Isolation and Identification of Bacteria and Fungi Associated With Rots of *Citrullus Lanatus* and *Capsicum Frutescence* in Sokoto Markets

Garba H. Danladi, Sanusi Muhammad, and Sule S. Manga

Abstract**—**A study was carried out to determine the causative micro-organisms of rots in *C. lanatus* and *C. frutense* from Ramin-Kura and Vegetable/Meat markets in Sokoto Metropolis, Nigeria. Potato dextrose and Nutrient agars were used for cultures of fungi and bacteria respectively, while macroscopy, microscopy and biochemical characterization according to the methods of Cheesebrough, 2000 were used in identification. Fungi belonging to six species including *Aspergillus niger* with seventeen occurrences; *A. flavus*, twelve; *Fusarium oxysporium*, thirteen; *F. solani*, ten; *Rhizopus stolonifer*, six, and *Mucor mucor*, nine occurrences, were isolated. Bacteria isolated include *Pseudomonas syringae*, nine occurrences; *Erwinia carotovora*, nine occurences and *Erwinia amylovora* with six occurrences. Patogenicity test revealed *Aspergillus* spp., *Erwinia* spp. and *F. oxysporium* rotted the vegetables within three days of inoculation while *F. solani* and *P. syringae* took 4 -5 days, respectively. The most common rot fungi were *A. niger* and *F. oxysporium*, while the least occurring fungi and bacteria were *R. stolonifer* and *E. amylovora*, respectively. A study on their possible control methods will help the marketers conserve resources lost as a result of the microbes' activities.

Keywords**—** vegetable rots, micro-organisms, markets, sokoto

I. Introduction

FARMERS and marketers of vegetables are faced with serious losses due to attack on the commodities by rot pathogens. The losses may start as heavy yield losses [1]. These usually take place from the farms, continue in transit and never stop even during display for sale at the markets. Post - harvest bacterial soft rot losses have been estimated to vary between 15 – 30% of the harvested crop [2]. The rots include fruit blotch, pimples, fruit cracking/scarring, cross stitch, belly rot, bacterial rind necrosis, among others [3]. This research is aimed at identifying microbes associated with the rots of two vegetables in the Sokoto urban markets of Ramin Kura and Central/Meat markets.

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II. Materials And Methods

Sterilization of glasswares was by use of hot air oven at 161^{o}C for 1hr; inoculation tables, and needles, knives and other equipment with 70% alcohol.

Potato dextrose agar was used for fungal cultures. It was prepared according to manufacturer's instructions; 39g of the agar was dissolved in 1000mls of distilled water and autoclaved at 121^{o}C for 15 minutes, allowed to cool sufficiently before pouring on petri dishes (plates).

2mm^2 of the diseased sample was then inoculated on the dishes aseptically. Fungal growths were observed, and colonies were later subcultured to get pure isolates. Fungi were identified based on macroscopic and microscopic features, and compared with standard mycology atlas. Where features proved difficult to appear, slide cultures were performed and daily observation of growth and appearances of the fungus was undertaken, until the fungus was identified.

Nutrient agar was used for bacteria cultures, and it was prepared according to manufacturer's instructions; 28g in 1000ml distilled water and autoclaved at 121^{o}C for 15mins. It was then allowed to cool to 47^{o}C before pouring to 90mm petri dishes, then kept 24hrs to solidify.

2mm of the suspected rotted samples were plated on the prepared media, and the bacterial and fungal growths were observed. The colonies were further sub cultured in order to obtain pure cultures, while the fungi that proved difficult to observe were further subjected to slide cultures and daily observations were carried out.

Gram staining was later carried out to identify morphological appearance of the bacteria (cocci or bacilli, gram negative or positive). This was followed by series of biochemical tests according to the methods of Cheesebrough, 2000 [4], which include; urease, catalase, Indole, starch hydrolysis, TSI, citrate, motility and MR and Voge's Proskauer tests[2]. Data obtained was compared with standard Bergeys manual of determinative bacteriology [5] to identify the bacteria.

Pathogenicity tests were carried out on all isolated fungi to verify the authenticity and capability of the isolated fungi and bacteria to cause rots on the two vegetables under study.

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III. Results

Based on the growth of the fungi on the culture media and the structures that were observed under the microscope to reveal the type of hyphal growth, fruiting bodies and if present, the types of resting spores, sevral fungal species were isolate.dhowever, after conducting pathogenicity test on fresh and healthy vegetables, six different species of fungi were confirmed to be pathogenic on both *Citrullus lanatus* and *Capsicum frutescence*. These include *A. niger*, *A. flavus*, *F. oxysporium*, *F. solani*, *R. stolonifer* and *M. mucor* (Table I). *A. niger*, *A. flavus*, *F. oxysporium* rotted *C. lanatus* within 72hrs of inoculation, while *F. solani*, and *R. stolonifer*, *M. mucor* took 4 – 6 days respectively to achieve sufficient tissue macerations.

TABLE I
IDENTIFICATION OF FUNGI

Macroscopy	Microscopy	Organism
Black colony, powdery With diffused hyphae in media	Smooth- walled stipe, conidiosphores radiate and terminate in vesicle	*A. niger*
Light green and Powdery colonies	Rough and coarse aerial hyphae present with simple sporangiophore which are shaped globose.	*A. flavus*
Brownish colonies with profuse aerial growth.	Aerial hyphae present with simple sporangiophore which has sub-globose rough zoospores	*M. mucor*
Snowhite colonies with aerial growth	Sparsed mycelia, 2septate micro-conidia, with macro-conidia 3 – 5 septate present in bunches of conidiophores.	*F. oxysporium*
Green, dense and floccose colonies with aerial growth	Abundant micro-conidia present, 3-5 septate slightly Curved macroconidia present in conidiophores, smooth-walled chlamydospores	*F. solani*
Whitish colony later turns brownish	Brown-black, globose sporangia, rhizoids also present and zygospores.	*R. stolonifer*

TABLE II
OCCURRENCE OF FUNGI PATHOGENS ON *CITRULLUS LANATUS*

Pathogen	RK1	RK2	RK3	V/M1	V/M2	V/M3
A. Niger	+ - -	+ + +	+ - +	+ + -	+ - -	+ - -
A. Flavus	- - -	+ - -	- + -	+ + +	- - -	- - -
F. oxysporium	- + -	- + -	+ - -	+ + -	- - -	- - +
F. solani	- - -	- - -	- - +	- - -	- + +	+ + -
R. stolonifer	+ - -	- - -	- - -	- + -	+ - -	- + -
M. mucor	- - +	- - +	- - -	- - -	- + -	- - -

Key: RK = Ramin Kura Market; V/M = Vegetable and Meat Market;
+ = Pathogen present; - = Pathogen absent

TABLE III
OCCURRENCE OF FUNGI PATHOGENS IN *CAPSICUM FRUTESCENCE*

Pathogen	RK1	RK2	RK3	V/M1	V/M2	V/M3
A. Niger	+ - -	+ - -	- + -	- + +	- + -	+ - -
A. Flavus	- + -	+ + -	- - -	+ - -	+ - -	+ - +
F. oxysporium	- - +	- - +	+ - +	- - -	- + +	- + -
F. solani	+ - -	- - -	+ + -	- + -	- + -	- - +
R. stolonifer	- - -	- - -	+ - -	+ - -	- - -	- - -
M. mucor	+ - +	- + +	- - -	- - -	- - +	- + -

Key: RK = Ramin Kura Market; V/M = Vegetable and Meat Market;
+ = Pathogen present; - = Pathogen absent

PLATEI
Aspergillus niger from the three sits of Ramin Kura

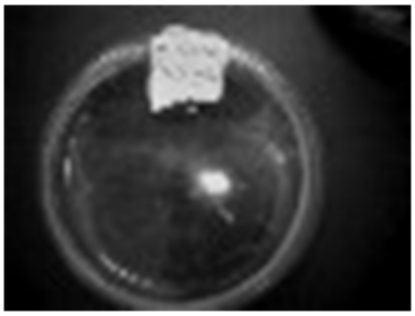
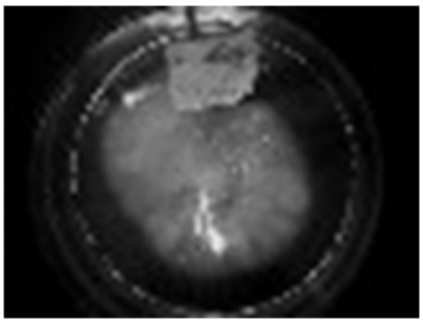
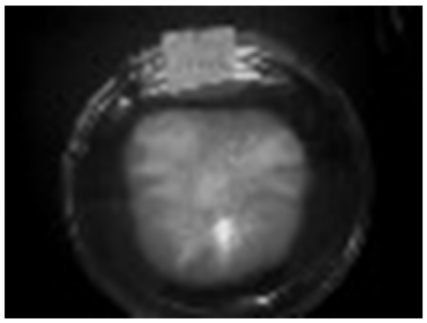


PLATE II
Aspergillus niger froM Vegetable and Meat Market



PLATEIII
Fusarium oxysporium from Ramin kura Market



PLATEIV
Fusarium oxysporium from Vegetable and Meat Market

According to the color taken from the stains, the bacteria were identified as either gram positive or negative. Their reactions to the series of tests were also positive or negative. A cumulative of the various results was compiled and the various bacteria were identified (Table IV). After pathogenicity tests, three bacteria species were identified as capable of being responsible for the rots of two vegetables. These bacteria were; *Pseudomonas syringae*, *Erwinia carotovora* and *Erwinia amylovora*

TABLE IV
BIOCHEMICAL ANANYSIS OF ROT ORGANISMS

Gram stain	-	-	-
Oxidative reduction	+	-	-
Indole production	-	-	-
Metyl red	+		
Voges' Proskauer	+	+	-
Citrate (simmons)	+		
Hydrogen sulphide	-	+	+
Urease hydrolysis	+	-	+
Motility	+	+	+
Lactose	-		
Maltose	-	-	-
Sucrose	-	-	-
Catalase		+	
Pathogen	*P. syringae*	*E. carotovora*	*E. amylovora*

The occurrences of bacteria pathogens in *Citrullus lanatus* samples obtained from the various sales points in the two markets is shown in TABLE V, as follows;

P. syringae had occurrences in two plates out of the three cultured from Rk1 and pne occurrence each in plates from RK3 and v/M3. There were no *P. syringae* pathogens isolated from RK2, V/M1 and V/M2.

E. amylovora was not isolated from samples in Rk1, RK3 and V/M1, but the pathogen was observed in RK2, V/M2and V/M3, occurring on one plate in each instance.

E. carotovora had two occurrences in plates from RK1, and one occurrence each from plates containing the samples from RK2, V/M1, V/M2 and V/M3, while the pathogen was absent in all samples obtained from RK3.

TABLE V
OCCURRENCE OF BACTERIA PATHOGENS IN *CITRULLUS LANATUS*

Pathogen	RK1	RK2	RK3	V/M1	V/M2	V/M3
P. syringae	+ + -	- - -	- - +	- - -	- - -	- - +
E. amylovora	- - -	- + -	- - -	- - -	- + -	+ - -
E. carotovora	- + +	- - +	- - -	- + -	+ - -	- + -

Key: RK = Ramin Kura Market; V/M = Vegetable and Meat Market;
+ = Pathogen present; - = Pathogen absent

TABLE VI
OCCURRENCE OF BACTERIA PATHOGENS IN *CAPSICUM FRUTESCENCE*

Pathogen	RK1	RK2	RK3	V/M1	V/M2	V/M3
P. syringae	- - -	- + -	- - -	- + +	+ - -	- + -
E. amylovora	- + -	- - -	- - +	- - -	- + -	- - -
E. carotovora	- - -	- - +	- + -	- - -	- - -	- + -

Key: RK = Ramin Kura Market; V/M = Vegetable and Meat Market;
+ = Pathogen present; - = Pathogen absent

Isolation and pathogenicity tests of bacteria from *C. frutescenses* obtained from the points of sales in the markets revealed that Ramin Kura (RK), had only one occurrence of *P. syringae* in RK2, while all the other points did not reveal presence of the pathogen. RK1 also had the presence of both *E. amylovora* and *E. carotovora* on one plate only, in each of points RK1 and RK2, respectively (TABLE VI).

The same table also showed the varying degrees of pathogen presence in Vegetable and Meat Market (V/M). Only *P. syringae* was isolated from V/M1, while the other two bacteria pathogens were absent in all plate of tis point. One occurrence was recorded for each of *P. Syrigae* and *E. amylovora* from point V/M2, wile *E. carotovora* was absent. The third point, V/M3 also had only single appearances, but of P. syringae and E. caorotovora, where only the second plates had the growth of the pathogens, while all other plates, had no pathogenic bacterial growth in them. The third organism, E. amylovora was absent from all plates containing the samples from V/M3.

PLATEI shows the pathogen, *Aspergillus niger* isolated from all three points of sales in Vegetable and Meat market, while PLATE II shows the same organism from a plate in Ramin Kura market.

PLATE III is a picture of *Fusarium oxysporium* isolated from Ramin Kura market, while the same organism also isolated but from a point in Vegetable and Meat market is as shown in PLATE IV.

IV. DISCUSSIONS

From the observed tables, the rots arising from the activities of fungi are more destructive than those by bacteria. Two fungi species; *A. niger*, and *F. oxysporium*t were noticed on almost all the points of sales. *A. flavus* and *F. solani* also have a relatively wide spread of infection in both markets, but *Mucor* species and *Rhizopus* sp which are usually opportunistic, may have been responsible for some of the results observed especially in Vegetable and meat market for *C. lanatus*, by *R. stolonifer*. The infection by *Mucor* is however noticed sporadically on some plates, in almost all the points, but hardly is it more than on two plates from a single sales point.

In the case of bacteria, *P. syringae* is the only species that was isolated from two plates at a single sales point in RK1 of *C. lanatus* and V/M1 of *C. frutescence.*

The infection of all pathogens is usually made possible due to the occurrence of the pathogens right from the fields [6]. It was also observed that *E. carotovora* can be disseminated into new infection sites by irrigation water, and thereafter enter the produce by wounding [7]. It has also been observed to be a plant pathogen with diverse host range, including many agriculturally and scientifically important plant species. It produces pectolytic enzymes that hydrolyse pectin between individual plant cells which causes the cells to separate [6].

E. carotovoa and P. fluorescence cause very destructive soft rots. They enter through wounds caused by insect bites or bruising at harvestor transport times. Once in the plant tissues, the bacteria also produce pectolytic enzymes which result in tissue marceration [8]; [9]; [10] observed *E. carotovora* to be one of the major soft rot causing bacteria in the tropics. They also reported that it is of great importance both in field, as well as transit and storage, causing heavy economic losses to various vegetables.

Also, vegetables coming from the field may be already

infected by the pathogens, although they may not show visible symptoms at harvest time, but these organisms will later cause severe damage because of high temperature, humidity, light, air and poor transport management practices [11]. They also reported that in post-harvest soft rot of Bell Pepper, decay greatly increased as a result of inoculation with *Erwinia* during transport and marketing, especially with high temperatures. Higashio and Yamada observed that under high temperature and poor transportation system, in countries like Indonesia and Singapore can result in heavy produce losses [14], while Farrar et al., reported that potato in Kene county is affected by *E. carotovora* [15].

The practice of stockpiling produce during display or offloading at the markets may also aid in making sure that temperatures rise.

Above; Bell pepper and tomato on display

Below; watermelon (both under tent shade)

When bruises occur during such handling times, the pathogens will generally have entry points. Offloading systems in the markets in which case few people are saddled with the manual offloading of the products can make them be in hurry to finish with one contract and move on to another in quick time to be able to make more cash. This usually results in careless dropping of the produce on the ground which can result in injuries or breakage of the vegetable, thereby serving as entry points or the pathogens.

The storage facilities at the market are made of thatched materials. This means that when rain falls, the vegetables are soaked directly, and the resultant rise in temperatures after the rains ensures that adequate humidity is provide for the pathogens. In other storage sites, they are made of shops which are poorly ventilated. Unsold products are usually crammed into such shops, mixing infected and healthy vegetables, and resulting in temperature and humidity rise.

Post-harvest diseases can cause the complete loss of harvested products as reported by Charmyne Smith in [12].

Soft rot losses have also been reported by Caponis and Butterfield on cucumber and Bell Pepper, in metropolitan Naw York [13].

Control measures for fruit rot of C. lanatus by the use of lime skin extract has also been carried out [16], after isolation of *F. solani* as one of the causative agents for the fruit rot. The treatments were found to have the capability to increase shelve life of the fruit up to 14 days.

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