

ALLELOPATHY EXHIBITED BY *IMPERATA CYLINDRICA* (L.) P. BEAUV.

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Abstract

Cold and hot water extracts from fresh and dry plant parts; natural rain leachates, litter and soil collected from underneath the grass invariably and significantly reduced the germination and early growth of *Setaria italica*, *Dichanthium annulatum*, *Chrysopogon montanus*, *Medicago polymorpha* and *Pinus roxburghii*. The number and biomass of root nodules in *Medicago polymorpha*, *M. minima* and *Melilotus indica* decreased in the *Imperata*-affected soils. Caffeic, ferulic, p-hydroxybenzoic, p-coumaric, vanillic, chlorogenic and syringic acids were identified as the allelopathic agents. Allelopathy by *I. cylindrica* seems to be one of the factors responsible for interfering with the regeneration of *P. roxburghii*.

Introduction

Allelopathy governs the community dynamics, pattern and productivity in natural and agroecosystems. Many grasses are known to exhibit allelopathy to preclude the associated species by reducing their regeneration, growth and yield (Hussain *et al.*, 1982, 1984; Rice, 1984). *Cenchrus ciliaris* allelopathically retards the germination and growth of susceptible species (Hussain & Anjum, 1981). Hussain & Gadoon (1981) Qureshi & Hussain (1980) and Begum & Hussain (1980) demonstrated the allelopathic effects of many grasses.

Imperata cylindrica (L.) P. Beauv., an aggressive perennial grass, is distributed throughout Pakistan from plains upto 2000 meters in the chir-pine zone. It is a common weed and a wasteland species especially on poorly drained soils. It is a strong competitive and serious weed in Nigeria (Oladokun, 1978) and elsewhere (Seth, 1970). Harlan (1956, 1975) reported that *I. cylindrica* suppresses the seedling growth and root sprouting of forest trees and associated species to gain dominance in the climax forest. Such *Imperata* dominated habitats ultimately face degradation (Harlan, 1975). Sajise & Lale (1975) observed that *I. cylindrica* reduces the growth of *Stylosanthus guyanensis* in mixed cultures. Mendoza (1978) reported that *Imperata* hampers the regeneration of forests in Philippine. Beg (personal communication) suspected that *I. cylindrica* encroaches and checks the regeneration of chir-pine (*Pinus roxburghii*) in the subtropical forests in Pakistan.

The role of allelopathy in forestry, community dynamics and the aforesaid evidences concerning aggressive invasion followed by suppression and replacement of the species by *I. cylindrica* hint upon the possibility of allelopathy. The present paper, therefore, reports the allelopathic effects of this grass against *P. roxburghii* and other natural associates.

Materials and Methods

Field Evidences: A general survey of the area indicated that *Imperata*-dominated sites are separated from other herbaceous plants by a transitional mixed zone. Two such localities were selected for phytosociological analysis. In each of the localities, three zones were analyzed using five, 0.5 x .5 m quadrats. Density, frequency and coverage of the recorded species were used to calculate Importance values.

Ten individuals of every recorded species were randomly collected from three zones in each of the two localities to determine height, fresh and dry mass. Plants were oven dried at 65°C for 72 h. The results of both the localities were averaged.

Soils were collected at 0-15 cm levels from all the 3 zones from both the localities and mixed to get a composite sample for each zone. Soils were analyzed for the texture (Bouyocous, 1936), pH, soluble salts, phosphorus, potassium, organic matter and CaCO₃ following Jackson (1962).

Allelopathic Study: Inflorescences, shoots (leaves and stems) and roots (including rhizomes) from mature *Imperata* were oven dried at 65°C for 72 h. Glassware was sterilized at 170°C for 4 h while heat labile materials were autoclaved at 15 p.s.i. at 120°C for 15 min.

Extracts were obtained by soaking 5 g plant material in 100 ml distilled water for 24 h at 25°C, filtered and stored at 5-10°C when not used. However, the extracts were generally used within a week. Seeds of *Setaria italica*, *Dichanthium annulatum*, *Chrysopogon montanus*, *Medicago polymorpha* and *Pinus roxburghii* were sown on twice folded whatman No.1 filter paper seed-beds in Petri dishes. Tests were moistened with the respective extracts. Control received distilled water. The dishes were incubated at 25°C in dark for 48 h for *S. italica*; 72 h for *D. annulatum*, *C. montanus* and *M. polymorpha* and 15 days in the case of *P. roxburghii*. There were 10 replicates, each with 10 seeds. The dishes were regularly checked for moisture. This procedure would be referred to as the "Standard filter paper bioassay" in the subsequent study. The results were statistically analysed using "t and z" tests (Cox, 1967).

Aqueous Extract Bioassay: (a) Aqueous extracts from dried and crushed inflorescences, shoots and roots were used against the afore-mentioned test species following the standard filter paper bioassay. Germination and radicle growth was recorded at the end. (b) Extracts were obtained by soaking 5 g fresh shoots or roots in 100 ml distilled water as described earlier and used against the same test species. (c) Hot water extracts were obtained by boiling 5 g dry shoots or roots in 100 ml distilled water for 3 min, filtered, cooled and tested against the aforesaid test species.

Natural Rain Leachate Bioassay: Fifteen g dried shoots or roots were loosely crushed and separately taken in large funnels containing single sheet of filter paper. A flask was kept below the funnel for the collection of rain leachate. The direct entry of the rain water into the collecting flasks was strictly avoided. This leachate collecting assembly was kept under the natural rain on a one m high chips bench after it had rained for about 1/2 h to avoid aerial contamination and splash water. The slow drizzle continued for almost 8 h, thus providing sufficient time for leaching of the phytotoxins. Direct rain water was simultaneously collected for comparison. Germination and radicle growth of test species grown in rain leachates, direct rain water

and distilled water was recorded at the end of bioassay. A portion of the rain leachate was reserved for the identification of phytotoxins.

Mulching Experiment: Plastic pots, 14 x 7 cm were filled with equal volume of coarse river sand which had been thoroughly washed and sterilized at 170°C for 4 h before use. Ten seeds of the aforesaid test species, except *P. roxburghii*, were independently sown in each of the 10 pots. Test pot soils were topped with 1 g finely crushed shoots or roots. Control were similarly treated except the grass mulch was replaced with fine pieces of filter paper. Each pot was provided with 60 ml distilled water and incubated at 30°C. After 4 days, the pots were transferred to 16 h photoperiod at room temperature (25-30°C) and 40 ml Hoagland's solution was provided to each pot. After a week, germination was recorded and 5 healthy, uniform, equi-distant seedlings were left in each pot. Height, fresh and drymass of the seedlings was determined after another 10 days. Seedlings were oven dried at 65°C for 72 h.

Effects on Nodulation: Twenty flowering plants of *Medicago polymorpha*, *M. minima* and *Melilotus indica* were carefully rooted out from places with or without the dominance of *Imperata*. The number of nodules in each plant in each of the treatments were counted and separated for fresh weight determination. They were oven dried at 65°C for 72 h to get dry weight. Fresh and dry mass of roots and shoots was separately recorded. The haeme colour of the nodules were visually compared (Murthy & Shihora, 1977).

Soil Residual Toxicity: Soils from places with or without the dominance of *Imperata* were collected, air dried, sieved through 2 mm mesh and used against the aforesaid test species using soil-extract and soil bed bioassays following our standard techniques (Hussain & Gadoon, 1981; Hussain *et al.*, 1979). Ten replicate dishes each with 10 seeds were incubated at 25°C as before. Germination and early growth was recorded.

Identification of Phytotoxins: Aqueous extracts from shoots were obtained by shaking 10 g shoots in 200 ml distilled water for 2 h. Natural rain leachate collected previously was also used. Both, aqueous extracts and rain leachates were concentrated to 1/3 of their original volume in rotavapor at low pressure and acidified to pH 2.5 with 1 N HCl. The concentrates were then extracted three times with 60 ml of ether each time by refluxing for 15-20 min. The three ether fractions were combined and dried. The residue was dissolved in 2.5 ml 100% ethanol and used for spotting the Whatman No.1 chromatography paper. Aqueous fractions were discarded.

The chromatograms were developed following Lodhi (1975) and Lodhi & Rice (1971) in two dimension with *n*-butanol-acetic acid-water (63:10:27, v/v/v), BAW, followed by 6% aqueous acetic acid, 6% A.A. The chromatograms were inspected with short (2537 Å) and long (3360 Å) ultra violet light. Compounds were marked with UV light, and subsequently eluted with 95% ethanol. The elutes were reduced to dryness in vacuo, taken up in 3 ml 95% ethanol and rechromatographed in one dimension on Whatman No.1 paper in three different solvent systems: BAW, 6% AA and iso-propanol-butanol-water (140: 20:60, v/v/v), IBW. The R_f in various solvent systems, colours in UV light, colours after spraying with diazotized *p*-nitroaniline, diazotized sulfanilic acid and potassium fericyanid-ferric chloride (Lodhi, 1975;

Naqvi, 1976) were recorded and compared with the standard markers which were co-chromatographed using the same solvent systems.

Results

Field Evidences: The importance values of all the species were clearly lower in the *Imperata* dominated localities. Both, the species and the total density of all the associated species were far less in association with *Imperata* (Table 1). The total number of individuals were 72 in the *Imperata*, 344 in the transitional and 512 in the herb zones (Table 1).

Table 1. Importance values (IV), Height, Fresh and dry mass of the associated species in *Imperata* zone (IZ), Transitional zone (TZ) and herb zone (HZ).

Species	IV			Height (cm)			Fresh mass (mg)			Dry mass (mg)		
	IZ	TZ	HZ	IZ	TZ	HZ	IZ	TZ	HZ	IZ	TZ	HZ
<i>Imperata cylindrica</i>	210.69	93.71	19.62	49	38	--	9.03	7.33	--	4.38	3.00	--
<i>Dichanthium annulatum</i>	16.87	31.20	22.43	42	47	57	7.07	8.56	8.00	2.04	3.26	3.25
<i>Erythraea rammosissima</i>	36.64	13.22	34.27	15	13	15	3.53	3.88	3.77	1.02	1.00	1.85
<i>Medicago polymorpha</i>	23.54	13.55	23.77	6	5	6	0.89	2.00	1.09	0.32	0.61	0.48
<i>M. minima</i>	--	6.81	5.76	--	--	6	--	--	3.52	--	--	1.16
<i>Cynodon dactylon</i>	12.16	74.76	47.88	4	5	6	2.78	3.02	3.00	1.10	1.98	2.00
<i>Loptochloa</i> sp.	--	26.98	82.28	--	5	6	--	3.02	3.00	--	2.11	2.33
<i>Oxalis corniculata</i>	--	15.60	7.15	--	7	7	--	1.18	1.24	--	0.20	0.38
<i>Ruellia tuberosa</i>	--	12.94	12.01	--	10	17	--	17.20	18.20	--	3.52	4.23
<i>Conyza bonariensis</i>	--	--	7.10	--	--	4	--	--	3.02	--	--	1.10
<i>Stellaria media</i>	--	--	12.67	--	--	4	--	--	5.11	--	--	1.00
<i>Taraxacum officinale</i>	--	--	13.57	--	--	10	--	--	7.25	--	--	4.00
<i>Senebiera didyma</i>	--	11.84	10.61	--	--	8	--	--	5.11	--	--	2.53

Table 2. Soil analysis of the three zones.

Zones	Texture			Textural class	Soluble salts (%)	EC (dSm ⁻¹)	Available P ₂ O ₅ (ppm)	Exchangeable K ₂ O (ppm)	Organic matter (%)	CaCO ₃ (%)
	Clay	Silt	Sand							
<i>Imperata</i> zone	50.6	30	15.11	Clay	.12	.35	24	360	1.41	7.25
<i>Transitional</i> zone	46.5	35	15.95	Clay	.12	.37	11	330	1.31	7.59
<i>Herb zone</i>	52.9	30	16.00	Clay	.13	.35	19	340	1.38	7.00

ECX10³ = Electrical Conductivity (dSm⁻¹)

Height of the associated species remained unaffected while significant reduction in the fresh and dry mass of the associated species were observed (Table 1). Soil analysis did not show any major differences in chemical and physical characteristics of different zones (Table 2).

Aqueous Extract Bioassay: The germination of all test species, except *Pinus* in roots, was significantly reduced by the various extracts. *Chrysopogon* was inhibited more than rest of the species especially by shoots and inflorescences (Fig.1). Radicle growth of all the test species in various extracts was severely arrested. Inflorescences and shoots were more inhibitory than the roots (Fig.1). *Chrysopogon* and *Dichanthium* were more susceptible to various extracts.

Excepting the germination of *Medicago* and *Pinus* and radicle growth of the later species in roots; all the remaining species were significantly suppressed in their germination and early growth in all the treatments (Fig.1). *Dichanthium* and *Chrysopogon* were more susceptible than other species.

The germination and radicle growth of all test species, except the germination of *Pinus* and radicle growth of *Setaria* were not affected in root extracts. Whereas the remaining test species exhibited retarded germination and growth in hot water extracts. *Setaria*, *Medicago* and *Pinus* were comparatively less susceptible than others (Fig.1). Phytotoxins were extractable from the dried and fresh materials with water at room temperature and also in the boiling water. Boiling reduces the time required for releasing phytotoxins under laboratory conditions.

Natural Rain Leachate Bioassay: Distilled and simple rain waters were not toxic to the test species. Rain leachates significantly inhibited germination and early growth of susceptible test species suggesting the leaching of toxins from grass. The germina-

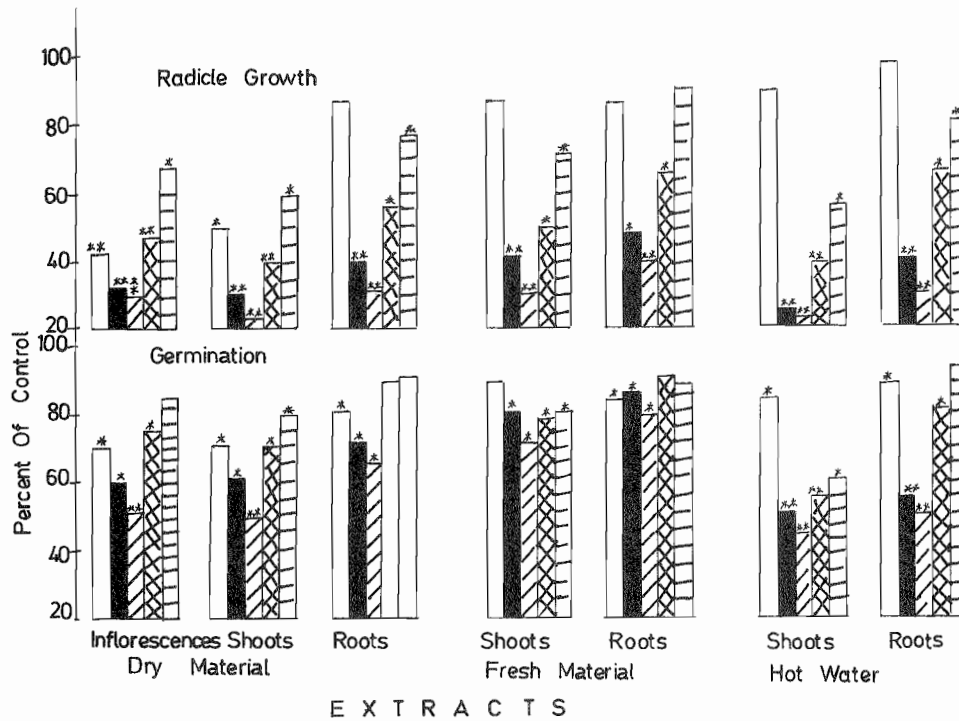


Fig.1. Germination and radicle growth of *Setaria ualica* (Blank), *Dichanthium annulatum* (Solid), *Chrysopogon montanus* (Thatched), *Medicago polymorpha* (Crossad) and *Pinus roxburghii* (Horizontally lined) in aqueous extracts of *Imperata cylindrica*. Each value is a mean of 10 replicates, each with 10 seeds and is expressed as % of control.

* & **: Significant at $P = 0.05$ and 0.01 (Z and t- tests for germination and radicle growth, respectively).

tion of all the test species, except *Setaria*, *Medicago* and *Pinus* in roots, decreased in the rain leachates (Table 3). *Dichanthium* and *Chrysopogon* were more susceptible than the others. Excepting the radicle growth of *Setaria* in both the leachates and that of *Chrysopogon* in roots only, all the remaining species were suppressed in their growth in rain leachates.

Mulching Experiment: Germination, height, fresh and drymass of all the test species were severely retarded in growth medium. However, *Setaria* remained unaffected in most cases (Fig.2). The reduction of biomass was significant in the test condition than their height.

Effect on Nodulation: Fresh and drymass of shoots and roots, the number of nodules per plant, fresh and drymass of nodules of all the three species significantly decreased with *Imperata* (Table 4). The nodules in the control sites were pinkish red while those from test plots were brownish or brownish-red in colour.

Table 3. Effect of natural rain leachate of *Imperata* on the germination and early growth of test species (n = 10).

Test species	Distilled water	Direct Rain water	% of control	Shoot leachate	% of control	Root leachate	% of control
GERMINATION (%)^a							
<i>Setaria italica</i>	76	80	106.66	78	104.00	80	106.66
<i>Dichanthium annulatum</i>	96	94	97.92	60	62.50*	78	81.25*
<i>Chrysopogon montanus</i>	74	76	102.70	54	92.97	62	83.50*
<i>Medicago polymorpha</i>	80	78	97.50	70	88.50	76	95.00
<i>Pinus roxburghii</i>	74	74	100.00	66	89.19	70	94.59
RADICLE GROWTH (mm)^b							
<i>Setaria italica</i>	13.00	15.01	115.46	17.28	132.92	18.90	145.38
<i>Dichanthium annulatum</i>	16.59	15.98	96.32	11.52	69.44*	10.83	65.28*
<i>Chrysopogon montanus</i>	7.39	8.01	108.39	6.00	81.19*	7.11	96.21
<i>Medicago polymorpha</i>	11.96	11.25	94.06	6.09	50.92*	7.89	65.97*
<i>Pinus roxburghii</i>	10.58	9.89	93.48	6.01	56.81*	7.73	73.06*

* Significant at P = 0.05 (a = Z test and b = T-test)

Soil Residual Toxicity: The germination of *Dichanthium* and *Chrysopogon* and radicle growth of all the test species was inhibited by *Imperata* affected soils, especially in the soil-beds (Fig.3). *Chrysopogon*, *Medicago* and *Dichanthium* were more susceptible than *Setaria* and *Pinus*.

Identification of Phytotoxins: Caffeic, *p*-coumaric, *p*-hydroxybenzoic, syringic, chlorogenic, ferulic and vanillic acids were identified as the possible phytotoxic principles in aqueous extracts and rain leachates. All these compounds are proven inhibitors of germination and growth (Naqvi, 1976; Lodhi 1975; Rasmussen & Enhellig, 1977; Rice, 1984), therefore, no effort was made in the present study to assay their phytotoxicity against the test species.

Discussion

The field observations indicated that *I. cylindrica*- dominated patches have relatively few species with poor density and growth under identical habitat conditions. No clear differences were observed in the chemical and physical status of soils with or without *Imperata* dominance. The observed reduced number of associated species

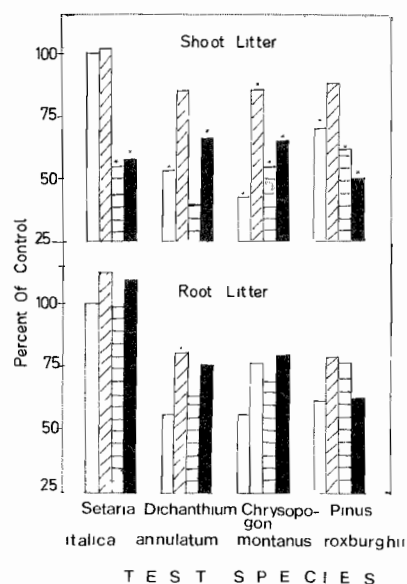


Fig.2. Effect of shoot and root litter on the germination (Blank), Height (Hatched), fresh mass (Horizontally lined) and drymass (Solid) of test species in mulching experiment. Each value, expressed as % of control, is a mean of 10 replicates each with 5 seedlings. Germination is a mean of 10 replicates each with 10 seeds.

*: Significant at $P = 0.05$ (Z- and t- tests for germination and other parameters, respectively).

and their poor growth was primarily due to some allelopathic interaction. *Cenchrus ciliaris* and *Bothriochloa pertusa* allelopathically suppress and preclude the associated species (Hussain *et al.*, 1982). This is also true for *Imperata* as the aqueous extracts were inhibitory to test species. Germination and early growth was inhibited in most instances in the various treatments due to the presence of water leachable toxins. This agrees with Hussain *et al.*, (1984) and Hussain & Anjum (1981) who reported retarded germination and growth of test species by other grasses. The toxins from *Imperata* were easily leachable in cold and hot water both from fresh and dry plants. Boiling the plant material reduced the time required for the release of toxins without denaturing them. Lodhi (1975) also demonstrated phytotoxicity of hot-water extracts. The findings also agree with other workers (Begum & Hussain, 1980; Qureshi & Hussain, 1980; Naqvi & Muller, 1975; Rice, 1984) who reported phytotoxicity to be part and species specific having an independent effect on various physiological processes. Natural and/or irrigation waters help leach and transport the toxins into the soil. Test species growing in rain leachates exhibited retarded growth due to the presence of water soluble toxins in amounts sufficient to cause suppression. The released inhibitory substances make their way into the nearby soil and accumulate to express toxicity against coexisting and/or sequentially occurring species. The incorporated litter rendered the potentially good growth medium undesirable. The litter

Table 4. Effect of *Imperata* on the fresh, drymass and nodulation of three leguminous species (n = 20).

	<i>Medicago polymorpha</i>			<i>Medicago minima</i>			<i>Melilotus indica</i>		
	Control	Test	% of control	Control	Test	% of control	Control	Test	% of control
<i>Shoots</i>									
Fresh weight (mg)	1140	840	73.43*	950	780	82.11*	3720	2200	59.14*
Dry weight (mg)	280	190	67.86*	230	134	58.26*	600	290	48.33*
<i>Roots</i>									
Fresh weight (mg)	59	44	74.58*	45	29	64.44*	180	150	83.33*
Dry weight (mg)	26	10	38.46*	19	9	47.37*	100	59	59.00*
<i>Nodules</i>									
Number	10.60	5.50	51.84*	7.88	4.95	62.82*	26.50	13.11	49.47*
Fresh weight (mg) per plant	6.86	4.83	70.41*	5.11	3.99	78.08*	17.59	10.89	60.20*
Dry weight (mg) per plant	1.83	0.21	11.48*	1.22	0.51	41.81*	7.25	5.10	70.34*

* Significantly different from control at P: 0.05 (t-test)

from *Eragrostis* (Hussain *et al.*, 1984), *Dichanthium* (Dirvi & Hussain, 1979), and *Datura* (Hussain *et al.*, 1979) and many other grasses (Rice, 1984; Putnam & Tang, 1987) exhibited similar soil-toxicity and our findings agree with them. Lodhi (1975) reported soil-plant phytotoxicity due to allelopathy. *Imperata*-affected soils always proved undesirable for the growth of test species. Soil beneath other grasses also proved inhibitory to test species (Dirvi & Hussain, 1979; Hussain & Gadoon, 1981; Qureshi & Hussain, 1980; Hussain, *et al.*, 1984). The identification of caffeic *p*-coumaric, *p*-hydroxybenzoic, syringic, chlorogenic and vanillic acids from aqueous extracts and rain leachates confirmed the allelopathic nature of *Imperata*. These toxins can accumulate in soils to create undesirable habitat. Lodhi (1975) and Rice (1984) isolated inhibitors from soil beneath allelopathic plants. We, therefore, suggest that the *Imperata* soils received and accumulated the above mentioned toxins in the same way. Rasmussen & Einhellig (1977), Naqvi (1976) and Rice (1984) have

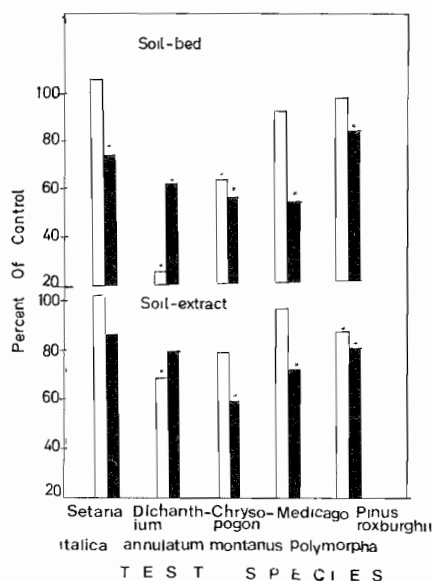


Fig.3. Germination (Blank) and radicle growth (Solid) of test species in soil extract and soil-bed bioassays. Each value is a mean of 10 replicates, each with 10 seeds. All values expressed as % of control.

*: Significant at $P = 0.05$ (Z test for germination and t-test for radicle growth).

confirmed the toxicity of these compounds. *I. cylindrica* arrested the nodulation of *Melilotus* and *Medicago* in mixed culture. Fewer unhealthy nodules with low haemoglobin contents would decrease the nitrogen fixing capability of the nodulated plants. This might also indirectly accelerate the exclusion of species requiring high nitrogen. *Aristida* inhibited nodulation in *Indigofera* (Murthy & Shihora, 1977). Rice (1971, 1984) reported inhibition of nodulation and nitrification due to allelopathy and our results also support their findings.

The present study strongly supports the views that suppression of root sprouting and seedling growth of fruit trees (Harlan, 1956, 1975), *Stylosanthes* (Sajise & Lales, 1975) and slow regeneration of forest seedlings in Philippine (Mendoza, 1978) are primarily due to allelopathy. The observed delayed and/or reduced regeneration of chirpine (Beg, personal communication) can also be attributed to allelopathy. Allelopathy by *I. cylindrica* is an important factor in decreasing the productivity of forests.

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