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Allelopathic effects of Eucalyptus globulus Labill. on seed germination and seedling growth of eggplant (Solanum melongena L.)

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ABSTRACT: Eucalyptus spp. are known to contain various substances that are allelopathic. The objective of this study was to determine allelopathic effects of various solvent extracts (aqueous, methanolic, ethyl acetate, acetonic and benzene) of Eucalyptus globulus leaves in five concentrations (0, 1.25, 2.5, 5 and 10 g/L) on seed germination and early seedling growth of eggplant cv. Black Beauty. The experiment was carried out using a completely randomized design arranged in factorial scheme with three replicates. The results showed that all extracts had inhibitory effects on germination and seedling growth although degree of their inhibitions was different. Methanolic extract had the highest inhibitory effect on germination percentage and rate, root and shoot lengths and fresh and dry weights of eggplant seedlings whereas aqueous extract followed by acetonic extract had the lowest inhibitory effects. In all extracts, the reduction in germination and growth of eggplant seedlings increased significantly with increasing concentration. The results suggest that leaf extracts of E. globulus had potent allelopathic activity although the activity differed depending on extract and concentration.

***Keywords*:** Allelopathy, Eggplant, Eucalyptus globulus, Germination, Growth

INTRODUCTION

Agroforestry is the integration of trees and shrubs into farming landscapes to increase the farm productivity and sustainability of farming systems (Fikreyesus et al., 2011). The inhibitory effect of one plant by another through releasing allelochemicals that is called “Allelopathy” has been well known (Djanaguiraman et al., 2005). Therefore, it seems essential that the allelopathic compatibilitiy of crops with trees should be checked that trees have not harmful effects on associated crops. It has been shown that *Eucalyptus* species have strong allelopathic activity among examined plants (Gliessman, 2007). *Eucalyptus* spp. belong to Myrtaceae family and are indigenous to Australia (May and Ash, 1990). They have been widely introduced into countries throughout the world (Turnbull, 1999) and have been cultivated in dry and saline areas of south of Iran and in Mazandaran located on north of Iran (Ziaebrahimi et al., 2007). Large area of ground surface beneath of eucalyptus remains bare and is limited understory vegetation growth (El-Darier, 2002). It has been shown that where eucalyptus stand is replaced by the agricultural crop, that crop will not grow well, at least for a number of years (Fikreyesus et al., 2011). (Del Moral and Muller ,1970) reported that annual vegetation adjacent to *Eucalyptus camaldulensis* is often severely inhibited by allelochemicals released by this species. The allelopathic effects of *Eucalyptus* spp. have been studied extensively (Alves et al., 1999; Bajwa and Nazi, 2005; El-khawas and Shehata, 2005). They are considered as one of the strongest allelopathic trees that have most number of allelochemicals (El-Darier, 2002; Ziaebrahimi et al., 2007). The allelopathic effect of eucalyptus has been attributed to the production of several volatile terpenes and phenolic acids (Djanaguiraman et al., 2005; Florentine and Fox, 2003; Sasikumar et al., 2002). Phytotoxic phenolic substances in leaf extracts of E. globulus produce volatile terpenoids that have inhibitory effects on germination and seedling growth of various crops (Sasikumar et al., 2002; Florentine and Fox, 2003). Phytotoxic substances are released into the soil ecosystem through volatilization, root exudation and leaching from the foliage (Djanaguiraman et al., 2005). These chemicals exhibit a wide range of mode of action. They may influence cell division and elongation, growth substances, enzymatic activities, respiration, photosynthesis or chlorophyll content, stomata and membrane integrity, water relations and mineral uptake (El-khawas and Shehata, 2005; Mohamadi and Rajaie, 2009).

Many studies have evaluated the allelopathic effects of Eucalyptus species and confirmed the strong inhibitory effects of eucalyptus extracts on some crops (Zhang and Shenglei, 2010). Leaf extract of eucalyptus inhibited seed germination and reduced root and shoot lengths of cucumber and maximum inhibition was observed in higher concentrations of extract (Allolli and Narayanareddy, 2000). The leaf leachate of E. globulus inhibited germination and growth of rice, sorghum and blackgram (Djanaguiraman et al., 2005). Moreover, the extract of E. globulus inhibited germination and seedling growth of greengram and cowpea (Djanaguiraman et al., 2002) and blackgram (Sasikumar et al., 2002; Djanaguiraman et al., 2002). (El-khawas and Shehata ,2005) found that leaf extract of E. globulus inhibited germination of maize and kidney-bean. The allelopathic effect of extract from E. camaldulensis was tested on tomato; the extract significantly inhibited germination and growth of this plant (Fikreyesus et al., 2011).

Although the inhibitory effects of Eucalyptus spp. have been investigated on some crops, information on allelopathic effects of E. globulus on eggplant is scanty. Hence, the present study was conducted to determine the influence of aqueous, methanolic, ethyl acetate, acetonic and benzene extracts of E. globulus leaves on seed germination and seedling growth of eggplant cv. Black Beauty.

MATERIALS AND METHODS

Preparation of eucalyptus extracts

Fresh samples of leaves of *E. globulus* were collected in early 2012 from Botanical garden of Islamic Azad University, Fasa Branch, Fars, Iran. The samples were air-dried in shade and then powdered using grinder. For preparation of extracts, various solvents (water, methanol, acetone, ethyl acetate and benzene) in the ratio of 1:5 (weight: volume) mixed leaf powder and each mixture was placed on shaker for 1 hour and kept in 4ºC for 24 hours then placed on shaker for 1 hour again. The mixture of each extract was filtered through filter paper Whatman no. 42 and each extract was dried using rotary evaporator model 'RV 10'. Finally, for each extract, various concentrations including 0, 1.25, 2.5, 5 and 10 g/L were prepared by using representative solvent.

Seed bioassay

Seeds of Eggplant cv. Black Beauty were obtained from reliable seed shop. The eggplant seeds of uniform color and size were surface sterilized with 1.5% sodium hypochlorite for 20 minutes and then thoroughly washed with tap water for several times followed by distilled water. Twenty seeds were germinated in Petri-dishes containing filter paper Whatman number 1 and added five ml of each extract at concencetrations 0, 1.25, 2.5, 5 and 10 g/L. The Petri-dishes were kept in germinator in 24ºC and darkness. Seeds were considered germinated when root length was 1-2 mm and the number of germinated seeds was recorded every 24 hours until 14 days. Germination rate according to (Maguire ,1962), germination percentage, root and shoot lengths and fresh and dry weights of samples were recorded after 14 days.

Experimental design and data analysis

The experimental design was a completely randomized design, with 25 treatments arranged in a factorial scheme with three replicates. All experimental data were subjected to analysis of variance by using MSTAT-C program and comparison of means was performed using least significant difference (LSD) at the 5% level of probability ($p<0.05$).

RESULTS AND DISCUSSION

Seed germination

As shown in Table 1, among various solvent extracts, maximum germination percentage and germination rate were observed in aqueous extract (64.34% and 1.28 number in day, respectively) while the minimum was found in methanolic extract (12% and 0.14number in day, respectively). The study revealed that the inhibitory effect of extracts increased with increasing extract concentration (Table 2). The highest inhibitory effect on germination percentage and rate were found in concentration of 10g/L (12% and 0.20 number in day, respectively) while the lowest was found in control (64.33% and 1.33 number in day, respectively) (Table 2). A significant interaction was found between concentration and various solvent extracts on seed germination. The magnitude of reduction in germination percentage and rate in all extracts were proportional with extract concentration. The highest germination percentage and rate were found in acetonic and aqueous extracts at control treatment. On the other hand, germination didn’t happened in benzene extract at concentrations higher than1.25 g/L (Table 3).

Table1. Effect of various solvent extracts of *E. globulus* leaf on seed germination and seedling growth of eggplant cv. Black Beauty

Type of extract	Germination percentage	Germination rate (number in day)	Root length (mm)	Shoot length (mm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Methanolic	12	0.14	2.45	2.30	3.25	0.50
Acetonic	21.67	0.64	10.71	9.98	13.15	1.79
Ethyl acetat	20.35	0.27	5.25	3.10	6.68	0.70
Benzene	15.67	0.25	4.61	3.76	5.17	0.75
Aqueous	64.34	1.28	23.52	20.97	26.67	2.97
LSD (5%)	6.23	0.07	0.21	0.24	1.28	0.22

Table2. Effect of various concentrations of E. globulus leaf extracts on seed germination and seedling growth of eggplant cv. Black Beauty

Extract concentration (g/L)	Germination percentage	Germination rate (number in day)	Root length (mm)	Shoot length (mm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
0	64.33	1.33	22.84	23.13	25.93	2.53
1.25	21.67	0.39	7.90	6.01	9.33	1.51
2.5	20.35	0.40	8.74	6.10	10.22	1.32
5	15.67	0.26	4.42	3.77	5.50	0.75
10	12.00	0.20	2.64	2.00	3.94	0.62
LSD (5%)	6.23	0.07	0.21	0.24	1.28	0.22

Table 3. Effects of various concentrations of E. globulus leaf extracts on germination percentage and germination rate of eggplant cv. Black Beauty

Type of extract	Germination percentage					Germination rate (number in day)				
	Extract concentration (g/L)					Extract concentration (g/L)				
	0	1.25	2.5	5	10	0	1.25	2.5	5	10
Methanolic	31.67	6.67	1.67	1.67	3.33	0.47	0.08	0.04	0.04	0.07
Acetonic	91.67	21.67	31.67	10.00	3.33	2.24	0.30	0.49	0.13	0.04
Ethyl acetate	40.00	13.37	5.00	10.00	0.00	0.80	0.31	0.14	0.11	0.00
Benzene	67.33	11.67	0.00	0.00	0.00	1.11	0.15	0.00	0.00	0.00
Aqueous	90.00	55.00	63.40	56.70	53.33	2.05	1.09	1.33	1.00	0.91
LSD (5%)	6.23					0.07				

Root and shoot lengths

Table 1 shows that methanolic extract significantly inhibited root and shoot growth while aqueous extract had the lowest inhibitory effect. According to Table 2, higher concentrations induced greater phytotoxicity, so shortest root and shoot lengths were found in concentration of 10 g/L that were 2.64 and 2mm, respectively. The interaction between concentration and solvent extracts on root and shoot lengths was significant. Maximum root and shoot lengths were found in aqueous and acetonic extract in control treatment (Table 4). Methanolic extract in all concentrations except control as well as benzene extract at concentrations higher than 1.25 g/L suppressed root and shoot lengths completely. Similar results were recorded by ethyl acetate at 5 and 10 g/ L (Table 4).

Table 4. Effects of various concentrations of *E. globulus* leaf extracts on root and shoot lengths of eggplant cv. Black Beauty

Type of extract	Root length (mm)					Shoot length (mm)				
	Extract concentration (g/lit)					Extract concentration (g/lit)				
	0	1.25	2.5	5	10	0	1.25	2.5	5	10
Methanolic	22.25	0.00	0.00	0.00	0.00	11.15	0.00	0.00	0.00	0.00
Acetonic	31.27	8.11	12.08	2.08	0.00	35.30	6.78	5.91	1.92	0.00
Ethyl acetate	16.26	2.50	7.50	0.00	0.00	14.99	1.67	3.33	0.00	0.00
Benzene	19.33	3.74	0.00	0.00	0.00	17.16	1.67	0.00	0.00	0.00
Aqueous	35.06	25.25	24.12	20.03	13.22	36.67	19.94	21.28	16.94	10.00
LSD (5%)			0.21					0.24		

Fresh and dry weights

Methanolic extract had the highest inhibitory effect on both fresh and dry weights of eggplant seedlings cv. Black Beauty (3.25 and 0.50 mg, respectively) while the highest fresh and dry weights were observed in aqueous extract (26.67 and 2.97 mg, respectively) (Table 1). It was found that higher concentrations had pronounced inhibitory effects on seedlings fresh and dry weights (Table 2). Table 5 shows interaction between type of solvent extracts and extract concentrations. In general, in all extracts, higher concentrations induced allelopathic effects. Maximum fresh and dry weights were observed in untreated control. However, fresh and dry weights of eggplant seedlings were zero at concentrations up to 1.25, 2.5 and 5 g/L in methanolic, benzene and ethyl acetate extracts, respectively.

Table 5. Effects of various concentrations of E. globulus leaf extracts on fresh and dry weights of seedlings of eggplant cv. Black Beauty

Type of extract	Seedling fresh weight (mg)					Seedling dry weight (mg)				
	Extract concentration (g/lit)					Extract concentration (g/lit)				
	0	1.25	2.5	5	10	0	1.25	2.5	5	10
Methanolic	16.39	0.00	0.00	0.00	0.00	2.53	0.00	0.00	0.00	0.00
Acetonic	35.00	12.22	15.22	3.33	0.00	2.43	2.58	3.13	0.83	0.00
Ethyl acetate	19.11	4.17	10.00	0.00	0.00	1.85	1.00	0.66	0.00	0.00
Benzene	22.50	3.33	0.00	0.00	0.00	2.85	0.90	0.00	0.00	0.00
Aqueous	36.67	26.92	25.86	24.18	19.70	2.97	3.08	2.79	2.91	3.08
LSD (5%)			1.28					0.22		

DISCUSSION

The results of this study showed that all solvent extracts had allelopathic effects on germination and seedling growth of eggplant cv. Black Beauty and inhibition increased with increasing concentration. These results agree to those obtained by (Mohamadi and Rajaie ,2009) that studied the effect of leaf extract of E. camaldulensis on sorghum and kidney bean. The results of present study were similar to those of (Malik ,2004), (El-Khawas and Shehata ,2005 and Yamagushi et al., 2011) that studied allelopathic effects of E. globulus leaf extract on germination and seedling growth of some vegetable and crop plants.

According to our results, various solvent extracts reduced germination percentage and germination rate and the highest inhibition was observed in highest concentration (10g/L). Similar results were obtained in tomato by (Fikreyesus et al., 2000), in wheat by (Khan et al., 2008) and in cucumber by (Allolli and Narayanareddy, 2000) through leaf extract of various Eucalyptus species. Several phenolic compounds such as caffeic, coumaric, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids have been identified in leaf extracts of three Eucalyptus hybrids (Chapius-Lardy et al., 2002) that have allelopathic potential (Rice, 1984) and can inhibit the activity of gibberellic acid (GA). GA is known to regulate enzymatic activity and conversion of reserved materials to transferable materials during seed germination and embryo growth (Das et al., 2012). Therefore, it is possible that these processes should inhibited by eucalyptus leaf extracts.

The lengths of root and shoot were greatly reduced in all leaf extracts of E. globulus and inhibition increased with increasing concentration. (Zhang and Shenglei ,2010) reported that the length of radicles and plumules of radish, cucumber and chinese cabbage treated with litter extracts of three Eucalyptus species were shorter than control and higher concentration induced greater phytotoxicity. In addition, leaf extract of E. camaldulensis decreased root and shoot lengths of tomato (Fikreyesus et al., 2011).

Fresh and dry weights were also reduced significantly in all extracts in current study. These results are in agreement to those obtained by (Djanaguiraman et al., 2005) who found that seedling dry matter of rice, sorghum and blackgram significantly reduced by leaf leachate of E. globulus and highest inhibition was observed in highest concentration. Moreover, (El-Darier ,2002) reported that dry weights of broad bean and maize reduced by leaf-litter water extract of E. rostrata. Fresh and dry weights of three wheat cultivars decreased in response to aqueous eucalyptus extract (Ziaebrahimi et al., 2007).The interference with nutrient uptake and reduction in nutrient accumulation is one of the most effective mechanisms of phenolic compounds action (El-Darier, 2005). This concept was confirmed by (El-Darier ,2005) who showed that allelopathic compounds released from eucalyptus leaf extract suppressed the uptake of N, P and K by broad bean and maize. Many polyphenols have catechol groups and at higher concentrations can chelate divalent or trivalent metal ions. Therefore, they inhibit ion uptake and thus cause reduction in seedling dry matter (Crawley, 1997).

The exact mechanism that germination and seedling growth are affected by eucalyptus leaf extracts is not known. However, studies show that besides phenolics, terpenoids, particularly monoterpenes are the main components of essential oils of Eucalyptus spp. that interfere in mitosis of growing cells and respiration and reduce photosynthesis (Batish et al., 2004). Inhibition of these vital processes can reduce or even inhibit seed germination and seedling growth.

From the present study, it can be concluded that various solvent extracts of leaves of E. globulus had allelopathic effects on germination and seedling growth of eggplant cv. Black Beauty. The extracts reduced germination and growth of seedlings and this inhibitory effect increased with increasing extract concentration. Inhibitory effect of various solvent extracts was not equal and highest inhibition was observed in methanolic extract while the lowest one was observed in aqueous and acetonic extracts. This study revealed the allelopathic potential of leaf extract of E. globulus on eggplant cv. Black Beauty. Further study must be done to know the allelochemical of the extracts.

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