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# PHYTOTOXIC ACTIVITY OF FUNGAL PATHOGENS INCITING IVY GOURD FRUIT

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**ABSTRACT**

Ivy gourd fruit rots caused by *Bipolaris tetramera* and *Geotrichum candidus. B. tetramera* and *G. candidus* was grown on Richard's medium and the culture filtrate was assayed for phytotoxic activity. Toxins was observed from the culture filtrates of tested pathogens and proved inhibitory for cereals, pulses, oil and vegetables seeds, which is toxic to percent seed germination. *B. tetramera* was found high toxic on sorghum seed (10%) and low toxic for sunflower seed (90%). *G. candidus* was found high toxic on maize seed (10%) while low toxic on fenugreek seed (80%).

**Keywords: Ivy Gourd, *Bipolaris tetramera* and *Geotrichum candidus*, Phytotoxic Activity, Crop Seeds**

## INTRODUCTION

Ivy gourd, *Coccinia indica* (Whight & Arn.) is a Cucurbitaceous vegetable grown for its edible fruits. It has got therapeutic value especially in the treatment of diabetics, bronchitis and skin disease **[1]**. The juice of the roots and leaves is considered to be a useful treatment for diabetes **[2]**. The juice of the stem is dripped into the eyes to treat cataract **[3]**. The disease causing organisms enter into the host tissue through mechanical pressure exerted by the growing germ tube or dissolving the host cell wall through secretion of toxins or enzymes **[4]**. Experiments were therefore carried out to study the phytotoxin by fungal pathogens associated with Ivy

gourd fruit rot disease and the role of these toxin in pathogenesis.

## MATERIAL AND METHODS

### Phytotoxin Activity

*Bipolaris tetramera* and *Geotrichum candidus* was isolated from *C. indica* fruit rot. *Bipolaris tetramera* & *Geotrichum candidus* (Carbendazim sensitive and resistant) isolates were grown on Richard's medium (broth) (Sucrose-35gm, Potassium Nitrate- 10gm, Potassium Dihydrogen Phosphate-5gm, Magnesium Sulphate-2.5gm, Ferric Chloride-0.02gm, Distilled water- 1000ml). Twenty five ml of Richard's broth was poured in 100 ml conical flasks. The flasks along with medium were autoclaved at 15 lbs for 20 minutes. The flasks were allowed to cool and after cooling, the flasks were inoculated with 1 ml spore suspension of pathogens from 7 days old cultures grown on PDA slants. The flasks were incubated for 9 days at 27 ± 2 $^0$C. After incubation period, flasks were harvested by filtration of their contents through Whatman filter paper No.1. The filtrates were collected in pre sterilized conical flasks and considered as crude toxin preparations.These preparations were tested for their toxicity by the methods of **[5]**.

### Phytotoxin Assay

The toxicity of culture filtrates was determined by using seed germination method. Healthy seeds of crop plants viz. Wheat (*Triticum aestivum*), Rice (*Oryza sativa*), Sorghum (*Sorghum bicolor*), Maize (*Zea mays*), Pigeon pea (*Cajanus cajan*), Chick pea (*Cicer arietinum* ), Groundnut (*Arachis hypogaea*), Sunflower (*Helianthus annus*), Spinach (*Spinacia oleracea*) and Fenugreek (*Trigonella foenum-graecum*) were selected and surface sterilized by treating with 0.1% Mercuric chloride ($HgCl_2$) solution and followed by repeated washing with sterilized distilled water. Surface sterilized seeds were soaked in crude toxin preparation for 24 hours. Then soaked seeds were placed on moist blotter paper in sterilized petriplates. Seeds soaked similarly in freshly uninoculated liquid medium served as control. Percent germination of seeds was observed and recorded the data **[6, 7]**.

## RESULTS AND DISCUSSION

### Production of Phytotoxin

The detection of phytotoxin from the pathogens was examined by treating seeds of crop plants in culture filtrates. The treated seeds were allowed for germination under *in vitro* condition. The culture filtrate of pathogens showed effects on seed germination. This indicated that extracellular metabolites in culture filtrate were toxic.

Culture filtrate of pathogens exhibited their effects on the seed germination. The culture

filtrate of isolated pathogens were grown on Glucose nitrate medium for seven days and tested for seed germination of 10 crops belonging to cereals, pulses, oils and vegetables seed. The results were recorded and presented in the **Table 1**. The obtained results indicated that culture filtrate of pathogens proved to be inhibitory for germination of crop plant seeds.

***Bipolaris tetramera***

Culture filtrate of isolates, *B. tetramera* was showed high toxicity for germination against sorghum (*Sorghum bicolor*) seed (00 & 10%) followed by spinach (*Spinacia oleracea*) seed (10 & 20%). In case of sunflower (*Helianthus annus*) seed was low toxic for germination (80 & 90 %) followed by rice (*Oryza sativa*) seed (70 & 80%) and chick pea (*Cicer arietinum*) seed (60 & 80%).

***Geotrichum candidus***

Culture filtrate of isolates, *G. candidus* was showed high toxicity for germination against maize (*Zea mays*) seed (00 & 10 %) followed by pigeon pea (*Cajanus cajan*) seed (10 & 20 %), groundnut (*Arachis hypogaea*) seed (10 & 20%) and spinach (*Spinacia oleracea*) seed (10 & 20 %). In case of wheat seed (*Triticum aestivum*) showed low toxic for germination (70 & 90 %), followed by chick pea seed (*Cicer arietinum*) (80& 90%) and fenugreek seed (*Trigonella foenum-graecum*) (80 & 90 %).

Overall results indicated that, except two cases in culture filtrate of pathogens proved to be inhibitory for the seed germination of crop plant seeds **(Figure 1)**. The culture filtrate of isolated pathogens showed inhibitory effects on the seed germination of crop plants. Resistant isolates culture filtrates showed low toxic than that of sensitive one.

Phytotoxins produced by fungi are often released into the artificial medium in very low amounts, causing difficulties in their isolation and purification; hence, prepare larger amounts of the growth medium to cultivate the pathogen so that we may obtain a greater yield of the compounds **[8, 9]**. Solvent extraction to recover organic compounds with novel activity has been considered one of the most effective methods to isolate phytotoxins from other fungal metabolites **[10]**. *R. solani* toxin is heat stable because the culture filtrate still showed high toxicity after exposure to a temperature of 121°C for 30 min **[11]**.

Several formae speciales of *F. oxysporum* produce secondary metabolites that are toxic to plants and are involved in wilt diseases. These include fusaric acid produced by *F. oxysporum* f. sp. *cubense* on banana, beauvericin produced by *F. oxysporum* f. sp. *melonis* on muskmelon and a polypeptide toxin produced by *F. oxysporum* f. sp. *vasinfectum* on cotton **[12, 13]**. Fungal

filtrates from *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria terreus* and *Alternaria alternata* were reported to reduce germination percentage of soybean seeds when seeds were soaked in the filtrate for 24 h **[14]**. Although there are numerous precedents for the production of toxins by species of *Alternaria*, little work has been reported on phytotoxic metabolites produced by *Alternaria brassicicola*. It was reported that methanol extracts of the culture filtrates of the fungus were phytotoxic **[15]**. It was found that spore germination fluids of the fungus were phytotoxic **[16]**. It was reported that the filtrate of *A.niger*, *Penicillium chrysogenum* and *Macrophomina phaseolina* inhibited percentage seed germination, with increase in filtrate age, soaking time in all fungal filtrates **[17]**. It means that metabolites are discharged by the tested fungi in the media in which they were grown.

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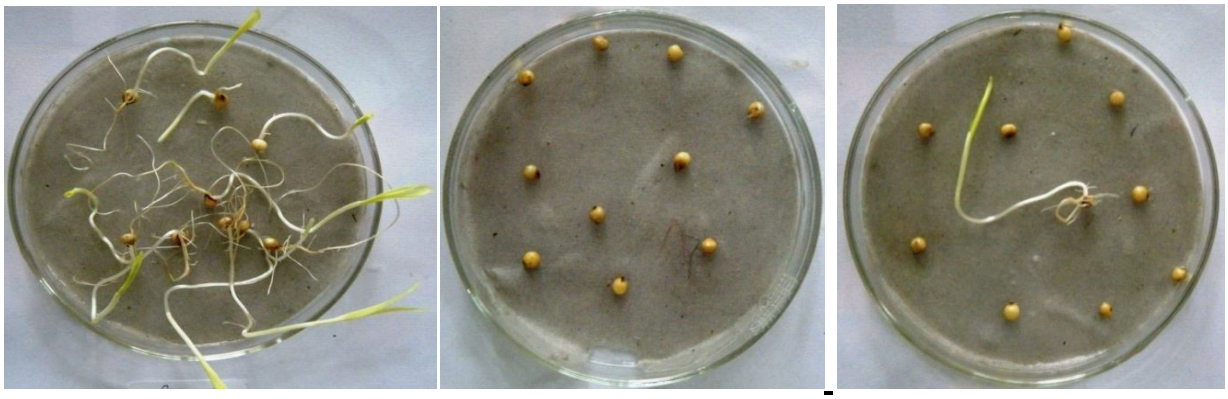
**Table 1: Effect of Culture Filtrate of Pathogens on Seed Germination of Crop Plants**

| S. No. | Crop Seeds | Isolates | Control (%) | Seed Germination (%) | |
|---|---|---|---|---|---|
| | | | | *B. tetramera* | *G. candidus* |
| 1 | Wheat (*Triticum aestivum*) | S | 100 | 20 (80) | 70 (30) |
| | | R | | 40 (60) | 90 (10) |
| 2 | Rice (*Oryza sativa*) | S | 100 | 70 (30) | 30 (70) |
| | | R | | 80 (20) | 50 (50) |
| 3 | Sorghum (*Sorghum bicolor*) | S | 90 | 00* (100) | 40 (60) |
| | | R | | 10 ( 90) | 70 (30) |
| 4 | Maize (*Zea mays*) | S | 100 | 20 (80) | 00* (100) |
| | | R | | 60 (40) | 10 (90) |
| 5 | Pigeon pea (*Cajanus cajan*) | S | 100 | 50 (50) | 10 (90) |
| | | R | | 70 (30) | 20 (80) |
| 6 | Chick pea (*Cicer arietinum* ) | S | 100 | 60 (40) | 80 (20) |
| | | R | | 80 (20) | 90 (10) |
| 7 | Groundnut (*Arachis hypogaea*) | S | 100 | 30 (70) | 10 (90) |
| | | R | | 50 (50) | 20 (80) |
| 8 | Sunflower (*Helianthus annus*) | S | 100 | 80 (20) | 30 (70) |
| | | R | | 90 (10) | 40 (60) |
| 9 | Spinach (*Spinacia oleracea*) | S | 100 | 10 (90) | 10 (90) |
| | | R | | 20 (80) | 20 (80) |
| 10 | Fenugreek (*Trigonella foenum-graecum* ) | S | 90 | 40 (60) | 80 (20) |
| | | R | | 50 (50) | 90 (10) |
| SEm ± | | | | 8.42 | 9.68 |
| | | | | 8.33 | 10.33 |
| CD @5% | | | | 19.00 | 21.89 |
| | | | | 18.33 | 23.35 |

**NOTE: S = Sensitive, R= Resistant, Figures in Parentheses are % value of toxicity**

*A. ipolaris tetramera*



**Control (Jowar)** **High toxic (00%)** **Low toxic (10%)**

*B. Geotrichum candidus*

**Control (Maize)** **High toxic (00%)** **Low toxic (10%)**

**Figure 1: Phototoxic Activity of Pathogens on Seed Germination Crop Plant**