



EVALUATION OF EFFECTIVENESS OF TAGETES ERECTA EXTRACT IN CONTROLLING CITRUS NEMATODE TYLENCHULUS SEMIPENETRANS IN WASIT PROVINCE

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ABSTRACT

The study has been conducted in the laboratory of college of science – university of wasit in cooperation with the ministry of science and technology- directorate of agriculture research to evaluate the effect plant extract of *Tagetes erecta* in controlling of citrus nematode *Tylenchulus semipenetrans* in wasit province. The samples had been collected from citrus orchard in Wasit Province – Badrah districts and the sample includes infected roots and soil, 1.5 m away from the tree in 5- 15 cm depth and then stored in refrigerator at 4 °C. The results of this study had been revealed that the water and alcohol extracts of *Tagetes erecta* at all used concentrations affect the larvae of *Tylenchulus semipenetrans* and the effect was proportion with the concentration and exposure time and the mortality percentages of larvae treated with water and alcohol extracts were 35.66% and 34.30% respectively after 24 hours and increased to 50.68%, 58.30 % respectively after 72 hour. The highest mortality percentages of larvae were recorded at the extract concentration 2000 ppm which were 79.92%, 83.50% in the case of water and alcohol extracts respectively, while the concentration 500ppm recorded lowest percentages which were 33.54%, 38.25% respectively. The results of the study of the effect of water and alcohol extracts of *Tagetes erecta* on the eggs hatching showed that the extracts at all used concentrations affect the hatching of eggs significantly as compared with control and the rates of eggs hatching in the case of water and alcohol extracts were 8.80%, 7.40% respectively after two days of treatment and increased to 22.25%, 21.70% respectively after 8 days.

KEYWORDS: Citrus nematode, *Tagetes erecta*, Biocontrol.

INTRODUCTION

Citrus plants are important economic plants in the subtropical and Middle Eastern regions. This plant represent is the largest genus in the Rutaceae family, and is the most traded horticultural product in the world. The productivity of this crop is gradually increasing, in 1979 were 13. 292.610 tons, While the production increased in 2009 to 112. 910.151 tons.^[1] Citrus trees are infected with many snake worms, but nematodes (*Tylenchulus semipenetrans cobb*) are the most damaging pests to citrus.^[2,3] Indicates that citrus plants are infected with more than 200 species of nematode, and that the most dangerous of these invasive species. The risk of this nematode is not only to infect citrus plants, but also to have a large family host, and they infect on many other plant species such as olives, grapes, khaki and pears.^[4] Noted that citrus fruits caused production losses in grape groves (grapevines) *Vitisvinifera* ranged from 15 – 25% and that the severity of the losses was proportional to the population density of the nematode in the soil.^[5] referred

that the nematode *Tylenchulus semipenetrans* is the more important nematode in the olive (*Olea europaea*L.) orchards in Chile. It is difficult to control this nematode by one method but the use of more than one method or bioagent is important to manage this nematode.^[6,7] Indicates that using *Trichoderma harzianum*, *T. viride* and *Bacillus subtilis* bacteria achieved good results in the fight against nematodes. The number of eggs and females has been reduced for these nematodes as well as improved plant growth standards.^[8] Refer to using *Pseudomonas fluorescens*, *Pochonia chlamydosporia* and *Trichoderma harzianum* have achieved good results in controlling the citrus fruits and reducing their damage and decreasing the population density of nematode by 70.49%, in addition to increasing productivity to five Times. Another study referred to the efficacy of the plant digest and some organic substances in the control of the citrus nematode in the laboratory and field. Other studies mentioned.^[9,10] that the mushroom *Paecilomyces lilacinus* has achieved good results in combating this scourge.^[11]

So because of the importance of this nematode and the activities of *Tagetes erecta* this study was carried out to evaluate the effects of aqueous and alcohol extract of *Tagetes erecta* on citrus nematode.

MATERIALS AND METHODS

Samples collection

The samples were collected in November from citrus orchard in wasit province .Soil samples and roots infested from 5-15 cm depth and 5 cm away from the infested citrus tree. The eggs larvae of nematode had been extracted from infected roots and soil by sieving method.

Tagetes erecta plants were obtained from the university of Wasit – college of agriculture. The plants were washed and dried in laboratory conditions. After drying, plants were grinded by using an electric grinderand the powder kept in dry condition for extraction.^[12]

Water extract preparation

Water extract was prepare by soaked 100 gm of dry plant material with 400 ml of distilled water and then left at room temperature for 24 hr. The mixture filtrated through adouble-fold muslin cloth and centrifuged with 3000 rpm for 10 minute. The extract was filtered through millipore filter (0.45µm) and then kept at 4C⁰for using.

Alcohol extract preparation

This extract was prepared by soaked 100 gm of dry powder of plant with 500 ml of ethanol (concentration 70%) in flask (1000ml) for 24 hr under room temperature and then centrifuged with 3000rpm for 15 minute. The extract was filtered by filter paper (What man No.1) then the filtrate evaporated by air drier at 37 C⁰ to obtain condense solution and then the solution kept at 4C⁰ for using.^[13] Stock solution prepared by the adding 1ml of condense solution to 100 ml of distilled water and then series of concentration were prepare which include 500, 1000, 1500, 2000 ppm.

Effect of plant extract on the larvae of *T. semipentrans*

The effect of water and alcohol extract on larvae of *T. semipentrans* had been done by preparing series of concentrations (500, 1000, 1500, 2000 ppm), and then

5ml from each concentration were added to 5 cm diameter petriplate contain 1ml of larval suspension (200 larvae/ml). The petriplates incubated at 25±2C⁰. Each treatment was replicated three times with completely randomized design. The mortality of larvae was estimated after 24 and 48 hr.^[14]

Effect of plant extract on the eggs hatching of *T. semipentrans*

The effect of water and alcohol extract on the eggs hatching had been done by preparing series of concentrations (500 , 1000 , 1500 , 2000 ppm), and then 5ml from each concentration were added to 5 cm diameter petriplate contain 1ml of eggs suspension (200 eggs/ml). The petriplates incubated at 25±2C⁰ .Each treatment was replicated two times with completely randomized design. The percentages of eggs hatching was estimated after 2, 4, 6 and 8 days.^[15]

Statistical analysis

The data were subjected to an Analysis of Variance (ANOVA) procedures using Statistical Analysis System (GenStat) and completely randomized design.^[15]

RESULTS AND DISCUSSION

Effect of plant extract on the larvae of *T. semipentrans*

Effect of water and alcohol extract of *Tagetes erecta* on the larvae (J2) of *T. semipentrans* (table 1) shown that the water and alcohol extract at all concentrations affect the viability of J2 and caused significant mortality of *T. semipentrans* J2 as compared with the control treatment. The percentages of juvenile mortality that treated with water and alcohol extract were 35.66% ,4.30 % respectively after 24 hr and 45.20%52.25% after 48 hr respectively. The percentages of juvenile mortality increase after 72 hr and became 50.68% in the case of water extract and in the case of alcohol extract 58.30%. Effect of plant extract (water and alcohol extracts) had been increased with the increasing of the concentration and the highest percentage of juvenile mortality was recorded in the case of the concentration 2000 ppm after 72 which was 94.5% , and also the effect of plant extract (water and alcohol extracts) had been increased with the increasing of the exposure time.

Table 1: Effect of extract of *Tagetes erecta* on the mortality of larvae of *T. semipentrans*.

Concentration ppm	(%)Mortality of larvae by water extract after			Rate	(%)Mortality of larvae by alcohol extract after			Rate
	24 hr.	48 hr.	72 hr.		24 hr.	48 hr.	72 hr.	
control	0	0	0	0	0	0	0	0
500	24.92	34.70	41.00	33.54	23.00	41.75	50.00	38.25
1000	34.89	49.38	54.65	46.31	33.00	60.00	69.50	54.17
1500	49.92	60.70	67.77	59.46	48.00	71.00	77.50	65.50
2000	68.55	81.20	90.00	79.92	67.50	88.50	94.50	83.50
Rate	35.66	45.20	50.68		34.30	52.25	58.30	
LSD 0.05	Con.	Time	C x T	LSD	Con.	Time	C x T	
	2.54	1.97	4.40	0.05	1.50	1.16	1.59	

The results of this study revealed that the effect of extract was increased with increasing of concentration and this may be due to the increase of the nematicidal material in the high concentration.

Results of this study agrees with the findings of^[16] who found that the effect of plant extract(*Moringa oleifera*) on the juvenile of *M. javanica* had been increased with the increasing of concentration and the highest percentage of juvenile mortality was recorded in the case of the concentration 100% which was 30% and the lowest was recorded in the case of the concentration 25% which was 16%.^[17] referred to the effect of the leaf extracts of three plants on the larval stage of *Tylenchulus semipenetrans* and they recorded that the effect of all extracts was increased with the increasing of the concentration and the exposure period.

The nematicidal effect of the extract of *Tagetes erecta* extract on the citrus nematode *Tylenchulus semipenetrans* may be due to the presence of toxic compounds like Flavonoids, Patuletin, Quercetagenin, Quercetin and their derivatives like Bithienyl and Carotenoid Lutein and the percentages of juvenile mortality were 75 – 100%.^[18,19] Results of this study also agrees with the results of^[20] who found that the alcohol extract of *Tagetes erecta* was more effective than the water extract in controlling the citrus nematode *Tylenchulus semipenetrans* and the effect increase with the increasing of the concentration.

Recent study^[21] revealed that the population of *M. javanica* in soil was reduced significantly when it treated with the extract of *Tagetes erecta* and the effect was proportional with concentration of extract.

Similar recent study^[22] revealed that the effect of extract of the African marigold (*Tagetes erecta* L.) on the larvae

of *Meloidogyne incognita* had been increased with the increasing of the concentration and the exposure time. Another studies were recorded the increase of the effect of medicinal plant extracts on the larvae of nematode with the increasing of the concentration of extracts and the exposure period.^[23,24]

Effect of plant extract on the eggs hatching of *T. semipenetrans*

The results of study of the effect of water and alcohol extract of *Tagetes erecta* on the eggs hatching of *T. semipenetrans* (table 2) revealed that the water and alcohol extracts at all concentrations affect the hatching of eggs significantly as compared with the control treatment. The result showed that the alcohol extract was more effective than the water extract.

The results revealed that the rate of percentages of eggs hatching that treated with water and alcohol extract were 16.04% and 13.63% respectively at the concentration 500 ppm and the rate percentages reduced to 11.88% and 9.75% at the concentration 2000 ppm. The results also revealed that the rate of percentages of eggs hatching that treated with water and alcohol extract were 8.80% and 7.40% respectively after 2 days while the rate of percentages of eggs hatching were 22.25% and 21.70% respectively after 8 days. The results also showed that the lowest percentages of eggs hatching (more inhibition) had been recorded in the case of alcohol extract at the concentration 2000ppm which was 2.75% after two days while in the case of water extract was 6% at the same concentration and after the same period. The results revealed that the eggs hatching decrease with increasing of the concentration of extract but increase with increasing of exposure time.

Table 2: Effect of extract of *Tagetes erecta* on the eggs hatching of *T. semipenetrans*.

Concentration ppm	(%) Eggs hatching treated with water extract after				Rate	(%) Eggs hatching treated with water extract after				Rate
	2 days	4 days	6 days	8 days		2 days	4 days	6 days	8 days	
Control	13.00	18.00	22.25	25.00	19.56	15.00	19.03	22.75	26.50	20.82
500	9.70	13.00	17.90	23.55	16.04	8.00	9.78	14.75	22.00	13.63
1000	8.55	11.25	16.50	22.18	14.62	6.50	8.00	14.25	21.00	12.44
1500	6.75	9.00	14.50	20.50	12.69	4.75	7.27	13.00	20.00	11.26
2000	6.00	8.00	13.50	20.00	11.88	2.75	6.00	11.25	19.00	9.75
Rate	8.80	11.85	16.93	22.25		7.40	10.02	15.20	21.70	
LSD 0.05		Con.	Time	C x T	LSD 0.05		Con.	Time	C x T	
		0.65	0.58	1.30			1.32	1.18	2.63	

It is clear from this result that the eggs hatching decrease with the increasing of the concentration and this may be due to the increase in the quantities of active materials that has nematicidal effect and this result was agree with

the finding of^[25] who found that the effect of the extract of *Tagetes erecta* on the egg hatching was increased with the increasing of the concentration of extract.

Similar study revealed that the extract of *Tagetes lucida* had been inhibited the eggs hatching of the nematode *Meloidogyne incognita* and showed that the percentages of inhibition was increase with the increasing of the concentration of extract and the percentages of inhibition were 86.6 % and 74.8 % at the concentration 100% and 50% respectively.^[26] Recent study revealed that the effect of *Tagetes erecta* on the eggs hatching of nematode is due to the presence of 30 volatile organic compounds.^[27] Another study showed that the effect of some medicinal plant affect the eggs hatching of the nematode and the effect of the extract had been increased with increasing of the concentration of extract.^[28]

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