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## **Studies on occurrence of the post-harvest fungi on papaya fruit and eco-friendly management**

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### **Abstract**

In present investigation emphasis has been given on to study the post-harvest fungi associated with the papaya fruit. An eco-friendly approach was followed to control post-harvest fungi. For this purpose leaf extract of some medicinal plants, essential oils, plant gums, fruit rind and *Trichoderma viride* were used to screen against selected post-harvest fungi. It was found that, leaf extracts of *Annona squamosa*, *Azadirachta indica*, *Datura strominum*, and *Vitex nigundo*; essential oils of tulsi, mustard, castor, neem, clove and eucalyptus; plant gums of *Acacia arabica*, *Acacia chundra*, *Boswellia serrata*, and *Terminalia arjuna* and fruit rinds of *Manilkara sapota*, *Magnifera indica*, *Citrus sinensis*, *Citrus reticulata*, and *Punica granatum* found to be inhibitory for the post-harvest fungal growth. On the other hand, *Trichoderma viride* showed antagonistic activity against all the post-harvest fungi except *Rhizopus stolonifer*.

**Keywords:** papaya fruit, post-harvest fungi, eco-friendly management and antagonistic activity

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### **Introduction**

Papaya (*Carica papaya* L.) a native of tropical America, is grown through the tropics and subtropics for its melon like fruits (Alvarez and Nishijima, 1987) <sup>[1]</sup>. Papaya fruits are very susceptible to disease caused by many microorganisms especially fungi, as papaya fruit is high in moisture and nutrients (Sankat and Maharaj, 1997) <sup>[20]</sup>. Development of fungal infection during the post infection phase and can depend upon the physiological age of the fruit, mechanical injuries and storage environment. Fungi derived food and energy from the substrate upon which they grow in nature. In order to culture the fungus *in vitro*, there is no ideal substrate or artificial medium upon which all fungi can isolate, grow and reproduce. Therefore, in present investigation experiments were carried out to study the occurrence of the post-harvest fungi on papaya fruits and their control. Post-harvest fungi are commonly controlled by synthetic chemicals; however, most of the fungicides of this group create several side effects in the forms of carcinogenicity, teratogenicity, and residual toxicity (Bajaj, 1975 and Edward, 1973) <sup>[2, 6]</sup>. Therefore, some alternative biodegradable chemical control measures should be discovered to replace synthetic pesticides for pest management without creating pesticidal pollution. Natural fungicides are free from environmental toxicity as compared to synthetic compound (Hooda and Srivastava, 1998) <sup>[8]</sup>. Natural products are less phytotoxic, easily biodegradable and more systematic (Saxena et al., 2005) <sup>[21]</sup>. Therefore, in second part emphasis has been given on eco-friendly management of these storage fungi by essential oils and gums of some medicinal plants. The adaptation of a sustainable agricultural practice, using strategies that are environmentally eco-friendly, less dependent on agricultural chemicals is gaining worldwide recognition. One of the key elements of such sustainable agriculture is the application of biocontrol agents. *Trichoderma* spp. is antagonistic by nature with rich resource and a broad action scope. In this regard experiments were carried out to screen the antagonistic nature of *Trichoderma viride* against post-harvest fungi.

### **Materials and Method**

#### **Isolation of post-harvest fungi from papaya fruit**

Co-I, Co-II, Taiwan, Washington and Local varieties of papaya fruit were collected from fruit markets of Aurangabad and adjoining places to study the incidence of post-harvest fungi on rotted papaya fruits. For isolation agar media were used. Out of 67 isolated fungal species ten dominant fungi *viz.* *Alternaria alternata*, *Fusarium equiseti*, *Aspergillus flavus*, *Fusarium moniliforme*, *Aspergillus niger*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *Penicillium digitatum*, *Curvularia lunata* and *Rhizopus stolonifer* were selected for further study.

#### **Eco-friendly management of post-harvest fungi**

##### **By using botanicals**

Fungi toxicity of plant extracts was studied by the poisoned food technique described by Nene and Thapliyal (1993) <sup>[17]</sup> Glucose nitrate medium was prepared in flasks and sterilized. To this medium the requisite quantity of the plant extracts was added. Plant extracts was prepared by collecting fresh leaves of different plants, washed thoroughly with distilled water and grinded in distilled water. The plant extracts was thoroughly mixed by stirring. The medium was then autoclaved at 15 lbs pressure for 20 minutes. After cooling the medium, fungi were inoculated in aseptic condition and incubated for 6 days at room temperature, suitable checks were kept where the fungi grown under the same condition on glucose nitrate without plant extract. Mycelial growth of the fungi compared with check, was taken as a measure of the fungal toxicity. The same method was implemented to study the fungi toxicity of plant product/ plant parts/ byproducts like essential oils, plant gums and fruit rinds.

##### **Biological control by using antagonistic microorganisms**

To study the antagonistic potential of *Trichoderma viride* against post-harvest fungi an agar disc (5mm) containing mycelium of

*Trichoderma viride* was inoculated at the centre of PDA poured petri plates and culture discs of the test fungi were placed 3 cm from the center. Petri plates were inoculated for a week at  $25 \pm 1^\circ\text{C}$ . In the control experiment a sterile agar disc (1.2 cm diam) was placed in the dish. At the end of the incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control (Edington *et al*, 1971) [5] as follows:

$$\frac{C-T}{C} \times 100 = \% \text{ Inhibition of radial mycelial growth}$$

Where, C = radial growth measurement of the pathogen in control  
T = radial growth of the pathogen in the presence of *Trichoderma viride*

## Results and Discussion

### Antifungal activity of leaf extract

It was found that the leaf extracts of all selected plants inhibited the growth of *Colletotrichum gloeosporioides* and *Curvularia lunata*. Leaf extracts of *Annona squamosa*, *Azadirachta indica*, *Datura strominum*, and *Vitex nigundo* were found to be fatal for the growth of *Alternaria alternata*. *Fusarium oxysporum* was hampered in presence of leaf extract of *Annona squamosa*, *Azadirachta indica*, *Datura strominum*, *Murraya koenigii* and *Piper betel*. Leaf extract of *Azadirachta indica* and *Piper betel* were found to be fatal for growth of *Aspergillus flavus*. *Aspergillus niger* was unable to grow in presence of *Annona squamosa* and *Jatropha curcus*. Leaf extract of *Annona squamosa*, *Jatropha curcus* and *Murraya koenigii* was inhibited the growth of *Fusarium equiseti* and *Fusarium moniliforme*. *Penicillium digitatum* was hampered in presence of leaf extract of *Annona squamosa*, *Jatropha curcus*, *Murraya koenigii* and *Vitex nigundo* (Table 1). Kakde *et al.* (2011) [4, 10, 11] reported that, aqueous extract of *Eucalyptus angophoroides* retarded the growth of *Alternaria dianthicola*, *Curvularia pellescens*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum*. Singh and Prasada (1993) [23] found that leaf extract of *Azadirachta indica* and *Ocimum sanctum* inhibited the growth of *Fusarium oxysporum*. Similarly, Manoharachary and Gourinath (1991) [13] found that aqueous leaf extract of *Eucalyptus lonceolatus* was inhibitory for the germination and growth of *Curvularia lunata*, *Cylindrocarpon lichenicola* and *Fusarium solani*. Recently, Meena *et al.*, (2010) [15] tested leaf extract of ten medicinal plants against *Alternaria cucumerina*.

### Antifungal activity of essential oils

It was found that tulsi, mustard, castor, neem, clove and eucalyptus oils inhibited the growth of all fungi (Table 2). Locke (1995) [12]. Reported that in field *Alternaria alternata*, *Aspergillus niger* and *Fusarium oxysporum* has been completely controlled by using 2-10% neem oil. Somda *et al.*, (2007) [24] tested essential oils of *Azadirachta indica* and *Eucalyptus camaldulensis* against *Fusarium moniliforme*, *Phoma sorghina* and *Colletotrichum graminicola* and reported that extent of

inhibition depends on the concentration of essential oils. Similarly, Oxenham *et al.* (2005) [18]. Found that tulsi oil was inhibitory for the growth of phytopathogenic fungi as well as storage fungi.

### Antifungal activity of medicinal plant gums

It was found that in the presence of powder of all the medicinal plant gums *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Curvularia lunata* and *Fusarium oxysporum* showed inhibition of the growth. *Fusarium equiseti* showed moderate inhibitory growth in presence of the gums of *Acacia arabica*, *Acacia chundra*, *Boswellia serrata*, and *Terminalia arjuna* (Table 3). Marques *et al* [14]. (1992) observed that cashew tree gum inhibited the growth of 10 out of 25 fungal samples, including *Aspergillus flavus*, *Penicillium implicatum*, *Colletotrichum musae* and *Verticillium sp.* Similarly, Torquato *et al.* (2004) [26] Tested cashew tree gum for their antimicrobial activity against bacteria, yeast and fungi. They found the only antimicrobial effect of cashew gum was against *S. cerevisiae*. Chavan and Kakde (2011) [4, 10, 11] found that gum of *Terminalia arjuna*, *Acacia arabica* and *Butea monosperma* inhibited the growth of storage fungi.

### Antifungal activity of fruit rinds

It was observed that the growth of *Colletotrichum gloeosporioides* and *Fusarium equiseti* were showed the complete inhibition in selected fruit rinds. *Manilkara sapota*, *Magnifera indica*. *Citrus sinensis*, *Citrus reticulate*, and *Punica granatum* showed maximum inhibition of *Alternaria alternata*. *Curvularia lunata* showed moderate inhibition in presence of fruit rinds of *Manilkara sapota*, *Citrus sinensis*, *Citrus reticulate* and *Punica granatum*, while *Fusarium oxysporum*, showed maximum inhibition in presence of fruit rinds of *Manilkara sapota*, *Magnifera indica*, *Citrus sinensis*, and *Citrus reticulate* (Table 4).

### Antagonistic activity of *Trichoderma viride*

Antagonistic activity of *Trichoderma viride* studied against the growth of ten post-harvest fungi. The antagonism studied on nutrient agar medium by inoculating the fungi against *Trichoderma viride* the plate were incubated for six days at  $25 \pm ^\circ\text{C}$ . It was found that except *Rhizopus stolonifer* the growth of all the tested fungi was inhibited due to *Trichoderma viride*. Among these the growth of *Penicillium digitatum*, *Alternaria alternata*, and *Curvularia lunata* was restricted by *Trichoderma viride* (Table 5). Hussain *et al.* (2009) [9] found that *Trichoderma harzianum* reduced the growth of *Alternaria alternata* by 67.07%. On the other hand, Howell (2003) [7] reported the interaction between *Trichoderma viride* and *Rhizoctonia solani*, *Macrophomina phaseolina* and *Rhizopus oryzae* by different mechanism. Sempere and Santa marina (2007) [22] analyzed *Trichoderma harzianum* as possible biocontrol agent of *Alternaria alternata* under different environmental conditions. Kakde and Chavan (2011) [4, 10, 11]. Found that, growth of *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Curvularia lunata* was retarded due to *Trichoderma virid*

**Table 1:** Effect of leaf extracts (10% concentration) on production of post-harvest fungi

Leaf extracts	Dry weight of mycelium (mg)									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
<i>Annona squamosa</i>	0.041	0.185	0.086	0.194	0.048	0.101	0.131	0.023	0.087	0.212
<i>Azadirachta indica</i>	0.074	0.114	0.242	0.099	0.066	0.172	0.183	0.059	0.201	0.232
<i>Datura strominum</i>	0.068	0.189	0.116	0.068	0.087	0.183	0.194	0.125	0.200	0.198
<i>Jatropha curcus</i>	0.88	0.228	0.095	0.068	0.104	0.226	0.102	0.151	0.094	0.198
<i>Murraya koenigii</i>	0.157	0.214	0.106	0.087	0.080	0.137	0.153	0.116	0.090	0.211
<i>Piper betel</i>	0.147	0.133	0.176	0.061	0.087	0.126	0.178	0.071	0.176	0.313
<i>Vitex nigundo</i>	0.039	0.192	0.193	0.007	0.094	0.174	0.168	0.146	0.128	0.224
Control	0.142	0.162	0.118	0.132	0.128	0.161	0.156	0.143	0.156	0.146

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*

**Table 2:** Effect of different essential oils (10% concentration) on production of post-harvest fungi

Oils	Dry weight of mycelium (mg)									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
Castor	0.029	0.068	0.065	0.032	0.028	0.029	0.026	0.028	0.035	0.036
Eucalyptus	0.033	0.079	0.077	0.073	0.054	0.058	0.067	0.064	0.066	0.070
Mustard	0.030	0.054	0.046	0.036	0.032	0.039	0.036	0.034	0.040	0.036
Neem	0.029	0.036	0.083	0.065	0.036	0.063	0.075	0.066	0.070	0.064
Tulsi	0.034	0.036	0.038	0.033	0.035	0.039	0.045	0.038	0.044	0.048
Clove	0.023	0.046	0.072	0.034	0.038	0.052	0.062	0.064	0.068	0.062
Control	0.142	0.162	0.118	0.132	0.128	0.161	0.156	0.143	0.156	0.146

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*

**Table 3:** Effect of different plant gums on (10% concentration) production of post-harvest fungi

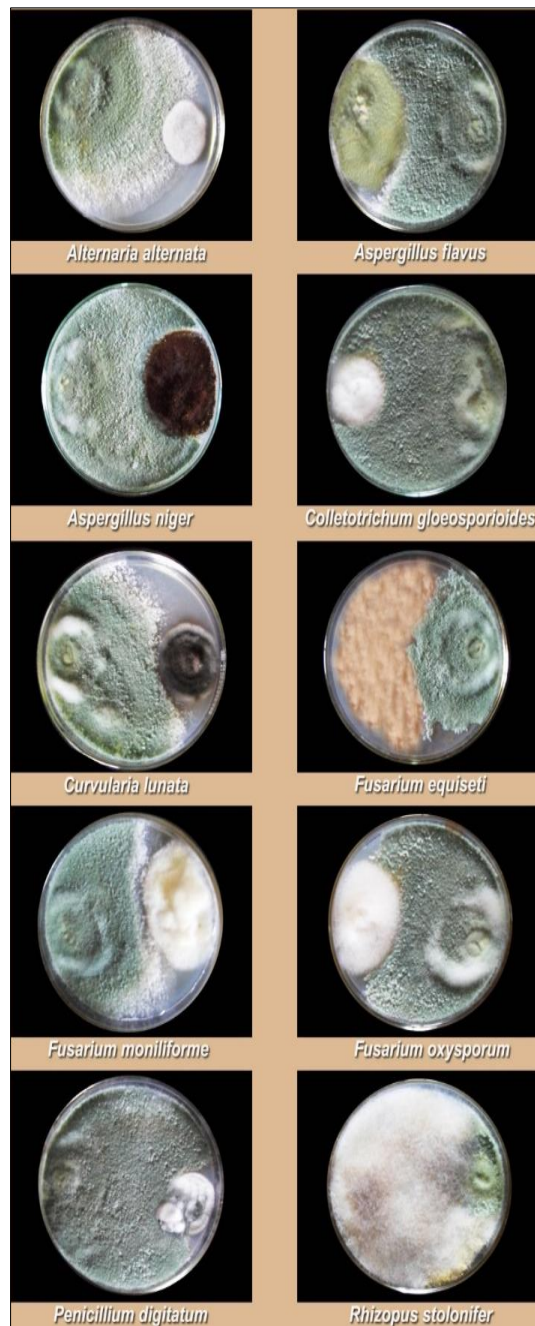
Plant gums	Dry weight of mycelium (mg)									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
<i>Acacia arabica</i>	0.043	0.121	0.117	0.043	0.027	0.127	0.118	0.038	0.082	0.206
<i>Acacia chundra</i>	0.047	0.194	0.088	0.079	0.093	0.091	0.094	0.103	0.093	0.131
<i>Boswellia sierata</i>	0.051	0.338	0.145	0.041	0.034	0.112	0.133	0.122	0.144	0.194
<i>Magnifera indica</i>	0.053	0.148	0.155	0.129	0.107	0.164	0.162	0.111	0.193	0.183
<i>Terminalia arjuna</i>	0.041	0.116	0.146	0.032	0.056	0.103	0.039	0.049	0.036	0.183
Control	0.142	0.162	0.118	0.132	0.128	0.161	0.156	0.143	0.156	0.146

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*

**Table 4:** Effect of fruit rinds on (10% concentration) production of post-harvest fungi

Fruit rinds	Dry weight of mycelium (mg)									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
<i>Manilkara Sapota</i>	0.041	0.155	0.252	0.065	0.047	0.097	0.081	0.097	0.201	0.305
<i>Magnifera indica</i>	0.111	0.164	0.157	0.067	0.138	0.070	0.074	0.052	0.195	0.164
<i>Citrus reticulata</i>	0.065	0.191	0.099	0.076	0.077	0.062	0.052	0.064	0.190	0.290
<i>Citrus Sinensis</i>	0.055	0.176	0.170	0.041	0.057	0.118	0.053	0.054	0.146	0.160
<i>Carica papaya</i>	0.156	0.224	0.101	0.097	0.132	0.129	0.109	0.156	0.278	0.248
<i>Pomegranate</i>	0.091	0.188	0.179	0.123	0.118	0.098	0.177	0.212	0.188	0.217
Control	0.142	0.162	0.118	0.132	0.128	0.161	0.156	0.143	0.156	0.146

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*



**Fig 1:** Photograph showing antagonistic activity of *Trichoderma viride*

**Table 5:** Antagonistic activity of *Trichoderma viride* against storage fungi

Fungi	(C)	(T)	% Inhibition
<i>Alternaria alternata</i>	7.4	2.3	68.91
<i>Aspergillus flavus</i>	7.2	4.6	36.11
<i>Aspergillus niger</i>	7.5	4.4	41.33
<i>Colletotrichum gloeosporioides</i>	6.4	2.6	59.37
<i>Curvularia lunata</i>	7.2	2.8	61.11
<i>Fusarium equiseti</i>	7.4	6.6	10.81
<i>Fusarium moniliforme</i>	7.2	3.8	47.22
<i>Fusarium oxysporum</i>	7.8	3.4	56.41
<i>Penicillium digitatum</i>	7.5	2.4	72.00
<i>Rhizopus stolonifer</i>	7.1	6.9	2.8

Radial growth (mm) in control set (C)

Radial growth (mm) in treated set (T)

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