African Journal of Agricultural Research Vol. 5(12), pp. 1531-1535, 18 June, 2010
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X ©2010 Academic Journals

Full Length Research Paper

Antifungal effects of pawpaw seed extracts and papain on post harvest *Carica papaya* L. fruit rot

Nwinyi, Obinna Chukwuemeka* and Abikoye Busola Anthonia

Department of Biological Sciences, School of Natural and Applied Sciences, College of Science and Technology, Covenant University, Km 10 Idiroko Road, P. M. B. 1023, Ota, Ogun State, Nigeria.

Accepted 25 May, 2010

Increasingly, public debate on ban of use of synthetic chemicals for pest control has been unabated, due basically to the hazards posed by such chemicals to the ecosystem and environment. Biological control using natural products presents as alternative and a viable means of control of pests. Effects of extracts from *Carica papaya.* L (seed and papain) on mycelial reduction of the most occurring fungal pathogen causing pawpaw fruit rot were investigated. Different fungi isolated were *Rhizopus* spp, *Aspergillus* spp and *Mucor* spp. The aqueous seed extract and papain exhibited remarkable mycelial inhibition with mean zones of inhibitions between (0.23 - 1.73 mm). Using ANOVA at 5% (P < 0.05) there seem to be no significant difference in activity between the extracts (aqueous seed extract and papain).The importance of these findings is hinged on non- chemical means of shelf life elongation of harvested pawpaw fruit in Africa.

Key words: *Carica papaya* L., aqueous seed extract, fungi, papain, pawpaw fruit rot.

INTRODUCTION

In Africa, fruit production has become an integral part of daily life as a source of food, vitamins and minerals. It serves as a means of trade and barter, food security and employment. In Nigeria, pawpaw is one of the most popular, cheapest, economically important fruit tree grown and consumed for its nutritional content (Baiyewu and Amusa, 2005). Pawpaw (*Carica papaya* L.) belongs to the family *Caricaceae* with over 22 species and only one member of the genus *Carica* that is cultivated as a fruit tree while the other three genera (*Cyclicomorpha, Jarilla* and *Jacaratia* are grown primarily as ornamentals (Burkhill,1966; Storey, 1985; Badillo,2000). Pawpaw (*C. papaya .L)* is short lived, with a hollow unbranched herbaceous stem. It is dual or multipurpose early bearing, space conserving crop. Papaya may be monoceious or hermaphrodite. Female trees have been found to differ in the amounts of the compounds produced. For instance, phenolic compounds tend to be higher in male plants than female plants.

According to the reports of Okeniyi et al. (2007) *C. papaya L*. fruit and seeds have antihelminthic and antimoeboic activities. Externally, the latex (papain) is irritant, dermatogenic and vesicant. The roots extracts have been reported to inhibit fungal growth, especially *Candida albicans* (Giordani and Siepai, 1991). From previous studies, papaya leaves have been reported to contain carpaine which has high antioxidant content that may be helpful for the prevention of atherosclerosis, diabetic heart disease, heart attacks and strokes. Pawpaw (*C. papaya L.)* improves the immune system and prevents illnesses such as recurrent ear infections, colds and flu.

Pawpaw (*C. papaya L.)* extracts have exhibited inhibitory effects on gram-positive bacteria and gram-negative bacteria. These organisms include: *Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterobacter cloacae* and *Proteus vulgaris* (Emeruwa, 1982). In humans, following treatment with antibiotics, papaya juice helps to restore normal intestinal flora destroyed by the antibiotics. Pawpaw (*C. papaya L.)* peels serve as poultice for treatment of skin wounds that do not heal quickly. Pawpaw fruit stimulates milk production in weaning mothers, prevents constipation, nausea, morning sickness, motion sickness, cataract formation, chronic obstructive pulmonary disease,

*Corresponding author. E-mail:nwinyiobinna@gmail.com. Tel: +234 (0)8037027786.

diverticulosis and hypertension. *C. papaya L.* contains enzymes such as papain, lipase and chymopapain. The papain and chymopapain can induce allergies when inhaled accidentally and Lipase, a hydrolase, is considered as a “naturally immobilized” biocatalyst. The levels of these enzymes vary in the fruit, latex, seeds, leaves and roots. As with many tropical crops, pawpaw fruits are beset with problems of field and storage rot. *C. papaya L.* is host to various species of pests and pathogens. Plant pathologists have reported about 39 arthropods that infest papaya (Singh and Sirohi, 1977; Morton, 1987). Papaya fruit fly (*Toxotrypana curvicauda*) is one of the principal insect pests that affect *C. papaya* throughout the tropical and sub tropical areas. Others include *Tetranychus urticae*, *Brevipalpaus carlifornicus* which cause damage by penetrating plant tissue. Nematodes namely *Rotylenchulus reniformis, Meloidogyne* spp*, Helicotylenchus dihysteria*, have been reported to cause root disease in *papaya*. Fungal pathogens such as *Phytophthora palmivora* causes root and fruit rot in *papaya*, *Collectricum gloerosporoides* causes anthracnose, *Asperisporium caricae* causes black spot in *papaya*.

Since the mid 1950s bacterial species such as *Pseudomonas carica papayae* have been known to cause small circular to angular dark green water soaked lesions on the lower surface of papaya leaves. *Erwinia cloacae* a gram negative rod shaped facultative anaerobe causes internal yellowing of leaves.

In developing countries, post harvest losses have been reported to be between 40 - 100% (Oludemokun, 1976). The losses are mainly due to decay, physiological disorders and mechanical injury. According to Kuthe and Spoerhase (1974), Gupta and Pathak (1986), Baiyewu (1994), *Aspergillus niger, Rhizopus nigricans, Aspergillus flavus, Rhizopus oryzae* and *Fusarium moniliforme* of fungal origin are responsible for post-harvest losses in pawpaw. Besides the economic losses to pawpaw fruit marketers, the rotten fruits could cause serious health hazards to consumers (Eaton and Groopman, 1994). Some of the harmful metabolites produced by pawpaw spoilage organisms include: Ochratoxins, Fumonisins and Aflatoxins produced by *Aspergillus spp.* These toxins when ingested by humans may cause severe effects in respiratory tract which may lead to bronchitis and liver dysfunction (Funnell et al., 1973; Krogh, 1992; Prasad, 1992; Eaton and Groopman, 1994). Thus the effects of these toxins are of serious global consequence particularly in the developing countries were there are shortages of food and medical infrastructures. According to F. A. O. (2004), *C. papaya L.* is mainly grown (> 90%) and consumed in developing countries. It is fast becoming an important fruit internationally both as a fresh fruit and as processed products. Thus, spoilage of such fruits further undermines the food scarcity and health of such percentage of the human population that rely on it as a source of vitamins.

Chemical pesticides and fungicides have been used to increase yield and protect pawpaw fruits crops. However, chemicals pose hazards to ecosystem through induced resistance against target organisms and undue inundation of the environment with organic pollutants. Biological control presents a better alternative with relative amount of cheapness, no side effects and reduced resistance (Okigbo and Ikediugwu, 2000; Okigbo, 2003; Okigbo, 2004; Okigbo, 2005; Okigbo and Nmeka, 2005).

In Nigeria, a number of traditional higher plants have been noted for their pesticidal properties and some of these have been screened biologically and phytochemically for their activity and chemical constitution (Okigbo and Emoghene, 2004; Okigbo and Ogbonnaya, 2006). The plant extracts are useful in the control of plant diseases (Okwute, 1992; Okigbo and Nmeka, 2005). The goal of the present study is to determine the antifungal potential of papain and aqueous seed extracts against organisms associated with *C. Papaya L.* fruit spoilage.

MATERIALS AND METHODS

Collection of sample

Mature pawpaw fruits of different ripening stages were obtained from Sango Ota market Ogun state, Nigeria. Samples were placed in a clean polyethylene bags and allowed to continue decay at about temperature of 25 - 28˚C.

Preparation of papain extracts

The papain extract was sourced from mature green female tree. About 3 cm deep incisions on the shoot of the mature female tree *(C. papaya L.)* were made using a sterile scalpel to bleed milky latex fluid (papain) into a 200 ml beaker. Bleeding was carried out in the early hours of the day before sunrise. For bleeding, female trees are preferred to hermaphrodite tree as the produce higher amount of exudates.

Preparation of aqueous seed extract

Decaying fruits from the samples were cut open using a sterile scalpel. The seeds were extracted and rinsed in sterile distilled water. Rinsed seeds were placed on an aluminum foil and milled using a ceramic mortar and pestle into semi – pulverized mashed form. This was further homogenized using a blender (Binatone Model BLG401). Blended extracts were filtered using Whatman No. 1 filter paper.

Isolation of spoilage fungi from rotted pawpaw

3.4 x 3.4 x 3 mm sized pieces of decaying portion of the pawpaw fruits were incised from the advancing edge of the rot. This was sterilized using 70% alcohol for 2 min, blotted dry with sterile tissue paper and placed on sterile Sabouraud Dextrose Agar (SDA) and incubated at room temperature for 3 days. Fungal growths associated with the rot affected tissue were identified and their frequency of occurrence determined as outlined by (Okigbo and Ikediugwu, 2000).

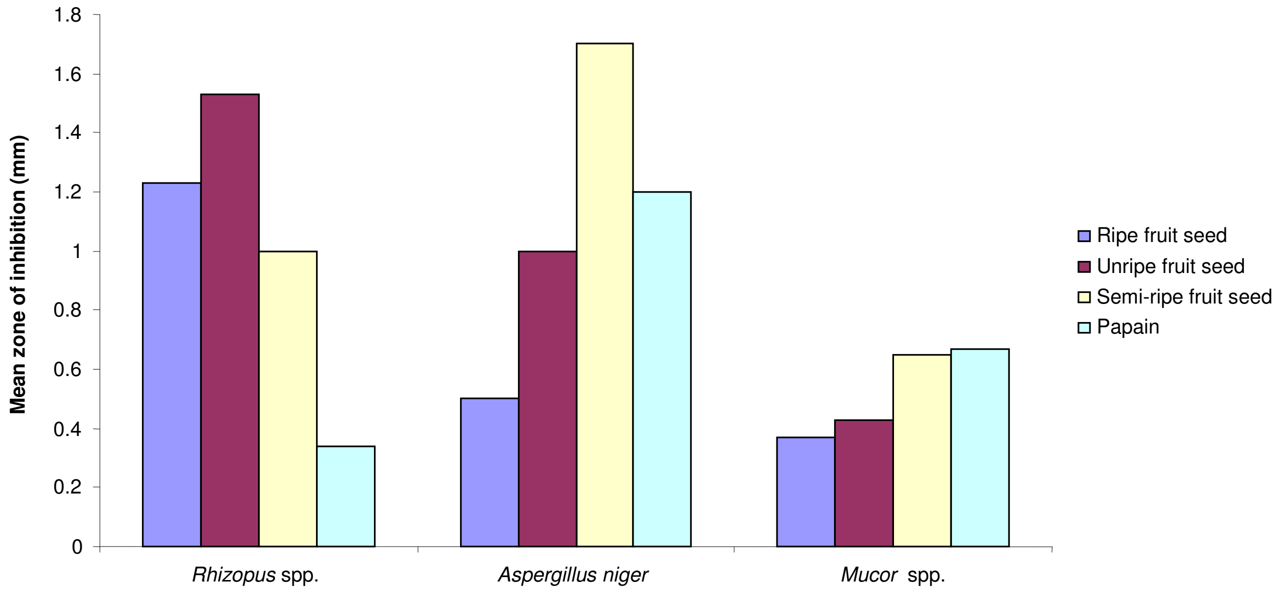

Figure 1. The mean zones of inhibition of the different fungi isolates to the different extracts from the seed and papain.

Determination of antifungal activity

The antifungal activities of the extracts were determined by placing one disc (3 mm diameter) of a 3 day old culture of the pathogens in each of the triplicates Petri - dishes (11 cm diameter) with 160 ml Sabouraud Dextrose Agar (SDA) medium and 3 ml of the seed aqueous extract and the papain respectively. The control experiments were set up with 3 ml of sterile distilled water using same media. This was done in triplicate plates and was incubated at room temperatures (28 ± 2°C) for three days. Daily measurements for three days of the mycelia extension of the cultures were determined by measuring culture along diameters and comparing with mycelial growth of the control. The difference in their diameters reflects the extent of inhibition by the extracts (seed extracts and papain).

Identification of fungal isolates

Three groups of species which occurred more frequently (90%) were identified in the mature papaya samples. The pure culture of these isolates was presumptively identified on the basis of their morphological and cultural state of growth. These were compared with standard reference organisms (Barnett and Hunter, 1972).

RESULTS

Three groups of fungi species which were isolated from the surface of the decaying samples were *Rhizopus* spp, *A. niger* and *Mucor* spp. Figure 1 shows the mean zone of inhibition of the 3 fungal isolates against ripe, unripe, semi-ripe seed extracts and papain extract. For *A. niger* the mean zones of inhibitions for ripe pawpaw fruit seed was 0.50 mm, unripe pawpaw fruit seed 1.00 mm, semi-ripe pawpaw fruit seed 1.70 mm, papain 1.20 mm and control too numerous to count (TNTC).

For *Mucor* spp. the zones of inhibitions for ripe pawpaw fruit seed was 0.37 mm, unripe pawpaw fruit seed 0.43 mm, semi-ripe pawpaw fruit seed 0.65 mm, papain 0.67 mm and control also was too numerous to count (TNTC). For *Rhizopus* spp. 1.23 mm ripe pawpaw fruit seed, unripe pawpaw fruit seed 1.53 mm, semi-ripe pawpaw fruit seed, 1.00 mm, papain 0.34 mm and control too numerous to count (TNTC).

Result of this study showed that, when *A. niger* mycelia were inhibited more with the semi-ripe seed extract and papain extract when compared with ripe seed extracts and Unripe seed extracts in Figure 1. For *Rhizopus* spp the seed extract from the unripe pawpaw inhibited its mycelial growth significantly when compared to *A. niger* and *Mucor* spp. Generally the *Mucor* spp exhibited a low level of mycelial reduction against the extracts when compared with the measurement of the extent of inhibition by the other extracts (Figure 1). Using ANOVA at 5% ($P < 0.05$) significant level, the null hypothesis Ho: $\mu_1 = \mu_2 = \mu_3 = \mu_4$ versus H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4$ of the mean of extracts. Thus the decision is to accept the null hypothesis were $F_{cal} < F_{tab}$, were F_{cal} calculated from Table 1 is 4.07 and F_{cal} 1.31.

DISCUSSION

From this study three different fungi associated with the rot of C. *papaya L.* were noted.These include *Rhizopus* spp*., Mucor spp., A. niger.* These isolates confirmed previous reports of (Gupta and Pathak, 1986 and Bayiewu, 1994) that isolated A. *flavus*, *Rhizopus* spp, and *Botryodiplodia and Cuvularia* spp. From the results, the

Table 1. Statistical calculation showing the mean of the extracts (3 days), the ANOVA.

	R s.E	U sE	S s E	P.E	Control
A. niger (mm)	0.50	1.00	1.70	1.20	TNTC
Rhizopus spp. (mm)	1.23	1.53	1.00	0 .34	TNTC
Mucor spp (mm)	0.37	0.43	0.65	0.67	TNTC
x	0.70	0.99	1.12	0.74	TNTC
	S^2_1 = 0.2149	S^2_2 = 0.3207	S^2_3 = 0.2859	S^2_4 = 0.1861	

Source	Sum of squares	d.f	Mean square	F value
Between	0.1215	3	0.0405	
Within	0.2474	8	0.0309	1.3107
Total				

F_{tab} = 4.07F_{cal} = 1.3107, $F_{3,8}$ (0.05) = 4.07
RsE = Ripe seed extract, TNTC = too numerous to count, UsE = Unripe seed extract, x = mean of extracts,
SsE = Semi ripe seed extract, S^2 = variance, P.E = Papain extract.

seed extracts (semi–ripe 1.70 mm and unripe 1.53 mm, respectively) showed more remarkable effect than the papain exudates (0.34 mm). This may possibly due to the active compounds found within the seed of pawpaw that is, glycosides and caricin. Other essential biologically active compounds include alkaloids, carpaine, pseudocarpaine, flavanols, butanoic acid, tannins, linalool, benzlglucosinolate, cis and trans-linalool, terpenoids, alpha- palmitic acid. Some of these compounds are effective super oxide antioxidants with ability to inhibit mycelial growth by reacting with cell wall components of these fungi. The low activity of the papain on the mycelial growth may possibly be due to dense nature of the exudates. It was observed that few minutes after bleeding for the papain exudates it tend to thicken , thus becoming more gummy. This possibly may slow the enzymes activity of papain on the mycelial growth of the fungal isolates.

From research findings, it has been noted that lytic enzymes found on pawpaw extracts may have target site on the cell wall of these isolated fungi. The mode of action may possibly be by attack on the sugar residues on the cell of these fungal species. According to Yoshio and Yoshio (1981), glucose was detected as a main sugar component in the cell wall of *A. niger* whereas in *Rhizopus* spp. glucosamine and N-acetylglucosamine were the major components. Hence the clue to remarkable inhibitory effects exhibited by the extracts may be attributed to this mode of action. Statistically, there is no difference in terms of activity between the various extracts such that an extract is preferred over other in the extracts used in this research work. Thus from the current findings, either of the extracts may be prepared to help stem the effects of post harvest losses caused by these isolated fungi.

One of the major post harvest diseases of *C. papaya* ‘anthracnose’ effect have been controlled by prochloraz or priopiconazolle during storage and transportation (Sepiah, 1993). However, some of the fungicides utilized often have residual effect on the treated fruits. Hot water treatments in combination with fungicides tend to improve the efficiency in controlling anthracnose. However, hot water dip treatments accelerate ripening in fruits (Paull, 1990); and the use of fungicide for extended periods may cause the emergence of fungicide – resistance strains of the fungus. Thus, a cost effective and reliable alternative is imperative. Irradiation presents an alternative but the draw back is on the high cost and prejudice by consumers against irradiated food. Several attempts with limited results have also been experienced by the use of other physical means of *C. papaya* preservation (Sivakumaret al., 2002; Gamagae et al., 2004). From the present investigation, there exists the possibility of use of extracts of pawpaw (seed extracts and papain) as means of preserving the shelf life of this all important fruit crop. This approach to plant disease management is economically viable with less environmental risk and can be exploited using biotechnological tools for industrial production which may substitute other synthetic fungicides.

REFERENCES

Badillo VM (2000). Carica L vs Vasconcella st-Hil(Caricaceae) con la rehabilitacion de else ultimo. Ernstia. 10: 74-79.

Baiyewu RU (1994). Fungi associated with fruit rot of pawpaw (*Carica papaya* L.) in South-Western Nigeria Ph.D. Thesis, University of Ibadan, Nigeria.

Baiyewu RA, Amusa NA (2005). The Effect of temperature and Relative Humidity on pawpaw fruit rot in South- Western Nigeria. World. J. Agric. Sci., 1(1): 80-83.

Barnett HC, Hunter BB (1972). Illustrated Genera of Imperfect Fungi, 3rd edition. Minneapolis Burgress Publishing Company Minneapolis, MN. pp. 241.

Burkhill IH (1966). A dictionary of the Economic products of the Malay Peninsula (2nd edn) Malay Ministry of Agriculture and Cooperatives. Kuala Lumpur.

Eaton DL, Groopman JD (1994). The toxicology of Aflatoxins, Academic press, New york, NY. pp. 383-424.

Emeruwa AC (1982). Antibacterial substances from *Carica papaya* fruit extract, J. Nat. Products, 45: 123-127.

FAO (2004). Food and Agriculture Organization of United Nations.
Funnell DI, Borthast RJ, Lillehij EB, Paterson RE (1973). Bright greenish- yellow fluorescence and associated fungi in corn naturally contaminated with Aflatoxin. Cereal. Chem., 50: 404-414.
Gamagae SU, Sivakumar D, Wijesundera RLC (2004). Evaluation of Post-harvest application of sodium bicarbonate incorporated wax formulation and *Candida oleophila* for the control of anthracnose of papaya. Crop Protection, 23: 575-579.
Giordani R, Siepai OM (1991). Antifungal action of *Carica papaya* latex isolation of fungal cell wall hydrolyzing enzymes Mycoses, 34: 469-477.
Gupta AK, Pathak VN (1986). Survey of fruit market for papaya fruit rot by fungi pathogens. Ind. J. Mycol., 16: 152-154.
Krogh P (1992). Adverse effect of Mycotoxins on human health in: seed pathology. In Mathur,S.B and Jorgensen,J (eds) Proceedings of the seminar,20-25 June 1988,Copenhagen, Denmark. pp. 149-157.
Kuthe GA, Spoerhase H (1974). Cultivation and use of pawpaw (*Carica papaya* L.). Tropen Lan Writ, 75: 129-139.
Morton JF (1987). *Carica papaya* L. in Fruits of warm climates. Creative resources inc.winterville,N.C.
Okeniyi JA, Ogunlesi TA, Oyelami OA, Adeyemi LA (2007). Effectiveness of dried *Carica papaya* seeds against human intestinal parasitosis: A pilot study. J. Med. Food. 10: 194-196.
Okigbo RN, Nmeka IA (2005). Control of yam tuber with leaf extracts of Xylopia aethiopica and Zingiber officinale. Afr. J. Biotechnol. 4(8): 804-807.
Okigbo RN (2005). Biological control of postharvest fungal rot of yams (Dioscorea spp.) with Bacillus Subitlis. Mycopathologia. 159(2): 307-314.
Okigbo RN (2004). A review of biological control methods for post harvest yams Dioscorea spp.) in storage in South Eastern Nigeria. KMITL. Sci. J., 4(1): 207-215.
Okigbo RN (2003). Fungi associated with peels of post harvest yams in storage. Global J. Pure Appl. Sci., 9: 19-23.
Okigbo RN, Ikediugwu FEO (2000). Studies on biological control of post harvest rot of yams (*Dioscorea* spp.) with *Trichoderma viride.* J. Phytopathol., 148: 351-355.
Okigbo RN, Ogbonnaya UO (2006). Antifungal effects of two tropical plant extracts (*Ocimum gratissimum* and *Afromomum melegueta* on post harvest yam (*Discorea* spp.) rot. Afr. J. Biotechnol., 5(9): 727-731.
Okigbo RN, Emoghene AO (2004). Antifungal activity of leaf extracts of some plant species on Mycosphaerella fijiensis Morelet, the causal organism of black sigatoka disease of Banana (Musa acuminata) KMITL. Sci. J., 4: 20-31.
Okwute SK (1992). Plant –derived pesticidal and antimicrobial agents for use in agriculture: A review of phytochemical and biological studies on some Nigerian plants. J. Agric. Sci. Technol., 2(1): 62-70.
Oludemokun AA (1976). Effect of temperature and relative humidity on mould deterioration of stored Kolanuts. Plant Report 60: 1008-1010.
Paull RE (1990). Post harvest heat treatments and fruit ripening. Post harvest News and Information, 1: 355-363.
Prasad R (1992). Some factors affecting herbicidal activity of glyphosate in relation to adjuvants and droplet size. Am. Soc. Testing Mater., 1112: 247-257.
Sepiah M (1993). Efficacy of propiconazole against fungi causing post harvest diseases on Eksotika papaya In: Champ BR, Highley E, Post harvest Handling of tropical fruits ACIAR conference proceedings Chiangmai Thailand. 50: 455-457.
Singh ID, Sirohi SC (1977). Breeding new varieties of *papaya* in India In: Niijjar GJ (Ed) Fruit breeding in India: Symposium of fruit crop improvement, Oxfordand IBH Publishers,New Delhi. pp. 187-192.
Sivakumar D, Hewarathgamagae NK, Wilson Wijeratnam RS, Wijesundera RLC (2002). Effect of ammonium carbonate and sodium bicarbonate on anthracnose of *papaya*. Phytoparasitica. 30(5): 486-492.
Storey WB (1985). *Carica papya* .In Halevy AH (Ed) CRC Handbook of flowering (VolII) CRC Press Inca.Boca.Raton Florida.
Yoshio T, Yoshio T (1981). Investigation of the structure of *Rhizopus* cell wall with lytic enzymes. Agric. Biol. Chem., 45(7): 1569-1575.