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EFFECT OF ANTIBIOTICS AND VITAMINS ON AMYLASE PRODUCTION BY SEED BORNE FUNGI OF MAIZE

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Abstract:

Production of extracellular hydrolytic enzyme by seed born fungi has a role during the process of seed deterioration and has been considered helpful to their invasion and colonization. Amylolitic fungi are mainly responsible for amylase production and spoilage of food. In present investigation ten amylolitic fungi viz. *Alternaria alternate, Aspergillus flavus, A. nigar, A. terreus,Curvularia lunata,Helminthasporium tetramera, Fusarium oxysporum, Penicillium notatum, Rhizoctonia solani Trichoderma viride,* were selected for amylase production and effect of antibiotics and vitamins were studied.Tetracycline, Amoxicillin, Cefepime and Mebendazole were stimulated amylase production *in Alternaria alternate, Aspergillus nigar,Aspergillus terreus.* Riboflavin stimulates amylase production

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in *Aspergillus flavus A. nigar*, whereas Ascrorbic acid showed inhibitory effect on amylase production.

Key words: Amylolitic fungi, inhibition, stimulation, amylase production, Antibiotics, Vitamins.

Introduction:

Maize (Zea mays L.) is a cereal crop widely cultivated throughout the world and greater weight of maize is produced each and every year than any other grain. The United States produces almost half of the world harvest whereas, other countries which grow maize are as wide spread as China, Brazil, France, Indonesia, Japan, Koriya, Taiwan, México, Egypt, Malaysia, Colombia, South Africa and India. Major consuming Nations of corn are China and USA. There has been continuous increase in the consumption demand of corn mainly owning to increase in the demand from meat and starch sector. There is growing requirement of maize from poultry sector where it is being used as feed. Important Nations as the major exporter of corn are USA followed by Argentina, Brazil, China, South Africa and Ukraine. USA dominates the International trade of corn as an exporter. Nutritionally corn seeds are very rich in its contain. Common Indian verities of corn contain starch 72%, Protein 5.8-13.7%, Fruit Fat 3-18%, Crude fiber 2.9% and Ash 1.3% approximately. During postharvest condition maize seeds gets infected by dominating fungi like*Alternaria alternata, Aspergillus flavus, A. niger, A. terrus, Curvularia lunata, Fusarium oxysporum, Helminthosporium tetramerae, Penicillium notatum, Rhizoctonia soloni* and *Trichoderma viride*, and during their infection ,these fungi secretes hydrolytic enzyme amylase cause a great economic loss.In order to study the effect of different antibiotics and vitamins on amylase production, nine different antibiotics and four commonly used vitamins at 100ppm concentration were tested in ten seed borne fungi.Bharswadkar (2003) reported that ampicillin proved stimulatory for amylase production in *Curvularia lunata, Drechslera tetramera, Fusarium oxysporum, Penicillium purpurogenum* and *Trichoderma viride*, tetramycin was stimulatory for *Aspergillus flavus, Drechslera tetramera* and *Penicillium purpurogenum*. The alpha-amylase activityincreased

significantly during 10 days ofincubation with *A. flavus, A. niger* and *A.versicolor*. Highest alpha-amylase activity wasrecorded in *A. glaucus*inoculated seeds whereaslowest alpha-amylase activity was recorded in*Penicillium* sp. inoculated seeds. Among different fungi,*A. flavus* was the mostdeteriorative

Materials and Methods:

Production of amylase was studied by growing the fungi in liquid medium containingglucose 1%,KNO_3, 0.25% KH_2PO_4 ,0.1% and $MgSO_4.7H_2O$ 0.05%, pH of the medium was adjusted at 5.5, twenty five ml of medium was poured in 100ml conical flasks autoclaved and inoculated separately with 0.1ml spore suspension of fungi which were grown for 7 days on PDA slants. Unless otherwise stated, the flasks were incubated for 6 days at 25= 1 °C with diurnal periodicity of light. On7th day, flasks were harvested by filtering the contents through Whatman filter No. 1 The filtrates were collected in presterilized bottles and termed as crude enzyme preparation.

Assay method for amylase enzymes (cup- plate method):

Determination of amylase activity was done with the help of plate method which was adopted by Singh and Saxena (1982), where 20 ml of starch agar assay medium (soluble starch -10gm, Na_2HPO_4-2.84gm, NaCl-0.35gm, Agaragar 20gm, distilled water 1000mland pH6.9) waspoured in each petriplate. On solidification of the medium, acavity (08mm diameter) was made in the center with the help of a cork borer (No.4) and was filled with filtrates (crude enzyme preparation) of the test fungi. The plates were incubated at 28 °C for 24 hours, and then they were flooded with Lugol's iodine solution as indicator. A clear, notiblue, circular zone obtained surrounding the central cavity; diameter of the zone was measured (mm) as the amylase activity zone .Similar procedure followed for the control except pouring of culture filtrate in central cavity instead of the act

Results and Discussion:

1) **Effect of antibiotics on amylase production:** Nine different antibiotics at 100 ppm concentration were employed against ten seed borne fungi for amylase production. It is clear form

the results given in table no. 01 (graph no. 01 and photo plate), that in the presence of Tetracycline, Amoxicillin, Cefepime and Mebendazole amylase production was stimulated in *Alternaria alternata, Aspergillus niger, Aspergillus terrus Curvularia lunata, Helminthosporium tetramera.* Ampicillin inhibits amylase production in *Aspergillus terrus* and *Penicillium notatum*, Ciporofloxacin and Norfloxacin in *Aspergillus flavus, Aspergillus terrus* and *Fusarium oxysporum*; Levofloxacin in *Alternaria alternata, Aspergillus terrus* and *Fusarium oxysporum*. Ceftraxone, Cefepine and Mebendazole in *Fusarium oxysporum* inhibit amylase production, whereas higher stimulatory effect of Mebendazole in *Aspergillus niger* and *A. terrus*., Rao and Sharma (1978) tested tetramycin against rhizosphere mycoflora of cauliflower for amylase production and interestingly reported that it was found stimulatory. Similarly, Khairnar (1987) (2014) tested a group of antibiotics seeds mycoflora of bajra and found stimulatory nature of streptomycin and streptopenicillin for amylase production while, only hostacylin could showinhibitory action. Bhosale (1989) reported stimulatory effect of penicillium, streptomycin and aurenfungin in sorghum.

Table No. 01: Effect of Antibiotics on the production of amylase in seed borne fungi

Antibiotic sources 100 ppm conc.	Alal	Asfl	Asni	Aste	Cula	Fuox	Hete	Peno	Rhso	Trvi
Tetracycline	12	-	18	18	18	12	15	10	14	14
Ciprofloxacin	10	-	15	-	18	-	16	10	13	14
Amoxicillin	12	12	18	-	15	16	16	16	14	14
Norfloxacin	13	-	15	-	16	12	17	16	15	16
Levofloxacin	-	11	16	-	12	-	14	15	12	13
Ceftraxone.	12	10	18	10	14	-	14	15	13	12
Cefepine	13	10	20	12	15	-	17	14	14	15
Mebendazole	14	15	22	25	16	-	17	-	15	16
Ampicillin (Control)	10	10	16	-	15	12	16	-	14	15

Altal - *Alternariaalternata*
Asfl - *Aspergillusflavus*
Asni - *Aspergillusniger*
Aste - *Aspergillusterreus*
Cula - *Curvularialunata*
Fuox - *Fusariumoxysporum*
Hete - *Helminthosporiumtetramere*
Peno - *Penicilliumnotatum*
Rhso - *Rhizoctoniasolani*
Trvi - *Trichoderma viride*

2) Effect of vitamins on amylase production:

Vitamins are very important in many vital activities of microorganisms. Therefore to understand the effect of vitamins on amylase production four commonly used vitamins at 100 ppm. Concentrations were tested in ten seed borne fungi.

It is clear from the results given in table no. 02 that vitamin A for *Aspergillus niger*, Thiamine for *Aspergillus flavus, Aspergillus niger,* and *Trichoderma viride. Riboflavin* for *Aspergillus flavus, A. niger, Fusarium oxysporum* and *Penicillium notatum*, proved stimulatory for amylase production. It was very interesting to note that in all fungi Ascorbic acid proved inhibitory for amylase production as compared to control.

Table No. 2: Effect of vitamins on amylase production in seed borne fungi.

Sr. No	Name of the Fungi	Alal	Asfl	Asni	Aste	Cula	Fuox	Hete	Peno	Rhso	Trvi
		Enzyme activity zone in mm									
1	*Ascorbicacid*	19	21	23	23	17	19	20	21	18	18
2	*Vitamin A*	18	22	26	24	17	24	21	22	18	19
3	*Thiamin*	21	25	28	20	19	24	20	23	21	20
4	*Riboflavin*	16	25	26	24	17	25	20	24	20	18
5	*Control*	20	24	25	24	22	24	22	23	20	19

Fig.1: EFFECT OF VITAMINS ON PROTEASE PRODUCTION

Similar types of results reported (Jayaraman and Prasad 1971), Riboflavin for *Aspergillusterreus*Rathod (2007) noted riboflavin stimulatory for amylase production in *A. tenuissima*and *A. diantaicola.*

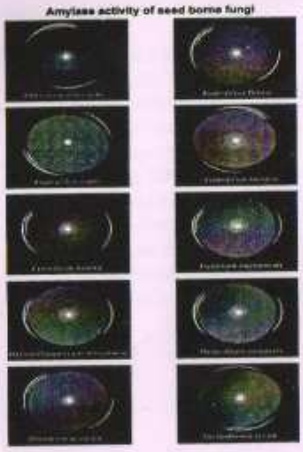
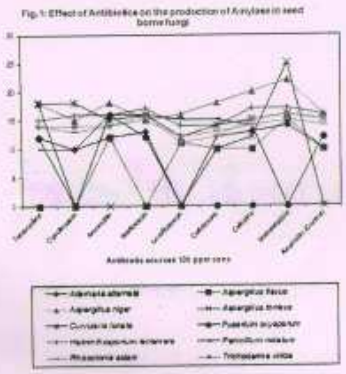


Photo plate



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