Indian J. Range Mgmt. 9 : 63-67, 1988 ISSN 0970—101 X

Impact of *Eucalyptus* on *Parthenium*—a Weed

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Abstract

The allelochemics from *Eucalyptus* leaves were fractionated using polar and non polar solvent systems. These allelochemics were tried on another allelopathic species i.e., *Parthenium hysterophorus* L. and were found quite effective in decreasing chlorophyll content and percent cell survival. Organic leachate and chlorofrom fractions were found least effective in killing the cells as well as in decreasing the macromolecules. Water fraction although quite effective at sub cellular and cellular level was not so effective at macromolecular level. Under the influence of allelochemics the water soluble carbohydrates were affected to maximum extent.

Introduction

Eucalyptus is an evergreen tall tree and has overshadowed the planting of indigenous species because of its fast growth rate. The leaves of *E. camaldulensis* produce large quantity of terpenes during hot, dry summer which is absorbed on dry soil in large amounts. The concentration of terpenes in litter zone soil were great enough to completely stop seed germination of herb species (del Moral and Muller 1970). Fog drip which is very common under *Eucalyptus* tree is responsible for the lack of annual herb growth under the canopy of *E. globulus*. The effect of litter is noticed to reduce groth and survival of seeds with no vegetation in *Eucalyptus* vicinity (del Moral and Muller 1969). In California, vegetation adjacent to naturalized stands of *E. camaldulensis* is inhibited severerly. Annual herbs rarely survive at maturity where *Eucalyptus* litter accumlates (del Moral and Muller 1970). From the foregoing it is clear that *Eucalyptus* contains allelochemics which are toxic to certain species. The present study was undertaken with a view to understand the mechanism of allelopathy of *Eucalyptus*, which, if possible, may be used as eradication means for an abnoxious weed—*Parthenium hysterophorus* L. which itself is allelopathic and allergic plant.

Materials and Methods

The leaves of *Eucalyptus* were collected from Botanical Garden, Punjab University, Chandigarh. The aqueous leachates, organic leachates and organic extract fractions were prepared from these leaves following the method of Anita *et al.* (1985). *Leachation*: Freshly colleted leaves were soaked in distilled water (pH 6.6, concentration: 0.5g/ml) for 10 h and filtered. One part of the clear filtrate was used as *aqueous leachate* while to the other part was added 50% HCl (1:1 v/v). The precipitates formed were centrifuged and washed 5-6 times with distilled water in a

Table 1: Effect of *Eucalyptus* extracts on chlorophyll content and cell survival in *Parthenium hysterophorus* L.

Treatment	Concentration	Total chlorophyll content (mg/gm fresh wt.)	% cell survival
Control	0	1.72	100
Aqueous leachate	1 gm/ml fr. wt.	1.42	49.77
Organic leachate	1 g/ml fr. wt.	1.28	69.50
Pet ether frn.	0.1%	1.60	44.29
Methanolic frn.	0.1%	1.54	44.03
Chloroform frn.	0.1%	1.20	65.02
Water frn.	0.33g/ml dry wt.	1.07	42.60
Eucalytus oil	0.1%	1.49	43.04

span of 24. Finally the precipitates were dried. The requisite weight of dried precipitates was dissolved in few drops of ethyl alcohol and the concentration was made with water. It has been referred to as *Organic leachate.*

Extraction: Shade dried leaves of *Eucalyptus* were powdered and kept in petroleum ether (pet. ether) for 24. The pet. ether was decanted off from the residue (marc), the solvent (pet. ether) was evaporated on the boiling water bath. Weighed amount of residue was dissolved in 0.5 ml xylene. A drop of tween-20 was added as surfactant and the volume made with distilled water. This has been called as *pet. ether fraction.*

The marc, on the other hand was soaked in methanol for 24 h and filtered. From one half of the filtrate, the solvent was evaporated over hot water bath. Weighed amount of residue was dissolved in 1-2 drops of methanol and the final volume was made with distilled water. This has been referred to as *methanol fraction.* The other half after evaporation of MeOH was partitioned between chloroform and water to get $CHCl_3$ and *water fraction*, respectively. Chloroform was evaporated over hot water bath. Residue was dissolved in methanol and the final volume was made to 100 ml with distilled water. Water fraction was used as such after making required concentration with distilled water.

Eucalyptus oil was extracted using oil trap and subject to fractional distillation. It was dissolved in 1-2 drops of ethyl alcohol and a drop of tween-20 was added as surfactant. The final volume was made with distilled water. This is *Eucalyptus* oil treatment.

Treatment: The plants of *Parthenium hysterophorus* L. two months old from the date of seedling emergence were selected. These were given a fine mist of aerial spray (50 ml/plant) of aqueous leachate/organic leachate/organic extract fractions/oil for three consecutive evenings. On the following morning of the last spray, the leaves

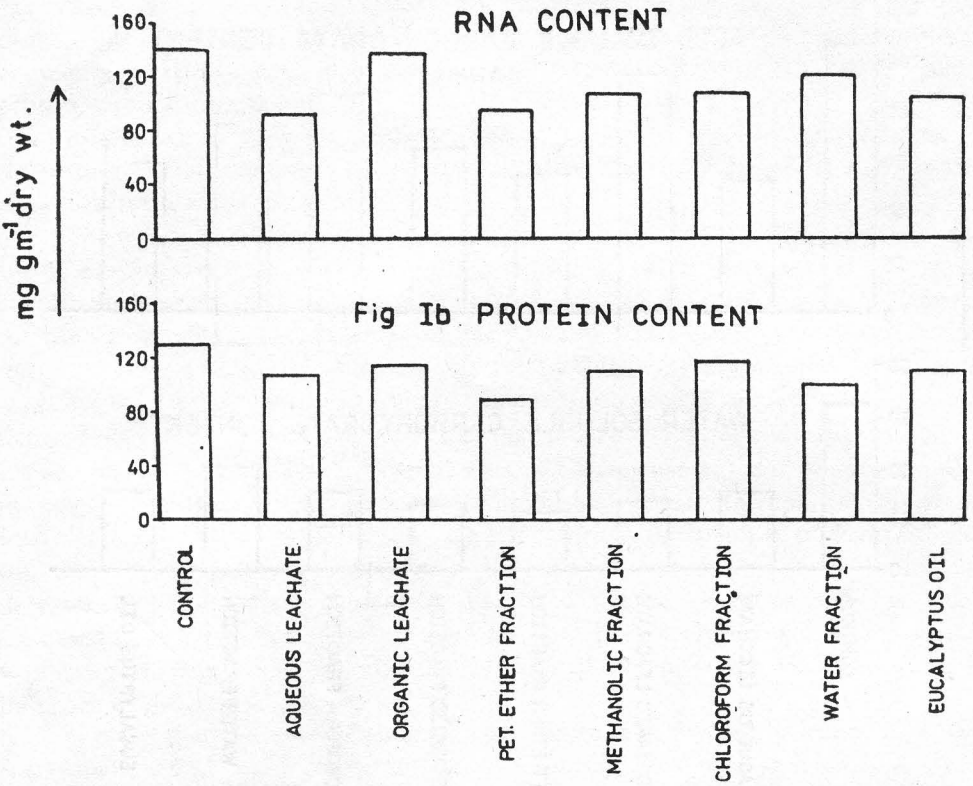

Fig. 1: Effect of *Eucalyptus* extracts on RNA and protein content in *Parthenium hysterophorus* L.

were plucked, washed and utilized for different tests. Distilled water spray served as control.

Cell survival of treated as well as control leaves was calculated following the method of Steponkus and Lanphear (1957) involving use of 2, 3, 5-triphenyl tetrazolium chloride. The chlorophyll content was estimated following the use of dimethyl sulphoxide (DMSO) after the method described by Hiscox and Istraelstam (1979).

The treated as well as control leaves were crushed in acetone. These were freed from pigments, water and phenol and air dried. The contents of total soluble proteins, RNA and carbohydrates (nutritive as well as structural) were colorimetrically estimated from dried powder by methods of Lowry *et al* (1951), Mezbaum (1939) and Loewus (1952), respectively.

Results and Discussion

The study of the allelochemics of *Eucalyptus* leaves on *Parthenium* revealed that the cell survival (an indicative of respiratory ability) of *Parthenium* leaves gets affected almost in all the cases (Table 1). However, most effective were the aqueous leachates, petroleum ether frcation, methanolic fraction and water fraction. The organic component of aqueous leachates (organic leachate) was seen not that effective as was the aqueous leachates. It shows that toxins are highly water soluble, effective and

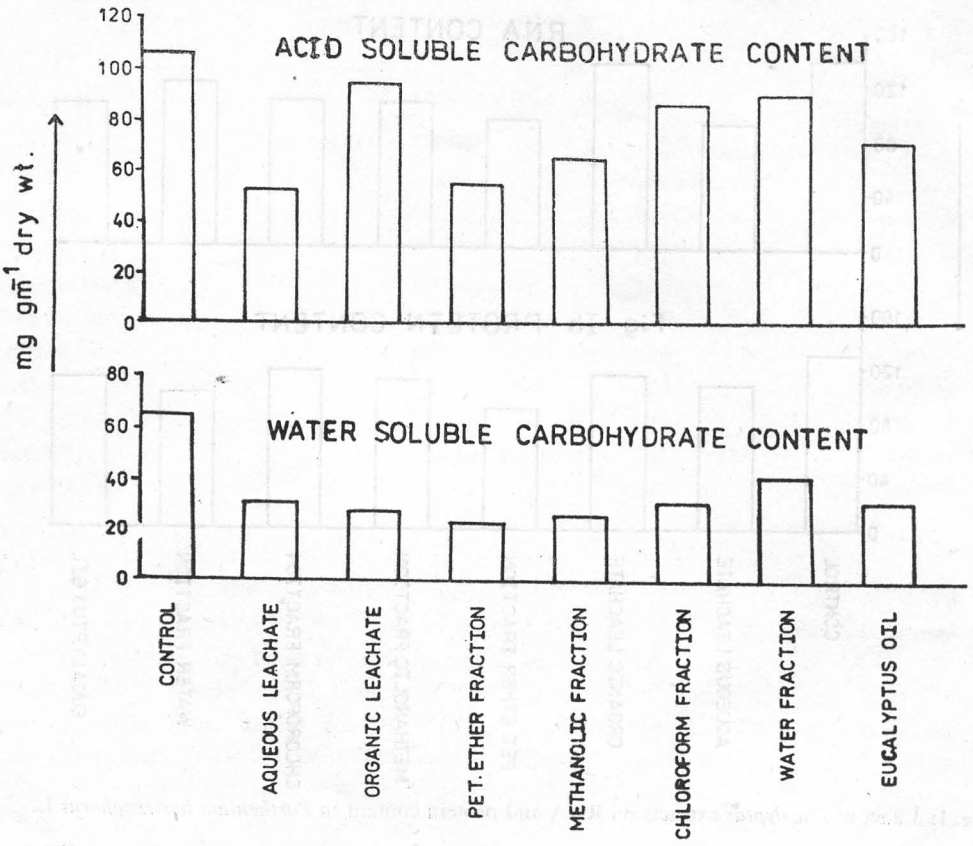

Fig. 2: Effect of *Eucalyptus* exracts on carbohydrate content in *Parthenium hysterophorus* L.

occur in their glycosidic form. Neiman (1952), Mandava (1985), and Anita and Kohli (1987) reported that many compounds having glycosidic group attached to them are easily dissolved in water and are effective, *Eucalyptus* oil also decreased cell survival showing, thereby, the presence of volatile alleochemics.

Water fraetion and the chloroform fraction derived from the methanol affected the chlorophpyll content more than any other extract fraction (Table 1). It is not clear whether this reduction in the chorophyll is a metabolic function due to inhibition of fresh synthesis or a reason of enhancement of chlorophyll degradation. Petroleum ether and methanolic extract fractions (the fractions derived in non polar solvent) effective in reducing the percent cell survival were not effective in reducing the chlorophyll content (Table 1). This also supports the specificity of allelochemics towards physiological function as is seen in *Lantana* (Kohli *et al.* 1987). One thing is, however, clear that apart from this specificity of allelochemics to physiological function, their action is specific to the responding plant too. The extract functions which are not effective in *Lantana* (Kohli *et al.* 1987) are effective in *Parthenium* or vice versa.

The aqueous leachates, petrolem ether fraction, methanolic fraction, chloroform fraction, water fraction and *Eucalyptus* oil decrease the total RNA content (Fig. 1). This shows that the allelochemic(s) exert their effect on *P. hysterophorus* by inhibiting

the mRNA synthesis. On the contrary, not much effect on the content of total soluble protein was noticed (Fig. 1). The decrease in RNA content with no effect on protein content points that it is possibly the free formed mRNA which gets utilized in protein synthesis inhibition.

Compared to aqueous leachate, the organic leachate was less effective in decreasing the macromolecules confirming that allelochemics which occur in their glycosidic form are water soluble.

The water soluble carbohydrates in response to the extract fractions were seen to have decreased appreciably (Fig. 2). Similar extent of decrease was seen in acid soluble saccharides, but in response to aqueous leachates, petroleum ether fraction, methanolic fraction and *Eucalyptus* oil, the decrease was more (Fig. 2). The fall in the nutritive and structural saccharides in response to the *Eucalyptus* allelochemic suggests their involvement in energy compensation needed to maintain the survival demand, especially in the light of the fact that these allelochemics drastically reduced the survival of the cells. The activity of α—and β—amytase was seen to have decreased in response to the extract fractions (unpublished data), thereby, showing that hydrolysis of starch gets decreased. On the contrary, the quantity of acid and water souluble carbohydrates was also seen to have decreased. This suggests that the decrease in carbohydrate content is due to decreased synthesis and not due to increased hydrolysis. The statement that no new carbohydrates are being formed, gets support from the results of the chlorophyll centent which decreased under the influence of different allelochemics.

It is felt that *Eucalyptus* contains two or more than two allelochimcs and these exert their, effect on *P. hysterophorus* by inhibiting 'denovo' synthesis of RNA and carbohydrates.

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