Incidence of Fruit Fly Rot of Watermelon *(Citrullus lanatus L)* of Nigeria

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ABSTRACT

The incidence of fruit fly (Bactocera curcubitae) rot of water melon and its potential to transmit fungi associated with pre-and post-harvest decay of water melon fruit was investigated in Port Harcourt Area of Nigeria. The fly was collected, identified and counted to determine its population level. The study implicated Penicillium oxalicum,(4.25cm) severity Aspergillus niger, (16.0cm) Aspergillus tamarii (3.55cm) and Cephalosporium species (3.25cm) combined of all fungi (2.75cm) and control (0.50cm) in the rot of the fruits in the area. Both the incidence and severity of rot indicated the following descending order of frequency and pathogencity: combination of all the fungi, Penicillium oxalicum, Cephalosporium species, Aspergillus niger and Aspergillus flavus. These fungi, apart from Aspergillus niger, were associated with the common pests of the crop, Bactocera curcubitae (fruit fly) are said to be introduced into the fruits by the insect during oviposition. The spores of the fungi were recovered from the gut of the insect after dissecting the intestine of the insect. This work is pioneering and suggests that a reduction in fruit fly population will consequently reduce water melon fruit rot. The importance of fruitfly management in order to control disease epidemics of water melon fruits.

KEYWORDS: *Fruit fly (Bactocera curcubitae), insect gut, exuviae, Watermelon (Citrullus lanatus)*

INTRODUCTION

Water melon (Citrullus lanatus) . It is believed that Citrullus lanatus (watermelon) is native of tropical Africa (Cobely, 1965; Masefield el al., 1969, Kirkbrid, 1993). Its cultivation began in ancient Egypt and Sudan then spread from there to other countries via the Mediterranean, Near East and Asia. From immune-chemical data the crop is thought to have originated from its semi cultivated variety Cordophanus found in Sudan (Fursa and Gavrilyuk, 1990). The long association of Citrullus, and many other cucurbits crops with human settlements has indicated that there is no clear demarcation between wild relatives and crop varieties (Heiser, 1969). However, the crop is also known in the tropical and subtropical Africa with an abundance of sunshine.

Watermelon is now cultivated in many parts of the tropics (Cobley, 1965; Masfield et al, 1969). The fruits are an important source of vitamins, minerals, carotenes and proteins. It is also a rich source of natural Iycopene, a carotenoid of great importance because of its antioxidant property (Denton, 2004). Highly prized oil is now being extracted from seeds of watermelon. This oil is used for cooking, cosmetic purposes and is of importance in pharmaceutical industry (Denton, 2004).

Global production of watermelon has increased from 30 million tons (2. 1 million/ha) in 1992 to 81 million tons (3.2 million/ ha) in 2002 (Denton, 2004). In Nigeria the growing of watermelon is increasing, especially in the North areas under irrigation.

Despite the importance of the crop, entomological and pathological work remains a serious limiting factor in obtaining good quality food and high yield of watermelon (Inayatullah et al, 1991; Rabindranath and Pillia 1986). In Rivers State of Nigeria, the melon fruit-fly (Bactrocera cucrubitae Coq) is a common pests of watermelon.

Adult fruit flies feed predominantly on ripe and wounded fruits (Hendrichs and Hendrichs, 1990). Direct damage to fruits were caused by female flies during oviposition, when the fruit skin is pierced by the insect to lay eggs which often lead to fruit decay caused by fungal pathogens (Grove et al., 1997). Watermelon fruit fly, is an important pests of cucurbit fruits in the world which Rivers State of Nigeria is not an exception (Dhillon et al., 2005). It is also the most common and destructive pests of cucurbits throughout Indo-Pakistan subcontinent for over 50% (Jainttu et al., 2008).

Inayatullah, et al., (1991) stressed that the relationship between the fruit infestation and density of melon fruit-fly has not been properly established. However, other higher insect species have been reported to suffer high infection level caused by fungi from Entomophthorales. Entomophthora muscae infects several species of crops e.g. Onion flies Delia antigua, wheat bulb fly Delia coarctata (Carruthers et al., 1985; Wilding and Laucker, 1974; Eilenberg and Philipsen, 1988). Similarly, Tolypocladium cylindrosporum, Beauveria bassiana and Metarhizium anisopliae have been reported to be pathogemic to house fly (Steinkraus et al., 1990; Barson et al., 1994; Castillo, et al., 2000 ; Watson, et al., 1995).

Hewitt (1974) also found Rhizopus and Penicillium incidences in vineyards adjoining plum orchards. The findings indicated that fruit files numbers, and the proportion flies that vectored a fruit decay causing fungus, tended to increase during the season in orchards and vineyards which suggest that fruit flies can be an important source of primary fungal inoculums.

Cayol et al., (1994) reported that the external and internal mode of transmission of R. stolonifer by C. Capitata was proved in-vitro. The external mode of transmission involved the mechanical transfer of conidia on the fruitfly's body. Scanning electron microscopes showed that the spores R. stolonifer were carried on the proboscis, head, tarsus and legs of the fruit fly. The internal mode of transmission involving partial (regurgitation) or total (faces) transmit through the digestive tract, was defined as semi-persistent where spores remained viable after passage through the digestive tract (Harris and Maramorosch, 1980).

Louis et al., (1996) demonstrated the ability of the vinegar fly (Drosophila melanogaster Meig) as vector to B. cinerea. Conidia of the fungus can be carried on the fly surface and through intestinal transit as reported for Rhizopus transmitted by D. melanogaster (Louis et al., 1989). When conidia germinated in the insect crop, they developed into mycelium and differentiated into micro sclerotia (fungal survival structures) that the flies can carry for their entire life (Louis et al., 1996; Fermaud and LeMenn (1989): Fermaud and Giboulot, 1992) reported that moth larvae of Lobesia botrana carried viable conidia of B. cinerea externally or internally. Fermaud and LeMenn (1992) further stated that the introduction of conidia into wounds by L. Botrana is important in the initiation of rot during the stages before versaison.

Fruit flies need carbohydrate, lipids and proteins to perform the biological activities necessary for survival and reproduction (Bateman, 1972). This suggests that the melon fruit fly may carry the inocula on its exuviae or in the gut and drop them on suitable fruits where infection could be initiated or introduced into the fruits during feeding and oviposition.

Miyatake et al., (1993) investigated the oviposition punctures in curcubit fruits and their damage caused by the sterile female fruit fly and observed that damaged caused by larvae contaminated the fruits with frass, providing entry points of rot fungi. This worm agrees with their findings. The water melon fruit fly therefore punctured the fruits during oviposition and introduced the fungi from its gut into the wound so initiated. Similarly, the wound might permit other aerial spores as secondary invaders.

Mucor and Penicillium species have been reported as toxic to insects (Brooks and Raun, 1965; Miczielski and Machowicz – Stafaniak, 1977). Engelbrecht, et al., (2004) reported the presence of fungi on adult male Mediterranean fruit insect (Ceratitis capitata).

Objectives of the study

This research therefore aims at:

a) Isolating and identifying the fungi that cause fruit rot of water melon.
b) Establishing the pathogenic relationship between the water melon fruit-fly and melon fruits.
c) Assessing the effects of crude extracts of water melon fruits on the survival growth of fungal dislodged from insect gut.

MATERIALS AND METHODS

Experimental site

Two field experiments were conducted in 2010 and 2011 at the Teaching and Research Farm, Rivers State University of Science and Technology, Port Harcourt, Nigeria at Latitude $4^0$42' and $4^0$48' N and Longitude $6^0$15' and 7.25^0 E on an elevation of 18 meters above sea level, a mean annual temperature of 27^0C and an annual rainfall of 2000–2476mm. (Tariah et al 1991)

Field Studies

The land, 41m x 21m, covered by grasses, herbs and shrubs was ploughed with tractor-drawn plough and then harrowed in 2010. Four seeds of water melon, Citrullus lanatus c.v. Sugar-Baby, were sown at 1.2 x 1.2m apart on each plot, 7m x 4m, replicated five times, giving a total of 20 plots with rows of water melon having 5 plants per row. The seedlings were later thinned to two plants per stand at three weeks after sowing to give 40 Plants/plot and a plant population of 9292 plants per hectare. A basal fertilizer (NPK 15: 15:15) application was made at the rate of 200Kg/ha to the experimental field. The experimental design was Randomised Complete Block (RCB) design. The following parameter/characters were assessed: number fruit blemishes. Number of rotted fruits was estimated by the number of fruits that rotted before and after maturity of the fruit while percentage of marketable fruits was determined by the number of fruits without blemish as percentage of the total number of mature fruits per plot, while fruit blemishes were assessed by the ratings of Draper (1976).

The fungi responsible for rot of the fruits were isolated using Potato dextrose agar (PDA) medium. In this method, five partially rotted fruits were selected and portions from each fruit were cut towards the periphery of the rotted tissues. They were plated on sterile potato dextrose agar in Petri dishes and examined for fungal growth for 7 days. The fungi were identified by means of spore morphology, habit of hyphae and colour of mycelium produced (Chukunda et al 2013). The fungi were tested for pathogenicity using relatively healthy fruits,

inoculating the fruits with sterile PDA discs represented the control. All treated fruits were incubated at 28 ± 1^0C for 14 days at the end of which the fruits were cut open through the point of inoculation and examined for rot. Diameter of rot was measured using transparent meter rule. Two diameter measurements were taken and the mean recorded.

In another study, fifteen fruit flies were collected using insect nets. The flies were surface – sterilised in 70% ethanol for 15 minutes. They were later dissected in Ringer's solution and the gut contents transferred into sterile 25ml-capacity Erlenmeyer flasks containing 2ml sterile distilled water. The gut was agitated to dislodge available spores and 0.1ml of the gut contents was placed on a glass slide with the aid of a sterile dropping pipette, a drop of methylene blue was added to the suspension. It was covered with a cover-slip and observed in a light microscope for germinated conidia. The number of germinated spores was computed for 2ml and converted to a percentage. The treatment was replicated five times. The aliquot was also plated on PDA by serial dilution method and pure cultures were prepared for each colony for identification.

The exuviae of five fruit flies were removed, surface-sterilized in 70% ethanol and separately soaked in sterile distilled water for 48 hours. A drop of the water-extract was placed on a sterile glass slide with the aid of sterile inoculating wire loop. Spores of each fungus were separately dislodged into the sex tract of the exuviae on separate slides. The slides were placed in a sterile Petri dish containing sterile moist Scottie tissues, these were left on the laboratory bench (28 ± 1^0C) for 24 hours at the end of which a drop of methylene blue was added to the slide and covered with sterile cover-slips. The number of germinated spores was observed by means of light microscope and recorded as a percentage of the total spores assessed. The treatment was replicated five times. Crude water extracts of water melon was extracted using an electric blender and the filtrates separated using a cheese cloth and the concentration was 100%, and 0% served as control (distilled water) to assess the survival rate of the fungal spores dislodged from the insects.

RESULTS AND DISCUSSION

Isolation and Identification of Fungal Responsible for Fruit Rot of Water melon

This work shows that the following fungi were implicated with the fruit rot of water melon; Penicillium oxalicum, Aspergillus niger, Aspergillus tamarii and Cephalosporium species. The frequency of occurrence of the fungi indicated that Penicillium oxalicum was highest (100%) followed by Cephalospsorium species (36%), Aspergillus niger (16%) and least Aspergillus tamarii (12%).

Also shows that the severity of infection of the fungi was of the following descending order; Combination of fungi, Penicillium oxalicum, Aspergillus tamarii, Cephalosporum spp, Aspergillus niger. However, the control exhibited rot within 0.5cm which could perhaps be as a result of physiological diseases rather than pathogenic.

The fungi earlier mentioned have been implicated as major organisms associated with post harvest decay of melon fruits which is in consonance with the findings of Fourie and Holz, (1985a),Swart and Holz, (1991) and Fourie, et al.,(2002) that Penicillium spp, Aspergillus spp, Alternaria, Mucor spp, Rhizopus spp and Botrytis cinerea are fruit borne pathogens. Randhawa et al (1991) earlier implicated Cephalosporium sp., Fusaruim oxysporum and Penicillium oxalicum in the fruits rot of water melon. This is in line with the present findings.

Table 1: Fruits Blemishes of Fruitfly of Watermelon

S/No	Intensity of blemish	Ratings

1.	0.5	1
2.	6 – 20	5
3.	24 – 35	10
4.	36 – 49	15
5.	50 and above	20

The mean of the five sampled fruits on the intensity of blemishes was determined using Drasper (1976) index.

Table 2: Frequency and Pathogenicity of Fungi Isolated from Rotted Fruits of Watermelon

S/No	Fungi Isolated	% Frequency	Severity (cm)
1.	Penicillium oxalicum	100	4.25
2.	Aspergillus tamarii	12	3.55
3.	Aspergillus niger	16	3.08
4.	Cephalosporium sp.	36	3.25
5.	Combination of all fungi	NA*	4.75
6.	Control	NA	0.50

*NA: Not Applicable

This work indicates that apart from Aspergillus niger which was not implicated in the rot of the fruits of water melon, almost all the fungi isolated from the rotted fruits were recovered from the guts of the melon fruits fly (Bactocera curcubitae). The results on the in vivo germination of the spore in the gut of the insects and water extracts of the exuviae of the insects showed zero percentage germination of the spores in both media (Table 2?).

Table 3: Percentage Fungal germination dislodged from the gut and exuviae of water melon Fruitfly (Bactocera curcuibitae)

S/No	Fungi isolated	% Germination	
		Gut	Exuviae
1.	Penicillium oxalicum	0.00	0.00
2.	Aspergillus tamarii	0.00	0.00
3.	Aspergillus niger	0.00	0.00
4.	Cephalosporium spp.	0.00	0.00

Table 4: Effects of water melon extracts on the growth of fungal Dislodged from Insect Gut

		(%) Fungal growth	
S/No	Fungi isolated	PDA (Amended)	PDA (Control)
1.	Penicillium oxalicum	5.20^{b}	2.30^{b}

2.	Aspergillus tamarii	7.60[a]	4.20[a]
3.	Aspergillus niger	8.00[a]	3.45[a]
4.	Cephalosporium sp.	6.30[b]	2.20[b]

Mean values within columns with the same letter do not differ significantly by DMRT at 5% probability level.

CONCLUSION

This research indicates that water melon fruit fly, Bactocera curcubitae was a carrier of most of the fungal pathogens of water melon fruits in Port Harcourt Area of Nigeria. This work suggests that any pesticide capable of reducing the population of the melon fruit fly will also be able to reduce the water melon fruit rot. This work is novel and pioneering in implicating the fruit fly as a carrier of the fungal pathogens of water melon fruits.

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