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Prevalence of Different Post Harvest Rots of Papaya in Subtropical Zone of Himachal Pradesh

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Abstract: Papaya "The Common Man's Fruit" is grown throughout the tropics and sub tropics and is relished for its good taste and medicinal properties. Papaya fruits are highly susceptible to various post harvest rots. During the present investigation, different post harvest rots of papaya were found to be prevalent throughout the year at five fruit markets surveyed in district Hamirpur, Himachal Pradesh during May, 2019 to April, 2020. The pathogens associated were identified as *Colletotrichum fructicola*, *C. truncatum*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Fusarium pallidoroseum* and *Alternaria* sp. to cause various post harvest rots in the region. Among these rots, Fusarium rot caused by *F. pallidoroseum* was the most prevalent followed by anthracnose caused by *C. fructicola*. During pathogenicity experiments, an incubation period of 3 days each was recorded in case of *C. fructicola, C. truncatum* and *F. pallidoroseum* while, that of 1, 2 and 4 days was recorded in case of *R. stolonifer, A. flavus* and *Alternaria* sp., respectively indicating the confirmed association of these pathogens with the rots. Symptoms of each rot varied initially, but ultimately lead to rotting of the fruit.

keywords: Post harvest rots, Papaya, Symptoms, *Rhizopus stolonifer*, *Aspergillus flavus*, *Fusarium pallidoroseum*, *Alternaria* sp, *Colletotrichum fructicola*

Papaya (*Carica papaya* L.) is a native of tropical America and is grown throughout the tropics and subtropics for its melon like fruit and ranks third in importance among fruits. Papaya contains sugars and nutrients at high level and low pH values make them susceptible to fungal decay (Singh and Sharma 2007). Due to high moisture and nutrient content, papaya is highly susceptible to microbial spoilage caused by fungi, bacteria, yeast and moulds. During the post-harvest period fungal and bacterial diseases cause heavy loss (Yahaya and Mardiyya 2019). Various workers have reported the incidence of different post harvest rots *viz*., anthracnose, fusarium rot, stem end rot, rhizopus rot, aspergillus rot and penicillium rot to be prevalent in papaya (Hamim et al 2014, Velenzuela et al 2015, Saini et al 2017, Helal et al 2018) but, the incidence of anthracnose was highest followed by Fusarium rot. The major portion of papaya production i.e. 40-60 per cent losses of the total production at various papaya growing regions are reported due to perishable nature despite a large production with the highest productivity among all the fruits grown in India (Prasad and Paul, 2021). Anthracnose alone has been reported to cause huge losses in production which may approximate to 90 per cent (Velenzuela et al 2015). Many important postharvest fungi have been reported to be associated with papaya out of which, *Colletotrichum gloeosporioides*, causing anthracnose is the most common and widespread pathogen of papaya around the world (da Silva and Michereff 2013, Patel 2013, Chowdhury et al 2014, Velenzuela et al 2015, Yohannes et al 2015, Saini et al 2017). Anthracnose of papaya caused by *C. truncatum* was reported by Ramapersad (2011), Torezz – Calzada et al (2018) and dos Santos Vieria et al (2019). Other species of *Colletotrichum* are associated with post harvest rots in papaya include *C. fructicola* (Marquez-Zequera et al 2018) and *C. dematium* (Helal et al 2018). At local level, fungi such as *Fusarium* spp, *Alternaria solani*, *Rhizopus stolonifer*, *Penicillium digitatum* , *Guignardia* spp., *Cercospora papayae Botryodiplodia theobromae, Phomopsis caricae-papayae*, *Mycosphaerella* spp. and *Phytophthora palmivora* are other important postharvest fungi associated with papaya (Chowdhury et al 2014, Akinro et al 2015, Kadam et al 2019, Sharma, 2015 , Helal et al 2018). Keeping in view the importance of the different post harvest rots in papaya, present investigations were conducted with the objectives to conduct survey and surveillance of local fruit markets in sub-tropical zone of Himachal Pradesh to record the incidence and abundance of post-harvest rots of papaya.

MATERIAL AND METHODS

The present investigations were conducted during 2018-2020 in the Plant Pathology Laboratory, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur.

Survey and surveillance: The routine survey and surveillance was conducted from May, 2019 to April, 2020 in the local fruit markets *viz*., Hamirpur, Nadaun, Sujanpur, Sulagwan and Barsar of district Hamirpur (Himachal Pradesh) to record the incidence (%) of different post harvest rots of papaya. From each market, 15 diseased fruits were collected and brought to laboratory for identification of a particular rot and pathogen associated with it. The associated pathogen was preliminarily identified on the basis of microscopic characters and symptoms produced by it. Disease abundance of each rot was then calculated. Disease incidence (%) and disease abundance (%) were calculated.

Isolation and maintenance of the associated pathogen (s): Associated pathogens were isolated from diseased fruits of papaya by single spore isolation method (Tuite 1969). Pure cultures of fungal pathogens thus obtained were further purified and maintained by using hyphal tip method on PDA slants under aseptic condition and stored in refrigerator at 4-5ºC for further studies.

Identification of associated pathogen(s): The pathogen(s) were preliminarily identified on basis of cultural and morphological characteristics. Three of the cultures were further got identified from ITCC, New Delhi and two of the cultures were identified from National Centre of Fungal Taxonomy. However, one pathogen associated with the rot exhibiting very low abundance was identified on the bases of its microscopic characters up to genus level only.

Pathogenicity of associated pathogen(s): For the pathogenicity experiments, healthy mature fruits of papaya were selected from the papaya plants or procured from the local market, washed thoroughly with tap water, swabbed with sodium hypochlorite (1 part sodium hypochlorite and 3 parts distilled water) for 30 seconds and washed with sterile distilled water to remove the traces of sodium hypochlorite. The inoculated fruits were placed in plastic trays and covered with transparent polythene sheet internally sprayed with sterilized distilled water so as to maintain the appropriate humidity. These trays were then incubated at room temperature and data were recorded in terms of incubation period (days).

RESULTS AND DISCUSSION

Survey and surveillance: Post harvest rots were prevalent in all the areas surveyed throughout the year (Table 1). The mean incidence of post-harvest rots was be minimum (15.77%) in May, 2019 which increased drastically in June, 2019 (100%) and remained at the same level from the June, 2019 to December, 2019, at all the locations surveyed. In January, 2020, the mean incidence of the disease (77.03%) started declining and reduced to 34.00 per cent by April, 2020. On an average, mean disease incidence (81.11%) was maximum at fruit market Hamirpur followed by Nadaun (79.46%) and Barsar (77.17%) while, minimum mean disease incidence (73.91%) was at Sujanpur followed by Sulagwan (76.00%) (Fig. 4. 2). All five fungal species *viz., Colletotrichum fructicola, C. truncatum*, *Rhizopus stolonifer, Aspergillus flavus* and *Fusarium pallidoroseum* were predominantly associated with the post harvest rots in papaya (Table 2). However, in addition to these, *Alternaria* sp. was associated to a limited extent during three months of the year, only (Fig. 1). Three of the cultures were got identified from ITCC, New Delhi as *R. stolonifer, A. flavus, F.*

Table 1. Frequency of post-harvest rots of papaya at different locations in Himachal Pradesh

Month	Disease incidence (%) at location					
	Hamirpur	Nadaun	Sujanpur	Sulagwan	Barsar	Average
May, 2019	15.38	18.18	11.11	23.08	11.11	15.77
June, 2019	100	100	100	100	100	100
July, 2019	100	100	100	100	100	100
August, 2019	100	100	100	100	100	100
September, 2019	100	100	100	100	100	100
October, 2019	100	100	100	100	100	100
November, 2019	100	100	100	100	100	100
December, 2019	100	100	100	100	100	100
January, 2020	85.71	75.00	80.00	66.67	77.78	77.03
February, 2020	66.67	44.44	62.50	50.00	60.00	56.72
March, 2020	55.55	71.42	62.50	50.00	57.14	59.32
April, 2020	50.00	44.44	33.33	22.22	20.00	34.00
Average	81.11	79.46	79.12	76.00	77.17	78.57

pallidoroseum under ITCC No. 11309.20, 11310.20 and 11308.20, respectively and two cultures were got identified from National Centre of Fungal Taxonomy as *C. fructicola and C. truncatum* under Id No. 9013.20 and 9014.20, respectively. However, *Alternaria* sp. was identified on the basis of mycelium and spore structures etc. within the laboratory itself. *C. fructicola* was responsible for causing post harvest rot throughout the year from May, 2019 to April, 2020 except in June, 2019 while, *C. truncatum* was associated with post harvest rots in of September to December, 2019 only. *R. stolonifer* was present and caused post harvest rot from June to August, 2019, while, *A. flavus* was from May to November, 2019 which disappeared in December, 2019 and reappeared in April, 2020. *F. pallidoroseum* was associated in post-harvest rots throughout the year except in June, 2019. However, *Alternaria* sp. present from July to September, 2019 only.

The maximum mean abundance (59.17) was in F. *pallidoroseum* followed by *C. fructicola* (39.48) while, minimum mean abundance (1.61) was in *Alternaria* sp followed by *R. stolonifer* (19.64) (Table 3). *C. fructicola* was present throughout the year except in June, 2019. Irrespective of the locations, maximum mean abundance (86.11%) of the pathogen was in July, 2019 followed by August (78.70%) reducing up to 14.87 per cent in April, 2020 while, minimum mean abundance (11.00%) was in May, 2019. *C. truncatum* was present from September to December, 2019. Maximum mean abundance (83.31%) of the pathogen was in September, 2019 followed by October, 2019 (74.78%) reducing to 74.17 per cent in November, 2019 while minimum mean abundance (66.10%) was in December, 2019. *R. stolonifer* was recorded to cause post harvest rot from June to August, 2019. Irrespective of the location of survey, maximum mean abundance (85.50%) of the pathogen was in June, 2019 followed by 77.79 per cent in July, 2019 and reducing to 72.43 per cent in August, 2019. Rot caused by *A. flavus* was prevalent from May to November, 2019 and again in April, 2020. The maximum abundance of *A. flavus* was observed in July averaging 100 per cent at all the locations surveyed followed by June, 2019 (54.10%) while, minimum mean abundance (10.50%) was recorded in May, *F. pallidoroseum* was prevalent throughout the year except June, 2019. The mean maximum abundance (86.44%) of the pathogen was recorded in March, 2020 followed by February (84.85%) and January, 2020 (83.47%) while, minimum (17.20%) was in July, 2019. Rot caused by *Alternaria* sp. was prevalent from July, 2019 to September, 2019. The maximum average abundance (12.06%) of the pathogen was in August, 2019 while, the minimum (2.50%) was in September, 2019. Fusarium rot caused by *F. pallidoroseum* was the predominant rot at all the five locations surveyed, as its mean abundance was highest at each location (Fig. 2). Anthracnose caused by *C. fructicola* was next highest in terms of mean abundance at each location surveyed. However, *Alternaria* rot was least abundant at all the locations surveyed. Rest of the rots exhibited intermediate range of abundance at various locations surveyed in district Hamirpur.

Symptomatology: The symptoms of *C. fructicola* causing anthracnose were recorded as dark brown to black coloured sunken water soaked spots in which orange coloured acervuli were visible in the form of concentric rings (Fig. 3). However, *C. truncatum* produced dark brown to black sunken lesions with black coloured acervuli in concentric rings. In Rhizopus rot, a soft rot was observed which developed white to grayish black fluffy mycelium bearing sporangia. *A. flavus*

Table 2. Association of different pathogens with post harvest rots of papaya during the year 2019-20

Month	Pathogens associated
May, 2019	*Colletotrichum fructicola, Aspergillus flavus* and *Fusarium pallidoroseum*
June, 2019	*Rhizopus stolonifer* and *A. flavus*
July, 2019	*C. fructicola, A. flavus, R. stolonifer, F. pallidoroseum* and *Alternaria* sp.
August, 2019	*C. fructicola, A. flavus , R. stolonifer , F. pallidoroseum* and *Alternaria* sp.
September, 2019	*C. fructicola, C. truncatum, A. flavus* and *F. pallidoroseum* and *Alternaria* sp.
October, 2019	*C. fructicola, C. truncatum, A. flavus, and F. pallidoroseum*
November, 2019	*C. fructicola, C. truncatum, A. flavus* and *F. pallidoroseum*
December, 2019	*C. fructicola, C. truncatum* and *F. pallidoroseum*
January, 2020	*C. fructicola* and *F. pallidoroseum*
February, 2020	*C. fructicola* and *F. pallidoroseum*
March, 2020	*C. fructicola* and *F. pallidoroseum*
April, 2020	*C. fructicola, A. flavus* and *F. pallidoroseum*

produced soft rot bearing white mycelium with suppressed growth and green coloured conidial mass. In case of Fusarium rot, symptoms developed as sunken watery spots with white to yellowish or pinkish mycelial growth which later became soft with time. Symptoms of *Alternaria* sp. were recorded as brown semicircular to circular spots covered with dark brown mycelium (Fig. 3).

Pathogenicity: Pathogenicity tests were conducted by inoculating the pure culture bits of these pathogens on young uninfected/healthy papaya fruits and incubation period was recorded in each case (Table 4). The incubation period of 3 days each was in *C. fructicola, C. truncatum* and *F. pallidoroseum* while, 1, 2 and 4 days was recorded in *R. stolonifer*, *A. flavus* and *Alternaria* sp., respectively. Symptoms developed after artificial inoculation of pathogens on papaya fruit produced symptoms similar to natural symptoms on the fruits thus confirming their pathogenicity (Fig. 4). During present studies, various post harvest rots were found to be prevalent throughout the year being maximum from June to December. Rahman et al (2008) also reported an incidence of post-harvest rots in papaya to be as high as 90-98 per cent in Malaysia. Pooja et al (2012) reported the presence of *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *C. gloeosporioides, F. moniliforme*, *F.*

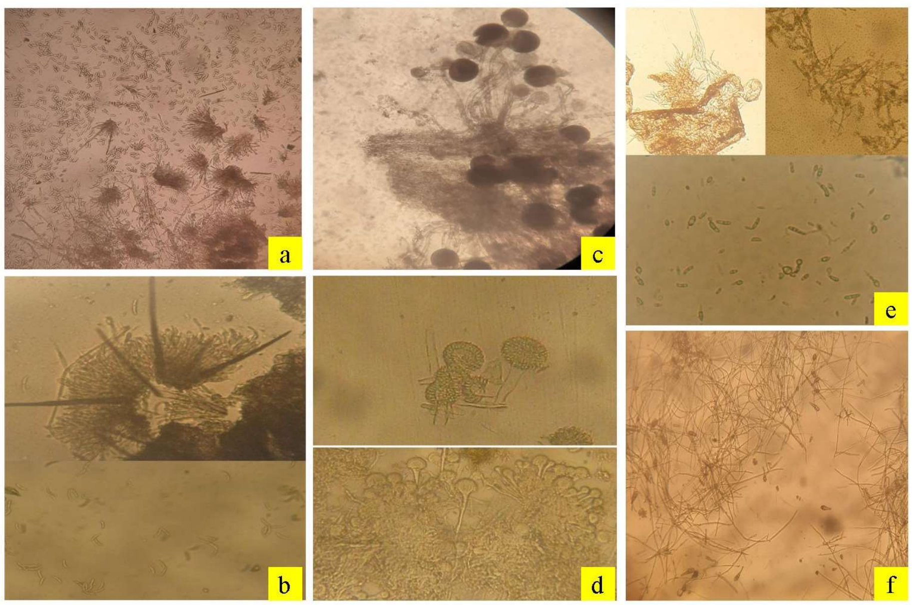

Fig. 1. Microscopic observation of a) *Colletotrichum fructicola* , b) *C. truncatum*, c) *Rhizopus stolonifer*, d) *Aspergillus flavus*, e) *Fusarium pallidoroseum* and *Alternaria* sp.

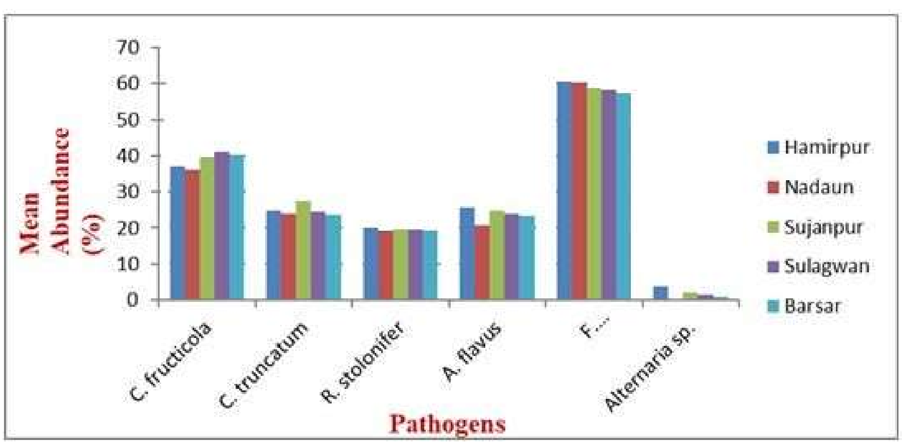

Fig. 2. Mean abundance of different post harvest rot of papaya at various locations of district Hamirpur during 2019-20

oxysporum and *R. stolonifer* in infected papaya fruits and highest total incidence and abundance of *A. flavus* in rainy season (July-Oct) and *F. moniliforme* during winter (November-February). The association of *C. truncatum* with the post harvest rot of papaya is confirmed by the findings of earlier researcher (Aktaruzzaman et al 2018, Torrez – Calzada et al 2018, dos Santos Vieria et al (2019) However, *C. fructicola* was associated with post harvest rots of papaya (Phoulivong et al 2012, Marquez-Zequera et al 2018) . Association of *Colletotrichum* spp. with the papaya fruit rots was recorded to be throughout the year except in June 2019. Patel (2013) also reported maximum prevalence of fruit rot in papaya was due to *Colletotrichum* sp.

The variation in abundance of different pathogens during various months of the year can be attributed to the fact that favorable conditions for the growth and development of pathogens vary according to the climatic conditions especially, temperature and relative humidity. These findings are also supported to certain extent by the findings of Pooja et al (2012) where *A. flavus* to be prevalent in the rainy season (July to October) and *Fusarium* sp. was abundant in winter months (November-February). Hamim et al (2014) reported the abundance of fusarium rot and anthracnose of papaya during November, 2012 to May, 2013 in Bangladesh. The symptoms of various post harvest rots recorded during present studies were similar to the symptoms of various rots observed in papaya by many workers earlier (Moraes et al 2013, Sharma and Kulshrestha 2015, Aktaruzzaman et al 2018).

The incubation of 1 to 4 days was recorded in different pathogenicity experiments. Vivekananth (2006) also reported an incubation period of three to four days in papaya fruits inoculated with *Colletotrichum* sp. Than et al (2008) inoculated chilli fruits artificially with *C capsici* as well as *C gloeosporioides* and reported that lesions appeared in 3 days after inoculation with wound/drop inoculation method. Popat (2013) also reported that symptoms appeared in 2-4 days in

Table 3. Average abundance of various post harvest rot of papaya in different months during 2019-20

Month	Disease abundance (%) at locations					
	C. fructicola	*C. truncatum*	*R. stolonifer*	*A. flavus*	*F. pallidoroseum*	*Alternaria* sp.
May, 2019	11.00	0.00	0.00	10.50	76.50	0.00
June, 2019	0.00	0.00	85.50	54.10	0.00	0.00
July, 2019	86.11	0.00	77.79	100.00	17.20	4.72
August, 2019	78.70	0.00	72.43	40.74	40.33	12.06
September, 2019	70.79	83.31	0.00	23.05	50.89	2.50
October, 2019	57.26	74.78	0.00	22.33	53.47	0.00
November, 2019	56.36	74.17	0.00	19.11	66.33	0.00
December, 2019	51.01	66.10	0.00	0.00	70.62	0.00
January, 2020	16.72	0.00	0.00	0.00	83.47	0.00
February, 2020	15.88	0.00	0.00	0.00	84.85	0.00
March, 2020	15.09	0.00	0.00	0.00	86.44	0.00
April, 2020	14.87	0.00	0.00	13.67	79.97	0.00
Average	39.48	24.86	19.64	23.63	59.17	1.61

Table 4. Symptoms and incubation period of various post harvest rots of papaya prevalent in district Hamirpur (HP)

Rot	Symptoms	Incubation period (days)
Anthracnose (*Colletotrichum fructicola)*	Dark brown to black sunken water soaked spots with orange coloured acervuli in the form of concentric rings.	3
Anthracnose (*Colletotrichum truncatum)*	Dark brown to black sunken lesions with black coloured acervuli in concentric rings.	3
Rhizopus rot	Soft, water soaked lesions with white to grayish black fluffy mycelium bearing sporangia	1
Aspergillus rot	Water soaked lesions with green coloured conidial mass.	2
Fusarium rot	Sunken, watery spots with white to yellowish and pinkish wavy mycelium later turning into soft rot.	3
Alternaria rot	Brown, semicircular to circular spots with dark mycelium.	4

Fig. 3. Symptoms of *a) C. fructicola, b) C. truncatum, c) R. stolonifer, d) A. flavus, e) F. pallidoroseum and f) Alternara* sp.

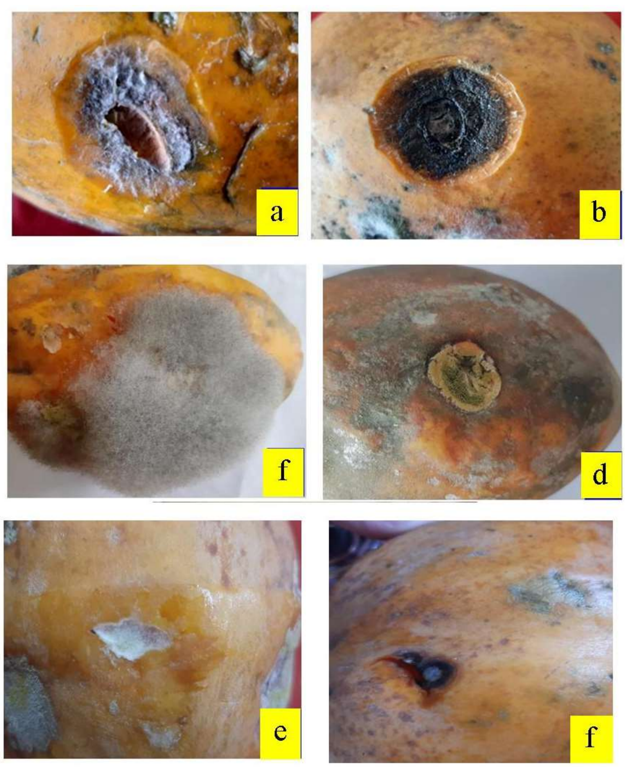

Fig. 4. Pathogenicity test of a) *C. fructicola, b) C. truncatum, c) R. stolonifer, d) A. flavus, e) F. pallidoroseum and f) Alternara* sp.

papaya fruits inoculated with *R. stolonifer* while, in 4-6 days when inoculated with *Alternaria* sp. Bautista-Banos et al (2008) also reported that *R. stolonifer* produced infection symptoms after 24 to 48 h in tomato in the presence of adequate temperature. Gore et al (2016) further strengthened findings and reported that symptoms of *Fusarium* sp. and *Alternaria* sp. appear in 2 to 4 days after inoculation on papaya fruits. The incubation period recorded in papaya fruits inoculated with *A. flavus* was in conformity with Louis et al (2013) who recorded an incubation period of 2 days in different plants inoculated with *Aspergillus* sp.

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