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PHYTOTOXIC EFFECTS OF *Eucalyptus globulus* LEAF EXTRACT ON *Solanum nigrum*

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ABSTRACT. *Allelopathic effects of Eucalyptus species have been widely reported and considered as a natural way for sustainable weed management. However, in most of these reports the essential oils and aqueous extracts have been evaluated. The present study was conducted in order to evaluate the phytotoxic effects of aqueous extract along with various organic extracts of E. globulus leaves against Solanum nigrum weed. This experiment was carried out as a completely randomized design with factorial layout. Studied factors were various solvent extracts (aqueous, methanolic, ethyl acetate, acetonic and benzene) and different concentrations of extracts (0, 1.25, 2.5, 5 and 10 gram per liter). Results indicated that with increasing of extract concentration germination percentage and rate, root and shoot lengths and fresh and dry weights significantly decreased. Among the extracts tested, the highest inhibitory effect was observed with methanolic and ethyl acetate extracts and the lowest ones were seen in aquoues and acetonic extracts. According to the results, methanolic and ethyl acetate extracts had the strong inhibitory effect on germination at higher concentrations (5 and 10g/l) while they could completely inhibited seedling growth of S. nigrum even at lower concentration (2.5g/l). Therefore, it is possible to use methanolic and ethyl acetate extracts for control of S. nigrum weed and it can be considered in management program of weed control.*

KEYWORDS*: Eucalyptus globulus, Solanum nigrum, phytotoxicity, seed germination, seedling growth*

INTRODUCTION

Weed infestation in agricultural fields results in reduction of quality and quantity of crops and huge economic losses (Rassaeifar et al. 2013, Singh et al. 2005). There are several methods for weed control including mechanical, chemical and biological methods (Rassaeifar et al. 2013). However, the intensive use of chemical herbicides has resulted in development of herbicidal resistance amongst weed and negative impacts on the environment (such as groundwater contamination) and human health (Sodaeizadeh and Hosseini 2012, Verdeguer et al. 2009). To overcome these problems, efforts are being made to reduce the reliance on chemical herbicides (Singh et al. 2005) and to produce biological herbicides with environmental coexistence and desirable herbicidal action (Rassaeifar et al. 2013, Singh et al. 2003). In recent years, the use of allelopathic plants and their products as a sustainable management strategy for crop protection against weeds has been widely noticed (Singh et al. 2003, Sodaeizadeh and Hosseini 2012). Different plants have allelochemicals that could be used for suppressing weeds growth (Singh et al. 2003, Mubeen et al. 2012). Eucalyptus (Myrtaceae), a native to Australia, is one of the potential allelopathic plants having a number of allelochemicals (Ziaebrahimi et al. 2007, El-Darier 2002) and its essential oils possess pesticidal activity (Batish et al. 2008). It has been shown that *Eucalyptus* species have strong allelopathic activity (Gliessman 2007) and it 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, Setia et al. 2007). The phytotoxic effects of eucalyptus species have been evaluated against a number of weed species. For example, essential oil of *Eucalyptus camaldulensis* suppressed germination and seedling growth of *Amaranthus hybrid* and *Portulaca oleracea* (Verdeguer et al. 2009). The herbicidal activity of *Eucalyptus globulus* essential oil against *Amaranthus blitoides*, *Cynodon doctylon* (Rassaeifar et al. 2013), and *Parthenium hysterophorus* (Kohli et al. 1998), of *E. citriodora* against *Cassia occidentalis*, *Echinochloa crus-galli, Phalaris minor, Parthenium hysterophorus* and *Amaranthus virids* (Batish et al. 2004, 2007, Kohli et al.

1998), and of *E. tereticornis* against *Amaranthus viridis* (Kaur et al. 2011) has also been documented.

The aim of the present work was to study the various solvents extracts of *Eucalyptus globulus* leaf in order to know if these extracts have phytotoxic effects against *Solanum nigrum* (a problematic weed in Mideast region) germination and seedling growth and which of the solvents has the strongest inhibitory effect.

MATERIALS AND METHODS

Preparation of *E. globulus* extract

Leaves of *E. globulus* were collected from 12-year-old trees growing in Botanical garden of Islamic Azad University, Fasa branch, Fars, Iran. After air drying at 25°C and powdering using grinder, the powdered leaves were mixed with various solvents (water, methanol, acetone, ethyl acetate and benzene) in the ratio of 1:5 (w/v) and the mixtures were placed on shaker for 1 hour. Then the mixture was kept at 4ºC for 24 h and placed on shaker for 1 h again. The obtained mixture was filtered using Whatman # 42 filter paper and the extracts were concentrated to dryness using rotary evaporator (RV 10 model). The yields of aqueous, methanol, acetone, ethyl acetate and benzene extracts were 9.77, 12.89, 6.45, 4.36, and 3.52 respectively. The working concentrations of 0, 1.25, 2.5, 5 and 10 g/l were prepared using the representative solvents and the effect of these concentrations was tested on *Solanum nigrum*.

Preparation and planting of *S. nigrum* seeds

Uniform healthy seeds of *S. nigrum* were collected locally from university campus. Seeds were first disinfected using a 1.5% solution of sodium hypochlorite and washed with tap water several times followed by distilled water. Sets of 20 seeds each, with three replicates per treatment, were placed in Petri dishes (9 cm diameter) between 2 layers of filter paper (Whatman # 1) and 5 ml of each extract was added. All the Petri dishes were kept in a growth chamber maintained at darkness at 24ºC temperature for one week and then they were brought to a light regime with 16 h light and 8 h darkness. To evaluate the phytotoxic activity of the extracts and their inhibitory effects on germination and seedling growth, data were recorded after 14 days.

Statistical analysis

The experiments were conducted in a completely randomized design with a factorial arrangement of 5×5 and with three replications. The data collected were analyzed statistically using MSTATC software and the means compared by least significant differences (LSD) test ($p<0.05$).

RESULTS

Effect of extracts on germination of *S. nigrum* seeds

The various leaf extracts of *E. globulus* had different degrees of inhibition on germination percentage and rate of *S. nigrum* seeds. The maximum inhibitory activity was observed with methanolic extract and the minimum effect was seen with aqueous extract (Table 1). The phytotoxic effects of *E. globulus* extracts on seed germination depended on their concentrations, the inhibition was stronger at the higher concentrations (Table 2). The methanolic extract showed high phytotoxicity against *S. nigrum* and no seed germinated at 10g/l treatment of extract (Table 3). Ethyl acetate extract also had the strong inhibitory effect on seed germination, especially at higher concentrations (5 and 10 g/l). On the other hand, aqueous extract had minimum inhibitory effect on seed germination even at 10 g/l concentration so that no significant differences were observed among various concentrations of this extract.

Table 1. Effect of various solvents extract of *E. globulus* leaf on seed germination and seedling growth of *Solanum nigrum.*

Type of solvent	Germination percentage	Germination rate (number in day)	Root length (mm)	Shoot length (mm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Methanol	36.00	0.99	7.28	6.34	2.42	0.19
Acetone	49.50	1.17	14.09	9.34	4.13	0.38
Ethyl acetate	42.33	1.01	3.17	2.97	1.67	0.14
Benzene	63.17	1.66	14.22	12.58	5.04	0.35
Water	93.00	3.26	13.21	21.87	8.55	0.52
LSD (5%)	17.34	0.39	0.27	0.32	1.05	0.08

Table 2. Effect of various concentrations of *E. globulus* leaf extracts on seed germination and seedling growth of *Solanum nigrum.*

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	95.33	3.18	28.78	24.06	9.45	0.58
1.25	69.33	1.85	13.34	16.81	6.74	0.54
2.5	58.50	1.45	6.85	9.51	3.91	0.31
5	38.00	1.01	2.99	2.81	1.71	0.17
10	22.83	0.61	0.00	0.00	0.00	0.00
LSD (5%)	17.34	0.39	0.27	0.32	1.05	0.08

Table 3. Interaction between type of solvents and various concentrations of *E. globulus* leaf extract on germination percentage and germination rate of *Solanum nigrum.*

Type of solvent	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
Methanol	90.00	53.33	21.67	15.00	0.00	3.00	1.29	0.41	0.25	0.00
Acetone	94.17	67.50	65.00	18.33	2.50	3.23	1.22	1.05	0.31	0.07
Ethyl acetate	98.00	60.00	48.33	13.33	1.66	3.17	1.10	0.74	0.04	0.00
Benzene	98.33	66.67	57.50	63.33	30.00	2.55	2.22	1.78	1.23	0.51
Water	95.83	99.17	100.0	90.00	80.00	3.93	3.43	3.27	3.22	2.45
LSD (5%)			17.24					0.39		

Effects of extracts on root and shoot lengths of *S. nigrum*

For initial seedling growth, all various extracts had different effects on shoot and root lengths of *S. nigrum*. The stronger inhibitory effect on root and shoot lengths were found in ethyl acetate extract followed by methanolic extract (Table 1). Moreover, in all extracts higher concentrations caused greater reduction of root and shoot growth. So, seedling growth ceased completely at 10 g/l concentration (Table 2). Ethyl acetate and methanolic extracts had the strongest inhibitory effect on root and shoot lengths (Table

4). Both ethyl acetate and methanolic extracts inhibited seedling growth at 2.5, 5 and 10 g/l concentrations, while aqueous, acetonic and benzene extracts inhibited seedling growth at highest concentration (10 g/l).

Table 4. Interaction between type of solvents and various concentrations of *E. globulus* leaf extract on root and shoot lengths of *Solanum nigrum* seedling.

Type of solvent	Root length (mm)					Shoot length (mm)				
	Extract concentration (g/l)					Extract concentration (g/l)				
	0	1.25	2.5	5	10	0	1.25	2.5	5	10
Methanol	33.30	3.10	0.00	0.00	0.00	25.70	6.00	0.00	0.00	0.00
Acetone	38.03	12.20	13.80	6.40	0.00	24.33	10.23	8.30	4.27	0.00
Ethyl acetate	12.63	3.20	0.00	0.00	0.00	10.13	4.70	0.00	0.00	0.00
Benzene	25.97	29.23	9.53	6.37	0.00	27.33	21.67	10.03	3.87	0.00
Water	33.97	18.97	10.93	2.20	0.00	32.80	41.43	29.20	5.93	0.00
LSD (5%)	0.27					0.32				

Effects of extracts on fresh and dry weights of *S.nigrum* seedlings

Similar to root and shoot lengths, for fresh and dry weights, ethyl acetate and methanolic extracts caused greater phytotoxicity in comparison with other extracts (Table 1). In all extracts, decrease in fresh and dry weights of seedlings became greater with increase in concentration, so that the minimum fresh and dry weights of *S. nigrum* seedlings were observed at 10g/l concentration (Table 2). The interactions between type of solvents and various extract concentrations of *E. globulus* on fresh and dry weights of *S. nigrum* seedling are presented in Table 5. Methanolic and ethyl acetate extracts showed the highest inhibitory effect on fresh and dry weights, so that in both of them, fresh and dry weights of seedlings were 0 at concentrations of above 1.25 g/l. On the other hand, aqueous extract had minimum inhibition on weights of seedlings.

DISCUSSION

The results of present study showed that leaf extracts of *E. globulus* had

Table 5. Interaction between type of solvents and various concentrations of *E. globulus* leaf extract on fresh and dry weights of *Solanum nigrum* seedling.

Type of solvent	Seedling fresh weight (mg)					Seedling dry weight (mg)				
	Extract concentration (g/l)					Extract concentration (g/l)				
	0	1.25	2.5	5	10	0	1.25	2.5	5	10
Methanol	8.63	3.46	0.00	0.00	0.00	0.46	0.52	0.00	0.00	0.00
Acetone	9.83	4.90	4.10	1.83	0.00	0.53	0.60	0.53	0.23	0.00
Ethyl acetate	5.60	2.73	0.00	0.00	0.00	0.53	0.16	0.00	0.00	0.00
Benzene	9.97	8.43	5.07	1.73	0.00	0.56	0.53	0.46	0.20	0.00
Water	13.20	14.17	10.40	5.00	0.00	0.80	0.86	0.53	0.40	0.00
LSD (5%)	1.05					0.09				

phytotoxic effects on germination and seedling growth of *S. nigrum* seeds. The phytotoxicity of volatile oils and extracts of different *Eucalyptus* species has been reported against many weedy species (Saberi et al. 2013, Azizi and Fuji 2006, Singh et al. 2002, Batish et al. 2004, 2007, Kohli et al. 1998; Setia et al. 2007). In present study, the leaf extracts of *E. globulus* had varying degrees of phytotoxicity against *S. nigrum*. Methanolic and ethyl acetate extracts had maximum inhibitory effects while aqueous extract had least inhibition. Solubility of allelochemicals is one of the major factors determining their phytotoxicity (Kaur et al. 2012). The results of this study demonstrated that lipophilic substances that dissolve readily in organic solvents like methanol and ethyl acetate had more phytotoxic activity than water soluble allelochemicals. However Abrahim et al (2000) found that relatively more lipophilic monoterpenes exhibited less activity compared to water soluble oxygenated monoterpenes towards germination and root growth, despite the fact they had a higher activity on oxidative metabolism of isolated mitochondria of *Zea mays*. Therefore, it is possible that water, methanol and ethyl acetate soluble substances have different degrees of phytotoxicity probably because of different mode of action.

In this study, the inhibitory effects of extracts on germination and seedling growth of *S. nigrum* were increased with increase in concentration especially in methanolic and ethyl acetate extracts. Rassaeifar et al. (2013)

reported that the length of radicles and plumules of *Amaranthus blitoides* and *Cynodon dactylon* treated with essential oil extracted from leaves of *E. globulus* were shorter than control and higher concentration induced greater phytotoxicity (Rassaeifar et al. 2013). In addition, essential oil of *E. camaldulensis* decreased seedling length of *Amaranthus hybridus* and *Portulaca oleracea* (Verdeguer et al. 2009). Niakan and Saberi (2009) also found that aqueous extract of *E. camaldulensis* reduced fresh and dry weights of *Phalaris* seedlings. The results of present study are similar to above mentioned findings. A number of volatile and non-volatile allelochemicals have been reported to be released from eucalypt trees and involved in allelopathic effects of tree (Kohli 1990). Several phenolic compounds such as caffeic, coumaric, gallic, gentisic, hydroxybenzoic, syringic, ferulic and vanillic acids have been identified in the leaves and understorey soil of eucalyptus plantations(Kohli 1990) and methanol and aqueous leaf extracts of three *Eucalyptus* hybrids (Chapius-Lardy et al. 2002) that have allelopathic potential (Rice 1984). These compounds have been reported to cause clogging of stomata, enhanced electrolyte leakage and impairment of photosynthetic and energy machinery (Kaur et al. 2012). The leaf volatile oils of *E. tereticornis* caused a significant reduction in early seedling growth and vigor, respiration and photosynthetic pigments of *Amaranthus viridis* (Kaur et al. 2011). The allelopathic effects of eucalyptus are also due to inhibition of some physiological process such as nutrient uptake, cell division, synthesis of carbohydrates, proteins and nucleic acids and phosphorylation pathways (Sasikumar et al. 2002). These inhibitory effects can mediate by phenolic compounds (El-Darier 2002). Therefore, these phenolic compounds reduce seed germination and growth of seedlings (Crawley 1997). It has been reported that total phenolic content of methanolic leaf extracts of three eucalyptus hybrids was higher than aqueous leaf extracts (Chapius-Lardy et al. 2002). Therefore, the higher inhibitory effect of methanol extract can be due to higher amounts of phenolic compounds. This conclusion is in agreement with the results of present study.

CONCLUSION

The result of this study showed that different extracts of *E. globulus* leaf had varying degrees of inhibition on germination and seedling growth of *S. nigrum* weed and more inhibition was seen with higher concentrations. In present study, methanolic and ethyl acetate extracts had the strongest inhibitory effects. Therefore, it is possible to use these two extracts as a component for production of bioherbicides. However further works are needed for greenhouse and field test and chemical characterization of these extracts.

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