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POST-HARVEST FUNGAL DISEASES OF PAWPAW (*CARICA PAPAYA L.*) FRUITS AND SEEDS IN NIGERIA

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ABSTRACT

Post-harvest fungal diseases of pawpaw (*Carica papaya* L.) fruits sold in Mile 3 Market, Port Harcourt were investigated bi-weekly for sixteen weeks using the Standard Blotter Method. The following fungi were isolated from the tissues of diseased fruits: *Fusarium solani, Phoma carica-papaya Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Cladosporium herbarum, Colletotrichum dematium, Fusarium moniliforme, Phomopsis carica-papaya, Penicillium sp., and Rhizopus stolonifer.* Seeds of diseased fruits were also tested for health using the Standard Blotter Method and all the fungi isolated from the fruit tissues were found to be seed-borne except *F. moniliforme.* Pathogenicity test of all the fungi isolated from ripe fruit tissues and seeds were carried out on mature green pawpaw fruits and they were found to be pathogenic. *Rhizopus stolonifer* and *F. solani* caused the greatest rot on the fruits. Agar discs (3.0mm, diameter) of these fungi were subjected to hot water treatment at varying periods of time, (40°C for 30 mins., 50°C for 20 mins., and 60°C for 10 mins.) to determine the efficacy of the treatments on the pathogens. The hot water treatments could not eliminate the pathogens; instead their lineal growths on PDA medium were just inhibited, when compared with their controls. A first report of seed-borne fungi of pawpaw and their pathogenicity is provided.

KEY WORDS: Pawpaw fruits, seed-borne fungi, pathosenicity test, hot water treatment.

INTRODUCTION

Pawpaw is propagated by seed sown directly in the field. Seeds are taken from the ripe fruits, dried and then planted in the field. Pawpaw trees produce mature fruits about 10 months after planting and then bear the year around (Hartmann *et. al.,* 1988). Pawpaw fruits may be consumed fresh alone or in mixed fruit salads or may be made into juice, pickles, preservatives or jelly (Kochhar, 1986). The ripe fresh fruits are used for making jam, ice cream flavouring, crystallized fruit or canned in syrup (Schery, 1972). Ripe fruits contain about 8-10% sugar and are rich in vitamins A, B1, B2 and C. Immature fruits can be cooked and eaten as vegetables or tapped for its latex known as papain (Kochhar, 1986). Papain is used for tenderizing of chewing gum, in the shrinkage of wood and in curing pyrorrhoea, a disease of gums (Kochhar, 1986).

Unfortunately the increase in productivity of this economic fruit is hampered by dangerous field and storage fungal diseases. Snowdon, (1990) reported important post-harvest diseases of pawpaw found in pawpaw growing countries of the world. These diseases include: anthracnose, black rot, Phytophthora rot, Rhizopus rot, stem-end rot, Alternaria rot, Aspergillus rot, blue mould rot, Cercospora rot, Fusarium rot, pink mould rot, greasy spot, purple stain and Stemphylium rot. These diseases need to be control to reduce post-harvest losses and increase market values.

There are two ways of improving the out put of food production. These involve increasing crop productivity and avoiding crop failures (Neergaard, 1970). This duality holds true, particularly in relation to the fundamental demand for better healthy seeds. Fungicides in wax and heat treatment are the two ways used to control post-harvest diseases of pawpaw fruits (Alvarez and Nishijima, 1987; Rirka and Douglas, 1991) but because of the environmental effects of chemicals, heat treatment is preferable even though it retards ripening (Couey *et. al.* 1984). More than 50% of pawpaw fruits sold in our market are never bought and eaten due to post-harvest rot diseases of pawpaw caused mainly by fungal pathogens. Some of these fungi are known to be seed-borne and seed-transmitted (Neergaard, 1979, Snowdon, 1990). Since pawpaw is propagated by seed sown directly in the field, seeds serve an channels through which these seed-borne fungi can be introduced in disease-free fields and other crops where mixed cropping is practised.

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This study was therefore undertaken:

- To isolate and identify fungi associated with post-harvest diseases of pawpaw fruits and seeds.
- To determine the pathogenic potentials of these fungi.
- To determine the effects of hot water treatment on these fungi (pathogens).

MATERIALS AND METHODS

Source of Pawpaw Fruits

The pawpaw fruits used in the study were purchased from Mile 3 market located at Bishop Okoye Street off Ikwerre Road, Diobu, Port Harcourt. The fruits were bought biweekly for four months from different pawpaw sellers in the market.

Isolation and Identification of Fungi from Ripe Diseased Pawpaw Tissues

The ripe diseased fruits were swabbed with 2% sodium hypochlorite (NaCLO) to remove surface contaminants. Then diseased portions including healthy areas were carefully cut out with a sterile kitchen knife and sliced into small pieces (2x2mm). The cut pieces were placed equidistantly in Petri dishes containing three layers of sterile filter papers soaked in sterile distilled water (ISTA, 1976). Five pieces were plated per Petri dish and five dishes were used per fruit. A total of five fruits were used per biweekly study. After plating, the Petri dishes were incubated for 7days at room temperature (25°C ± 2°C). At the end of the incubation period, the tissues were examined under a Wild M5A Stereobinocular Microscope (5-50X) for fungal growth and identification. Temporary slides were made and observed under a research compound microscope (5-100X) to confirm identification. Identifications were made following fungal descriptions by Barnett and Hunter (1991), Booth (1977) and Street (1975).

Isolation and Identification of Fungi from Seeds of Ripe Diseased Pawpaw Fruits

Seeds of ripe diseased pawpaw fruits used for the skin tissue test were tested for seed-borne fungi. The seeds of each fruit were scrapped out into Petri dishes. With a sterile forceps, seeds were picked and placed into Petri dishes containing three layers of sterile filter papers soaked in sterile distilled water (ISTA 1976). Twenty-five seeds were plated per Petri dish and three dishes were used for each pawpaw fruit in each biweekly study. The dishes were incubated as previously described. On the 7th day, seeds were examined for fungal growth and identified as previously stated.

Pathogenicity Test of All Isolated Fungi

Pure cultures of 11 fungi (*A. flavus, A. niger; theobromae, C. herbarum, C. dematium, moniliforme, F. solani, Phoma carica-papa Phomopsis carica-papaya, Penicillium sp. and stolonifer*) isolated from ripe diseased pawp fruits and their seeds we inoculated into healthy-looking, unripe, matu pawpaw fruits. The aim was to determine th pathogenic potential. Two pawpaw fruits we used for each fungal treatme and control. The fruits were surface sterilised wi cotton wool soaked in 70% ethanol and sterilised cork borer (Size 3) was used to bor three holes (stem end, middle and tail-end) pe pawpaw fruit. The same cork borer, was flame and after cooling, used to bore out inoculi disc from 7-day old fungal cultures grown on potat dextrose agar (PDA) medium. The inoculum discs of each fungus were inoculated into the three pawpaw fruits holes made with the cork borer. Vaseline cream was used to seal the surfaces of the fruits to avoid any external contamination. Cotton wool was used to cover the areas where vaseline cream was applied. Two of the unripe healthy-looking pawpaw fruits were inoculated with agar plugs without any fungus. These served as control. All the inoculated fruits were incubated for 7 days at room temperature (25°C ± 2°C). On the 7th day, the pawpaw fruits (treated and control) were excised horizontally with a sterile kitchen knife and the magnitude or intensity of rot examined, assessed and recorded. The fungi that caused rot were re-isolated in pure culture, characterized and compared with original cultures of fungi isolated from diseased fruits bought from the market.

Effects of Hot Water Treatments on the Isolated Fungal Pathogens

All the eleven fungi isolated were subjected to hot water treatment at varying periods of time (40°C for 30mins., 50°C for 20mins., and 60°C for 10mins.) to determine the effects of hot water treatment on the fungi. Small McCarthney bottles with caps removed were divided into sets of three and three sets were used for each fungal treatment. A sterile cork borer (Size 3) was used to bore out discs of each fungus grown on PDA in Petri dishes. The fungal discs were put into the bottles and hot water was added according to the different temperature regimes. Four discs, of each fungus were put per bottle. Hot water, boiled to 100°C was allowed to cool down to the desired temperatures before pouring it onto the bottles containing the discs. The temperature of the water was maintained by putting the bottles in temperature-controlled water baths. At the end of each treatment period, water was decanted from

Table I. Fungi Isolated From Ripe Diseased Pawpaw Fruits In Four Months Studies

Fungi Isolated	Wk.2	Wk.4	Wk.6	Wk.8	Wk.10	Wk.12	Wk.14	Wk.16
Aspergillus flavus	-	-	-	+	-	-	-	-
Aspergillus niger	-	-	-	+	-	-	+	-
Botryodiplodia theobromae	-	+	-	+	+	+	+	-
Cladosporium herbarum	-	-	-	+	+	+	-	-
Colletotrichum dematium	+	+	+	+	+	+	+	+
Fusarium solani	+	+	+	+	+	+	+	+
Fusarium moniliforme	-	+	-	-	-	-	-	-
Phoma caricae-papaya	-	+	+	-	+	-	+	-
Phomopsis caricae-papaya	-	-	-	+	+	-	-	+
Rhizopus stolonifer	+	+	+	+	+	+	+	+

+ means present and – means absent.

the bottles and the fungal discs plated one disc per dish on PDA medium in Petri dishes. Fungal discs without hot water treatments served as controls. The dishes were incubated in an incubator (25°C ± 2°C) for seven days. At the end of the incubation period, the discs were examined for fungal growth and results recorded.

RESULTS

Fungi Isolated From Diseased Pawaw Fruit

The fungi isolated from diseased ripe pawpaw fruits in the bi-weekly studies are presented in Table I. Results showed that *F. solani, R. stolonifer* and *C. dematium* were isolated throughout the bi-weekly studies, followed by *B. theobromae* and *Phomopsis carica-papaya* which occurred 5 times out of the eight bi-weekly studies. *Aspergillus flavus* and *C. herbarum* were identified only in three of the eight bi-weekly studies, while *Aspergillus niger* and *F. moniliforme* were identified only once in all the eight bi-weekly studies (Table I).

Fungi Isolated From Seeds Of Diseased Ripe Pawpaw Fruits

The same fungi that were isolated from the pawpaw fruits (Table I) were also isolated from the seeds in the bi-weekly studies (Table 2). *Fusarium solani* gave the highest occurrence of fungi on pawpaw seeds. It infected 707 seeds out of a total of 3000 seeds tested. *Rhizopus stolonifer* was recorded on 174 seeds, *C. herbarum* on 55 seeds and *B. theobromae* on 53 seeds, out of a total of 3000 tested seeds. *Colletotrichum dematium, Phoma carica-papaya* and *Phomopsis carica-papaya* were identified at very low frequencies. *Aspergillus niger* appeared only on 5 seeds. *Penicillium sp. and A. flavus* were recorded on 2 and 1 seed(s) respectively.

Pathogenicity Studies of Fungi Isolated From Diseased Pawpaw Fruits Seeds

Results of pathogenicity studies showed that all the fungi tested have the ability to cause rot at varying degrees of severity. No symptoms were observed on the control fruits inoculated with agar plugs only. *Fusarium solani* caused small, dry lesions around the inoculated areas of fruits. These were later covered with a white, compact, fluffy, mycelial mat. *Phomopsis carica-papaya* caused wet rot. The entire inoculated areas were soft and translucent. *Phoma carica-papaya* caused stem-end rot while *Colletotrichum dematium* caused anthracnose lesion with white discolouration of pawpaw flesh. *Botryodiplodia theobromae* caused soft, charcoal rot at the stem-end and the fungus penetrated the inner tissues. *Rhizopus stolonifer* was the most destructive of all the fungi. The fungus invaded the entire fruit with mass of coarse, grey mycelium with black macroscopic sporangia. It caused watery fruit rot. *Aspergillus* and *Penicillium* spp. caused the least rot damage when compared with rot damages caused by the other fungi. *Aspergillus flavus* developed yellowish or greenish soft rot at the

inoculated areas while *A. niger* caused dark-brown soft rot at the inoculated areas. *Penicillium* sp. caused blue-grey colouration with mild soft rot at the inoculated areas.

Effects of Hot Water Treatments on the Pathogens

Hot water treatment at specific time periods did not control the growth of the pathogens. The hot water treatments only retarded the growth rates of the pathogens. However, fruits that were treated with hot water for 10mins. at 60°C gave the greatest retardation of fungi followed by fruits treated for 20mins. at 50°C. Hot water treatment also retarded ripening of treat fruits when compared with control fruits.

DISCUSSION

The fungi

Aspergillus flavus, A. niger, B. theobromae, C. dematium, C. herbarum, F. moniliforme, F. solani, Phoma carica-papaya, Phomopsis carica-papaya, Penicillium sp., and R. stolonifer associated with the pawpaw fruits and seeds were found to be pathogenic. These fungi have been reported to cause serious pawpaw fruit diseases in many pawpaw growing countries of the world (Snowdon, 1990; Srivastava, 1977). These fungi affect the market value and nutrient levels of the fruits (Snowdon, 1990).

Fungi identified on seeds is the first report of seed-borne fungi of pawpaw fruits reported in

Table 2. Seed – Borne Fungi Isolated From Ripe-Diseased Pawpaw Seeds

Fungi Isolated	Wk.2	Wk.4	Wk.6	Wk.8	Wk.10	Wk.12	Wk.14	Wk.16	Total no of fungi isolated in the 8 bi-weekly expe-riments.	Mean of fungal occur-rence
Aspergillus flavus	-	-	-	-	1	-	-	-	1	0.13
Aspergillus niger	-	-	5	-	-	-	-	-	5	0.63
Botryodiplodia theobromae	-	37	2	-	7	7	-	-	53	6.63
Cladosporium herbarum	-	29	19	1	2	-	-	4	55	6.88
Colletotrichum dematium	-	-	4	5	-	20	16	-	45	5.63
Fusarium solani	56	115	75	98	128	73	64	98	707	88.38
Phoma caricae	-	18	-	5	5	-	-	-	28	3.50
Phomopsis caricaepapaya	-	-	-	6	19	15	-	4	44	5.50
Penicillium sp.	2	-	-	-	-	-	-	-	2	0.25
Rhizopus stolonifer	5	15	4	5	3	92	43	7	174	21.75

25 seeds were plated per Petri dish and three dishes were used for each fruit seeds in each biweekly study. - Means absent.

Nigeria. The pawpaw seeds are means of propagating pawpaw. If the seeds are infected by these seed-borne fungi, the planting values of the crop will be affected. In addition, infected seeds serve as means of spreading the pathogens to disease-free fields and to other crops, where mixed cropping is practised. Rirka and Douglas (1991) reported that hot water temperatures and time of 40°C for 30mins., 50°C for 20mins. and 60°C for 10mins. controlled these fungi. The findings of this work contradict this accession. The differences in result may be due to factors such as physiological, ecological and varietal differences, as well as age of the pawpaw fruits. Ages of the plants bearing the fruits can also influence this disparity in results. Proper storage of pawpaw fruits after harvest has continued to pose a serious problem to growers of this crop in every pawpaw growing country (Snowdon, 1990).

Therefore adequate care should be taken during harvesting to avoid bruises and/or wounds on the pawpaw skin since most of the pathogens have been reported to penetrate the fruits through wounds and bruises (Alvarez and Nishijima, 1987). It has also been found that rot can be spread through contact of healthy fruits with infected fruits (Kulshrestha *et. al.* 1976). Therefore healthy fruits should be separated from infected fruits. Results of this study has shown that hot water immersion for 10mins. at 60°C followed by proper handling of fruits to prevent bruises will substantially reduce post-harvest decay and extend storage life of unripe fruits during surface shipment to other places. Hot water treatment also was found to retard ripening. This is important because it provides excellent inhibition of post-harvest disease problems of fruits in transit (Alvarez and Nishijima, 1987). Unripe pawpaw fruits are more resistant to rot pathogens than ripe fruits (Alvarez and Nishijima 1987).

REFERENCES

Alvarez, A.M. and Nishijima, W.T., 1987. Post-harvest diseases of papaya. Plant Disease 71: 681-686.

Barnett, H. L. and Hunter, B. B., 1991. Illustrated Genera Of Imperfect Fungi. (4th Edition) Macmillan Publishing Company, New York.

Booth, C., 1977. Fusarium-Laboratory Guide to the Identification of the Major Species. Commonwealth Myeological Institute, Kew, Surrey, England. 58p.

Couey, H. M., Alvarez, A. M. and Nelson, M. G., 1984. Comparison of hot water spray and immersion treatments for control of post-harvest decay of papaya. Plant Disease 68: 436-437.

Hartmann, H. T., Kofranek, A. M., Rubatzky, V.E. and Flocker, W. J., 1988. Plant Science:

ISTA, 1976. International Seed Testing Association. International Rules for Seed Testing. ISTA Secretariat, Zurich, Switzer land.

Growth, Development, and Utilization of Cultivated Plants. (2nd Edition). Prentice-Hall Inc. Englewood, Cliffs, New Jersey. 674p.

Kochhar, S. L., 1986. Tropical Crops. A Text Book of Economic Botany. Mcmillian Publishing Company, London. 467p.

Kulshrestha, D. D., Mathur, S. B. and Neergaard, P., 1976. Identification of Seed-borne species of *Colletotrichum*. *Friesia* 11:116-125

Schery, R. W., 1972. Plant and Man (2nd Edition). Prentice-Hall Inc. Englewood, Cliff, New Jersy. 657p.

Snowdon, Anna, 1990. A Colour Atlas of Post-harvest Diseases and Disorders of Fruits and Vegetables. Vol. I: General Introduction and Fruits. Wolfe Scientific Ltd., London. 302p.

Street Sr. R. B., 1975. The Diagnosis of Plant Diseases: A Field and Laboratory Manual Emphasizing the most Practical Methods for Rapid Identification. University of Arizona Press, Tucson, Arizona. 11.11p.

Srivastava, M.P. and Tandon, R. N., 1977. Post-harvest diseases of papaya. PAN 17:51-54.

Rirka, B. G. and Douglas, J. P., 1991. Post-harvest heat treatment of fresh fruits and vegetables for decay control. Plant Disease 75(11):1085-1089.

Neergaard, P., 1970. Seed Pathology: International Cooperation and Organization. Seed Science and Technology. 35:19-42.

Neergaard, P., 1979. Seed Pathology Vols. I & II, MacMillian Press Ltd; London 839p.