Control of Post-Harvest Bacterial Soft Rot of Potato Using Aqueous Fruit Extract of Christmas Melon (*Laganaria breviflorus*)

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Abstract

Managing the post-harvest bacterial soft rot of potato with chemicals can lead to severe health issues. During this study, an attempt was made to use aqueous Christmas melon (Laganaria breviflorus) fruit extract to control bacteria soft rot of potato. The experiment was carried out in the Pathology Laboratory of the Federal College of Horticulture, Dadin-kowa, Gombe, Nigeria in 2022, and laid in a Completely Randomized Design with four replications. The treatments used were the different concentrations of the aqueous Christmas melon fruit extract comprising (stock solution), (80% concentrated CMFE), (60% concentrated CMFE), (40% concentrated CMFE), (20% concentrated CMFE) and Control (pathogen only without treatment). The bacterium was isolated from infected potato tuber and was plated on Nutrient Agar (NA). Pathogenicity test was carried out to confirm the pathogen before the In-vivo study. Peeled fruits of L. breviflorus were fermented for seven days in distilled water. The inoculated tubers were treated with aqueous Christmas melon fruit extract and incubated for six days at 27°C. Graphpad Prism version 9 software package was used for the analysis. The results revealed that L. breviflorus aqueous fruit extracts did not protect the potato tuber against bacteria soft rot, and therefore cannot serve as substitute for synthetic pesticide in controlling bacteria soft rot of potato caused by Pectobacterium carotovora ssp carotovora. Further study is recommended to be carried out using organic solvents such as n-hexane, methanol, etc to extract the botanical pesticide from Christmas melon fruit as there might be possibility of getting a positive result.

Keywords: *Aqueous extract, Erwinia carotovora, Laganaria breviflorus, Pathogenicity, Potato.*

Introduction

Potato (*Solanum tuberosum*, L.) is one of the most important crops in the world from the point of view of local consumption and export (Sahi *et al*., 2007). Cultivated potato is often prone to microbial infections. One of the most common diseases in potatoes is bacterial soft rot or blackleg, caused by the pathogenic strain *Pectobacterium carotovora ssp carotovora* which disseminated into the plant and tubers through the vascular vessels (Des essarts *et al*., 2016).

Several strategies have been developed to control potato soft rot. Physical methods improved the management and selection of healthy potato tubers prior to culture or storage as they allow the control of superficial fungal pathogens. However, these methods are expensive, time consuming and failed to eradicate vascular pathogenic bacteria (Czajkowski *et al*., 2011). Chemical strategies using copper sulphate, dimethylammonium chloride, sodium hypochloride, formaldehyde and antibiotics (streptomycin and derivatives) have also been used to abolish potato bacteria (Graciagarza *et al*., 2002) but they are no longer recommended because of their high impact on environmental and human health as well as the risk for selecting multidrug-resistant bacterial strains (Jess *et al*., 2014). In recent decades, essential oils and plant extracts have been screened for their ability in controlling the pathogenic strain *P. carotovorum* (Vasinauskiene *et al*., 2006).

Wide range of chemicals and antibiotics are in use against potato tuber soft rot with somewhat satisfactory results. However, prolonged use of antibiotics led to the bacterial adaptation, resulting in the development of multidrug resistance in bacteria leading to several human health complications. This has significantly limited the use of antibiotics, warranting alternative strategies to combat rot causing microbes. Moreover, low doses of antibiotics use on vegetables during storage, considering human health, make pathogenic bacteria resistant to them.
The emergence of antibiotic resistance in pathogenic bacteria has led to renewed interest in exploring the potential of plant derived pesticides as an alternative strategy to combat these microbes which are safe, effective, economical, without any side effects and are readily available in nature. As target sites of action is highly diverse in case of these plant extracts, it is difficult for microorganisms to create resistance against these (Thompson *et al*., 2013; Rahman *et al*., 2011). Amina and Yetunde (2021) reported that *Laganaria breviflorus* fruit extracts demonstrated a potential in reducing toxigenic fungi viz *Aspergillus nidulans, A. parasiticus, Fusarium specie* and *Penicillium chrysogenum* which showed susceptibility to the whole fruit extract of *Laganaria breviflorus.* Growth inhibition of *Erwinia carotovora* by aqueous extracts of *Allium sativum* has been previously reported by Akbar *et al*. 2014. In-vitro growth inhibition of *Erwinia carotovora* by aqueous extracts of *Azadirachta indica* and *Eucalyptus spp*. was also reported (Akbar *et al.*, 2014; Opara and Agugo, 2014; Simeon and Abubakar, 2014).
This study, therefore, was carried out to determine the effect of Aqueous Christmas Melon Fruit Extract for the control of bacterial soft rot of potato tuber caused by *Pectobacterium. Carotovorum subspp carotovorum*.

Materials and Methods

Study Location

The research was carried out in the pathology laboratory of the Federal College of Horticulture Dadin-Kowa in Gombe State, Nigeria. Dadin-kowa is located at latitude 10°-18° N, longitude 11°-18° E and altitude 434 m above sea level and lies in the Sudan savanna belt and characterized by a single peak of rainy season with an average rain fall of 800 mm and mean daily temperatures of 32 °c (Kowal and Knabe, 1972).

Preparation of Christmas Melon Fruit Extract

Chrismas melon (*Laganaria Breviflorus)* fruits were thoroughly washed, peeled and sliced into bucket. Distilled water was added in a ratio of 1:2 (1kg of Christmas melon to 2-litres of distilled water). The bucket was covered with a breathing net and placed in a dark ventilated room for seven days for fermentation to take place. The setup was being stirred every morning using a stick. The fermented fruits were filtered using muslin cloth and stored in rubber container prior to use.

Figure 1: *Laganaria breviflorus* fruits

Figure 2: *Laganaria breviflorus* fruits extract

Collection of Tuber Soft Rot Sample

Infected Nicola potato tubers with soft rot symptoms were purchased from Gombe market and transported to the Laboratory in a sterile zip lock bag.

Inoculum Preparation

The pathogen, *Pectobacterium carotovora ssp carotovora* was isolated from soft rot infected potato tubers. The bacteria were cultured and preserved on Nutrient Agar prior to In-vivo treatment applications.

Media Preparation

Laboratory bench was sterilized with 70% Ethanol. 8.4 g of Nutrient Agar media was weighted by using sensitive balance on filter papers and dissolved in 300 ml of distilled water. The solution was autoclaved at 121^{o}C for 19 minutes. The media was poured in the petri dishes and left to solidify. Before the media solidify, 1 ml of the bacteria (tuber soft rot) suspension was added to the media, and after solidification was incubated at 30^{0}C for 48 hours.

Pathogenicity Test

Three healthy potato tubers were surface sterilized in 10% sodium hypochlorite solution for three minutes and thereafter rinsed in five changes of sterile tap water. The Tubers were allowed to dry at room temperature. The tubers were wounded by punching 3 holes of about 5 mm deep using lancet. Thereafter, the tubers were inoculated by submerging in the bacteria cell suspension (1×10^8 cfu/ml). The inoculated tubers were stored in a rubber container with moist tissue papers on the bottom in order to keep the appropriate humid conditions. They were incubated at 30^{o}C according to Bdilya and Bashir, (2006). The rotten tissue was further plated on sterilised Nutrient Agar (NA) and the growth was sub-cultured to get a pure sample which was compared with the original isolates and confirmed.

In-vivo (Tuber) Inoculation

Healthy potato tubers were selected from the lot and sterilized in 10% sodium hypochlorite solution for 3 minutes, and thereafter was rinsed in sterile tap water up to five changes and dried at room temperature. Each tuber was wounded at the depth of 5mm with sterile lancet and artificially inoculated by submerging in the bacterial suspension for ten minutes and thereafter was allowed to dry at room temperature for thirty minutes.

In-vivo Treatment Application

The inoculated tubers were submerged in the 100% CMFE, 80% CMFE, 60% CMFE, 40% CMFE and 20% CMFE respectively for 10 minutes and dried at room temperature.

Experimental Setup

After the treatment application with the plant extracts, the tubers were placed in surface sterilized plastic containers (about 17 x 11 x 5 cm) with lids at the rate of three tubers per container, replicated four times for each treatment. Moist tissue paper was placed at the bottom of each container to maintain relative humidity. The control treatments were setup in the same manner but without treatment (CMFE) application. The containers were arranged on shelves in an incubator and incubated for six days at 30°C.

Observations were recorded on soft rot incidence and severity, disease reduction and weight loss on 2nd,4th and 6th day of storage.

$$\text{Incidence of soft rot} = \frac{\text{Number of infected tubers}}{\text{Total number of tubers observed}} \times 100$$

Tuber soft rot Severity: The severity of tuber soft rot was determined using a scale of 0–5 as described by Bdliya and Langerfeld (2005b)

where:

0 = no symptom of rot

1 = 1–15% of tuber rotten

2 = 16–30% of tuber rotten

3 = 31–45% of tuber rotten

4 = 46–60% of tuber rotten

5 = ≥ 61% of tuber rotten.

The severity was computed using the formula:

$$S = \frac{\sum n}{N \times 5} \times 100$$

where:

S = severity of tuber rot

(%) ∑n = the sum of individual ratings

N = total number of potato tubers assessed
5 = highest score on the severity scale.

Percentage of Disease Reduction (PDR)

The percentage of disease reduction (PDR) was calculated according to the following formula as previously described (Hajhamed *et al*., 2007).

$$PDR = \frac{Ack - Atr}{Ack} \times 100$$

Where:

Ack = Disease severity in control

Atr = Disease severity in treated potato tubers

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using CRD (Completely Randomized Design) and the mean differences were declared significant at 5% level of probability using the standard error of means as described by Gomez and Gomez (1984). Graphpad Prism version 9 software package was used for the analysis.

Results and Discussion

Result

Effect of Aqueous Christmas Melon Fruit Extract on Incidence, Severity and Disease Reduction of Bacteria Soft Rot Caused by *Pectobacterium carotovora* on Stored Potato Tubers after 6-days of Storage.

After 6-days of storage, all the treatments recorded 100% incidence (Fig:3). The severity was tremendously high with 100% CMFE (stock solution) giving 95.56% soft rot severity while the rest of the treatments gave 100% soft rot severity (Fig:4). The treatments were not significantly better compared to the control. Similarly, there was drastic decline in disease reduction with only 4.44% obtained from 100% CMFE, while the other treatments gave 0.00% disease reduction similar to control (Fig:5).

Discussion

The aqueous Christmas melon fruit extract (CMFE) exhibited low efficacy against *Pectobacterium carotovora* as observed during tuber storage. Results obtained after 6 days of tuber storage gave soft rot mean incidence of 100% across the different levels of concentration (treatments) and mean severities of 95.56% for 100% CMFE (stock solution) and 100% mean severity for 80, 60, 40 and 20% CMFE respectively, which was not significantly better than the control (pathogen only and no treatment). Also, the percentage disease reduction after 6-days of tuber storage was 4.44% for 100% CMFE (stock solution) and 0.00% disease reduction for 80, 60, 40 and 20% CMFE respectively.

The study, however, showed that the aqueous Christmas melon fruit extract did not offer any protection to the potato tubers against the bacteria soft rot thus, not significantly reducing the severity of the disease. A thorough search of literature could not reveal any reports regarding control of soft rot caused by *Pectobacterium carotovora* in particular using aqueous extracts of Christmas melon fruit. But, Badmos and Mamood (2021) reported that *Laganaria breviflorus* fruit extracts demonstrated a potential in reducing toxigenic fungi viz *Aspergillus nidulans, A. parasiticus, Fusarium specie* and *Penicillium chrysogenum* which showed susceptibility to the whole fruit extract of *Laganaria breviflorus.* Outstanding efficacy of neem seed kernel (*Azadirachta indica*) against post-harvest bacterial soft rot on stored potato tubers caused by *Pectobacterium carotovora* (*Erwinia carotovora)* has already been reported by Bdilya and Bashir (2006).

Similarly, Viswanath *et al*., (2019) reported that aqueous extracts of *Syzygium aromaticum* and *Salix alba* (leaves) were found more effective than *Azadirachta indica* in decreasing the incidence and severity of post-harvest soft rot caused by *Pectobacterium carotovora* (*Erwinia carotovora)* up to one week of storage, which proved the potential of plant extracts of *Syzygium aromaticum* and *Salix alba* for their antimicrobial activity and possibility of developing their use against post-harvest soft rot of vegetables in further research, which are eco-friendly in nature.

Aqueous extract of leaves, barks, fruits and green husks of *J. regia* revealed broad spectrum antibacterial activity against *Pseudomonas aeruginosa* (another potato tuber soft rot causative agent) (Deshpande *et al*., 2011).

Conclusion

The results of this study reviewed that Aqueous extract of Christmas melon fruit did not have potential in protecting stored potato tuber against bacteria soft rot cause by *Pectobacterium carotovora* subsp. *Carotovora*, and as such cannot serve as a substitute for chemical pesticides in the management of bacteria soft rot potato tuber. So far, no study has revealed the use of *Laganaria breviflorus* in storing potato against *Pectobacterium carotovora* (*Erwinia carotovora)* in potato tuber; this is the first of its kind. Although it is widely used in folklore medicine in West Africa, particularly in Nigeria as antibacterial and antiviral herbal remedies especially amongst poultry farmers. Further study is recommended to be carried out using organic solvents such as n-hexane, methanol, etc to extract the botanical pesticide from Christmas melon fruit as there might be possibility of getting a positive result.

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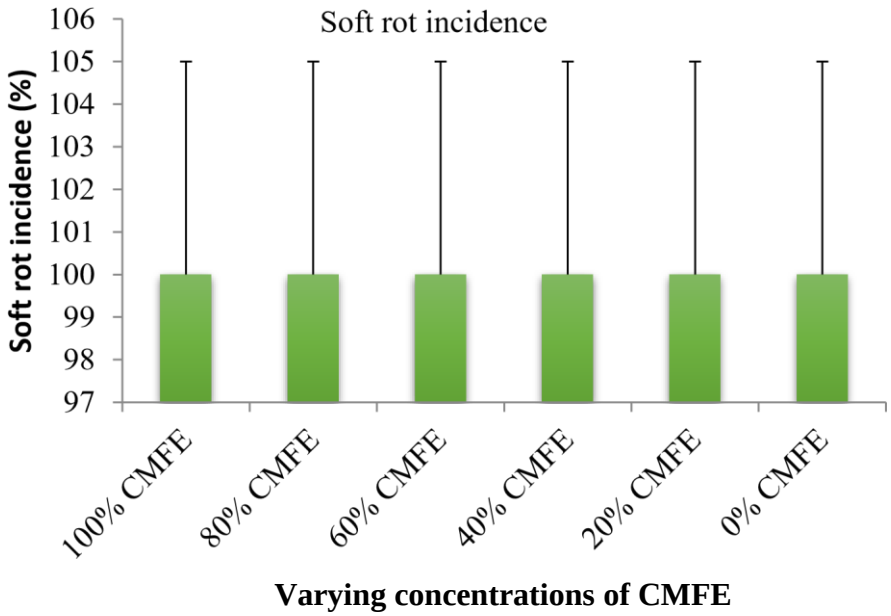

Figure 3. Soft rot incidence 6 days after storage (In-vivo). Bars indicate standard error of means at 5% probability level.

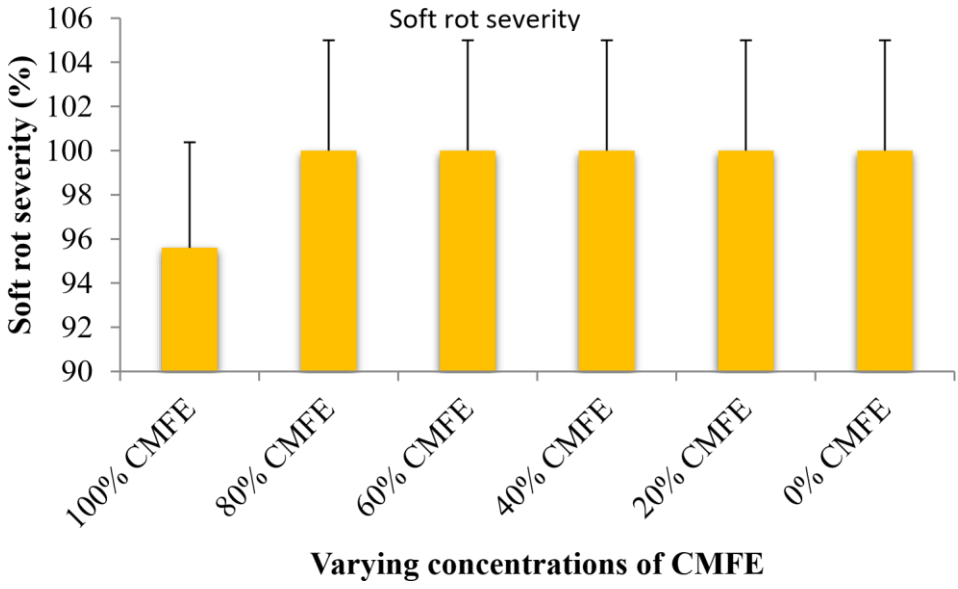

Figure 4. Soft rot severity 6 days after storage (In-vivo). Bars indicate standard error of means at 5% probability level.

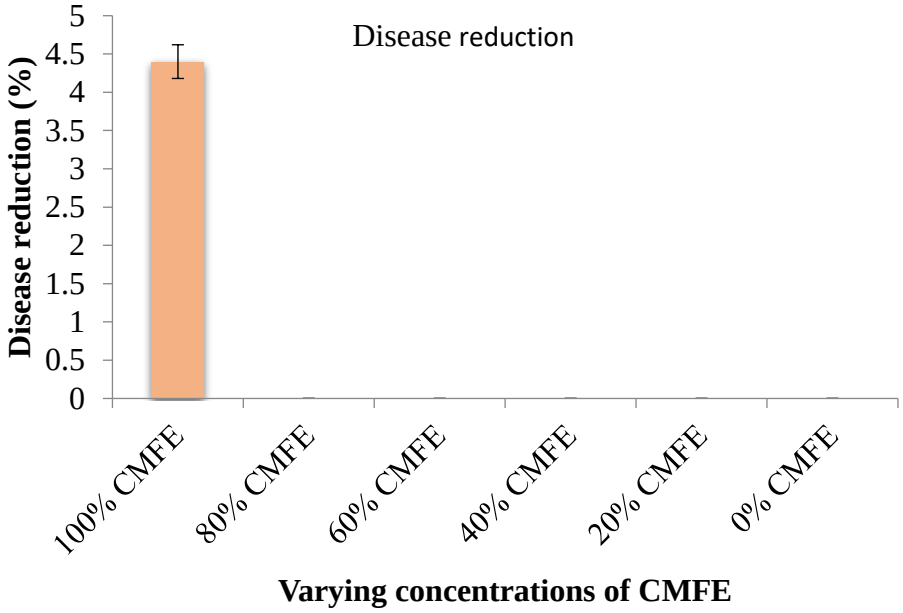

Figure 5. Soft rot disease reduction 6 days after storage (In-vivo). Bars indicate standard error of means at 5% probability level.