

PHYSIO-MORPHOLOGICAL RESPONSES OF NATIVE *ACACIA NILOTICA* TO *EUCALYPTUS* ALLELOPATHY

F.A. BUGHIO^{1*}, S.M. MANGRIO¹, S.A. ABRO¹, T.M. JAHANGIR² AND HADI BUX¹

¹Institute of Plant Sciences, University of Sindh, Jamshoro, Sindh, Pakistan

²Institute of Advanced Research Studies in Chemical Sciences, University of Sindh, Jamshoro, Sindh, Pakistan

*Corresponding author's e-mail: farooquealibughio@yahoo.com

Abstract

Allelopathic effects of exotic *Eucalyptus camaldulensis* Dehnh. have been widely reported and are considered the major factor limiting the establishment of native tree species in local forests. A lab study was conducted to investigate the allelopathic effects of *Eucalyptus camaldulensis* on native trees and *Acacia nilotica* was selected as bioassay plant. Since *Eucalyptus* leaf litter enters in the soil environment either in the form of solid matter or as aqueous extract. The allelopathic impacts of these two forms were assessed in laboratory experiment conducted at Green House of Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. In a pot experiment the leaf litter was applied in total 6 concentrations along a control, no leaf litter. The three concentrations of ground solid leaf litter were mixed with soil in 10, 20 and 30%. Similarly, the aqueous stock solution of 1:20 was prepared and dilutions were made in 10, 20 and 30%. The plant parameters such as percent germination, relative germination ratio (RGR), percentage mortality rate (MR), seedling vigour index (SVI), chlorophyll content (mg/g f. wt.), relative elongation of shoot (RERs), relative elongation of root (RERr), relative biomass ratio (RBR), fresh and dry weight (g) was calculated. Soil samples were taken from laboratory experiments and analyzed for pH, soil salinity, organic matter, N, P, K, and Na. Results suggest that *Eucalyptus* leaf litter and leaf litter aqueous extract significantly reduced the frequency of percent germination and relative germination ratio, *Eucalyptus* leaf litter show high mortality rate than leaf litter extract, reduced seedling vigour index, chlorophyll content, relative elongation of shoot and relative elongation of root, also reduced the fresh and dry weight of the plant. The soil pH decreased with increasing concentration and soil salinity increased with increasing the concentration. While organic matter, nitrogen, phosphorus and potassium concentrations increased in soil with increasing concentration. The solid leaf litter was found more dangerous than aqueous extracts.

Introduction

The exotic invasive species are considered to be the second largest cause of biodiversity loss after habitat destruction (Schwartz *et al.*, 1996; Shinwari *et al.*, 2012). The negative impact of such species on native biodiversity has been well documented (D'Antonio & Mahall 1991; Ridenour & Callaway, 2001). Exotic invasive plants have been found to change the composition of native communities and been associated with reduced native plant diversity (Bone *et al.*, 1997; Ferreira & Marques, 1998; Shinwari & Qaisar, 2011). Many native forests have been destroyed by the introduction and large-scale planting of fast growing exotic tree species. Due to the invasion of exotic species some native species have become endangered and the ecosystem services provided by native forests are diminishing (Islam *et al.*, 1999; Foroughbakhch *et al.*, 2001; Sangha & Jalota, 2005). Most of the exotic plant effects reported have been identified as caused by allelopathic interaction which resulted in interference with physiological and biochemical processes in plants, due to chemicals released by exotic plants (Alhammedi, 2008). The Australian native *Eucalyptus camaldulensis* was first introduced in Pakistan in 1843, but no substantial plantations were made until 1950. A rise in *Eucalyptus* plantations seemed during 1970s, when it was primarily used for the reclamation of saline and waterlogged soils. The plantations were boosted at large scale in 1985 by farmer motivation through social forestry projects (Tanvir, 1996).

The *Eucalyptus* is now considered a threat to both plant and animal biodiversity because it reduces the

growth of under-story and adjacent plant communities by secreting allelochemicals in the soil and declining the avifaunal and other animal populations by habitat loss. The allelopathic effects of *Eucalyptus* have been studied extensively by various workers for example; Del Moral & Muller, 1970; Willis, 1999; Sasikumar *et al.*, 2001; Bajwa & Naz, 2005; El-Khawas & Shehata, 2005 etc. It has been found that *Eucalyptus* species release volatile compounds such as benzoic, cinnamic and phenolic acids, which inhibit growth of crops and weeds growing near it (Sasikumar *et al.*, 2001; Kohli *et al.*, 1998). Phenolic acids and volatile oils released from the leaves, bark, and roots of certain *Eucalyptus* spp. have deleterious effects on other plant species (Sasikumar *et al.*, 2002; Florentine & Fox, 2003). According to studies made by Yamane *et al.*, (1992) all the basic plant processes such as hormonal balance, protein synthesis, respiration, photosynthesis, chlorophyll formation, permeability and plant water relations may be disturbed by allelopathy. The continuous planting of *Eucalyptus* in monoculture may cause accumulation of allelochemicals in soil, which result in soil degradation and loss of productivity (El-Khawas & Shehata, 2005; Forrester *et al.*, 2006).

Eucalyptus produces prolific litter in the form of dropping leaves and naturally flecked off bark. If allelochemicals are released from *Eucalyptus* leaf litter its accumulation in the soil could result in poor development of native flora. In Pakistan *Eucalyptus* is mostly grown on crop field banks, as part of agro-forestry, on road sides, along fish ponds in aquaculture and as monoculture stands in forests. It has been observed that most of the farmers and forest managers destroy *Eucalyptus* leaf litter, when in excess, or by leaving it on ground, leaf litter mixes in

soil directly as solid material or as leaf litter extract when field is irrigated. From this it is assumed that the *Eucalyptus* leaf litter may enter in soil environment in two ways i.e., solid leaf litter and leaf litter extract. Thus the present study is designed primarily to investigate the allelopathic impacts of these two media on the germination and growth of native *Acacia nilotica* (L.) Delile. and the changes caused by them in the soil environment in terms of nutrient availability.

Materials and Methods

Collection of leaf litter: The dropped dried leaves of *Eucalyptus camaldulensis* Dehnh., were collected from *Eucalyptus camaldulensis* monoculture stands growing at Pai-Forest, District: Shaheed Benazirabad, Sindh, Pakistan. The leaves were washed thoroughly with distilled water to remove dust and dried for one week at room temperature. The dried leaves were ground, passed through 2.0 mm sieve and stored at room temperature. This powder was further divided into two equal parts of 2kg each. The one part was directly used in soil as leaf litter (w/w) and second part was used to formulate leaf litter extract (w/v) respectively.

Preparation of Treatments: For *Eucalyptus* leaf litter treatment ground powder of leaves was mixed in three concentrations i.e. 10, 20 and 30% (w/w) in sandy-silt soil. For *Eucalyptus* leaf litter extract treatment, the ground leaves powder was soaked in deionized distilled water in the ratio of 1:20 (w/v) and kept for 72 hours. The extract was filtered through a double layer muslin cloth (Fikreyes *et al.*, 2011) and designated as stock solution of 100% concentration. From this stock solution other concentrations viz., 10, 20 and 30% (v/v) were prepared by diluting it with distilled water (Hussain & Gadoon, 1981).

Experimental design and replications: The experiment was conducted in the Green House of Institute of Plant Sciences, University of Sindh, Jamshoro. The minimum and maximum inner environmental conditions of Green House were temperature 32.2-38°C, light 3620-6500 lux, heat index 43.2-50.5, humidity % 45.7-72.6, altitude 172m. Total 06 treatments (02 formulations and 03 concentrations) and a control each replicated four times were applied in randomized complete block design (Table 1).

Table 1. The scheme of treatments applied in the experiment.

Treatments/formulations	Concentrations
T0= Control	Soil without leaf litter or extracts
T1= Leaf litter	C1= Soil mixed with 10% dry leaf litter (w/w)
	C2= Soil mixed with 20% dry leaf litter (w/w)
	C3= Soil mixed with 30% dry leaf litter (w/w)
T2= Leaf litter extract	C1= Soil mixed with 10% leaf litter aqueous extract (w/v)
	C2= Soil mixed with 20% leaf litter aqueous extract (w/v)
	C3= Soil mixed with 30% leaf litter aqueous extract (w/v)

Germination & growth: The native *Acacia nilotica* (L.) Delile., tree was selected as bioassay plant. The seeds were collected from *Acacia nilotica* (L.) Delile., trees growing at Pai-Forest, District: Shaheed Benazirabad, Sindh, Pakistan. The seeds were subjected to physical scarification with sand paper and were sown in 8 inch diameter earthen pots filled with sandy-silt soil (soil pH 8.0, salinity 350 mg/kg, organic matter 0.34%, N 0.014%, P 2.14ppm, K 38 ppm, Na 200 ppm). The 20 seeds per treatment per replication were used. The seed germination was regularly recorded after each 24 hours until the last germination reached in control. The plants were harvested after two months. The seedling growth (cm) and biomass, fresh and dry weight (g) were obtained after the oven drying to constant weight at 65°C for 24 hours was recorded on harvest.

Chlorophyll content: The total chlorophyll content (mg/g f. wt.) was determined according to the method of (Arnon, 1949). The fresh leaves weighed 0.2g were cut and extracted overnight with 80% acetone at 0-4°C. The extracts were centrifuged at 100,000xg for 5 minutes. Absorbance of the supernatant was read at 645 and 663 nm using a Lambda 35 UV-V Spectrophotometer.

Soil nutrient analysis: The composite soil samples were taken from each treatment and taken to laboratory. The soil was analyzed for pH (JENWAY 3510 pH meter), soil

salinity (WTW EC Meter), Organic matter (Walkley & Black, 1934), Nitrogen (Kjeldahal method), Potassium and Sodium (Flame Photometer Model 400), Phosphorus (PC Spectro Lovibond Company).

Statistical analysis: The data was statistically analyzed for ANOVA and Pearson correlation tests. The differences between the means were computed using Tuckey's pair wise comparison test at 0.05. All the statistical analysis has been done using Minitab®.

The germination and growth ratios were obtained through the formulas as under:

1. Percentage germination; (Scott *et al.*, 1984)

$$G \% = \frac{Gn}{GN} \times 100$$

where *Gn* is the number of seeds germinated after 14 days and *GN* is the total number of seeds sown.

2. Percentage mortality rate;

$$MR = \frac{MRn}{GN} \times 100$$

where *MR* is percentage mortality rate, *MRn* is the number of seeds died after germination and *GN* is the total number of seeds germinated.

3. The relative germination ratio; (Rho & Kil, 1986).

$$RGR = \frac{GRt}{GRc} \times 100$$

where *RGR* is the relative germination ratio, *GRt* is the germination ratio of plants under treatment and *GRc* is the germination ratio of plants under control.

4. Relative elongation of shoot; (Rho & Kil, 1986).

$$RERs = \frac{MLS_t}{MLSc} \times 100$$

RERs is the relative elongation ratio of shoot, *MLS_t* is the mean length of shoot of plant under treatments and *MLSc* is the mean length of shoot of plant under control.

5. Relative elongation of root;

$$RERr = \frac{MLR_t}{MLRc} \times 100$$

RERr is the relative elongation ratio of root, *MLR_t* is the mean length of root of plant under treatments and *MLRc* is the mean length of root of plant under control.

6. Relative biomass ratio;

$$RBR = \frac{MBt}{MBc} \times 100$$

where *RBR* is relative biomass ratio, *MBt* is the mean biomass of plant under treatments and *MBc* is mean biomass of plant under control.

7. Seedling Vigour Index; (Abdul-Baki & Anderson, 1993)

SVI = Germination percentage (Shoot length + Root length)

Results

Germination and mortality: The allelopathic effects of *Eucalyptus* leaf litter and leaf litter extract significantly

reduced the percentage germination of *Acacia* seeds when compared to control in which seeds attained the highest germination. The highest percentage germination in case of treatments was recorded in leaf litter extract followed by leaf litter (Table 2). However the leaf litter has more severe effects on germination percentage than leaf litter extract and as the leaf litter decompose in soil its effect increases. It was found that as the concentration of each formulation increased the percentage germination decreased significantly, showing the negative correlation between concentration and germination percentage. Similarly the relative germination ratio (RGR) was found significantly high in leaf litter extract than leaf litter (Fig. 1).

The *Eucalyptus* treatments also induced mortality, calculated in percentage seedlings died after germination. The leaf litter when more decomposed, plants become yellow in colour and their growth is stunted and induce the mortality of seedlings. The highest mortality rate was found in leaf litter than leaf litter extract (Fig. 2).

Shoot and root length: All the *Eucalyptus* formulations significantly decreased the shoot length. The more decrease was found in leaf litter than leaf litter extract. But the difference between these two formulations was non-significant. Similarly shoot length reduced significantly as the concentration of the formulations increased. The highest concentration reduced highest shoot growth and lowest concentration reduced smallest. This relationship is confirmed by the Pearson correlation analysis which shows the negative correlation between shoot growth and concentration (Table 2).

The root length was also reduced significantly by *Eucalyptus* formulations and the more reduction was found in leaf litter than leaf litter extract. The concentration - root length correlation was negative and highest reduction in root length was observed under the highest concentration of either formulation applied (Table 2).

The analysis of relative elongation ratio of shoot (RERs) was highest in leaf litter extract, while the lowest values were found that of leaf litter (Fig. 3). Also the relative elongation ratio of root (RERr) was higher in leaf litter extract than leaf litter (Fig. 4).

Table 2. The allelopathic effects of various *E. camaldulensis* treatments on various plant parameters. The values are the mean of four replications.

Treatments	Plant parameters				
	Germination %	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
T0	98.13	36.75	18.00	11.22	5.05
T1	81.88	12.38	12.50	2.05	1.13
T2	63.75	10.75	9.75	1.78	0.93
T3	56.88	10.25	6.00	1.53	0.80
T4	91.88	12.38	11.50	2.65	1.35
T5	83.13	11.50	9.75	1.63	0.78
T6	75.00	9.50	9.25	1.30	0.50
Mean	78.664	14.7871	10.9642	3.1657	1.5057
LSD _{0.05}	1.08	1.32	1.02	0.22	0.20
Pearson correlation coefficient	-0.195	-0.648	-0.624	-0.641	-0.678

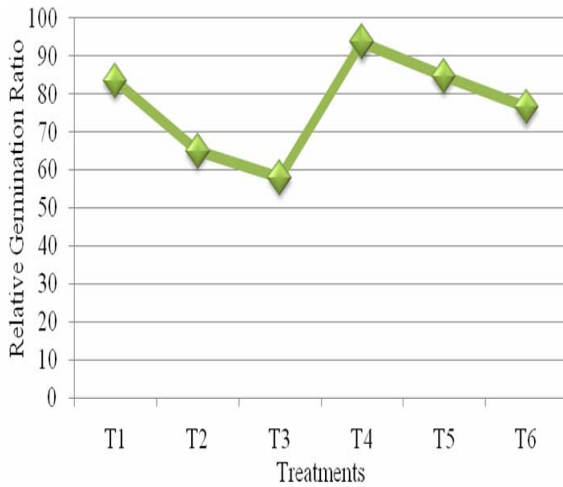


Fig. 1. Effect of *E. camaldulensis* on relative germination ratio (RGR) of *Acacia nilotica*.

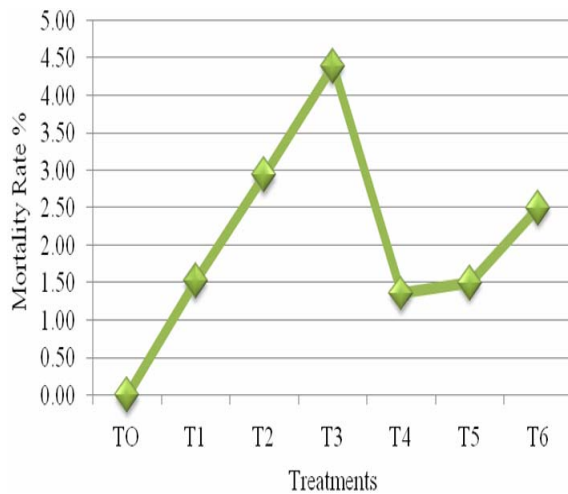


Fig. 2. Effect of *E. camaldulensis* on percentage mortality rate of *A. nilotica*.

Biomass (g): The *Eucalyptus* formulations showed the significant decrease in fresh & dry biomass of *Acacia* seedlings as compared to the control. There is no significant difference between leaf litter and leaf litter extract, but as the concentration increases, fresh and dry biomass of both the formulations decreased. The reduction in fresh biomass was observed in leaf litter extract followed by leaf litter. Similarly dry biomass was reduced in leaf litter extract followed by leaf litter (Table 2).

The relative biomass ratio (RBR) calculated in percentage (Fig. 5), the leaf litter has more values than leaf litter extract. In both formulations, the concentration effect was significantly different at $p < 0.05$ and dry biomass was negatively correlated.

Chlorophyll content: The total chlorophyll content (mg/g f. wt.) was decreased significantly in all the treatments as compared to the control. Like other plant parameters the leaf litter has more negative effects on chlorophyll content than leaf litter extract (Fig. 6).

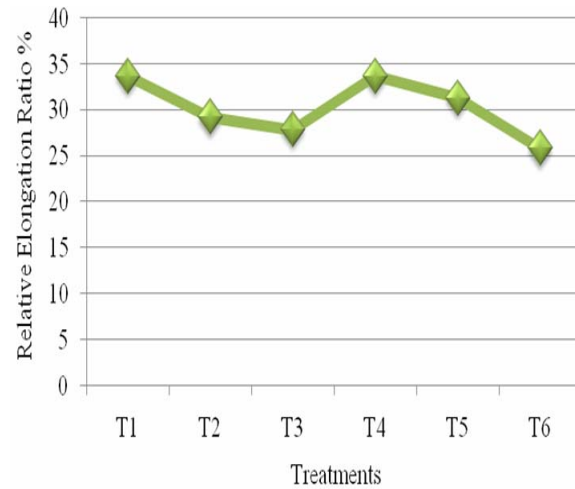


Fig. 3. Effect of *E. camaldulensis* on relative elongation ratio of shoot (RERs) of *Acacia nilotica*.

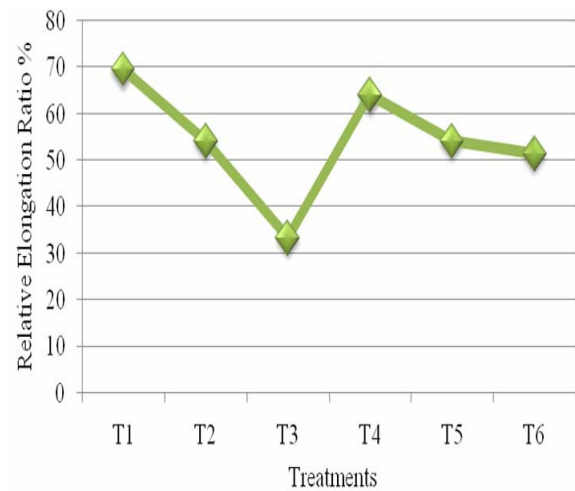


Fig. 4. Effect of *E. camaldulensis* on relative elongation ratio of root (RERR) of *Acacia nilotica*.

Seedling vigour index (SVI): The seedling vigour index was significantly reduced in all treatments as compared to the control, it was also noted that as the concentration increases the SVI decreases in both the formulations. The SVI was greater in leaf litter extract than leaf litter (Fig. 7).

Soil analysis: The soil pH decreased in leaf litter and leaf litter extract treatments as compared to the control, as the concentration of the both formulations increases, the pH decreased. Soil salinity increases as the concentration of both the formulations increases except T4 where it is less than control. The organic matter was greater in both formulations than control and greater in leaf litter than leaf litter extract. Nitrogen was also greater in both formulations than control, increased with increasing of concentration and slightly greater in leaf litter. P, K, Na was also greater in both formulations than control, increased with increasing of concentration and greater in leaf litter extract (Table 3).

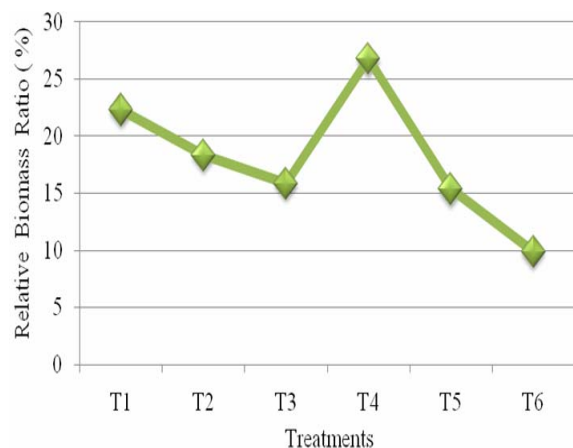


Fig. 5. Effect of *E. camaldulensis* on the relative biomass ratio (RBR) of *Acacia nilotica*.

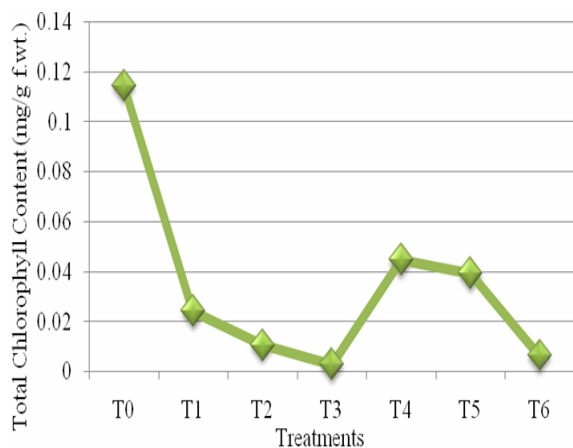


Fig. 6. Effect of *E. camaldulensis* on total chlorophyll content (mg/g f. wt.) of *Acacia nilotica*.

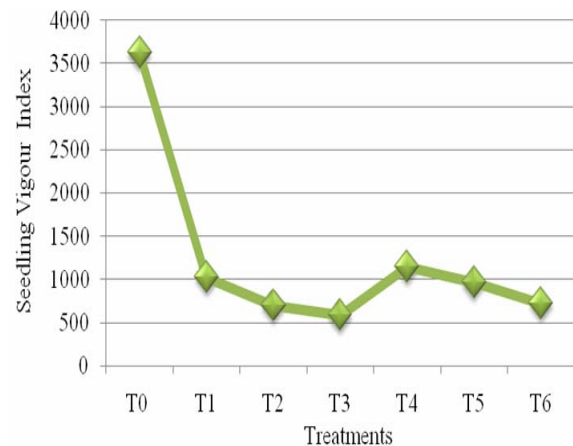


Fig. 7. Effect of *E. camaldulensis* on seedling vigour index (SVI) of *Acacia nilotica*.

Discussion

The results clearly indicate the inhibitive effect of *E. camaldulensis* leaf litter and leaf litter extract on percent

germination and relative germination ratio of *A. nilotica*. *E. camaldulensis* also induce seedling percentage mortality rate. The results also indicate that the allelopathy is a concentration dependent process, when the concentration increases the extent of the inhibition also increases. The effect of leaf litter was more severe on these plant parameters than leaf litter extract. The leaf litter as more decomposed it has more inhibitive effect on plant parameters studied. These results are agreed with the work of Niakan & Saberi (2009) who reported that the most of the growth parameters of *Phalaris* were decreased when exposed to different amounts of decompose and water extracts of *E. camaldulensis* leaves, the reduction was more severe by decomposed leaves of *E. camaldulensis*. Kil & Lovett (1999) reported the inhibition of seed germination and seedling growth of some herbaceous plants such as chick pea, maize and pea by aqueous leaf extracts of *E. camaldulensis*. Muhammad *et al.*, (2008) also reported that leaf extract of *E. camaldulensis* have allelopathic effect on seed germination and seedling growth of wheat (*Triticum aestivum* L.). The allelopathic effects of *Eucalyptus* may be attributed to the water soluble inhibitors present in its leaves (Al-Mousawi & Al-Naib, 1975). The inhibition of germination is dependent on the concentration of the extract, it may be due to the entry of water soluble allelochemicals in to the seed, which retards the germination and growth (Suseelamma & Venkataraju, 1994; Gilani *et al.*, 2007). Djanaguiraman *et al.*, (2002) observed that the aqueous extract of *E. globulus* caused inhibition of seed germination in greengram, blackgram and cowpea. Porwal & Mundra (1993) reported similar allelopathic effects of purple nut sedge and barnyard grass on germination and seedling growth of rice and black gram. Fikreyesus *et al.*, (2011) observed that the extracts of *E. camaldulensis* delayed as well as hindered the germination significantly in the receptor plant compared to the control. *Eucalyptus* is toxic, due to allelopathic properties which serve to reduce germination of other plants (Khan *et al.*, 2005; Shiming, 2005). Ahmed *et al.*, (2008) reported that with the increased application of *E. camaldulensis* leaf litter percentage, germination rate of the bioassay crops reduced. Padhy *et al.*, (2000) also reported the suppressing effects of *Eucalyptus* leaf leachates on germination and seedling growth of finger millet. The allelochemicals have harmful effects on crops in the ecosystem resulting in the reduction and delaying of germination, mortality of seedlings and reduction in growth and yield (Mcworthier, 1984; Herro & Callaway, 2003). Aqueous extract of some plants inhibited germination (Pratley *et al.*, 1996) and induce mortality of plants (Eyini *et al.*, 1996). *Eucalyptus* trees might negatively impact seed germination and growth of native species (Everett, 2000; Duarte *et al.*, 2006).

The shoot and root length was also reduced in all treatments (Table 2). These results are correlated with Lawan *et al.*, (2011) who reported that aqueous extract of *E. camaldulensis* has highest inhibitory effect on root length of *Arachis hypogea*. The aqueous extracts of seeds, leaf, root of *Ageratum conyzoides* delayed the germination and decreased the root and shoot elongation in chickpea (Angiras *et al.*, 1988). Aqueous extract of some plants inhibit seedling growth (Athanasova, 1996), root and shoot growth (Das & Bandyopadhyay, 2011). The root leachate of *Anagallis arvensis* decreased the radical length of mung bean, which showed allelopathic effect of the weed (Salam *et al.*, 2011).

Table 3. The soil analysis showing the allelopathic effect of various *Eucalyptus camaldulensis* treatments.

Treatments	Soil parameters						
	pH	Salinity (mg/Kg)	OM (%)	N (%)	P (ppm)	K (ppm)	Na (ppm)
T0	8.20	220	0.44	0.020	2.40	56	100
T1	8.10	260	8.10	0.11	9.30	75	100
T2	8.00	297	9.94	0.13	10.23	84	104
T3	7.80	300	11.20	0.14	10.50	97	135
T4	8.00	190	1.50	0.061	12.10	175	100
T5	7.90	387	3.28	0.064	13.87	300	200
T6	7.80	600	6.30	0.065	14.50	610	400
Initial Soil							
	8.0	938	0.34	0.014	2.14	38	475

The fresh and dry weights were also reduced in all treatments (Table 2). These results are correlated with several authors, Khan *et al.*, (2008) reported that aqueous extract of *E. camaldulensis* reduced the fresh weight and dry weight of wheat seedlings. The chemicals like phenolics, terpenoids and alkaloids and their derivatives found in plants are potential inhibitors of germination, seedlings growth, fresh weights and dry weights (Herro & Callaway, 2003; Siddiqui & Zaman, 2004; Siddiqui & Zaman, 2005). Similar findings were reported by Sanginga & Swift, 1992; Lisanewok & Michelson, 1993; Khan *et al.*, (1999) who found that *Eucalyptus* extract reduces the fresh weight and dry weights of maize seedlings. The reduction in biomass may be due to stunted and reduced seedling growth (Tripathi *et al.*, 1999, 2000). *E. globulus* leachate decreased the dry matter in black gram and rice, the magnitude of the reduction in the crops was proportionate with the leachate concentration (Djanaguiraman *et al.*, 2005).

The total chlorophyll concentration was also reduced significantly in all treatments (Fig. 6). These results are correlated with several authors, such as Djanaguiraman *et al.*, (2005) reported that in black gram, chlorophyll a was reduced by 50%, chlorophyll b by 138% and total chlorophyll by 71% in 20% concentrated leaf leachates of *E. globulus*. Abu-Romman (2011) reported that photosynthetic pigments in *Capsicum annum* seedlings were significantly and negatively affected by treatment with *Achillea biebersteinni*, chlorophyll a, chlorophyll b and total chlorophyll decreased with increasing leachate concentration. These photosynthetic pigments of *Acacia nilotica* seedlings were reduced by allelochemical stress (Fig. 6). Reduction in chlorophyll a, chlorophyll b, total chlorophyll was previously reported as a result of allelochemical stress (Singh *et al.*, 2009; Ervin & Wetzel, 2000; Moradshahi *et al.*, 2003). A correlation between photosynthetic alteration and the action of some allelochemical compounds was shown in previous works (Einhellig, 1986; Hejl *et al.*, 1993) being the disruption of electron transport chain one of the most usual ways for affecting photosynthesis by allelochemical compounds (John, 1982; Al-Khatib *et al.*, 1992; Nimbale *et al.*, 1996; Gonzelz *et al.*, 1998). Mersie & Singh (1993) found a direct relation between the application of some phenolic

acids (vanillic, chlorogenic, ferulic and p-coumaric) on *Abutilon theophrasti* Medik and an inhibition of the photosynthetic rate. Jayakumar *et al.*, (1990) observed reduction in chlorophyll content of crops treated with extracts of abscised leaves of *Eucalyptus*, which hints the possibility of poor photosynthesis and in turn poor plant growth.

The *Eucalyptus* leaf litter and leaf litter extract also caused significant reduction in seedling vigour index of *Acacia nilotica* (Fig. 7). These results are correlated with Djanaguiraman *et al.*, (2002) who found a similar type of result that *E. globulus* reduced the vigour index in greengram, blackgram and cowpea. A similar inhibitory effect of *Digera muricata* on sorghum was reported by (Karthiyayini *et al.*, 2003). Mubeen *et al.*, (2011) who found significantly minimum seedling vigour index (SVI) for rice seeds which were soaked in leaf extract of *Trianthema portulacastrum*. Sasikumar *et al.*, (2001) reported that leachates of bark, fresh leaves and leaf litter of four *Eucalyptus* species revealed significant reduction in germination and vigour index of redgram compared to the control. Das *et al.*, (2012) reported that with the increasing the concentration of leachate of *E. globulus* and *Shorea robusta* the vigour index of the *Cicer arietinum* decreased.

The OM, N, P, K, Na concentrations were greater in *Eucalyptus* leaf litter treatments than control but the growth of tested plant was higher in control. It is because of the phenolic compounds not present in control soil. When *Eucalyptus* leaf litter and leaf litter extract was added with soil in different concentrations, phenolic compounds released in soil and come in contact with roots of test plants and may alter its absorption capacity for water and minerals, cell division and other physiological functions. The allelochemicals can alter the rate at which ions are absorbed by plants. A reduction in both macro- and micronutrients are encountered in the presence of phenolic acids (Rice, 1974). El-Khatib *et al.*, (2004) reported that the decrease in dry matter production of *Triticum pyramidal* was due to phenolics and alkaloids from the extract of *Chenopodium murale* which affect the cell division, causing a reduction in the root cell growth. This may lead to a decrease in mineral uptake, nutrient absorption and the transport of nutrients from the root to other plant parts. This

reduction in growth and development also resulted in the reduction of dry matter production.

The above results also agreed with findings of (Anon., 1971) which reported that when plants were subjected to an allelopathic regime, plants showed impoverished appearance and suffering from acute deficiency of minor and major elements. The tissues of those plants were observed to be low in essential mineral nutrients, even the soil in which they were growing has an adequate supply of mineral nutrients. Such deficiency response might be due to an impairment of the ability of the affected plants to absorb sufficient nutrients for normal growth from the soil medium or an inability of affected plants to transport mineral nutrients to the shoots. The allelopathic compounds in soil come in contact with the roots of tested plant and may alter its absorption capacity for water and minerals, cell division and other physiological functions (Majeed *et al.*, 2012).

Conclusion

Based on the results present above it is concluded that allelopathy is a concentration-dependent phenomenon, as the concentration of the *E. camaldulensis* treatments increases, its effect increases. The leaf litter and leaf litter extract affected all the parameters as compared to the control.

References

- Abdul-Baki, A. and J.D. Anderson. 1993. Vigour determination in soybean seed by multiple criteria. *Crop Sci.*, 13: 630-633.
- Ahmed, R., A.T.M.R. Hoque and M.K. Hossain. 2008. Allelopathic effects of leaf litters of *Eucalyptus camaldulensis* on some forest and agricultural crops. *Journal of Forestry Research*, 19(1): 19-24.
- Abu-Romman, S. 2011. Allelopathic potential of *Achillea biebersteinii* Afan. (Asteraceae). *World Applied Sciences Journal*, 15(7): 947- 952.
- Alhammadi, A.S.A. 2008. Allelopathic effect of *Tagetes minuta* L. water extracts on seeds germination and seedling root growth of *Acacia asak*. *Ass. Univ. Bull. Environ. Res.* 11:17-24.
- Al-Khatib, K. Mink, G.I. and R. Parker. 1992. Detection and tracking of air born herbicide by using bio-indicator plants. *Proceedings Western Society of Weed Science*, 45: 27-31.
- Al-Mousawi, A.H. and F.A.G. Al-Naib. 1975. Volatile growth inhibitors produced by *Eucalyptus microtheca*. *Bull. Biol. Res. Cen.*, 7: 17-23.
- Angiras, N.N., S.D. Singh and C.M. Singh. 1988. Allelopathic effects of some weeds on germination and growth of chickpea. *Ind. J. Weed Sci.*, 20: 85-87.
- Anonymous. 1971. Biochemical Interactions among Plants. Natl. Acad. Sci. Washington, DC. pp, 12.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts: Polyphenylperoxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1-15.
- Athanassova, D.P. 1996. Allelopathic effect of *Amaranthus retroflexus* L. on weeds and crops. Seisieme Conterence du columa. *Journees Internationales Sr La Luttr contre les mauviases herbs*, Reims, France. 437- 442.
- Bajwa, R. and I. Naz. 2005. Allelopathic effects of *Eucalyptus citriodora* on growth, nodulation and AM colonization of *Vigna radiata* (L.) Wilczek. *Allelopathy J.*, 15: 237-246.
- Bone, R., M. Lawrence and Z. Magombo. 1997. The effect of a *Eucalyptus camaldulensis* Dehnh., plantation on native woodland recovery on Ulumba Mountain, southern Malawi. *Forest ecology and management*, 99: 83-99.
- D'Antonio, C.M. and B.E. Mahall. 1991. Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. *Am. J. Bot.*, 78: 885-894.
- Das, C. and A. Bandyopadhyay. 2011. Searching for allelopathic potential of *Shorea robusta* Gaertn.f. leaf. *Bionature.*, 31: 29-35.
- Das, C.R., N.K. Mondal, P. Aditya, J.K. Datta , A. Banerjee and K. Das. 2012. Allelopathic potentialities of leachates of leaf litter of some selected tree species on gram seeds under laboratory conditions. *Asian. J. Exp. Biol. Sci.*, 3(1): 59-65.
- Del Moral, R. and C.H. Muller. 1970. The allelopathic effects of *Eucalyptus camaldulensis*. *Am. Midl. Nat.*, 83: 254-282.
- Djanaguiraman, M., P. Ravishankar and U. Bangarusamy. 2002. Effect of *Eucalyptus globulus* on greengram, blackgram and cowpea. *Allelopathy J.*, 10: 157-62.
- Djanaguiraman, M., R. Vaidyanathan, J. Annie Sheeba, D. Durga Devi and U. Bangarusamy. 2005. Physiological responses of *Eucalyptus globulus* leaf leachate on seedling physiology of rice, sorghum and blackgram. *International Journal of Agriculture & Biology*, 7(1): 35-38.
- Duarte, N.F., E.U. Bucek, D. Karam, N. Sa and M.R.M. Scotti. 2006. Mixed field plantation of native and exotic species in semi-arid Brazil. *Aus. J. Bot.*, 54: 755-764.
- Einhellig, F.A. 1986. Mechanisms and modes of action of allelochemicals. In "The Sciences of Allelopathy" (Eds.): P.R. Putnam and C.S. Tang. pp. 171-188. Wiley Interscience, New York.
- El-Khatib, A.A., A.K. Hegazy and H.K. Galal. 2004. Does allelopathy have a role in the ecology of *Chenopodium murale*? *Annals of Botany Fennici.*, 41: 37-45.O
- EL-Khawas and Shehata. 2005. The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.). *Plants Biotechnology*, 4: 23-34.
- Ervin, G.N. and R.G. Wetzel. 2000. Allelochemical autotoxicity in the emergent wetland macrophyte *Juncus effuses* (Juncaceae). *Am. J. Bot.*, 87: 853-860.
- Everett, R.A. 2000. Patterns and pathways of biological invasions. *Trends in Ecology and Evolution*, 15: 177-178.
- Eyini, M., A.U. Maheswari, T. Chandra and M. Jayakumar. 1996. Allelopathic effects of leguminous plants leaf extracts on some weeds and corn. *Allelopathy J.*, 3(1): 85-88.
- Ferreira, R.L. and M.M. Marques. 1998. Litter fauna of arthropods of areas with monoculture of *Eucalyptus* and heterogeneous secondary forest. *Anais da Sociedade Entomológica do Brasil*, 27: 395-403.
- Fikreyes, S., Z. Kebebew, A. Nebiyu, N. Zeleke and S. Bogale. 2011. Allelopathic effects of *Eucalyptus camaldulensis* Dehnh., on germination and growth of tomato. *Am. Euras. J. Agric. Environ. Sci.*, 11: 600-608.
- Florentine, S.K. and J.E.D. Fox. 2003. Allelopathic effects of *Eucalyptus victrix* L. on *Eucalyptus* species and grasses. *Allelopathy J.*, 11: 77-83.
- Foroughbakhch, F., L.A. Hauad, A.E. Cespedes, E.E. Ponce and N. Gonzalez. 2001. Evaluation of 15 indigenous and introduced species for reforestation and agro forestry in north eastern Mexico. *Agro forest system*, 51: 231-221.
- Forrester, D.I., J. Bauhus, A.L. Cowie and J.K. Vanclay. 2006. Mixed-species plantations of *Eucalyptus* with nitrogen-fixing trees: A review, *Forest Ecology and Management*, 233: 211-230.
- Gilani, S.A., A. Kikuchi, Z.K. Shinwari, Z.I. Khattak and W.N. Watanabe. 2007. Phytochemical, pharmacological, and ethnobotanical studies of *Rhazya stricta* Decne. *Phytother. Res.*, 21: 301-307.

- Gonzalez, V.M., J. Kazimir, C. Nimbai, L.A. Weston and G.M. Cheniae. 1998. Inhibition of a photosystem II electron transfer reaction by sorgoleone, a natural product. *Journal of Agriculture and Food Chemistry*, 45: 1415-1521.
- Hejl, A.M., F.A. Einhellig and J.A. Rasmussen. 1993. Effects of Juglone on growth, photosynthesis and respiration. *Journal of Chemical Ecology*, 19: 559-568.
- Herro, J.L. and R.M. Calaway. 2003. Allelopathy and exotic plant invasion. *Plant and Soil*, 256: 29-39.
- Hussain, F. and M.A. Gadoon. 1981. Allelopathic effects of *Sorghum vulgare* Pers. *Oecologia Berl.*, 51: 284-288.
- Islam, K.R., M. Kamaluddin, M.K. Bhuiyan and A. Badrudin. 1999. Comparative performance of exotic and indigenous forest species for tropical semi evergreen degraded forest land reforestation in Chittagong, Bangladesh. *Land Degradation & Development*, 10(3): 241-249.
- Jayakumar, M., M. Eyini and A. Pannarselvam. 1990. Allelopathic effects of *Eucalyptus globulus* Labill., in groundnut and corn. *Comparative physiol. Ecol.*, 15: 109-113.
- John, J. B. St.1982. Effects of herbicides on the lipid composition of plant membranes. In: *Biochemical Responses Induced by Herbicides*, pp. 97-109.
- Karthiyayini, R.K., N.R. Ponnamal and B. Rajesh. 2003. Effects of *Digera muricata* L., on germination and seedling growth of *Sorghum bicolor* L. varieties. *Allelopathy J.*, 12: 89-94.
- Khan, M.A., M.U. Rashid and M.S. Baloch. 1999. Allelopathic effect of *Eucalyptus* on maize crop. *Sarhad J. Agric.* 15: 393-397.
- Khan, M.A., K.B. Marwat, G. Hassan and Z. Hussain. 2005. Bioherbicidal effects of tree extracts on seed germination and growth of crops and weeds. *Pak. J. Weed Sci. Res.*, 11: 89-94.
- Khan, M. A., I. Hussain, and E. A. Khan. 2008. Allelopathic Effects of *Eucalyptus (Eucalyptus camaldulensis L.)* on Germination and Seedling Growth of Wheat (*Triticum aestivum L.*). *Pak. J. Weed Sci. Res.*, 14(1-2): 9-18.
- Kil, B.S. and J.V. Lovett. 1999. The research of allelopathy in Australia: A review. In: (Eds.): Indirjit. Dakshini, K.M.M and C.L. Foy. *Principles and Practices in Plant Ecology: Allelochemical Interactions*, London, pp. 221-236.
- Kohli, R.K., D.R. Batish and H.P. Singh. 1998. Eucalypt oils for the control of parthenium (*Parthenium hysterophorus L.*). *Crop Prot.*, 17: 119-122.
- Lawan, S.A., K. Suleiman and D.N. Iortsuum. 2011. Effects of allelochemicals of some *Eucalyptus* species on germination and radicle growth of *Arachis hypogea*. *Bayero Journal of Pure and Applied Sciences*, 4(1): 59-62.
- Lisanework, N. and A. Michelson. 1993. Allelopathy in agroforestry systems. The effects of leaf extracts of *Eucalyptus* species on three crops. *Agroforestry Systems*, 21(1): 63-74.
- Majeed, A., Z. Chaudhry and Z. Muhammad. 2012. Allelopathic assessment of fresh aqueous extracts of *Chenopodium album L.*, for growth and yield of wheat (*Triticum aestivum L.*). *Pak. J. Bot.*, 44(1): 165-167.
- Mcworthier, C.G. 1984. Future needs in weed science. *Weed Sci.*, 32: 850-855.
- Mersie, W. and M. Singh. 1993. Phenolic acids affect photosynthesis and protein synthesis by isolated leaf cells of velvet-leaf. *Journal of Chemical Ecology*, 19: 1293-1301.
- Moradshahi, A., H. Ghadiri and F. Ebarhimikia. 2003. Allelopathic effects of crude volatile oil and aqueous extracts of *Eucalyptus camaldulensis* Dehnh. leaves on crops and weeds. *Allelopathy J.*, 12: 189-196.
- Mubeen, K., M.A. Nadeem, A. Tanveer and Z.A. Zahir. 2011. Allelopathic effects of aqueous extracts of weeds on the germination and seedling growth of rice (*Oryza sativa L.*). *Pak. J. Life Soc. Sci.*, 9(1): 87-92.
- Muhammad, A.K., H. Iqtidar and A.K. Ejaz. 2008. Allelopathic effects of *Eucalyptus camaldulensis L.*, on germination and seedling growth of wheat (*Triticum aestivum L.*). *Pak. J. Weed Sci. Res.*, 14(1-2): 9-18.
- Niakan, M. and K. Saberi. 2009. Effects of *Eucalyptus* allelopathy on growth characters and antioxidant enzymes activity in Phalaris weed. *Asian Journal of Plant Sciences*, 8(6): 440-446.
- Nimbai, C.L., L.A. Weston, C.N. Yerkes and S.C. Weller. 1996. Herbicidal activity and site of action of the natural product sorgoleone. *Proceedings- Weed Science Society of America*, 36-56.
- Padhy, B., P.K. Pattnaik and A.K. Tripathy. 2000. Allelopathic potential of *Eucalyptus* leaf litter leachates on germination and seedling growth of finger millet. *Allelopathy J.*, 7: 69-78.
- Porwal, M.K. and S.L. Mundra. 1993. Allelopathic effects of purple nut sedge and barnyard grass on germination and seedling growth of blackgram and paddy. In: *Proceedings International Symposium on weeds*, Hisar, India: Indian Society of Weed Science. 3: 65-67.
- Pratley, J.E., P. Dowling and R. Medd. 1996. Allelopathy in annual grasses. Wild oats, annual ryegrass and vulpia. *Proceedings of a workshop held at Orange*, New South Wales, Australia, 1: 213-214.
- Rho, B.J. and Kil, B.S. 1986. Influence of phytotoxin from *Pinus rigida* on the selected plants. *J Na Sci Wonkwang Uni.*, 5: 19-27.
- Rice, E.L. 1974. *Allelopathy*. Academic Press.
- Ridenour, W.M. and R.M. Callaway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia*, 126: 444-450.
- Salam, I.U., M. Ahmed and S.T. Ali. 2011. Allelopathic effect of scarlet pimpernel (*Anagallis Arvensis*) on seed germination and radical elongation of mung bean and pearl millet. *Pak. J. Bot.*, 43(1): 351-355.
- Sangha K.K. and R.K. Jalota. 2005. Value of Ecological Services of Exotic *Eucalyptus tereticornis* and Native *Dalbergia sissoo* Tree Plantations of North-Western India. *Conservat Soc.*, 3: 92-109.
- Sanginga, N. and M.J. Swift. 1992. Nutritional effects of *Eucalyptus* litter on the growth of maize (*Zea mays L.*). *Agric. Ecosystems and Environ.*, 41(1): 55-65.
- Sasikumar, K., C. Vijayalakshmi and K.T. Parthiban. 2001. Allelopathic effects of four *Eucalyptus* species on redgram (*Cajanus cajan L.*). *Journal of Tropical Agriculture*, 39: 134-138.
- Sasikumar, K., C. Vijayalakshmi and K.T. Parthiban. 2002. Allelopathic effects of *Eucalyptus* on blackgram (*Phaseolus mungo L.*). *Allelopathy Journal*, 9: 205-214.
- Schwartz, M.W., D.J. Porter, J.M. Randall and K.E. Lyons. 1996. Impact of non indigenous plants. Pages 1203-1218 in *Sierra Nevada Ecosystem Project: Final Report to Congress. Volume 2. Assessments and scientific basis for management options*. Centers for Water and Wild and Resources, University of California, Davis, California, USA.
- Scott, S.J., R.A. Jones and W.A. William. 1984. Review of data analysis methods for seed germination. *Crop Science*, 24: 1192-1199.
- Shiming, L. 2005. Allelopathy in south china agroecosystems. *Proceedings of the 4th World Congress on Allelopathy*, Wagga, NSW, Australia.

- Shinwari, Z.K. and M. Qaisar. 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot.*, 43(Special Issue): 5-10.
- Shinwari, Z.K., S.A. Gilani and A.L. Khan. 2012. Biodiversity loss, emerging infectious diseases and impact on human and crops. *Pak. J. Bot.*, 44(Special Issue): 137-142.
- Siddiqui, Z.S. and A.U. Zaman. 2004. Effects of systemic fungicide (benlate) on germination, seedling growth, biomass and phenolic contents of two different varieties of *Zea mays*. *Pak. J. Bot.* 36: 577-582.
- Siddiqui, Z.S. and A.U. Zaman. 2005. Effects of *Capsicum* leachates on germination, seedling growth and chlorophyll accumulation in *Vigna radiata* L. Wilczek. Seedlings. *Pak. J. Bot.*, 37(4): 941-947.
- Singh, A., D. Singh and N. Singh. 2009. Allelochemical stress produced by aqueous leachate of *Nicotiana plumbaginifolia* Viv. *Plant Growth Regul.*, 58: 163-178.
- Suseelamma, M. and R.R. Venkataraju. 1994. Effect of *Digera muricata* (L) Mart. Extracts on germination and seedling growth of groundnut. *Allelopathy J.*, 1: 53-7.
- Tanvir, A. 1996. *Eucalyptus* in Pakistan. Report submitted to FAOs regional expert consultation on *Eucalyptus* Vol II.
- Tripathy, S., A. Tripathy and D.C. Kori. 1999. Allelopathic evaluation of *Tectona grandis* leaf, root and soil aqueous extracts on soybean. *Indian J. Forestry*, 22: 366-74.
- Tripathy, S., A. Tripathy, D.C. Kori and S. Paroha. 2000. The effects of *Dalbergia sissoo* extracts, rhizobium and nitrogen on germination, growth and yield of *Vigna radiata*. *Allelopathy J.*, 7: 255-63
- Walkley, A. and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.*, 37: 29-38.
- Willis, R. 1999. Australian studies on allelopathy in eucalyptus: a review. In: (Eds.): Inderjit, Dakshini, K.M.M., Foy, C.L. Principles and Practices in Plant Ecology: Allelochemical Interactions. CRC Press, pp. 201-219.
- Yamane, A., H. Nishimusa and J. Mizutani. 1992. Allelopathy of yellow field cress. (*Rorippa sylvestris*). Identification and characterization of phytotoxic constituents. *J. Chem. Ecol.*, 18: 683-691.

(Received for publication 1 September 2012)