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DUAL EFFECTS OF LEAF EXTRACTS OF *EUCALYPTUS CITRIODORA* ON CONTROLLING PURSLANE AND ROOT-KNOT NEMATODE IN SUNFLOWER

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Abstract: Experiments were conducted under laboratory and greenhouse conditions to investigate the allelopathic activity of aqueous extracts of dry and fresh leaves of *Eucalyptus citriodora* on purslane weed growth and nematode *Meloidogyne incognita* infecting sunflower plants cv. Giza 102. A Petri dish biotest showed that the aqueous extracts significantly reduced purslane (*Portulaca oleracea* L.) seedling length, with the degree of inhibition being dependent on the extract concentration. The fresh and dry leaf extracts of *E. citriodora* standard solution "S" caused the highest net mortality percentage of 100% after 72 hrs of exposure. Greenhouse studies in 2008 and 2009, indicated the greatest significant inhibition in purslane growth as well as the number of galls and egg masses of infecting nematode affected the increase in sunflower growth and yield. The studies indicated increase in the endogenous contents of total phenols in purslane tissues. This increase, correlated with growth inhibition due to treatment with leaf extract of *E. citriodora*. Chemical analysis indicated an increase in the contents of carbohydrates, protein and oil in sunflower seeds. The analysis of fatty acid composition by Gas Liquid Chromatography (GLC) indicated increases in the percentage of oleic and linoleic acid in sunflower seeds when fresh leaf extract of *E. citriodora* was used. A high-performance liquid chromatography analysis showed that the following acids; caffeic, ferulic, coumaric, benzoic, vanelic, chlorogenic, and hydroxybenzoic were present in *Eucalyptus* extracts.

Key words: allelopathy, *Portulaca oleracea, Meloidogyne incognita*, phenolic acids, *Helianthus annus,* fresh leaf extract, dry leaf extract

INTRODUCTION

Sunflower (*Helianthus annus* L.) is one of the most important oil crops. It is considered an economic and nutritious crop supplying oils which are very essential in the diet, especially in the diets of those from developing countries. Sunflower seeds are the third largest source of vegetable oil worldwide. According to the annual statistical report of the Ministry of Agriculture and Land Reclamation in Egypt, it was grown on about 1497.5 ha. Increased yield could be achieved through introducing high yield producing cultivars and effective management of weeds, diseases and pests. Weeds are considered to be a serious problem because they compete for water, nutrients, light, and space. Weeds reduce crop growth and yield (Hussein 2001). In addition, weeds are part of the ecology of a field and can have other effects, such as serving as a reservoir for insects (Marshall *et al*. 2003), diseases (Ramappa *et al*. 1998), and nematodes (Venkatesh *et al*. 2000). Losses of millions of dollars are recorded all over the world due to reduction in crop yield caused by many weeds species (Webster 2004).

Nematodes are important pests causing severe damage and great yield losses in the most important oil crops in Egypt (Mohamed 2005; El-Hamshary *et al*. 2006). Several species of nematodes attack sunflower but the most severe damage is caused by the root-knot nematodes, *Meloidogyne* spp., (Mohamed 2005; Korayem *et al*. 2006; Youssef *et al*. 2008). Plant-parasitic nematodes that damage crops can also reproduce on weeds (Davis and Webster 2005). These nematodes have a wide range of hosts including purslane weed. The interaction of weeds and nematodes recorded high values in yield losses (Koenning *et al*. 1999). For this reason, indiscriminate amount of synthetic herbicides or nematocide are being used worldwide to control weeds and nematodes. If the trend of increasing dependence on the heavy use of chemicals for weeds and pest control is to be reversed, then the practice of reducing or delaying the use of pesticides is worth exploring. Such a strategy could also lead to improved water quality and reduced environmental contamination, besides, reducing the hazards of pesticide residues in food and soil.

Allelopathy arises from plants that produce allelochemicals released by leaching, exudation, volatilization, or decomposition. Some of these compounds, at certain concentrations, are phytotoxic to receiving organisms, but

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at other concentrations they are also stimulating. Among the most commonly found allelochemicals, cinnamic and benzoic acids, flavonoids, and various terpenes are listed (Singh *et al*. 2003). The *Eucalyptus* species possess high allelopathic activity (May and Ash 1990; Singh *et al*. 2005). The extracts of four species of *Eucalyptus* including *E. citriodora* L. reduced the percentage of radish germination, root and shoot lengths (Luo *et al*. 1995). Also, volatile oils from *E. citriodora* drastically affected *Phalaris minor, Chenopodium album, Echinochloa crus-galli* and *Amaranthus* spp. (Singh *et al*. 2005). Moreover, some plant extracts possess multifunctional allelopathic effects *i.e.* have an inhibitory effect on insects, at the same time inhibiting the growth of various acceptor plant species like radish, ryegrass, and barnyard grass, and on pathogens such as rice banded sclerotial blight and phytothora root rot of pepper (Lu 1999; Zhang *et al*. 2002).

The objective of the present work was to investigate the possible role of allelopathic compounds contained in fresh and dry leaf extracts of *E. citriodora* on the germination and early growth of purslane. The objective was also to investigate the effect of the extracts on the root-knot nematode *M. incognita* (laboratory test), and the interference between the selected weed purslane and *M. incognita* on sunflower plants in greenhouse conditions.

MATERIALS AND METHODS

Laboratory test

E. citriodora used as the donor of allelochemicals for the experiments was collected from Egyptian gardens.

Weed

Samples of 10, 15, 20 or 25 g of fresh leaves of *E. citriodora* were washed under tap water, then with distilled water, to remove dust. Next, the samples were transferred into labelled bottles, and 100 ml of sterile, deionized distilled water was added to each bottle. The mixture was then shaken well by hand and soaked for 48 h at room temperature. It was then filtered to get 10, 15, 20 and 25% extract concentrations. The same weight of fresh leaves were oven-dried at 40°C to obtain the corresponding dry weight then ground to fine powder . The powder was then transferred into bottles to which 100 ml of sterile, deionized distilled water was added for a 48 hour extraction, as described above. The produced extracts were collected and filtered through filter paper Whatman No.1. The extracts were used in the assay of allelopathic activity against purslane as follow: A Petri dish biotest was carried out for screening the different concentrations of the previous aqueous extracts of *E. citriodora* leaves on germination and seedling growth of purslane (*Portulaca oleracea).* Twenty seeds of purslane weed were germinated in Petri dishes containing 1-layer filter paper Whatman No. 3 moistened with 6 ml of the aqueous extracts of fresh and dry leaves of *E. citriodora* in concentrations of: 0 (distilled water), 10, 15, 20 and 25% fresh leaf extracts and corresponding dry leaf extracts as follow: 3.134, 4.702, 6.270 and 7.850%. The germination was carried out in the laboratory in May with average maximum and minimum temperatures of 32±1°C and 17.5±1°C. Each treatment was represented by five replicates; each Petri dish represented one replicate. Five days later 2 ml of the tested extracts were added. Records on percentage of germination, seedling root and shoot length of purslane weed were taken 10 days after germination. The experiment was repeated twice at one week intervals and the presented results are the mean of the two experiments.

Nematode

Aqueous plant extracts of fresh and dry leaves of *E. citriodora* (25 and 7.850%) were prepared as above and termed as standard solution (S). Other dilutions, such as: 1:2 and 1:10 were prepared from the (S) solution by adding distilled water.

Nematode inoculums

Second-stage juveniles (J_2) of *M. incognita* were obtained from pure culture maintained in the glasshouse on tomato (*Lycopersicum esculentum* L. cv. Peto UC 82). Infected tomato roots bearing egg masses were incubated in water for three days at 30±5°C and hatched. J_2 were collected and counted. The number of J_2 was 300 J_2 per ml. Nine ml of each aqueous extract was added to 1 ml of nematode suspension containing 300 J_2 of *M. incognita* in 50 ml plastic capsule. Distilled water was used as the control. Each treatment was replicated five times. The numbers of alive and dead nematodes were counted under a light microscope after 24, 48 and 72 h exposure to the extract at 25°C. The nematode mortality percentage was calculated for each treatment. Nematodes were considered alive if they moved or appeared as a winding shape, and they were considered dead if they adopted a straight shape and were immobile. Then, the nematodes in each concentration were transferred to distilled water for 48 h to ascertain whether dead nematodes regained mobility or not. The corrected nematode mortality percent was calculated according to Abbott's Formula:

$$\text{Mortality [\%]} = m\text{-}n/100\text{-}n \times 100$$

where:
m and n indicate (%) mortality in treatments and the control

Pot experiment

This investigation was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, during the two successive summer seasons of 2008 and 2009 based on results of the preliminary laboratory test. The seeds of sunflower plants *(Helianthus annus* L) cv. (Giza 102) were obtained from the Agricultural Research Centre, Ministry of agriculture, Giza. Seeds were sown 2 cm deep, and germinated under the average maximum and minimum temperature of 35.5±1 and 18.5±1°C. The pots which had a 30 cm diameter and 30 cm height contained equal amounts of sieved soil (clay and sand; 2:1 v/v). All pots were infested with a constant weight of seeds of purslane: *Portulaca oleracea* L. (broad-leaved weed). Weed seeds (10 seeds) were sown simultaneously and mixed thoroughly at a depth of 2 cm in the soil. Thinning of sunflower was done after 2 weeks so that 3 homo-

geneous seedlings were left per pot. Routine fertilizers were added as a mixture of calcium super phosphate and ammonium sulfate, representing sources of N and P. One week later (21 days after sowing), the plants were inoculated with 2,000 newly hatched second stage juveniles (J_2)/pot of *M. incognita* obtained from pure culture raised in glasshouse grown tomato (*Lycopersicum esculentum* cv. Peto UC 82). In addition there were the control pots; unweeded (healthy) and free weed (healthy), free weed (infected) and unweeded (infected). Based on the preliminary work (Petri dish biotest), the fresh leaf extract was used at a concentration of 25% and the corresponding dry leaf extract, as follows.

Preparation of the extract

Fresh leaves of *E. citriodora* (500 g) were washed with tap water, then with distilled water, to remove dust. Leaves were transferred to labelled beakers to which 2000 ml distilled water was added, and allowed to soak for 48 hours. Then the produced extracts were collected and filtered through very fine mesh and pressed carefully for complete extraction. This step was repeated with the corresponding dry (oven dried at 40°C) finely powdered leaves. The process was repeated according to the quantity of the extract needed but always the extract was freshly prepared. Both foliar and soil treatments were applied three times during the three weeks, starting from 21-days-old plants.

Foliar application

The following aqueous extracts were applied at a rate of 250 ml/pot:
a – Aqueous extract of fresh leaves of *E. citriodora* of 25%,
b – Aqueous extract of corresponding dry leaves of 7.85%.

Soil treatments

The previous aqueous extracts were added to the soil at a rate of 250 ml/kg soil in addition to the control treatments as follow (a) untreated free of weed plants (healthy plants *i.e.* not infected with nematodes), (b) untreated free of weed plants infected with *M. incognita*, (c) the unweeded healthy control (not infected) and (d) the infected unweeded control. The experiment consisted of 8 treatments including 4 control treatments as previously mentioned. Each treatment was represented by 9 pots. The pots were distributed in a complete randomized design. Weed samples were taken from three pots at each stage, and data was recorded.

Allelopathic effects on weed

Plants of the infested weed were collected from each pot, 51 and 81 days after sowing. Weed samples were taken from three pots at each stage.

Allelopathic effects on nematodes

After one month (51 days after sowing sunflower) and two months (81 days after sowing sunflower) from inoculation, the plants and weed were carefully uprooted. Roots of each replicate were cut into small pieces (1/2 mm) in Petri dishes. Then the root pieces were, examined under stereoscopic microscope for counting the number of galls and egg-masses on the entire root system.

Allelopathic effects on sunflower

The following data were collected for sunflower plant growth parameters and were recorded for each individual plant, 51 and 81 days after sowing. Fresh plants were oven dried at 60°C for determination of dry weight (g/plant). At the end of the season, head diameter (cm), fresh and dry weights of sunflower heads/plant (g) were taken. Sunflower yield which is represented in this work by dry matter of heads, was calculated for each treatment.

Determination of total phenols in purslane weed

Total phenolic compounds in purslane weed were extracted from dry finely ground tissues (at 60°C). Total phenols were determined colorimetrically according to the method defined by Snell and Snell (1953) using Folin and Ciocalteu phenol reagent.

Determination of total carbohydrate contents in sunflower seeds

Total carbohydrate were extracted from dry finely ground sunflower seeds according to Herbert *et al*. (1971) and estimated colourimetrically by the phenol-sulphoric acid method (Montogomery 1961).

Determination of protein contents in sunflower seeds

Protein contents were determined in dried seeds according to the method of Lowery *et al.* (1951).

Determination of oil contents in sunflower seeds

Oil content of dried seeds was determined as described by AOCS (1964). A known weight of ground sunflower seeds was imbedded in 50 ml of petroleum ether and allowed to extract using Soxhlet apparatus.

Determination of fatty acids in extracted oils

The extracted oil was methylated by refluxing in a water bath for 90 min at 90°C with 1 ml sulphuric acid in methanol and benzene (2:1 v/v). Afterwards 5ml petroleum ether (40/60) was added (Harborne 1984). After shaking, the petroleum ether fraction was evaporated to dryness, the residue was dissolved in 1ml petroleum ether and analyzed with GLC by Instrument Agilent Technologies 6890N GC system with oven temp programmed to 70–220°C, initial time 2 min and 20 min final time using capillary column HP-5 5% phenyl siloxane, L = 30 min, d = 320 μm, film thickness = 0.25 μm, detector temp. 300°C FID (flame ionization detector) with flow 3 ml/min and carrier gas N_2, H_2 at 30 ml/min and air.

Phenolic acids contents by HPLC of the experimented extracts

Phenolic acids in the tested aqueous extracts of fresh and dry leaves of *E. citriodora* were extracted as follow: 5 g of fresh or dry leaves were immersed in 100 ml distilled water for 48 hours, and filtered. The filtrate was subjected to separation by HPLC with the following: mobile phase acetonitrile (86%), Buffer 14% (pot. dihydrogen phosphate: phosphoric acid, 2:1 v/v), flow rate 1ml/

min; Agilent 1100 series (Waldborn, Germany), quarternary pump (G1311A), Degasser (G1322A), Thermostated Autosamples (G1329A), variable wave length detector (G1314A); column: Zorbax 300SB C18 column (Agilent Technologies, USA). Separation of different phenolic acids was carried out at 254, 280, and 320 nm wave lengths.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA). The mean values of particular combinations were compared by Fishers Least Significant Difference (LSD) at the 0.05 levels of significance (Snedecor and Cochran 1980).

RESULTS

Laboratory tests

Effect of water extracts on germination and early seedling growth

Germination percentage, seedling root, and shoot length of purslane weed were significantly reduced by both fresh and dry leaf extracts of *E. citriodora*, as compared to the untreated control (Table 1). The effect, however, depended on extract concentrations. Seedling root length was completely inhibited by 25% of fresh leaf extract of *E. citriodora*. Maximum inhibition in seedling shoot length reached 59% with the 25% fresh leaf extract, in comparison to the untreated control.

Effect of water extracts on root-knot nematode

The efficacy of two aqueous extracts of fresh and dry leaves of *E. citriodora* on *M. incognita* mortality, under laboratory conditions, is presented in table 2. Data reveal that fresh and dry leaf extracts of *E. citriodora*, standard solution "S" (25%) caused the highest net mortality percentages of 100 and 100% after 72 hrs of exposure, respectively.

Pot experiments

Effect of water extracts on weed growth

Both extracts of fresh and dry leaves of *E. citriodora* significantly inhibited purslane growth (Table 3). The toxic effect of both extracts depended on the type of the extract and method of application. With all treatments, inhibitory effects on fresh weight of purslane as compared to the respective controls were found after 51 and 81 days from sowing. In general, foliar treatments were more toxic. Data in table 3 also indicate that foliar treatments of fresh and dry leaf extracts induced maximum inhibitions in fresh weight of purslane. These treatments resulted in 53.3 and 50% inhibition, 81 days after sowing. Maximum reduction in dry weight of purslane reached 58% by foliar treatment of fresh leaf extract of *E. citriodora*, 81 days after sowing, in comparison to the untreated control.

Changes in phenolic contents in weed

The amount of phenolic acids in purslane dried tissues correlated with the growth inhibition of the respective

Table 1. Effect of aqueous extracts of fresh and dry leaves of *E. citriodora* on germination, seedling root and shoot length [mm] of purslane weed

Aqueous leaf extract	Concentration	Germination [%]	Root length	Shoot length
Fresh	0	100	36.90	32.80
	10%	78	9.94	28.40
	15%	70	6.00	22.20
	20%	40	2.20	17.40
	25%	35	0.00	13.50
Dry	3.26%	100	17.20	30.80
	4.70%	80	15.40	25.20
	6.20%	76	11.70	23.30
	7.85%	60	5.90	20.70
LSD at 5%		1.732	0.932	1.775

Table 2. Effect of aqueous extracts of fresh and dry leaves of *E. citriodora* leaves on survival of the root-knot nematode *M. incognita*

Plant material	Aqueous leaf extract	Extract concentration	Mortality [%]			Recovery [%]	Net mortality [%]
			24 h	48 h	72 h		
E. citriodora	fresh	S	96	98	100	0.0	100
		S/2	79	84	86	0.0	86
		S/10	56	60	68	10.0	58
	dry	S	97	100	100	0.0	100
		S/2	90	93	97	0.0	97
		S/10	65	70	72	8.0	64
Distilled water		0	0.0	0.0	0.0	0.0	0.0

S = 25%; S/2 = 12.5%; S/10 = 0.78%; values are averages of five replicates
LSD at 5% level to compare:
Extract (E): 2.6; ExC: 3.9; Concentration (C): 1.7; ExH: ns
Hours (H): 2.1; CxH: ns; ExCxH: ns
ns – non significant

treatments (Table 3). Generally, all treatments increased total phenol content significantly, over the untreated control. Spraying treatment resulted in relatively higher contents than soil treatments. Maximum growth inhibition in purslane was concomitant with maximum total phenolic acids content. Total phenolic acids content in purslane, when sprayed with fresh leaf extract of *E. citriodora,* exceeded those found in the untreated control by 278.4%, after 81 days.

Effect of water extracts on root-knot nematode

The results revealed that the two aqueous extracts of fresh and dry leaves of *E. citriodora* significantly inhibited *M. incognita* infecting sunflower cv. Giza 102 and purslane weed (Table 4) no matter whether treatment was done by foliar spraying or soil drench. These inhibitions depended on the type of the extract and method of application. In general, fresh leaf extract was more effective. The inhibition in number of galls reached 76.5, and 100% in egg masses on roots by fresh leaf extract, 51 days after sowing**.** The corresponding results exerted by dry extract of *E. citriodora* were 76.5 and 90%. In addition the recorded inhibition in number of galls, and egg masses were 88.1 and 100% by fresh extract, 81 days after sowing, and 85.7, 96.7% by dry extract in comparison to the respective controls. Similar trends were recorded in number of galls, and egg masses by the soil drench treatment**,** although it was less effective (Table 4).

Effect of water extracts on growth of sunflower plants

Growth parameters

All extract treatments induced significant increase in dry weight of sunflower as compared to the untreated unweeded infected plants (Table 5). The increase in dry matter reached maximum value with foliar treatment of fresh leaf extract of *E. citriodora*, 81 days from sowing (93.5%), over untreated unweeded infected plants. However, purslane weed competition (unweeded plants) in addition to *M. incognita* infection (infected plants) reduced plant dry weight by 45.5 and 50% in the free of weed healthy plants, 81 days after sowing.

Sunflower yield/plant

The capability of *E. citriodora* leaf extract to increase head diameter was different depending on type of the extract. Spraying fresh leaf extract was more effective in increasing head diameter (116%), although there were high significant increases by all treatments, over the unweeded infected control (Table 5). The fresh and dry weight of sunflower heads significantly increased over the infected unweeded control when fresh or dry leaf extract were used. The results indicated a highly significant increase especially when spraying treatments were used. Higher significant increases in fresh and dry weight of sunflower heads (yield/plant) were recorded by spraying fresh leaf extract. The heads reached 215 and 212%, over unweeded

Table 3. Effect of aqueous extracts of fresh and dry leaves of *E. citriodora* on the growth of purslane weed and total phenol contents (mg/g dry weight) (average of the two seasons)

Treatments		Extract conc. [%]	Fresh weight g/pot		Dry weight g/pot		Total phenol	
			51 DAS	81 DAS	51 DAS	81 DAS	51 DAS	81 DAS
Spraying	fresh leaf extract	25	17.30	28.00	0.402	1.681	20.47	50.67
	dry leaf ext.	7.85	18.76	30.00	0.508	2.953	17.15	40.15
Soil treatment	fresh leaf extract	25	17.80	42.00	1.312	3.250	14.61	33.39
	dry leaf ext.	7.85	31.5	45.50	2.520	3.651	14.27	33.26
The controls	unwedded (Infected)		39.00	54.50	3.503	3.856	3.34	14.65
	unwedded (healthy)		42.50	60.00	3.710	4.003	3.29	13.39
	free weed		–	–	–	–	–	–
LSD at 5%			2.348	3.348	0.147	0.193	0.926	1.556

DAS – Days after sowing; ext. – extract

Table 4. Effect of aqueous extracts of fresh and dry leaves of *E. citriodora* on number of galls and egg masses of the root-knot nematode, *M. incognita,* on both purslane and sunflower (average of the two seasons)

Treatments		Extract conc. [%]	No. of galls				No. of egg masses			
			sunflower		purslane		sunflower		purslane	
			51 DAS	81 DAS	51 DAS	81 DAS	51 DAS	81 DAS	51 DAS	81 DAS
Spraying	fresh leaf ext.	25	4.00	5.00	2.00	2.00	0.00	0.00	0.00	0.00
	dry leaf ext.	7.85	4.00	6.00	2.00	3.00	1.00	1.00	0.00	0.00
Soil treatment	fresh leaf ext.	25	4.00	5.00	3.00	3.00	1.00	3.00	0.00	0.00
	dry leaf ext.	7.85	6.00	12.00	3.00	5.00	1.00	3.00	0.00	1.00
The controls	unweeded healthy	–	–	–	–	–	–	–	–	-
	unweeded infected	–	13.00	24.00	6.00	10.00	6.00	15.00	3.00	6.00
	weed free infected	–	17.00	42.00	–	–	10.00	30.00	–	-
	weed free healthy	–	–	–	–	–	–	–	–	-
LSD at 5%		–	1.792	3.176	1.421	1.588	1.584	2.891	0.294	0.666

conc. – concentration; ext. – extract

infected control. The most significant increase in fresh and dry weight of heads/plant was obtained with weed free healthy plants (302.65 and 297.44%, over the infected unweeded control) as shown in table 5.

Effect of water extracts on some chemical constituents of sunflower seeds

Total carbohydrates

Total carbohydrates in the sunflower seeds increased significantly with spraying or soil applied leaf extracts of *E. citriodora* (Table 5). Leaf extracts, when used as spraying treatments, were more effective. The increase in total carbohydrate content was noticeable in the seeds of the fresh leaf sprayed plants as compared to the infected untreated unweeded plants.

Total protein contents

All treatments presented in table 5 show significant increases in the total protein content in the sunflower seeds. Treatments with different extracts caused a significant increase in protein contents. This increase was obtained with all extracts and all applications. It did not matter whether they were prepared from fresh or dry leaves. The highest protein content was measured in the untreated free weed healthy plants.

Total oil content

The results in table 5 show that the total oil content was affected by different applications of leaf extracts of *E. citriodora.* A highly significant increase was observed with all treatments; fresh or dry. Maximum content was measured when using a spray of fresh leaf extracts (Table 5).

Fatty acid composition

The fatty acid composition is useful for evaluating product quality. GLC analysis of sunflower seed oil using 7 standards, indicated that fatty acid quantities were affected by different treatments (Table 6). Oleic and linoleic acids (unsaturated fatty acids) were the predominant fatty acids most affected by all treatments. Palmitic acid was the most abundant saturated fatty acid. Both

Table 5. Effect of aqueous extracts of fresh and dry leaves of *E. citriodora* on growth, yield (g/plant) and some chemical constituents of sunflower (mg/g dry weight)(average of the two seasons)

Treatments		Extract conc. [%]	Dry weight [g/plant]		Yield/Plant			Some chemical constituents		
			51 DAS	81 DAS	head diam. [cm]	FW of head	DW of head	carbo-hydrate contents	protein contents	oil contents
Spraying	fresh leaf ext.	25	4.85	10.70	10.80	89.00	18.33	234.78	151.60	440.78
	dry leaf ext.	7.85	3.85	9.00	8.33	79.50	16.58	208.29	129.8	367.26
Soil treatment	fresh leaf ext.	25	3.15	8.00	8.03	45.83	11.97	192.41	125.60	360.43
	dry leaf ext.	7.85	3.00	7.00	7.00	37.50	10.42	202.32	120.20	345.78
The controls	unweeded healthy	–	2.65	6.66	6.00	29.00	5.97	127.33	81.80	235.28
	unweeded Infected	–	2.50	5.53	5.00	28.25	5.87	120.03	45.40	161.47
	weed free infected	–	2.90	6.00	5.50	28.75	6.28	121.33	85.80	207.82
	weed free healthy	–	5.80	11.05	12.33	113.75	23.33	267.31	154.60	444.51
LSD at 5%		–	0.397	0.842	0.694	3.963	0.864	4.126	4.447	6.689

head diam. – head diameter; FW of head – fresh weight of head; DW of head – dry weight of head; conc. – concentration; ext. – extract

Table 6. Effect of aqueous extracts of fresh and dry leaves of *E. citriodora* on fatty acids percentage in extracted oils of sunflower seeds (average of the two seasons)

Treatments		Extract conc. [%]	Saturated fatty acids [%]			Unsaturated fatty acids [%]			
			16:0	18:0	20:0	18:1	18:2	18:3	20:1
Spraying	fresh leaf ext.	25	7.90	0.00	0.00	64.34	25.25	2.49	0.00
	dry leaf ext.	7.85	10.16	0.67	0.93	55.30	26.89	4.81	1.20
Soil treatment	fresh leaf ext.	25	12.31	0.00	0.00	56.84	29.18	0.11	0.00
	dry leaf ext.	7.85	9.63	0.86	0.00	56.74	26.05	6.01	0.69
The controls	unweeded healthy	–	8.43	0.00	0.31	50.39	38.53	1.19	1.11
	unweeded Infected	–	25.91	11.13	1.14	8.80	37.37	14.30	1.32
	weed free infected	–	8.97	3.11	0.51	42.10	26.23	18.45	0.60
	weed free healthy	–	6.81	0.00	0.26	65.90	23.47	3.12	0.42

16:0 palmitic acid; 18:0 stearic acid; 18:1 oleic acid; 18:2 linoleic acid; 18:3 linoleneic acid; 20.0 arachidic acid; 20:1 Gadoleic acid; conc. – concentration; ext. – extract

Table 7. Percentage of phenolic acid content in leaf extracts of *E. citriodora*

Phenolic acids	Ferulic	Coumaric	Benzoic	Vanelic	Chlorogenic	Caffiec	Gallic	Hydroxybenzoic
Fresh leaf extract	23.57	20.26	33.44	7.55	5.66	5.12	4.37	0.00
Dry leaf extract	33.34	8.69	13.34	10.34	26.93	13.85	7.25	8.09

saturated and unsaturated fatty acids were affected by leaf extracts; either fresh or dry. Oleic acid decreased in the oil obtained from the yielded seeds of the untreated unweeded infected plants compared with that obtained from the healthy and treated plants. On the contrary, both linoleic and linolenic acids increased compared with that obtained from the healthy and treated plants.

DISCUSSION

Weeds and nematodes are two of the major constraints to plant production worldwide. Understanding plant interactions is important to reduce the dependency on pesticides in future cropping systems. The term allelopathy has been applied to plant/plant biochemical interactions that cause detrimental effects (Chon *et al*. 2003). Hence, allelopathy is a natural and environmental-friendly technique which may prove to be a unique tool for pest management and thereby increase crop yields. Allelopathy offers potential for selective biological pest management through release of allelochemicals from leaves, flowers, seeds, stems, and roots of living or decomposed plant materials (Weston 1996).

The results of the present investigation indicated that growth of purslane was affected by all spray treatments or soil treatment 51 and 81days after sowing compared to the respective controls (Table 3). The degree of inhibition varied, however, according to the type of aqueous plant extracts and method of application. The results revealed that foliar treatments of leaf extract of *E. citriodora* were more phytotoxic to purslane. Generally, different plant extracts were found to suppress growth of different weed species (Cheema *et al*. 2003; El-Rokiek *et al.* 2006). Allelopathic activity of released compounds of different *Eucalyptus* species has been well recognized (May and Ash 1990; Singh *et al.* 2005). In addition, increasing inhibitory growth action by the fresh leaf extract was previously mentioned by Al-Naib and Al-Mousawi (1976). Table 3, also reveals that the endogenous contents of total phenols in purslane tissues increased with increasing growth inhibition, as compared to the control. The increased phenol content is often a characteristic of stress (Nemat Alla and Younis 1995; El-Rokiek 2007; El-Rokiek and Aid 2009).

The results also indicate that aqueous extracts of *E. citriodora* leaves were effective in reducing galls and egg masses of *M. incognita* on roots of sunflower and purslane weed (Table 4). In this connection, *Simmondsia chinensis*, *Azadirabta ndica*, *Withania somnifera*, *Tagetes erecta* and *E. citriodora* proved to be toxic to root-knot nematode, *M. incognita* (El-Nagdi 2005; Khan *et al*. 2008). In addition, some plant extracts possess multifunctional effects of allelopathy i.e. have an inhibiting effect on insect, and at the same time inhibit the growth of some weeds (Lu 1999; Zhang *et al.* 2002).

The results of the present study reveal that different aqueous extracts of *E. citriodora* have indirect positive effects on sunflower growth (dry weight). These positive effects are seen in the reduction of competitive purslane weed as well as reduction in number of galls and egg masses of *M. incognita*.

The above mentioned increase in growth of sunflower plants also explains the increased yield (Table 5). It should be noted, that the increase in sunflower growth treated with different aqueous extracts of *E. citriodora* leaves was accompanied by a corresponding decrease in the fresh and dry weights of purslane weed. Sunflower growth and biomass accumulation in heads was stimulated by some treatments with *E. citriodora.* These findings were contrary to those found for purslane weed. The sunflower growth and biomass accumulation in heads might simply be the result of a good sunflower competitive ability against weed plant, and absence of nematodes.

Several scientists found that inhibition of weed growth increased the competitive ability of crop plants, and consequently increased growth and yield (Aly *et al*. 2001; Alonso-Prados *et al*. 2002; Sanchez *et al.* 2003; Stephanie *et al*. 2004). The increase in both growth and yield of sunflower can also be attributed to the control of root-knot nematode *M. incognita* (El-Nagdi 2005; Mohamed 2005; Korayem *et al*. 2006). Increase in different metabolic activities such as total carbohydrates, protein and oil content in sunflower seeds (Table 5) can be possibly explained by the increased growth and yield of sunflower.

The fatty acid composition of the oil (quality) seems to have improved as oleic acid increased and linolenic decreased (Table 6). This may indicate that the product (oil) is resistant to autoxidation and more suitable for frying (Shahidi 1999).

The results also indicate that spraying treatments were more effective than soil treatments. The effectiveness of spraying may be due to the adsorbtion of allelochemicals by soil particles, decomposed by microorganisms, or moved with water. Both, abiotic and microbial decomposition will have significant effects on the concentration of allelochemicals reaching other plants (Inderjit 2002). *E. citriodora* extract was effective on controlling both weed and nematode. Analyses of the fresh and dry leaf extracts by HPLC indicated the presence of ferulic, coumaric, benzoic, vanelic, chlorogenic, caffiec, gallic, hydroxybenzoic acids in the extracts (Table 7). The highest amount of benzoic acid was present in fresh leaf extracts. The difference in the individual contents may explain why weed and root-knot nematode responded differently to these extracts. In a similar comparison, Chon *et al*. (2003), Singh *et al*. (2003), Chon and Kim (2004) attributed the highly allelopathic, herbicidal potential of some plant extracts to the presence of causative allelopathic substances e.g. coumarin, *o*-coumaric acid, *p*-coumaric acid, benzoic acid, p-hydroxybenzoic acid, and ferulic acid. In addition, El-Rokiek and Aid (2009) attributed the different responses to fresh and dry leaf extract, to the difference in the constituents of the essential oil contents.

CONCLUSIONS

The results from this study indicate the usefulness of allelopathic activity of *Eucalyptus* for elaborating method(s) to combat purslane and root-knot nematode in sunflower cultivation under sustainable and organic agriculture. Nonetheless, further studies performed under various field conditions are still required.

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POLISH SUMMARY

SKUTECZNOŚĆ WYCIĄGU Z LIŚCI DRZEWA EUKALIPTUSOWEGO *EUCALYPTUS CITRIODORA* W ZWALCZANIU PORTULAKI SIEWNEJ I GUZAKA POŁUDNIOWEGO W UPRAWIE SŁONECZNIKA

Przeprowadzono doświadczenia w warunkach laboratoryjnych i szklarniowych w celu zbadania allelopatycznego działania wodnych wyciągów z suchych i świeżych liści eukaliptusa cytrynowego *Eucalyptus citrodiora* na wzrost chwastów portulaki siewnej (*Portulaca oleracea* L.) oraz nicień – guzak południowy (*Meloidogyne incognita*) infekujący rośliny słonecznika. Do doświadczeń wykorzystano odmianę słonecznika Giza 102. Wyniki biologicznego testu na płytkach Petriego wykazały, że wodne wyciągi istotnie ograniczały wzrost siewek portulaki siewnej, a stopień inhibicji był zależny od koncentracji wyciągu. Wyciąg ze świeżych i suchych liści *E. citriodora* zastosowany jako standardowy roztwór „S" powodował najwyższą śmiertelność guzaka południowego (100%) po 72 godzinach ekspozycji. Na podstawie wyników badań szklarniowych wykonanych w latach 2008–2009 stwierdzono, że silne inhibujące działanie testowanego wyciągu zarówno na rozwój portulaki siewnej jak też liczbę galasów i masę jaj guzaka spowodowało lepszy rozwój roślin i wzrost plonu słonecznika. Ponadto, stwierdzono wzrost całkowitej zawartości fenoli w tkankach portulaki skorelowany z zahamowaniem wzrostu i rozwoju chwastów po zastosowaniu wyciągu z *E. citriodora*. Chemiczna analiza nasion słonecznika wykazała zwiększoną zawartość węglowodanów, białka i oleju. Analiza składu kwasów tłuszczowych wykonana techniką GLC wykazała wzrost procentowego udziału kwasów olejowych i linolenowych w nasionach słonecznika w przypadku zastosowania wyciągu ze świeżych liści eukaliptusa cytrynowego. Analiza składu kwasów tłuszczowych w testowanych wyciągach z liści eukaliptusa wykonana techniką HCLP wykazała obecność następujących kwasów: kofeinowy, ferulowy, kumarunowy, benzoesowy, waniliowy, chlorogeniczny i hydrobenzoesowy.