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CANABIS SATIVA L. IS ALLELOPATHIC

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Aqueous extracts from various parts; litter and rain-leachates and volatiles from shoots of *Canabis sativa* L. significantly retarded either germination, radicle growth, fresh and dry biomass or moisture contents of *Sorghum bicolor, Trigonella foenum-graeceum, Vigna mungo, Trifolium resupinatum and Brassica compestris* in various bioassays. Caffeic, ferulic, p-OH-benzoic, benzoic and coumaric acids were identified as the phytotoxins.

Key words: *Canabis sativa L.*, Weed, Allelopathy, Inhibitors.

Introduction

Canabis sativa L. grows from the plains upto 2000 meters in Pakistan as a weedy plant. Some Pakistani weeds exhibit allelopathy [1-10]. The importance of allelopathy in natural and agricultural ecosystems cannot be underrated [11-14]. No reference is, however, available on the allelopathic effects of *Canabis sativa*. The present investigation was, therefore, undertaken to determine its allelopathic potential against some crop species and to identify the allelopathic agents.

Materials and Methods

Canabis sativa plants were collected and separated into leaves, stems and roots and dried in shade at room temperature (25-30°). Germination and radicle growth of the test species was recorded after 72 hr. incubation at 25°. Five replicated petri dishes with 10 seeds were used for all experiments. The results were statistically analyzed using *z* and *t* tests following Cox [21].

1. Relative toxicity. Five and 10 gm crushed and dried leaves, stems and roots were separately soaked in 100 ml Hoagland's nutrient solution at 25° for 24 hr. and filtered. The extracts were then used against *Trifolium resupinatum, Sorghum bicolor, Trigonella foenum-graceum, Vigna mungo* and *Brassica campestris* following Hussain and Gadoon [15] and Hussain [4]. The test and control solutions were adjusted to pH 6.5 to avoid osmotic effects.

2. Litter-bed bioassay. One gm fresh or dried crushed shoots were tested by evenly placing in a petri plate and topping with a single sheet of Whatman No. 1 filter paper following Hussain *et. al.* [1, 10]. Control was similarly prepared by using five pieces of filter papers.

3. Soil residual toxicity. Test and control soils were, respectively, collected from within and without *Canabis* thickets upto 15 cm depth, dried and used as the growth medium for the aforesaid test species following soil extract and bed bioassays [2, 4, 7].

4. Aqueous culture experiment. One month old seedlings of the above mentioned test species were used in this experiment as described by Dirvi and Hussain [16] and Hussain *et. al.* [17]. Plant extracts were prepared in 5:100, shoot: distilled water ratio. The solutions were adjusted to pH 6.5 to avoid osmotic effects.

5. Rain-leachate bioassay. Artificial rain leachate was collected following Naqvi and Muller [18] and Hussain *et. al* [16]. While natural rain leachate was collected from underneath the *Canabis* thickets in May, 1987. A portion of which was concentrated to 1/4 of its original volume at 50°. These leachates were used against the test species following our standard procedures [16, 18].

6. Volatile inhibitor(s) bioassay. The effects of volatiles eminating from *Canabis* shoots was assayed following Ahmed *et. al.* [3]. Seeds of *Brassica compestris* and *Trifolium resupinatum* were used as the test species.

7. Identification of phytotoxins. Ten percent aqueous shoot extracts were concentrated to 1/3 of its original volume and pH was adjusted to 2.5 with 1N HCl. It was extracted, developed in 6% AA and BAW fractioned and sprayed with reagents and the phytotoxins identified following Naqvi [19] and Lodhi [20].

Results and Discussion

1. Relative toxicity. Germination of *Trifolium, Trigonella* and *Brassica* was significantly reduced in leaf extracts while the radicle growth was retarded by all the extracts. However, the radicle growth of *Trifolium* in root extracts and *Sorghum* radicle growth in leaves was not inhibited (Fig. 1 and 2).

2. Litter-bed bioassay. Fresh litter retarded the germination and radicle growth of *Trifolium* only. The germination and growth of all the text species, except

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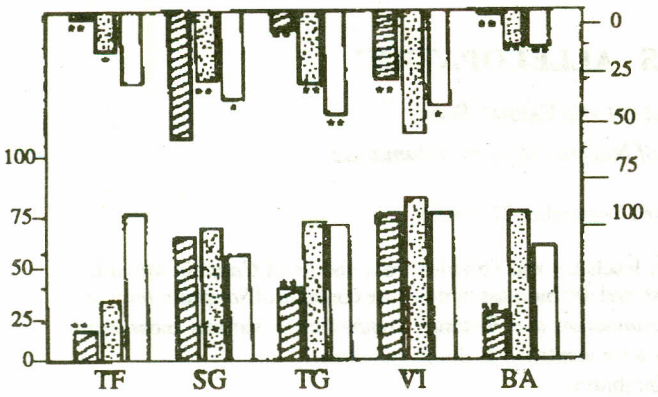

Fig. 1. Germination and radicle growth of *Trifolium resupinatum* (TF), *Sorghum biocolor* (SG), *Trigonella foenum-graceum* (TG), *Vigna mungo* (VI) and *Brassica campestris* (BA) in aqueous extracts, 5 g. x 100 ml. concentration of leaves (thatched), stems (dotted) and roots (open). Each value expressed as percent of control is mean of 5 replicates each with 10 seeds.
*Significant at P<0.05; **Significant at P<0.01

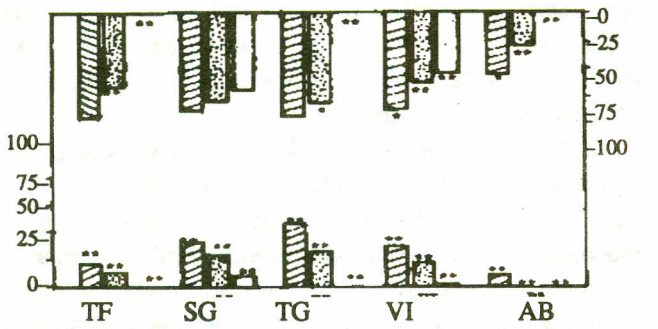

Fig. 2. Germination and radicle growth of *Trifolium resupinatum* (TF), *Sorghum biocolor* (SG), *Trigonella foenum-graceum* (TG), *Vigna mungo* (VI) and *Brassica campestris* (BA) in aqueous extracts, 10 g x 100 ml. concentration of leaves (thatched), stems (dotted) and roots (open). Each value expressed as percent of control is mean of 5 replicates each with 10 seeds.
*Significant at P<0.05; **Significant at P<0.01

germination of *Sorghum*, was significantly reduced in dry litter-beds (Table 1).

3. Soil residual toxicity. The radicle growth of *Brassica* and *Vigna* decreased to 69 and 70%, respectively in the affected soils.

4. Aqueous culture experiment. The fresh, dry weight and moisture contents of all the test species, except the dry weight of *Trifolium* and *Vigna*, were reduced in the nutrient medium containing plant extracts (Table 2).

TABLE 1. EFFECT OF DRY AND FRESH LITTER OF *CANABIS SATIVA* ON THE GERMINATION AND RADICLE GROWTH OF TEST SPECIES. EACH VALUE IS A MEAN OF 5 REPLICATES, EACH WITH 10 SEEDS.

	Trifolium resupi-natum	*Sorghum bicolor*	*Trigonella foenum-graceum*	*Vigna mungo*	*Brassica campestris*
GERMINATION (%)					
Control	88.00	78.00	98.00	66.00	96.00
Fresh litter	68.00	84.00	100.00	90.00	100.00
% of control	77.00*	107.69	102.04	121.21	104.16
Dry litter	16.00	80.00	66.00	58.00	16.00
% of control	18.18**	102.56	67.34**	87.87*	16.66**
RADICLE GROWTH (MM)					
Control	11.44	28.44	23.42	17.88	31.04
Fresh litter	07.96	33.44	24.70	22.48	29.82
% of control	69.58*	117.58	105.46	125.72	96.06
Dry litter	00.38	07.98	02.56	04.60	00.93
% of control	03.32**	28.05**	10.93**	25.72	02.99**

* and ** significantly different from control at P=0.05 and 0.01, respectively.

Table 2. Biomass and Moisture Contents of Test Species in Aqueous Culture Experiment. Each Value is a Mean of 10 Replicates, Each with One Seedling.

Test species	Observations	Control	± SD	Test	± SD	% of control
Trifolium resupinatum	Fresh weight (mg)	00.41	00.13	00.21	00.10	51.22*
	Dry weight (mg)	00.06	01.33	00.08	02.91	133.33
	Moisture (%)	577.34	274.36	165.87	45.74	28.73*
Sorghum bicolor	Fresh weight (mg)	00.46	00.21	00.25	00.15	54.34*
	Dry weight (mg)	00.09	03.22	00.08	03.26	88.88
	Moisture (%)	410.14	110.81	211.40	87.32	51.54**
Trigonella foenum-graceum	Fresh weight (mg)	00.53	00.23	00.14	06.92	26.41**
	Dry weight (mg)	00.09	00.04	00.06	02.42	66.66*
	Moisture (%)	564.67	103.04	117.35	56.92	20.78**
Vigna mungo	Fresh weight (mg)	00.37	00.16	00.18	05.93	48.64**
	Dry weight (mg)	00.08	03.15	00.08	02.44	100.00
Brassica campestris	Fresh weight (mg)	01.94	00.27	00.40	00.19	20.61**
	Dry weight (mg0	00.22	02.69	00.19	03.83	86.36
	Moisture (%)	766.50	17.77	126.14	146.02	16.45**

* and ** Significantly different from control at P=0.05 and 0.01, respectively.

5. Rain-leachate bioassay. There was an insignificant decrease in the germination of *Sorghum* by the artificial rain-leachate (Table 3). The radicle growth of all the test species, with the exception of *Vigna*, got reduced in the rain leachates (Table 3).

6. Volatile inhibitors bioassay. The radicle growth of *Brassica* and *Trifolium* declined to 66 and 78%, respectively, in the *Canabis* micro-environment.

7. Identification of phytotoxins. Caffeic, ferulic, p-OH-benzoic, benzoic and coumaric acids were identified as the inhibitors in the shoot extracts. These water soluble substances are allelopathic agents [12, 13, 19, 20].

The inhibitory effects vary with the toxins involved, its concentration, susceptibility of species and habitat conditions. *Canabis* exhibits allelopathy under natural conditions. Natural and irrigation water transport-phytotoxins to the nearby soil to render it undesirable. The findings agree with other workers [1, 4, 10, 12, 13, 20] who have observed similar soil-plant phytotoxicity for various other allelopathic plants. The productivity of the susceptible species reduces due to allelopathy. The aqueous extracts from *Canabis* exhibited differential phytotoxicity against the test species. This agrees with early workers [2-9, 11– 13, 18] who reported similar findings for other plants. Sometimes a slight stimulated growth was observed in certain test species in low concentration of extract. This is in conformity with Rice [12], Putnam and Tang [13] and Waller [14] who reported that low concentration of phytotoxins might increase the growth of certain species. *Canabis* affected soil did not show much inhibitory effects due to the reason that the soil was collected during rainy season which might have reduced the concentration of the deposited toxins. The lack of accumulation of phytotoxins and soil washing by rains is an important factor in reducing the inhibitory effects. Ahmed *et. al.* [13] and Inam *et. al.* [9] reported poor soil-plant phytotoxicity due to soil leaching by rains. The findings suggest that the habitat conditions are important in determining allelopathy. This view is also supported by the results obtained from rain leachate experiment where no inhibitory effects could be demonstrated in low concentrated. The leachability of toxins from soil renders it ineffective under natural conditions. This agrees with Naqvi and Muller [18] and Hussain *et. al.* [6, 7, 16, 17] who observed that accumulation of phytotoxins to a physiologically active level in the habitat is important in manifesting allelopathy. The reduction of biomass and moisture contents indicate that phytotoxins prevent the affected species to utilize the available habitat resources as the solutions were osmotically inactive. The wilting of seedlings in nutrient rich growth

TABLE 3. EFFECT OF ARTIFICIAL AND NATURAL RAIN LEACHATES ON THE GERMINATION AND RADICLE GROWTH OF TEST SPECIES. EACH VALUE EXPRESSED AS % OF CONTROL IS A MEAN OF 5 REPLICATES, EACH WITH 10 SEEDS. (X1 AND X4 REPRESENT THE NON-CONCENTRATED AND 4 TIMES CONCENTRATED NATURAL RAIN LEACHATES).

Test species	Artificial rain leachate	Natural rain X1	Rain leachate X4
GERMINATION			
Trifolium resupinatum	97.56	105.26	86.48
Sorghum bicolor	85.00	89.58	73.80*
Trigonella foenum-graecium	100.00	100.00	100.00
Vigna mungo	80.00	107.31	100.00
Brassica campestris	89.13	97.91	93.75
RADICLE GROWTH			
Trifolium resupinatum	58.36**	118.16	46.51**
Sorghum bicolor	80.10*	95.27	78.58*
Trigonella foenum-graecium	62.81**	116.72	39.45**
Vigna mungo	98.72	110.40	101.38
Brassica campestris	58.67**	115.93	33.61**

* and ** Significantly different from control at P=0.05 and 0.01, respectively.

medium supports this view. Similar results have been reported [1, 8, 12,16] elsewhere. Substances volatilizing from *Canabis* decreased the seedling growth as it is an effective mechanism in aromatic species [3, 7, 12]. The present findings suggest that *C. sativa* is potentially allelopathic owing to the presence of caffeic, ferulic, benzoic, P-OH-benzoic and coumaric acids in shoot extracts. These allelopathic agents are water extractable [12, 14]. The litter of this species will therefore, retard the growth of associated crop species owing to allelopathy. However, the associated habitat conditions might alter the allelopathic stress.

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