J. Phytol. Res. 25(1) : 69-70, 2012

STUDIES ON PHYSIOLOGY OF STORAGE FUNGI OF SOME MEDICINAL SEEDS: IV-PRODUCTION OF AMYLASE

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Production of amylase by storage fungi of 9 medicinal seeds was investigated. These fungi were *Aspergillus carbonarius, A. flavus, A. niger, Cladosporium cladosporioides, Fusarium oxysporum, Penicillium corylophilum, Pythium indigoferae* and *Rhizopus oryzae* .The 8 storage fungi were cultivated in glucose nitrate and starch nitrate liquid medium and the culture filtrates exhibited the macerating enzyme activity; indicating thereby that the enzyme production was adaptive in nature.

Keywords: Amylase; Medicinal seeds; Storage fungi.

The seeds are known to be infested by fungi and other microbes. The fungi are known to produce various extra cellular metabolites like enzymes, toxins, acids, growth regulators etc. Enzymes produced by seed borne fungi are involved in seed deterioration and break down the complex ingredients of the seeds into other compounds, thereby affecting the viability and nutritive value of the seeds. In such type of seed deterioration, the enzymes amylase, cellulases and pectinases play an important role[1-3]. The seeds that are used as medicines are also infested by fungi during storage. In our earlier studies 32 storage fungi were recorded on the medicinal seeds of *Azadirachta indica, Butea monosperma, Holarrhena pubescence, Madhuca longifolia, Plantago ovata, Pongamia pinnata, Semecarpus anacardium, Tectona grandis* and *Thespesia populnea*[4]. These storage fungi degrade the active medicinal components of the seeds and significantly reduce them. In addition, these fungi reduce its market value and affect germination. Therefore, during present investigation enzyme amylase, which is known to degrade starch, was studied.

Eight dominant storage fungi viz, *Aspergillus carbonarius, A. flavus, A. niger, Cladosporium cladosporioides, Fusarium oxysporum, Penicillium corylophilum, Pythium indigoferae* and *Rhizopus oryzae;* isolated from the medicinal seeds of 9 different plants, mentioned above, were selected for the present studies.

The fungi were grown separately on starch nitrate liquid medium (Starch 1 %, KNO_3 0.25 %, KH_2PO_4 0.1 %, $MgSO_4.7H_20$ 0.05 %, distilled water to volume). 25 ml of the medium was poured in 250 ml conical flasks, sterilized and inoculated with the standard spore suspension of the respective fungus. The flasks were incubated 25 ±2 °C for 7 days and the culture filtrate was collected by filtering the contents through Whatman filter paper No. 1. The culture filtrate collected after 7 days incubation was used as "crude enzyme solution". Determination of amylase enzyme activity was done with the help of cup-plate method[5], where 25 ml of starch agar assay medium (soluble starch 10 gm, Na_2HPO_4 2.84 gm, NaCl 0.35 gm, Agar- Agar 20 gm, distilled water 1000 ml, pH 6.9) was poured in each petriplate. On solidifying the medium, a cavity (of 8 mm diameter) was made in the center with the help of a cork borer (No.4) and was filled with 0.1 ml culture filtrate (crude enzyme solution). The petriplates were incubated at 25 ±2 °C for 24 hours, then they were flooded with Lugol's iodine solution as an indicator ((Lugol's iodine was prepared as Iodine 1 gm, KI 2 gm, distilled water 300 ml)[6]. A clear non blue circular zone was obtained surrounding the central cavity, the diameter measured (mm) as the amylase enzyme activity.

It is observed from table 1 that all the 8 fungi studied, secreted the enzyme amylase. The production of amylase was observed only in SN medium (containing starch) and not in GN medium. Therefore, the production of amylase enzyme by 8 storage fungi was adaptive in nature. The activity was found to be maximum in the culture filtrate of *Aspergillus flavus* followed by the culture filtrate of *Penicillum corylophilum.* It is also observed that there is no correlation between the fungal growth and amylase activity.

Amylase produced by fungi play very important role in seed deterioration. The seeds which mainly contain starch, are degraded by fungi having amylase producing

Table 1. Amylase enzyme activity by some storage fungi of medicinal seeds in GN and SN medium.

S. No.	Fungi	Initial pH	Final pH in GN medium	Dry wt. of mycelium in GN medium (mg)	GN-cult. filt.- Activity zone (in mm)	Final pH in SN medium	Dry wt. of mycelium in SN medium (mg)	SA-cult. filt.- Activity zone (in mm)
1	*Aspergillus carbonarius*	5.6	4.8	77	--	5.4	52	25.00
2	*A. flavus*	5.6	4.5	112	--	4.8	88	36.33
3	*A. niger*	5.6	5.2	65	--	4.6	76	32.35
4	*Cladosporium cladosporioides*	5.6	4.9	75	--	5.2	58	25.50
5	*Fusarium oxysporum*	5.6	4.8	104	--	5.0	42	16.50
6	*Penicillium corylophilum*	5.6	5.5	72	--	4.4	80	35.15
7	*Pythium indigoferae*	5.6	5.0	70	--	4.6	28	10.00
8	*Rhizopus oryzae*	5.6	4.8	115	--	5.0	40	15.66

ability. During present study, all the 8 storage fungi were found to possess the ability for the production of enzyme amylase. The enzyme production was found to be adaptive. Similar observation has been made by Pawar and Papdiwal[7]. During present investigation, it was observed that there is no correlation between growth and amylase production; which has also been reported by Pawar and Papdiwal[7].

Extracellular synthesis of two mould fungi- *Aspergillus flavus* and *Penicillium purpurescence* has been observed by Olama and Sabry[8]. Storage fungi of Rice seed *viz. Aspergillus flavus, A. glaucas, A. niger, A. versicolor* and *Penicillium* sp. were studied for a-amylase activity. Earlier it was found that all these fungi showed the enzyme activity[9]. Among different fungi *A. flavus* was the most deteriorative of rice quality followed by *A. glaucas* and *A. versicolor.* The differential activity of this fungus may be due to production of different types of secondary metabolites. Sager *et al.*[10], observed amylase production by 27 fungi. Six highly pathogenic fungi (*Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Phytopthora,* sp. and *Rhizoctonia solani*) were isolated from different medicinal plants by Jadhav and Shinde[11]. All these fungi produced amylase, and sucrose was found stimulatory for amylase production.

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