-5.
  71. Amer A, Mehlhorn H. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitology Research*. 2006; 99(4):478-490.
  72. Tyagi BK, Ramnath T, Shahi AK. Evaluation of repellency of *Tagetes minuta* (Family: Compositae) against the vector mosquitoes *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* (L.). *International Pest Control*. 1997; 39:184-185.
  73. López SB, López ML, Aragón LM, Tereschuk ML, Slanis AC, Feresin GE, et al. Composition and anti-insect activity of essential oils from *Tagetes* L. species (Asteraceae, Helenieae) on *Ceratitis capitata* Wiedemann and *Triatoma infestans* Klug. *Journal of Agricultural and Food Chemistry*. 2011; 59(10):5286-5292.
  74. Adekunle OK, Acharya R, Singh B. Toxicity of pure compounds isolated from *Tagetes minuta* oil to *Meloidogyne incognita*. *Australasian Plant Disease Notes*. 2007; 2:101-104.
  75. Singh AU, Prasad D. Management of plant-parasitic nematodes by the use of botanicals. *Journal of Plant Physiology & Pathology*. 2014; 2(1):1-10.
  76. Macedo TF, Oliveira LM, Camurca-Vasconcelos AL, Ribeiro WL, Santos JM, Morais SM, et al. *In vitro* effects of *Coriandrum sativum*, *Tagetes minuta*, *Alpinia zerumbet* and *Lantana camara* essential oils on *Haemonchus contortus*. *Revista Brasileira de Parasitologia Veterinária*. 2013; 22(4):463-469.
  77. Fischer NH. The function of mono and sesquiterpenes as plant germination and growth regulators. In: Putnam A R, Tang C S (Eds.), *The science of allelopathy*. John Wiley & Sons (Wiley-Interscience), Inc., New York, 1986, 317.
  78. Seigler DS. Chemistry and mechanisms of allelopathic interactions. *Agronomy Journal*. 1996; 88:876-885.
  79. Sangeetha C, Baskar P. Allelopathy in weed management: a critical review. *African Journal of Agricultural Research*. 2015; 10(9):1004-1015.
  80. Scrivanti LR, Zunino MP, Zygadlo JA. *Tagetes minuta* and *Schinus molle* essential oils as allelopathic agents. *Biochemical Systematics and Ecology*. 2003; 31(6):563-

- 572.
81. Arora K, Batish DR, Singh PH, Kohli RK. Allelopathic potential of the essential oil of wild marigold (*Tagetes minuta* L.) against some invasive weeds. *Journal of Agriculture and Environmental Sciences*. 2015; 3:56-60.
  82. Amri I, Hamrouni L, Hanana M, Jamoussi B. Reviews on phytotoxic effects of essential oils and their individual components: news approach for weeds management. *International Journal of Applied Biology and Pharmaceutical Technology*. 2013; 4:96-114.
  83. Lee S, Shim K, Kil J. Phytotoxic effect of aqueous extracts and essential oils from southern marigold (*Tagetes minuta*). *New Zealand Journal of Crop and Horticultural Science*. 2015; 30:161-169.
  84. Kyarimpa C, Omolo IN, Kabasa JD, Nagawa CB, Wasswa J, Kikawa CR. Evaluation of anti-oxidant properties in essential oil and solvent extracts from *Tagetes minuta*. *African Journal of Pure and Applied Chemistry*. 2015; 9(5):98-104.
  85. Moon JK, Shibamoto T. Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry*. 2009; 57:1655-1666.
  86. Helle W, Anne SB, Malterud KE. Antioxidant activity in extracts from coriander. *Food Chemistry*. 2004; 88:293-297.
  87. Manjamalai A, Berlin VM. Antioxidant Activity of Essential Oils from *Wedelia chinensis* (Osbeck) *in vitro* and *in vivo* lung cancer bearing C57BL/6 mice. *Asian Pacific Journal of Cancer Prevention*. 2013; 13:3065-3071.
  88. Mahmoud GL. Biological effects, antioxidant and anticancer activities of marigold and basil essential oils. *Journal of Medicinal Plants Research*. 2013; 7(10):561-572.
  89. Ruiz C, Cachay M, Domínguez M, Velásquez C, Espinoza G, Ventosilla P, *et al.* Chemical composition, antioxidant and mosquito larvicidal activities of essential oils from *Tagetes filifolia*, *Tagetes minuta* and *Tagetes elliptica* from Perú. *Planta Medica*. 2011; 77(12):30.
  90. Stewart BW, Wild CP. World Cancer Report 2014. International Agency for Research on Cancer. World Health Organization, 2014.
  91. Gordaliza M. Natural products as leads to anticancer drugs. *Clinical and Translational Oncology*. 2007; 9(12):767-776.
  92. Heinrich M, Bremner P. Ethnobotany and ethnopharmacy - their role for anti-cancer drug development. *Current Drug Targets*. 2006; 7(3):239-245.
  93. Bayala B, Bassole IH, Scifo R, Gnoula C, Morel L, Lobaccaro JA, *et al.* Anticancer activity of essential oils and their chemical components-a review. *American Journal of Cancer Research*. 2014; 4(6):591-607.
  94. Ali NA, Sharopov FS, Al-Kaf AG, Hill GM, Arnold N, Al-Sokari SS, *et al.* Composition of essential oil from *Tagetes minuta* and its cytotoxic, antioxidant and antimicrobial activities. *Natural Product Communications*. 2014; 9(2):265-268.
  95. Murata S, Shiragami R, Kosugi C, Tezuka T, Yamazaki M, Hirano A, *et al.* Antitumor effect of 1, 8-cineole against colon cancer. *Oncology reports*. 2013; 30(6):2647-2652.
  96. Martijena ID, Garcia DA, Marin RH, Perillo MA, Zygadlo JP. Anxiogenic-like and antidepressant like effects of the essential oil from *Tagetes minuta*, *Fitoterapia*. 1998; 69:155-160.
  97. Chandhoke N, Ghatak BJ. Studies on *Tagetes minuta*: some pharmacological actions of the essential oil. *Indian Journal of Medical Research*. 1969; 57:864-876.
  98. Karimian P, Kavooosi G, Amirghofran Z. Anti-oxidative and anti-inflammatory effects of *Tagetes minuta* essential oil in activated macrophages. *Asian Pacific Journal of Tropical Biomedicine*. 2014; 4(3):219-227.
  99. Koul O, Walia S, Dhaliwal GS. Essential oils as green pesticides: potential and constraints. *Biopesticides International*. 2008; 4(1):63-84.
  100. Lis-Balchin M. Aromatherapy science: a guide for healthcare professionals. Pharmaceutical press, London, 2006, 75.
  101. Panda H. Cultivation and Utilization of Aromatic Plants. Asia Pacific Business Press Inc. New Delhi, 2005, 149.
  102. Scientific Committee on Consumer Safety (SCCS). Opinion on the fragrance ingredients *Tagetes minuta* and *T. patula* extracts and essential oils (phototoxicity only), 2015 [[http://ec.europa.eu/health/scientific\\_committees/consumer\\_safety/docs/sccs\\_o\\_172.pdf](http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_172.pdf)]. Visited on 16 June, 2016.