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STUDIES ON PRODUCTION OF HYDROLYTIC ENZYMES (CELLULASES) BY SOIL BORNE FUNGI CAUSING STEM ROT AND WILT IN GROUNDNUT

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ABSTRACT

An attempt has been made to observe production of cellulases enzyme by *Sclerotium rolfsii* Sacc. causing stem rot of Groundnut and *Fusarium oxysporum* Schlechtend emend Sny. & Hans causing wilt of Groundnut. From the results obtained it is evident that both the fungi produced cellulases in both substrate medium and GN medium in both the test varieties (TAG-24 & SB-XI). In case of *Sclerotium rolfsii* maximum cellulases production was observed in substrate medium in variety TAG-24 as well as in SB-XI. It was noticed that cellulases production was more in Groundnut variety TAG-24 than SB-XI in substrate medium while in GN medium in both the varieties cellulases production was similar. In case of *Fusarium oxysporum* maximum cellulases production was observed in substrate medium in variety TAG-24 as well as in SB-XI. It was noticed that cellulases production was more in Groundnut variety TAG-24 than SB-XI in substrate medium while in GN medium in both the varieties cellulases production was similar.

KEYWORDS: Soil borne, Groundnut, Cellulases, Biodeterioration.

INTRODUCTION

Groundnut crop suffers from diseases to a much larger extent than other crops. From its early stage of development to maturity it is attacked by various pathogens. The majorities are caused by fungi and several of them are yield reducers in certain regions and seasons (Mayee, 1995). In India it is attacked by over 55 pathogens including viruses (Mishra & Ghewande, 1989). Among these, stem rot caused by *Sclerotium rolfsii* Sacc. and *Fusarium oxysporum* Schlechtend emend Sny. & Hans are much important.

These pathogens alone and in association are known to produce different types of hydrolytic enzymes in field as well as during storage which is termed as seed biodeterioration. It is estimated that about 4% of the world's grains are lost due to biodeterioration caused by microorganisms (Clerke, 1966).

It is clear from the literature that the degree of biodeterioration has been found to be directly related with efficiency of seed moulds to produce hydrolytic enzymes.

Therefore some common soil borne fungal isolates of Groundnut variety TAG-24 and SB-XI were screened for their ability to produce the hydrolytic enzymes (cellulases).

MATERIALS AND METHOD

I. Groundnut varieties

Groundnut cultivars used in the present study are TAG-24 and SB-XI.

II. Isolation and identification of pathogen causing stem rot in Groundnut

Diseased samples showing typical symptoms of stem rot i.e. wilting of total plants, white mycelial growth at collar region of plant were selected and used as sample source for the isolation of causative agent. Infected portion of stem was cut into small pieces with sterilized scalpel, cleaned with distilled water, then surface sterilized with 0.1% $HgCl_2$ solution for 30 second and again washed thrice with sterile distilled water. Small 1 to 2 pieces were transferred aseptically on Potato Dextrose Agar (PDA) plates containing Chloramphenicol (30 mg/100 ml) with the help of sterilized forceps under aseptic condition (Appolinaire, 2004). Inoculated Petri plates were incubated at room temperature for 7 days for growth of the pathogen.

Identification and cultural characters of *S. rolfsii* Sacc. were confirmed with the help of ICRSAT Information Bulletin No. 36, (1992) and Mesquita, (2007).

III. Pathogenicity of *S. rolfsii* Sacc.

i) Mass production of *Sclerotium rolfsii* Sacc.

Mass multiplication of *S. rolfsii* was carried out in Potato Dextrose broth at room temperature for 3 weeks (Ordentlich *et al.,* 1988) and then the numbers of sclerotia produced were used for the preparation sick pots.

ii) Preparation of sick pots

Sterilized soil sand (1:1) mixture was artificially infested with sclerotia of *S. rolfsii* at 1 sclerotia/ gm soil (Fouzia Yaqub and Saleem Shahzad, 2005). Six pots were disinfected with 5 % $CuSO_4$ solution and out of 6 pots, three pots were filled with inoculated soil at 150 gm soil per pot and three pots filled with sterilized uninoculated soil, were maintained as control. The pots containing inoculum were incubated for 15 days at room temperature, frequently stirred and watered for colonization of fungus in the soil. Surface disinfected seeds of Groundnut varieties TAG-24 and SB XI were sown in plastic pots. All these pots were kept at room temperature and watered regularly. Observations were recorded on germination and mortality of the plants. This experiment was conducted in triplicates.

IV. Isolation and identification of pathogen causing wilt in Groundnut

Isolation of the pathogen was conducted from roots of wilting and root rotted groundnut plants at different stages of plant growth. Root of diseased plants was washed carefully under tap water to remove the adhering soil particles. The washed roots were cut into small pieces and surface sterilized by immersing the root pieces in 0.1 % $HgCl_2$ for 2 to 3 minutes, washed 2 to 3 times with sterile distilled water to remove any residues of $HgCl_2$. The washed root pieces were dried between two sterilized filter paper, then transferred to potato dextrose agar (PDA) amended with 0.1 ml of 0.1 % $HgCl_2$ and Streptomycin sulfate (0.01%) in Petri dish and incubated at 25 ± 2°C for 7 days (El-Sayed *et al.,* 2008). The growing fungi were individually transferred to PDA medium. Pure cultures of fungi were obtained using hyphal tip technique. Pure cultures of the isolated fungi were subjected to identification based on morphological and cultural characteristics, transferred to PDA slants and kept in refrigerator at 4°C for further use.

Identification and cultural characters of *Fusarium oxysporum* Schlechtend emend Sny. & Hans. were confirmed according to Ahmed and Reddy, (1993).

V. Pathogenicity of *Fusarium oxysporum* Schlechtend emend Sny. & Hans.

i) Mass production of *Fusarium oxysporum* Schlechtend emend Sny. & Hans.

For plant inoculation, mycelium taken from the edge of the colony was transferred to 150 ml of Potato Dextrose Broth (PDB) and incubated at room temperature for 5 days in a rotary incubator (120 rpm) (Fakher *et al.,* 2006). The liquid culture was filtered and conidial suspension was adjusted to10^7 spores/ml. The spore suspension produced was used for the preparation sick pots.

ii) Preparation of sick pots

The preparation of sick pots was made by following the method as described by Fakher *et al.,* (2006).

For this surface disinfected seeds of groundnut varieties, TAG-24, and SB XI were planted in plastic pots containing sterilized soil sand mixture (1:1) incubated at room temperature. Fifteen days after their emergence pots were infested with 100ml of conidial suspension (10^7 spores/ ml). Similarly control plants were treated with100 ml of sterile distilled water. All these pots were kept at room temperature and watered regularly. Observations were recorded for germination and mortality of the plants up to 60 days. This experiment was conducted in triplicates.

VI. Production of cellulases by *Sclerotium rolfsii* **causing stem rot and** *Fusarium oxysporum* **causing wilt in Groundnut variety TAG-24 and SB-XI**

An attempt has been made to observe the production of cellulases by *Sclerotium rolfsii* Sacc. The enzyme assay was made by the method described by Denison and Koehn (1979).

Table 1: Production of Hydrolytic enzymes (cellulases) by soil borne fungi *Sclerotium rolfsii Sacc.* and *Fusarium oxysporum Schlechtend* emend *Sny.* & Hans.

Sr. No.	Common soil borne fungi of Groundnut	Groundnut variety TAG-24		Groundnut variety SB-XI	
		Cellulases* production		Cellulases* production	
		GN medium	Substrate medium	GN medium	Substrate medium
1	*Sclerotium rolfsii* Sacc.	0.90	2.69	0.90	1.02
2	*Fusarium oxysporum* Schlechtend emend Sny. & Hans.	0.63	1.32	0.63	1.00

**= Expressed as O.D. at 560 nm*

Photo Plate 1A: Groundnut plant showing symptoms of stem rot.

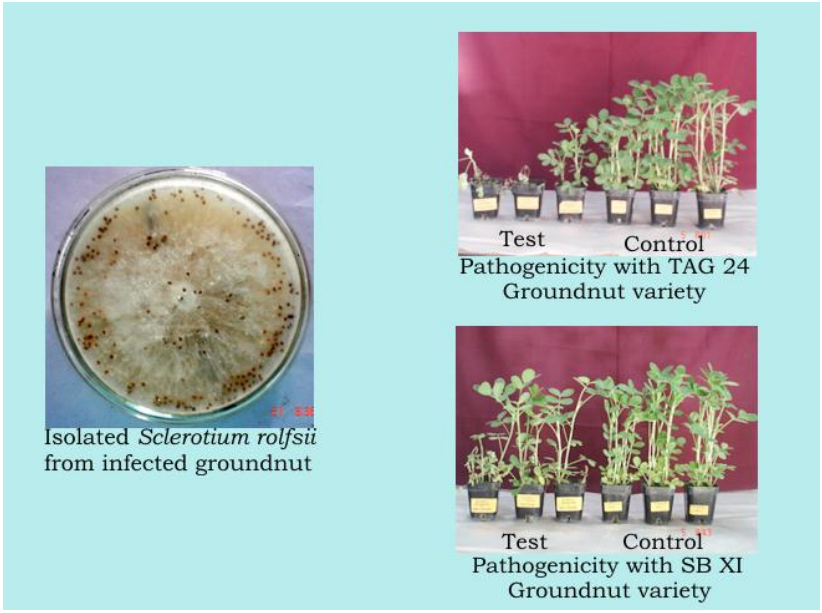

Photo Plate 1B: Isolation and Pathogenicity of *Sclerotium rolfsii* Sacc.

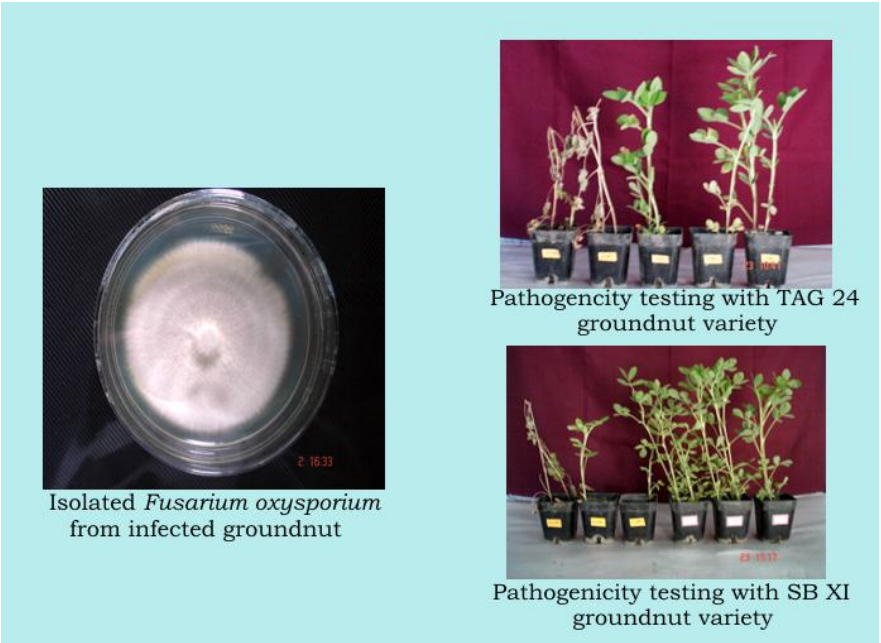

Photo Plate 2: Isolation and Pathogenicity of *Fusarium oxysporum* Schlechtend emend Sny. & Hans.

RESULTS AND DISCUSSION

I) Isolation and identification of pathogen causing stem rot in Groundnut

After 7 days incubation on PDA plates it was observed that the fungus produced abundant white septate mycelium, 1.5–3.0 µm diameter with clamp connections at each septation, aerial hyphae and also numerous spherical, or ellipsoidal, white sclerotia turning brown on maturation. Based on these morphological and cultural characteristic, the disease causing organism was identified as *Sclerotium rolfsii* Sacc. The results are presented in Photo Plate: 1 A & B.

II) Pathogenicity Tests of *Sclerotium rolfsii*

It was observed that in both the test varieties isolated strain of *Sclerotium* showed reduction in percent germination. Amongst the germinated seeds, initially yellowing of basal leaves was evident followed by drooping of leaves and wilting of plant. Some of the plants remained upright (Photo Plate: 1 B). It was also observed that white mycelium and small sclerotia were present at the base of infected plants (Photo Plate: 1B). While uninoculated plants (control) remained healthy and free of signs and symptoms of disease. Among the infected plants mortality was calculated and was found to be 64.28 % and 28.57% in TAG 24 and SB XI, respectively.

To prove Koch's postulates, the diseased plants were removed and infected portion of stem was cut into small pieces and was used further for isolation of causative agent of the disease on PDA using standard microbiological method. The result obtained revealed that the isolated organism when compared with inoculated strain was found to be same. These results are indicative of the fact that *Sclerotium rolfsii* Sacc. used for artificial inoculation was a potent pathogen of Groundnut showing typical symptoms of stem rot.

III) Isolation and identification of pathogen causing wilt in Groundnut

The Potato Dextrose Agar plates incubated with wilted Groundnut sample for the isolation of phytopathogen when was analyzed after 7 days showed white colored mycelium, bearing microconidia, macroconidia and chlamydospores which were observed under microscope. Based on morphological and cultural characteristic, the disease causing organism was identified as *Fusarium oxysporum* Schlechtend emend Sny. & Hans**.** The results are presented in Photo Plate-2.

IV) Pathogenicity Tests of *Fusarium oxysporum*

From the results presented in Photo Plate-2, it was observed that the artificially inoculated seeds of both the varieties viz. TAG 24 and SB XI showed first sign of wilting only after 45 days of incubation. It was found that initially lower leaves started wilting and then extended to the upper leaves and finally the whole plant was wilted (56 days), on the contrary the uninoculated (control) plant of both varieties have no sign of wilting and remained healthy, indicating that the tested isolate was potent pathogen causing wilting of Groundnut varieties. It was also recorded that infection wise pathogen also reduced the per cent germination of both the varieties TAG-24 (38.46 %) and SB XI (66.66 %).

To fulfill Koch's postulates, diseased plants pieces were then transferred to potato dextrose agar (PDA) plates amended with 0.1 ml $HgCl_2$ (0.1 %) and Streptomycin sulfate (0.01%) and incubated at 25±2 ˚C for 4-7 days. The colony characteristic and microscopic observation of the re-isolated fungus when compared with the original isolate, found to be the same.

V) Production of Hydrolytic enzymes (cellulases) by soil borne fungi *Sclerotium rolfsii* Sacc. and *Fusarium oxysporum* Schlechtend emend Sny, & Hans. An attempt has been made to observe production of cellulases enzyme by *Sclerotium rolfsii* Sacc. causing stem rot of Groundnut and *Fusarium oxysporum* Schlechtend emend Sny. & Hans causing wilt of Groundnut. The results are mentioned in table-1. From the results obtained it is evident that *Sclerotium rolfsii* Sacc. and *Fusarium oxysporum* Schlechtend emend Sny. & Hans produced cellulases in both the media in both the test varieties.

In case of *Sclerotium rolfsii* Sacc maximum cellulases production was observed in substrate medium in variety TAG-24 as well as in SB-XI. It was noticed that cellulases production was more in Groundnut variety TAG-24 than SB-XI in substrate medium while in GN medium in both the varieties cellulases production was similar.

In case of *Fusarium oxysporum* Schlechtend emend Sny. & Hans maximum cellulases production was observed in substrate medium in variety TAG-24 as well as in SB-XI. It was noticed that cellulases production was more in Groundnut variety TAG-24 than SB-XI in substrate medium while in GN medium in both the varieties cellulases production was similar.

CONCLUSION

The soil borne fungi were found to be capable of producing hydrolytic enzymes like cellulases. This clearly suggests well equipped enzyme make up of these fungi to degrade and utilize any kind of storage chemical present in the oil seeds. However, degree of enzyme production was found to be variable among the mycoflora. This may be related with their adaptation potential which might be different in these fungi.

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