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# Studies on the effect of seed leachates and seed extracts of Bhindi on the germination of spores of some pre-dominant fungi of rhizosphere

**EUSHAH ALI AND K. N. BORA**
*Department of Botany; Gauhati University, Guwahati-781014, Assam*

The present paper deals with the study on the effect of seed teachates and seed extracts of Bhindi on the pereentage germination of spores of sume predominant fungi of rhizosphere. The experimental results revaled that the effect of seed leachates and seed extracts on the percentage germination of spores of the test fungi was mostly low and different as compared to the control. It was observed from the results that the germination percentage of the fungal spores decreased gradually with the increase in concentration of seed leachates and seed extract. In case of seed leachates treatment, the percentage of spore germination of *Aspergillus niger* was found maximum (72%) at 25% concentration and the lowest was showed by *Trichoderma viride* (26%) at 100% concentration. In case of seed extract treatment, the maximum percentage of spore germination was showed by *Aspergillus niger* (66%) at 25% concentration and the minimum was showed by the spores of *Mucor hiemalis* (23%) at 100% concentration. The experimental results also showed that the percentage of spore germination of fungi was found higher in the seed leachates in comparison to seed extract.

The inhibitory effect of the seed leachates and seed extracts on the germination of the test fungi may be due to some chemical components present in seed leachates and seed extract.

**Key words:** Seed leachates, seed extracts, fungi, rhizosphere, inhibitory effect, antifungal substances

## INTRODUCTION

The association of certain fungi with cereal grains has been reported by various workers (Roychoudhury, 1968; De Tempe, 1960). It is generally known that the seed borne fungi cause severe damage to the seeds in various ways, causing loss in the percentage of germination, retarding healthy growth and development of seedling and consequently it leads to the loss in yield of crops. But all fungi have not the capacity to damage all kinds of seeds. The seed exudates are composed of a wide variety of substances like amino acids, sugars, vitamins and other phenolic compounds, some of which influence the growth of pathogens and other inhibit the pore germination. Seed leachates effect the seed borne fungi contributing to their nutritional status prior to penetration or inhibiting their saprophytic or pathogenic activity.

Seed leachates have been reported to cause inhibitory effect on seed borne fungi (Ark and Thomson, 1958; Srivastava and Mishra, 1971). The resistance capacity of seeds may depend on the toxic substances produced as a reaction to infection. Resistance may be based on preformed substances that may act directly against the parasite or indirectly by acting on associated microorganisms. Seeds are attacked by the various fungi, but not all the pathogen can damage the seeds internally due to presence of certain antifungal substances present in the seeds internally due to presence of certain antifungal substances present in the seed (Purohit and Bohra, 1999). Suriachandranselvan *et. al.* (2004) have reported that certain seed borne fungi posses somew toxic principles, which help them to invade the seed coat and damage the seed tissues. Several workers have reported the inhibitory effect of seed leachates and seed extract on the spore

germination of certain fungi (Chandi Ram *et al.* 1999; Purohit and Bohra, 1999; Das, 2005; Mishra and Kanaujia, 1973).

The present investigation has therefore, been carried out to study the effect of seed leachates and seed extract of Bhindi on the germination of spores of some predominant fungi of rhizosphere.

## MATERIALS AND METHODS

The seeds of Bhindi (variety—Parbhani Kranti) was taken from "Assam Seed Corporation", Guwahati, in polythene bag for the present investigation. The experiment was carried out at the Department of Botany, Gauhati University during the crop season in 2008. The seed leachates of Bhindi was prepared following the method as described by Saxena and Gupta (1982). For this 10 g Bhindi seeds were surface sterilized with 0.1% $HgCl_2$ solution for one minute and washed thoroughly with several changes of sterile distilled water (40°C). The seeds were washed again with sterile warm (70-80°C) water and soaked in 100 ml sterilized distilled water in 250 ml conical flasks. After 24 hrs, the flasks were gently hand shaken for 20-25 minutes and filtered. The liquids thus obtained were made concentrated to a volume of 10 ml on water bath at 50°C. This concentrated liquid is called as "Seed-leachates." Different concentrations (25%, 50%, 75% and 100%) of seeds leachates were prepared with sterile distilled water and takne for further experiment.

For the preparation of seed extract, 10 g surface sterilized seeds were crushed in mortar and soaked in 100 ml sterilized distilled water in 250 ml conical flasks. After 24-hrs, the flasks were gently hand shaken for 25-30 minutes and filtered. The filtrates were made concentrated to a volume of 10 ml on water bath at 50°C. Different concentrations (25%, 50%, 75% and 100%) were made with sterilized distilled water.

The pre-dominant rhizosphere fungi of Bhindi taken for the present investigation were *Aspergillus flavus, A. niger, Fusarium moniliformae, Penicillium oxalicum, Mucor hiemalis and Trichoderma viride.* These tested fungal single spore culture were grown on Czapeck's Dox Agar medium. After 15 days of incubation, the fungal spores of different fungi were collected for the experiment. The effect of seed leachates and seed extract on fungal spore germination were studied on cavity slide by hanging drop method. Four cavities slides were filled with different concentrations of seed leachates and seed extracts separately and teh spores of fifferent fungi were placed in the leachate and extract in such a way that 20-25 spores per microscopic field (under 150x magnification of the microscope) could be easily observed. Spore suspension in 0.1% sucrose solution served as control. Four replicates were taken for each experiment. All the glass slides were kept in sterilized Petriplates and were incubated at 27±1°C for 24 hrs. To maintain the humidity of the Petriplates, 10 ml sterile distilled water was poured into the Petriplates. After completion of incubation period of 24 hrs, the slides were taken out, the percentage of spore germination on four microscopic fields was observed, and the average numbers were recorded.

## RESULTS AND DISCUSSION

The results (Tables land 2) showed that the effect of seed leachates and extracts on the percentage germination of spores of the tested fungi was mostly low and different as compared to the control. It was observed from the the results (Tables 1 and 2) that the germination percentage of the fungal spores decreased gradually with the increase in concentrations of seed leachates and seed extracts. In case of seed leachates treatment, the percentage of spore germination of *Asperium niger* was found maximum (72%) at 25% concentrationfollowed by *Fusarium moniliformae* (60%), and *Aspergillus flavus* (56%). The lowest spore germination ws showed by *Trichoderma viride* (26%) followed by *Penicillium oxalicum* (28%) at 100% concentration of the seed leachates, while in control set all the test fungi showed best germination *i.e.* 87 to 93%.

In case of seed extract, it was found (Table 2) that all the test fungi in control set, showed their best germination i.e. 89 to 94%. The maximum percentage of spore germination was showed by *Aspergillus niger* (66%) at 25% concentration followed by. *Fusarium moniliformae* (53%) and *Aspergillus flavus* (50%) while the minimum spore germination was recorded by the spores of *Mucor hiemalis* (23%) at 100% concentrations of the seed extract followed by *Penicillum oxalicum* (24%) and *Trichoderma viride* (25%). It ws observed (Table 1)

**Table 1 :** Effect of seed-leachates of bhindi on the germination of spores of some pre-dominant fungi of rhizosphere. (Results represent the average number of spore germination in percentage)

| Fungal species | Concentration of seed leachates (%) | | | | Control (0.1%) sucrose solution) |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| *Aspergillus flavus* | 56 | 48 | 40 | 30 | 90 |
| *Aspergillus niger* | 72 | 63 | 53 | 48 | 92 |
| *Fusarium moniliformae* | 60 | 51 | 42 | 35 | 93 |
| *Penicillium oxalicum* | 55 | 47 | 38 | 28 | 90 |
| *Mucor hiemalis* | 49 | 43 | 36 | 32 | 87 |
| *Trichoderma viride* | 47 | 36 | 29 | 26 | 91 |

**Table 2 :** Effect of seed extract of bhindi on the germination of spores of some pre-dominant fungi of rhizosphere. (Results represent the average number of spore germination in percentage).

| Fungal species | Concentration of seed leachates (%) | | | | Control (0.1%) sucrose solution) |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| *Aspergillus flavus* | 50 | 45 | 32 | 26 | 92 |
| *Aspergillus niger* | 66 | 60 | 53 | 40 | 94 |
| *Fusarium moniliformae* | 53 | 47 | 40 | 28 | 90 |
| *Penicillium oxalicum* | 48 | 40 | 34 | 24 | 88 |
| *Mucor hiemalis* | 42 | 37 | 29 | 23 | 91 |
| *Trichoderma viride* | 40 | 30 | 26 | 25 | 89 |

that the maximum inhibition of spores germination was recorded by *Trichoderma viride* (74%) at 100% concentration of seed leachates while at 100% concentration of seed extract, the maximum inhibition was showed by *Mucor hiemalis* (77%). The present findings are similar with the findings of Purohit and Bohra (1999). Purohit and Bohra (1999) have studied the effect of seed leachates of spiece plants on spore germination of some seed borne fungi and reported that *Aspergillus niger* was least effected by seed-coat leachates and showed germination between 76% to 87% and the greater inhibition in spore germination of *Alternaria alternata* and *Curvularia lunata* was observed in seed leachates, whereas the control set, all the test fungi showed best germination i.e. from 83 to 100%. The experimental results (Table 1 and 2) showed that the seed leachates and seed extracts of Bhindi inhibited the spore germination of the tested fungi in different concentrations as compared to the control which may be due to the presence of certain antifungal substances or chemical components in seed coat. It was also revealed that the germination percentage of the fungal spores decreased gradually with increase in concentrations of seed leachates and seed extract. Similar results are also made by Kumar and Jalali (1984) and Das (2005). Mishra and Kananujia (1973) have reported that the inhibitory effect of seed leachates of oil seed is due to the presence of certain anti-fungal substances presence in the seed coat and the inhibitory effect varied differently against seed borne fungi and at the lower concentrations the effect is mild, while in higher concentrations, it reduces the fungal spore germination drastically.

The experimental results (Table 3) showed that the percentage of spore germination of fungi is higher in the seed leachates in comparison to seed extract of Bhindi. The present findings are in agreement with the findings of Das (2005). Das (2005) has studied the effect of seed leachates and seed extract on the spore germination of fungi in Chilli seds and has reported that both the seed leachates and seed extract have some inhibitory effect in spore germination of *Alternaria alternata, Aspergillus niger, Colletrotrichum capsici, Fusarium* sp. and *Penicillium* sp. The germination percentage of fungal spores decreases gradually with the increase concentration of seed leachates and seed extract. Das (2005) has also reported that the percentage of spore germination of fungi is higher in seed leachates in comparison to seed extract.

**Table 3 :** Effect of seed-leachates and seed extracts of bhindi 100% concentration on the inhibition percentage of spore germination of some predominant fungi of rhizosphere.

| Fungal species | Seed leachates (100% concentration) | Seed extract (100% concentration) |
|---|---|---|
| *Aspergillus flavus* | 70 | 74 |
| *A. niger* | 52 | 60 |
| *Fusarium moniliformae* | 65 | 72 |
| *Penicillium oxalicum* | 72 | 76 |
| *Mucor hiemalis* | 68 | 77 |
| *Trichoderma viride* | 74 | 75 |

The retarding effect of the seed leachates and seed extracts used in the present investigation on the germination of certain fungal spores may be due to some chemical components present in the seed leachates and seed extracts. These chemical components may be amino acids, sugars and some antifungal substances.

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## REFERENCES

Ali, E. 2000 *Microbiological Studies of Rhizosphere Mycoflora of Bhindi (Abelmoschus esculentus* (L.) Moench) *Plants. M Phil. Dissertation,* Gauhati University.

Ark, P.A. and Thomson, J.P. 1958. Antibiotic properties of the seeds of wheat and barley. *Plant Dis. Reptr.* **42:** 959-962.

Chandi, Ram; Maheswari, S.K. and Gupta, P.C. 1999. The effect of seed coat leachates and seed extract on seed mycoflora of bottle gourd and sponge ground. *Agri. Sci. Digest.* **19**(1): 426.

Das, R. 2005. *Studies on certain aspects of infection of chilli [Capsicum annuum* (I.)] fruits by fungi. Ph.D. Thesis, Gauhati University.

De Tempe, J. 1960. Routine methods for determining the health condition of seeds in the seed testing station. *Proc. Inttl. Seed Test Asso.* **26:** 27-60.

Kumar, A. and Jalali, B.L. 1984. Differential effects of seed leachates on seed-borne pathogens associated with chickpea *(Cicer arietinum). Indian Phytopath.* **38(1):** 99-103.

Kundaswami, D.; Kesavan, R.; Ramaswamy, K. and Prasad, N.W. 1974. Occurrence of microbial inhibition in the exudates of certain leguminous seeds, *Indian J. of Microbiol.,* **14:** 25-30.

Mishra, R.P. and Kanauja, R.S. 1973. Studies on certain aspects of seed-borne fungi II Seed borne fungi of certain oil-seeds. *Indian Phytopath.* **26:** 184-294.

Purohit. P. and Bohra. A. 1999 Effect of seed leachates of spice plants on spore germination of some seed-borne fungi. *J. Mycol. Pl. Pathol.* **29(1):** 134-135.

Roychoudhury, S.P. 1968. Role of plant patholegy in crop production in India. *Indian Phytopath.* **21:** 1-13.

Saxena, R.M. and Gupta, J.S. 1982. Effect of seed leachates on spore germination of seed horne fungi of *Vigna radiata. Indian Phytopath.* **35(2)** 230-240.

Srivastava, V.B. and Mishra, R.R. 1971. Rhizosphere fungal variation in susceptible and healthy wheat. *Indian Phytopath.* **24:** 874-786.

Suriachandraselvan, M; Salalrajan. F. and Aiyanathan, K.E.A. 2004. Seed and root exudates of sunflower in relation to host susceptibility to charcoal rot. *J. Mycol. Pl. Pathol.* **34(2):** 585-587.

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