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Isolation and Identification of Fungi Associated With Spoilage of Mango Fruit (M.s.), India

Botany

KEYWORDS : Mango (Mangifera indica L.) Post harvest, diseases, fungal pathogen.

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ABSTRACT

Post harvest diseases of mango minimize the fruit quality and cause heavy losses, thus it does not meet the required standard and it causes economic loss in international market due to the considerable difference between export and local prices. In the present study the fruits were collected from different places of Marathwada region. The isolation of Fungi from different Varieties of mango was carried out on potato dextrose agar (PDA). It was found that after harvesting eight species of fungi associated with fruit decay. The most common fungi found were Alternaria alternata, Aspergillus falvus,Aspergillus fumigatus, Aspergillus niger, Botryodiplodia theobromae, Collectrichum gloeosporide, Penicillum chrysoenum, Rhizopus stolongifer. Pathogenicity test carried out revealed that all the fungi isolates were pathogenic. The fungi associated with the spoilage of the fruit colonial and morphological characteristics. Therefore this investigation gives a brief account of rotting of fruit were identified based on their and their infection organism.

INTRODUCTION:

Fruits constitute an important item of our food and they play a vital role in the human diet through the supply of vitamin and mineral. The recommended quantity of fruit to be consumed by a normal healthy adult is 230g/ day while the present per capita consumption of fruit is reported to be below 160g/day (Veeraraga Vathatham et al., 1996). Among the various fruit grown in India, mango is one of the most important tropical fruit. India ranks first in the world production of mango with a share of 54.3 percent.

Post harvest disease of fruit represent one of the most severe causes of loss of production (Eckert, 1977; Derbyshrine and Shipway, 1978; Harvey, 1978 and Coursey, 1983). The high moisture content of mango fruit make them highly susceptible to the attack of pathogen. Major loss of harvested mango fruit is caused due to fungi. The disease caused during pre harvest of fruit also responsible for the lowering the quality and market value of the fruit. Post spoilage of fruit may be due susceptibility of fruit to the fungal growth. Susceptibility of fruit to the attack of fungi is varying according to season, cutivar, mechanical, injuries and handling at the time of harvesting as well as by post-harvest treatment and storage condition. (Derbyshrine and Shipway, 1978 and Dennis, 1983). The most important post harvest disease of mango fruit are Anthracnose, Stem end rot, *Aspergillus niger* rot, soft rot, *Alternaria* rot (Arya 1993 and Philip 2002). Sangchote (1987), reported that *Colletotrichum gloeosporioids, Botryodiplodia theobromae, Dothiorella dominican, D. Mangifferae, Phomopsis mangiferae* and *Aspergillus niger* are common post harvest pathogen of mangoes in Thailand. Species belonging to *Rhizopus, Aspergillus, Colletotrichum, Botrydiplodia, Phompsis* and *Diplodia* have been reported to cause rotting of fruit during transit, storage and marketing injuries (Dasgupta and Bhatt, 1946, Kanitkar and Uppal, 1939; Thakur and Chenulu, 1970; Laxinarayan and Reddy, 1975; Thakur, 1972). Fungal pathogen involved in mango rotting after harvest include *Collectorichum gloeosporioides* responsible for mango anthraenose, *Alternaria alternata* and *Alternaria* tenuissima that cause alternariose, *Botryodiplodia theobromae* and *Dothiorell* Spp. responsible for stem end rot phoma mangiferae (Dodd et al., 1997; Arauz, 2000).

The high moisture content of mango fruit make them highly susceptible to attack of pathogen. Fungal infection alone were causing 17 percent losses annually in Warangal. Lele et al., (1975) and pathak (1980) reported that many fungal pathogen are attacking the mango fruit and majority of them are wound pathogen and causing extensive damage. Similar loss of harvest mango crop in India has been estimated to be about 40% by Singh (1960). The extent of loss caused by the individual pathogen has also seldom been estimated. Srivastava et al., (1965) while surveying the markets situated at different localities in India have recorded that the post harvest loss in mango varieties due to Aspergillus rot is 4.35 % Colletotrichum rot 5.-15% and Botryodplodia rot 6.5 -20%.

In developing countries, post harvest losses are often more severe due to inadequate storage and transportation facilities. Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post harvest storage and marketing condition or after purchasing by the consumer. Fruit contain high levels of sugar and nutrients element and their low PH values make them particularly desirable to fungal decayed (Singh and Sharma 2007). Studies by li-cohen and Bruhn (2002) had shown that fungi can survive and / or grown on fresh produce and that the nutrient content (Carbohydrate, Protein and fat) of fresh produce support pathogens.

Fruit are however affected by a wide array of microorganism causing its decay. These microorganism, under the influence of environmental factors, pose a serious threat to fruits production. Spoilage refers to any change in the condition of food in changes may be accompanied by the food becomes becomes less palatable, or even toxic, these spoilage alterations in taste, smell, appearance or texture (Akinmusire 2011) fungi that typically produce more diverse and greater amount of extracellular depolymerases successfully attack and spoil both fruits and vegetables. (Barth et al., 2009). Fungi in particular produce an abundance of extracellular pectinase and hemicellulase that are important factors for fungal spoilage (Meiedes and Lorenses, 2004). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas and Katasoudas, 2005).

Spoilage microorganism can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and post harvesting handling, or during storage and distribution (Barth et al., 2009). Those some type of soil borne spoilage microbes that occur on produce are

the same spoilage microorganism that are present on harvesting equipment on handling equipment in the parking house, in the storage facility, and on distribution chain (Barth et al.,2009). Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide dramatic reduction in yield loss due to spoilage at all subsequent steps in the food –to-fork continuum (Eckert and Ogawa 1988; Barth et al,. 2009).

Although available literatures revealed that the importance of fruit is increasing daily. The incidence of microbial attack on this fruit demands attention. Over the years, there has been an increase in the need to identify and isolate the fungi associated with their spoilage. The aim of this study was to isolate and identify the fungi that are associated with the spoilage of different varieties of mango fruit.

MATERIAL AND METHOD:

I) Isolation of Fungi from rotten mango fruit : Infected fruit of different mango varieties viz kesar local (Beed, Jalna, Nanded and Aurangabad) were collected from different districts of Marathwada region of Maharashtra State. Small pieces, measuring 2mm^2 each of infected tissue were cut off from fruit with the help of sterile sharp knife. Pieces of diseased fruit were washed with tap water and surface sterilized with 1% sodium hypochloride solution from 2 min, washed twice with sterilized distilled water and then dried using sterile filter paper.The pieces were separately transferred to sterilized petri–dishes containing potato dextrose agar (PDA) medium and incubated at 25°C for 10 days.

II) Identification of fungi isolates: Petri-dishes were observed daily and colonies of fungi were chosen.The isolated fungi were purified using single spore technique and then kept in a refrigerator on PDA medium (Gams et al.,1998). Pure colonies of fungal isolates were identified according to Ellis(1971)). Symptoms were confirmed by Koch's postulates.

III) Patongencity Test: Three healthy fruits from each sample were surface sterilized with 90% ethanol, and incisions were made on them using sterile 4mm cork borer; was use to cut pellets of agar containing the culture of fungal mycelia of the isolates. These fungi were then inoculated into the hole created on the healthy fruit in a laminar flow chamber. The inoculated wound was sealed with petroleum jelly. Two control with incision but not inoculated were established. The inoculated fruits and the controls were placed in a clean polythene bag (One fruit per bag) each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at 28°C for 5,10,15,20 and 25 days of incubation period. In another experiment such fruits were kept at different temp like 10°C, 20°C, 30°C, 40°C. and 50°C. Inoculated fruit were observed for symptom development. The casual agents were re-isolated from the infected fruits and compared with the original isolates (Akinmusire 2011). Pathogenicity experiment was also conducted with respect to different stages of mango fruit ripening like green stage, yellow stage and orange stage.

EXPERIMENTAL RESULT AND DISCUSSION:

i) Isolation of fungi from different varieties of mango fruit:

different colonies observed at the end of the procedure necessary for the isolation and identification of fungi associated with different varieties of mango, kesar,local (Beed,Jalna,Nanded,Aurangabad)

Table -1 : Isolation of Fungi from different varieties of Mango.

Disease	Causal organism	Varieties of Mango					
		Kesar	Local Beed	Local Jalna	Local Nanded	Local A'bad	
Alternaria rot.	Alternaria alternata	-	+	++	_	+	
Aspergillus falvus rot.	Aspergillus flavus	+	++	++	+	++	
Aspergillus fumigatus rot	Aspergillus fumigatus	++	+	_	++	++	
Aspergillus niger rot.	Aspergillus niger	++	+	++	++	++	
Stemend rot.	Botryodiplodia theobrame	+	++	+	+	+++	
Antnracose	Colletoricum gloeosporiodes	+++	+	+++	++	+	
Penicillium rot	Penicillium Chrysogenum	++	+	_	++	++	
Soft rot.	. Rhizopus Stolonifer	++	++	++	+++	+++	

Note: - = Absent, + = Minimum, ++ = Maximum +++ = Abundant

The fungal colonies spoiled the mango fruit cousing their deterioration. Occurrence of fungi isolates associated with the spoilage of different varities of mango fruit is shown in table No.1.

It shows that a total no.of 8 fungi isolates were obtained from different varities of mango fruit which were identified as *Alternaria Alternata, Aspergillus flavus, Aspergillus fumigatus,Aspergillusniger, Botryodiplodia theobromae, Colletotracum gloeosporioides, Penicillum crysogenum, Rhizopus stolonifer.* It is the clear from the table that Kesar variety of mango showed maximum incidents of fungi A. fumigates, A.niger, Penicillum crysogenum, Rhizopus stolonifer. Kesar and local Jalna variety showed abundant incident of Colletotricum goleosporiodes. Aurangabad variety show abundant incident of Botryodiplodi theobromae. Rhizopus stolinifer abundant on local Nanded and A,bad variety. Alternaria alternate occurred on Kesar , local Jalna,local A,bad while , A. fumigates, penicillum cryosogenum occurred on all varities except local Jalna .

It is clear from the table that, Kesar variety of mango showed maximum incidence of Fungi Aspergillus fumigatus, Aspergillus niger, Penicillium chrysogenum, Rhizopus stolenfier and abundant incidence of Colletotricum gloeosporiodes were found on all mango varieties. Local Aurangabad variety showed abundant incidence of fungi Botryodiplodia theobromae. Kesar and local Jalna variety showed abundant incidence of Colletotricum goleosporiodes. Rhizopus stolenifer abundant on local Nanded and local A'bad variety. Allernaria alternata occured on kesar, local Jalna and local Aurangabad while, Aspergillus fumigatus and penicillium chrysogenum occured on all varieties except local Jalna.

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Pathogencity Test:

ii) Effect of ripening stages on mango fruit rotting:

In order to study the effect of ripening stages on mango fruit rotting spore suspension of test fungi was inoculated in different stages of mango viz green, yellow and orange and results are given in table No.2.

It is clear from the table that as fruit ripening increased, disease severity was also increased. At green stage, Aspergillus niger showed maximum rotting while Collectotrichum gloeosporiodes showed severe rotting. At yellow stage maximum rotting was observed Aspergillus flovus, Botryodiplodia theobromae and Colletotricum gloeosporioides. At rippened stage Aspergillus niger, Colletotrichum sp. and Botryodiplodia theobromae showed sever rotting.

Table 2 : Effect of different ripening stages on mango fruit rotting.

Fungi	Stages		
	Green	Yellow	Orange
1. Aspergillus flavus	+	++	++
2. Aspergillus niger	++	+	+++
3. Colletoricum gloeosporiodes	+++	++	+++
4. Botryodiplodia theobrame	+	++	+++
5. Penicillium Chrysogenum	-	+	++
6. Rhizopus Stolenifer	+	+	++

Note : - = Rotting Absent + = Minimum Rotting ++ = Maximum Rotting +++ = Severe Rotting.

Effect of different incubation periods on mango fruit rotting:

Spore suspension of test fungi was inoculated in healthy mango fruit and was kept fro 10,15,20 and 25 days in order to study the effect of different incubation periods on mango. Fruit rotting and results are summarized in table 3.

It is clear from the table that as incubation period increased mango fruit rotting was also increased. Rotting was began after 10 days up to some extent rotting was caused by Aspergillus niger, Botryodiplodia theobromae and Rhizopus Stolenifer after 10 and 15 days. Aspergillus niger, Botryodiplodia theobromae and colletotrichum gloeosporioides showed severe rotting to mango fruit after 20 and 25 days.

Table 3 : Effect of different incubation period on mango fruit rotting.

Fungi	Incubation period (Days)				
	5	10	15	20	25
1. Aspergillus flavus	--	+	+	++	+++
2. Aspergillus niger	--	++	+++	+++	+++
3. Colletoricum gloeosporiodes	--	+	--	+++	+++
4. Botryodiplodia theobrame	--	++	++	+++	+++
5. Penicillium Chrysogenum	--	+	--	++	++
6. Rhizopus Stolenifer	--	++	++	++	++

Note: - = Rotting Absent + = Minimum Rotting ++ = Moderate Rotting +++ = Severe Rotting.

Effect of different temperature on mango fruit rotting:

In order to study the effect of different temperature on mango fruit rotting spore suspension of test Fungi was inoculated in healthy mango fruit and was kept at 10 °C, 20°C, 30 °C, 40° C. and 50°C. temperature and results are given in table No.4.

It is clear from the table that 30° temperature was found to be favorable for Aspergillus niger, colletotrichum gloeosporioides and Botryodiplodia theobromaeto cause sever rotting to mango fruit. Only Aspergillus niger showed sever rotting to mango fruit at 40° C on the other hand, Botryodiplodia theobrmae and Rhizopus stolonifer showed moderate rotting at 40°

Table 4 : Effect of different temperature on mango fruit rotting.

Fungi	**Temperature** (°C)				
	10	20	30	40	50
1. Aspergillus flavus	--	++	++	+	--
2. Aspergillus niger	+	+	+++	+++	+
3. Colletoricum gloeosporiodes	+	++	+++	--	--
4. Botryodiplodia theobrame	+	++	+++	++	+
5. Penicillium Chrysogenum	--	+	++	--	--
6. Rhizopus Stolenifer	--	++	++	++	--

Note: - = Rotting Absent + = Minimum Rotting ++ = Moderate Rotting +++ = Severe Rotting.

In this study all the fungi isolates were found to be pathogenic on all fruits. The rot symptoms obtained were similar to those observed previously on the fruit when subjected to identification procedures by examining their morphological colonial characteristics. The pathogencity test showed that each infected fruit gave the initial organism that caused the spoilage of fruit.

Similar results are reported by at different grade of ripening of banana. Similarly, staghellini and Aragaki (1966) reported that spores C. gloeosporiodes causes infection only on unwounded detached mature fruits. This clearly indicates that green fruit do not meet the nutritional requirement of the fungus. The antfungal activity or enzyme potency is more at green fruit stage as compare with fully ripening fruit. It may possible that some toxin may be produced in the green fruit which later on disappear in the stage or become inactive in the ripened fruits. Tandon and Mishra (1969) reported that the infected results of the microorganism reduced the market value and nutrional value of fruit may be due to its physical appearance or may be due to the stored products.

From obtained results it is clear that maximum mango fruit rotting was at 30° C. present finding confirm the earlier report of Lukose and Singh (1997) on pomegranate rot, Baiyewu and Amusa (2005) on papaya rot, Singh and Sumbali (2007) on apple rot; suggested maximum fungal rot between the temperature of 30-35° C. Establishment and progress of post – harvest fruit diseases is depend upon the availability of a suitable temperature. The optimum temperature which favor the growth and sporulation of a pathogen invitro culture is generally also suitable for the development and spread of the corresponding fruit rot (Ghos , 1998).

Pathak and Srivastava (1969), Prasad and Sinha (1981), Meah et al., 1999 and Patil and Pathak (1993). Observed that the fruit incubated at 10° C did not develop any symptoms of Botryodiplodia rot of mango fruit. highest fruit incubated 25°C spore germination was highest at 25°C while at 10°C. germination did not occur after 6 and 24 hours. While Patel and Pathak (1995) reported that 30°C temp responsible for severity in Botryodiplodia rot of guava fruit.

Jadeja (2000) also revealed that at 30° C temperature was found to be optimum or stem end rot development. Stem end rot hampered at low temperature and it did not develop up to 15°C Sumia et al., (2006) found that Botryodiplodia rot of mango fruit was sever at 25°C -30°C and at high humidity.

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