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# Impact of vitamins, amino acids and antibiotics on the activity of cellulase produced by post-harvest fungi of *Manilkara achras* m.(sapota)

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**ABSTRACT**

In present research investigation effect of antibiotics and vitamins on the activity of enzyme cellulase of post-harvest fungi of sapota fruits were studied. It was found that vitamins, amino acids and antibiotics significantly inhibit cellulase action of post-harvest sapota fungi. Vitamins, Folic acid and Riboflavin has been retarded cellulase action. It was observed that Arginine monochloride and Threonine inhibited enzyme action in *Rhizoctonia solani* (7mm, 8mm) and *Geotrichum candidum* (8mm, 10mm). It is interesting to note that Ampicillin inhibited cellulase activity in all the tested fungi.



## Introduction

Sapota (Manilkara achras M.) belongs to the family sapotaceae and is an economically important edible fruit crop cultivated in tropical and subtropical regions of the world. It is native of Southern Mexico and Central America (Popenoe, 1974). During storage conditions sapota gets infected by several fungal diseases like Sour rot (Geotrichum candidum), Cladosporium rot (Cladosporium oxysporum), Blue mould rot (Penicillium itallicum) (Mickelbart, 1996). Rhizopus rot, Aspergillus niger rot (Bakar and Karim, 1990) etc. In the present investigation impact of vitamins, amino acids and antibiotics on the activity of cellulase of storage fungi viz. Aspergillus niger, Geotrichum candidum and Rhizoctonia solani were studied. Cellulase production in post-harvest fungi of sapota is directly related with reduction in quality of fruits.

## Materials And Methods

In present investigation three post-harvest sapota fungi Aspergillus niger, Geotrichum candidum and Rhizoctonia solani were grown on liquid medium (pH 5.6) containing CMC (Carboxy Methyl Cellulose)-10gm, Potasium Nitrate-0.25 %, $KH_2PO_4$ -0.1%, $MgSO_4.7H_20$-0.05%, Distilled water -1000ml: 100ppm each vitamins, amino acids and antibiotics separately in 1000ml distilled water.

Twenty five ml of the medium was poured in 100 conical flasks. These conical flasks were autoclaved at 15 lbs for 1 hour and allowed to cool. After this the flasks were inoculated with 1.0 ml spore suspension obtained from 7 days old culture of isolated fungi on PDA slants. Three replications were made for each species. The inoculated flasks were incubated at 27 ± $2^0$ C for 7 days in BOD incubator. Fungi inoculated in CMC medium without vitamins, amino acids and antibiotics were treated as control. After the incubation period the flasks were harvested by filtering the content through Whatman filter paper No. 1. The filtrate obtained were collected in presterilized bottles and considered as crude enzyme preparation. Cellulase enzyme activity was assayed by cup-plate method followed by Dingle et al. (1953) and Szecsi (1969) was used. The assay medium contains 1% CMC and 2% difco agar, was poured in Petri plate (20ml/plate) and allowed to solidify in the centre; a 6 mm diameter cup/cavity was made with pre-sterilized cork borer (No.4). The cup was filled with 0.1m culture filtrate and incubated at room temperature for 48 hours. The activity zone was developed flooding the plates with 3% lead acetate solution (10-15ml/plate). Milky white coloured activity zones were clearly seen on removing lead acetate solution with distilled water after a period of 30 minutes. The diameter of zone was measured in mm. Cultures of Aspergillus niger, Geotrichum candidum and Rhizoctonia solani were used in this study, isolated from post-harvest infected ripe sapota fruits of Kalipatti, Kutchh and Cricket ball cultivars during storage condition. The data was statistically analyzed for C.D. following Panse and Sukhatme(1978).

## Results and Discussion

It was observed that vitamins like Folic acid and Riboflavin has been retarded cellulase action while Ascorbic acid, Thiamine and Pyridoxine induced cellulase action. Ascorbic acid stimulated cellulase production in Geotrichum candidum (13mm) and Aspergillus niger (11mm) whereas it inhibited cellulase production in Rhizoctonia solani (8mm). Thiamin was proved to be stimulatory for Aspergillus niger (12mm) and Rhizoctonia solani (11mm). Pyridoxine inhibited cellulase production in Rhizoctonia solani (9mm) whereas it proved stimulatory in Geotrichum candidum (14mm) and Aspergillus niger (12mm). Table 1 and fig.1.

It was observed that amino acids like Arginine monochloride and Threonine inhibited enzyme action in Rhizoctonia solani (7mm, 8mm) and Geotrichum candidum (8mm, 10mm). Aspartic acid induced production of cellulase in Aspergillus niger (10mm) as compared to control (9mm). Table 2 and fig.2.

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It was observed that in the presence of antibiotic, Mox cellulase production was stimulated in Geotrichum candidum (20mm) while in Rhizoctonia soloni (18mm) stimulation of cellulase production was due to Terramycin. Streptomycin showed stimulation of cellulase production in Aspergillus niger (20mm). In Aspergillus niger (18mm) and Rhizoctonia soloni (17mm), Doxycyclin proved stimulatory for cellulase production. It is interesting to note that Ampicillin inhibited cellulase activity in all the tested fungi. Table 3 and fig.3.

All the three tested fungi showed presence of hydrolytic enzymes, cellulase which provide the fungi chemical means of entrance into the host and a process whereby nutrients can be digested.

Rathod (2011) found vitamins like Folic acid and Riboflavin has been retarded cellulase action while Ascorbic acid, Thiamine and Pyridoxine induced cellulase action in post harvest sapota fungi. Gadgile and Chavan(2010) reported that Ampicillin, Terramycin and significantly inhibited cellulase action of all the post-harvest fungi. This knowledge may prove useful to control enzyme activity and growth of post harvest sapota fungi by limiting the enzyme activity. And such nature of inhibition in different sources may be recommended for the production of different systematic fungicides which will be helpful to control post harvest sapota fungi

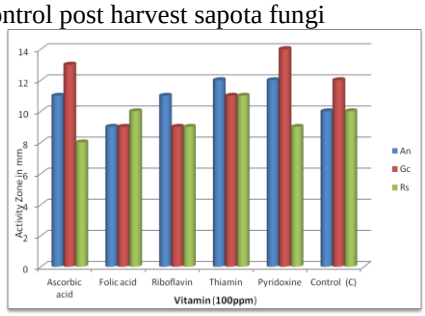



**Fig. 12: Effect of vitamin sources on cellulase production. An= Aspergillus niger, Gc= Geotrichum candidum, Rs= Rhizoctonia solani**

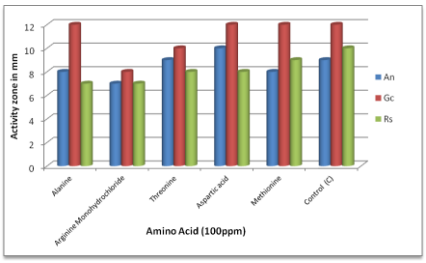



**Fig. 12: Effect of Amino acid sources on cellulase production.**

An= Aspergillus niger, Gc= Geotrichum candidum, Rs= Rhizoctonia solani

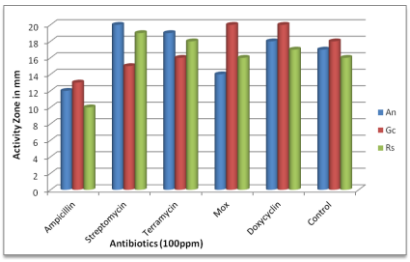
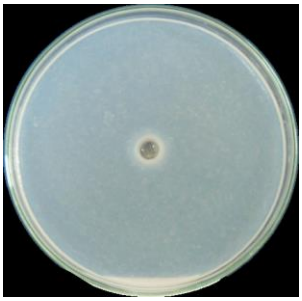



**Fig. 12: Effect of Antibiotics sources on cellulase production.**

An= Aspergillus niger, Gc= Geotrichum candidum, Rs= Rhizoctonia solani

A



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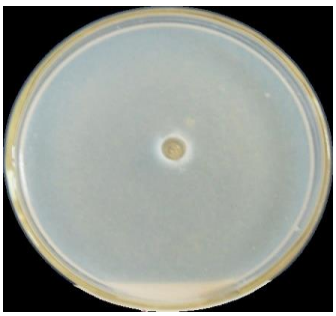

C

Effect Vitamin on cellulase production (cup-plate method); plates showing the zone (mm) of cellulase activity of A.niger (A), G.candidum (B) and R.solani (C).

**Table 1: Effect of vitamin sources on cellulase production**

| Vitamins sources (100 ppm conc.) | Fungi(mm) | | |
|---|---|---|---|
| | An | Gc | Rs |
| Ascorbic acid | 11 | 13 | 08 |
| Folic acid | 09 | 09 | 10 |
| Riboflavin | 11 | 09 | 09 |
| Thiamin | 12 | 11 | 11 |
| Pyridoxine | 12 | 14 | 09 |
| Control (C) | 10 | 12 | 10 |
| SEm± | 0.47 | 0.78 | 0.42 |
| CD(P=0.05) | 1.207 | 2.00 | 1.07 |

SEm± - standard error of mean

An= Aspergillus niger, Gc= Geotrichum candidum, Rs= Rhizoctonia solani

**Table 2: Effect of amino acids on cellulase production**

| Amino acids sources (100 ppm conc.) | Fungi(mm) | | |
|---|---|---|---|
| | An | Gc | Rs |
| Alanine | 08 | 12 | 07 |
| Arginine Monohydrochloride | 07 | 08 | 07 |
| Threonine | 09 | 10 | 08 |
| Aspartic acid | 10 | 12 | 08 |
| Methionine | 08 | 12 | 09 |
| Control (C) | 09 | 12 | 10 |
| SEm± | 0.45 | 0.8 | 0.50 |
| CD (P=0.05) | 1.156 | 2.056 | 1.28 |

SEm± - standard error of mean

An= Aspergillus niger, Gc= Geotrichum candidum, Rs= Rhizoctonia solani

**Table 3: Effect of antibiotics on cellulase production**

| Antibiotics (100 ppm conc.) | Fungi(mm) | | |
|---|---|---|---|
| | An | Gc | Rs |
| Ampicillin | 12 | 13 | 10 |
| Streptomycin | 20 | 15 | 19 |
| Terramycin | 19 | 16 | 18 |
| Mox | 14 | 20 | 16 |
| Doxycyclin | 18 | 20 | 17 |
| Control | 17 | 18 | 16 |
| SEm± | 1.22 | 1.15 | 1.29 |
| CD(P=0.05) | 3.13 | 2.95 | 3.31 |

SEm± - standard error of mean

An= Aspergillus niger, Gc= Geotrichum candidum, Rs= Rhizoctonia solani

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