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Effect of nitrogen and phosphorus sources on amylase production in seed born fungi of maize

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Abstract
Starch degrading amylolytic enzymes are of great significance in biotechnological application ranging from food, fermentation, and textile to paper industries. Amylase enzyme action of ten dominating fungi viz. *Alternaria alternata, Aspergillus flavus, A niger, A terrus, Curvularia lunata, Fusarium oxysporum, Helminthosporium tetramera, Penicillium notatum, Rhizoctonia solani* & *Trichoderma viride* isolated from different varieties of maize seeds were studies under the influences of nitrogen & phosphorus sources. The results are very helpful to minimize the bio-deterioration of maize seeds in different storage condition.

Keywords: Amylase, maize seed, seed borne fungi

INTRODUCTION

Production of extracellular hydrolytic enzyme by seeds born fungi has a role during the process of seed deterioration and has been considered helpful to their invasion and colonization. However, Vidhyasekharan et al (1966) [1] claimed that production of extracellular hydrolytic enzymes by *Fusarium moniliforme* and *Aspergillus flavus* was found to be responsible in the spoilage of paddy seeds. Fungi associated with seed in ill storage condition uses the seed content and release the extracellular hydrolytic enzymes. Such enzymatic activities cause bio-deterioration of seeds. Among inorganic nitrogen sources, potassium nitrate and sodium nitrate supported amylase production in case of *Aspergillus awamori* (Musaeva, 1966 [2] & Shilova 1967 [3]). *A tamari*, *Aspergillus flavus*, *A fumigatus*, *Percillum italicum* (Singh and Agrawal, 1981 [4]). Peptone and organic nitrogen sources, was found to be stimulatory in *Aspergillus awamori* (Musaeva 1966 [2]), in some seed born fungi of Bajra (Khairnar, 1967 [5]), Bhosale (1989) [6] reported that, gelatin and urea proved inhibitory amylase production in *Curvularia lunata* and *Fusarium oxysporum*.

The phosphorus sources used in the based medium, dipotassium hydrogen phosphate at very low concentration favoured amylase production in *Lentinus edodes* (El zalaki et al 1980) [7] and potassium dihydrogen phosphate in *Aspergillus niger* (Mahamood et al. 1978) [8].

Considering the fact attempt has been made to study the impact of nutritional factors on amylase activity of seed borne fungi.

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Materials and Methods

Monosporic culture of *Alternaria alternate, Aspergillus flavus*, *A niger, A terrus*, *Curvularia lunata*, *Fusarium oxysporum*, *Helminthosporium tetramera*, *Penicillium notatum*, *Rhizoctonia solani* & *Trichoderma viride* isolated from different varieties of maize seeds and maintained on PDA slants.

Production of amylase was studied by growing the test fungi in liquid medium containing soluble Starch 1%, KNO_3 0.25%, KH_2PO_4 0.1% and $MgSO_4.7H_2O$ 0.05%, PH of medium was adjusted at 5.5. Twenty five ml of the medium was poured in 100ml conical flasks autoclaved and inoculated separately with 01ml spore suspension of the test fungi which were grown for 7 days on PDA slants. The flasks were incubated for 6 days at 25±1°c with diurnal periodicity of light on 7th day, the flasks were harvested by filtering the contents through whatman filter no. 1, and the filtrates were collected in pre-sterilized bottles and termed as crude enzyme preparation.

Determination of amylase activity was done with the help of cup plate method which was adopted by Singh and Saxena (1982) [9], where 20ml of starch assay medium (Soluble starch - 10gm, Na_2HPO_4 - 2.84 gm, NaCl - 0.35 gm, Agaragar - 20 gm, distilled water 1000ml and PH 6.9) was poured in each Petriplate. On solidification of the medium a cavity (08 mm diameter) was made in the centre with the help of a corkborer (No. 4) and was filled with 1ml culture filtrates (crude enzyme preparation) of the test fungi. The plates were incubated at 28ºC for 24 hours, than they were flooded with Lugol's iodine solution as an indicator. A clear non blue, circular zone obtained surrounding the central cavity; diameter was measured (mm) as the amylase activity zone similar procedure followed for the control. The KNO_3 served as control in the starch nitrate containing sources and in phosphorus sources potassium dihydrogen phosphate as control.

RESULTS AND DISCUSSION

From table 1 and graph 1 reveals that calcium nitrate was stimulatory for amylase production in *Alternaria alternata*, *Aspergillus flavus*, *A niger, A terrus* and *Helminthosporium tetramera* where as sodium nitrate was found to be stimulatory for *Alternaria alternata*, *Aspergillus niger*, *A terrus*, *Curvularia lunata*, *Fusarium oxysporum*, *Helminthosporium tetramera*.

Sodium nitrate was stimulatory for amylase production in *Aspergillus niger*, *A terrus*. In ammonia form ammonium oxalate showed stimulatory effect for amylase production in *Aspergillus niger*, *A terrus* and *Curvularia lunata* all other forms of nitrogen sources showed minimum production of amylase in all ten tested fungi. Organic nitrogen sources are preferred for the production of α-amylase. A maximum α -amylase production was supported by yeast extract, peptone or beef extract (Hamilton et al., 1999 [10], Emanuilova and Toda, 1984 [11], Krishnan and Chandra, 1982 [12], Hayashida et al., 1988 [13]). Urea & Casein strongly inhibited amylase production in all fungi. Gelatin stimulated the amylase production in *Alternaria alternata*, *Aspergillus niger*, where as peptone stimulated the amylase production in *Curvularia lunata, Helminthosporium tetramera*. About relationship between growth and amylase production, there was no any clear cut difference among them. However, *Aspergillus* genera viz *Aspergillus flavus*, *A niger*, *A terrus* showed maximum amylase production in the presence of calcium nitrate and sodium nitrate than any other nitrogen source.

It is clear from the data given in table 2 and graph 2 that in all fungi, potassium dihydrogen phosphate (control) was found to be better than the other phosphorus sources, on the contrary ammonium bi-phosphate for *Alternaria alternata*, *Trichoderma viride*, *Rhizoctonia solani* showed minimum production of amylase. Whereas *Aspergillus flavus, A niger* and *A terrus* showed maximum amylase production in potassium dihydrogen phosphate (control) followed by sodium dihydrogen phosphate.

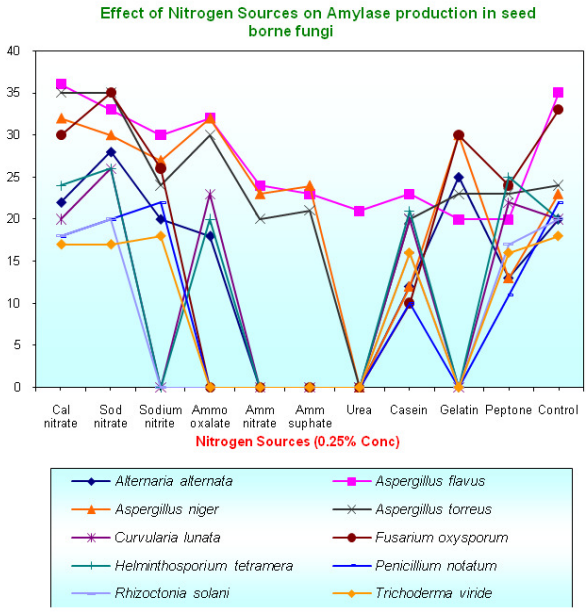
Table 1. Effect of Nitrogen Sources on Amylase production in seed borne fungi

Nitrogen Sources (0.25% conc)	Fungi									
	Alal	**Asfl**	**Asni**	**Aste**	**Culu**	**Fuox**	**Hete**	**Peno**	**Rhso**	**Trvi**
A] Nitrates										
1] Cal nitrate	22	36	32	35	20	30	24	18	18	17
2] Sod nitrate	28	33	30	35	26	35	26	20	20	17
B] Nitrites										
Sodium nitrite	20	30	27	24	-	26	-	22	-	18
C] Ammonia forms										
1] Ammo oxalate	18	32	32	30	23	-	20	-	-	-
2] Amm chloride	-	-	-	-	-	-	-	-	-	-
3] Amm nitrate	-	24	23	20	-	-	-	-	-	-
4] Amm sulphate	-	23	24	21	-	-	-	-	-	-
D] Amids										
Urea	-	21	-	-	-	-	-	-	-	-
E] Organic forms										
1] Casein	12	23	12	20	20	10	21	10	16	16
2] Gelatin	25	20	30	23	-	30	-	-	-	-
3] Peptone	13	20	13	23	22	24	25	11	17	16
Control										
(Pot nitrate KNO3)	20	35	23	24	20	33	20	22	20	18

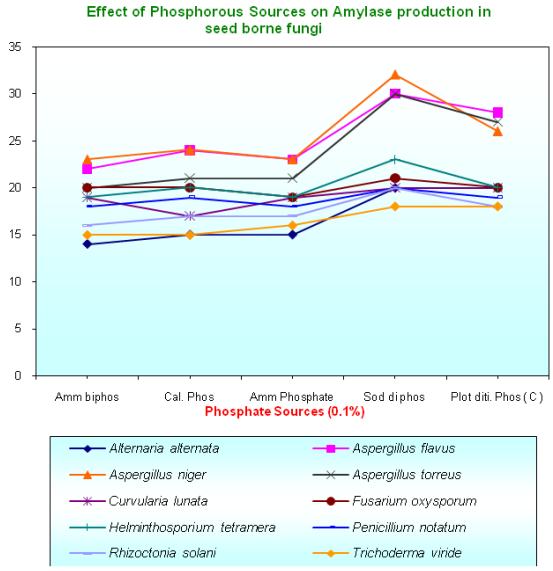
Table 2. Effect of Phosphorous sources on Amylase production in seed borne fungi

PhosphateSources (0.1%)	Alal	Asfl	Asni	Aste	Culu	Fuox	Heyr	Peno	Rhso	Trvi
Phosphate Sources										
1] Amm biphos	14	22	23	20	19	20	19	18	16	15
2] Cal. Phos	15	24	24	21	17	20	20	19	17	15
3] Amm Phosphate	15	23	23	21	19	19	19	18	17	16
4] Plot diti.phos (C)	20	30	32	30	20	21	23	20	20	18
5] Sod di phos	20	28	26	27	20	20	20	19	18	18

Altal – *Alternaria alternata*
Asfl – *Aspergillus flavus*
Asni – *Aspergillus niger*
Aste - *Aspergillus terus*
Culu - *Curvularia lunata*
Fuox – *Fusarium oxysporum*
Hetr – *Helminthosporium tetramere*
Peno – *Penicillium notatum*
Rhzo – *Rhizoctonia solani*
Trvi – *Trichoderma viride*



Graph 1.



Graph 2.

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