Journal of Tropical Forest Science 11(4):801–808 (1999)

ALLELOPATHIC EFFECT OF *LEUCAENA LEUCOCEPHALA* ON *ZEA MAYS*

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Received February 1998

SINGH, H. P., BATISH, D. R. & KOHLI, R. K. 1999. Allelopathic effect of *Leucaena leucocephala* on *Zea mays*. Allelopathic effect of leaves and litter of *Leucaena leucocepahala* was examined on *Zea mays* through various greenhouse and laboratory studies. The extracts of dried leaves as well as litter were found to be inhibitory to germination and seedling growth of *Z. mays* under laboratory conditions. Further, the amendment of leaf powder and litter in the soil as mulch or soil mixture caused detrimental effect on root and shoot growth and biomass which increased with increase in the amount of litter/leaf powder applied. The inhibitory effect was greater when the material was amended as soil mixture. Litter was found to be more inhibitory than leaf powder in both laboratory and greenhouse studies. The amount of phenolics (a well known allelochemical group) was determined from different soil horizons under *Leucaena* plantations as well as its leaves. Maximum amount was found in leaves followed by the A_{00}- soil horizon containing partially degraded leaf litter of *Leucaena*.

Key words: Allelopathy - *Leucaena* leaf mulch - litter - phenolics - growth performance

SINGH, H. P. , BATISH, D. R. & KOHLI, R. K. 1999. Kesan alelopati *Leucaena leucocephala* terhadap *Zea mays*. Kesan alelopati daun dan sarap *Leucaena leucocephala* diuji dengan melakukan pelbagai kajian di rumah hijau dan di makmal. Ekstrak daun kering dan juga sarap didapati rencat terhadap percambahan dan pertumbuhan anak benih *Z. mays* di bawah keadaan makmal. Seterusnya, pindaan serbuk daun dan sarap di dalam tanah sebagai sungkup atau campuran tanah menjejaskan akar dan pertumbuhan pucuk dan biojisim yang bertambah dengan bertambahnya jumlah sarap/serbuk daun yang digunakan. Kesan rencat adalah lebih besar apabila bahan tersebut dipinda sebagai campuran tanah. Sarap didapati lebih rencat berbanding dengan serbuk daun dalam kedua-dua kajian di makmal dan di rumah hijau. Banyaknya fenol (satu kumpulan alelokimia yang sangat dikenali) ditentukan daripada horizon tanah yang berbeza serta daun di bawah ladang *Leucaena*. Jumlah maksimum di dalam daun diikuti dengan A_{00}- horizon tanah yang mengandungi sarap daun pokok *Leucaena* separuh usang.

Introduction

Many socio-economic problems have arisen due to over-exploitation of natural resources caused by rapidly increasing population. Agricultural land and forests are under tremendous pressure to cater to these increasing demands. Diversification of modern agriculture by integrating fast-growing multi-purpose woody perennials has, therefore, been envisaged so as to achieve increased agricultural production as well as that of timber, fuel, wood and forage, etc. in a sustainable way.

Leucaena leucocephala (Lam.) de Wit (hereafter referred to as *Leucaena*), commonly known as subabul in India, is a fast-growing versatile leguminous tree widely distributed in the tropics (Brewbaker 1987). It is grown extensively under various afforestation programmes and in agricultural fields either in alleys or along field margins as boundary plantations or even scattered. As regards the interaction of this tree with agricultural crops or other vegetation, both positive as well as negative reports are available. Some reports indicate that tree prunings placed as mulch in agricultural field improve the physical characteristics of soil and thus, increase the productivity (Budelman 1989, Tian *et al.* 1993). On the other hand, reduction in the growth and yield of crops growing either in close proximity to tree lines or alleys cropped with it has been reported (Karim *et al.* 1991, Rishi & Dhillon 1996). Likewise, Suresh and Vinaya Rai (1988) and Chou and Kuo (1986) reported exclusion of ground vegetation under this tree and ascribed this to allelopathy which refers to the negative effect (but positive too, in some cases) of one plant on the other through the release of chemical substances into the environment (Molisch 1937, Rice 1984, Janovicek *et al.* 1997). This phenomenon may play a significant role in determining the interactions of *Leucaena* with agricultural crops as well. We, therefore, hypothesise that the allelopathic chemicals (=allelochemicals) of the tree which are released in the soil through fresh intact leaves and the litter (comprising mainly dead leaves and small twigs which fall in plenty and are in various stages of decomposition) exert a negative effect on the crops and understorey vegetation. The present study was, therefore, undertaken to investigate the

(i) effect of *Leucaena* leaves and litter extracts and their amendment in the soil on the growth and biomass of *Zea mays* (maize)— an important agroforestry combination, and
(ii) amount of phenolics in the fresh leaves, the litter enriched top organic layer of the soil and the soil beneath the organic layer.

Material and methods

Collection of material

Fresh leaves and litter were collected from the 6 ± 1-y-old trees of *Leucaena* growing near the agricultural fields on the outskirts of Chandigarh (30°42' N, 76° 54' E; 333 m a.s.l.). Because of its perennial and evergreen nature, *Leucaena* produces litter throughout the year and forms an organic horizon (comprising upper A_{00} - horizon having freshly fallen raw litter, and the lower A_0-horizon consisting of finely degraded litter mixed with the soil). Soil samples were collected from the A_{00}-, A_0- and A-horizons (i.e. just below the organic layer comprising a mixture of mineral matrix). The soil was also collected from the upper surface (3-5 cm depth) of *Leucaena* free site for the purpose of amendment. The sampling was done in triplicate and the samples of leaves, litter and soil were air dried, milled, sieved and filled in polythene bags for further studies. For the bioassay studies, pure line certified seeds of *Zea mays* var. EH-114 were used.

Preparation of extracts from Leucaena leaves and litter

Aqueous extracts of leaf and litter were prepared by soaking 10 g dried and powdered material of each in 500 ml of pure water (conductivity < 0.05 µS, obtained through Millipore RO - Milli Q Water Purification System) for 24 h and 25 °C so as to get an extract of 2 % concentration. The extracts were filtered through a muslin cloth followed by Whatman # 1 filter paper. Each of these leaf or litter extracts was diluted to 1% and kept at 4 °C until used.

Amendment of soil with Leucaena leaf and litter

Three kilogram of the *Leucaena* free soil was filled in the 10 × 10 × 15 inch earthenware pots. The powdered *Leucaena* leaves and litter were amended in this soil at the rate of 12, 24, 36 and 48 g per pot equivalent to 2.5, 5, 7.5 and 10 t ha^{-1}. Amendment of leaf as well as litter was done in the following two ways in order to simulate the natural conditions, i.e.

a) as surface mulch (equivalent to A_{00}- horizon as already described), and
b) as soil-powder mixture (equivalent to A_0- horizon).

A set of pots amended in a similar way but with peat moss (an inert plant material) in place of leaf or litter powder served as control. Three pots were maintained for each treatment and the entire set-up was placed in a greenhouse in a completely randomised block design.

Determination of phenolic content

The amount of total phenolics was determined from the A_{00}-, A_0- and A-horizons of the soil collected from the *Leucaena* inhabited area and the leaf powder using Folin-Ciocalteu reagent as per the method of Swain and Hillis (1959). In addition, phenolics were extracted from the top A_{00}- layer using the sodium salt of EDTA following the method of Kaminsky and Muller (1977). Two concentrations of these extracted phenolics (0.5 and 1.0 mg ml^{-1}) were prepared in pure water after initially dissolving the requisite amount in a few drops of ethyl alcohol. These were used for the germination and growth studies on *Z. mays* under laboratory conditions.

Germination and growth studies

Under laboratory conditions

The germination studies were carried out in 6" diameter Petri dishes lined with Whatman # 1 filter paper and underlined with a thin absorbent cotton wad. These were moistened with 10 ml of each of the extract or phenolic solutions. Twenty-five seeds of *Z. mays* imbibed for 10 h in the respective solutions were then equidistantly placed on the Petri dishes. Four replications were maintained for each treatment. Treatment in a similar manner with pure water instead of extracts served as control. All the Petri dishes were maintained at 25 ± 1°C and 75 ± 2% humidity in a seed germinator. After a period of 10 days, percentage germination, seedling length and dry weight of seedlings were measured. The experiment was repeated twice.

Under greenhouse conditions

Five seeds of *Z. mays* were sown per pot amended with leaf and litter powder as described above. After germination, these were thinned to two seedlings per pot. After eight weeks, the plants were uprooted, their root and shoot length were measured and biomass of root and shoot was determined by the oven-drying method (drying at 80 °C for 24 h). Three replications were made for each treatment and the pots were arranged in a randomised manner.

Statistical analysis

The different treatments were subjected to one-way ANOVA and the comparisons were made using Duncan's multiple range test (Duncan 1955). Correlation coefficients (r values) were also calculated between parameter value and respective concentrations.

Results and discussion

The extracts of *Leucaena* leaves and litter in concentrations as low as 1 or 2% reduced the germination and seedling growth of *Z. mays* under laboratory conditions (Table 1). The litter extracts were more inhibitory than the fresh leaves. Further, the amendment of soil collected from *Leucaena* free site with leaf or litter powder reduced growth (root and shoot length) and biomass of *Z. mays* compared to peat amended control (Figures 1a-d). Here too, the litter was more inhibitory than the fresh leaves, irrespective of the mode of application. The amendment in the form of soil mixture caused more reduction than as surface mulch. With an increasing rate of amendment, a further decrease in the growth and biomass of *Z. mays* was noticed as is also evidenced by reciprocal and statistically significant values of r between concentration and effect (Figures 1a-d). The study, therefore, reveals a strong allelopathic effect of leaves as well as litter of *Leucaena* on *Z. mays*.

Table 1. Effect of leaf and litter extracts of *Leucaena* on the germination and initial growth of *Zea mays*

Treatment		Germination (%)	Seedling length (cm)	Biomass (mg)
Control (water)		100[a]	17.8[a]	92.4[a]
Leaf powder	1 %	86.3[b]	13.8[b]	46.3[b]
	2 %	68.3[c]	6.4[d]	15.2[d]
Litter powder	1 %	85.0[b]	10.0[c]	35.6[c]
	2 %	61.0[c]	5.6[d]	17.2[d]

Different letters in a column represent significant difference at 5% level applying Duncan's multiple range test.

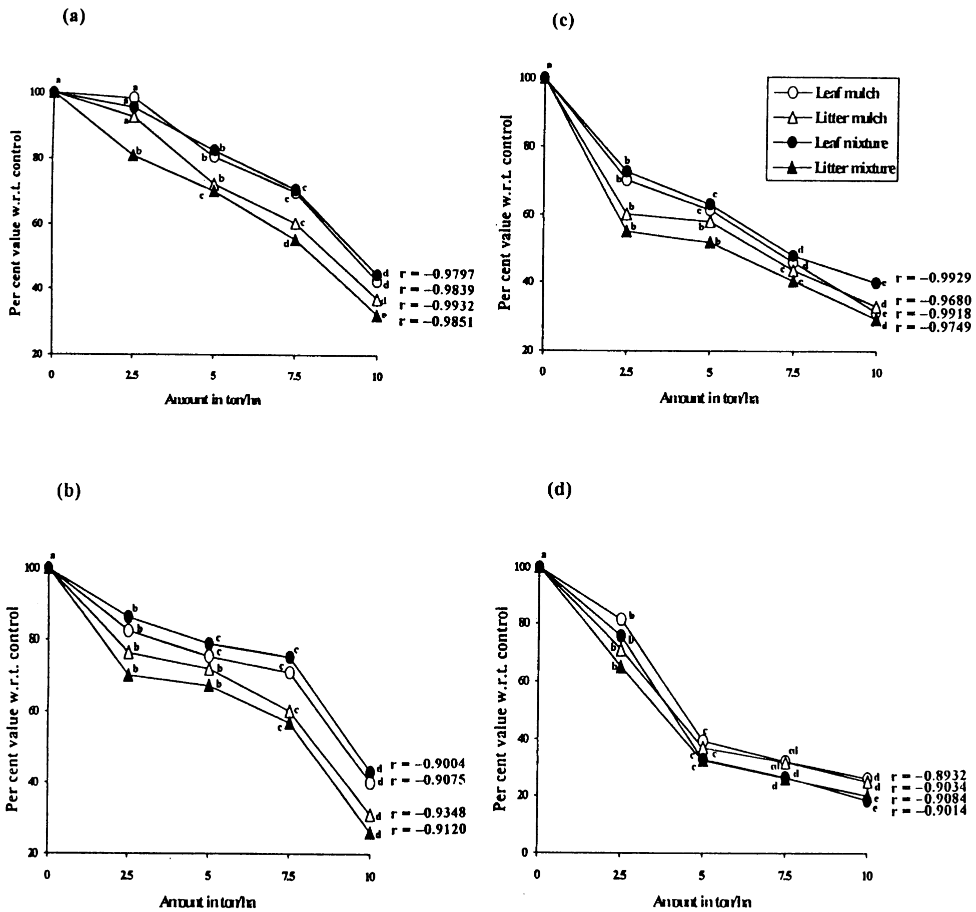

Figure 1. Effect of varying concentrations of leaf and litter of *Leucaena leucocephala* amended in the soil as mulch or soil mixture on the (a) root length, (b) shoot length, (c) root biomass, and (d) shoot biomass of *Zea mays*

Similar letters along a curve represent insignificant difference at p = 0.05 level applying Duncan's multiple range test. *r* along each curve represents correlation coefficient between different concentrations and value of parameter.

In nature, *Leucaena* leaves fall throughout the year and form a characteristic litter layer dominated by its leaves besides pods and seeds. It hardly supports any understorey vegetation except its own seedlings (Chou & Kuo 1986). The growth of *Leucaena* is fast although the tree is not very hardy. Even its branches keep falling with the pressure from strong winds and may exert a negative effect on adjoining crops and vegetation. *Leucaena* when grown as a hedge tree is pruned periodically and the prunings placed in the fields with a motive to incorporate the nutrients in the soil. Facelli and Pickett (1991) have reported that plant litter placed as mulch has an important impact on nutrient availability and their accumulation in the soil. However, a number of reports indicate that several allelochemicals are released upon leachation or decomposition from the litter (Blashke 1979, Rice 1984, Kuiters 1990, Chou & Leu 1992, Gonzalez *et al.* 1995). A number of reports are available which indicate that litter of several trees/shrubs cause significant toxicity to the understorey and field crops (Younger *et al.* 1980, Wilt *et al.* 1988, Molina *et al.* 1991, Inderjit & Mallik 1996, Singh 1996). Jobidon (1986) reported the reduction in seed germination of grasses by the leaf and litter extracts of coniferous trees. The allelochemicals upon release may cause toxic effect on the vegetation or prevent the emergence and growth of crop plants. In some cases litter mixed in soil significantly reduced growth and biomass accumulation of test plants and even the addition of fertilisers failed to compensate it (Sidhu & Hans 1988, Mallik 1996).

Our studies further show that phenolics are present in considerable amount in *Leucaena* leaves, the top soil layer containing un- and partially decomposed litter (equivalent to A_{00}-horizon) and decomposed litter-soil mixture (equivalent to A_0-horizon) and the humus free soil (i.e. A_0-horizon) in the order of leaves > A_{00}-soil horizon > A_0-soil horizon > A-horizon (Table 2). Chou and Kuo (1986) have reported the presence of phenolic acids, quercetin and mimosine in the leaf and litter of *Leucaena*. Chaturvedi and Jha (1992) reported that the reduction in the germination and radicle growth of rice seedlings in response to leaf leachates was due to the presence of mimosine in the leaves. In the present study, however, total phenolic content was studied rather than individual compounds because the allelochemicals act additively or synergistically but not singly (Einhellig 1995). The phenolics collected from the uppermost soil layer (A_{00}-layer) were tested for their phytotoxicity towards *Z. mays* and seen to inhibit germination and growth under laboratory conditions (Table 3). The presence of phenolics may also affect the nutrient status of *Leucaena* as phenolics, in general, are known to alter the accumulation, availability and uptake of the nutrients in plants (Appel 1993).

Table 2. Amount of phenolics extracted from the leaves of *Leucaena* and different soil horizons

Material	Amount (mg $100g^{1}$)
Leaves	77.30 ± 2.35
A_{00}-horizon	45.18 ± 1.45
A_0-horizon	28.64 ± 0.50
A-horizon	12.38 ± 0.21

Table 3. Effect of different concentrations of phenolics extracted from the A_{00}- horizon under *L. leucocephala* on the germination and initial growth of *Z. mays* in the laboratory. Values are with respect to control.

Concentration (mg ml^{-1})	Germination (%)	Seedling length (cm)	Seedling dry weight (mg)
0 (Control)	100^{a}	100^{a}	100^{a}
0.5	85^{b}	66.47^{b}	45.4^{b}
1.0	47^{c}	25.41^{c}	35.3^{c}

Similar letters in a column represent insignificant difference at 5% level applying Duncan's multiple range test. Values in control: seedling length, 15.45 ± 1.32 cm; seedling dry weight, 88.72 ± 3.58 mg.

It is, therefore, concluded that the presence of phenolics in the *Leucaena* litter and leaves is largely responsible for allelopathic effect on *Z. mays*.

Acknowledgements

The first auhor is thankful to the Centre for Scientific and Industrial Research (CSIR), New Delhi, for financial assistance.

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