EFFECT OF CARBOHYDRATE AND NITROGEN SOURCES ON CELLULASE ACTIVITY OF FUNGI FROM VEGETABLE WASTE

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Abstract: At market places and agricultural area the people negligees towards the degraded vegetable waste which left behind. Particularly these wastes vegetable impact on environment pollution. Fungi superior for degradation and utilization of vegetables waste and convert it into the biocompost. Many microorganisms are capable of degrading the utilizing cellulose and hemicelluloses as carbon and energy sources. In the present study Carbohydrate and nitrogen used as the source and study were made the effect of these sources on cellulase production of ten dominating fungi. The Cellulase production activity was assayed by cup-plate method. In the present investigation, isolated fungi were determined by the production of cellulase. In case of carbohydrate sources CMC (Carboxymethyl cellulose) and glucose showed maximum stimulatory effect on production of cellulase and minimum in fructose, sucrose and starch, in ten dominating fungi. In nitrogen sources potassium nitrate, peptone and ammonium sulphate showed highest cellulase production and lowest was found in sodium nitrite, sodium nitrate, ammonium phosphate and in casein. Production of cellulase activity of fungi were useful to degraded vegetable waste and shortly it convert into valuable product that is compost.

Key word: **Vegetable waste, Cellulase, Fungi, Carbohydrate sources, Nitrogen sources and Cup plate method.**

INTRODUCTION

Fruits and vegetable wastes have serious challenges to their existence and these may affected and get spoilage by pests, in adequate rainfall and fungal attack (Amusa, et al. 2002). In India, 20-30 % of the produce is spoiled in the markets (FAO, 2002 and Deka *et al*. 2006). A huge amount of these materials are left on farmlands to be decomposed by microorganism such as bacteria and fungi. The ability of some microorganisms to metabolize lignin and hemicelluloses (Silva, *et al.* 2005). Mostly saprophytic soil fungi act on the vegetable wastes. These wastes have major components as cellulose, starch, lignin, xylan, and pectin, can be used by several microorganisms as a source of energy for growth and as carbon source. The microorganism producing enzymes and other products with high commercial value (de Freitas, *et al.*, 2006 and Costa, *et al.* 2007). Annually, 830 Gt of renewable plant biomass is formed consisting mainly of cellulose and hemicelluloses (Rauscher, *et al*. 2006).

Trichoderma and *Aspergillus* are known to be cellulase producers and crude enzymes produced by these microorganisms are commercially available for agricultural use it was reported by (Peij et al. 1998). The capacity of thermophilic microorganisms to assimilate organic matter depends on their ability to produce the enzymes needs for degradation of the substrate (Tuomela, *et al.* 2000).

The degradation of cell wall, exocellular enzymes like cellulolytic, hemicellulolytic and proteolytic enzymes are produced which are capable of attacking each of the major polymeric components, Wheeler (1975). Apart from cell wall degrading enzymes secreted by a wide variety of saprophytic and phytopathogenic micro organisms showed by (Bailey and Pessa 1990) and Rombouts and Pilnik 1980). Production of these hydrolytic enzymes is influenced by various sources of nutrients. The carbohydrates are one of the prominent sources of nutrients which are responsible for production of hydrolytic enzymes, reported by (Waghmare, *et.al*, 2010 and kulkarni, *et. al* 2012)

In present studies attempts were made to study the effect of carbohydrate and nitrogen sources on production of cellulolytic enzymes by fungi which were isolated from vegetable wastes.

MATERIALS AND METHODS

Vegetable wastes were collected from agriculture field and market places from Jalna District of Maharashtra State. The fungi were isolated by Potato Dextrose Agar (PDA) medium. Identification of fungi was made with help of standard literature, (Mukadam 1997).

Ten dominating fungi were selected and maintained for further studies that is the cellulase activity.

Production of cellulase

Isolated fungi were grown on liquid medium containing 1% carboxymethyl cellulose (CMC), KNO_3 - 0.1 %, KH_2PO_4 - 0.1 % and $MgSO_4.7H_2O$ - 0.5 % & pH 6.0. Twenty five ml of the medium was poured in 100 conical flasks. These conical flasks were autoclaved at 15 lbs for 20 min and allowed to cool. After this the flasks were inoculated with 1 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 at room temperature. After the incubation period the flasks were harvested by filtering the content through Whatman filter paper No. 1. The obtained filtrate were collected in pre-sterilized bottles and considered as crude enzyme preparation.

Cellulase assay by Cup-plate method:

The cup plate method followed by (Dingle et al., 1953 and Szecsi 1969) was used. In this method, 20 ml of CMC agar assay medium (soluble CMC -1% and agar -2%) were incorporated in each pre sterilized petriplates. After solidifying the medium a cavity or hole was made in the centre with the help of a cork borer (8mm). The central cavity was filled with 1 ml crude enzyme (culture filtrate). The petriplates were incubated at 27^0 C for 24 hours. Then the plates were flooded with 3% lead acetate solution as an indicator. Keep the plates for 20 - 40 minutes, milky white colored activity zone are clearly seen after removing lead acetate solution with distilled water. The diameter of activity zone was measured which was resulted due to cellulase activity.

RESULT AND DISCUSSION

Vegetable waste were collected from agriculture and market places of Jalna District of Maharashtra State, and studied for the association of different fungi. Dominant fungi were observed viz., *Aspergillus niger*, *A. flavus, A. fumigatus Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum*, *Penicillium notatum*, *P. spp. Rhizopus stolanifer* and *Trichoderma harzianum.* These dominating fungi produce different types of hydrolyzing cellulolytic enzymes. Productions of enzymes were influenced by different sources and environmental factors. Here studied the effect of carbohydrate and nitrogen sources on the production of cellulase activity of these ten dominating fungi.

B) Cellulase production

Carbohydrate source

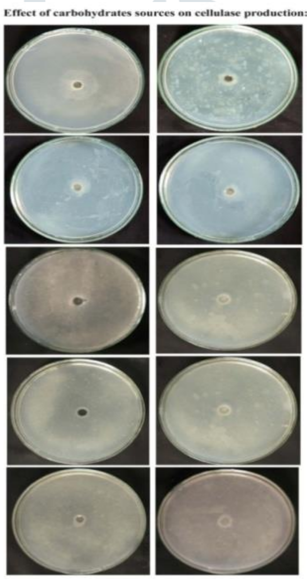
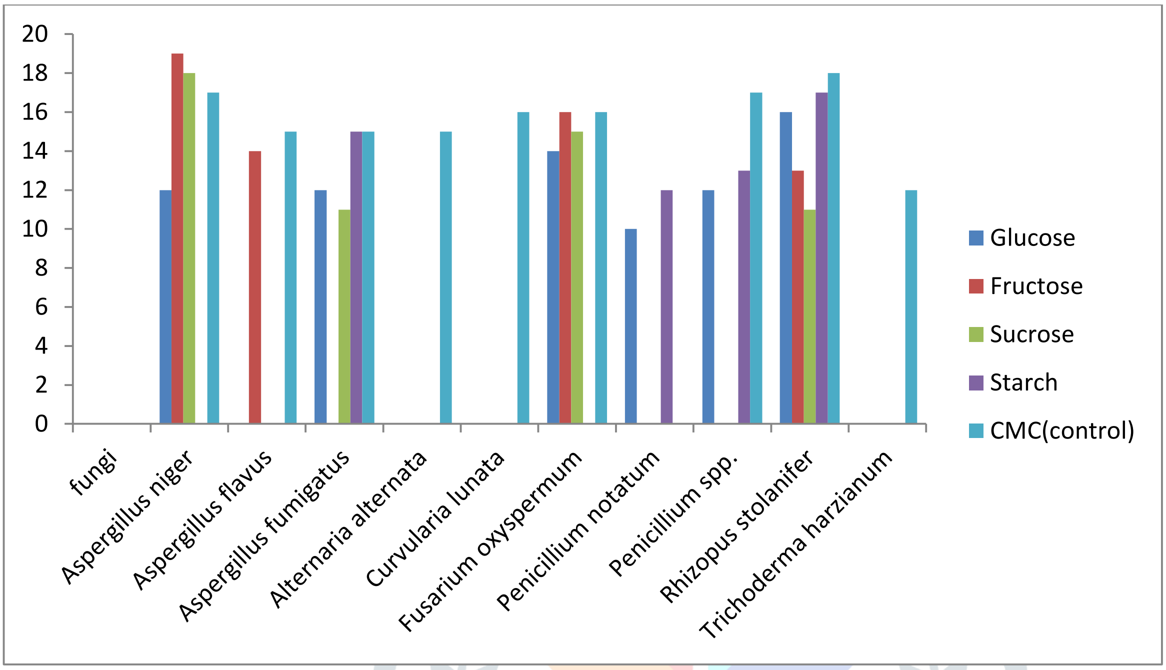


Table no. 1: Effect of carbohydrates sources on cellulase production

Carbohydrate effect(1%)	Glucose	Fructose	Sucrose	Starch	CMC(control)
Fungi	Zone diameter in mm				
Aspergillus niger	12	**19**	**18**	-	17
Aspergillus flavus	-	14	-	-	15
Aspergillus fumigatus	12	-	11	15	15
Alternaria alternata	-	-	-	-	15
Curvularia lunata	-	-	-	-	16
Fusarium oxysporum	14	16	15	-	16

Penicillium notatum	10	-	-	12	-
Penicillium spp.	12	-	-	13	17
Rhizopus stolanifer	**16**	13	11	**17**	**18**
Trichoderma harzianum	-	-	-	-	12

Graph 1: Effect of carbohydrates sources on cellulase production



In order to study the effect of carbohydrate and nitrogen sources on cellulase production, carbon sources other than glucose were tested by supplementing them individually in the basal medium from which two sources belongs to monosaccharides, one belongs to disaccharide and two polysaccharides were selected and tested at 0.5% concentration and the results are summarized in table 1. Basal medium containing CMC (carboxomethyl cellulase) served as control (Table no.1)

It was found that effect of monosaccharide like **glucose,** the highest cellulase produced by *Rhizopus stolanifer* (16mm) and lowest by *Penicillium notatum* (10mm). While moderate cellulase activity showed by *Fusarium oxysporum*, *Aspergillus niger*, *A. fumigatus*, *Penicillium spp.*. Cellulase productions were inhibited in *Aspergillus flavus*, *Alternaria alternata*, *Curvularia lunata*, and *Trichoderma harzianum.* Whereas **fructose** proved maximum stimulated production of cellulase in *Aspergillus niger* (19mm) and minimum cellulase production in *Rhizopus stolanifer* (13mm). Fructose also showed stimulatory effect in *Fusarium oxysporum*, *Aspergillus flavus.* Production of cellulase inhibited in case of *Aspergillus fumigatus*, *Alternaria alternata*, *Curvularia lunata,* Penicillium *spp.* and *Trichoderma harzianum.*

In disaccharide **sucrose** showed maximum stimulatory effect on cellulase production in *Aspergillus niger* (18mm) and minimum production detected in *Rhizopus stolanifer(*11mm) while *Aspergillus flavus*, *Alternaria alternata*, *Curvularia lunata*, *Penicillium spp.* and *Trichoderma harzianum,* had reduced of cellulase production.

In polysaccharide, effect of **starch** showed significant highest cellulase activity in *Rhizopus stolanifer* (17mm) and lowest activity were showed in *Curvularia lunata (*12mm*).* Moderate activity was showed in *Aspergillus fumigatus*, *Penicillium spp.*. Whereas in *Aspergillus niger, A. flavus*, *Alternaria alternata*, *Fusarium oxysporum* and *Trichoderma harzianum inhibited* of cellulase production. On CMC (control) *Rhizopus stolanifer (*18mm*)* showed maximum cellulase production followed by *Aspergillus niger*, *Penicillium species*, *Fusarium oxysporum*, *Aspergillus flavus*, *A. fumigatus*, *Alternaria alternata*, while in *Curvularia lunata* had inhibited to cellulase production, same result was reported by Wagh and Bhale (2014). Similarly shown by Niranjane (2007).

2)Nitrogen sources

Effect of Nitrogen sources on cellulase production:

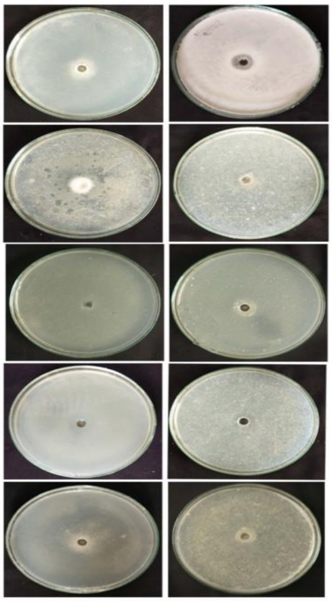
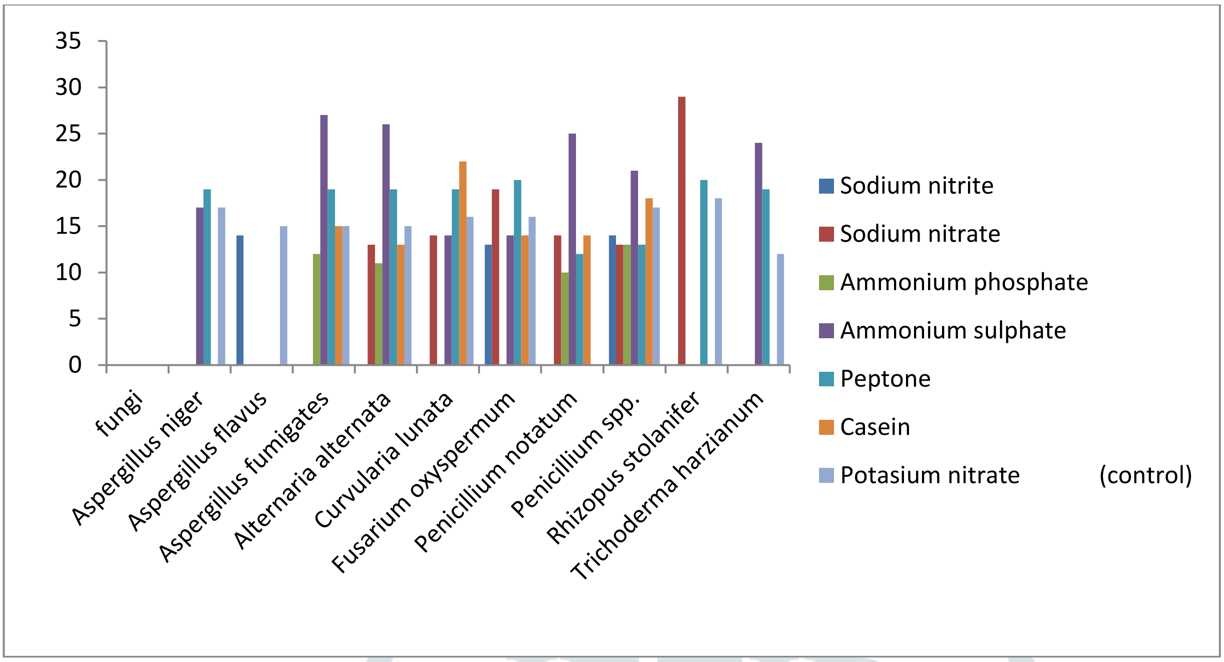


Table no. 2: Effect of nitrogen sources on cellulase production

Nitrogen effect 0.25%	Sodium nitrite	Sodium nitrate	Ammonium phosphate	Ammonium sulphate	Peptone	Casein	Potassium nitrate (control)
Fungi	Zone diameter in mm						
Aspergillus niger	-	-	-	17	19	-	17
Aspergillus flavus	14	-	-	-	-	-	15
Aspergillus fumigatus	-	-	12	**27**	19	15	15
Alternaria alternata	-	13	11	26	19	13	15
Curvularia lunata	-	14	-	14	19	**22**	16
Fusarium oxysporum	13	19	-	14	**20**	14	16
Penicillium notatum	-	14	10	25	12	14	-
Penicillium spp.	14	13	**13**	21	13	18	17
Rhizopus stolanifer	-	**29**	-	-	**20**	-	**18**
Trichoderma harzianum	-	-	-	24	19	-	12

Graph 2: Effect of nitrogen sources on cellulase production



Different nitrogen sources like nitrate, nitrite, ammonium and organic forms at 0.25% concentration were incorporated separately in the basal medium and their effect on cellulase production were recorded. Basal medium containing potassium nitrate served as control (Table no.2 & graph).

In nitrite form **sodium nitrite** inhibited production of cellulase in case of *Aspergillus niger, A. fumigatus, Alternaria alternata, Curvularia lunata, Penicillium notatum, Rhizopus stolanifer* and *Trichoderma harzianum* and stimulated in *Aspergillus flavus, Penicillium spp.* and *Fusarium oxysporum.*

In nitrate form **sodium nitrate** showed maximum production of cellulase in *Rhizopus stolanifer* (29mm) and minimum in *Penicillium spp. (*13mm*)*. Whereas sodium nitrate in *Fusarium oxysporum, Curvularia lunata, Penicillium notatum, Alternaria alternata were* stimulated and inhibited in *Aspergillus niger, A. flavus, A. fumigatus and Trichoderma harzianum.* In **potassium nitrate** most of all dominating fungi showed stimulated production of cellulase except *Penicillium notatum*.

In ammonium form **ammonium phosphate** proved stimulatory effect on cellulose production in *Penicillium species* (13mm), *Aspergillus fumigatus* (12mm) *Alternaria alternata (*11mm*)* and *Penicillium notatum (*10mm*)* and it was inhibited in *Aspergillus niger, A. flavus, Curvularia lunata, Fusarium oxysporum, Rhizopus stolanifer* and *Trichoderma harzianum.* **Ammonium sulphate** showed maximum stimulate of cellulase production in all dominating fungi except *Aspergillus flavus* and *Rhizopus stolanifer*.

In organic form **peptone** produced maximum cellulase in *Fusarium oxysporum* (20mm) and *Rhizopus stolanifer* (20mm) whereas in *Penicillium notatum* (12mm) minimum cellulase production. Peptone also stimulated cellulase in *Aspergillus niger A. fumigatus Alternaria alternata, Curvularia lunata, Trichoderma harzianum* and *Penicillium spp*. and inhibited in *Aspergillus flavus*. **Casein** proved maximum cellulase production in *Curvularia lunata* (22mm) and minimum stimulated in *Alternaria alternata* (13mm) and also stimulated in *Penicillium species, Aspergillus fumigatus, Fusarium oxysporum, Penicillium notatum*. Production of cellulase completely inhibited in *Aspergillus niger, A. flavus, Rhizopus stolanifer* and *Trichoderma harzianum*. All results were compared with potassium nitrate as control. Same result reported by Wagh and Bhale (2014). Similarly showed by Gautam, et al. (2010).

CONCLUSION

From the result, the CMC (control) was found to be better than the other carbohydrate for production of cellulase by all test fungi. The *Rhizopus stolanifer* and *Aspergillus niger* showed maximum cellulase production in all carbohydrates. *Alternaria alternata, Curvularia lunata* and *Trichoderma harzianum* clearly inhibited by carbohydrates except CMC. In nitrogen sources, peptone, potassium nitrate and ammonium sulphate maximum stimulatory for all fungi and minimum in sodium nitrate, sodium nitrite, ammonium phosphate and casein. The highest cellulase production showed in *Aspergillus fumigatus, Alternaria alternata, Penicillium notatum* and *Fusarium oxysporum* and minimum in *Aspergillus niger, Curvularia lunata Penicillium spp., Rhizopus stolanifer* and *Trichoderma viride*. Production of cellulase completely inhibited in *Aspergillus flavus* except *Potassium nitrate*. From the investigation conclude that the carbohydrate and nitrogen sources affect the cellulase activity of tested fungi. It also conclude that there is a relation between degradation of vegetable waste and production of cellulase by dominating fungi.

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