EFFECT OF NUTRITIONAL SOURCES ON PECTINASE PRODUCTION OF FUNGI FROM VEGETABLE WASTES

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Abstract- **Pectinases enzymes are responsible for the biological degradation of pectin. Pectin a large molecular weight polysaccharide found in plants cell wall. Pectinases are produced by microorganisms such as bacteria and fungi. These enzymes are used in industry to improve the production of fruit juices, fruits texture, plant fiber processing and tea. In present study the production of pectinase by ten dominating fungal species associated with vegetable waste were tested with carbohydrate, nitrogen, phosphorous and sulphur medium. Pectinase activity was determined by viscometrical method. The result pectinase productions revealed containing nutritional source broth medium. The fungi at different time intervals pectinase enzymatic activity representing percent loss of viscosity. The effect of carbohydrate source like pectin and glucose showed highest pectinase activity by *Curvularia lunata* and *Aspergillus niger,* nitrogen source like Ammonium phosphate was responsible for maximum production of pectinase by *Rhizopus stolonifer,* phosphorous source like ammonium phosphate maximum production were recorded by *Penicillium sp.* in and sulphur source like zinc sulphate maximum pectinase activity were showed by *Fusarium oxysporum* at different interval time 60 min.**

Key word*:* Nutritional source medium, Ostwald viscometer, fungi, pectinase activity, vegetable waste.

INTRODUCTION

The vegetables get spoiled by fungi, these fungi are known to destroy vegetables and an under threat due to fungi in all over vegetables at market areas. The contamination of fruits and vegetables by fungi could also produces cause of poor handling practices in food supply chain, storage conditions, distribution, marketing, practices and transportation [8]. In India, 20-30 % of the produce is spoiled in the markets [4], [3]. Fruits and vegetables spoiled with mycotoxin producing fungal species such as *Aspergillus, Fusarium* etc. These are dangerous for human health, because they produce mycotoxin which is carciniogenic in nature [15], [6].

Fungi produce intracellular as well as extracellular enzymes. All fungi carbon compounds synthesized by other living organisms. Complex compounds like cellulose, pectin, and converted in simple compound with the help of extracellular enzyme secrete by fungi [7].

Application of pectinases in food processing has prolonged significantly in recent years mainly in extraction, clarification and stabilization process [18]. Pectinases have major role in fruit and vegetable juice industry, they are produced by fungi mainly from *Aspergillus sp*. due to its distinct role in food industries. Pectinases are upcoming enzymes that are involved in pectin degradation so these enzymes are very important in commercial sector food and agricultural sectors due to their extensive applications. The global sale of pectinases is 25% of the industrial enzymes annually. Other than these applications they have inclusive and widespread application in wine industry, paper and pulp industry preferably in making Japanese paper [5]

Application of agro-industrial residues as carbon sources in enzyme production processes reduces the cost of production, and also helps in solving problems with their disposal [9]. Such residues have yielded good results in the production of pectinase [17].

In present investigation attempts were made to study the pectinase enzymes activity of dominating fungi and effect of different nutritional factors on their activity.

MATERIAL AND METHODS

Production of pectinase

Production of pectinase was made by growing the fungi in liquid medium containing pectin – 10gm, KNO_3 – 0.25%, KH_2PO_4 – 0.1%, $MgSO_4.7H_2O$ – 0.05%, pH – 5.0. Out of which 25 ml of medium was poured in 100 ml Erlenmeyer conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml standard spores suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25^0C with diurnal periodicity of light. On 7^{th} day, the flasks were harvested by filtering the contents through Whatman filter paper no.1. The filtrates were collected in the presterilized bottles and termed as crude enzyme.

Assay for pectinase

Pectinase activity was assayed by viscometric method [12] as viscosity loss % after 60 minutes. The Ostwald's viscometer was thoroughly cleaned with distilled water and dried before use. 6ml of pectin in 2ml of 0.2 M acetate buffer (pH 5.2) and 4ml of enzyme source were taken in viscometer and were thoroughly mixed and incubated at 250C temperature. The efflux time of the mixture at 0, 20, 40 and 60 minutes was recorded with the help of stop watch.

The percent loss of viscosity was calculated by using the formula,

$$\text{Percent loss of viscosity} = \frac{T_0 - T_x}{T_0 - T_w} \times 100$$

Where, T_0 = Flow time in seconds at zero time

T_x = Flow time of the reaction mixture at time T

T_w = Flow time of distilled water

Statistical analysis

All the results were statistically analyzed using analysis of variance, means of the treatments were compared using the least significant difference (C.D., p = 0.05) which allowed determination of significance between different applications [10], [11].

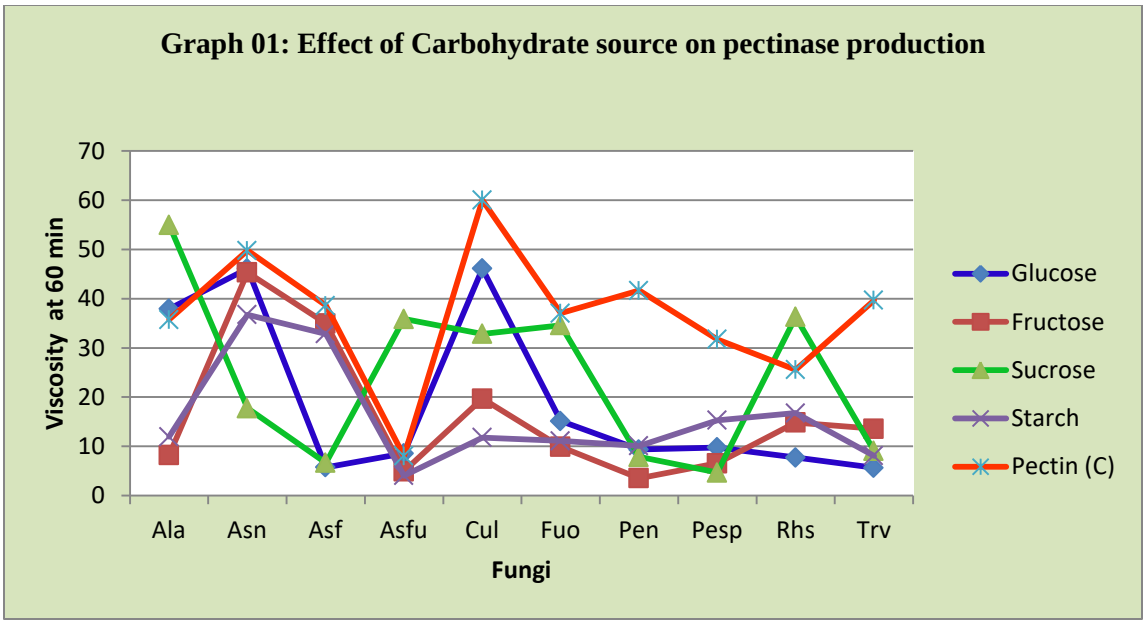
RESULTS AND DISCUSSION

In present investigation production of Pectinase by ten dominating fungal species associated with Vegetable waste were tested on nutritional source like, carbohydrates, nitrogen, phosphorous and sulphur medium. On the contrary of this pectinase activity of *Alternaria alternata, Aspergillus niger, A. flavus, A. fumigatus, Curvularia lunata, Fusarium oxysporum, Penicillium notatum, P.spp, Rhizopus stolanifer* and *Trichoderma viride* was stimulated due to pectin.

Nutritional factor

Table 1: Effect of carbohydrate source on pectinase production

Fungi	Carbohydrate sources (1%)					S.E.	C.D. (p = 0.05)
	Glucose	Fructose	Sucrose	Starch	Pectin (C)		
	Viscosity duration at 60 min						
Alternaria alternata	37.92	8.23	54.97	11.97	35.75	8.71	24.21
Aspergillus niger	46.12	45.31	17.73	36.76	49.77	5.76	16.01
Aspergillus flavus	5.76	34.95	6.66	32.84	38.61	7.23	20.10
Aspergillus fumigatus	8.59	4.95	35.86	4.05	8.04	5.95	16.54
Curvularia lunata	46.10	19.68	32.86	11.81	60.00	8.72	24.24
Fusarium oxysporum	15.13	9.92	34.59	11.11	37.04	5.90	16.40
Penicillium notatum	9.35	3.55	7.87	10.11	41.66	6.88	19.13
Penicillium sp.	9.78	6.57	4.69	15.31	31.73	4.87	13.54
Rhizopus stolonifer	7.73	14.85	36.30	16.77	25.57	4.92	13.68
Trichoderma viride	5.65	13.57	9.09	8.08	39.68	6.25	17.38



Ala- *Alternaria alternata* Asn- *Aspergillus niger* Asf- *Aspergillus flavus*

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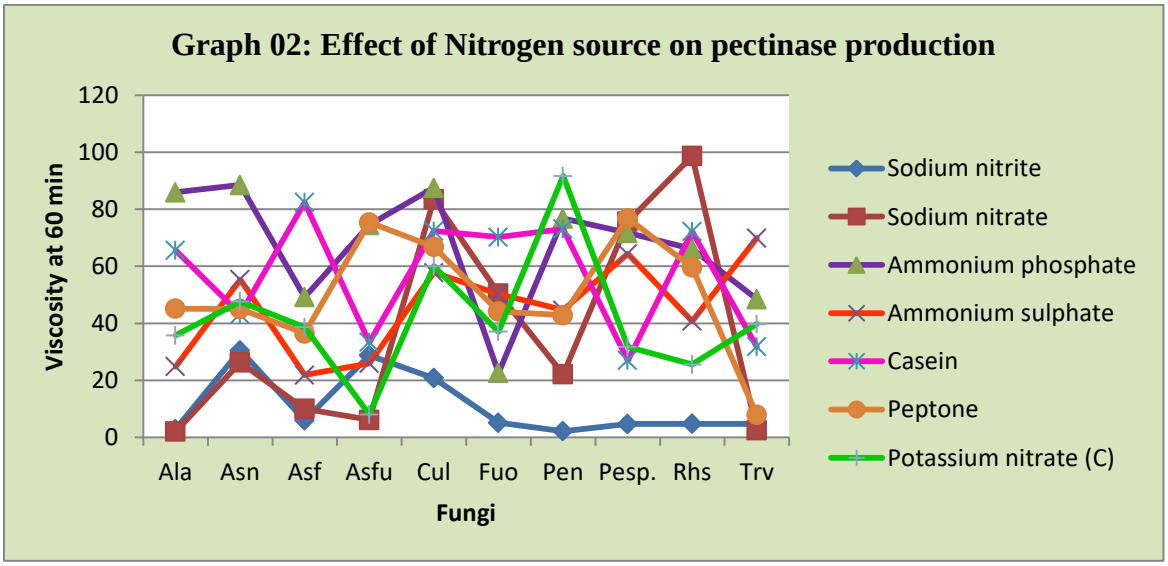
i) Effect of carbohydrates source on pectinase production

It was observed that (table 01 and graph 01) maximum pectinase enzyme production showed by pectin source. The highest pectinase production was recorded by *Curvularia lunata* followed by *Aspergillus niger* in pectin and glucose sources. It also Glucose showed stimulatory effect to pectinase production as compare to other sources. Whereas fructose and starch showed inhibited effect on pectinase production in all testing fungi except *Aspergillus niger* and *Aspergillus flavus*. On the other hand sucrose did not showed enzyme activity by *Penicillium notatum*, *Penicillium sp*. and *Trichoderma viride* except *Alternaria alternata*. Similar work was done by [13], in the study effect of carbon and nitrogen sources on pectinase enzyme by fungi on mango fruit. It also supported [1], he was work done on pectinase enzyme production by *Penicillium chrysogenum.*

Table 2: Effect of nitrogen source on pectinase production

Fungi	Nitrogen sources (0.25%)							S. E.	C. D. (p = 0.05)
	Sodium nitrite	Sodium nitrate	Ammonium phosphate	Ammonium Sulphate	Casein	Peptone	Potassium nitrate (C)		
	Viscosity duration at (60 min)								
Alternaria alternata	2.90	2.05	85.90	24.83	65.54	45.11	35.75	11.76	28.80
Aspergillus niger	30.48	26.27	88.49	55.33	43.18	45.00	47.59	7.72	18.92
Aspergillus flavus	6.00	10.03	49.15	21.85	82.37	36.42	38.61	9.88	24.20
Aspergillus fumigatus	28.75	6.13	74.50	25.94	33.38	75.35	8.04	10.76	26.37
Curvularia lunata	20.80	83.52	87.50	57.78	72.36	66.81	60.0	8.36	20.47
Fusarium oxysporum	5.15	50.47	22.60	50.24	70.20	44.14	37.04	7.97	19.53
Penicillium notatum	2.19	22.12	76.72	44.62	73.07	42.85	91.66	12.07	29.58
Penicillium spp.	4.67	75.67	71.66	64.39	26.94	76.81	31.73	10.88	26.66
Rhizopus stolanifer	4.75	98.65	66.06	40.78	72.18	59.60	25.57	11.85	29.02
Trichoderma viride	4.66	2.57	48.44	69.86	31.79	7.90	39.68	9.64	23.62



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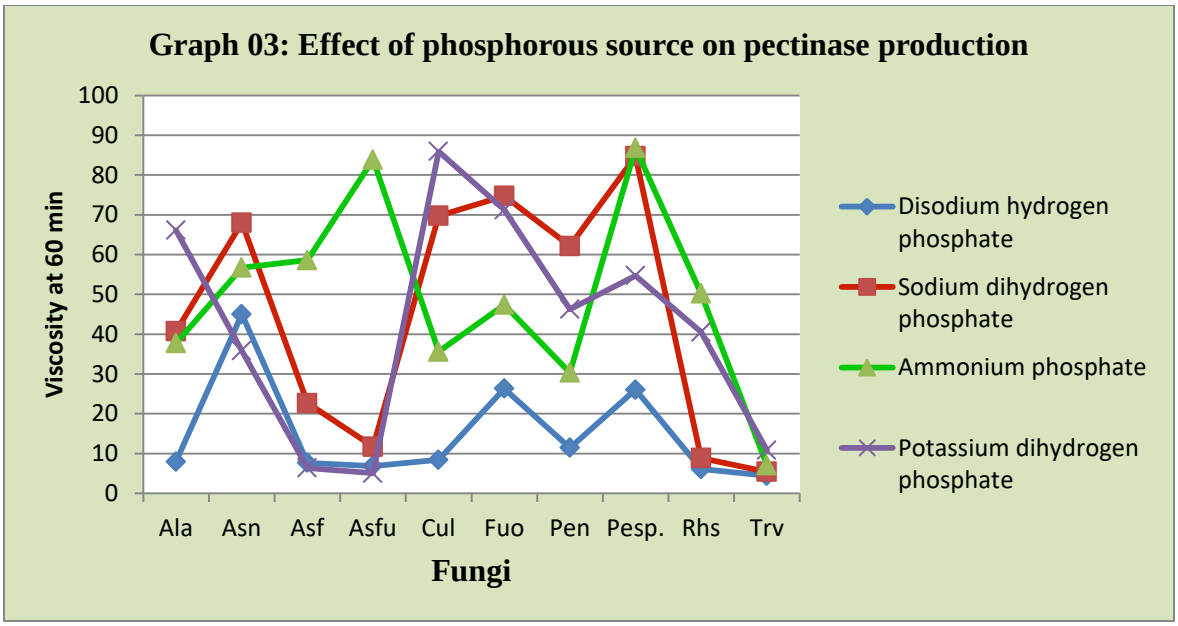
ii) Effect of nitrogen source on pectinase production

Seven nitrogen sources, at 0.25% concentration were used for pectinase production of fungi. The results are mention in the table 2 and graph 2.

It was clear from the table that source like ammonium phosphate proved significant stimulate to the pectinase production as compare to other sources. Casein, ammonium sulphate and peptone source also showed stimulatory effect. The highest pectinase production were recorded by *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum* in sodium nitrate, ammonium phosphate, casein and potassium nitrate respectively. Potassium nitrate showed moderate effect on pectinase enzyme activity for all tested fungi except *Aspergillus fumigatus*. The sodium nitrite showed inhibited effect on enzyme production in all testing fungi whereas sodium nitrate showed inhibition effect on pectinase production with *Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata* and *Trichoderma viride.* Similar work made by [**2]** on enzymatic activity of *Trichoderma species.*

Table3: Effect of phosphorous source on pectinase production

Fungi	Phosphorous sources (0.1%)				S. E.	C. D. (p = 0.05)
	Disodium hydrogen phosphate	Sodium dihydrogen phosphate	Ammonium phosphate	Potassium dihydrogen phosphate (C)		
	Viscosity duration at 60 min					
Alternaria alternata	7.94	40.71	37.76	66.15	11.91	37.89
Aspergillus niger	45.01	68.04	56.73	35.83	7.00	22.27
Aspergillus flavus	7.67	22.68	58.62	6.41	12.17	38.69
Aspergillus fumigatus	6.87	11.75	83.87	5.06	19.05	60.57
Curvularia lunata	8.46	69.76	35.63	85.92	17.35	55.18
Fusarium oxysporum	26.32	74.75	47.41	71.25	11.31	35.96
Penicillium notatum	11.47	62.17	30.35	46.29	10.86	34.53
Penicillium spp.	26.04	84.72	86.80	54.71	14.36	45.65
Rhizopus stolanifer	6.12	8.88	50.22	40.54	11.13	35.38
Trichoderma viride	4.43	5.44	7.02	10.80	1.40	4.45



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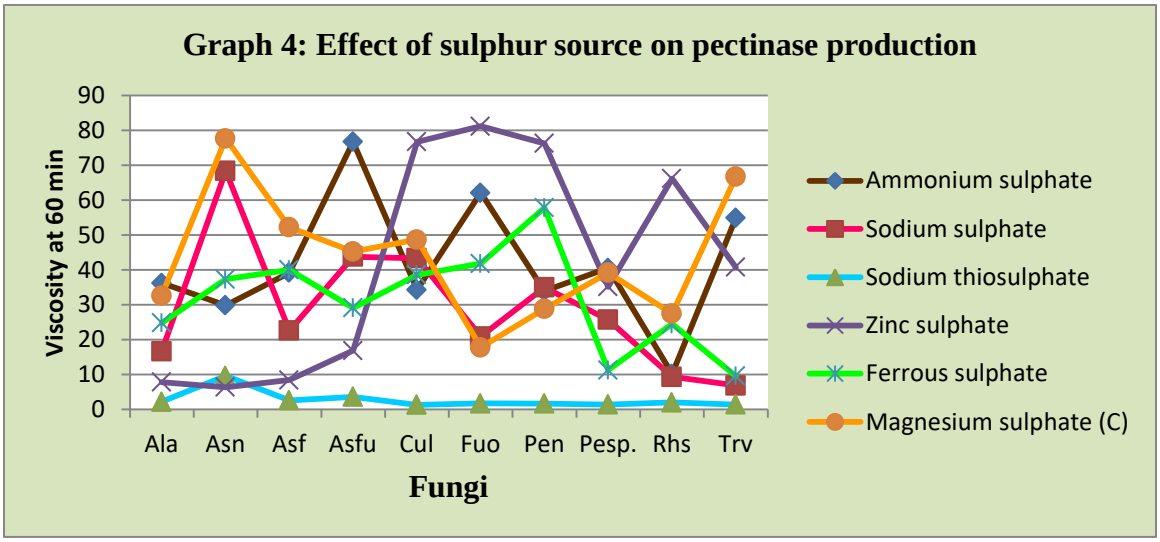
iii) Effect of phosphorous source on pectinase production

Four different phosphorous sources of 0.1% concentration were added in to the basal medium and their effect on pectinase production was studied. The results are given in table 3 and graph 3.

It was found that ammonium phosphate and sodium dihydrogen phosphate significant for the pectinase production they enhance the enzyme activity in all tested fungi except *Trichoderma viride*. The highest pectinase production were recorded by *Penicillium sp*. in ammonium phosphate. The potassium dihydrogen phosphate showed stimulatory effect for pectinase production except *Aspergillus flavus*, *Aspergillus fumigatus* and *Trichoderma viride*. Whereas disodium hydrogen phosphate were inhibited to pectinase enzyme production except *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium sp*.. Also supported work was made by [**14]** effect of nutritional sources on pectinase enzyme due to fungi from post harvest papaya fruits.

Table 4: Effect of sulphur source on pectinase production

Fungi	Sulphur sources (0.5%)						S. E.	C. D. (p = 0.05)
	Ammonium sulphate	Sodium sulphate	Sodium thiosulphate	Zinc sulphate	Ferrous sulphate	Magnesium sulphate (C)		
	Viscosity duration at 60 min							
Alternaria alternata	36.20	16.66	2.15	7.80	24.77	32.60	5.55	14.25
Aspergillus niger	29.82	68.35	9.58	6.34	37.27	77.63	12.07	31.01
Aspergillus flavus	39.16	22.58	2.58	8.40	40.00	52.21	7.99	20.52
Aspergillus fumigatus	76.66	43.75	3.58	16.81	29.07	45.20	10.43	26.81
Curvularia lunata	34.28	43.28	1.27	76.69	38.52	48.67	9.94	25.55
Fusarium oxysporum	62.06	20.86	1.71	81.15	41.79	17.74	12.21	31.39
Penicillium notatum	33.87	34.95	1.65	76.22	57.80	28.81	10.46	26.87
Penicillium sp.	40.49	25.68	1.39	35.15	11.25	39.16	6.55	16.84
Rhizopus stolanifer	10.15	9.33	1.96	66.13	24.46	27.43	9.45	24.27
Trichoderma viride	54.85	6.82	1.39	40.71	9.56	66.66	11.33	29.11



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Trv- *Trichoderma viride*

iv) Effect of sulphur source on pectinase enzyme production

Six different sulphur sources at 0.05% concentration were added in to the basal medium and their effect on production of pectinase was studied and the results are given in table 4 and graph 4.

It was observed that sodium thiosulphate were retarded to pectinase enzyme activity with selected fungi. The maximum pectinase were produced in magnesium sulphate by tested fungi. The highest pectinase production was recorded by *Fusarium oxysporum* and *Curvularia lunata* in zinc sulphate. Ammonium sulphate was stimulate the pectinase production in *Aspergillus fumigatus, Alternaria alternata, Fusarium oxysporum* and *Penicillium sp*.. It also sodium sulphate, ferrous sulphate, zinc sulphate and ammonium sulphate showed moderate pectinase production by tested fungi. Similar work was done by [**16]**, on screened pectinase producing fungi which isolated from spoiled fruity vegetables.

ACKNOWLEDGEMENT

Authors are thankful to Principal of Lal Bahadur Shastri Senior College, Partur for providing all necessary facilities.

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