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RESEARCH ARTICLE

Studies on diversity, Amylase Production by Seed-borne fungi of Pearl millet and their Control Measures

Khairnar DN

Plant Pathology Research Laboratory, Department of Botany, KAANMS, Arts, Commerce & Science College Satana-423301, Maharashtra, India.

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ABSTRACT

Twenty three fungal species were found associated with seeds of eight cultivars of pearl millet. Maximum fungi were reported from seeds of var. BJ-104 and ICMS-7703.Starch nitrate medium was more favourable than glucose nitrate and bajra flour nitrate medium for amylase production by fungi. Maximum amylase production was produced by *Aspergillus flavus, Curvularia pallecens* and nil by *Cladosporium herbarium.* Amylase production was completely inhibited by Foltaf and Zincop and they followed by Hexafer-10, Dithane M-45, Zineb-75, Bavistin, Captan and Dithante Z-78.

Key Words: Seed-borne fungi , Pearl millet, Amylase, control.

INTRODUCTION

The seed-borne fungi of pearl millet (*Pennisetum typhoides* (Burm.) Stapf and Hutt) were earlier studied by Sharma and Basuchaudhary (1975), Gupta (1976), Konde et al. (1980); Prasad and Narayan (1981); Khairnar and Mukadam (1989); Khairnar (2013). Cereal grains are rich in starch and therefore its degradation should by only due to the amylases produced by microorganisms. Unfortunately in the literature information for this aspect is very scanty. However, amylase production and seed damage by seed-borne fungi have been correlated in *Vigna radiata* (Charya and Reddy, 1980) and also in mouldy maize grains (Olutiola, 1982) and Prasad (1979) has noted significant production of amylase and cellulose by coriander seed-borne fungi.

The present investigations were carried out to detect the seed-borne fungi of pearl millet cultivars, viz. African A-1, BJ-104, BK-560, ICMS-7703, Local, MBH-110 and WCC-75 by different seed health testing methods and to study their amylase production by seed moulds and control by seed dressing fungicides.

MATERIALS AND METHODS

Seed samples of pearl millet varieties African A-1, BJ-104, BK-560, ICMS-7703, MBH-110, WCC-75, X-5 and Local were collected in three random samples (half kg each) from fields, various store houses and markets. A composite sample of this was prepared by mixing the individual samples, preserved in cloth bags at laboratory temperature during the studies.

Standard blotter and agar plate method with Wakmans acid agar medium were used as recommended by ISTA (1966) for the isolation of seed-borne fungi of pearl millet (glucose 10g, KH2O4 1g, MgSO4 0.5g, agar agar 20g and distilled water 1000 ml. pH 5.6) Four hundred seeds were used in each case. Seeds used for experiments were untreated and pretreated with 01.% HgCl2 solution. In agar plate method, ten seeds were plated in each plate The plates were incubated at 28-30 C under alternate light darkness condition for seven days.

Production of amylase(s) was studied by growing the fungi on liquid medium at pH 5.5, containing starch 1 % , KNO3 0.25%, KH2PO4 0.1% and MgSO4, 7H2O 0.05 %. 25ml of the medium was powered in 100 ml conical flasks and autoclaved at 15 lbs pressure for 30 min. Then the flasks were inoculated separately with 1 ml spore suspensions of the fungi which were grown for 7 days PDA slants. Unless otherwise stated, the flasks were incubated for 6 days at 25C with diurnal periodicity of light. On the 7th day, the flasks were harvested by filtering the contents through whatman filter paper No. 1. The filtrates were collected in pre-sterilized culture filtrate bottles and termed as crude enzyme preparation.

Assay method: Determination of amylase (s) activity was done with the help of cup-plate method which was adopted by Sundarasingh and Saksena (1982), where 25 ml of starch agar assay medium (soluble starch 10gm, Na2HPO4 2.84 g, NaCl 0.35 g, agar agar 20 g and distilled water 1000 ml at pH 6,9) was poured in each petri-plate. On solidifying the medium, with the help of a cork borer (No. 4) a activity (8 mm diameter) was made in the centre and was filled with 1 ml culture filtrate/ the plates were incubated at 28C After 48 hours, the plates were flooded with Lugol's iodine solution as indicator. A clear, non-blue, circular zone was obtained surrounding to the central cavity, the diameter which was measured (CM) as the amylase activity zone. Similar procedure was followed for the control except the pouring of autoclaved culture filtrate in the central cavity instead of the active enzyme.

RESULTS AND DISCUSSIONS

It is clear from the results summarized twenty three fungal species appeared in the seeds of eight different cultivars by treated and untreated seeds by agar plate methods. Results summarized in Table 1.

Table 1: effect of three liquid media on amylase production by seed borne fungi of pearl millet

Seed-borne fungi	Activity zone in CM		
	Glucose nitrate medium	Starch nitrate medium	Bajra flour nitrate medium
Alternaria alternata	1.4	2.1	1.8
A.tenuis	1.6	2.5	2.4
Aspergillus flavus	1.7	3.5	1.9
A.fumigatus	0.0	2.3	2.3
A.nidulans	1.8	2.2	1.7
A.niger	2.2	1.6	0.9
Cladosporium herbarum	0.0	0.0	0.0
Curvularia lunata	1.5	2.5.	2.0
C.pallescens	1.6	3.2	1.7
C.penniseti	1.3	1.8	1.8
Drechslera longirostrata	1.6	1.6	1.9
D.rostrata	1.7	1.9	1.6
D.tetramera	2.4	2.0	1.6
Fusarium moniliforme	2.0	2.3	2.0
F.oxysporum	0.0	1.9	1.5
F.semitectum	1.4	2.0	2.1
Penicillium oxalicum	0.0	2.0	2.0
Pythiu;m sp.	2.5	2.7	2.1
Memboniella echinata	0.0	1.0	1.0
Rhizopus nigricans	1.8	2.1	1.0
Rhizoctonia solani	1.9	2.5	2.4
Torula herbarum	0.0	1.0	1.5

Table 2: Effect of fungicides on amylase production

Fungicides (100 ppm)	Activity zone in CM		
	A. flavus	*C. pallescens*	*F. moniliforme*
Blitox 50 W	2.6	1.9	2.1
Bavistin	1.9	2.0	1.0
Captan	1.8	2.4	2.4
Dithane M-45	1.6	1.0	1.1
Dithane Z-78	1.4	2.0	1.2
Foltaf	0.0	0.0	0.0
Hexafer-10	2.0	1.5	0.0
Hexaferb	1.6	2.3	1.4
Zincop	0.0	0.0	0.0
Zineb-75	1.3	1.6	2.1
Control (whthout fungicide)	3.2	3.0	1.9

A. flavus : Aspergillus flavus | *C. pallescens: Curvularia pallescens* | *F. moniliforme : Fusarium moniliforme*

Aspergillus fumigatus, Cladosporium herbarum, Fusarium oxysporum, Penicillium oxalicum, Memnoiella echinata and *Torula harbarum* were unable to produce amylase in the absence of starch (substrate) i.e. in glucose nitrate medium. On the other hand in the absence of starch amylase production was shown only by the species of *Alternaria, Curvularia Drechslera, Aspergillus, Fusarium, Pythium, Rhizopus* and *Rhizoctonea*. Among theree media, starch nitrate medium was found to be superior for amylase production to all the fungi except to *Cladosporium herbarum*. Bajra flour nitrate medium proves to be next superior medium to some of the fungi.

It is found that *Aspergillus flavus and Curvularia pallescens* are produce maximum amylase production in starch nitrate medium, and hence these two fungi can cause high deterioration in seeds and seeds become more viable and they also show high percentage of germination inhibition.

Different promising fungicides at 100 ppm concentration were tested against *Aspergillus flavus, Curvularia palescens* and *Fusarium moniliforme* for amylase production.

It is clear from the table 2 that amylase production was completely inhibited due to foltaf and zincop in all the three fungi which was followed by Hexafer-10, Dithane M-45, Bavistin Zineb 75, Captan, Dithane Z-78 and Hexaferb.

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