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EFFECT OF PHOSPHOROUS AND SULPHUR SOURCES ON CELLULASE ACTIVITY OF FUNGI FROM VEGETABLE WASTE

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ABSTRACT The market places and agricultural area were polluted due to vegetable wastes and also impact on environment pollution. Fungi play important role in degradation and utilization of vegetables waste and convert it into the biocompost. Many fungi are capable to degrading and utilizing cellulose, hemicelluloses as a carbon and energy sources. There are many nutritional, salts and physical factors impact on the cellulase production. In the present study phosphorous and sulphur 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 made by cup-plate method. In the present investigation, isolated fungi were determined by the production of cellulase. Very interesting results was found in case of phosphorous sources like, potassium dihydrogen phosphate highest cellulase production showed by Rhizopus stolonifer and lowest showed by Alternaria alternata. Sodium dihydrogen phosphate and ammonium phosphate was proved stimulatory for cellulase enzyme production in selected ten dominating fungi. In case of sulphur source Rhizopus stolonifer showed were maximum cellulase production in zinc sulphate followed by Aspergillus niger, Penicillium species. The minimum cellulase enzyme production was recorded by Curvularia lunata in the presence of sodium sulphate. Production of cellulase activity of fungi were useful to degraded vegetable waste and shortly it convert into valuable product that is compost.

KEYWORDS : Vegetable waste, Cellulase, Fungi, Phosphorous sources, Sulphur sources and Cup plate method.

INTRODUCTION:

Fungi are the major sources of cellulase production for industrial applications, few of them have been extensively investigated because they produce on large scale these enzymes extracellulary. In the world, vegetables spoilage are often more severe at storage conditions and transportation facilities. Fungal infection may occur during the transport, storage and marketing conditions, or after purchasing by the consumer and it resulted to vegetables spoilage (Rajmane and Korekar 2012).The spoilage management is a challenging task. So, there is a need for an effective waste disposal method for converting this waste into some valuable form. Physical, chemical, and biological decompositions break the cellulose in spoilage and result in the enhancing of the nutrient content of soil (Kamara *et al*., 2015). Biological decomposition is the main and efficient decomposition method in which bacterial and fungal spores speed up the decomposition of wastes. Microbial decomposition enhances nutrient content, nitrogen fixing, phosphorous solublization, and cellulose decomposed into final product (Nusrat Iqbal 2021). Most commonly known bio-decomposers are fungi which include *Humicola*, *Trichoderma*, and *PenicilliumAspergillus* (Gautamet al., 2009). The market sale value of soil microbes is increasing nowadays (Sandhu 2010).

Vegetables were gets infected and spoiled by several fungi, during their infection, these fungi secretes their biological weapons, that is enzyme, like cellulase and pectinase which causes spoilage of vegetables and fruits (Rathod and Chavan 2013). Cellulase is a complex enzyme, act to convert complex carbohydrates present in lignocellulosic biomass into glucose efficiently cellulolytic enzymes are the main biological weapons by which fungi can break down the cellulosic substances of the host cell wall (Holker *et al.,* 2004).Theproduction of these hydrolytic enzymes is influenced by various sources of nutrients, salts and physical factors(Amer Ahmed and Aasia Bibi, 2018).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 study attempt was made on the effect of phosphorous and sulphur sources on cellulolytic enzymes activity by fungi which were isolated from vegetable wastes.

MATERIALAND METHODS:

Collection of Materials and Isolation of Fungi:

Vegetable wastes were collected from agriculture field and market places of various localities of Jalna District of Maharashtra State. The fungi were isolated by using agar plate method. In this method Potato Dextrose Agar (PDA) were used .Identification of fungi were made with help of standard literature, D. S. Mukadam (1997).

In the present investigation ten dominating fungi like, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Penicillium notatum, Penicillium species, Rhizopus stolanifer and Trichoderma hargianum were studied by using effect of phosphorous and sulphur sources on cellulase production.

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 % and distilled water 1000ml, pH was maintained 6.0. In order to study the effect of phosphorus sources and sulphur sources were added to above basal medium. 25 ml of the medium was poured in 100 ml 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 culture of isolated dominating 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 (culture filtrate).

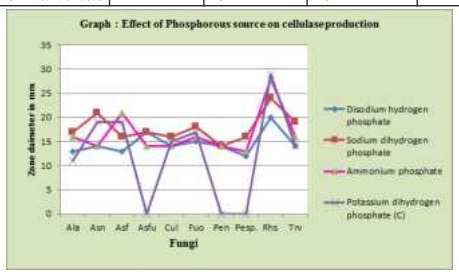
Cellulase assay by Cup-plate method:

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 solidify 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 27^0C for 24 hours. Then the plates were flooded with 3% lead acetate solution as an indicator. Keep the plate for 20 - 40 minutes, milky white colored activity zone was clearly seen after removing lead acetate solution with distilled water. The diameter of activity zone was measured which was resulted due to cellulase activity.

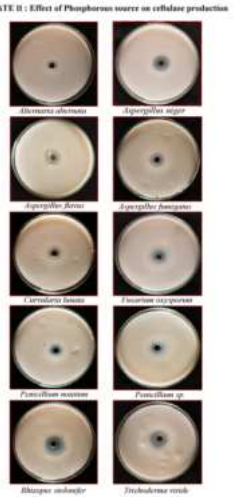
Table: Effect of phosphorous source on cellulase production

Fungi	Phosphorous sources (0.1%)			
	Disodium hydrogen phosphate	Sodium dihydrogen phosphate	Ammonium phosphate	Potassium dihydrogen phosphate
	Zone diameter in mm			
Alternaria alternata	13	17	16	11
Aspergillus niger	14	21	14	19
Aspergillus flavus	13	16	21	19
Aspergillus fumigatus	17	17	14	-

Curvularia lunata	14	16	14	15
Fusarium oxysporum	15	18	16	17
Penicillium notatum	14	14	14	-
Penicillium sp.	12	16	13	-
Rhizopus stolonifer	20	24	28	19
Trichoderma viride	14	19	16	14



Ala- *Alternaria alternata* Asn- *Aspergillus niger* Asf- *Aspergillus flavus* Asfu- *Aspergillus fumigatus* Cul- *Curvularia lunata*, Fuo- *Fusarium oxysporum* Pen- *Penicillium notatum* Pensp- *Penicillium sp*. Rhs- *Rhizopus stolonifer* Trv- *Trichoderma viride*



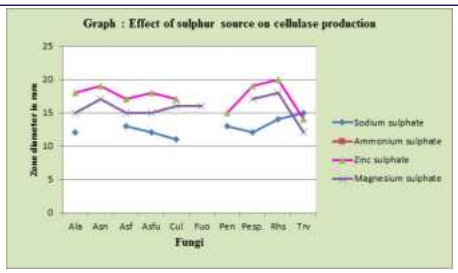
i) Effect of phosphorus sources on cellulase production

Four different sources of phosphorus were studied against the cellulase enzymes production in ten selected fungi and results are given in table, graph and photoplate.

It was observed from the result that the highest cellulase production by *Rhizopus stolonifer* and lowest recorded by *Alternaria alternata* in the presence of Potassium dihydrogen phosphate. The sodium dihydrogen phosphate and ammonium phosphate was proved stimulatory for cellulase enzyme production. Disodium hydrogen phosphate and potassium dihydrogen phosphate reduced enzyme production as compare to Sodium dihydrogen phosphate and ammonium phosphate. The sodium dihydrogen and ammonium phosphate were best phosphorous source for the production of cellulase by testing fungi. Similar study made by (Rathod and Chavan 2010), Gadgile and Chavan (2009) and Rathod (2010).

Table : Effect of sulphur source on cellulase production

Fungi	Sulphur (0.5%)			
	Sodium sulphate	Ammonium sulphate	Zinc sulphate	Magnesium sulphate (**C**)
	Zone diameter in mm			
Alternaria alternata	12	-	18	15
Aspergillus niger	-	-	19	17
Aspergillus flavus	13	-	17	15
Aspergillus fumigatus	12	-	18	15
Curvularia lunata	11	-	17	16
Fusarium oxysporum	-	-	-	16
Penicillium notatum	1	-	15	-
Penicillium sp.	12	-	19	17
Rhizopus stolonifer	14	-	20	18
Trichoderma viride	15	-	14	12



Ala- *Alternaria alternata* Asn- *Aspergillus niger* Asf- *Aspergillus flavus* Asfu- *Aspergillus fumigatus* Cul- *Curvularia lunata*, Fuo- *Fusarium oxysporum* Pen- *Penicillium notatum* Pensp- *Penicillium sp*. Rhs- *Rhizopus stolonifer* Trv- *Trichoderma viride*

Effect of sulphur sources on cellulase production.

Four different sources of sulphur were tested against the cellulase, production in ten dominant fungi and results are given in table and graph.

It was interesting to note that all four sources of sulphur play more or less role in the enzymes production. *Rhizopus stolonifer* showed were maximum cellulase production in zinc sulphate followed by *Aspergillus niger*, *Penicillium* species. The minimum cellulase enzyme production were recorded by *Curvularia lunata* in the presence of sodium sulphate. *Fusarium oxysporum* did not show cellulase enzyme production in all three sulphate source except magnesium sulphate. Whereas ammonium sulphate totally inhibited of cellulase enzyme production in the all selected fungi. Significant cellulase production showed by zinc sulphate followed by magnesium sulphate. Sodium sulphate were showed minimum cellulase production as compare to zinc sulphate and magnesium sulphate. Similar work done by Bhale and Rajkonda (2012), Thiyam and Sharma (2013), Wagh and Bhale (2014),

CONCLUSION

Fungi have the advantage over other microbes because of their ability to secrete large quantities of biomass degrading enzymes when grown on cheap substrates.Cellulase can be used in waste management, cellulase benefits in minimizing the effect of celluose waste on environment and driving the conversion of the pollutants to an alternative source of energy (Gautam etal.2011).

From the result, the potassium dihydrogen phosphate (control) was found to be better than the other phosphorous for production of cellulase by all test fungi. The *Rhizopus stolanifer* showed maximum cellulase production. The sodium dihydrogen and ammonium phosphate were best phosphorous source for the production of cellulase. In sulphur sources, like zinc sulphate maximum cellulase showed by *Rhizopus stolanifer* and minimum showed by *Curvularia lunata* in the presence of sodium sulphate. Whereas ammonium sulphate totally inhibited of cellulase enzyme production in the all selected fungi. Significant cellulase production showed by zinc sulphate From the investigation conclude that the phosphorous and sulphur sources affect the cellulase activity of tested fungi, most of phosphorous and sulphur sources enhance the the enzyme activity.

Production of cellulase activity of fungi were useful to degraded vegetable waste and shortly it convert into valuable product that is compost.

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REFERANCES:

1) Amer Ahmed & Aasia Bibi (2018). Fungal Cellulase: Production And Apllications: Minireview, International Journal of Health and Life Science, vol 4 Issue 1,pp 19-36. http://grdspublishing.org/
2) Bhale U. N. and Rajkonda J. N. 2012.Enzymatic activity of *Trichoderma species. Novus Natural Science Research,*1(4):1-8.
3) Dingle J.W., Reid, W. and Solomas G.L., 1953. The enzyme degradation of pectin a n d other polysaccharides-II. Application of the cup plate assay to the estimation of enzyme, *Journal of Science Food Agriculture,*4:149-155.
4) Gadgile D. P. and Chavan A. M. 2009.Impact of nutritional sources on the activity of enzyme cellulase produced by post harvest fungi isolated from mango fruits. *Bioinfolet-A Quarterly Journal of Life sciences.*6(3):227-229.
5) Gautam SP, Bundela PS, Pandey AK, Jain RK. 2009. Biodegradation and recycling o f

urban solid waste. American Journal of Environmental Sciences,:5:45-60.
6) Gautam SP,Bundela PS, Pandey AK,Khan J, Awasthi MK, Sarsaiya S. 2011.Optimization for the production of cellulase enzyme from Municipal solid waste residue by two novel cellulolytic fungi. Biotechnology Research International, pp 8, https://doi.org/10.4061/2011/810425
7) Holker, U., M. Höfer and J. Lenz, 2004. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Appl. Microbiol. Biotechnol., 64: 175-186.
8) Iqbal, N.. Agrawal A Dubey, S., & Kumar, J. 2021 Role of Decomposers in Agricultural Waste Management, Intech Open, doi: 10.5772/intechopen.93816.
9) Kamara AH, Kamar S, Kamara MS. 2015. Effect of rice straw biochar on soil quality and the early growth and biomass yield of two rice varieties. Journal of Agricultural Sciences,;6:798-806.
10) Kulkarni,A. U., Chavan A. M., Kasare, U. T., & Pawar, S.M., 2012. Effect of nitrogen and phosphorous sources on amylase production in seed born fungi of maize. *Current Botany* Vol3.no.4.
11) Rajmane S. D. and Korekar S. L.2012. Cellulase enzyme production of post-harvest fungi under the influence of carbon and nitrogen sources, *Current Botany*, 3(2): 13-15.
12) Rathod G. M. (2010) Studies on postharvest diseases on papaya. Thesis, submitted to *Dr. Babasaheb Ambedkar Marathwada University* Aurangabad.
13) Rathod Gulab M. and Chavan Ashok M. 2013. Extra-cellular cellulase enzyme production by post-harvest fungi under the influence of physical factors *Elixir Appl. Botany* 54: 12774-12777.
14) Rathod Sulochana R. and Chavan Ashok M.2010. Extracellular hydrolytic enzymes action of *Alternaria* species under the influence of different nutritional sources, *Journal of Ecobiotechnology* 2(6): 57-62.
15) Sandhu HS. Wratten SD Cullen R. 2010. Organic agriculture and ecosystem services, Environmental Science & Policy,:13:1-7.
16) Thiyam Benkee and Sharma G.D. 2013.Isolation and identification of fungi associated with local fruits of Barak Valley, Assam. *Current World Environment* 8(2): 319-322.
17) Wagh P. M. and Bhale U. N. 2014.Potentials of nutritional factors on production of cellulase enzyme bypost-harvest fungal pathogens on sapodilla fruit *Current Biotica*7(4): 256-265.
18) Waghmare, B. M. , Shinde S. R. , Sumanth G. T. And Gorgile V. T. 2010. Effect o f Carbohydrates on production of hydrolytic enzymes in different species of *Fusarium. International Journal of plant science,* Vol.5 Issue 2:577-578.