

ORIGINAL ARTICLE

***Eucalyptus citriodora* leaf extract as a source of allelochemicals for weed control in pea fields compared with some chemical herbicides**

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Abstract

Two field experiments were established at the Agricultural Experimental Station of the National Research Centre at Nubaria, Beheira Governorate, Egypt to study the herbicidal potential of the leaf extract of *Eucalyptus citriodora* at 5, 10, 15, 20 and 25% compared to two hand hoeing, unweeded treatments and the chemical herbicides Bentazon + Clethodium, Bentazon + Fluazifop-P-butyl and Butralin on pea plants and associated weeds. The results indicated that two hand hoeing achieved the maximum weed depression as expressed by the dry matter of total weeds. The dry matter of total weeds decreased by 95.08 to 94.77% as compared with unweeded treatment 50 and 70 days after sowing (DAS) followed by Butralin (93.93–94.65%), Bentazon + Clethodium (93.26–94.07%), Bentazon + Fluazifop-P-butyl (91.82–92.77%) and leaf extract of *Eucalyptus* at 25% (91.61–91.95%). Furthermore, the reduction in weed development was accompanied by enhanced pea growth and yield. The results revealed that two hand hoeing was the best treatment to increase plant height, shoot dry weight and SPAD value at 50 and 70 DAS. Also, two hand hoeing produced the maximum values of pod length and number of seeds/pod. The results also indicated that Bentazon + Clethodium treatment gave observable values [recorded 72.96% in pod yield (ton · fed.⁻¹) over that of unweeded control] of number of pod/plant, weight of pod/plant, seed yield/fed and protein percentage. Also, the results revealed great increases in the growth of pea as well as yield due to treatment with *E. citriodora* dry leaf extract at 25%. [recorded 64.8% in in pod yield (ton · fed.⁻¹) over that of unweeded control]. So, the results indicated using Bentazon + Clethodium as well as *E. citriodora* dry leaf extract at 25% to control weeds associated with pea plants. The authors suggested application of *E. citriodora* dry leaf extract at 25% in controlling weeds associated with pea plants as a safe method that avoids environmental contamination.

Keywords: allelopathy, *Eucalyptus citriodora*, herbicides, leaf extract, *Pisum sativum*, weeds

Introduction

Pea, as a vegetable crop, is a legume which grows in non-stressful soil and is an important winter vegetable crop for domestic consumption and export. Yield losses in pea were significantly reduced. A 20 to 40% reduction in pea yield due to competition of weeds has been reported in previous weed competition studies in peas (Wall *et al.* 1991; Blackshaw and O'Donovan 1993; Ahmed *et al.* 2014). Therefore, it is important

to pay attention to the safe control of weeds in pea fields. Recent approaches in agricultural production have been trying to use natural and safe materials to control weeds, insects, nematodes, etc. to decrease the harmful effects of synthetic chemicals (herbicides, pesticides, nematicides and fungicides) and at the same time increase the production and quality of different crops. The allelopathic phenomenon is based on the

fact that plants produce numerous secondary bio-products. When these compounds are released into the surrounding environment, either by leaching exudation or through decomposition processes, they can have positive or negative effects on the growth of other plants (Cheema *et al.* 2013).

Allelopathy may be employed for weed control in field crops by combining cropping, intercropping, surface mulch, soil incorporation of plant residues, allelopathic aqueous extracts, combined application of allelopathic aqueous extracts with lower herbicide doses and crop rotation. In addition, some crops, such as rye, buckwheat, black mustard and sorghum-sudan grass hybrids can also be used for controlling different weeds. May and Ash (1990) and Singh *et al.* (2005) reported that the eucalyptus species possess high allelopathic activity. Volatile oils from *E. citriodora* significantly affected *Phalaris minor*, *Chenopodium album*, *Echinochloa crus-galli* and *Amaranthus* spp. (Singh *et al.* 2005). Also, different amounts of aqueous extracts and decompose of *Eucalyptus* leaves decreased most growth parameters of *phalaris* especially in the leaf decompose (Niakan and Sabari 2009).

Cao and Luo (2005) reported that aqueous extracts from bark and leaves and volatiles from leaves of *E. citriodora* showed allelopathic effects on the growth of nine species, including the weeds: *Bidens pilosa*, *Digitaria pertenuis*, *Eragrostics cilianensis*, *Setaria geniculata* and crops such as corn, rice, cucumber and bean. Studies were carried out to explore the effect of volatile oils from *E. citriodora* to control weeds such as: *P. minor*, *Ch. album*, *E. crus-galli*, *Ageratum conyzoides*, *Parthenium hysterophorus* and *Amaranthus* spp. In laboratory bioassay germination, the seedling length of weed plants was drastically affected by *E. citriodora* leaf extract (Batish *et al.* 2004). El-Rokiek and Eid (2009) and El-Rokiek and El-Nagdi (2011) reported that the leaf water extract of *E. citriodora* decreased the growth of *Avena fatua* and *Portulaca oleracea*. Many researchers have reported that hand hoeing achieved the maximum weed depression, as expressed by the lowest dry matter of weeds in many crops (El-Metwally and

Dawood 2017; El-Metwally *et al.* 2017a). Also, hand hoeing was the best treatment in raising pea yield and its components (El-Metwally and Saad El-Deen 2003).

Previous studies carried out in the greenhouse of the National Research Centre (Egypt) using leaf extracts of *E. citriodora* have shown inhibitory activity against weeds especially by spraying (El-Rokiek and Eid 2009; El-Rokiek and El-Nagdi 2011). To confirm previous research, further studies must be carried out under field conditions. Consequently, the present study was aimed to examine further applications of *Eucalyptus citriodora* leaf extract to control weeds in pea fields.

Materials and Methods

Experimental procedures

Two field experiments were carried out during the 2016/2017 and 2017/2018 seasons at the Experimental Station of the National Research Centre at Nobariya, Behaira Governorate, Egypt, to compare the herbicidal potential of the dry leaf extract of *E. citriodora* compared with some chemical herbicides, Bentazon + Clethodum, Bentazon + Fluazifop-P-butyl and Butraline, in controlling weeds growing in pea fields. The soil of the experiments was sandy. Mechanical and chemical analyses (Cottenie *et al.* 1982) of the soil were carried out before planting and are presented in Table 1.

Preparation of allelopathic materials

Eucalyptus citriodora leaves were collected from Egyptian gardens, washed with tap water, then with distilled water to eliminate dust, then allowed to dry in the shade. *E. citriodora* dry leaves were finely powdered by an electric mill.

Preparation of the aqueous extract

The dry, finely powdered leaves of *E. citriodora* (1,500 g) were transferred to labeled beakers to which 6 l of distilled water were added and allowed to soak for 48 h. Then the produced extract was collected and

Table 1. Some physical and chemical properties of the soil used

Sand [%]		Silt [%]	Clay [%]	Texture		O.M. [%]	CaCO ₃	
72.3		18.7	9	sandy		0.16	7.00	
pH (1 : 2 : 5)	EC [ds · m ⁻¹]	Cations [meq · l ⁻¹]				Anions [meq · l ⁻¹]		
		Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻⁻
7.5	0.20	3.2	1.2	1.1	0.25	2.3	0.80	5.29
Macronutrients [mg · 100 g ⁻¹ soil]			Micronutrients [mg · kg ⁻¹]					
N	P	K	Fe		Mn	Zn	Cu	
14.5	9.20	16.0	7.36		3.19	1.66	3.0	

O.M. – organic matter, EC – electric conductivity

filtered through a very fine mesh and pushed through the mesh carefully for complete extraction. The produced extract was at a 25% concentration. Part of the extract remained as it was (2 l of 25%) and the remaining extract was diluted with distilled water, to concentrations of 5, 10, 15 and 20% for each extract. The process of extraction was repeated according to need to ensure that the extracts were fresh. The prepared aqueous extracts with different concentrations were sprayed at a rate of 500 ml · plot⁻¹ area. Spraying was applied two times during the 2 weeks, starting with 15-day-old plants.

The experiment was carried out with a complete randomized block design with four replications. Weed control treatments were as follows:

1. Extract of *Eucalyptus* at 5%,
2. Extract of *Eucalyptus* at 10%,
3. Extract of *Eucalyptus* at 15%,
4. Extract of *Eucalyptus* at 20%,
5. Extract of *Eucalyptus* at 25%,
6. Bentazon + Clethodium,
7. Bentazon + Fluazifop-P-butyl,
8. Butraline,
9. Two hand hoeing 21 and 35 days after sowing,
10. Unweeded.

Common, trade, and chemical names, rate and time of application of used herbicides are shown in Table 2.

Agronomic practices

Herbicides were applied with a knapsack sprayer equipped with one nozzle boom. The water volume was 200 l · fed⁻¹ (fed = 4,200 m²). Two hand hoeing was applied at 21 and 35 days after sowing. Sprinkler irrigation was used. Each treatment plot consisted of 5 ridges (10 m long and 2.1 m wide). The treatment plot areas were 21 m². Peas were sown in three sides of a ridge, 5 cm apart. Seeds of pea cultivar (Master B) were sown on 6 November in both seasons. The previous summer crop was peanut (*Arachis hypogaea* L.). All agronomic practices for growing pea were done as recommended.

Assessments

Weeds and pea data

Weeds

After 50 and 70 days from sowing in both seasons, frame of wood area in one m² is used and thrown randomly within each experimental unit and collect weeds within the frame and then were identified and classified into two groups (broadleaved, grasses and total weeds were recorded). The dry weight of each group was determined.

Pea crop

Vegetative growth: Fifty and 70 days after sowing, samples of five random plants were taken from experimental plots for recording plant height (cm), shoot dry weight (g) and SPAD value of four pea leaves was determined according to a chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan, Minolta Co., 2013).

Yield traits: At harvest 10 pea plants were randomly chosen from each plot to estimate the number of pods/plant, pods dry weight/plant (g), number of seeds/pod and 100-green seed weight (g). Moreover, whole plants of the experimental unit were harvested for recording pod yield · ha⁻¹.

Seed quality: Total crude protein, phosphorus and potassium were determined according to Cottenie *et al.* (1982).

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) according to Gomez and Gomez (1984), using CoStat Software Program Version 6.303 (2004) and LSD at 0.05 level of significance was used for the comparison between means.

Table 2. Common, trade and chemical names, rate and time of application of herbicides used

Common name	Trade name	Chemical name	Rate of application	Time of application
Clethodium	select super 12.5% EC	(±)-2-[(E)-1-[(E)-3-chloroallyloxyimino] propyl]-5-[2-(ethylthio)propyl]-3-hydroxycyclohex-2-enone	0.625 l · ha ⁻¹	30 days after sowing
Bentazon	Basagran 48% AS	3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide	1.25 l · ha ⁻¹	20 days after sowing
Butralin	Amex, 820 (48% EC)	4-(1, 1dimethylethyl)-N- 1-methyl propyl)-2, 6-dinitrobenzenamine	2.40 · ha ⁻¹ (a.i.)	pre-emergence
Fluazifop-P-butyl	Fusilade 12.5% EC	butyl 2-[4-[5-(trifluoromethyl)pyridin-2-yl]oxyphenoxy]propanoate	1.25 l · ha ⁻¹	30 days after sowing

Results and Discussion

Weed growth

Results in Table 3 and Figures 1, 2, 3 and 4 reveal that both the natural extract of *E. citriodora* and chemical herbicides significantly decreased the dry weight of broad leaved, narrow-leaved and total weeds 50 and 70 days after sowing as compared to the unweeded check. Two hand hoeing was the most effective against broad leaved and narrow-leaved weeds. Butraline came in second, followed by Bentazon + Clethodium and Bentazon + Fluazifop-P-butyl. The maximum significant reductions in total dry weight of weeds were obtained by two hand hoeing (95.08–94.77%) followed by Butraline (93.93–94.65%), Bentazon + Clethodium (93.26–94.07%), Bentazon + Fusilade (91.82–92.77%) and extracts of *E. citriodora* at 25% (91.61–91.95%) in comparison with unweeded treatment 50 and 70 days after sowing, respectively. The results also indicated that there were no significant differences between two hand hoeing, Butraline and Bentazon + Clethodium on the dry weight of total weeds 70 days after sowing. These reductions may be due to the inhibitory effect of herbicides and hand hoeing treatments on growth and development of weeds. These results are in general agreement with those recorded by Rassaeifar *et al.* (2013), Khan *et al.* (2015), Abou El-Ghit (2016), El-Metwally (2016), El-Metwally *et al.* (2017b).

Pea crop

Pea growth

Results in Table 4 reveal significant increases in plant height, shoot dry weight and SPAD values of peas when the natural extract of *E. citriodora* and chemical

herbicide treatments were applied 50 and 70 days after sowing in comparison to unweeded treatment. Two hand hoeing and Butraline gave the maximum values of pea growth without significant differences between them. Applications of both the natural extract of *E. citriodora* and chemical herbicide treatments were effective in controlling weeds (Table 3) and consequently decreased their competition against peas and accordingly the reduction in weed growth was accompanied by increases in bean growth as compared to unweeded treatment. There were no significant differences between Bentazon + Clethodium, Bentazon + Fluazifop-butyl and the extract of *E. citriodora* at 25% treatments on growth characters. These results are in accordance with those recorded by Cheema *et al.* (2003), El-Metwally and Saad El-Deen (2003), Nawaz *et al.* (2014), Fakkar and El-Dakkak (2015), Chaubey *et al.* (2016).

Yield traits

The data in Table 5 reveal that both the natural extract of *E. citriodora* and chemical herbicide treatments induced significant increases in yield traits. Butraline treatment significantly increased pod length and the number of pods/plant as compared to the unweeded treatment. Bentazon + Clethodium treatment gave the highest value in weight of pods/plant. In addition, hand hoeing provided the greatest values of the number of seeds/pod, 100-green seed weight and seed yield ton/fed. In contrast, the minimum values of pea yield traits were recorded with unweeded plots. Two hand hoeing, Bentazon + Clethodium followed by Butraline, Bentazon + Fluazifop-P-butyl and the extract of *E. citriodora* at 25% treatments produced high values of seed yield ton/fed. These treatments significantly increased seed yield ton/fed over the unweeded check by 73.85, 72.97, 70.77, 70.33 and 64.84%, respectively.

Table 3. Effect of the leaf extract of *Eucalyptus citriodora* and some chemical herbicides on the dry weight of pea weeds ($\text{g} \cdot \text{m}^{-2}$) 50 and 70 days after sowing (combined analysis of two seasons)

Treatments	50 days after sowing			70 days after sowing		
	broad-leaved	grasses	total	broad-leaved	grasses	total
<i>Eucalyptus</i> at 5%	18.92	15.22	34.13	23.83	28.90	51.07
<i>Eucalyptus</i> at 10%	8.78	13.93	22.72	20.02	20.58	40.53
<i>Eucalyptus</i> at 15%	5.93	9.08	15.02	11.73	10.98	22.72
<i>Eucalyptus</i> at 20%	4.53	6.17	10.70	7.68	7.08	14.77
<i>Eucalyptus</i> at 25%	3.95	5.20	9.15	6.22	6.58	12.80
Bentazon + Fluazifop-P-butyl	4.05	4.87	8.92	5.40	6.08	12.10
Bentazon + Clethodium	4.23	3.12	7.35	5.02	4.40	9.42
Butraline	2.97	3.65	6.62	4.32	4.18	8.53
Two hand hoeing	2.70	2.67	5.37	4.14	4.17	8.31
Unweeded	53.72	55.38	109.10	74.53	84.38	158.92
LSD 0.05	1.23	1.10	1.6	1.54	1.50	2.69



Fig. 1. Two hand hoeing



Fig. 2. Bentazon + Clethodium



Fig. 3. Extract of *Eucalyptus* at 25%



Fig. 4. Unweeded treatment

Table 4. Effects of the leaf extract of *Eucalyptus citriodora* and some chemical herbicides on growth parameters of pea 50 and 70 days after sowing (combined analysis of two seasons)

Treatments	50 days after sowing			70 days after sowing		
	plant height [cm]	shoot dry weight [g]	SPAD value	plant height [cm]	shoot dry weight [g]	SPAD value
<i>Eucalyptus</i> at 5%	36.38	1.73	38.57	46.33	4.78	38.07
<i>Eucalyptus</i> at 10%	37.45	1.82	37.60	47.27	4.99	39.17
<i>Eucalyptus</i> at 15%	39.93	1.93	39.13	50.15	5.13	40.00
<i>Eucalyptus</i> at 20%	40.30	2.07	40.40	52.38	5.28	40.18
<i>Eucalyptus</i> at 25%	41.35	2.13	40.18	52.55	5.45	40.60
Bentazon + Fluazifop-P-butyl	42.37	2.13	40.27	53.33	5.91	40.92
Bentazon + Clethodium	42.63	2.16	40.40	54.23	5.94	41.30
Butraline	43.17	2.19	40.65	54.37	5.97	41.60
Two hand hoeing	44.35	2.22	40.72	55.28	6.35	42.18
Unweeded	26.78	1.69	36.03	39.83	4.21	34.75

Table 5. Effects of the leaf extract of *Eucalyptus citriodora* and some chemical herbicides on yield and yield attributes as well as chemical composition of pea seeds (combined analysis of two seasons)

Treatments	Pod length [cm]	No. of pod/plant	Weight of pod/plant [g]	No. of seeds/pod	Weight of 100 green seed [g]	Pod yield [ton · fed ⁻¹]	Protein [%]	P [%]	K [%]
<i>Eucalyptus</i> at 5%	7.12	4.43	15.25	4.84	39.59	4.72	21.92	0.27	1.57
<i>Eucalyptus</i> at 10%	7.62	4.69	16.66	5.19	40.95	5.57	21.90	0.28	1.55
<i>Eucalyptus</i> at 15%	8.66	5.25	19.77	5.79	46.13	6.12	22.13	0.30	1.68
<i>Eucalyptus</i> at 20%	8.72	5.93	25.13	5.98	49.94	7.33	22.26	0.31	1.79
<i>Eucalyptus</i> at 25%	8.80	6.45	25.64	6.28	57.04	7.50	22.73	0.31	1.60
Bentazon+ Fluazifop-P-butyl	8.89	7.79	28.13	6.96	52.22	7.75	22.99	0.32	1.88
Bentazon + Clethodium	9.00	8.41	31.05	6.89	51.11	7.87	23.47	0.33	1.98
Butraline	9.20	8.51	30.20	7.23	51.94	7.77	23.53	0.34	2.11
Two hand hoeing	9.12	8.43	30.07	7.45	52.60	7.91	23.34	0.34	2.03
Unweeded	6.57	4.23	14.22	4.61	37.07	4.55	21.06	0.24	1.49
LSD 0.05	0.39	0.25	1.13	0.42	6.03	0.36	2.23	ns	ns

ns – not significant

The increase in yield attributes by the natural extract of *E. citriodora* and chemical herbicide treatments may be due to the good control of weeds which consequently minimizes weed competition, creating a good chance for pea growth and increased yield and yield attributes. The promoting effect of both the natural extract of *E. citriodora* and chemical herbicide treatments on growth characters of pea plants are reflected by increased yield attributes of pea as well as seed yield. There were no significant differences between the application of two hand hoeing, different herbicides and the extract of eucalyptus at 25% on most characters of yield and yield attributes. Similar results have been reported by Fakkar and El-Dakkak (2015) and Chaubey *et al.* (2016).

Seed quality

It is well known that pea seeds contain about 19 to 23% protein (Harmankaya *et al.* 2010), 0.13 to 0.23 phosphorus and 1.01 to 1.14 % potassium (Kandil 2015).

The results in Table 5 reveal that both the natural extract of *E. citriodora* and chemical herbicide treatments significantly increased protein percentage in comparison to that recorded in pea seeds of unweeded plots. The highest value of the protein percentage was recorded with Butraline treatment. Bentazon + Clethodium came in second, followed by two hand hoeing without significant differences between them. The lowest protein percentage was found in peas from untreated plots. There were no significant differences between all weed control treatments in phosphorus and potassium characters. The increase in protein percentage may be due to less competition of growth factors (nutrients, water and light) through limiting weed infestation with hoeing or the natural extract of *E. citriodora*

and chemical herbicide treatments. So, decreasing the competition between weeds and pea plants in turn increased the uptake of different nutrients. These results coincide with those reported by Fakkar and El-Dakkak (2015) and Chaubey *et al.* (2016).

In general, in accordance to the above results, several workers have shown that controlling weeds by different herbicides reduced weed/plant competition and consequently, increased product income (Abdelhamid and El-Metwally 2008; El-Metwally *et al.* 2010).

In an attempt to reduce environmental pollution and reduce the resistance of weeds to herbicides, several workers have controlled weeds in crop plants using natural extracts of different plants (Batish *et al.* 2006; Cheema *et al.* 2013; El-Rokiek *et al.* 2016, 2018). The inhibition in weed growth by such natural extracts may be attributed to the presence of some causative allelochemicals (Chon *et al.* 2003). In previous work by El-Rokiek and Eid (2009), chromatographic analysis of the volatile oils of dry leaves of *E. citriodora* revealed the presence of major constituents of α -terpineol, for example, α -pinene, terpinene, citronellal, citronellol, borneol and linalool. The authors discussed the inhibition in weed growth in light of the presence of those substances. Similar conclusions were made by Haibin *et al.* (2009) and El-Rokiek *et al.* (2018). Analyses of the dry leaf extract of *E. citriodora* by HPLC indicated the presence of ferulic, coumaric, benzoic, vanilic, chlorogenic, caffeic, gallic, hydroxybenzoic acids (El-Rokiek and El-Nagdi 2011). The authors attributed the herbicidal potential against weeds to those phenolic acids, supporting the findings of Chon and Kim (2004). The results revealed that the best treatment in pod yield/fed. was 7.87 ton/fed. with using a combined treatment

of Bentazon + Clethodim (Table 5). The corresponding results with spraying *E. citriodora* leaf extract was 7.50 although the difference between the two results was significant, the authors suggested using safe methods as the allelopathy only (herein, *E. citriodora* leaf extract) in controlling weeds in a strategy to minimize crop damage and environmental pollution. The direct incorporation of allelopathic plant materials into rice fields clearly reduced the weed interference (Xuan *et al.* 2005; Khanh *et al.* 2006).

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