PHYTOTOXICITY OF *EUCALYPTUS MICROTHECA* F. MUELL. ON *PENNISETUM GLAUCUM* CV. BARI-HAIRY

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Abstract

Allelopathic effect of *Eucalyptus microtheca* F. Muell. was studied in laboratory experiments. The aqueous extracts from different dried plant parts, soaked for 48 hours, inhibited radicle growth, plumule growth and seed germination of *Pennisetum glaucum* cv. BARI-Hairy. However, no serious inhibition of seed germination was occurred. The toxicity varied from part to part and was related to concentration and soaking duration. Root exudates were highly toxic to the radicle growth followed by leaves, stem and whole plant material. For the growth of plumule, leaves were found to be highly toxic followed by whole plant, roots and stem. Enhancing effect on the growth of radicle was not observed. However, it was observed on the growth of plumule by roots and stem extracts only.

Introduction

Allelopathy is derived from two Greek words "Allelon" meaning mutual and "Pathos" meaning harm, i.e., the injurious effect of one plant upon another. The allelopathic behaviour of some plants has also been selective in some cases, i.e., retarding the growth of some species and/or enhancing the growth of others (Bisla *et al.*, 1992; Hasegawa, 1993; Patil, 1994; Macharia & Peffley, 1995; Ambika & Vidya, 1996; Gilani *et al.*, 2000). It means that some plants are highly specific in producing bio-chemicals, which are selectively toxic; in some cases, they have rather enhancing effect. Thus these recent reports about some toxic plants stimulating the growth is contradictory to the definition of allelopathy.

During the course of evolution, plants have developed the ability to produce chemicals either inhibitory or stimulatory which have not only enhanced their ability to compete but also given them an edge over others in struggle for existence. These chemicals are known as allelochemicals or allelopathins. They are present in plant roots (Tsuchiya *et al.*, 1994), stem (Smith & Martin, 1994), leaves (Goel *et al.*, 1994), flowers or inflorescence (Chou & Leu, 1992), pollens (Murphy & Aarsen, 1995), fruits (Ambika & Vidya, 1996) and seeds

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(Wardle *et al.*, 1991). Their concentrations vary with age, season, and plant parts. The plants produce them through decomposition of residues, root exudates, leachates and volatilization. Recent research has revealed that there are some plants producing chemicals which are more effective in promoting growth of the other plants than gibberellin or IAA (Hasegawa, 1993).

Material and Methods

The plant material was collected from *Eucalyptus microtheca* trees growing in PFI, Peshawar. The allelopathic effect of cold water extracts from dry leaves, roots, stem and whole plant was tested on the germination and growth of *Pennisetum glaucum* (L.) R.Br. cv. BARI-Hairy in the laboratory of the Department of Botany, Islamia College, Peshawar. Plant material was collected and washed thoroughly with running water to remove dust and other undesired organic material. It was dried at 100°C in an oven for 24 hours and then the material was crushed by an electric chopper, washed and soaked straight away for dry material treatment. In whole plant treatment, equal weights of crushed leaves, roots, stem and fresh fruits were mixed thoroughly for homogeneity.

Dry Material Bioassay

Dry leaves, roots stem and whole plant material weighing 5, 10 and 15 g each were soaked in separate beakers each containing 100 ml. doubly distilled cold water for 48 hours. Extracts were filtered using Whatman filter paper No.1. The filtrates were either used straight away or stored in a refrigerator at 8°C for further use. The effect of aqueous extracts was tested against *Pennisetum glaucum* using standard filter paper bioassay techniques following Khan (1982), Chaghtai *et al.* (1985, 1986, 1988), Chaghtai (1992) and Gilani *et al.* (2000).

The germination and growth of *P.glaucum,* the test species, were studied in 9 cm. diameter sterilized petridishes each lined with two layers of Whatman filter paper No.1. The filter paper forming the seed bed was soaked with 3ml. of plant extracts and healthy seeds of *P. glaucum* which had been sterilized before by rinsing them with 2% Hg Cl_2 solution for a minute or so and then washing them with running water several times, were uniformly spread on the filter paper. The petridishes were wrapped in tin foil and incubated for 24, 48 and 72 hours in an oven at 26°C (±2). Distilled water was used as a control. Each treatment was replicated five times. The observations on the following parameters of the plants were recorded: (1) Percent germination. (2) Length of radicle and (3) length of plumule. The results were expressed as percent of control. The data were statistically analysed by applying 't' test to determine the significance of the results obtained.

Results and Discussion

1. Roots

a. Radicle growth

All the concentrations showed inhibitory effects in all incubation periods (Table 1) (Chaghtai *et al.*, 1986, 1988; Gilani *et al.*, 2000, 2002a). It was statistically significant in 66% treatments (Table 2). Toxicity increased with increasing concentration (Chaghtai *et al.*, 1986, 1988; Gilani *et al.*, 2002a). However, it was decreased with increasing incubation periods showing that the resistance of the test plant to the toxicity of the chemicals produced, increased as it grows more (Gilani *et al.*, 2002a). Toxicity of the allelochemicals was statistically significant in 56% cases at 24 hours soaking period in dry plant materials (Gilani *et al.*, 2002a). The results indicated that the longer soaking periods are more toxic than the shorter soaking periods. Fresh plant material though produced inhibitory effects in all the treatments at 48 hours soaking period but it was statistically significant in 44% treatments (Gilani *et al.*, 2000b). Thus, dry root exudates proved to be more toxic than the fresh root exudates to the radicle growth showing the high concentration of toxic allelochemicals in dry than the fresh plant material on weight basis (Chaghtai *et al.*, 1985, 1987, 1988; Gilani *et al.*, 2002a).

b. Plumule growth

Both the inhibitory and stimulatory effects on the growth of plumule were more pronounced than the radicle growth (Table 1) (Gilani *et al.*, 2000, 2002a & b). In 78% cases, inhibitory effect was observed but it was statistically significant in 67% treatments (Table 2). Stimulatory effect was observed in 22% cases. Toxicity increased with increasing concentration (Gilani *et al.*, 2002a). Stimulatory effect on plumule growth was only observed in 48 hours incubation period (Table 1). At 24 hours soaking period, the exudates from dry roots retarded the growth of plumule in 67% treatments and stimulated in 33% cases. These results are in conformity with Gilani *et al.* (2002a). Thus, current findings showed that the longer soaking periods produced more toxicity than the shorter soaking periods. Inhibitory effect of fresh root extracts was observed in 78% cases and stimulatory effect in 22% cases but statistically significant inhibitory effect was observed in 56% treatments. Comparing, statistically significant inhibitory effects of fresh roots on the plumule growth (56%) with dry roots (67%) at 48 hours soaking period, leachates from dry roots were relatively more toxic to the growth of plumule than those from fresh roots. Similar observations were reported by Chaghtai *et al.* (1985, 1987) and Gilani *et al.* (2002a).

c. Seed germination

Seed germination was inhibited in 56% cases (Table 3). The rate of germination of seeds was not more than 15% (Table 3). At 24 hours soaking period, it was inhibited in 67% cases. Longer soaking periods produced no toxic effect on the germination of seeds but produced toxicity to the growth of radicle and plumule. Thus, it can be concluded that growth of radicle and plumule may be inhibited by the root extracts but seeds can safely be germinated.

2. Stem

a. Radicle growth

All the incubation periods exhibited inhibitory effects on the radicle growth at all concentrations which is in conformity with the findings of others (Table 1) (Gilani *et al.*, 2002a). The toxic effect does not follow a uniform pattern (Ahmed *et al.*, 1984). Exudates from the fresh stem of *E. microtheca* sokaed for 48 hours, produced detrimental effect in 67% treatments on the test plant (Gilani *et al.*, 2002b) while, in dried stem extracts it was 100% (Table 2). In 66% treatments, inhibitory effect was statistically significant, while in 34% treatments, it happened to be insignificant (Table 2). Thus dry stem extracts were found to be more toxic to the test plant than the fresh stem extracts and it was also observed by Heiset (1990) and Gilani *et al.* (2002a).

Dry stem extracts of *E. microtheca* produced detrimental effect in all the concentrations and incubation periods at 24 hours soaking period but it was statistically significant in 44% cases. By comparing the current findings at 48 hours soaking period, the longer soaking periods produced more toxicity which is contrary to the findings of others (Chaghtai *et al.*, 1988; Gilani *et al.*, 2002b).

b. Plumule growth

Besides inhibitory effect on plumule growth, enhancing effect was also observed. Significant inhibitory effect in all the concentrations was observed in 24 hours incubation period only (Table 1). However, both the effects did not show a uniform pattern and were selective (Ahmed *et al.*, 1984; Gilani *et al.*, 2002a). The plumule growth was stimulated in 33% treatments (Table 2). These results indicated that the toxic allelochemicals are volatile in nature and unable to show their action. It also indicates the slow diffusion of toxic allelochemicals.

c. Seed germination

Slight inhibitory effect was observed in 78% cases (Table 3). However, a relatively low rate of inhibition of seed germination (0-10%) was also observed

(Table 3). In 22% cases, no inhibition of seed germination occurred. Gilani *et al.* (2002b) also reported that the rate of inhibition of *E. microtheca* on *Pennisetum glaucum* was 0-5% in 44% treatments with 24 hours soaking treatment in case of fresh stem extracts. Thus comparing the current findings, the longer soaking periods (48 hours) produced more toxicity than the shorter soaking periods. The dry stem exudates proved to be more toxic to the germination of seeds than the fresh stem extracts.

3. Leaves

a. Radicle growth

All the concentrations exhibited inhibitory effect at all the incubation periods (Table 1). Statistically, both significant and insignificant inhibitory effects were noticed in 56% and 44% cases, respectively (Table 2). Toxicity increased with increasing concentration (Khanum *et al.*, 1979; Chaghtai *et al.*, 1987, 1988). However, it was decreased with increasing incubation period. Probably, the toxic chemicals showed their detrimental effects in early stages and with the passage of time, the deleterious effect decreased indicating that the toxins were volatile in nature.

Dry leaves extracts at 24 hours soaking period, though produced toxicity in all the treatments, but it was statistically significant in 89% treatments (Gilani *et al.*, 2002a). Comparing these findings with the present study, the relative toxicity of dried leaves extract decreased with increasing soaking period, showing the volatile nature of the toxic allelochemicals Gilani *et al.* (2002b) observed that fresh leaves extracts produced both the inhibitory and enhancing effects on the radicle growth. The results of the present study revealed that the dried leaves were more toxic to the radicle growth of test plant than the fresh leaves extracts.

b. Plumule growth

All the concentrations of dried leaves extracts showed statistically significant inhibitory effect on plumule growth at all the incubation periods (Tables 1 & 2). Similar results were also reported by Gilani *et al.* (2002a) who studied the toxicity of dried leaves extracts of *E. microtheca* on *Pennisetum glaucum*, at 24 hours soaking period. The author observed both the inhibitory and enhancing effects of fresh leaves extracts on plumule growth at 48 h soaking period. Thus, the dried leaves were found to be more toxic to the test plant than fresh leaves. However, the toxic effect does not follow a uniform pattern (Ahmed *et al.*, 1984; Gilani *et al.*, 2002a).

c. Seed germination

Inhibitory effect was observed in 78% cases (Table 3). The rate of inhibition of seed germination was 0-15% (Table 3). Gilani *et al.* (2002a) observed inhibitory effect on seed germination in 33% treatments at 24 hours soaking period, in which the rate of inhibition was not more than 15%. Thus, longer soaking periods produced more toxicity, however, no serious inhibitory effect on the germination of seeds was observed.

4. Whole plant

a. Radicle growth

All the treatments inhibited growth of the plumule (Saxena, 1990; Gilani *et al.*, 2002a). Statistically, insignificant suppressing effect was recorded in 89% cases, while in 11% treatments the inhibitory effect was significant (Tables 1 & 2). However, inhibitory effect did not follow a uniform pattern and the effect was selective (Hussain *et al.*, 1984; Chaghtai *et al.*, 1986, 1988; Gilani *et al.*, 2002a). At 24 hours soaking period, the radicle growth was inhibited in all the treatments, but it was statistically significant in 33% cases (Gilani *et al.*, 2002a). Comparing to the current results, the shorter soaking periods produced more toxicity which is contrary to the findings of others (Hussain *et al.*, 1984; Chaghtai *et al.*, 1986, 1988). In longer soaking periods, the inhibitory effect of phytotoxins was considerably reduced probably by the loss of some fraction of volatile and chemically unstable toxic substances (Friedman *et al.*, 1977; Chaghtai *et al.*, 1988; Gilani *et al.*, 2002b). Fresh whole plant extracts produced more deleterious effect than the fresh whole plant extracts (Gilani *et al.*, 2002a).

b. Plumule growth

The extracts from the whole plant in all the combinations caused inhibition of the plumule growth which is in line with the findings of Saxena (1990) and Gilani *et al.* (2002). Statistically, significant inhibitory effect was observed in 89% treatments, while in 11% cases, insignificant inhibitory effect was recorded (Tables 1 & 2). However, inhibitory effect did not follow a uniform pattern and the trend was disarrayed which is contrary to the findings of Gilani *et al.* (2002a) who found that the toxicity increased with concentration, at 24 hours soaking period, for the dried whole plant extracts of *E. microtheca*. Gilani *et al.* (2002a) found that statistically significant inhibitory effect was 67%, in addition to the inhibitory effect in all the combinations at 24 hours soaking period for the dried whole plant exudates. Thus, longer soaking periods produced more toxicity to the growth of plumule. At 48 hours soaking period, fresh whole plant extracts

produced both the inhibitory and exhilarating effects, as observed by Gilani *et al.* (2002b). Thus, the dried whole plant extracts produced more toxicity than the fresh whole plant extracts (Gilani *et al.*, 2002a).

c. Seed Germination

Both inhibitory and stimulatory effects on seed germination were observed in 33% and 22% treatments respectively (Table 3), which is in line with the findings of Gilani *et al.* (2002a). In 45% treatments, seed germination remained unaffected. The rate of inhibition was not more than 10%, while the rate of stimulation was upto 19% (Table 3). In the present study, all the combinations of plant parts extracts of *E. microtheca* inhibited the growth of radicle. However, comparing the percentage of statistically significant inhibitory effect, the highest toxicity was induced by root exudates followed by leaves, stem and whole plant (Table 2) as also observed by Saxena (1990) and Tsuchiya *et al.* (1994). However, it is contrary to certain reports where leaves extracts showed maximum inhibition (Bisla *et al.*, 1992; Goel *et al.*, 1994). All the plant parts failed to produce any enhancing effect on the growth of radicle.

By comparing the effects of dried roots, stem, leaves and whole plant extracts in all concentrations and incubation periods, the leaves were found to be more toxic to the plumule growth than any other plant parts (Bisla *et al.*, 1992; Goel *et al.*, 1994). Order of toxicity in plumule growth was: leaves (100%), roots (100%) and stem (67%). Stimulatory effect was observed in the roots and stem extracts in 22% and 33% cases respectively. Thus, the stem extracts were found to be more stimulating than the roots extracts. The treatments, which have shown the insignificant inhibitory effect may be due to relatively low concentration of the allelochemicals (Chaghtai *et al.*, 1988).

The exudate from any plant part failed to produce statistically significant toxic effect on the germination of seeds, however, the extracts from leaves produced relatively more insignificant toxic effect than the other plant part (Tables 1 & 3). The order of toxicity for seed germination was: leaves (78%), stem (78%), roots (56%) and whole plant (33%). The rate of inhibition of the germination of seeds in leaves was comparatively more than the stem exudates (Table 3).

Table 1. Effect of aqueous extracts from various dried parts of *Eucalyptus microtheca*, soaked for 48 hours on germination, radicle and plumule growth of *Pennisetum glaucum*. Data is expressed as % of control; figures in parenthesis represent% germination

Parts	Conc	Incubation periods					
		Radicle Growth			Plumule Growth		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Roots	5	54.04* (95)	61.84 (100)	86.3 (100)	36.11* (95)	137.77 (100)	50.85* (100)
	10	30.80* (85)	58.36* (95)	80.87 (100)	25* (85)	130.17 (95)	63.85 (100)
	15	28.12* (90)	57.06*(100)	56.1* (95)	16.66* (90)	29.31* (100)	33* (95)
Stem	5	30.78* (95)	79.73 (95)	84.18 (90)	30.71* (95)	132.36 (95)	114.18 (90)
	10	16.04* (90)	86.84 (90)	74.73 (95)	12.85* (90)	88.15 (90)	96.82 (95)
	15	19.77* (95)	89.33 (100)	50.52* (100)	21.42* (95)	129.38 (100)	77.64 (100)
Leaves	5	48.07* (95)	82.09 (100)	82.56 (95)	18.33* (95)	45.45* (100)	35.85* (95)
	10	29.23* (90)	58.77 (100)	66.03 (90)	15* (90)	28.97* (100)	17.02* (90)
	15	28.07* (95)	37.5* (85)	49.5* (100)	18.33* (95)	13.63* (85)	16.8* (100)
W.P.	5	75 (106.25)	87.05 (100)	90.55 (95)	50* (106.25)	57.29* (100)	94.05 (95)
	10	59.61 (100)	57.96*(100)	71.14 (90)	50* (100)	35.41* (100)	54.72* (90)
	15	61.53 (119)	70.11 (95)	64.27 (100)	53.57* (119)	42.18* (95)	42.65* (100)

* significant at 5% level.

Key: Conc: Concentration in percent; P.Parts: Plant Parts; W.P: Whole Plant.

Table 2. Stimulatory and inhibitory effects of exudates from fresh parts of *Eucalyptus microtheca* on the growth of radicle and plumule of *Pennisetum glaucum* at 48 hrs. soaking period

Plant Parts	Radicle growth				Plumule growth			
	Inhibitory effect (%)			Stimulatory effect (%)	Inhibitory effect (%)			Stimulatory effect (%)
	Total	Significant	Non-Significant		Total	Significant	Non-Significant	
Roots	100	66	34	0	78	67	11	22
Stem	100	44	56	0	67	34	33	33
Leaves	100	56	44	0	100	100	0	0
W.P.	100	11	89	0	100	89	33	0

Table 3. Effect of aqueous extracts from various fresh parts of *Eucalyptus microtheca* in all concentrations at 48 hours soaking period on the seed germination of *Pennisetum glaucum*

Plant parts	No effect (%)	Inhibitory effect (%)	Rate of inhibition (%)	Stimulatory effect (%)	Rate of stimulation (%)
Roots	44	56	0-15	0	0
Stem	22	78	0-10	0	0
Leaves	22	78	0-15	0	0
Whole Plant	45	33	0-10	22	19

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