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**Plant Pathology**

# SEED LEACHATES AND INHIBITION OF CERTAIN SEED-BORNE PATHOGENIC FUNGI OF BAJRA (*PENNISETUM TYPHOIDES*)

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The interaction of eight pathogenic fungi with seed was studied by sowing spores of fungi in the seed leachates of bajra collected after 12, 18, 24 and 30 hr and the effect of leachates on conidial germination of the 8 test organisms was seen. All the samples of seed leachates inhibited conidial germination to varying degree. Seed leachates were analysed for amino acids, organic acids, sugars and phenols to locate the inhibitory substance, if any. Total phenols did not correspond with the inhibitory activity of seed leachates but p-coumaric acid, known to be germination inhibitor, was detected in all the four samples. Besides, nine amino acids, known to have therapeutic effect on fungus diseases, and five organic acids were detected in the leachates (malic acid being present in 3 samples). Five sugars were also detected in seed leachates but stimulation of conidial germination was not observed.

## INTRODUCTION

As mentioned in earlier publication (Mathur *et al.*, 1973) 8 fungi, associated with bajra seeds, are pathogenic causing leafspot symptoms or seed mortality. In sowing practice the fungi carried with the seed, evidently come into interaction with seed leachates. Experiments were therefore, carried out to find out the effect of seed leachates on the spore germination of the pathogens and to locate the presence of inhibitory substance, if any.

## MATERIALS AND METHODS

About 1500 seeds of local bajra were sown in moist sterilized neutral sand in three petri dishes @ 500 seeds/petri dish and the seed leachates were collected after 12, 18, 24 and 30 hr. Seeds in the petri dishes were washed successively with 80% ethanol, 50% ethanol and then with double distilled water. The combined washings of one petri dish were collected in one porcelain dish, alcohol was evaporated on steam bath and the leachates were dried in an oven at $60(\pm 1)°C$. Leachates from one porcelain dish were dissolved in 15cc of water and were tested for inhibitory effects on conidial germination of 8 pathogenic fungi viz., *Chaetomium globosum, Curvularia penniseti,*

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*Drechslera longirostrata, D. papendorfii, D. rostrata* Drechslera state of *Cochliobolus spicifer, Fusarium equiseti* and *F. fusarioides* isolated from bajra seed as well as from diseased leaves in the bajra fields. Conidia were harvested from sporulating cultures of the fungi, washed by centrifugation at 2500 rpm and then incubated in hanging drops of leachates at 28(±1)°C for period previously estimated for the fungal spores to germinate in distilled water. Control sets in hanging drops of distilled water were run simultaneously for each fungus. The percentage conidial inhibition was calculated in relation to the control.

Amino acids, organic acids and sugars were detected by thin layer chromatography following Randeroth (1965), the solvent and markers were used according to Block *et al.* (1955). Total phenols were determined according to Bhatia *et al.* (1972) and the presence of three phenols i.e. chlorogenic acid, p-coumaric acid and ferulic aci known to be germination inhibitors, was tested by descending paper chromatography, following Jindal (1972). Amino acids, organic acids, sugars and phenols were identified by comparing the Rf values with those of known ones.

## RESULTS

The seed leachates collected at 4 different periods inhibited conidial germination of 8 fungal species. The response of fungal spore germination of all the fungi towards a particular leachate is expressed in Figs. 1-4 while the comparative inhibitory effect of different leachates on one fungus is depicted in Figs. 5 & 6.

Total phenols on the basis of optical density was maximum in 30 hr. leachates and minimum in 18 hr and 24 hr leachates (Table I)

TABLE I

*Total phenolic content* in the seed leachates of bajra*

| Period of leachate collection (in hr) | Total phenolic contents in terms of optical density |
|---|---|
| 12 | 0.553 |
| 18 | 0.469 |
| 24 | 0.469 |
| 30 | 0.678 |

*Obseved on a Carlzeiss calorimeter at 725 nm.

The results of seed leachate analysis are enumerated in Table II.

TABLE II

*Seed leachate analysis*

| | seed leachate collection | | | |
|---|---|---|---|---|
| | 12 | 18 | 24 | 30 hr |
| **SUGARS** | | | | |
| 1. Arabinose | — | + | — | — |
| 2. Fructose | + | + | — | — |
| 3. Galactose | — | — | + | + |
| 4. Ribose | + | + | — | — |
| 5. Xylose | — | — | + | — |
| 6. Unknown-1 (Rf. 0.203) | + | — | — | — |
| 7. Unknown-2 (Rf. 0.248) | — | — | — | + |
| **AMINO ACIDS** | | | | |
| 1. Alanine | — | — | + | — |
| 2. Aminobutyric acid | + | + | + | — |
| 3. Glutamic acid | — | — | + | — |
| 4. Histidine | — | + | — | + |
| 5. Isoleucine | + | + | — | + |
| 6. Leucine | — | + | + | — |
| 7. Phenylalanine | + | + | — | + |
| 8. Tryptophane | + | — | — | + |
| 9. Valine | — | — | + | + |
| 10. Unknown (Rf. 0.746) | + | — | + | — |
| **ORGANIC ACIDS** | | | | |
| 1. Adipic acid | — | — | — | + |
| 2. Ascorbic acid | + | + | — | — |
| 3. Glutaric acid | — | + | — | — |
| 4. Malic acid | + | + | + | — |
| 5. Sebacic acid | — | — | + | — |
| 6. Unknown-1 (Rf. 0.532) | — | — | + | — |
| 7. Unknawn-2 (Rf. 0.568) | — | — | — | + |
| **PHENOLS** | | | | |
| 1. p-coumaric acid | + | + | + | + |
| 2. Chlorogenic acid | — | — | — | — |
| 3. Ferulic acid | — | — | — | — |

+indicates presence —indicates absence

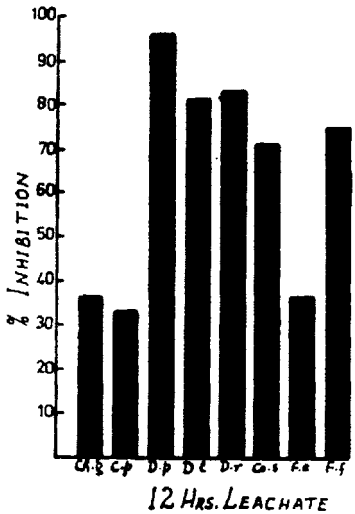


Fig. 1

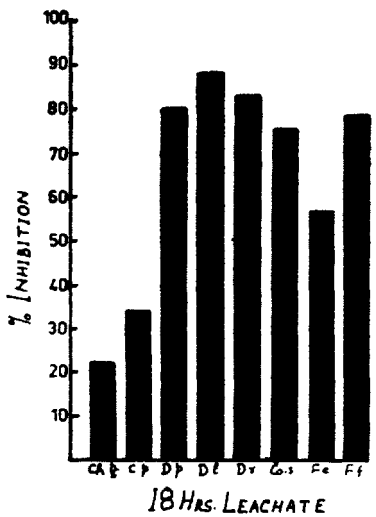


Fig. 2

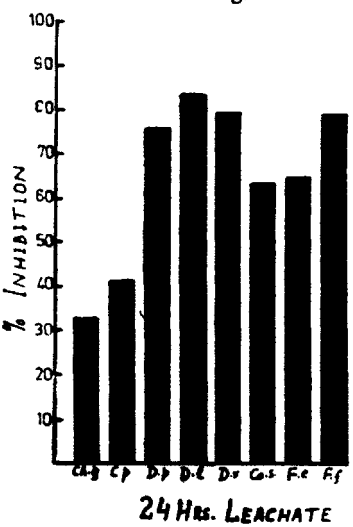


Fig. 3

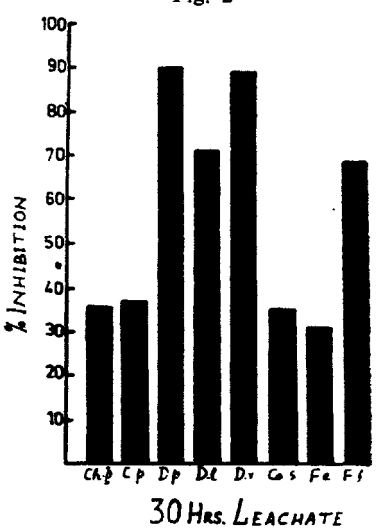


Fig. 4

Ch.g., *Chaetomium globosum*; C.p., *Curvularia penniseti*; D. p., *Drechslera papendorfii*; D.l., *Drechslera longirostrata*; D.r., *Drechslera rostrata*; Cos., *Cochliobolus spicifer*; F.e., *Fusarium equiseti*; F.f., *Fusarium fusarioides*.

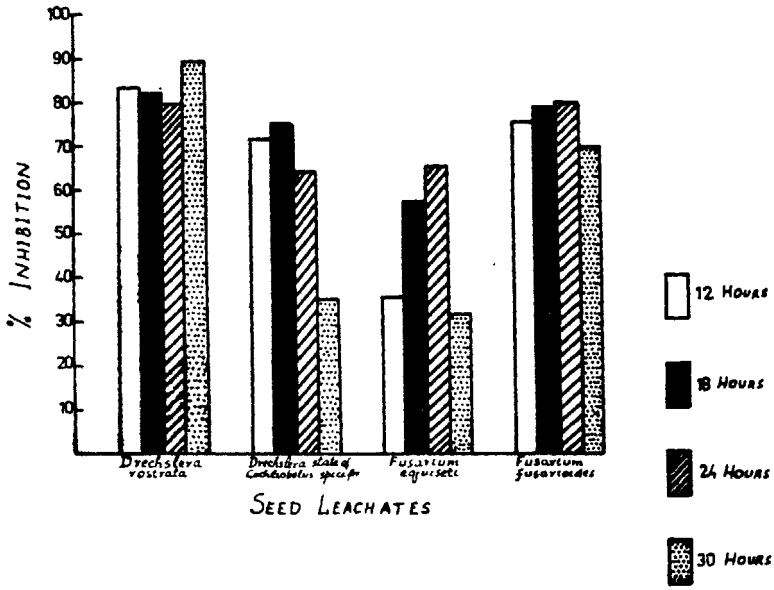
% Inhibition
100
90
80
70
60
50
40
30
20
10
Drechslera rostrata
Drechslera state of Cochliobolus, spicifer
Fusarium equiseti
Fusarium fusarioides
Seed Leachates
12 Hours
18 Hours
24 Hours
30 Hours

Fig. 5

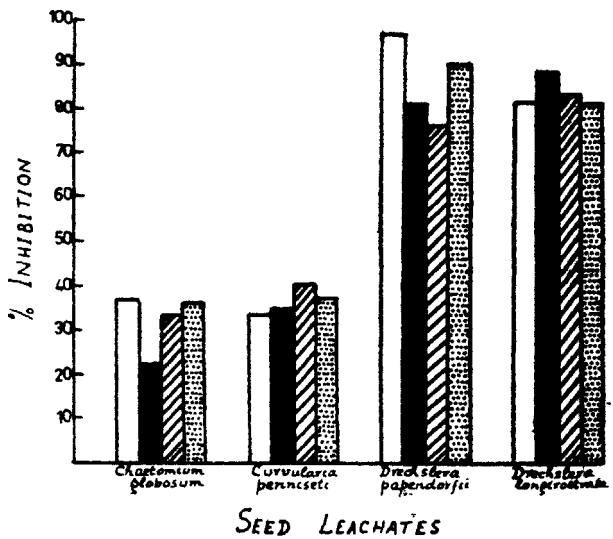
% Inhibition
100
90
80
70
60
50
40
30
20
10
Chaetomium globosum
Curvularia pennisetii
Drechslera papendorfii
Drechslera longirostrata
Seed Leachates

Fig. 6

Seven sugars (5 known and 2 unknown) and 10 amino acids (9 known and 1 unknown) were observed in the seed leachates. Of the 5 organic acids leached out of the seeds, malic acid was present in 3 samples (12, 18 and 24 hr). Amongst the 3 phenols tested p-coumaric acid was present in all the 4 leachates. The phenol gave coffee colour flourescence under UV, purple flourescence in UV ammonia and reddish brown reaction with diazotized sulfanilic acid.

## Discussion

Chemical barriers inhibit or interfere with the spore germination of fungi and these are materials often exuded by the seeds. Seed leachates of bajra collected after 12, 18, 24 and 30 hr inhibited the spore germination to varying degree in 8 fungal species viz., *Chaetomium globosum, Curvularia penniseti, Drechslera longirostrata, D. papendorfii, D. rostrata,* Drechsler state of *Cochliobolus spicifer, Fusarium equiseti* and *F. fusarioides.* In earlier reports seed leachates of 7 types of oilseeds inhibited conidial germination of 8 fungal species viz., *Rhizopus nigricans, Mucor hiemalis, Choanephora cucurbitarum, Aspergillus flavus, Penicillium* sp., *Curvularia lunata, Alternaria tenuis* and *Fusarium nivale* to varying degree depending upon the seeds and fungal species (Misra & Kannaujia, 1973). Seed exudates of cumin inhibited spore germination of *Curvularia lunata, Alternaria solani* and *Colletotrichum capsici* (Chaturvedi *et al.*, 1974) and of sorghum, tomato and finger millet inhibited, appreciably, conidial germination of *Helminthosporium oryzae* (Balsubramanian & Rangaswami, 1967).

The presence of antifungal substance in the seed coat might be the reason for the inhibition of certain fungi (Srivastava & Misra, 1971). Amino acids, organic acids and sugars were qualitatively analysed and total phenols were quantitatively determined in the bajra seed exudates. Malic acid was commonest organic acid detected in 3 out of 4 samples of leachates. Malic acid has been shown to be inhibitory to *Mycosphaerella pinodes,* the causal organism of gram blight (Hafiz, 1952) and also inhibited, completely spore germination of *Alternaria alternata* (Khanna, 1974). Since it was not detected in 30 hr leachates of bajra seeds which was inhibitory towards all the fungi, malic acid may not be solely responsible for the conidial inhibition in the present studies.

Nine known amino acids viz., aminobutyric acid, isoleucine, phenylalanine, tryptophane, histidine, leucine, glutamic acid, alanine and valine were detected in bajra seed leachates. Certain amino acids like alanine, valine, leucine, aspartic acid asparagine and glutamic acid have been reported to be associated with inhibition of germination because they have been exuded, mostly, from the non-viable seeds of *Secale cereale, Hordeum vulgare* and *Triticum aestivum* (Borner, 1960). Eight amino acids viz., cystine, methionine, tryptophane, histidine, leucine, glutamic acid, phenylalanine, valine and glycine were tested on germination of spores of 10 isolates of *Alternaria alternata* and all of them inhibited germination to a greater or lesser extent (Khanna, 1974). All the amino acids leached out of bajra seeds were reported to have chemotherapeutic activity against fungus diseases (van Andel, 1966). Amino acids, therefore, may account to some extent, for the inhibitory activity of bajra seed leachates.

Total phenols were also found to be present in varying concentrations in the seed leachates of bajra but did not show any correlation with the percentage inhibition of

the conidial germination of the fungal parasites. Kandaswamy *et al.* (1974) also did not observe any correlation between the total phenolic contents of the seed exudates of pea, *Dolichos lablab*, *D. bifloru Cicer arietinum*, *Cyamopsis tetragonoloba*, *Vigna sinensis* and their inhibitory activity towards *Rhizobium*. Total phenolic content may not be a true criterion for explaining the inhibitory activity for all phenols react with Folin Dennis Reagent. But only a few phenols like chlorogenic acid, p-coumaric acid, ferulic acid etc., are known as germination inhibitors. In the bajra seed leachates p-coumaric acid was detected in all the 4 samples of leachates. Phenolic compounds have earlier been reported to be germination inhibitors. Smith (1971) infected the pods of tropical legumes with *Colletotrichum lindemuthianum* and collected the diffusates. The antifungal inhibitors appeared to be phenolic compounds. Kandaswamy *et al.* (1974) reported that seed leachates of 6 species of leguminous plants inhibited conidial germination of *Helminthosporium oryzae*, *Fusarium oxysporum*, *Aspergillus niger*, *A. tamarii* and *Alternaria* sp. and the inhibitory principle was found to be phenolic in nature. The exudates from resistant lines of pea, with pigmented seeds, inhibited sporulation of *Fusarium solani* f.sp. *pisi*, growth of *Pythium ultimum* and conidial germination of *Fusarium solani* and phenols were found to be present in the exudates (Kraft, 1974). Wang and Pinckard (1973) found that the spore germination of *Diploidia gossypina* was inhibited by all the 11 phenolic compounds tested while that of *Alternaria alternata* by all the 6 phenols (Khanna, 1974). The germination inhibitor in *Linum usitatissimum* was found to be phenolic in nature and p-coumaric acid, ferulic acid and p-hydroxybenzoic acid were identified in seed exudates (Borne, 1960). Ferulic acid was reported to be germination inhibitor in rice seed (Sircar & Sircar, 1966).

In the present investigations the inhibitory activity of the seed leachates towards fungal spore germination may be due to the exudation of phenolic compound namely, p-coumaric acid along with amino acids and malic acid. The variation in the inhibitory activity may be due to quantitative and qualitative differences in the phenols, amino acids and organic acids.

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