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IMPERATA CYLINDRICA (LINN.) P. BEAUV AFFECTS GERMINATION, EARLY GROWTH AND CELL DIVISION AND DEVELOPMENT IN SOME CROP SPECIES

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Imperata cylindrica (Linn.) P. Beauv., is a troublesome grass that may exclude associated species from common habitat. Laboratory studies indicated that the aqueous extracts, rain leachates and litter from shoots and rhizomes suppressed either germination, seedling growth or both of mustard *(Brassica campestris)*, Bajra *(Pennisetum americanum)* and letuce *(Lactuca sativa)*. The percentage of dividing cells and their sizes in root tips of test species were small under test condition. Rain leachates contained caffeic, *p*-coumaric, *p*-hydroxy-benzoic, syringic, chlorogenic, *iso*-chlorogenic, ferulic and vanillic acids and scopolin and scopoletin. The findings suggest that the decreased radicle growth may have resulted from inhibition of cell division and development. The observed aggression of this species may be attributable at least in parts to its allelopathic influences.

Key words: *Imperata cylindrica,* Allelopathy, Cell division inhibition.

Introduction

Imperata cylindrica (Linn.) P. Beauv., is a troublesome grass found through much of the world including Pakistan [1-3]. Harlan [4,5] observed that it suppresses seedling growth and sprouting of forest trees and might affect the regeneration of forest trees [6]. Sajise and Lales [7] reported that it retarded growth of *Stylosanthes* species in mixed cultures. Rice [8], Abdul-Wahab and Rice [9] and Eusson and Soerjani [10] concluded that extracts from its leaves retarded growth of cucumber. Subsequently, Hussain and Abidi [11] reported that it inhibited germination and seedling growth of many range grasses. Furthermore, it reduced root nodulation in species of *Melilotus and Medicago.*

Partial identification of phytotoxic substances from *I. cylindrica* have been reported [1,9]. There is still further need to identify phytotoxic principles in this grass. Although, it has been reported to retard the growth of many seedlings, how it affects radicle growth is still unknown. Many workers [11-16] have demonstrated that reduced growth of root tips or radicles occurs as a result of (i) inhibition or delay in cell division, (ii) poor development of cell after division and (iii) the combined effect of both processes. The present study was conducted to address (i) how aqueous extracts, rain leachates and litter from shoots and rhizomes, affected germination and early seedling growth of some selected crop species, (ii) if the division and development of cells in root tips were affected and (iii) to what phytotoxin might be involved.

Materials and Methods

Flowering plants of *I. cylindrica*, growing naturally in Peshawar University Campus, were carefully excavated and separated into shoots (leaves, stems and inflorescences) and rhizomes (including roots). They were air dried at room temp. (25-30°). Glassware was sterilized at 170° for 4 hrs. Heat labile materials were autoclaved at 110° under 115 lbs pressure for 1/2 hr. Seeds of mustard *(Brassica campestris)*, bajra *(Pennisetum americanum)*, and lettuce *(Lactuca sativa)* were used as the test species. Aqueous extracts or rain leachates were stored at 5-10°.

There were always 10 replicates, each with 10 seeds, unless otherwise stated. Replicates were arranged randomnly. Results for the growth parameters in various treatments were statistically compared with their respective control using t-tests while germination %ages were tested by z-tests [17]. Significant differences were accepted at $P = 0.05$.

Effect of aqueous extracts. Aqueous extracts were obtained by boiling 5 g shoots or rhizomes in 100 ml double distilled water for 5 min. Extracts were filtered and cooled to room temp. Seeds of test species were placed on 2-folds of Whatman No. 1 filter in petri dishes and moistened with either extract (test) or double distilled water (control). Germination, radicle and plumule growth were determined after 72 hrs. incubation at 26°.

Effect of rain-leachates. Crushed shoots or rhizomes (15 g) were seprately placed in large glass funnel on filter paper. The funnel was fixed to a stand and placed on 1m high bench during a slow drizzle. A flask was placed below the funnel for the collection of required volume of rain leaching through the plant material. Direct entry of rain water into the flasks was prevented. A similar arrangement was used, but without plant material, for the collection of rain water as a control.

Effect of rhizome exudates. Mature living plants of *I. cylindrica* were carefully dug up and rhizomes were thor-

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oughly washed with water and air dried between folds of filter papers. Four 26 x 10 cm plastic containers were alcohol sterilized. Rhizomes were then inserted between 2-folds of Whatman No. 1 filter paper, moistened with double distilled water and placed within the containers. Shoots did not touch the filter papers. These containers along with the plants were incubated at 26°. Filter papers without any plants in containers were simultaneously incubated for preparing the control. Plants were removed after 7 days and the filter papers dried. Twenty five seeds of every test species were placed on the *Imperata* affected (test) and un-affected (control) filter papers. They were moistened with equal amounts of double distilled water and incubated as before.

Effect of added litter. Dried shoots of rhizomes litter (1 g) was uniformly spread on top of 250g washed and moist coarse sand and incubated at 26°. This litter-added sand was air dried after 10 days and litter removed. Sand without litter was treated similarly for use in the control. Treated or un-treated sand (20 g) was taken in a petri dish for germination and seedlings growth of test species.

Effect on celll division and development. Root tips of mustard and bajra, grown in 5% aqueous extracts for 72 hrs were excised and treated with 0.01% colchicine solution for 2-3 hrs. and fixed in a 3:1 mixture of alcohol and glacial acetic acid for 12 hrs. They were then washed with 70% alcohol and stored at 5-10°. For staining, the root tips were hydrolyzed in I *N* HCl at 60° for 10-15 mins., then washed with distilled water and treated with basic fuchsin for 30 mins in air tight glass vials. Root tips turned either pinkish or purple violet. The violet tips of roots were then placed in a drop of aceto-carmin on a glass slide, gently warmed and squashed to spread the tissue for counting the dividing and non-dividing cells.

Root tips were excised and transferred to a concentrated solution of chloral hydrate to determine the effect on cell size. After 10-12 hrs of incubation at 25°, they were randomly squashed over slide. The number and size of cells were determined over fixed distance in between the 3-5th cortical layers. There were 10 tips (replicates), each with 10 counts.

Identification of phytotoxin. Rain leachates were concentrated in rotry evaporator under reduced pressure to 1/3 of its original volume. The concentrate was then acidified with I *N* HCl to pH 2.5 and extracted three times with ether by reflux shaking. Ether fractions were combined and evaporated to almost dryness in rotry evaporator. The residue was dissolved in 2 ml of 100% ethanol and used for spotting the Whatman No. 1 chromatographic paper. Aqueous fractions were discarded.

Chromatograms were developed following Lodhi [18], Lodhi and Rice [19] and Hussain and Abidi [1] in two dimensions with *n*-butanol-acetic acid-water (63:10:27, v/v/v; BAW) followed by 6% aqueous acetic acid (6% AA). The chromatograms were inspected under short wave length (2537A°) and long wave length (3360A°) UV light. Compounds were marked under UV light and subsequently eluted with 95% ethanol. The elutes were dried in vacuum, taken up in 3 ml 95% ethanol and rechromatographed in one dimension on Whatman No. 1 chromatographic paper in three different solvent systems: BAW, 6% AA and isopropanol-butanol-water (140:20:60, v/v/v,;IBW). The R_f values in various solvent systems, colours under UV lights, colours after spraying with diazotized sulfanilic acid, diazotized *p*-nitroanaline and potassium fericynide-ferric chloride were recorded and compared with standard markers (Fluka and Sigma Chemicals, USA) which were co-chromatographed using the same solvent systems.

Results and Discussion

Germination of mustard seeds in rhizome extracts, germination of bajra seeds in shoot extracts and germination of lettuce in both extracts was significantly reduced compared to their control (Table 1). Except for radicle growth of mustard in shoot extract and radicle growth of bajra in rhizome extract, all the test species had retarded radicle and plumule growth under test conditions (Table 1). Many other plants affect germination and seedling growth of susceptible species and our findings agree with them [1,9,12,20-21]. Lodhi [19] and Lodhi and Rice [17] demonstrated inhibitory effects of hot water extract from *Celtis*. Hussain *et al.*, [22] stated that water extracts from different *Ficus* species would inhibit germination and seedling growth of many crop species. The present

TABLE 1. EFFECT OF EXTRACT ON GERMINATION AND SEEDLING GROWTH OF TEST SPECIES. (EACH VALUE IS A MEAN OF 10 REPLICATES, EACH WITH 10 SEEDS).

Test species	Control	Rhizome (% of Control)	Shoot (% of Control)
GERMINATION (%)			
Mustard	91	75 (82)*	91 (100)
Bajra	81	77 (95)	60 (74)*
Lettuce	71	41 (58)*	24 (34)*
RADICLE GROWTH (mm)			
Mustard	10.1	7.7 (71)*	9.3 (92)
Bajra	24.3	21.4 (88)*	16.3 (67)*
Lettuce	6.1	2.6 (43)*	1.3 (21)*
PLUMULE GROWTH (mm)			
Mustard	1.6	1.1 (68)*	1.4 (84)
Bajra	8.7	6.2 (71)*	4.9 (56)*
Lettuce	1.3	0.4 (31)*	0.1 (8)*

* Significantly different from control at P = 0.05.

findings agree with other workers [6,7,14,15] who observed germination and growth inhibitors in aqueous extracts from other plants.

We conclude that *Imperata* has water soluble phytotoxin. This view is supported by Abdul Wahab and Rice [9], Eusson [20] Eusson and Soerjani [10] and Hussain and Abidi [1] who suspected some inhibitory mechanism in *Imperata*. Germination of bajra declined in rain leachate of rhizome, while seedling growth of all test species was significantly suppressed by either rhizome or shoot rain leachates (Table 2). Direct rain water had no inhibitory effect on any of the test species. Rain is an effective transporting agent of allelopathic substances from plants to soil [17].

Rhizome exudates from *Imperata* significantly depressed germination and early growth of bajra and mustard (Fig. 1). Rhizome extracts of *Imperata* reportedly are more toxic than shoot extracts [1,10,20]. It is possible that rhizome exudates render the soil less favourable for the growth of susceptible species.

TABLE 2. EFFECT OF RAIN LEACHATES ON GERMINATION AND EARLY SEEDLING GROWTH OF TEST SPECIES. (EACH VALUE IS A MEAN OF 10 REPLICATES, EACH WITH 10 SEEDS).

Test species	Rain water control	Rhizome (% of Control)	Shoot (% of Control)
GERMINATION (%)			
Mustard	97	90 (93)*	92 (95)
Bajra	71	70 (99)	55 (77)*
Lettuce	83	82 (99)	80 (96)*
RADICLE GROWTH (mm)			
Mustard	17.0	11.5 (68)*	8.5 (50)*
Bajra	22.6	18.3 (80)*	17.6 (78)*
Lettuce	7.9	6.3 (71)*	5.5 (69)*
PLUMULE GROWTH (mm)			
Mustard	3.2	2.8 (87)*	2.1 (66)*
Bajra	7.9	8.8 (112)	6.0 (76)
Lettuce	1.4	1.1 (81)*	1.0 (104)

* Significantly different from control at P = 0.05.

TABLE 3. EFFECT OF ADDED LITTER ON SEEDLLING GROWTH (RADICLE) OF TEST SPECIES. (EACH VALUE IS A MEAN OF 10 REPLICATES, EACH WITH 10 SEEDS.

Test species	Control	Shoot litter (% of control)	Rhizome litter (% of control)
Mustard	10.1	12.6 (125)	10.0 (99)
Bajra	24.4	11.6 (48)*	18.7 (77)*
Lettuce	6.1	4.3 (72)*	5.2 (85)*

* Significantly different from control at P = 0.05.

Litter usually decays to enhance soil fertility. However, this may not always be true as added *Imperata* litter made otherwise favourable soil less desirable for seedling growth of bajra and lettuce (Table 3) These findings agree with earlier workers [6,21] who reported phytotoxicity of soil caused by grass litter. Hussain and Abidi [10] also observed that *Imperata* litter retarded germination and growth of *Pinus roxburghii* and some grasses. The present findings along with those of

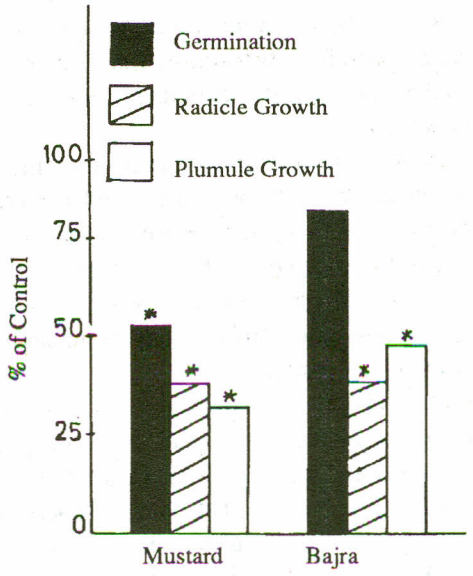

Fig. 1. Effect of rhizome (including roots) exudates on germination, radicle growth and plumule growth of Mustard and Bajra. Each value, a mean of 10 replicates, each with 10 seeds, is expressed as % of control. Bars with astrike (*) are significantly different from control at P = 0.05.

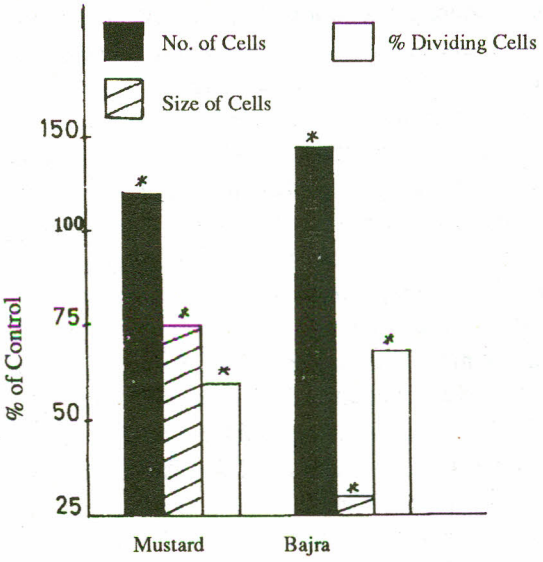

Fig. 2. Effect of hot water extract of *Imperata* on number and size of cells and % dividing cells of Mustard and Bajra. Each value is a mean of 100 cells. Bars with astrike (*) are significantly different from control at P = 0.05.

previous workers [1,5,9,10,20] establish that *Imperata* can retard seedling growth.

Radicle growth is diminished by reduced cell division, retarded development of cells or as a result of both these processes [12-16]. Extracts from *Imperata* reduced celll division and their sizes (Fig. 2). A greater number of cells per unit area were found in root tips grown in extracts from *Imperata*. Hussain *et al.* [12] observed that aqueous extracts from *Eragrostis* reduced division and development of root tip cells of *Allium cepa*.

Aqueous extracts from other plants retarded division and development of root tip cells and affected their anatomy [8,11,13-16]. Decreased radicle growth of test species was caused by the joint action of both reduced cell division and poor development of cells.

The identification of ferulic, syringic, caffeic, *p*-coumaric, chloregenic, iso-chlorogenic, *p*-hydroxy-benzoic, vanillic acids and scopolin and scopoletin in rain leachates of *Imperata* support our view about inhibitory nature of *Imperata*. Some of these phytotoxins have been identified from *Imperata* by other workers [1,8,9] and all of them are water soluble and proven inhibitors [8,9,16,18,19]. Buta and Spaulding [22] observed that one-day desicated leaves of *Festuca* contained 10 x more ABA than fresh leaves and that ABA was major inhibitor of growth in this grass. Although, we did not determined ABA in *Imperata* yet it could be one of the inhibitors in addition to the identified phytotoxins. Phytotoxic compounds in *Imperata* decrease germination and growth by reducing water and mineral uptake, depressing chlorophyll contents and thus photosynthesis, impaired stomatal functioning [8]. These phytotoxin accumulate gradually in the adjacent soil to make it less desirable. Rice [8] documented that nutrient cycling might be arrested by these phototoxins to reduce the availability of nutrients in the soil. Wacker *et al.*, [23] observed ferulic acid to inhibit mycorhizal growth which might also be one of the causes for decreased growth of test species in the *Imperata* - affected soil.

The present findings conclude that *Imperata cylindrica* releases phytotoxin to make the soil less favourable for susceptible plants in its vicinity. However, Krogmeier and Bremner [24] suggested that plant phenolics from other plants had no influence on the germination and growth of test species in soil. The allelopathic effects of a particular plant species depend upon the response of test species, nature and concentration of allelochemicals [25]. Many factors of the environment such as time and amount of precipitation, soil character and climatic conditions are important in manifesting allelopathy by a particular species. Further study is required to see if the allelopathic effects persist under natural condition and to quantify the allelopathic principles.

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