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Some Biological Activities of *Tagetes lucida* Plant Cultivated in Egypt

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ABSTRACT

Mexican tarragon (*Tagetes lucida*, Family Asteraceae) seeds were introduced to be cultivated as one of the medicinal plants in Egypt. This work aimed to study the antioxidant, antimicrobial, insecticidal and Nematocidal activities of *T. lucid*a plant. Antioxidant activity of the different concentrations of *T. lucida* extract was near that of ascorbic acid and increasing the used concentration increased the antioxidant activity. IC_{50} of *T. lucida* extract was 109 % of ascorbic acid which means that their IC_{50} were very close to each other. The essential oil of *T. lucida* was active against all tested microbial strains. *Candida albicans* and *Staphylcocus aureaus* were very sensitive to the essential oil than the other strains. Their inhibition zone diameters were more or less similar to that obtained with Streptomycin (10 mcg). The ethanolic extract of *T. lucida* showed high reduction against the aphid (*Aphis brassicae*) during the first six days after application. After nine days, the population of aphids started to increase. The ethanolic extract of *T. lucida*, inhibited ($P \leq 0.05$) motility, visible flexing of all plant-parasitic nematode genera tested. Immobility of *Meloidogyne incognita*-J2 and filiform stages of *Criconemella* spp., *Helicotylenchus* spp, and pratylenchus spp. was higher ($P \leq 0.05$) after 24 and 72 h in mg and mg/2 dilutions of *T. lucida* roots than their corresponding herbal parts. *M. incognita*-egg-hatching followed the same trend. From the nematological point of view, this study revealed that *T. lucida* is a promising starting and new material for the production of bio-nematicides in Egypt.

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INTRODUCTION

Tagetes lucida is an aromatic herb used as a spice, for medicine, and as insecticide [13]. Bioactive extracts of different plant parts exhibit nematicidal, fungicidal and insecticidal activities [33]. Mexican marigold can play an important role in food preservation, food preparation and as an excellent food spice. It is an important, nutritious plant and an effective herbal medicine as antifungal [12].

Aquino *et al.* [2] revealed that *T. lucida* methanol extract and some of its constituents showed a significant free-radical-scavenging effect in comparison to alpha-tocopherol and standard flavonols by using the DPPHo test. Olivero-Verbel *et al.* [28] reported that the antioxidant activity of the essential oil from *T. lucida* showed the lowest mean effective concentrations (EC_{50}), with values of 31.1, 37.9 and 94.9 micro g/ml, respectively. The main component for its essential oil was methyl chavicol (estragole) with 95.7%. Regalado *et al.* [30] measured the antioxidant capacity of *T. lucida* essential oil by two different *in vitro* assays (DPPH and TBARS) and significant activities were evidenced.

Caceres *et al.* [9] mentioned that *T. lucida* extracts showed anticandidal activity against *Candida albicans*. Caceres *et al.* [8] indicated that *T. lucida* exhibited antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Caceres *et al.* [10] evaluated acetone, alcohol and n-hexane extracts of leaves of *T. filifolia* and *T. lucida* for activity against the bacteria *Escherichia coli*, *Salmonella enteritidis* and *Shigella flexneri*. They found that the alcohol extracts were the most active. Caceres *et al.* [11] indicated that only the 10% tincture prepared from *T. lucida* gave positive results against *V. cholerae* 01, with the best activity obtained by using n-hexane extract. Cespedes *et al.* [12] concluded that *T. lucida*

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extracts act on phytopahtogenic fungi and the dimethoxy compounds showed a strong activity against fungal strains, especially *T. mentagrophytes* and *R. solani* (100% of inhibition at 125.0 and 250.0 micro g/ml, respectively). Hernandez *et al.* [16] stated that the ethyl acetate extract of *T. lucida* showed antibacterial activity against *Shigella boydii*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Sarcina lutea*, and four strains of *Vibrio cholerae*. Damian-Badillo *et al.* [14] conducted that the methanol-chloroform and ethyl acetate extracts from *T. lucida* had pronounced antifungal activity. Regalado *et al.* [30] revealed that the leaf essential oil of *Tagetes lucida* showed a moderate activity against *P. berghei* and *E. coli*.

Marotti *et al.* [23] revealed that *T. minuta* and *T. lucida* appeared to be the most promising *Tagetes* species, with high potential for use as biocidal crops for the implementation of pest control practices that are less harmful to human health and natural resources. Nerio *et al.* [27] evaluated the essential oil isolated from *T. lucida* plant for repellent activity against *Sitophilus zeamais* (Coleoptera: Curculionidae) using the area preference method. Most oil components were oxygenated monoterpenoids or phenolic compounds. The oil was repellent at doses between 0.063 and 0.503 micro L / cm^2. Ciccio [13] stated that Mexican Marigold (*T. lucida*) is used as insecticidal.

Siddiqui and Alam [31] mentioned that the mixed cultures of *T. lucida* inhibited the root-knot development caused by *M. incognita* on tomato and eggplant and reduced the populations of *Rotylenchulus reniformis* and *Tylenchorhynchus brassicae* on tomato, eggplant, cabbage and cauliflower. The root-exudates of *T. lucida* were also found to have nematicidal properties. Siddiqui and Alam [32] stated that all parts (flower, leaf, seed and roots) of *T. lucida* showed nematicidal activity, with flower extracts giving the greatest nematode mortality followed by seed, leaf and root extracts. Nematode mortality increased with increase in the concentration of extracts and the exposure period. Juvenile hatching of *Meloidogyne incognita* was also greatly inhibited by the extracts. Caballero-Gallardo *et al.* [7] concluded that essential oils from plants cultivated in Colombia i.e *T.lucida* are sources of repellents against *T. castaneum*.

The cultivation of *T. lucida* in Egypt was carried out for the first time in sand soil using sprinkler and dripping irrigation system [18]. This work aimed to study the antioxidant, antimicrobial, insecticidal and nematocidal activities of *T. lucid* plant cultivated in Egypt for the first time.

MATERIALS AND METHODS

Plant materials:

T. lucida seeds were imported from Canada and cultivated in the nursery at Elmyzan Company greenhouse (SEKEM, Bilbase, Sharkya) in seed trays then transplanted in the permanent field after 45 days. The agricultural practeces noramly applied for *Tagetes* species in Egypt were applied for *T. lucida* [18].

Essential oil extraction:

The essential oil of fresh herb *T. lucida* herb was extracted with hydro-distillation for 3 hours with Clevenger-type apparatus according to the Egyptian Pharmacopoeia. The resulted essential oil was dehydrated with anhydrous sodium sulphate and kept in the deep freezer for biological assay.

1. Antioxidant Activity:

2,20-Diphenyl-1-picrylhydrazyl (DPPH) Radical-Scavenging Method:

Samples of the air dried herb of *T. lucida* plant were extracted by maceration of 1 gm herb in 10 ml methanol for 24 h. in the room temperature. The mixture was filtered and the filtrate was kept for determination the anti oxidant activity. The antioxidant activity of *T. lucida* was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical DPPH Brand-Williams *et al*., [6]. Ascorbic acid (in the same concentration) was used as a reference. The mixtures were well shaken in a vortex (2500 rpm) for 1 min and then placed in a dark room. The decrease in absorbance at 517 nm was determined with a JENWAY 6315 spectrophotometer after 1 h for all samples. Methanol was used to zero the spectrophotometer.

Absorbance of the radical without sample was used as control. The amount of sample necessary to decrease the absorbance of DPPH (IC_{50}) by 50% was calculated graphically. The inhibition percentage of the DPPH radical was calculated according to the formula:

$\%I = [(A_B - A_S)/A_B] \times 100$

Where I=DPPH inhibition %, AB=absorbance of control sample (t=0 h), and AS = absorbance of a tested sample at the end of the reaction (t=1 h). Each assay was carried out in triplicate.

2. Antimicrobial activity:

Test organisms:

The organisms used were *Staphylococcus aureus* NRRRL B-313, *Bacillus subtilus* NRRL B-542, *Esherichia coli* NRRL B-210 and *Candida albicans* NRRL Y-477. These microorganisms were obtained from Northern Utilization Research and Development Division, US Department of Agriculture; Peoria, Illinois, USA

were selected for microbial assay as these are common pathogens. The pure cultures were maintained by routine such culturing at one week interval in nutrient agar and potato-dextrose agar slants for bacteria and fungi, respectively.

Testing for bacteria strains:

The essential oil was subjected to antimicrobial assay by measuring the inhibition zone diameter (IZD) using agar diffusion technique according to Bershtein *et al.* [4] with some modifications. Nutrient agar (for bacterial strains) and potato dextrose agar (for yeast of fungi strains) plates were prepared by pouring 20 ml. each seeded with 0.5 ml of 10^{-4} dilution of 24 hours old bacteria and yeast cultures in Petri dishes (9cm.) and allowed to solidify. Six equidistant holes were made in the agar plate using sterile cork borers (0.9mm).

A 100 ul volume of essential oil samples solutions containing 100, 10, 1 and 0.1 ug/ml dilution 1:1 (v/v) was added to the holes using sterile pipit. The test plates were refrigerated at 8^{o}c for 1h to facilitate diffusion and then incubated at 28-30^{o}c for 24-48h. After incubation the inhibition zones were measured and the effect was calculated as a mean of triplicate tests.

3. Insecticidal assay:

A field experiment was carried out to test the 70 % ethanolic extract of *T. lucida* on cabbage (*Brassica oleracea var. Capitata*) plants grown in Elmanawat village, Giza Governorate in October 2012. Five infested cabbage plants with aphid (*Aphis brassicae*) were used for the application of each treatment. The studied three treatments were 284 mg extract / 200 ml water, and 142 mg extract / 200 ml of water and 0 mg extract / 200 ml water (as a control). Spraying was carried out using hand sprayer (1.5 L capacity). Aphid numbers were counted before application and 3, 6, 9, 12 and 15 days after application.

4. Nematocidal assay:

The obtained ethanol extract (284 mg extract / 200 ml water) was termed as Standard (= S) and then S/2 dilution was prepared with distilled water using 0.05% Tween 80 as spreading agent. Populations of *Criconemella* spp., *Helicotylenchus* spp. and *Pratylenchus* spp. were extracted [19] from soil of tomato (*Solanum lycopersicum* L. cv. Super Strain B) field. Tomato roots with many exposed egg-masses were used to obtain *Meloidogyne incognita* eggs [17] from average sized and freshly picked eggmasses. The soil stages, juveniles and adults of nematodes and *M. incognita* eggs were separately transferred to oil solutions of the plants in 5 cm Petri dishes. *M. incognita*-second stage juveniles (J_2) used herein were obtained by incubation of nematode egg masses in tap water at 27°C in the dark. They were collected every 2 days and concentrated in small volumes of sterilized water by filtering through 1 µm filters (Whatman type) and collecting them after repeated washes [25]. Each treatment had eight replicates (dishes), each contained about 1000 nematode eggs or 30 soil stages of a single genus. The experiment was repeated once. Nematodes in distilled water and 0.05 Tween solution served as checks. The Petri dishes were kept at room temperature (27± 3^{0}C). Numbers of unhatched and *M. incognita* juveniles were daily recorded for 16 days and immobile soil stages of the other nematode genera were counted after 24 and 72 h [1]. Each time, the nematodes were transferred in aerated distilled water and then the active nematodes were counted after a day. Numbers of immobile nematodes at different treatments were subjected to analysis of variance and their averages were compared using Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

1. Antioxidant activity:

Scavenging activity:

The results of antioxidant activity of *T. lucida* herb extract using DPPH method are shown in Table (1). It is clear that antioxidant activity of the different concentrations of *T. lucida* extract were near that of ascorbic acid and increasing the used concentration increased the antioxidant activity. IC_{50} of *T. lucida* extract was 109% of ascorbic acid which means that their IC_{50} were very close to each other.

The antioxidant properties seem to be due to the phenolic compounds in *T. lucida* herb extract. Specific polyphenols are able to reduce free-radicals formation, to scavenge free radicals, particularly, superoxide and hydroxyl radicals, to reduce lipid peroxyl radicals and to inhibit lipid peroxidation [24].

Table 1: Antioxidant activity of the different concentrations and IC_{50} of *T. lucida* herb extract using DPPH method.

	DPPH % Inhibition (g / L)				
	5	10	20	50	IC_{50}
T. Lucida extract	84.75±0.28	86.87±0.36	87.54±0.82	88.39±0.21	0.60
Ascorbic acid	91.34±0.56	91.51±1.25	92.33±0.18	92.73±0.66	0.55

These results are in accordance with Aquino *et al.* [2], Olivero-Verbel *et al.* [28] and Regalado *et al.* [30] *on T. lucida* and Mahmoud [22] on *T. minuta.*

2. Antimicrobial activity of the essential oil of Tagetes lucida:

The essential oil of *T. lucida* was active against all tested microbial strains (Table, 2). *Candida albicans* and *Staphylococcus aureaus* were very sensitive to the essential oil than the other strains. Their inhibition zone diameter were more or less similar to that obtained with Streptomycin (10 mcg) as shown in Table (2). Same results were obtained by Damian-Badillo *et al.* [14] who mentioned that *T. lucida* extract inhibited the growth of fungi, but the ethyl acetate and methanol-chloroform extract from *T. lucida* inhibited all the fungi assayed: *Candida albicans*, *Colletotrichum lindemuthianum*, *Mucor circinelloides*, *Saccharomyces cerevisiae*, and *Sporothrix schenckii*, Avila-Sosa *et al.* [3] who stated that *T. lucida* ethanol extract showed an 84% inhibition of radial growth against *Colletotrichum gloeosporioides* and Regalado *et al.* [30] who showed that a moderate activity of *T. lucida* essential oil against *Plasmodium berghei* and *Escherichia coli.*

Table 2: Inhibition zone (diameter (cm)) of the essential oil of *T. lucida* for some microorganism.

Test organism	DEMSO	Hexane	Streptomycin 10 mcg / disk
24 h.			
Esherichia coli	1.6	1.4	1.3
Staphylococcus aureus	1.8	1.7	1.3
Candida albicans	1.5	1.2	2.0
Bacillus subtilus	1.6	1.4	2.2
72 h.			
Esherichia coli	1.6	1.5	3.4
Staphylococcus aureus	2.6	2.4	2.6
Candida albicans	2.3	2.6	3.0
Bacillus subtilus	1.6	1.4	3.2

3. Insecticidal activity:

Data in Table (3) and Fig (1) show that numbers of alive aphids decreased markedly after 3 days of application with the higher concentration (4 individuals/leaf). This numbers significantly increased after 9 days and later, while twelve and fifteen days after application, numbers of aphids showed significant increase. Lower concentration showed lower effect on alive aphid population in the all dates of recording. Parasitism by the parasitoid *Diaeretiella rapae* (a parasitoid on aphis), was slightly affected by the application of *T. lucida* extracts.

From the above mentioned results, it can be concluded that the ethanol extract of *T. lucida* showed high reduction against the aphid (*Aphis brassicae*) during the first six days after application. After nine days, the population of aphids started to increase, which mean that the effect of the *T. lucida* extract lost its effect. Another application is needed after 9 days from the 1st application to obtain long period of protection on cabbage against the infestation of aphids. The *T. lucida* extract showed low effect on the aphid parasitoid *D. rapae*. These results are in agreement with those of Nerio *et al.* [27] on *T. lucida* against *Sitophilus zeamais*, Marotti *et al.* [23] who revealed that *T. minuta* and *T. lucida* appeared to be the most promising *Tagetes* species, with high potential for use as biocidal crops for the implementation of pest control practices and Caballero-Gallerdo *et al.* [7] who concluded that EOs from *T. lucida* and other Colombian flora are important source of natural and potent repellents against *Tribolium castaneum*, being a plausible alternative to the current commercial repellents used to control this organism.

Table 3: Effect of *T. lucida* extract on the average no. of aphid (*Aphis brassicaceae)* on cabbage aphids before and after treatment.

Days		3 days	6 days	9 days	12 days	15 days
conc.						
2 mg	alive	4	25	27	58	72
1 mg		18	8	4	14	52
control		130	158	211	291	385
2 mg	dead	75	83	82	33	17
1 mg		95	93	92	51	43
control		7	9	9	11	19
2 mg	parasitism	3	4	4	7	12
1 mg		3	3	2	3	8
control		8	11	11	11	14

4. Nematocidal activity:

The herb and root from *T. lucida*, inhibited ($P \leq 0.05$) motility, visible flexing of all plant-parasitic nematode genera tested (Table, 4 and 5). Immobility of *Meloidogyne incognita*-J_2, and filiform stages of *Criconemella* spp., *Helicotylenchus* spp., and *pratylenchus* spp. was higher ($P \leq 0.05$) after both 24 and 72 h in S and S/2 dilutions of *T. lucida* roots than their corresponding herbal parts (Table, 4). *M. incognita*-egg-hatching followed the same trend (Table, 4). Such a trend may be explained by the fact that roots, compared to other plant parts, have been reported [23] to have the highest diversity and contents of thiophenes (from 64 to 100 % of the total thiophene amount). Among other *Tagetes* species tested, *T. lucida* and *T. tenuifolia* possessed the highest

amounts of total thiophenes (6717.3 and 6452.5 mg kg^{-1} dry weight respectively). Also, among different extraction methods carried out on *Tagetes erecta* plants, ethanol extract was found to be the most toxic to *M. incognita* J2 [29].

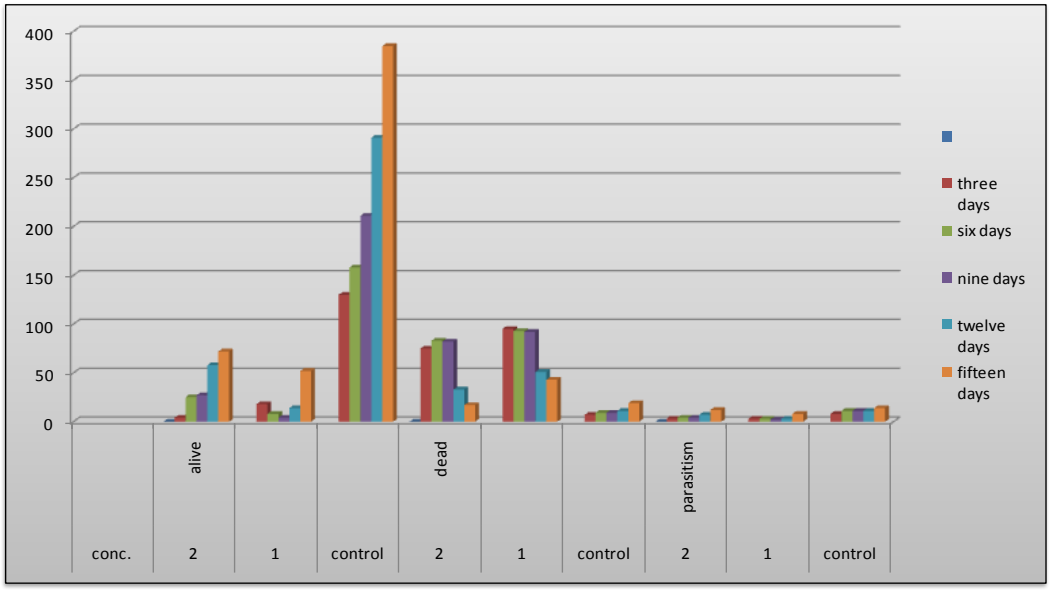

Fig. 1: Effect of *T. lucida* extract on the average no. of aphid (*Aphis brassicaceae)* on cabbage aphids before and after treatment.

Table 4: Effect of herb and root extract from Mexican marigold (*Tagetes lucida*) on percentage motility of soil stages of phytonematodes and their recovery in distilled water*.

Plant part or control	concentration	Average numbers of developmental stages in eggs		Inhibition of hatch %
		Embryos	larval stages	
Herb	S	610	112	72.2
Root	S	810	56	86.6
Herb	S/2	584	98	68.2
Root	S/2	645	103	74.8
Tween (0.05)	--	45	7	5.2
Distilled water	--	32	18	5.0

* Average of 16 replicates. In a column, numbers followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple-range test.

+ The plant extract was diluted with distilled water using 0.05% Tween 80 as spreading agent; concentration of any extract is the standard solution (S) or diluted to S/2.

Table 5: Effect of herb and root extract from *T. lucida* on development to hatching of *Meloidogyne incognita* eggs*.

Plant part or control	Concentration	*Meloidogyne incognita*		*Criconemella* spp.		*Helicotylenchus* spp.		*Pratylenchus* spp.		% recovery (total nematodes)	
		% immobility after									
		24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Herb	S	74 ab	91 b	71 b	81 bc	78 b	72 h	91 a	100 a	24	15
Root	S	83 a	100 a	88 a	96 a	87 a	83 b	97 a	100 a	15	6
Herb	S/2	62 c	78 c	63 c	74 c	67 c	100 a	83 b	100 a	33	22
Root	S/2	68 bc	83 bc	78 b	84 b	79 b	72 c	91 a	100 a	23	12
Tween 80 (0.05)	--	14 d	18 d	5 d	8 d	14 d	100 a	9 c	18 b	--	--
Distilled water	--	10 d	13 d	6 d	9 d	18 d	27 d	11 c	20 b	--	--

* Each value is an average of 16 replicates; concentration of any oil is the standard solution (S) or diluted to S/2.

These results are in general agree with those reported by Marotti *et al*. [23] who mentioned that *Tagetes* species produce thiophenes, polyacetylenic compounds that possess strong biocidal activity, thus making *Tagetes* plants very useful for suppressing nematode populations in the soil and as sources of natural pesticides. Moreover, having evaluated six *Tagetes* species for their morphophenological parameters and thiophene pattern in different plant parts, they concluded that *T. minuta* and *T. lucida* appeared to be the most promising *Tagetes* species, with high potential for use as biocidal crops for the implementation of pest control practices that are less

harmful to human health and natural resources. The percentage of nematode immotility increased with increase in the concentration of volatile oil and the exposure period (Table 29). Such an increase was also reported by Khan and Chindo [20], Korayem *et al*. [21], Abd-Elgawad and Omer [1], Bharadwaj and Sharma [5] and Moosavi [26] as a general trend concerning other plant extracts. From a nematological point of view, this study revealed that *T*agetes *lucida* is a promising starting and new material for the production of bio-nematicides in Egypt. However, further studies are necessary to obtain insights on rates and timing of application, as well as growth parameters of treated plants under greenhouse then field conditions.

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