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CONTROL OF SOFT ROT OF COCOYAM CORMS IN STORAGE USING SOME PLANT EXTRACTS

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ABSTRACT

Erwinia carotovora is a bacterium causing high percentage loss in the production of cocoyam by farmers in South Eastern Nigeria. Sequel to this, in vitro and in vivo studies was carried out with seven varieties of Colocasie esculenta and Xanthosoma sagitifollieum cocoyam (NCE1, NCE2, NCE3, NCE4, NXS1, NXS2, NXS3) in the Department of Plant Health Management laboratory in Michael Okpara University of Agriculture, Umudike to test their susceptibility and resistance to bacterial soft rot caused by Erwinia carotovora. The experiment was laid out in complete randomized design (CRD), replicated four times. Samples of infected corms were taken to the laboratory for isolation and the inoculum obtained was used in this trial. The cocoyam corms were opened with cork borer (3mm diameter) before introducing the bacterial inoculum but control was without the bacterial inoculum. Some plant extracts used for their effectiveness in controlling bacteria soft rot include: Chromolaena odorata, Cymbopogon citratus, Moringa oleifera, and Occimum gratissimum. Data collected showed that the control had least diameter rot (25.00cm) when compared with the treated corm varieties, for NCE4 (64.25cm) and NCE1 (66.75 cm) indicated susceptibility to the bacteria (Erwinia carotovora) however NXS3 had higher diameter rot (85.25 cm) and was found most susceptible to the bacteria infection (P=0.05). All plant extracts used were effective in the inhibition of bacteria soft rot in cocoyam, where Chromolena odorata extract (2.22 cm) had the best inhibition score on the growth and development of the bacteria followed by Occimum gratissimum (3.47 cm) whereas the untreated control (4.49 cm) had poorest bacteria soft rot inhibition. Results obtained indicated that the bacterium (Erwinia carotovora) isolated from infected cocoyam corms was the organism responsible for soft rot in cocoyam. Also, the plant extracts used inhibited the disease incidence of Erwinia carotovora soft rot and should be adopted by farmers in controlling bacteria soft rot in cocoyam, preference to synthetic chemicals.

Keywords: *Erwinia carotovora,* cocoyam corm, susceptibility, plant extracts, bacteria soft rot.

INTRODUCTION

Cocoyam (*Colocasia esculenta* (L) Scholt) a perennial monocotyledonous and herbaceous plant of the family *Araceae* (Onyeka, 2014), has high content of carbohydrate and provides edible starchy storage corms or cormels, in many regions in developing countries (Opara, 2002 and Ojinaka *et al.,*2009).

Cocoyam constitutes one of the basic food crops of major economic importance in the South Eastern Nigeria, ranking third after cassava and yam, in terms of total production, land area under crop cultivation and consumption (Chukwu and Nwosu, 2008). Most popular type of cocoyam in most South-eastern Nigerian is "*ede-uli"* in Igbo (*Colocasia esculenta*) which grows in marshy areas and its corms are used as soup thickening agents and the less popular type is called "*ede-oku"* in *Igbo* (*Xanthosoma sagittifolium*), whose corms could be boiled and eaten with various soups.

Cocoyam production in Nigeria in the last four decades, has been threatened and a drop of 11% was recorded between 2000 and 2004 due to several fields and storage bacterial diseases such as bacterial stem rot, bacterial wilt, bacterial stem gall etc (FAO, 2001; FAO, 2007; Kolchaar, 2006). Generally, rotting starts from the field and progresses in storage (Enyiukwu *et al*, 2004). Lack of good storage methods limits the availability of cocoyam throughout the year (Chukwu *et al*., 2008). Conventional storage methods have been found to be ineffective due to high percentage losses. In past decades the use of chemicals has helped in controlling cocoyam leaf blight and other bacterial disease but in recent time due to some identifiable problems (e.g persistence of chemical residues, biodegradation, phytotoxicity, pollution, development resistance in target organisms, high cost, at times non-availability and hazards to man and his environments renders them unattractive to adopt by farmers (Okigbo and Odurukwe, 2009). Shukla *et al*. (2012) reported that numerous *in vitro* studies have validated the efficacy of plant-derived pesticides in many branches of agriculture.

Erwinia belongs to the family Enterobacteriaceae, three commercially important soft rot *Erwinia* are *Erwinia carotovora* pv. *carotovora*, *Erwinia chrysanthemi* and *Erwinia carotovora* pv. *atroseptica* (Kharayat and Singh, 2015). *Erwinia atroseptica* is mainly restricted to temperate regions, whereas *Erwinia carotovora* is distributed in temperate and tropical zones worldwide and *Erwinia chrysanthemi* is found in sub-tropical as well as in warm temperate regions (He´lias *et al*., 2000). In agriculture, the microbes ceaselessly invade crops of potatoes and other vegetables in the fields or in storage and cause plant tissues to become soft and watery which eventually turn slimy and foul-smelling (Bell *et al*., 2004). On Cocoyam, bacterial soft rot symptoms often appear as a soft, watery rot of individual scales that may advance and rot the entire corm. A foul-smelling viscous fluid oozes from the neck when infected corms are squeezed (Howard and David, 2007). Fewer *in vivo* works have been done on stored products caused by moulds and storage bacteria. The main objectives of the study include; to Isolate and identify bacteria associated with the post-harvest damage of cocoyam corms in storage, to determine the resistance of cocoyam varieties in storage to bacterial disease and finally to evaluate the control measures to the bacterial soft rot disease.

MATERIALS AND METHODS

Experimental Location

The experiment was conducted at the Laboratory of College of Crop and Soil Sciences of

Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Umudike is located at latitude 5° 28¹ north and longitude 7° 35¹ and an altitude of 112m above sea level with an annual rainfall of 2200mm, it has an evaporation rate of 1531mm and the soil type is sandy loamy soil with relative humidity of 76% and temperature range of 25-35°C.

Source of Cocoyam

Healthy and infected cocoyam cormels were collected from the cocoyam barn of National Root Crops Research Institute (N.R.C.R.I.), Umudike, Nigeria. Two main varieties of cocoyam utilized in this research and were *Xanthosoma spp*: Ede Ocha *(NXS 001*), Ede uche (*NXS* 002), okorokoro (*NXS* 003), and *Colocasiae spp* : Coco India (NCE 001), Ede ofe green (NCE 002), Ede ofe purple (NCE 003), Nkpon (NCE 004) were stored in envelopes before taken to Plant Health Management Laboratory in Micheal Okpara University of Agriculture, Umudike.

Preparation of Plant Extracts

Four plant extracts were collected from the forest in Umuahia, Abia State. These are fresh leaves of *Chromolena odorata, Cymbopogon citratus, Occimum gratissimum* and *Moringa oleifera* (100 g, each), washed in the laboratory with tap water, air dried, grounded separately and each added to 10 ml of hot water. This was vigorously stirred and left to stand for 1hour. The solution was filtered and used as the extracts (Okigbo and Nmeka, 2005).

Preparation of Bacterial Inoculum

Fresh samples of cocoyam corms were washed and allowed to air dry. Thereafter a small piece of the diseased tissue was cut out between the boundary of the healthy and diseased sections (Okigbo and Nmeka, 2005) which was sterilized with 70% absolute ethanol and washed three times with sterile distilled water before being placed in Petri dish with a drop of sterile water, crushed with sterile rod and incubated at ambient temp for 30 minutes to enable the multiplication of the bacterium. The bacteria suspension obtained was streaked on nutrient agar media using a flamed wire loop and then incubated at 30°C for 48 hr.

Subculturing

When growth was established, subcultures were made using inoculum from the different organisms in the mixed cultures to obtain pure cultures. The Petri dish was properly sterilized and using the sterilized wire loop a portion the mixed culture was collected and streaked on the nutrient agar, then allowed to produce culture colonies (Ellis *et al*., 2007).

Pathogenicity Test

A fresh, healthy cocoyam was washed with tap water and distilled water, and sterilized with 70% ethanol. Cylindrical discs sizes were removed from the corms with a sterile 4 mm cork borer. A disc of 24 hour old cultures of the isolates was used to plug the holes created in the tubers and the disc of the tuber in the cork borer was replaced, then Vaseline was applied on the point of inoculation. This was done for all the isolates obtained in pure cultures (Okigbo and Nmeka, 2005).

Effect of Plant Extract on Bacterial Growth

The effect of plant extract on bacterial growth was determine by creating a four equal section on each Petri-dish, drawing two perpendicular lines at the bottom of the plate and the point of intersection indicating the centre of the plate was marked (Amadioha and Obi, 1999). About 2 ml of each plant extract was separately introduced into the Petri-dish containing nutrient agar media, mixed thoroughly and allowed to solidify. A disc (4 mm diameter) of the pure culture of *Erwinia carotovora* was placed on the extract, just at the point of intersection of the two lines drawn at the bottom of the petridish. Control experiments were set up by adding sterile water. Bacterial toxicity was recorded in terms of percentage colony inhibition and calculated.

Growth inhibition (%) = [(DC-DT)/DC] X 100 (Okigbo and Nmeka, 2005).

Where: DC = average diameter of control,

DT = average diameter of fungal colony with treatment.

Data Collection, Experimental Design and Data Analysis

The arrangement was laid out in a completely randomized design (CRD), while the experiment was replicated four times. Data was collected on the following parameters: Morphological appearance of bacterial culture and inhibition of the bacteria by the plant extract. Data collected was subjected to Analysis of Variance using (SAS package 9.1) model 2008

RESULTS AND DISCUSSION

The result obtained from table 1 showed that NXS3 gave the highest rate of rot (85.25cm) which was significant ($P \leq 0.05$) followed by NCE2 AND NXS2 (79.00cm) and (77.00cm) respectively, followed by NCE3 and NXS1 (72.00cm) and (71.00cm) and finally followed by NCE4 and NCE1 (64.25) and (66.75) which gave the least rate of rot of the cocoyam variety. The control which was a cocoyam opened with the cork borer, had a fixed diameter but without the introduction of the isolated bacteria (E*rwinia carotovora)* had the least diameter of rot comparably indicating that the bacteria (*Erwinia carotovora)* was inciting rot in cocoyam The result revealed that NCE4 and NCE1 were the most effective in the inhibition of rot incited by *Erwinia carotovora* in cocoyam corms.

TABLE1: Frequency mean inhibition diameter of *Erwinia carotovora* on the Rot of Cocoyam Varieties in Culture

Variety	Diameter of rot (cm)
NCE1	66.75
NCE2	79.00
NCE3	72.00

NCE4	64.25
NXS1	71.00
NXS2	77.00
NXS3	85.25
CONTROL	25.00
LSD(0.05)	4.71

TABLE 2: Effect of plant extracts on the diameter of rot of Bacteria (*Erwinia carotovora)* Pathogen on the Cocoyam Corms *in vivo*

EXTRACT	INHIBITION DIAMETER (cm)
Occimum gratissimum	3.47
Cymbopogon citrates	2.95
Moringa oleifera	2.83
Chromolena odorata	2.22
Control (sterile water)	4.49

The result obtained from the culture in table 2 above indicated that all the extracts used inhibited the growth and development of the bacteria (*Erwinia carotovora).* The study showed that all the plant extract used in the study significantly inhibited pathogen, C*hromolaena odorata* had the least *(*2.22cm*)* inhibition diameter on the culture and is significant (p≤ 0.05) different from other extracts and the control, this was followed by C*ymbopogon citratus* and *Moringa oleifera* (2.9cm)) (2.83cm) *followed* by *Occimum gratissimum (3.47cm)* then control (sterile water) (4.49cm*).* From the study *Chromolena odorata* was found to be the best in the control of bacteria rot in cocoyam

DISCUSSION

From the result obtained from Table 1 it indicated that varieties NCE4 and NCE1 were more resistant to the rot incited by *Erwinia carotovora* this might be due to genetic makeup of the other varieties (Okigbo and Nmeka, 2005).

In most cases bacteria gain entrance into cocoyam cormels through natural openings and wounds created during harvesting, transportation, handling and marketing. However, Okigbo and Nmeka (2005) stated that root and tuber crops at time of harvest may already be infested by pathogens derived from disease foliage, roots or mother tubers/cormels. Pathogenicity tests on the susceptible, healthy cocoyam varieties indicated that NXS3 had the highest susceptibility to rot of cocoyam caused by *Erwinia carotovora*

The result obtained from table 2 indicated that the plant extract used for this study significantly inhibited the effect and activities of *Erwinia carotovora,* similar to the findings of Nwachukwu and Osuji (2008), that was confirmed in this study. The *in vitro* study on culture showed that extracts from *C. odorata* inhibited bacterial colonies of *E. caratovora* when compared to the other plant extracts. The inhibition potential of *C. odorata* can be due to high levels of bacterotoxic substances like flavonoids, tannins and sapronin found in the extracts, as earlier reported from extraction works on *C. odorata* leaves by Akinmoladun *et al.* (2007). This work is similar to those reported by earlier workers (Amadioha, 2004; Opara and Wokocha, 2008) which showed that some indigenous plant species could serve as potential antimicrobial agents against bacterial pathogens. Opara and Obani (2010), observed in bacterial blight of egg-plant that plant extracts used proved to be as effective as standard synthetic pesticides such as antibiotics (streptomycin sulphate) and fungicide (copper oxychloride) and were even in some cases at par with these conventional chemicals usually used in disease reduction, indicating that some natural anti-microbial active ingredients are indeed contained in these plant extracts. Amadioha (2004), reported that the greater effectiveness of the extracts may be due to inherent chemical constituents or bioactive ingredients of the plant extracts. These active principles in the plant extracts he concluded contain anti-bacterial polyphenolic compounds. This study therefore elaborates on the potency of plant extract in the control of bacteria diseases in crops. Shukla *et al*. (2012) reported that numerous *in vitro* studies have validated the efficacy of plant-derived pesticides in many branches of agriculture.

Comparatively however, fewer *in vivo* works have been done on stored products caused by moulds and storage bacteria. These investigators however inferred that plant materials were fungitoxic and prophylactic against rots organisms. According to Okigbo *et al*. (2009b) many rot inducing organisms of cassava including *A. niger* were inhibited *in vitro* by plant-based fungicides from *A. meleguata* and *A. indica*. Also, Okigbo *et al.* (2009a) reported that *Allium sativum* exhibited the toxic effects on all the organisms assayed in a study while *O. gratissimum* retarded mycelial growth of the mycoflora by 64%. In another study, Amadioha and Markson (2007), found extracts of *Piper nigrum*, *Ageratum conyzoides* and *A. melegueta* to significantly arrest the mycelial growth and biomass development of *Botrydiplodia acerina* causal agent of rot of cassava *in vivo.* According to Taiga (2011), 40% aqueous extract of *Nicotinia tabacum* completely inhibited yam rot development (*Rhizopus stolonifer*) *in vivo*. In like manner, ash of plantain peels, aided in prolonging the shelf-life of bruised yam tubers (Nahunnaro, 2008).

CONCLUSION

The population increase in the world and demand for agricultural produce is on the increase. It is important to adopt disease control practices that will be affordable by the bulk of resource poor farmers in our country (Nigeria). Such plant protection strategy must be durable, compatible and integrable with the prevailing agricultural practices specific to our people. Therefore, the use of plant-derived pesticides in crop production meets these criteria and hence warrants their exploitation in crop production. Compared to synthetic chemicals extracts of plant origin offer the benefits of pre- and post-infection bacterotoxicity and leave no toxic load on produce. This makes them an input of choice particularly in organic farming and in low input conventional farming systems. Plant-derived pesticides require no pre-harvest interval before treated produce can be harvested and consumed. They are eco-compliant; being less likely to harm farm-friendly bees, butterflies and earthworms. Extracts of plant origin are biodegradable and hence are efficient tools in reducing or eliminating pesticides persistence problems in the environment. Because they offer wide ranging modes of action against pathogens, plant extracts aids in delaying resistance problems in agriculture and as such usable in both conventional and organic farming systems. Application of plant-derived pesticides not only in field interventions, but especially in storage will spell improvement in sustainable food production and food security programs of developing countries of the world with nearly 1 billion severely hungry people. Use of plant extracts to fight rots and spoilage diseases decimating stored agricultural products in sub-saharan African countries like Nigeria, undoubtedly reflects the least cost method of arresting phyto-bacterial diseases in the country.

RECOMMEDATION

The result obtained from this study has revealed the effect of bacteria on cocoyam corms in storage but it is unfortunate that more research work has not being done to understand the epidemiology of the bacteria (*Erwinia spp.*) hence, epidemiologist should carry out more research work to study the bacteria.

Due to the detrimental effect of chemicals used in agriculture, it is important to recommend the use of plant extract in the control of various phyto-pathological diseases in crops, which will ameliorate the harmful effect of chemicals used in agriculture.

The use of resistant varieties of cocoyam in breeding purpose should be encourage and more research should be conducted by plant breeders on the production of resistant varieties of cocoyam.

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