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EFFECTS OF NUTRITIONAL SOURCES ON CELLULOSE ENZYMES BY FUNGI OF ONION

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ABSTRACT

The present study deals with impact nutritional source on cellulase activity ofsome fungi. Fungi were isolated from different varieties of onions by agar plate method. 6 species of fungi were selected tostudy their cellulase enzyme activity. Different chemical and physical parameter such as carbohydrate, Nitrogen, sulphur, vitamins, physical parameter temperatureand P^Hwere studied in order to determine the optimum conditions forcellulase production of six fungi. The cellulase present in the broth was assayed bycup-plate method. It is observed that cellulase activity of fungi was found due carbohydrates and nitrogen sources.

Key words: *Cellulase activity, Chemical factors, onions fungi.*

INTRODUCTION:

Onion (*Allium cepa* L.) belongs to family Alliaceae is one of the important commercial vegetable crops grown throughout the world including India.It is used as row vegetable and in spices, also called as "Queen of kitchen. Onion bulb contains antiinflamentory, anticholestrol, anticancer and antioxidant compound quercetin (Augusti, 1996). Whatever the colour of the bulb, the taste does not depend on the color. Onions are pungent in nature. It contributes significantly to the human diet having a good source of minerals, vitamins, polyphenols and number of phytonutrients. These phytonutrients such as flavonoidsand Phenolicpresent in onion have been found to act as antioxidants to lower blood pressure and prevent some kinds of cancer (Yang *et al.*, 2004; SlimeStad*et al.*, 2007). It also possesses a high content of flavonoid and sulphur compounds, both of which have a high level of antioxidant activity (Griffiths *et al.*, 2002). Fungal and bacterial infection may take place during the growing season, during harvest time, handling and storage, during transport and marketing or even after purchase by the consumer (Dennis, 1983). Previous studies

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of Mba and Akueshi (2001) indicated that pathogenic infections affect the overall level of nutritional component in a plant. The research of Nwaukwu *et al*., (2012) emphasized the role pathogenic fungi affect the nutritional composition of the edible fruit. Considering this fact present work has been carried out to study the effectof production of cellulaseenzyme activity parameters of onion.

MATERIAL METHODS:

Production of cellulase was made by growing the fungi on liquid medium containing CMC – 10gm, KNO_3 – 0.25%, KH_2PO_4 – 0.1% and $MgSO_4.7H_2O$ – 0.05%, pH – 5.0. To study the impact of physical factors on cellulase enzyme production, treatments of different physical factors such as temperature and pH were given to above basal medium. In order to study the effect of different nutritional sources; carbon sources, nitrogen sources, sulphur sources and vitamins were added to above basal medium. Out of which 25ml of medium was poured in 100ml Erlenmeyer conical flasks and autoclaved at 15 lbs pressure for 15 minutes. The flasks on cooling were inoculated separately with 1 ml spore suspension of test fungi prepared from the 7 days old cultures grown on PDA slants. The flasks were inoculated for 6 days at 25± 10^C with diurnal periodicity of light. On the 7^{th} day the flasks were harvested by filter the contents through Whitman's filter paper No.1. The filtrate were collected in the pre-sterilized bottles and termed as crude enzyme.

Assay for Cellulase (Cup-Plate Method):

The cup-plate method followed by Dingle *et al.*, (1953) was used. The assay medium contains 1% CMC and 2% Difco agar, was poured in petri dish (20ml/plate) and allowed to solidify. In the center, a 6mm 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 24 hours. The activity zone was developed flooding plates with 3% lead acetate solution (10-15ml/plate). White coloured activity zones were clearly seen on removing lead acetate solution with distilled water after a period of 30minutes. The diameter of zone was measured in mm.

EXPERIMENTAL RESULTS

Effect of Carbohydrate sources on cellulase production:

Effect of different carbohydrate sources on cellulase production of selected fungi was studied by selecting different monosaccharides, disaccharides and polysaccharides and results are given (Graph.01)

Cellulase enzyme activity of all the fungi was reduced due to all the carbohydrate sources. As compared to other source, glucose and maltose hampered the cellulase activity of *Alternaria porri.* Cellulase activity of *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium digitatum* was minimum due to glucose. Similarly, sucrose was found to be responsible for decrease in cellulase activity of *Colletotrichum circinans* and *Penicillium digitatum.* Maltose caused lowest cellulase activity of *Alternaria porri*and *Penicillium digitatum.* In the case of *Botrytis squamosa* cellulase activity was reduced due to fructose and starch. Drastic hamper in cellulase activity was observed due to starch in case of *Colletotrichum circinans*.

Effect of Nitrogen sources on cellulase production:

Cellulase production of selected fungi was studied under the influence of different nitrogen sources and results are noted in (Graph.02)

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Nitrogen source such as potassium nitrate proved to be inhibitor for cellulase enzyme production in case of *Aspergillus niger*, *Colletotrichum circinans* and *Fusarium oxysporum*whereas, sodium nitrate hampered the cellulase action of *Alternaria porri* and *Penicillium digitatum.* Lowest cellulase production of *Penicillium digitatum* was also observed due to urea. Minimum cellulase enzyme production was observed in case of *Alternaria porri* and *Botrytis squamosa* due to peptone and gelatin.

Effect of sulphur sources on cellulase production:

Cellulase production of selected fungi was studied under the influence of different sulphur sources and results are noted in(Graph.03)

Lowest cellulase activity was observed in case of *Alternaria porri* due to Sodium thiosulphate, Ammonium sulphate and Sodium sulphate. Ferrous sulphate was responsible for lowest cellulase action of *Colletotrichum circinans, Fusarium oxysporum* and *Penicillium digitatum.* Cellulase activity of *Aspergillus niger* and *Colletotrichum circinans* was hampered due to sodium sulphate.

Effect of vitamins on cellulase production:

Vitamins sources were screened for the cellulase production of selected fungi and results are given in (Graph.04)

Vitamin such as Riboflavin and Pyridoxine lowered the cellulase production of *Alternaria porri*. Riboflavin was also observed to be responsible for lowest cellulase enzyme action of *Botrytis squamosa, Colletotrichum circinans* and *Fusarium oxysporumm.* It is interesting to state that, except riboflavin all the vitamins were found to be inducer for cellulase enzyme production for *Penicillium digitatum*.

Effect of temperature on cellulase production (Graph-05)

Effect of temperature on cellulase production by selected fungi was studied and results are noted in graph(-5.)

Cellulase enzyme production of all the fungi except *Aspergillus niger* was totally absent at 10^{o}C temperature. Similarly, in case of *Alternaria porri, Botrytis squamosa* and *Fusarium oxysporum* cellulase activity was also absent at 20^{o}C temperature. At 25^{o}C temperature cellulase production of *Botrytis squamosa* and *Fusarium oxysporum* was hampered. Only *Alternaria porri* was showed lowest cellulase action at 25^{o}Ctemperature.

Effect of pH on cellulase production(graph -06)

Effect of pH on cellulase production by selected fungi was studied and results are note in graph.(06).

pH 3.0 and 3.5 were responsible for complete reduction of cellulase action of all the selected fungi. 4.5 pH was observed to be responsible for minimum cellulase action of *Botrytis squamosa, Colletotrichum circinans* and *Fusarium oxysporum.*5.5 pH lowered the cellulase action of *Alternaria porri* and *Fusarium oxysporum*

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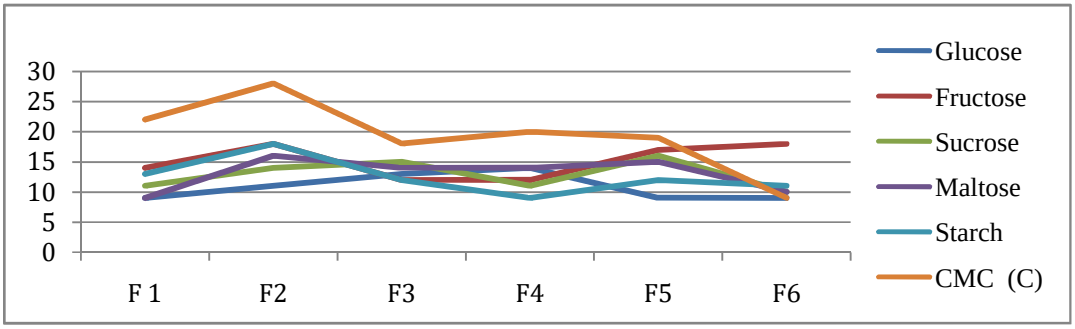
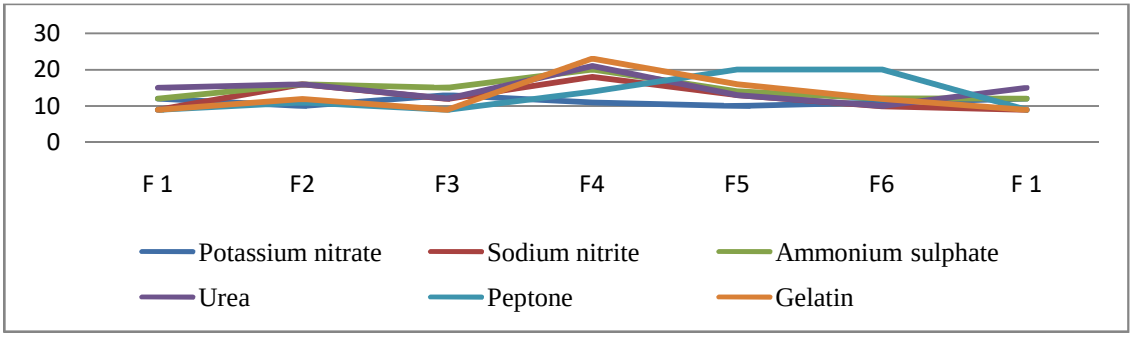
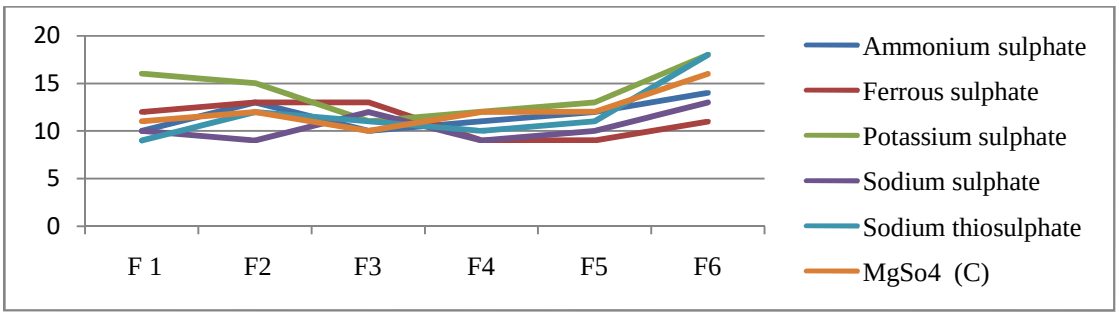
Graph 01,shoes Effect of Carbohydrate sources on cellulase production.



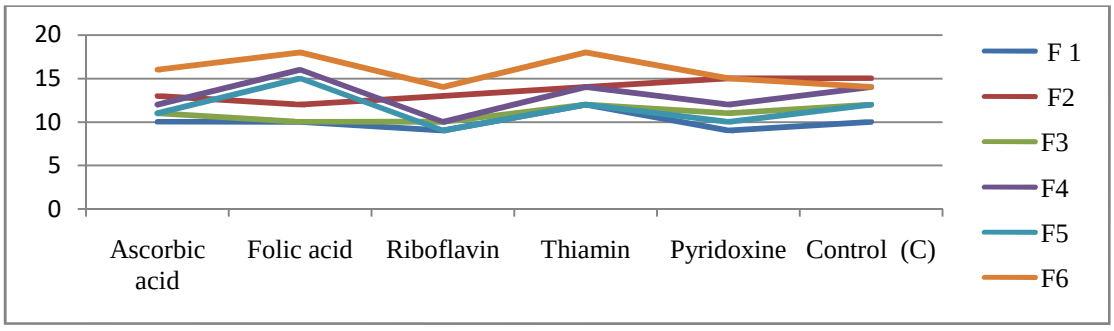
Graph 02,shoes Effect of Nitrogen sources on cellulase production.



Graph 03, Effect of sulphur sources on cellulase production.



Graph 04, shoes Effect of vitamin's on cellulase production.



Zone ofenzyme activity expressed in mm

Graph 05, Effect of temperature on cellulase production:

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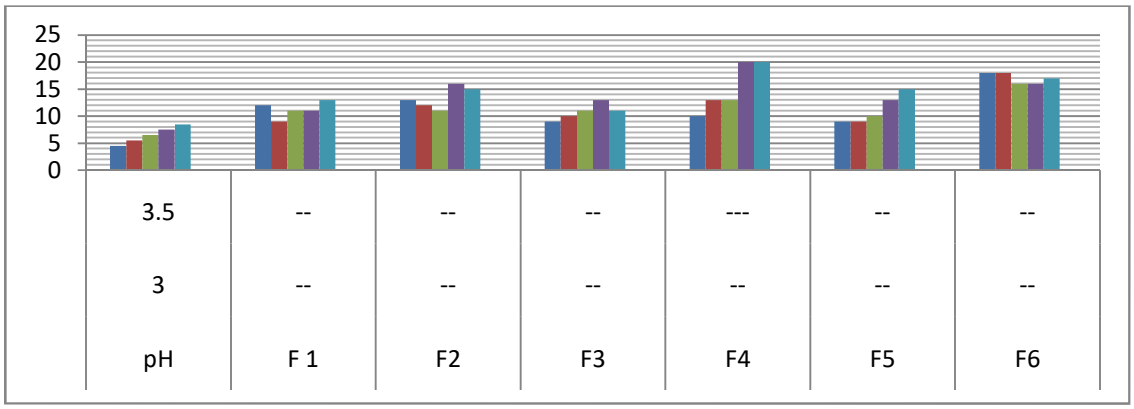
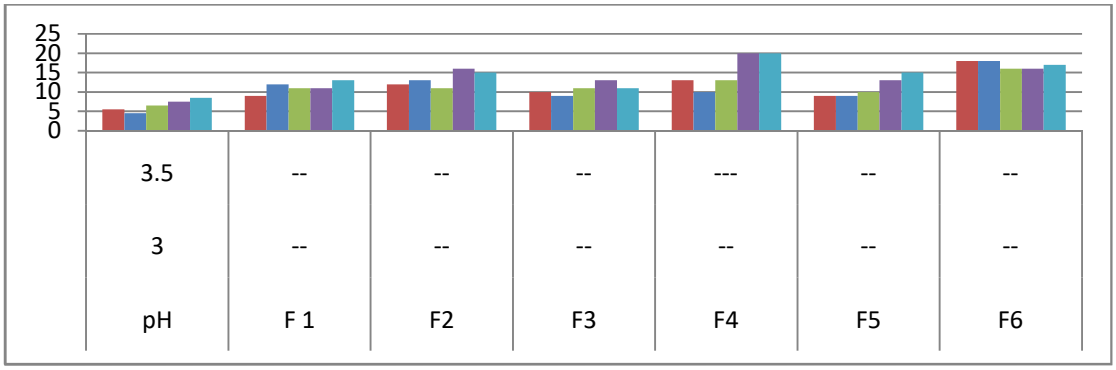


sGraph 06, Effect of P^H on cellulase production:



F1- Alternaria porri *F2- Aspergillus niger*

F3- Botrytis squamosa *F4- Colletotrichum circinans*

F5- Fusarium oxysporum F6- Penicillium digitatum

DISCUSSION:

Cellulose can be degraded by cellulase enzyme produced by cellulolytic bacteria and fungi. Studies were performed to understand the effect of some stimulatory as well as inhibitory compounds on cellulase production. Carbohydrate source such as, glucose hampered the cellulase activity of *Alternaria porri, Aspergillus niger, Fusarium oxysporum* and *Penicillium digitatum.* Similarly, sucrose was found to be responsible for decrease in cellulase activity of *Colletotrichum circinans* and *Penicillium digitatum.* Maltose caused lowest cellulase activity of *Alternaria porri*and *Penicillium digitatum.* In the case of *Botrytis squamosa* and *Colletotrichum circinans* cellulase activity was reduced due to fructose and starch.

From results it is revealed that nitrogen source like potassium nitrate, sodium nitrate, urea, peptone and gelatin were found to be responsible for reduction in cellulase production of selected fungi. Some sulphur sources and vitamins were also proved to be inhibitory as well as stimulatory for the cellulase production of selected fungi.

Gadgile and Chavan (2009) found that carboxy methyl cellulose, starch, ammonium phosphate, diammonium hydrogen phosphate, zinc sulphate, copper sulphate and ferrous sulphate inhibited the cellulase produced by the fungi isolated from mango.But Gadgile (2011a) reported somewhat different conclusions in case of

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mono and disaccharides. He found that among carbon sources fructose and sucrose significantly induced cellulase activity, while lactose, CMC and starch inhibited the cellulase activity of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. Mehta *et al.*, (1993) found that no antibiotic completely inhibited the cellulase activity in *F. oxysporum* and *F. moniliforme* however, amoxicillin was better than other antibiotics in inhibiting the cellulase activity in both the organisms. However, amoxicillin was better than other antibiotics in inhibiting the cellulase activity in both the organisms.

Physical parameters such as temperature and pH were also tested to study their effects on cellulase production by onion fungi. It was found that, Cellulase enzyme production of all the fungi except *Aspergillus niger* was totally absent at 10^{o}C temperature. Similarly, in case of *Alternaria porri, Botrytis squamosa* and *Fusarium oxysporum* cellulase activity also absent at 20^{o}C temperature. At 25^{o}C temperature cellulase production of *Botrytis squamosa* and *Fusarium oxysporum* was hampered. at pH 3.0 and 3.5 complete reduction of cellulase action of all the selected fungi was observed. 4.5 pH was observed to be responsible for minimum cellulase action of *Botrytis squamosa, Colletotrichum circinans* and *Fusarium oxysporum.* Several workers studied the cellulase production under the influence of different physical factors. Rathod (2010) revealed that alternate light and dark stimulated cellulase action in all tested fungi. Maximum cellulase activity of all post-harvest fungi was found in between 15-20th days of incubation period. Temperature range between 20-35^{o}C is more suitable for cellulase and pectinase production. At pH 5.0 to 7.0 pectinase and cellulase action of all tested fungi was optimum. Bhosale (1989), Sonwane (2002), Jadhav (2006), Rathod (2007) find more or less similar findings about the effect of temperature, pH and light on other hydrolytic enzyme of fungi.

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