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Extra- cellular Pectinase Activity of Post-harvest Fungi from Papaya Fruits in Presence of Different Influencing Factors

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Abstract

The present paper deals with the study of pectinase enzyme activity of post-harvest fungi of papaya fruits under the influence of nutritional factors, antibiotics, vitamins and fungicides. It was found that carboxy methyl cellulose (CMC), sodium sulphate; ferrous sulphate, barium chloride, sodium chloride, magnesium chloride, zinc, ferrous, manganese, copper, barium, fungicides and ampicillin inhibits the pectinase action of all tested post-harvest fungi while other factors induces the same.

Key words: Carbon, Phosphorous, Sulphur, Antibiotics, Vitamins, Papaya fruits, Post-harvest fungi, Pectinase activity

Introduction

Papaya (*Carica papaya* L) is an economically important fruit crop cultivated in tropical and subtropical regions of the world. During post- harvest condition papaya fruits gets infected by several fungi ,during their infection, these fungi secretes their biological weapons, that is, enzyme as like cellulase and pectinase which causes spoilage of fruits. Since very little information was available on the effect of carbon, nitrogen, phosphorous, sulphur sources, antibiotics, vitamins, fungicides and trace elements on pectinase activity of post- harvest fungi of papaya fruits, attempts were made to determine the impact of these influencing factors on pectinase activity of post- harvest fungi.

Material and Methods

Isolation of post- harvest fungi

Post- harvest fungi viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium moniliforme Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer* were isolated from papaya fruits and pure culture was maintained at 25^0 C.

Production of pectinase enzyme

Liquid medium at pH5.6 containing pectin -10g, KNO_3 -2.5g, KH_2PO_4- -1.0g, $MgSO_4$ – 0.5g and 1000ml distilled water was prepared. Twenty five ml of the medium was poured in 100ml conical flasks and autoclaved at 15 1bs pressure for 30 minutes and then on cooling, the flasks were inoculated by post- harvest fungi, separately with 1.0ml spore suspension of the fungi. To study the impact of carbon, nitrogen, phosphorous and sulphur each source was replaced by respective basic component of G .N. media while impact of antibiotics, vitamins, fungicides and trace elements were studied separately at 100 ppm. G.N. media containing pectin was served as control. These flasks were then allowed to incubate for 6 days at 25± 1°C with diurnal periodicity of light. On 7th day, flasks were harvested by filtering the contents through whatman filter paper no.1. The filtrates were collected in presterilized culture filtrate bottles separately, termed as crude enzyme.

Assay of pectinase enzyme

Pectinase enzyme activity was assayed by viscometer method as viscosity loss % after 60 minutes following Gadgile and Chavan (2009 a).

Results and Discussion

Table 1 Effect of carbohydrate sources on pectinase production

Carbohydrates	Fungi									
	Ala	**Asf**	**Asn**	**Cog**	**Cul**	**Fue**	**Fum**	**Fuo**	**Ped**	**Rhs**
Monosaccharide										
Glucose	42	54	56	43	43	58	50	58	58	54
Fructose	48	42	55	42	42	58	48	60	56	55
Xylose	44	52	53	46	46	56	43	54	64	58
Disaccharides										
Sucrose	56	53	58	43	38	55	52	53	54	39
Maltose	45	48	54	44	42	48	44	52	45	43
Polysaccharides										
CMC	23	46	36	38	28	40	33	42	44	36
Starch	36	52	58	62	57	59	53	62	62	60
Pectin (C)	56	58	60	56	52	58	57	43	56	52

Ala = *Alternaria alternata* Fue = *Fusarium equiseti*
Asf = *Aspergillus flavus* Fum = *Fusarium moniliforme*
Asn = *Aspergillus niger* Fuo = *Fusarium oxysporum*
Cog = *Colletotrichum gloeosporioides* Ped = *Penicillium digitatum*
Cul = *Curvularia lunata* Rhs = *Rhizopus stolonifer*

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

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Table 2 Effect of nitrogen sources on pectinase production

Nitrogen Sources	Fungi									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
Nitrate forms										
Sodium nitrate	56	62	70	53	56	58	54	59	65	56
Potassium nitrate	58	59	56	52	55	45	55	58	56	50
Nitrite forms										
Sodium nitrite	46	56	55	56	54	60	52	60	62	58
Ammonium forms										
Ammonium phosphate	58	50	52	52	42	59	58	58	60	54
Ammonium sulphate	52	54	54	54	58	61	54	56	54	52
Amide forms										
Urea	56	52	51	48	45	48	43	48	20	46
Organic forms										
Gelatin	52	54	58	54	42	46	50	40	38	59
Peptone	57	56	55	55	61	59	60	63	71	62
Casein	56	53	53	58	60	52	59	60	66	52
KNO3 (C)	58	59	56	53	55	45	55	58	56	50

Ala = Alternaria alternata
Asf = Aspergillus flavus
Asn = Aspergillus niger
Cog = Colletotrichum gloeosporioides
Cul =Curvularia lunata
Fue = Fusarium equiseti
Fum = Fusarium moniliforme
Fuo = Fusarium oxysporum
Ped = Penicillium digitatum
Rhs = Rhizopus stolonifer

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

Table 3 Effect of phosphorus sources on pectinase production

Phosphorus sources	Fungi									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
Sodium dihydrogen phosphate	42	53	54	46	42	48	45	46	58	45
Disodium hydrogen phosphate	45	51	52	56	54	52	54	55	52	54
Potassium hydrogen phosphate	46	54	57	42	55	45	47	52	54	46
Ammonium biphosphate	52	53	50	48	40	42	59	56	56	54
Ammonium phosphate	54	46	56	45	40	55	48	60	58	57
KH2(PO4)2 (C)	58	50	52	52	42	59	58	58	60	54

Ala = Alternaria alternata
Asf = Aspergillus flavus
Asn = Aspergillus niger
Cog = Colletotrichum gloeosporioides
Cul =Curvularia lunata
Fue = Fusarium equiseti
Fum = Fusarium moniliforme
Fuo = Fusarium oxysporum
Ped = Penicillium digitatum
Rhs = Rhizopus stolonifer

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

Table 4 Effect of sulphur sources on pectinase production

Sulphur sources	Fungi									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
Zink sulphate	40	42	47	42	45	46	47	44	49	56
Sodium sulphate	38	45	53	51	48	52	40	45	52	50
Sodium thiosulphate	56	56	48	48	40	48	53	44	43	58
Ferrous sulphate	42	46	43	45	44	46	42	40	46	42
Ammonium sulphate	55	53	45	50	42	50	44	55	54	54
Magnesium sulphate	54	40	54	42	41	53	54	45	42	54
Potassium sulphate	44	58	40	52	50	52	45	41	53	59
Control	56	54	58	53	52	54	48	56	54	53

Ala = Alternaria alternata
Asf = Aspergillus flavus
Asn = Aspergillus niger
Cog = Colletotrichum gloeosporioides
Cul =Curvularia lunata
Fue = Fusarium equiseti
Fum = Fusarium moniliforme
Fuo = Fusarium oxysporum
Ped = Penicillium digitatum
Rhs = Rhizopus stolonifer

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

Table 5 Effect of vitamins on pectinase production

Vitamins (100 ppm)	Fungi									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
Ascorbic acid	41	52	55	42	34	50	36	56	50	54
Folic acid	44	40	42	38	40	38	40	56	43	55
Riboflavin	39	42	43	40	42	59	42	42	60	40
Thiamin	36	43	59	38	40	38	33	54	45	39
Pyridoxine	33	40	59	39	33	46	42	53	48	44
Control	54	54	58	52	54	58	54	55	58	53

Ala = Alternaria alternata
Asf = Aspergillus flavus
Asn = Aspergillus niger
Cog = Colletotrichum gloeosporioides
Cul =Curvularia lunata
Fue = Fusarium equiseti
Fum = Fusarium moniliforme
Fuo = Fusarium oxysporum
Ped = Penicillium digitatum
Rhs = Rhizopus stolonifer

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

Table 6 Effect of fungicides on pectinase production

Fungicides	Fungi									
(100 ppm)	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
Capton	--	30	32	--	30	25	--	24	--	--
Dithane M-45	33	36	--	--	--	--	--	--	38	39
Benomyl	30	--	--	--	--	42	29	--	40	--
Dinocap	28	--	--	--	--	38	30	--	40	--
Dithane I-78	20	--	--	--	--	32	28	--	32	--
Control	50	52	54	52	48	52	49	51	53	50

Ala = Alternaria alternata
Asf = Aspergillus flavus
Asn = Aspergillus niger
Cog = Colletotrichum gloeosporioides
Cul =Curvularia lunata
Fue = Fusarium equiseti
Fum = Fusarium moniliforme
Fuo = Fusarium oxysporum
Ped = Penicillium digitatum
Rhs = Rhizopus stolonifer

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

Table 7 Effect of antibiotics on pectinase production

Antibiotics	**Fungi**									
(100 ppm)	**Ala**	**Asf**	**Asn**	**Cog**	**Cul**	**Fue**	**Fum**	**Fuo**	**Ped**	**Rhs**
Ampicillin	40	45	43	44	46	45	42	46	48	47
Streptomycin	52	58	56	54	56	50	43	48	52	49
Terramycin	55	54	55	55	55	50	46	52	49	51
Griseofolin	51	56	52	50	52	54	48	53	56	48
Doxycyclin	54	53	56	54	52	51	45	54	54	52
Control	53	52	54	53	54	52	54	56	53	52

Ala = Alternaria alternata
Asf = Aspergillus flavus
Asn = Aspergillus niger
Cog = Colletotrichum gloeosporioides
Cul =Curvularia lunata
Fue = Fusarium equiseti
Fum = Fusarium moniliforme
Fuo = Fusarium oxysporum
Ped = Penicillium digitatum
Rhs = Rhizopus stolonifer

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

Table 8 Effect of trace elements on pectinase production

Trace elements	**Fungi**									
(100 ppm)	**Ala**	**Asf**	**Asn**	**Cog**	**Cul**	**Fue**	**Fum**	**Fuo**	**Ped**	**Rhs**
Fe	40	42	43	46	44	39	34	40	46	35
Mn	48	46	51	52	56	44	40	49	53	42
Cu	48	42	45	33	45	48	37	35	28	34
Ba	53	40	41	51	42	38	29	42	23	36
Zn	29	41	39	26	41	33	39	37	42	39
Control	56	54	54	53	52	54	48	54	56	53

Ala = Alternaria alternata
Asf = Aspergillus flavus
Asn = Aspergillus niger
Cog = Colletotrichum gloeosporioides
Cul =Curvularia lunata
Fue = Fusarium equiseti
Fum = Fusarium moniliforme
Fuo = Fusarium oxysporum
Ped = Penicillium digitatum
Rhs = Rhizopus stolonifer

Enzyme activity expressed as viscosity loss (%) after 60 minutes.

It was observed from table 1 that, among monosaccharides xylose prove poor source of carbon as compared to glucose and fructose for pectinase production in all tested fungi while among disaccharides sucrose induce pectinase production in all fungi. Glucose stimulate pectinase action in *Fusarium oxysporum, Fusarium equiseti, Penicillium digitatum,* and *Rhizopus stolonifer* where as it inhibit the same in *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme, Curvularia lunata,* and *Colletotrichum gloeosporioides.* CMC and maltose deactivate pectinase action of all tested fungi. Starch stimulates pectinase action of *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium oxysporum, Penicillium digitatum,* and *Rhizopus stolonifer* while it retards the same of *Alternaria alternata, Aspergillus flavus, Aspergillus niger* and *Fusarium moniliforme.* There are more or less similar reports on impact of different carbohydrates sources on hydrolytic enzyme production. Adam and Deploey (1976) found that glucose along with starch stimulated amylase production in *Mucor pusillus* and *M. michei*, Charya and Reddy. (1983) in case of *Phoma exigua, Graphium penicillioides* and Khairnar (1987) in case of *Curvularia lunata, Alternaria tenuis* and *Aspergillus flavus* reported amylase production better in presence of starch than other carbohydrates. Gadgile and Chavan (2009a) reported that among carbon sources, fructose and sucrose induce post-harvest fungal cellulase enzyme activity of mango fruits. Kakde *et al.* (2009) found more or less similar results in case of lipase production by *Aspergillus niger, Fusarium oxysporum, Macrophomina phaseolina* and *Penicillium notatum* in different oil seeds.

It was also found that nitrogen sources like sodium nitrate favoured maximum production of pectinase in case of *Aspergillus niger, Penicillium digitatum, Aspergillus flavus*, *Fusarium equiseti, Fusarium oxysporum, Curvularia lunata,* and *Rhizopus stolonifer* where as it inhibit the same in *Colletotrichum gloeosporioides, Fusarium moniliforme* and *Alternaria alternata.* While in presence of sodium nitrite *Colletotrichum gloeosporioides*, *Penicillium digitatum, Fusarium oxysporum, Fusarium equiseti,* and *Rhizopus stolonifer* were showed maximum production of pectinase, where as it retard the same in *Alternaria alternata, Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata,* and *Fusarium moniliforme.* Among two ammonium forms, ammonium phosphate favoured maximum production of pectinase in *Penicillium digitatum, Fusarium equiseti, Alternaria alternata, Fusarium moniliforme, Fusarium oxysporum* and *Rhizopus stolonifer* where as it inhibit the pectinase action in *Curvularia lunata, Aspergillus flavus, Aspergillus niger* and *Colletotrichum gloeosporioides.* While ammonium sulphate induce the pectinase production of *Aspergillus flavus, Aspergillus niger*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium oxysporum* and *Rhizopus stolonifer*. Gelatin induces the pectinase activity in *Aspergillus niger*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* while peptone and casein activate pectinase in all fungi except *Alternaria alternata, Aspergillus flavus* and *Aspergillus niger* (Table 2).Where as Gadgile and Chavan (2009a) revealed that nitrogen sources in the form of urea and peptone significantly increased cellulase action of *Aspergillus niger, Colletotrichum gloeosporioides, Penicillium* sp*, Rhizopus stolonifer, A. fumigatus* and *A. flavus* from mango fruits they also reported that ammonium phosphate significantly reduced it. Nitrogen sources like peptone and casein increase significantly the lipase enzyme activity, but $(NH_4)_2PO_4$, $NaNO_2$ and urea showed reverse result (Kakde *et al.*, 2009).

Among phosphorous sources, sodium dihydrogen phosphate retard pectinase production in *Aspergillus niger, Curvularia lunata* and *Fusarium moniliforme,* where as Sodium dihydrogen phosphate was responsible for maximum pectinase production in *Rhizopus stolonifer, Alternaria alternata.* Pectinase production in *Alternaria alternata, Curvularia lunata* and *Fusarium oxysporum* were reduced by sodium hydrogen phosphate. *Aspergillus flavus* and *Penicillium digitatum* pectinase action was induced by disodium hydrogen phosphate. Potassium hydrogen phosphate inhibitory in *Alternaria alternata, Colletotrichum gloeosporioides* and *Fusarium moniliforme,* where as potassium hydrogen phosphate induced pectinase action of *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum* and *Fusarium equiseti.* Pectinase activity of *Curvularia lunata* and *Fusarium oxysporum* was retarded by ammonium phosphate where as it induce the pectinase production in *Aspergillus niger, Aspergillus flavus, Fusarium equiseti,* and *Fusarium moniliforme.* Ammonium biphosphate retarded pectinase action of *Curvularia lunata, Fusarium moniliforme, Aspergillus niger, Alternaria alternata, Colletotrichum gloeosporioides, Fusarium equiseti,* and *Penicillium digitatum,* where as ammonium biphosphate was responsible for induction of pectinase production in *Aspergillus flavus, Rhizopus stolonifer* and *Fusarium oxysporum* (Table 3). Sodium hydrogen orthophosphate as a Phosphorous source induced the enzyme activity, while diammonium phosphate and potassium hydrogen phosphate inhibited the enzyme activity. Dis. Sodium hydrogen ortho-dehydrates and di-sodium hydrogen phosphate did not affect the enzyme activity (Gadgile and Chavan, 2009a).

Table 4 shows that, zinc sulphate, sodium sulphate and ferrous sulphate deactivate the pectinase action of all selected fungi. It was also found that magnesium sulphate and potassium sulphate also inhibited the pectinase action in all selected fungi except *Rhizopus stolonifer.* Sodium thiosulphate inhibited the pectinase action of *Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer* while it was induced the same in *Alternaria alternata, Aspergillus flavus* and *Fusarium* moniliforme. Gadgile and Chavan (2009a) reported same results in case of cellulase action of post-harvest fungi from mango fruits. Kakde *et al.* (2009) reported that, zinc sulphate, ferrous sulphate, calcium sulphate, disodium sulphate, copper sulphate significantly inhibited lipase production
It was observed from the table 5 that ascorbic acid and folic acid inhibits the pectinase action of *Alternaria alternata*, *Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Penicillium digitatum, Fusarium equiseti,* and *Fusarium moniliforme.* While it induced the same of *Fusarium oxysporum* and *Rhizopus stolonifer.* Thiamin and pyridoxine retard pectinase action of *Alternaria alternata, Aspergillus flavus, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium moniliforme*, *Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer* where as these stimulate the same of *Aspergillus niger.* Riboflavin proved inhibitory for pectinase production in *Alternaria alternata*, *Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium moniliforme, Fusarium oxysporum,* and *Rhizopus stolonifer* while it accelerates the same of *Fusarium equiseti,* and *Penicillium digitatum* .Gadgile and Chavan (2009b) found that all tested vitamins induce pectinase action except pyridoxine inhibits activity of *Rhizopus stolonifer* and *Aspergillus niger*). Vitamin C significantly induces the cellulase production of *Penicillium* sp, *R.stolonifer, A.flavus*, while it significantly inhibits activity of *C. gloeosporioides.* There was no significant effect of vitamin C on activity of *A. niger* and *A.fumigatus.* Vitamin A, thiamin and riboflavin significantly increases enzyme action of all fungi. Pyridoxine decrease enzyme action of C*. gloeosporioides* and Penicillium sp. while it significantly increases enzyme activity of remaining fungi (Gadgile and Chavan, 2009c). Bhikane (1988) found that thiamine and nicotinic acid were induced protease action in *A. flavus*, while pyridoxine was retarded the protease activity in *Curvularia lunata*, *Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia soloni*.

Fungicides viz. capton, diathane M-45, benomyl, dinocap and diathane M-78 retard the pectinase production of all tested fungi. Capton totally retard the pectinase action of *Alternaria alternata, Colletotrichum gloeosporioides Fusarium moniliforme, Penicillium digitatum* and *Rhizopus stolonifer.* Diathane M-45 completely inhibited the pectinase action of *Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium moniliforme* and *Fusarium oxysporum.* It was also found that Benomyl, Dinocap and Diathane M-78 totally nil the pectinase activity of *Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium oxysporum* and *Rhizopus stolonifer* (Table 6). Jadhav (2006) reported that a fungicide such as bavistin, benomyl, captan, difoltan, diathane M- 45 and tilt inhibit amylase action while vitamins induce amylase activity of fungi on medicinal plants.

It is clear from table 7 that, it was found that ampicillin decrease the pectinase production of all fungi. Streptomycin and Terramycin retard the pectinase action of *Alternaria alternata, Fusarium moniliforme, Fusarium oxysporum, Penicillium digitatum, Fusarium equiseti,* and *Rhizopus stolonifer* where as these induces the same of *Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides* and *Curvularia lunata*. Griseofolin activates the pectinase action of *Aspergillus flavus, Fusarium equiseti,* and *Penicillium digitatum* while it decreases the same of *Alternaria alternata, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium moniliforme* and *Fusarium oxysporum.* Doxycyclin induced pectinase production of *Alternaria alternata, Aspergillus flavus, Aspergillus niger* and *Colletotrichum gloeosporioides* where as it retard the same of *Curvularia lunata, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum* and *Penicillium digitatum.* Where as Sulochana Rathod (2007) revealed that antibiotics did not affect the amylase, lipase and protease activity of seed borne fungi. Kesare (2008) reported effect of antibiotic at 100 ppm concentration on different seed borne fungi. Antibiotic like norflaxacium, ampicillin, trioflan, tetracycline and almox DT inhibited lipase action in species of *Aspergillus.*Almox DT significantly inhibited pectinase activity of *Penicillium* species and *Aspergillus flavus.* Hostacyclin significantly inhibited pectinase activity of *Aspergillus niger, A. flavus, A. fumigatus, Colletotrichum gloeosporioides, Rhizopus stolonifer* and *Penicillium* species, while antibiotic induced pectinase activity of some post harvest fungi of mango fruits (Gadgile and Chavan, 2009b).

Table 8 show that, trace elements like Fe Cu, Ba, and Zn retarded the pectinase action of *Alternaria alternata*, *Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer.* It was also found that Mn inhibited the pectinase production of *Alternaria alternata*, *Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer* while it induced the pectinase production of *Curvularia lunata.*

Conclusion

It can thus be, concluded that sources as like carboxy methyl cellulose (CMC), sodium sulphate; ferrous sulphate, barium chloride, sodium chloride, magnesium chloride, zinc, ferrous, manganese, copper, barium, fungicides and ampicillin retards the pectinase action This knowledge may prove useful to control enzyme activity and growth of post-harvest fungi on papaya fruits by limiting the enzyme activity.

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