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Evaluation of Phytotoxic Potential of Two *Aspergillus* Isolates by Germination Test

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Abstract: ***Seed borne pathogens can cause reduction in germination capacity of seeds resulting in crop losses. Many biologically active metabolites are produced by Aspergillus species some of which have herbicidal activity. This study was undertaken to study the phytotoxic effect of cell free culture filtrates of two Aspergillus isolates on germination of Vigna radiata seeds (green gram seeds). Fifteen-day old culture filtrates of both the Aspergillus isolates inhibited the germination of Vigna radiata seeds but CFCF of Aspergillus isolate #11 was more phytotoxic in comparison to Aspergillus isolate #14. Culture filtrate of both isolates was extracted with chloroform to obtain crude extract. The crude extract of both isolates was observed to be phytotoxic as they inhibited germination of Vigna radiata seeds. It was thus inferred that reduction in seed germination was due to phytotoxic metabolites produced by these isolates.***

Keywords: Phytotoxicity, Cell free culture filtrate, Inhibition and Germination

1. Introduction

Many plant pathogens which are seed borne cause seed borne diseases resulting in enormous crop losses [1]. Pathogens, which live on the surface or interior of seed such as bacteria, fungi or viruses, have the potential to spread disease to the subsequent crop. Of these, fungal contamination is the most important [2]. Such contaminants are fearsome since they affect the seeds before harvest time and may find optimal developing conditions when the seeds are stored leading to alteration of germination quality of these seeds [3].

Fungi of different genera have been reported to produce toxic metabolites which can reduce the viability of seeds, disrupt growth processes and development of plant organs [4] and [5]. The phytotoxins produced by fungi are often suitable for the pathogenesis or infection of weeds [6]. Fungi of *Aspergillus* species have been shown to be excellent sources of new natural chemicals [7] some of which have shown promising herbicidal activity. Asperal acid D from *Aspergillus alabamensis* showed higher plant growth inhibitory activity on wheat root and shoot elongation than terbutryn [8]. Three cichorine analogues with an isoindolinone skeleton obtained from *A. nidulans*, exhibited superior phytotoxicityto cichorine on the leaves of *Zea mays* and *Medicago polymorpha* [9]. Phytotoxicity of penicillic acid to corn seed during germination has been reported [3]. Penicillic acid was reported to inhibit the growth of young plant roots of rice [10] and of oat seedlings by lessening their respiration [11]. Aflatoxin B1, rubratoxin B and zearalenone are considered phytotoxic [12].

This work was undertaken to study whether the altered germination qualities of seeds were due to the growth of contaminating fungi or due to the phytotoxic action of metabolites produced by them.

2. Materials and Methods

Fungal material-
Aspergillus isolates #11 and #14 were isolated from soil samples of Jabalpur.

Seed Samples-
Vigna radiata seeds were purchased from local market of Jabalpur.
Growth medium- PDA of Hi Media was used for culturing both *Aspergillus* isolates.
Production of secondary metabolites-
Czapek-Dox medium supplemented with 1% casein was used for production of secondary metabolites of both *Aspergillus* isolates. Composition of medium is as follows- Sodium nitrate- 0.3%, Sucrose- 3%, $K_2HPO_4.3H_2O$- 0.13%, $MgSO_4.7H_2O$- 0.05%, KCl- 0.05%, $FeSO_4.7H_2O$- 0.001%, $CuSO_4.5H_2O$- 0.0005%, $ZnSO_4.7H_2O$- 0.001% and Casein- 1.0%.

100mL of medium was taken in 250mL conical flasks. Autoclaved media was inoculated with spore suspension of *Aspergillus* isolates #11 and #14 and incubated for 15 days at ambient temperature.

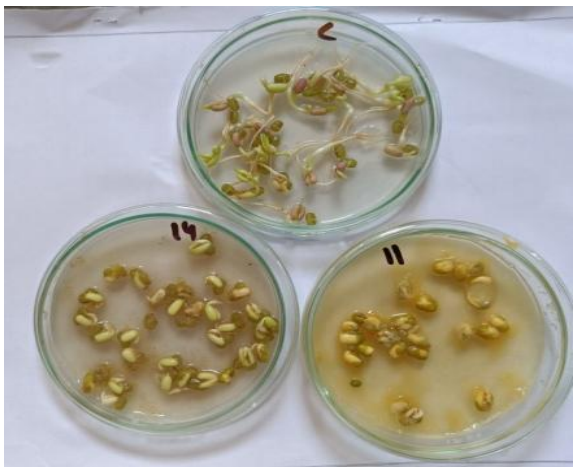
Preparation of cell free culture filtrates (CFCF)- Filtrates of cultures were obtained by filtration with Whatman filter paper.

Preparation of culture extracts- Filtrates of cultures were extracted two times with 100mL of chloroform. The combined solvent fractions were dried and then dissolved in 2.0mL of ethanol and the volume was made up to 20mL with distilled water.

Germination Tests- Germination tests were used for measurement of phytotoxicity. For both,culture filtrates and crude extracts 10 hypochlorite cleaned seeds were placed in agar containing sterilized petriplates and 10mL of test solution (culture filtrate or crude extract) was added to the petriplates. For control 10 mL of distilled water was used.

After four days germination occurred. Results were expressed as inhibition of root growth or as the decrease of root length relative to that of the controls.

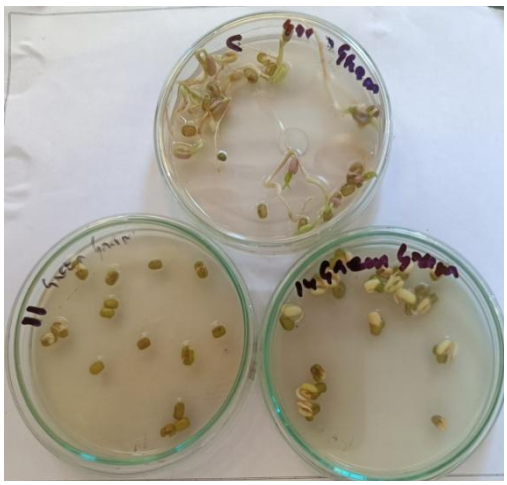
3. Result and Discussion



Phytotoxic effect of CFCF on germination of *Vigna radiata* seeds

Filtrates from two weeks old cultures of *Aspergillus* isolates #11 and #14 inhibited the germination of *Vigna radiata* seeds but filtrate of *Aspergillus* isolate #11 was more phytotoxic than that of *Aspergillus* isolate #14 because reduction in root length of *Vigna radiata* seeds was greater in isolate #11 CFCF treated seeds than those treated with CFCF of isolate #14.

To determine that the altered germination qualities of the seeds were due to the action of phytotoxic metabolites, the culture filtrates were extracted with chloroform and then dried. Residue was dissolved and then used for the germination test. The crude extracts were also observed to inhibit germination of *Vigna radiata* seeds. Seeds treated with crude extract of isolate #11 failed to germinate while seeds treated with crude extract of isolate #14 showed reductions in root length as compared to controls.



Phytotoxic effect of crude extract on germination of *Vigna radiate* seeds

Keromnes and Thouvenot [3] have reported inhibition of corn seed germination due to phytotoxic effects of penicillic acid. According to them the toxicity of penicillic acid is about 5% that of aflatoxin B1. Inhibitory effects of conidia suspension of *Aspergillus* and *Trichoderma* species on seed germination of *Bidens Pilosa* have been reported [13]. Secondary metabolites from *Aspergillus sparsus* have been shown to be phytotoxic against *Echinochloa crusgalli* and *Amaranthus retroflexus* [14].

The extracts from *Aspergillus* isolates #11 and #14 were toxic to *Vigna radiate* seeds during germination so these isolates produce phytotoxic metabolites which inhibit germination of seeds. Further studies need to be done to assess the nature of these phytotoxic metabolites which inhibited germination of *Vigna radiata* seeds.

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Author Profile

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