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Allelopathic Effects of *Berberis* Fruit Pulp Leachate on Germination of Some Crop Plants

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Germination and seedling growth of *Hordeum vulgare*, *Eleusine coracana* and *Dolichos biflorus* are inhibited by the fruit pulp extract of *Berberis*. The effect increased with increasing concentration of the leachate and was more marked on root growth. The inhibition of seed-germination was found to be associated with inhibition of amylase and acid-phosphatase activity in seeds. The acidic fruit pulp leachate contained two phenolic acids (chlorogenic and ferulic), one anthocyanin (cyanidin-3-glucoside) and one flavonol (quercetin-3-glucoside).

Key Words: Allelopathic effects, *Berberis*, Germination, Fruit pulp leachate

Introduction

The bushes of *Berberis* bearing edible fruits grow wild in Himalayas and are found in abundance along the edges of cultivated terraces with abundant fruiting during summers. The fruits are either eaten by villagers or they fell down in the fields. Following fruiting the terraces are put under cultivation of crop plants like *Eleusine coracana*, *Hordeum vulgare* and a few legumes. It has been observed invariably that germination of these crop plants along the edges of the terraces is either poor or the emerging seedlings are very stunted. Since *Berberis* form almost monospecific stands along the edges of these fields and fruits of these species are known to have sufficient amount of alkaloids and phenolics (Chandra & Purohit 1980, Khanduri & Purohit 1981), it was thought of considerable interest to find out if the fruits of this species have any allelopathic influence on the germination and growth of the crop plants sown in these fields. Since amylase and acid-phosphatase, two important hydrolytic enzymes, take part in starch and phosphate turn-over during seed germination (Mayer & Poljakoff-Mayber 1975) the effects of fruit pulp extract on the activity of these enzymes were studied to explain the mechanism of allelopathic effects of pulp extracts.

Materials and Methods

Fully ripened fruits of *Berberis (B. lycium* Royle) were collected from Pauri, Garhwal (1500 m). Local seed varieties of *Hordeum*

vulgare, *Eleusine coracana* and *Dolichos biflorus* were used as test materials.

Extraction of fruit pulp: The fruit pulp of *Berberis* was extracted following the method of Corcoran (1966) with slight modifications. Two hundred g of fully ripe fruits were put in 2l of 50% aqueous acetone and allowed to stand for 10 days at 0°C. The diffusate was decanted and concentrated under vacuum to a final volume of 200 ml. This aqueous residue was mixed with 10g of 2:1 mixture of celite and activated charcoal. After filtration the celite:charcoal mixture was washed with 250 ml of water. The filtrate and the washate were discarded and the celite:charcoal mixture was eluted with 100 ml of acetone. The acetone eluate was evaporated to dryness and the residue left was dissolved in water to get required concentration of partially purified inhibitor(s). For tentative identification the extract was subjected to chromatography on Whatman No. 1 chromatographic paper in n-butanol:acetic acid; water (4:1:5, top layer), 15% acetic acid, forestal (HCl:acetic acid:water, 3:30:10) and n-butanol:HCl (1:1, top layer) solvants. The developed chromatograms were screened in UV before and after fuming with ammonia and Rfs of the spots with all the solvents were recorded. Spots eluted in methanolic HCl (1%) or methanol were rechromatographed and the absorption spectra were determined by UV spectrophotometer (ECIL model GS 865 B).

Bioassay: Seeds of *Hordeum*, *Eleusine* and *Dolichos* species were germinated separately in two concentrations (100 and 400 ppm) of the extract. One set of seeds of each species was germinated in distilled water (control). For germination 100 seeds of each species were placed in separate Petri dish (15 cm diam.) lined with Whatman No. 1 filter paper (moistened with 2 ml of inhibitor(s) solution or distilled water). Same amount of solution was added after 24 hr to keep the filter paper moist. The experiment was performed at room temperature (24°C day and 20°C night) and light conditions. All the treatments mentioned above were replicated twice.

After every 24 hrs, data on germination as well as length of root and coleoptile/hypocotyl were recorded and 0.5g of seeds of each species were taken from each treatment to prepare a crude enzyme extract. These seeds were macerated separately in prechilled mortar containing 0.05 M tris-HCl buffer (*p*H 7.0). The homogenates were centrifuged at 1500 × g for 10 min and the supernatant was diluted to make the final volume 5 ml. This crude extract was used for the assay mentioned below.

Amylase assay: Method of Bernfeld (1955) was followed for amylase assay. The assay mixture consisted of 0.5 ml of 0.2 M citrate buffer (*p*H 6.5), 0.5 ml of 0.2% starch as substrate and 0.1 ml of enzyme extract (final volume 2 ml). Incubation was done at 30°C for 20 min and reaction was stopped by adding 3, 5 dinitrosalicyelic acid and by boiling the mixture for 7 min in a waterbath. Subsequently, the absorbance of the mixture was read at 540 nm on an Elico spectrophotometer. The amount of maltose was calculated with the help of standard curve. Average value of the replicates was taken to obtain the final data. Activity units are expressed as the amount of enzymes which liberated 1 μM of maltose/g fr. wt/min.

Acid phosphatase assay: Method of Baijal et al. (1972) using β-glycerophosphate as substrate was used for acid phosphatase assay. Colour development for the estimation of Pi released was done by the method of Fiske and Subbarow

(1925). Average value of the replicates was taken to obtain the final data. Activity units are expressed as the amount of enzyme which liberated 1 μM of Pi/g fr. wt/min.

Proteins were precipitated from 0.5 ml of homogenate by adding 0.5 ml of 10% TCA (w/v). After centrifugation the supernatant was used for Pi estimation according to the method of Fiske and Subbarow (1925).

Results

Effect of fruit pulp leachate on germination, length of root and coleoptile/hypocotyl: The data on germination of 3 species recorded at 24 hr interval (table 1) and root and shoot growth recorded after 72 hr (table 2) clearly shows that in all the 3 species, germination as well as elongation of root and coleoptile/hypocotyl is inhibited by the fruit pulp extract and the inhibitory effect became more pronounced with increase in the concentration of the partially purified extract. Out of 3 species treated, *Hordeum* was found to be more sensitive than other two and in fact, coleoptile growth was completely supressed by inhibitor (table 2). In general, the inhibition of growth was more in root than coleoptile/hypocotyl.

Amylase activity: There was marked reduction in the amylase activity in seeds of all the 3 species when germinated in presence of the extract (table 3) and the inhibition increased with the increase in concentration of the extract. However, the magnitude of inhibition varied from species to species and decreased with time.

Acid phosphatase and inorganic phosphorus: The presence of crude extract inhibited both acid phosphatase as well as Pi. The trend observed was similar to that described in the case of amylase activity.

Table 1 *Percentage af seed germination in* Hordeum, Eleusine *and* Dolichos *treated with partially purified fruit pulp extract of* Berberis

Concentration (ppm)	Hours after sowing		
	24 hr	48 hr	72 hr
Hordeum			
0	50	80	80
100	40	60	70
400	20	60	60
Eleusine			
0	0	98	100
100	0	98	98
400	0	70	75
Dolichos			
0	30	70	99
100	10	40	89
400	10	40	85

Table 2 *Root and coleoptile/hypocotyl lengths of* Hordeum, Eleusine *and* Dolichos *72 hr after germination in presence of* Berberis *fruit pulp extract*

Concentration (ppm)	Root length (cm ± SD)	Coleoptile/ hypocotyl length (cm ± SD)
Hordeum		
0	1.36 ± 0.54	0.50 ± 0.00
100	0.56 ± 0.10	0
400	0.10 ± 0.00	0
Eleusine		
0	0.74 ± 0.15	0.41 ± 0.10
100	0.53 ± 0.17	0.40 ± 0.11
400	0.20 ± 0.08	0.33 ± 0.13
Dolichos		
0	0.84 ± 0.14	0.30 ± 0.08
100	0.56 ± 0.15	0.30 ± 0.10
400	0.57 ± 0.11	0.15 ± 0.05

Table 3 *Effect of* Berberis *fruit extract on amylase (activity units) during seed germination of* Hordeum, Eleusine *and* Dolichos

Concentration (ppm)	Hours after sowing		
	24 hr	48 hr	72 hr
Hordeum			
0	19.54	22.45	9.49
100	18.26	20.45	8.94
400	16.80	16.98	8.94
Eleusine			
0	58.47	85.78	226.35
100	50.47	80.71	225.85
400	43.09	80.36	222.39
Dolichos			
0	116.90	73.09	51.16
100	87.71	95.02	50.40
400	58.47	51.16	29.23

Table 4 *Effect of* Berberis *fruit extract on acid phosphatase activity (activity units) during seed germination of* Hordeum, Eleusine *and* Dolichos

Concentration (ppm)	Hours after sowing		
	24 hr	48 hr	72 hr
Hordeum			
0	0.61	0.38	0.49
100	0.51	0.32	0.35
400	0.19	0.22	0.25
Eleusine			
0	0.25	0.45	0.61
100	0.32	0.46	0.61
400	0.19	0.35	0.48
Dolichos			
0	1.45	2.21	2.60
100	1.70	1.54	2.69
400	1.45	2.31	2.02

Table 5 *Effect of* Berberis *fruit extract on the inorganic phosphorus contents (μg/g fr. wt.) during seed germination of* Hordeum, Eleusine *and* Dolichos

Concentration (ppm)	Hours after sowing		
	24 hr	48 hr	72 hr
Hordeum			
0	16.92	21.54	28.00
100	16.92	18.46	13.85
400	20.00	18.46	12.31
Eleusine			
0	6.15	24.64	8.46
100	4.62	15.38	8.96
400	4.61	16.92	6.92
Dolichos			
0	6.15	29.23	5.77
100	9.23	20.00	5.77
400	6.15	7.69	4.62

Characterization of fruit pulp extract: Chromatographic studies show that there are four compounds in the extract (table 3). Out of the four, one was anthocyanin and spots 2 and 3, which are blue or blue-green in UV light and give blue and blue-green colour respectively when fumed with ammonia were phenolic acids. Fourth compound gave purple colour in UV and when fumed with ammonia. UV spectra and max of these compounds revealed that the compounds are cyanidin-3-glucoside, chlorogenic acid, ferulic acid and quercetin-3-glucoside respectively.

Discussion

The inhibitory properties of the fruit pulp extract of *Berberis* are evident from its effect on seed germination as well as seedling growth in all the three test plants. The lower activity of amylase and acid-phosphatase in the presence of extract also suggests that germination inhibition as well as seedling growth is related to its effect on these enzymes. Therefore, the inhibitor(s) extracted from *Berberis* adds to those reported earlier from several plants (Evaneri 1949, Hamberg 1961, Rodley 1961). In most of the cases these inhibitors have been considered as gibberellin-antagonists and generally phenolic in nature (Corcoran

Table 6 *Chromatographic properties of spots separated from* Berberis *fruit pulp extract*

Spot No.	Colour			Rf × 100				λMax (MeOH)	Compound
	Vis	UV	UV+NH_3	BAW	Forestal	AcOH	Bu–HCl		
1.	P	P	B	40	25	50	36	274, 524*	cyan-3-glucoside
2.	—	B	B	80	—	97	80	325	ferulic acid
3.	—	LBG	IBG	98	—	62	—	237, 330	chlorogenic acid
4.	—	Pr	YPr	94	15	47	48	256, 365	Quer-3-glucoside

* (solvent) MeOH-HCl, *P*, Pink; *B*, Blue; *G*, Green; *L*, Light; *I*, Intense; *Vis*, Visible; *UV*, Ultraviolet; *Pr*, Purple; Y, Yellow

1970). As is evident from the identification, *Berberis* fruit pulp extract has phenolic constituents. However, this differs from the inhibitory extracts of other plants as it is more effective on root growth than on shoot growth which leads to speculate that probably it does not come in the class which are considered gibberellin antagonists. Had it been so, its effect would have been more on coleoptile/hypocotyl growth. It seems that inhibitory effect of this extract is more pronounced during first 24 hr than during subsequent period of germination. Another characteristic of this extract is its inhibitory effect on the germination of *Berberis* seeds itself (unpublished). Quercetin, chlorogenic acid and ferulic acid are reported to be germination inhibitors (Harborne 1980).

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References

Baijal M, Singh S S, Shukla R N and Sanwal G G 1972 Enzymes of Banana plant, optimum conditions for extraction; *Phytochemistry* **11** 929–936

Bernfeld P 1955 Amylase α and β; *Methods Enzymol.* **1** 149–158

Chandra P and Purohit A N 1980 Berberine contents and alkaloid profile of *Berberis* species from different altitudes; *Biochem. Syst. Ecol.* **8** 379–380

Corcoran M R 1966 Reduction of amylase by an inhibitor from carob; *Pl. Physiol.* **41** 1265–1267

——— 1970 Inhibitors from carob *(Ceratonia siliqua* L.) II. Effect on growth induced by indoleacetic acid or gibberellins A_1, A_4, A_8, and A_7; *Pl. Physiol.* **46** 531–534

Evaneri M 1949 Germination inhibitors; *Bot. Rev.* **153** 153–194

Fiske C H and Subbarow Y 1925 The colorimetric determination of phosphorus; *J. Biol. Chem.* **66** 375–400

Hamberg T 1961 in *Encyclopedia of Plant Physiology* **14** 1162–1183

Harborne J B 1980 Plant Phenolics; in *Encyclopedia of Plant Physiology* (new series) **8** 330–402

Khanduri S K and Purohit A N 1981 Patterns of phenolics in *Berberis* species from different altitudes in Garhwal Himalaya; *Pl. Biochem. J.* **8** 17–24

Mayer A M and Poljakoff-Mayber A 1975 in *The Germination of Seed* (Oxford: Pergamon Press)

Radley M 1961 Gibberellin like substances in plants; *Nature* **191** 684–685

Sumere C F Van 1960 in *The Phenolics in Plants in Health and Diseases* pp 25 (Oxford: Pergamon Press)