TAGETES: A MULTIPURPOSE PLANT

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Abstract

Tagetes species, popularly known as marigold, are grown as ornamental plants and thrive in varied agroclimates. Bioactive extracts of different Tagetes parts exhibit nematocidal, fungicidal and insecticidal activity. Nematocidal activity of roots is attributed to thiennyls while the biocidal components of the essential oil from flowers and leaves are terpenoids. Also carotenoid pigments from Tagetes are useful in food coloring. In this paper, various uses of this multipurpose plant are reviewed.

Key words: Marigold, nematocidal, pesticidal, bactericidal, fungicidal, medicinal, pigments.

INTRODUCTION

A variety of Tagetes species are grown all over the world for ornamental purposes. In eastern countries cut flowers are used loose or in garlands for social and religious purposes. Hence, besides its use as an ornamental plant it is grown as a crop. Due to the short period needed for its cultivation it is conveniently grown as part of a multipurpose system, rotated with other agricultural or horticultural crops. It is also grown as a mixed crop on the borders with other plants (e.g. tomatoes) with beneficial effects to the latter. The plant also has some resistance to saline and other adverse conditions (Foy & Wheeler, 1979; Girwani et al., 1990; Goh & Haynes, 1978; Huang & Cox, 1988). Agricultural practices, especially chemical and biochemical treatments for improving the flower yield, have been reported (Al Badawy et al., 1993; Das et al., 1975; Gowda & Jayanthi, 1994; Hore & Sen, 1986; Kumar et al., 1991; Peat & Summerfield, 1977; Sharova & Savva, 1974; Smalley et al., 1993; Tsurushima & Date, 1971). Tissue culture studies are also being done (Cieniecka-Posupkiewicz & Szybinska, 1992).

The species Tagetes minuta erecta, patula and tenuifolia are most common. Other species referred to are often specific to a region. A number of chemotypes and variants are available. Over the last thirty years the number of reports on this plant have increased. While initial studies pertain to chemical and biochemical constituents of the plant and plant chemistry and biology in general, more recently interest has been in the bioactivity of various plant extracts and their isolates. Other applications, such as a natural colorant for food from the flower piments, have also emerged. Hence effects of extraction procedures on the quality and quantity of the extracts from the plant parts (root, flower and leaves) have become important.

Considering the current interest in naturally occurring, biodegradable botanicals for pesticide, pharmaceutical and other applications, in this paper a comprehensive review of the literature on this plant is presented in two sections. In the first section, bioactive components from different plant parts are considered for their nematocidal and insecticidal (e.g. against mosquito) effects and medicinal values. In the second section, Tagetes pigments and their values as food and feed colorants are reviewed.

BIOACTIVE COMPONENTS OF TAGETES

Botanicals from Tagetes sp.

The major category of phytochemicals of interest, terpenoids, flavonoids, alkaloids, polycyclic lignans and fatty acids, have been the subjects of studies by different groups, and a review on these, with 45 references, was presented by Rodriguez and Mayr (1975). Of these, the bioactivities of extracts containing thienyls and terpenes have been studied extensively and these are discussed in terms of nematocidal, fungicidal and insecticidal activities.

Nematocidal activity

Thienyls from Tagetes

Zechmeister and Sease (1974), reported that petals of the lemon variety of the common African marigold (Tagetes erecta) contained α-thienyl, the
quantity varying from 15–21 mg kg\(^{-1}\) of fresh petals extracted by methanol. They noted that it did not occur in other varieties of \(T. erecta\) grown simultaneously in the field and that it showed no provitamin A activity in the rat and was void of antioxidant property. In an extensive study on the constituents of \(T. erecta\) species in relation to thienyl, Bohman and Herbst (1963) reported the recovery of several bithienyls, besides terthienyl, from fresh ground roots of \(T. erecta\) and \(T. patula\) extracted by ether.

Uhlenbroek and Bijloo (19580 isolated polythienyls, including \(x\)-thienyl, from roots of \(T. erecta\) with alcohol and petroleum ether. These fluoresced blue under UV (340–350 nm). The nematocidal activity in a 6 days test \textit{in vitro} showed that several \(x\)-polythienyls in ppm doses were active against \textit{Heterodera rostochiensis}, \textit{Ditylenchus dipsaci} and \textit{Anguina tritici}. \(x\)-Terthienyl was most active, as 0.1, 0.2 and 0.5 ppm, respectively, were the minimum lethal doses for the above nematodes. The same authors also reported on the activity of 5-buty1-2,2' bithienyl against \textit{Panagrellus redivivus} at 3-125 m.l.d. (1959).

The U.V., visible, NMR and mass spectra of these nematocidal principles of \(T. erecta\) roots were reported by Horn and Lamberton (1963).

A number of other researchers subsequently studied the nematocidal action of \(T. erecta\) root extract as such, without isolating the polythienyl, or the action of the isolated active component. Swapr and Sharma (1967) showed \(T. erecta\) root extracts were lethal or inhibitory to the hatching of \textit{M. Javanica} and \textit{M. arenaria}. Spanish workers discussed the potential of natural nematocides from plants of the genus \(T. erecta\), especially \textit{T. hallisciens} roots, against \textit{Meloidogyne incognita} in light (Liliana et al., 1982).

In fact, since many plants belonging to the family compositeae were found to have the ability to suppress nematodal population, \(T. erecta\) has often been studied as a member of this family. Gommers (1973) studied the nematocidal principles in several compositeae members and reported that they suppressed soil populations of \textit{Pratylenchus penetrans} but did not always suppress the population of other plant-parasitising nematodes. Gommers and Voor in Tholt (1976) found that out of 175 species of compositeae tested, nearly 70 effectively suppressed populations of the nematode \(P. penetrans\). \(x\)-Terthienyl and 5-(3 butenc-1- enyl) 2-2' bithienc was detected not only in \(T. erecta\), \(T. patula\) and \(T. tenuifolia\), but also in other extracts of plants of this family.

\textbf{Effect of UV — irradiation on bioactivity}

The interesting effect of UV in enhancing the activity of thienyl has been reported by several workers. Gommers (1973) noted that day light (near UV irradiation) enhanced the nematocidal activity of the natural thienyls as well as two synthetic thiophenes \textit{in vivo} by a dose-dependent relation. These compounds apparently permeate from the compositeae root tissues into the nematodes in the root and soil. Cercarialic activity of \(x\)-terthienyl from roots of \(T. patula\) was examined with and without UV activation (Graham \textit{et al.}, 1980). In darkness, the lethal concentration for 100% mortality, (LC\textit{100}) for some digenetic trematode cercariae was 0.3 ppm. Under UV irradiation of 300–400 nm and incident intensity of \(5 \text{w m}^{-2}\), the LC\textit{100} value was reduced by a factor of 30 (to 0.01 ppm). A cercaricidal effect of aqueous infusion \((1:1000)\) of \(T. erecta\) root protected \textit{Phyla occidentalis} from bacterially complicated death but did not reduce cercariae to subsequent UV injury. The compound \(x\)-terthienyl being biodegradable leaves no long-lived residues and may be used for the control of schistosomal and other digenetic trematode parasites (Towers \textit{et al.}, 1984). The nematocidal activity against \textit{Meloidogyne incognita} was noted in a study on wood preservative composition. Japanese workers reported that \(x\)-terthienyl from \(T. microglossa\) and \(T. jaliisciensci\) had LC\textit{100} at 10 ppm in light (Liliana \textit{et al.}, 1982). In the dark it was less effective. Thiophene compounds extracted from \(T. microglossa\) and other synthetic thiophenes were also evaluated. In a study of photosensitive insecticidal, volatile, compounds from \(T. minuta\), marigold flower oil was irradiated for periods up to 24 h by UV radiation and the degradation profiles of thiophenes were determined (Wells \textit{et al.}, 1993). The most significant changes occurred after approximately 6 h of exposure. The \(\lambda\) and IV were the least stable thiophenes. This was attributed to the highly conjugated side chains which interact more strongly with UV-A radiation. The thiophenes included 2-2', 5-2'-terthiophene (I, 5-methyl-2,2' = 5', 2'-terthiophene (II), 5-(but. 3-ene-1-ynyl)-2-2'-biotiohphene (III) and 5-(but-3-ene-1-ynyl)-5'-methyl-2', 2'-biotiohphene (IV).

\textbf{Nematode control using Tagetes}

Although the nematocidal activity of polythienyls, especially \(x\)-terthienyl, is established, since the concentrations of these are low in \(T. erecta\) root extracts, the problem of actual utilisation at effective concentrations has to be solved. Daylight, which includes UV radiation, should enhance the activity sufficiently to render the plant itself effective to its surrounding soils. For example, planting \(T. erecta\) between tomato plants is useful, although not as potent as applying a synthetic nematocide. In an experiment in the Philippines, \(T. erecta\) root extract suppressed egg hatching, infectivity to tomato and development of \textit{M. incognita}, especially when the extract was not diluted with water (Ducusin \& Davide, 1972). It was also noted that although \(T. erecta\) planted as an inter-crop, showed some nematodal control, the tomato yield did not improve (Davide, 1979). Odobo-Owino (1993) studied the effects of \(T. minuta\) and other plants on the pathogenicity of root-infesting nematodes in Kenya. Tomato plants grown with the three nematicidal plants had significantly greater shoot growth and
fruit yield than control plants. Nematode control using marigolds and their nematocidal components, and their use in general control, have been reviewed with five references (Obayashi, 1977). Gommers and Bakker (1988) have reviewed the interaction of Tagetes species and parasitic nematodes and the actions of α-terthienyl and related thiophenes.

The commercial aspects of using such natural pesticides has to be evaluated in the light of increasing preference for organically-grown crops and environmental concerns.

For enhancing the yield of α-terthienyl from plants for use as a nematocide Shikoku Chem Corporation, Japan induced calluses in T. patula in a medium containing auxin and cytokinins and cultured the calluses in the presence of 0.01-1.0 per mg auxin. The terthienyl extracted from the cultural medium was shown to be active against Pratylenchus penetrans and Cornus multiflorus elegans (Fujimoto et al., 1989a). The same group infected the Tagetes with Agrobacterium rhizogenes, cultured rhizome roots of Tagetes and extracted the nematocides from these cultured roots using an organic solvent. P. patula was infected with A. rhizogenes for 2 wks and the resulting hairy roots were cultured in cactarans containing Murashige-Skoog medium at 25°C for 8 wks. Tips of these roots were further cultured in a similar medium at 21°C for 2 wks. Part of the roots was subcultured in a plant hormone. The resulting roots, when dried and extracted with hexane, produced an extract containing 0.94 μg α-terthienyl ml⁻¹, and this showed complete control of P. penetrans and C. elegans after 8 h of treatment (Fujimoto et al., 1989b).

Kyo et al. (1990) have also discussed production of nematotical compounds from hairy root cultures of T. patula by A. rhizogene induction. Hairy roots produced up to 15-1288 mg α-terthienyl per gram dry weight when grown in the dark, i.e. yields which corresponded to 0.15-12.7-fold those from intact roots. HPLC analysis showed that in such cultures besides α-terthienyl other nematocidal components were present.

Pyrethrins in Tagetes
Floral heads of T. minuta from two sites, and T. patula, contained 6.5, 2.1 and 2.4 mg of pyrethrin per gram (Kamal & Mangla, 1987). Studies extended to other plants indicated the effectiveness of the plants against insects in the order T. minuta > Pulicaria crispa > T. patula > P. minuta > P. augustifolia (Kamal et al., 1988).

Pyrethrins are very potent and safe insecticides and researchers have tried to synthesise them. But observations showed that synthetic pyrethrins (pyrethroids) were not as efficient as natural pyrethrins and insects developed resistance to them after some time.

It must be noted that in one instance a thiophene compound in a Tagetes flower extract was mistakenly identified as pyrethrin (Wells et al., 1993). Hence more work is needed to unequivocally establish the presence of pyrethrins in this extract.

Terpenoids from Tagetes
Both the flowers and leaves of Tagetes contain terpenoids, the components and composition of the extracts varying with the species. In phytochemical studies on Tagetes species, the intraspecific differences in the essential oils in T. minuta and T. tenuifolia were reported in plants from Hungary and elsewhere in 1984-85 (Hethey et al., 1987). The essential oil composition was noted to be species specific, but for ornamental variants of a given species variations only in concentrations of components not types of components were seen. β-Ocimene was found only in the essential oils of T. minuta (T. glandulifera) (30-40%) and T. tenuifolia (T. signata) (5-10%). The essential oil of T. patula was characterised by (Z) and (E)-ocimenes (13-4, 13-4% resp.), besides limonen, carophyllene, pipertone and pipernetone. A special component is contained in the oils of T. erecta amounting to 4-4-15-8% (M*152) besides pipertone and carophyllene. The characteristic components of the essential oil of T. lucida were linalool, estraqol (45%) and methyl eugenol. An analysis of volatile oil of T. argentina caberra by gas chromatography showed that it contained (Z)-acacimen (4-3-4-5-3%) and (E)-ocimene (37-29-40-38%). Smaller amounts of (Z)-β-octene (1-4-1-49%), (E)-β-octene (2-7-26%), dihydrotagetone (3.8-6-57%), (Z)-tagetone (1-29-1-77%) and E-tagetone (1-30-1-87%) were also found in the oil (Tyndall et al., 1993). Twenty seven constituents in T. erecta leaf oil were characterised by GC/MS. The major components were terpinolene (12-4%), E-β-octene (13-1%) piperitone (20%) and limonene (11-0%). The presence of indole was seen as a minor constituent not previously reported for this genus (Machado et al., 1994).

Activity against larvae and adult mosquitoes
A number of papers are now available on the repellent and biocidal activities of essential oils of Tagetes sp. against different mosquito species. The terpenoids are the main bioactive component, although the effect of terthienyl and pyrethrin has also been noted. (E)-ocimene was isolated as the mosquito larvicide of T. minuta, from fresh flowers and leaves both by steam distillation and solvent extraction (Naradu & Luteja, 1978). Bioassays showed that 40 ppm of (E)-ocimene was effective against Aedes aegypti larvae in 24 h. Jeffrey studied the mosquito larvicidal activity of the oil of marigold (Singer, 1987). Volatiles with insecticidal properties obtained from T. erecta, T. patula and T. minuta by soxhlet and simultaneous distillation-extraction (SSDE) procedures showed the fraction with the highest bioactivity to be isolated by SSDE using methylene chloride (Wells et al., 1992). Aedes aegypti
and *Anopheles stephensi* adults and larvae were the target organisms. *T. minuta* was the most effective of the three species studied. An extraction time of 10 h resulted in the highest activity and over 90% recovery of the oil. The volatiles were highly effective toward both larvae and adult mosquitoes and most of the activity was located in the flower.

A phytotoxicological evaluation of *T. erecta* was conducted with a petroleum ether (5%) in ethyl acetate fraction (Sharma & Saxena, 1994). The extract was toxic against second- and fourth-instar larvae of *A. stephensi* with LC₅₀ of 43 and 58 ppm for the two larvae, but did not affect the larval developmental period. It had a significant effect on the mortality and reduced the adult emergence of the vector. Tests of light-dependent toxicity of the extract of *T. erecta* and α-terthienyl towards mosquito larvae of *Culex tritaeniorhynchus* showed that exposure of the larvae to the extract in sunlight generated a high level of activity (Singh et al., 1987). There was no toxicity in the absence of sunlight. The effect was not due to photochemical conversion of any of the constituents of the plants into toxic products as an identical solution exposed to sunlight did not kill larvae that were later exposed to it.

**Bactericidal and fungicidal activity**

The thienyl, terpenoids and other classes of compounds which show nematocidal and larvicidal activity would also be expected to be active against various microorganisms. A few papers have referred to this.

Various *Tagetes* oils analyzed by GC/MS were shown to contain limonene, α-terpinolene, dihydrotagetene and ocimene, and seen to inhibit gram-positive bacteria and fungi (Hethelyi et al., 1988). Leaves of *T. minuta* and *T. filifolia* exhibited strong fungitoxicity by completely inhibiting mycelial growth of *Sclerotium cepivorum*, *Colletotrichum coccodes* and *Alternaria solani*. The active components were characterised by GC/MS. Two chemotypes (ocimene-rich and ocimone-rich) of *T. minuta* and an (E)-enethole-rich chemotype of *T. filifolia* exhibited mycelial inhibition at 5000, 3000 and 2000 ppm, respectively (Zygadlo et al., 1994).

**Medicinal value**

Anti-tumour screening of a random collection of *T. minuta* revealed that the whole flowering plants of *T. minuta* were significantly active against Lewis lung carcinoma in vivo (Ickes et al., 1973).

The hepatoprotective properties of preparations from spreading marigold have been noted. Extracts from the flowers of *T. patula* made with peach oil, alcohol and chloroform were investigated in rats having CCL₄-induced liver damage (Vasilenko et al., 1990). The protective effects were accompanied with a choleric action when using the oil extract.

The therapeutic use of some *Tagetes* species, their botanical, chemical pharmacodynamic and agronomical characteristics have been reviewed (10 references) (Szabo et al., 1975). The medical application, origin, geographical distribution, main morphological characteristics and structure of the more important ingredients and cultivation of *T. patula*, *T. erecta* and *T. minuta* are discussed in this review.

**TAGETES PIGMENTS**

Among a variety of plants used in different indigenous cultures for making tea, *Tagetes* finds a place. Daferriere et al. (1991) analysed the plants for mineral and nutritional content and tabulated the Fe, Cu, Zn, Ca and Mg concentrations for native teas made from shoots of *T. lucida* and *T. filifolia*. The dry *T. minuta* seed contains oil with 73% fatty acid. This can be extracted with hexane and meal prepared from the seed residues contains 25-14% crude protein but is low in available lysine (Wiese et al., 1992). However the major components of commercial interest are the pigments which can be used as natural colorants in food and feed. The two major classes of pigments present in *Tagetae* are the flavonoids and carotenoids.

**Flavonoids**

Flavonoids of the flowers and of the leaves of *T. erecta* were extracted with methanol (Morita, 1957). About 0.1% of the quercetin monoglucoside, tage tin, was isolated. Extraction of fresh *T. erecta* flowers yielded 0.4% quercetin (Sankara & Marayana, 1963).

Flavones of *T. patula* varieties growing in Romania were analysed by TLC and paper chromatography to establish the optimal conditions for identification and separation of flavones (Tarpo, 1969). Patuletin, quercetagetin and their glucosides patuletin and quercetagetrin were isolated. The flavonoids and fatty acids of *Tagetes* and their taxonomic significance have been reviewed (Rodriguez & Mabry, 1975). Patuletin and patuletin were isolated from the seeds of *T. patula* and quercetagetrin and quercetagetrin from seeds of *T. erecta* during the fruit-bearing stage (Kaloshina & Mazulin, 1983).

Myricetin 3 and 7-glucosides were identified in four species of *Tagetes*, and myricetin glucoside reported for the first time in *Asteraceae*. Flavonoids from *T. rupestris* were quercetin, quercetagetin, luteolin, and kaempferol glycosides (De Israleiv et al., 1994). The physical characteristics of yellow pigments from flowers or petals of eleven *T. erecta* and three *T. patula* varieties have been described (Kasumov, 1992). The petals contained 9–22% flavonoids.

The flavonoid composition of some plants of the Northern Caucasus including *Tagetes*, were analysed by Bandyukova and Shinkarenko (1964). A photometric method, based on the reaction of flavonoid azo compounds, was developed for the
determination of flavonoids. The authors suggest that the flavonoids can be used as reagents for the determination of sulfonamides and novocaine. Quercetin and genistein isolated from Japanese Saphora were active against Staphylococcus aureus and Escherichia coli, while kaempferol 3,7 dihydroxysides and kaempferol 3-rhamnoglycoside from rhododendrons had diuretic properties. In the light of this, further work could be done on the Tagetes flavonoids for their medicinal value.

Carotenoids
A Tagetes extract contained ~27% carotenoids with β carotene 0.4%, cryptoxanthin esters 1.5% and xanthophyll esters 86.1% (Benk et al., 1976). Thus the latter are the major carotenoid constituents. T. erecta petals had more carotenoid than did the seeds or sepals and 200 times more than yellow corn. Oven drying of the petals caused greater loss of carotenoids than shade drying. The process of purification of lutein fatty acid ester from marigold petals based on isopropanol precipitation has been described (Philip & Berry, 1976). The precipitated fraction contained 51.3% lutein esters.

Tagetes pigments as natural colorants
The Tagetes carotenoids are being used to enhance the yellow colour of chicken skin and eggs. The use of Tagetes meal from dried, ground, flower petals of T. erecta mixed with not more than 0.3% ethoxiquin is permitted in chicken feed under the US Federal Food Drug and Cosmetic Act (Anon., 1963, Anon., 1966). A US patent describes a method of production of xanthophyll and the use of this preparation as a supplement in bird feed (George, 1966). The pigment prepared from dried and ground flower petals of Tagetes is mixed in the feed at levels of 0.125–0.250%. The pigment index of egg yolk is 14–16 when the feed is mixed with Tagetes additive as compared to 2 for a pigment-free diet. Another patent pertains to the stabilisation of xanthophyll esters for use in chicken feed by partial saponification (80–90%). Losses in storage at 40°C for 75 days were only 5.2% (Salkin, 1976). The effects on egg yolk colour of the addition of T. erecta to the production rations of laying hens has also been reported by others (Dumanovsky et al., 1980; Narahari et al., 1981).

The effect of marigold xanthophyll supplementation on improving the yellow tail (Seriola quinqueradiata) discoloration in fish has also been tried. The effect of the dietary chromophyl-xanthophyll from Tagetes sp. was investigated. Xanthophyll concentration in the diet was tested at concentrations of 0, 15, 30, 60 ppm in the daily ration size of 2% body weight for 28 days. The pigmentation was most enhanced in fish fed the 60 ppm diet (Takki et al., 1961).

Addition of Tagetes pigments to drinks and foods has also been in vogue. Detection of Tagetes extract in orange concentrates using HPLC has been described (Benk et al., 1976; Philip & Berry, 1976; Wild & Dobrovolsky, 1976). Lutein fatty acid esters from petals are miscible with vegetable oil and this produces a yellow to orange colour in foods and the hue range can be extended to red by the addition of synthetic carotenoids (Philip & Berry, 1976). About 6% of carotenoids was obtained from air dried T. patula flowers. It comprises galene, lutein, lycopene, α-carotene, β-carotene and γ-carotene. After distillation of essential oil the dried residues of T. patula flowers were extracted with dichloroethane (1 part of residue with 10 parts of solvent at 18–20°C). The extracts contained mainly carotenoids with some flavonoid glycosides, aglycons and waxes. The latter was removed by chromatography to give carotenoid (6%) (Kasumov, 1991). The carotenoid extracts are acceptable for use in foods, pharmaceuticals, and cosmetics (Kasumov, 1984).

CONCLUSION
This review has indicated that the major bioactive components in Tagetes are polyphenyls and terpenoids. The compositions of extracts, from roots, foliage and flowers vary, with thiensyls being predominant in root extracts and terpenoids in flower and leaf oils. The constituents from different species and chemovariants differ in quality and quantities of active components. Also, the extraction conditions or methods affect the yield and composition of constituents. Further, the review has brought out the usefulness of carotenoid pigments of Tagetes in food colouring, essential oils or volatiles for nematocidal or biocidal value and Tagetes pigments as colorants. The beneficial effects of the flavonoids and carotenoids in pharmaceutical and other applications need further study.

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REFERENCES


