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Analysis of Phenolic Compounds and Allelopathic effects of *Eucalyptus* on *Prosopis cineraria*

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ABSTRACT

The *Eucalyptus* has been expansively planted in Pakistan in native forests because of its fast-growing and adaptability to different habitats. Allelopathic and ecological effects are receiving severe concern with the growth of more plantations of *Eucalyptus*. Since the *Eucalyptus* has allelopathic effects on neighboring plants. The effect of the exotic *Eucalyptus leaf litter and ash* on *Prosopis cineraria* germination and growth was observed. The three concentrations of leaf litter and ash 10, 20 and 30% were applied, as were the controls (without leaf litter and ash). The germination and growth parameters of *Prosopis cineraria* were analyzed. The phenolic acids were identified and quantified through HPLC from laboratory experiments soil. The laboratory experiments soil was analyzed for soil properties such as; EC, pH, OM, N, P, K, and Na. The germination was observed to be reduced in leaf litter and ash applied treatments. After germination, mortality of seedlings at the early stage was recorded in leaf litter ash applied treatments. Leaf number, shoot elongation ratio, length of shoot, root length, relative biomass ratio, amount of chlorophyll, fresh and dry weight of seedlings were found to have decreased. In soil mixed with leaf litter, nine phenolics; hypogallic acid, gallic acid, 2, 4, 6-trihydroxybenzoic acid, protocatechuic acid, pyrogallolaldehyde, caffeic acid, *m*-coumaric, *o*-coumaric and *p*-coumaric acid were analyzed through the HPLC. 2, 4, 6-trihydroxybenzoic acid was predominant with concentration of 34.51 mg/100g. In soil mixed with ash, gallic acid, protocatechuic acid and pyrogallolaldehyde were analyzed. Gallic acid was predominant in leaf litter ash 30% with concentration of 11.44 mg/100g. The pH of the soil decreased in leaf litter. OM, EC, K, N, P and Na have increased in treatments. Higher EC and Na were noted in the ash. A negative correlation was seen between concentration and the plant parameters studied. *Eucalyptus* has deleterious effects on the native species, *Prosopis cineraria*. Therefore, *Eucalyptus* should not be planted along with native trees or in native forests in Pakistan. Thus, it is also recommended that *Eucalyptus* trees be replaced with native species.

Keywords: *Eucalyptus camaldulensis*, Allelopathy, Leaf litter, Leaf litter ash, *Prosopis cineraria*, Phenolic compounds.

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INTRODUCTION

The exotic species, such as *Eucalyptus* are major causes of the destruction of habitats and loss of biodiversity (Schwartz *et al.*, 1996; Vitousek *et al.*, 1997). The introduction of exotic plants is the main cause of the decrease in native species. Exotic plants become invasive and form monocultures in the habitats; in this way, suppress the native species populations through the release of allelochemicals in their surroundings (Mooney *et al.*, 1986; Randall, 1996; BLM, 1998; Callaway & Aschehoug, 2000; Keane & Crawley, 2002; Reigosa *et al.*, 2002; Bais *et al.*, 2003; Thelen *et al.*, 2005; Callaway *et al.*, 2005). Allelopathic chemicals are released into surroundings through the decomposition of plant parts, exudation from roots, volatilization and leaching of plant parts (Babu & Kandasamy, 1997 and Ahmed *et al.*, 2008). *Eucalyptus* has caused various ecological problems, together with the release of chemical compounds into the soil (Kumar, 1991; Poore & Fries, 1985). The phenolic compounds; benzoic acid, cinnamic acid, flavonoids, terpenoids, and tannins are the main categories of allelochemicals. Phenolic compounds remain main chemicals with allelopathy potential (Duke *et al.*, 2000). Allelopathic compounds have been reported from *Eucalyptus camaldulensis* by several authors, studies by Del Moral and Muller in 1970. *Eucalyptus* releases the majority of allelochemicals from its leaves (Melkania, 1986; Marwat and Khan 2006; Khan *et al*., 2009). Ullah *et al*., 2023 identified seven phenolic compounds by HPLC from *Eucalyptus*-planted soils. Allelopathic compounds may inhibit photosynthesis, modify nutrient ion uptake and modify the soil properties (Booker *et al*., 1992; Barkosky and Einhellig, 1993; Hejl *et al*., 1993; Stinson *et al*., 2006 and Samreen *et al*., 2009). The phenolic acids can hinder the physiological processes of plants (Carballeira & Reigosa, 1999; Lorenzo *et al*., 2008).

Allelopathic substances (phenolic compounds) decrease the germination, growth and yield of plants. Also induce the seedling's mortality (Ghafar *et al*., 2000; Einhellig, 2002; Kumar *et al*., 2006; Zhou & Yu, 2006). Aziz and Shaukat (2014) reported that leaf litter aqueous extracts of *Digera muricata* have significantly decreased native plant germination. Kumar and Kumar in 2010 found the suppression of *Phaseolus mungo* germination and growth by *Parthenium hysterophorus* ash. The concentrations of ash extract, above 3% decreased all parameters studied. Shaddam *et al*., 2020 found that leaf extract of *Eucalyptus* negatively affects mung bean germination and growth. Maqbool *et al*., 2022 identified phenolic compounds from *Glycyrrhiza glabra*. They found that *Glycyrrhiza glabra* extract has phytotoxic effects on the germination and growth of *Phalaris minor*. Earlier research on *Eucalyptus* allelopathic potential was conducted on crops and weeds (Babu and Kandasamy, 1997).

Since, *Eucalyptus* is predominantly grown in native forests along with native trees. Subsequently, it is essential to evaluate the *Eucalyptus camaldulensis* Dehnh. effects of allelopathy on native tree, *Prosopis cineraria*. *Prosopis cineraria* is a commonly used agroforestry tree in Pakistan. It is a multipurpose, nitrogen-fixing tree and supports the understory vegetation. *Prosopis cineraria* provides fodder and a source of vegetables to many people in the world. Literature suggests that the effect of leaf litter and ash from *Eucalyptus camaldulensis* on native *Prosopis cineraria* was not evaluated. So, the study was conducted to determine phenolic compounds from *Eucalyptus camaldulensis* leaf litter and their effect on *Prosopis cineraria.*

MATERIALS AND METHODS

The leaf litter of *Eucalyptus camaldulensis* Dehnh. was collected from a reserve forest, Pai-Forest, District Shaheed Benazirabad, Pakistan. *Eucalyptus camaldulensis* Dehnh. identified, voucher specimen (No. 24712), deposited at the herbarium of the Institute of Plant Sciences, University of Sindh, Jamshoro. The leaf litter was completely air dried. Then grind to make powder. After grinding, it passed through a 2.0-mm sieve. Leaf litter concentrations, i.e., 10, 20, and 30%, were mixed with soil and in control (without leaf litter). For ash, the leaf litter was completely burned. Then it was passed through a 2.0-mm sieve. Three concentrations, i.e., 10, 20, and 30% of ash, were mixed with soil and control. There was a separate control for each experiment. The treatments were replicated four times and applied in a RCBD. Treatments are shown in Table 1.

Table 1. The preparation of treatments

Treatments	Concentrations (w/w)
T0	Soil only
T1	10% leaf litter
T2	20% leaf litter
T3	30% leaf litter
T4	10% ash
T5	20% ash
T6	30% ash

Germination and growth of Prosopis cineraria

Prosopis cineraria (L.) Druce. seeds were sown in earthen pots. Sandy-silt soil was filled with pots. The soil was analyzed for its properties, such as organic matter (0.34%), N (0.014%), pH (8.0), P (2.14 ppm), EC

(0.52 dS/m), K (38 ppm) and Na (200 ppm). *Prosopis cineraria* germination was noted. The mortality of seedlings was noted during the experiment. The plants were harvested after two months. The length of the shoot and root (cm) and the fresh weight of the seedlings (g) were recorded. Dry weight (g) was acquired through oven drying at 65 ºC for 24 h (Fikreyesus *et al*., 2011).

Following plant germination related parameters were calculated;

1. *Germination %:* (Scott *et al*., in 1984).

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

Gn=Total germination, *GN*=Total seeds

2. *Germination inhibition:* (revised after Hassannejad and Ghafarbi, 2013).

$$Inhibition\ \% = \frac{GST - GSC}{GSC} \times 100$$

GST= Germination of seeds in treatment, *GSC*= Germination of seeds in control

3. *Seedlings mortality*: (revised after Rho and Kil, 1986).

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

MRn=Mortality in treatment, *GN*= Total germination

4. *Relative germination ratio*: (Rho and Kil, 1986).

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

GRt=Germination ratio in treatment, *GRc*= Germination ratio in control

5. *Shoot relative elongation ratio:* (Rho and Kil, 1986).

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

MLSt=Mean length of shoot in treatment, *MLSc*= Mean length of shoot in control

6. *Root relative elongation ratio*: (Rho and Kil, 1986).

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

MLRt=Mean length of root in treatment, *MLRc*= Mean length of root in control

7. *Biomass*: (revised after Rho and Kil, 1986).

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

MBt=Mean biomass in treatment, *MBc*=Mean biomass in control

8. *Vigor index of seedlings:* (Abdul-Baki and Anderson, 1973)

$$SVI = (Shoot\ length + Root\ length) \times Germination\ percent$$

9. I*nhibition of seedling length %:* (modified after Hassannejad and Ghafarbi, 2013).

$$Inhibition\ \% = \frac{SLT - SLC}{SLC} \times 100$$

SLT=Seedling length in treatment, *SLC*=Seedling length in control

10. *Chlorophyll content* *(mg/g f. wt.):*

$$Chl\ a\left(\frac{mg}{g} f.wt.\right) = \left[12.7(OD\ 663) - 2.69(OD\ 645) \times \frac{V}{1000} \times W\right]$$

$$Chl\ b\left(\frac{mg}{g} f.wt.\right) = \left[22.9(OD\ 645) - 4.68(OD\ 663) \times \frac{V}{1000} \times W\right]$$

Soil analysis

Soil pH was investigated through a pH meter, Jenway 3510. WTW EC meter was used for analyzing soil electrical conductivity (EC). Amount of organic matter was examined through the Walkley and Black method. K and Na through the flame photometer, Model 400, P through the Spectrophotometer (U-2900UV/VIS, Hitachi, Japan), and N through the Kjeldahl method (*Jackson*, 1962).

Determination of phenolic compounds from soil

The HPLC profiling of phenolic compounds were carried out at the National Centre of Excellence in Analytical Chemistry (NCEAC), University of Sindh, Jamshoro, Sindh. All standards of phenolic compounds (protocatechuic acid, 2,4,6-trihydroxybenzoic acid, gallic acid, gentisic acid, protocatechualdehyde, pyrogallolaldehyde, hypogallic acid, *β*-resorcinolic acid, sinapic acid, vanillin, caffeic acid, vanillic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, *m*-coumaric acid, ferulic acid, chlorogenic acid, cinnamic acid, *o*-coumaric acid) of Tokyo Chemical Industry Ltd. (Japan) were used. Methanol (HPLC grade), ethylacetate, ethanol, acetonitrile and formic acid of Fischer Scientific (UK) were used. Sodium carbonate was purchased from Merck, Germany.

Preparation of sample:

Five grams of soil were weighed in a 50 mL glass beaker and 20 mL (80% methanol) was added. Then, using a magnetic bar measuring 10 x 2 mm, the soil and water mixture was stirred for ten minutes. Then, it was filtered and store at 4 ºC for further analysis.

HPLC-DAD profiling of phenolic compounds:

The phenolic compounds were separated out by using reported HPLC-DAD method of Memon *et al*., (2010). Briefly, Spectra system SCM 1000 (Thermo Finnigan, California, USA) liquid chromatograph, equipped with a vacuum degasser and a DAD system was used for analysis of phenolic acids. For separation, a Hypersil Gold C-18 (250 mm×4.6 mm, 5 µm) column (Thermo Corporation, USA) was used. Mobile phase contained 0.1% formic acid in water (B) and methanol (A). The flow rate was 0.7 mL/min. The volume of injection was 20 µL. UV detection was done at 270 nm. Chromoquest, Version 4.2 software was used for data acquisition and calculation. Phenolic acids identification was based on

UV spectrum and retention time of standards. The standards from 1 to 40 µg/mL concentrations were injected in HPLC-DAD system. The calibration curve for each standard compound was established. The concentrations of the compounds were calculated from peak area according to calibration curves.

Data analysis

The data was analyzed for analysis of variance. The Pearson correlation test was calculated. The significance level (0.05) was set to compare with significance probability value (*p*-value). The data was analyzed through Excel and MINITAB® software.

RESULTS

Germination

Prosopis cineraria germination, as indicated in Table. 2, decreased by leaf litter and ash as compared to control. As concentration of *Eucalyptus* increased, a significant decrease in percent germination was noted. The data analysis shows a negative correlation for various concentrations and germination percentages. *Eucalyptus* has significantly decreased the relative germination ratio (RGR) of *plant* (Figure 1). The germination rate was inhibited. Leaf litter has more inhibition effect as compared to ash (Figure 1). The ash induces mortality in seedlings after germination. Seedling mortality was also recorded in leaf litter-applied treatments (Figure 2). The analysis of variance of germination and growth parameters is given in table 3, it shows a significant difference between treatments.

Shoot and root

Length of the shoot of *Prosopis cineraria* was recorded as reduced by leaf litter and ash (Table 2). As the seedling's mortality occurs in ash, a greater decrease was found. The effect on the length of the shoot was noted to increase with more concentrations. A negative correlation was calculated among shoot length and concentrations. The length of the root was also decreased by *Eucalyptus* treatments. Ash was found to have a more suppressive effect on root length (Table 2). The ash significantly reduced the relative elongation ratio of shoot (RERs) of plants (Figure 3). Relative elongation ratio of root (RERr) was found to decrease in ash, followed by leaf litter (Figure 3). It was noted that as the leaf litter and ash concentrations increased, the effect was also found to increase. The seedling's length was inhibited by *Eucalyptus* treatments. Ash was found with more suppressive effect on the seedling's length (Figure 4). The extent of seedling length inhibition was related to the different concentrations.

Leaf number

Number of leaves of *Prosopis cineraria* seedlings was found to decrease with leaf litter and ash, as given in Table 2. A significant difference between the leaf litter and ash-applied treatments and the control was recorded. However, higher *Eucalyptus* concentrations have a higher effect on leaf number.

Plant fresh and dry weight (g)

As compared to control, *Eucalyptus* negatively affect the fresh and dry weight of seedlings *of Prosopis cineraria*, as shown in Table 2. Fresh and dry weights were found to significantly decrease as the concentration was increased.

The biomass ratio of seedlings, as shown in Figure 3, indicates a significant reduction in ash compared to leaf litter. The relative biomass ratio of *Prosopis cineraria* showed a considerable reduction with 20% and 30% concentrations of leaf litter and ash.

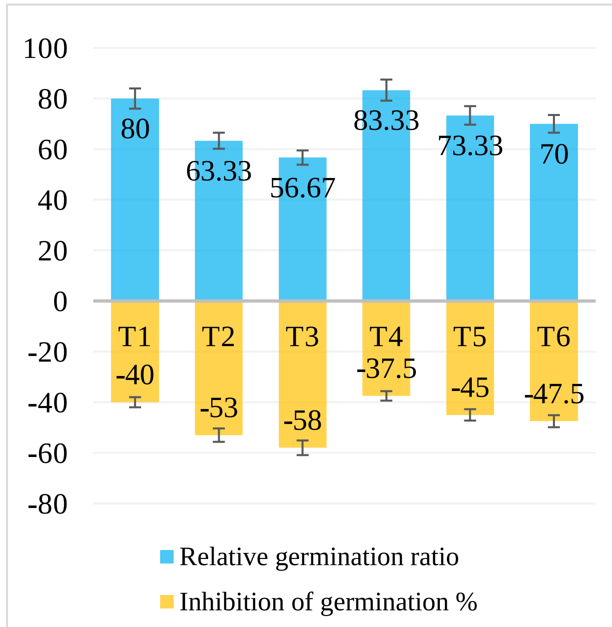

Figure 0-1. Effect of Eucalyptus on germination of *Prosopis cineraria.*

The chlorophyll content (mg/g f. wt.)

The amount of chlorophyll was recorded, shown in Figure 5. *Eucalyptus* has decreased the chlorophyll as observed in control. The ash was noted with a higher impact on the amount of chlorophyll than leaf litter.

Seedlings vigor index (SVI)

The vigor index of *Prosopis cineraria* seedlings was significantly reduced by the *Eucalyptus* leaf litter and ash-applied treatments, as presented in Figure 6.

Table 2. The effects of *Eucalyptus* leaf litter and ash on plant parameters.						
Treatments	Germination %	Leaf number	Shoot (cm)	Root (cm)	Fresh weight (g)	Dry weight (g)
T0	75	8.75	12.5	15.5	3.02	1.34
T1	60	4.38	6.13	4.13	1.08	0.28
T2	47.5	3.94	5.88	3.88	0.84	0.24
T3	42.5	4.19	5.5	3.50	0.63	0.20
T4	62.5	0.75	0.75	1.25	0.18	0.08
T5	55	0	0	0	0	0
T6	52.5	0	0	0	0	0
Mean	56.429	3.143	4.393	4.036	0.819	0.305
SD	13.393	3.249	4.343	5.496	1.041	0.452
Correlation coefficient	-0.339	-0.855	-0.919	-0.759	-0.829	-0.763

The results indicate that the vigor index of *Prosopis cineraria* decreases with the increasing concentration of leaf litter and ash. The vigor index was noted to be greater in leaf litter, followed by ash.

Phenolic acid analysis from soil applied with leaf litter and ash

The HPLC chromatogram showed separation of gallic acid in T0, control (Figure 7 a) with a concentration of 5.66 mg/100 g. In T1, five phenolic acids with the concentrations gallic acid (9.36 mg/100g), pyrogallolaldehyde (4.79 mg/100g), caffeic acid (1.21 mg/100g), hypogallic acid (7.39 mg/100g), and *p*-coumaric acid (1.76 mg/100g) were analyzed (Figure 7 b). In T2, eight phenolic acids: gallic acid (14.77 mg/100g), 2, 4, 6-trihydroxybenzoic acid (34.51 mg/100g), pyrogallolaldehyde (7.57 mg/100g), caffeic acid (12.54 mg/100g), protocatechuic acid (5.12 mg/100g), *m*-coumaric acid (2.01 mg/100g), *o*-coumaric acid (2.97 mg/100g) and *p*-coumaric acid (3.84 mg/100g), were analyzed (Figure 7 c). In T3, seven phenolic acids, gallic acid (16.32 mg/100g), protocatechuic acid (18.26 mg/100g), pyrogallolaldehyde (11.87 mg/100g), *p*-coumaric (6.66 mg/100g), caffeic (16.35 mg/100g), *o*-coumaric (3.0 mg/100g) and *m*-coumaric (1.76 mg/100g) were investigated (Figure 7 d). In T4, two phenolic acids with concentrations of gallic acid (4.74 mg/100g) and pyrogallolaldehyde (4.87 mg/100g) were examined (Figure 7 e). In T5, one phenolic acid, gallic acid (4.51 mg/100g) was analyzed (Figure 7 f). In T6, two phenolic acids, protocatechuic acid (3.03 mg/100 g) and gallic acid (11.44 mg/100g) were examined (Figure 7 g). The HPLC quantification of phenolic acids in soil is shown in figure 8.

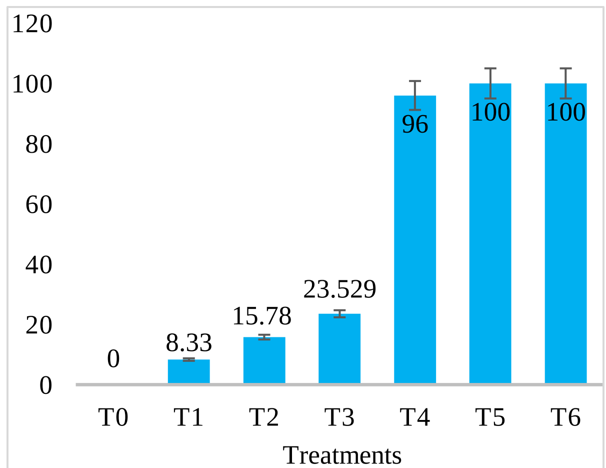

Figure 2. Effect of Eucalyptus on percentage mortality rate of Prosopis cineraria.

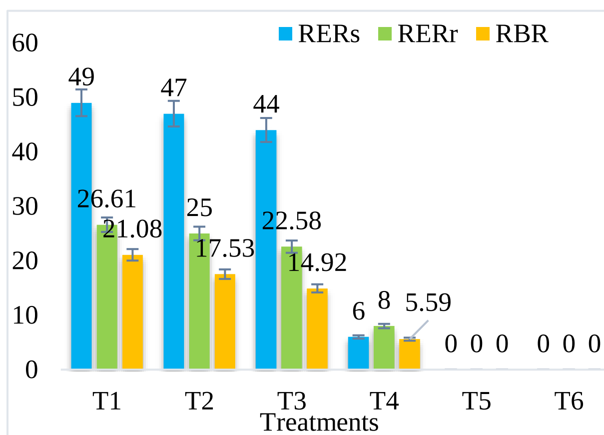

Figure 3. Effect of Eucalyptus on growth of *Prosopis cineraria*.

Table 3. One-Way analysis of variance (ANOVA) of various parameters.					
Source	**DF**	**SS**	**MS**	**F**	**P**
Germination	6	2742.86	457.14	17.81	0.010
Leaf No.	6	240.64	40.107	17.81	0.010
Shoot length	6	496.05	82.675	132.49	0.000
Root length	6	688.33	114.72	20.78	0.000
Fresh Weight	6	26.739	4.456	32.09	0.000
Dry weight	6	5.306	0.884	86.11	0.000

Analysis of soil

Eucalyptus leaf litter has slightly decreased the soil pH as compared to control. However, leaf ash has, to some extent, increased the soil pH. The leaf litter, has slightly increased the soil EC. Likewise, the leaf litter ash has greatly increased the soil EC. A higher amount of organic matter was noted in the treatments applied with leaf litter. The amount of phosphorus (P) and nitrogen (N) was noted in higher concentrations in leaf litter applied treatments. The amount of potassium (K) and sodium (Na) was observed in very high concentrations in leaf litter ash treatments. Still, the amount of K and Na was found to be higher in leaf litter as compared to the control (Table 4). The analysis of variance is given in table 5, it shows a significant difference between treatments.

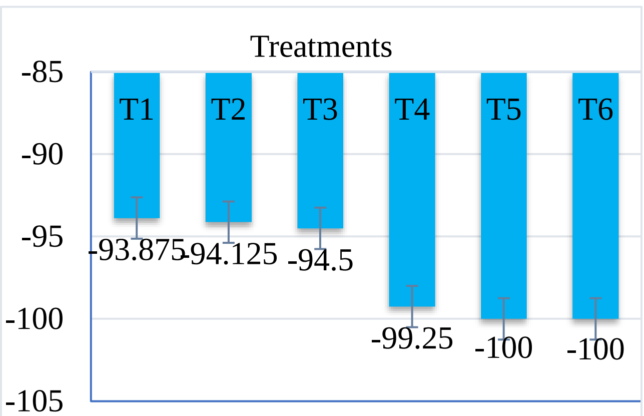

Figure 4. Effect of Eucalyptus on inhibition of seedling length of Prosopis cineraria.

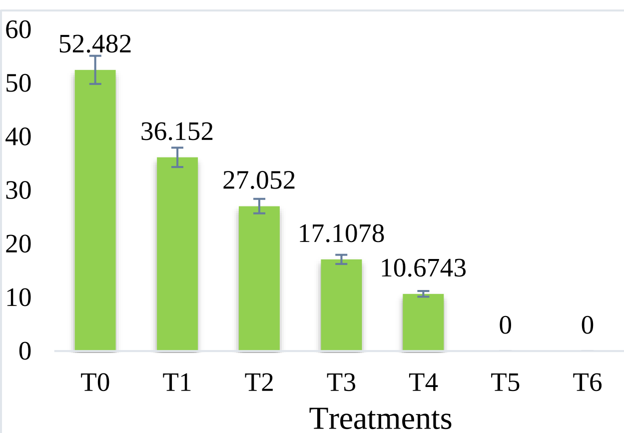

Figure 5. Effect of Eucalyptus on chlorophyll content (mg/g f. wt.) of Prosopis cineraria.

DISCUSSION

The results shown that *Eucalyptus* leaf litter and ash have an allelopathic effect on the studied plant, reduced

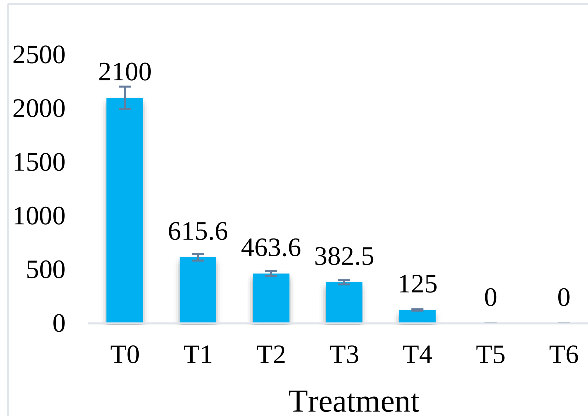

Figure 6. Effect of Eucalyptus on seedling vigor index (SVI) of Prosopis cineraria.

the germination of *Prosopis cineraria*. Induced the seedlings mortality of *Prosopis cineraria*. Many authors have found similar results of reduction of germination and growth of plants by *Eucalyptus*: such as; Khan *et al*., (2005); Shiming (2005); Duarte *et al., (*2006); Khan *et al*., (2009); Sirawdink *et al*., (2011); Bughio *et al*., (2013); Shaddam *et al*., (2020); Sobola *et al*., (2022). The allelopathic plant extract has suppressed the germination and also induced the mortality of seedlings (Eyini *et al*., 1996). The shoot and root length of the *Prosopis* seedlings were found to decrease with treatments applied with *Eucalyptus* leaf litter and ash. Same results were found in Bughio *et al*., (2013), *Eucalyptus* leaf litter decreased the root and shoot of *Acacia nilotica*. *Eucalyptus* has a negative effect on the root length of *Arachis hypogea* (Lawan *et al*., 2011). The biomass of *Prosopis* seedlings was decreased by *Eucalyptus* leaf litter and ash. Similar negative effects of *Eucalyptus* were reported by Bughio *et al*., (2013). The phenolic acids, alkaloids, terpenoids released by allelopathic plants reduce the growth, fresh weight of plants (Siddiqui and Zaman, 2005). Yaghmai *et al*., (2023) found that *Eucalyptus* significantly decreased the shoot length and biomass of cucumber and black nightshade. The amount of chlorophyll was also decreased in *Prosopis* seedlings. Djanaguiraman *et al*., (2005) reported a similar effect of *Eucalyptus* leaf litter. The leaf litter decreased the amount of chlorophyll in black

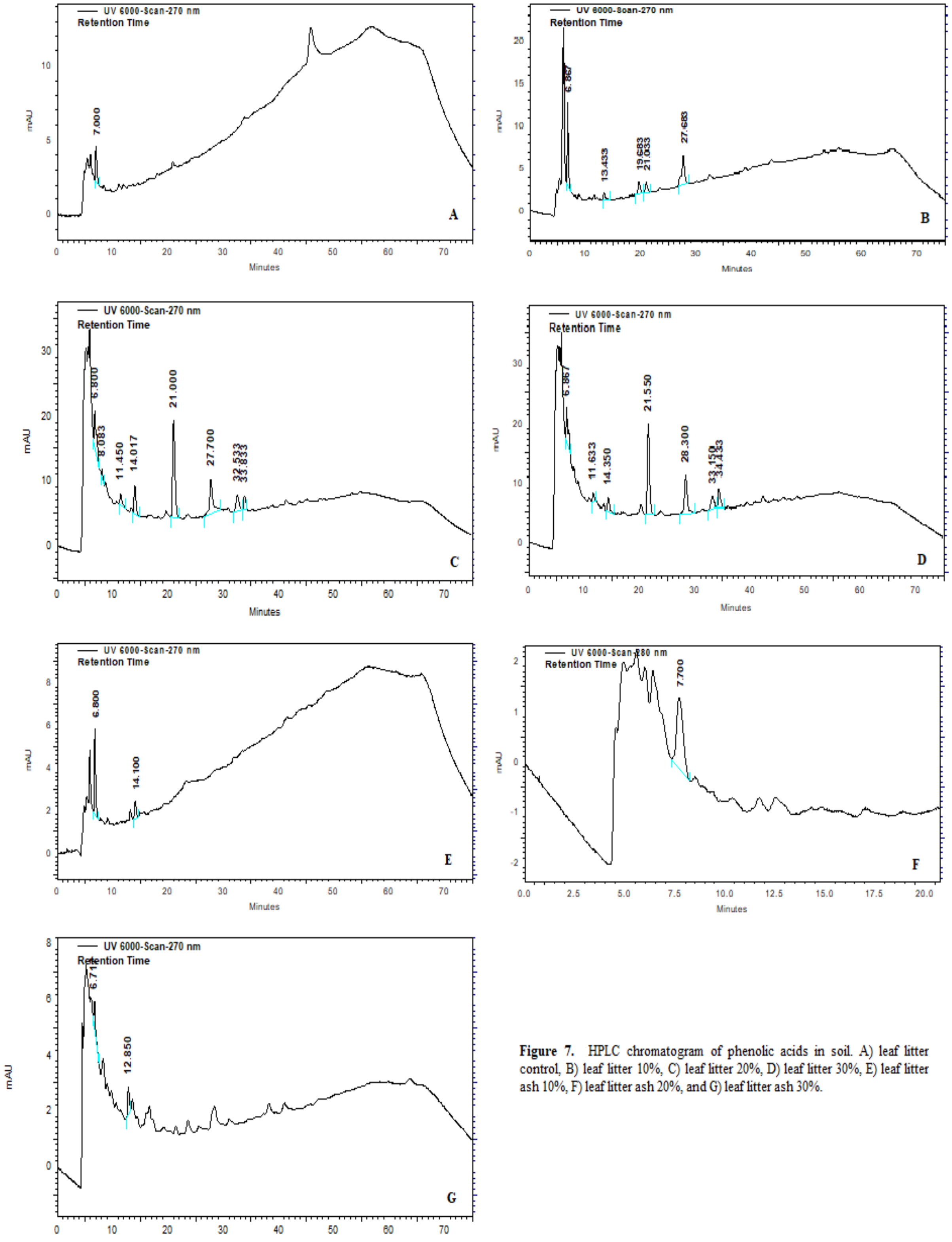

Figure 7. HPLC chromatogram of phenolic acids in soil. A) leaf litter control, B) leaf litter 10%, C) leaf litter 20%, D) leaf litter 30%, E) leaf litter ash 10%, F) leaf litter ash 20%, and G) leaf litter ash 30%.

Table 4. Effect of *Eucalyptus* leaf litter and ash on soil properties.

Treatments	pH	EC (dS/m)	OM (%)	N (%)	P (ppm)	K (ppm)	Na (ppm)
T0	8.1	0.332	0.39	0.0224	2.9	56	80
T1	8.0	0.395	8.2	0.112	9.2	74	90
T2	8.0	0.417	9.92	0.121	9.8	84	105
T3	7.9	0.432	10.9	0.126	10.1	96	128
T4	8.7	6.119	1.2	0.041	2.7	3600	2000
T5	9.3	7.535	2.19	0.0532	4.7	4000	2500
T6	9.4	8.940	3.67	0.066	6.7	4550	2920
Mean	**8.48**	**3.45**	**5.21**	**0.077**	**6.58**	**1780**	**1117.57**
St. Dev	0.646603	3.901136	4.364722	0.041884	3.208545	2141.206	1295.862

gram. Zhou and Yu, (2006) reported similar results, a reduction in the amount of chlorophyll by the activity of different allelochemicals. Bughio *et al.*, (2013) found the reduction in the amount of chlorophyll in *Acacia nilotica* by *Eucalyptus* leaf litter. The seedling vigor index was found to decrease with *Eucalyptus* leaf litter and ash. Sasikumar *et al.*, (2001); Djanaguiraman *et al.*, (2002) found a reduction in vigor index of plants by *Eucalyptus*. Karthiyayini *et al.*, (2003); Mubeen *et al.*, (2011); Das *et al.*, (2012) and Bughio *et al.*, (2013) also found the similar results. The phenolic compounds were analyzed from *Eucalyptus*. The phenolic compounds identified in *Eucalyptus* are well known for their allelopathic properties. Sasikumar *et al.*, (2001) identified phenolic acids from four *Eucalyptus species* such as gallic, hydroxybenzoic, syringic, *p*-coumaric, ferulic, catechol and vanillic acids. They studied the effect of identified phenolic acids on the germination and growth of redgram. Each individual phenolic compound has inhibited the germination and growth of redgram. Many authors, Jayakumar and Eyini, (1990); Sivagurunathan *et al.*, (1997) and Vaughan and Ord, (1990) identified phenolic compounds from *Eucalyptus* species. The amount of soil nutrients, was increased by *Eucalyptus* leaf litter and ash. Higher soil EC, potassium and sodium in leaf litter ash caused the 100% mortality of *Prosopis cineraria* seedlings. When the leaves are burned at low fire intensities, a large amount of the nutrients are returned to the ecosystem (Yang *et al.*, 2005). During the burning of leaf litter, phosphorus and nitrogen are volatilized at lower temperatures. The increase in nutrient concentration is due to the fact that some nutrients need high temperatures for volatilization (Jensen *et al.*, 2001). Leaves play a significant role in ecosystems due to their higher concentration of nutrients. *Eucalyptus* requires more nutrients as a fast-growing tree; it also returns to the soil in the form of leaf litter. *Eucalyptus* sheds large amounts of leaf litter that remain on the ground. Due to the presence of allelopathic compounds in leaf litter that release into the soil after decomposition, it inhibits the germination and growth of native trees in their surroundings. In native forests, under *Eucalyptus* canopies, the ground remains completely bare; it lacks native trees and understory vegetation. Due to wildfires, leaf litter burns and is converted into ash. It is also a common practice in Pakistan to burn the leaf litter of *Eucalyptus* in native forests since it accumulates in higher amounts under tree canopies. After fire, *Eucalyptus* species regenerate from seeds and through coppice. Thereby, *Eucalyptus* develops monoculture stands and displaces the native tree species. *Eucalyptus* in Pakistan is extensively planted in agriculture fields, in public parks, homes, along roadsides and in native forests. Due to its allelopathic and ecological effects, *Eucalyptus* is a threat to native trees/plants in Pakistan.

CONCLUSION

Prosopis cineraria, germination and growth were observed to be significantly reduced by leaf litter and

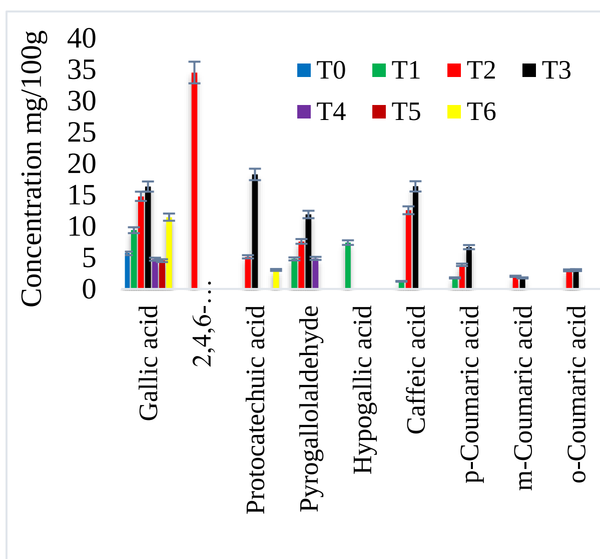

Figure 8. Quantification of phenolic acids in soil applied with leaf litter and ash.

ash-applied treatments. The mortality of seedlings was recorded in leaf litter ash. Higher EC, potassium and sodium were noted in the ash soil. A negative correlation was seen between concentrations and the plant parameters studied. In soil mixed with leaf litter, nine phenolic compounds and in leaf litter ash soil three phenolic compounds were identified and quantified. The 2, 4, 6-trihydroxybenzoic acid was predominant with a concentration of 34.51 mg/100g in leaf litter soil and gallic acid was predominant with a concentration of 11.44 mg/100g in ash soil. *Eucalyptus* has deleterious ecological effects on the native species, *Prosopis cineraria*. Therefore, *Eucalyptus* should not be planted along with native trees or in native forests in Pakistan. Thus, it is also recommended that *Eucalyptus* trees be replaced with native trees.

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