Advanced Journal of Plant Biology
Volume 4(2), pages 16-20, April 2023
Article Number: 8277BF001
ISSN: 2992-4928
https://doi.org/10.31248/AJPB2021.017
https://integrityresjournals.org/journal/AJPB

Full Length Research

Bacillus cereus, Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa and *Proteus mirabilis* associated with post-harvest storage of cocoyam (*Colocasia esculentus* L) corms rot: Occurrence and *in vitro* response to leaf extracts of *Vernonia amygdalina*

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Received 8th December, 2021; Accepted 25th January, 2022

ABSTRACT: Cocoyam is a root crop that is produced in regions of the tropical or sub-tropical developing countries. In certain countries like Ghana, cocoyam production is surplus but post-harvest losses are high due to mechanical damage of corms during harvest which predisposes the corms to microbial attack in storage. Antibacterial activities of the test plant were determined using agar diffusion method. Five bacteria strains were isolated from rotten cocoyam corm viz: *Bacillus cereus, Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa* and *Proteus mirabilis.* Cold aqueous extract of 20% of the test plant mostly inhibited *Staphylococcus aureus* (0.66) followed by *Bacillus cereus* (0.65). The least inhibited organisms being *Pseudomonas aeruginosa* (0.36) and *Proteus mirabilis* (0.38). Hot water extract (20%) of the test plant mostly inhibited *Bacillus cereus* (0.52) followed by *Staphylococcus aureus* (0.49). The results showed that higher concentrations of *V. amygdalina* extracts inhibited the growth of organisms more than lower concentrations. The use of plant based biocide against bacterial isolates proves efficacious as they are effective, affordable and are less harmful to man and the environment.

Keywords: Antibacterial, cocoyam, *V. amygdalina.*

INTRODUCTION

Cocoyam is an herbaceous plant belonging to the family Aracaceae. They are grown primarily for their edible starchy root, although all the plant parts are edible. Cultivated cocoyam as food crops belong to either the genus *Colocasia* or *Xanthosoma*. Colocasia is grown for its corm which is consumed after boiling, frying or roasting (Rao et al., 2010). The corms can be dried and used to make flour or sliced and fried to make chips. The leaves of the plant are also edible and are usually consumed as a vegetable after cooking in dishes such as stews. *Xanthosoma* species produce tubers much like potato and are boiled, baked, steamed or fried prior to consumption. The corm of some varieties is also consumed. Young leaves are eaten as a vegetable (CABI, 2014; Vaneker and Slaats, 2013).

Cocoyam thrives best when planted in full sunlight or partial shade. The plant can survive for short period of 10°C but will be damaged or killed by lower temperature

(MoFA, 2010). Cocoyam leaves are locally used for wrapping kolanut and bitter cola (Lawal, 2004). Braide and Nwaoguikpe (2011) reported dietary fibre of cocoyam as being aider of digestive system and easy passage of excreta. Cocoyam is prone to pest and disease attack which can account for 60% of corm loss (Opara and Obani, 2010). Microbial deterioration can be mitigated through the use of disease free planting material (Onwusu-Darko, 2014), weeding and treatment with cooper based copper pesticide (Opera, 2000). High moisture has been found to promote bacterial rot of cocoyam corms (Onwusu-Darko, 2014).

Vernonia amygdalina belonging to the family Asteraceaa (Ijeh and Ejike, 2011), perennial shrub or small tree of 2 to 5 m with petiolate leaf of about 6 mm diameter and elliptic shape (Farombi and Owoeye, 2011). The leaves are green with a characteristic odour and a bitter taste. No seeds are produced and the tree has to be distributed through cutting. The leaves are used for human consumption and washed before eating to get rid of the bitter taste. They are used as vegetable and stimulate the digestive system, as well as reduce fever. Furthermore, they used as local medicine against leech, which are transmitting bilharziose. Free living chimpanzees eat the leaves, if they have attacked by parasites. *Vernonia amygdalina* is also used instead of hops to make beer in Nigeria (Anonymous, 2000). Furthermore, *Vernonina amygdalina* is found in homes in villages as fence post and pot-herb (Anonymous, 1999). Antimicrobial activities of methanolic extract of *Vernonia amygdalina* against *E. coli*, *C. koseri*, *E. aerogenes, S. aureus* and *E. cloacae* clinical strains have been reported by Gashe and Zeleke (2017).

Therefore, this research is designed to investigate the anti bacterial effect of *Vernonia amygdalina* to mitigate various microbes that are associated with storage rot of cocoyam as botanical based bactericide against cocoyam isolates proves effective as they are accessible and less harmful.

MATERIALS AND METHODS

Study area

The experiment was carried out in the laboratory of Plant Science and Biotechnology Department, Faculty of Science, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. Ado-Ekiti has wet and warm seasons. The dry season is hot, muggy, and partly cloudy. Ado-Ekiti is located on latitude 7° 37' 15.9996'' N and longitude 5° 13' 17.0004'' E with annual average rainfall of 14.89 mm, temperature typically varies from 64°F to 90°F and is rarely below 58°F or above 95°F. A wet day is one with at least 0.04 inches of liquid or liquid-equivalent precipitation. The chance of wet days in Ado-Ekiti varies very signify-cantly throughout the year. The wet season lasts 6.3 months, from April 15 to October 25, with a greater than 43% chance of a given day being a wet day, 14.89 mm of rain and approximately 7 rainy days in the month. The month with the most wet days in Ado-Ekiti is September, with an average of 24.8 days with at least 0.04 inches of precipitation. The average humidity of Ado Ekiti is 18%.

Preparation and Sterilization of laboratory wares

All glass wares used for this experiment were washed using detergent in running water. They were air dried before sterilization in the autoclave. The inoculating chamber and other working surfaces were sterilized by swabbing with 70% alcohol.

Sample collection and identification

Fresh leaf samples of *V. amygdalina* were collected from a vegetation site in Ado Ekiti. The identity of the collected plant was authenticated in the herbarium unit of Ekiti State University, Ado Ekiti. The leaf samples were air dried at room temperature for two weeks before being pulverized. The powdered samples were stored in a clean air tight container in the laboratory before use.

Preparation of media

Twenty-eight (28 g) of nutrient agar was weighed on Melter weighing balance and poured into 100 ml beaker. The medium was dissolved by boiling in a water bath in order to give room for homogenization. This was later removed and allowed to cool down at room temperature before dispensing into sterile MacCartney bottles before it was autoclaved at 121°C for 15 minute.

Preparation of plant extracts

Hundred grams (100g) of test plant powder was weighed into 200 ml of distilled water and this was allowed to stand overnight at room temperature. This was later filtered using muslin cloth and the filtrate (served as the extract) was stored in the sterile bottle at 4°C.

Isolation of bacteria

After extraction, 1 ml of infected cocoyam corm broth was taken using syringe and dispensed into 9 ml of sterile water. This process was serially diluted and the final diluent was stored in the test tube and corked using cotton wool to avoid contamination.

Pathogenicity tests

Pathogenicity tests were carried out using established protocols and techniques in bacteriology. Healthy cocoyam corms were washed in sterile distilled water and surface sterilized with 0.1% ethanoic solution. A sterilized cork borer was used to cut (creating core) the corms and then cultures of the bacterial isolates were introduced into the openings and the cores were placed back. Petroleum jelly was smeared to completely seal the hole to guide against contamination. These were incubated for five (5) days. Upon observation of lesions, inoculums from the infected corms were taken and cultured. The symptoms were identical to those of naturally infected cocoyam.

Identification of organisms

Pure isolates obtained from the diseased cocoyam fruits were identified subjecting for identification purposes. Each isolate was subjected to macroscopic and microscopic examinations during which their structural features were observed. Identification of bacteria was based on the growth patterns, colour of culture and microscopic examinations of bacteria.

Determination of antibacterial activity of the test plant

Determination of antimicrobial effects of the test plant was by pour plate method. Molten nutrient agar was poured into sterile Petri dishes, allowed to stand, cool down to 45°C and the bacterial inoculum was streaked on the medium. Wells were punched into the agar gel using 4 mm cork borer and the wells were filled with 1 ml of the test plant extracts. The plates were incubated at 37°C for 24 hours. The antibacterial activity of the test plant was determined by measuring the diameter of the zone of inhibition using metre rule.

Data analysis

Data obtained from this study were subjected to analysis of variance (ANOVA) using SPSS. Means were separated using Duncan multiple range test (DMRT) at 5% level of significance ($p \leq 0.05$).

RESULTS AND DISCUSSION

Table 1 showed the effects of different concentrations of aqueous extracts of *V. amygdalina* against post-harvest bacteria isolated from rotten cocoyam corm as contaminants viz: *Bacillus cereus, Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa and Proteus mirabilis.* All the concentrations (5 to 20%) of the test plant mostly inhibited *Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa and Proteus mirabilis.* Cold aqueous extract of 20% of the test plant mostly inhibited *Staphylococcus aureus* (0.66) followed by *Bacillus cereus* (0.65). The least inhibited organisms being *Pseudomonas aeruginosa* (0.36) and *Proteus mirabilis* (0.38).

Table 2 showed that all the concentrations (5 to 20%) of the test plant mostly inhibited *Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa and Proteus mirabilis.* Cold aqueous extract of 20% of the test plant mostly inhibited *Bacillus cereus* (0.52) followed by *Staphylococcus aureus* (0.49) while the least inhibited organism was *A. hydrophila* (0.40).

Results obtained in this study further showed that cold aqueous extract of *V. amygdalina* leaf extract had significant bacteriacidal effect against the five species of bacteria used. The resistance shown by Staphylococcus aureus is similar to that of Nostro et al. (2000) and Ojala et al. (2000) who reported that gram-negative bacteria were not susceptible to plant extracts when compared with gram positive bacteria. This resistance of gram negative bacteria is said to be related to lipopolysaccharides in their outer membranes (Gao et al., 1999).

In contrast to this, Foster and Duke (1990) concluded that gram-positive organisms, because of their double cell wall component of mucopeptide and peptidoglycan, tends to resist adverse condition better than gram-negative organisms which may be the major cause why *S. aureus* proved to have the least inhibition than the other organisms. The inhibitory effect of 2.5 gl ml concentration on all the test organism shows that high concentrations are desirable for effective control as asserted by Calixto (2000). Compare with some of the antibiotic discs in the control, *V. amygdalina* has been shown to have very good antibiotic properties comparable to the conclusion of Calixto (2000) which should further be exploited. The results obtained in this work confirms the efficiency of some plant materials as natural anti-microbial and suggest the possibility of using them in drugs for treatment of infectious disease caused by test organisms (Obeidat et al., 2012). Plants produce antimicrobial agents as secondary metabolites for self-defense against pathogenic invasion (Meela, 2008). The use of plant extracts remains one of the major sources of natural products for a new therapy mainly in the developing countries as plant extracts cost less. It is also effective against a broad range of antibiotic resistant microbes. Additional benefit of using plant-based biocide is that, natural products have less adverse consequence on man and the environment (Chethana et al., 2012). Chemical application creates numerous environmental and attendant resistant by microbes. Nahunnaro (2008) reported high antibacterial effects of *V. amygdalina* against yam rot pathogens. Plant extracts have effectively controlled various phytopathogens. Amadioha (2000) successfully controlled rice blast pathogens both *in vitro* and *in vivo* using *A.*

Table 1. Effects of different concentrations of cold aqueous extracts of *V. amygdalina* against post-harvest bacteria of cocoyam corm.

Concentration of extracts %	Bacterial isolates				
	S. aureus	***A. hydrophila***	***P. aeruginosa***	***P. mirabilis***	***B. cereus***
Control	6.41^{c}	8.60^{b}	6.91^{c}	5.81^{c}	9.57^{a}
5	0.44^{c}	0.56^{a}	0.36^{c}	0.38^{b}	0.51^{b}
10	0.50^{c}	0.61^{a}	1.41^{a}	1.45^{c}	0.52^{b}
15	0.55^{c}	0.65^{c}	0.43^{c}	0.49^{d}	0.58^{b}
20	0.61^{b}	1.66^{c}	0.46^{c}	0.52^{c}	0.65^{c}
LSD	1.45	0.08	0.07	0.08	0.80

Values followed by the same letter are not significantly different at ($p<0.05$ at Fischer's LSD).

Table 2. Effects of different concentrations of hot aqueous extracts of *V. amygdalina* against post-harvest bacteria of cocoyam corm.

Concentration of extracts %	Bacterial isolates				
	S. aureus	***A. hydrophila***	***P. aeruginosa***	***P. mirabilis***	***B. cereus***
Control	6.41^{c}	8.60^{b}	6.91^{c}	5.81^{c}	9.57^{a}
5	0.31^{c}	0.42^{a}	0.32^{c}	0.34^{b}	0.42^{a}
10	0.34^{d}	0.44^{a}	1.36^{c}	1.43^{b}	0.44^{a}
15	0.37^{c}	0.46^{b}	0.40^{b}	0.46^{b}	0.48^{a}
20	0.40^{c}	1.49^{b}	0.42^{c}	0.48^{b}	0.52^{a}
LSD	1.45	0.05	0.05	0.04	0.91

Values followed by the same letter are not significantly different at ($p<0.05$ at Fischer's LSD).

indica. In Nigeria, plant extracts have been used by various researchers to mitigate plant diseases such as cowpea (Alabi et al., 2005), banana (Okigbo and Emoghene, 2004), yam (Okigbo and Nmeka, 2005), cocoyam (Nwachukwu and Osuji, 2008) and sweet potato (Amienyo and Ataga, 2007).

Both cold and hot aqueous extracts of *V. amygdalina* proved efficacious against isolated bacteria from rotten cocoyam corms. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) obtained in this study could be attributed to low concentration of active compounds and vice versa as this has been reported by Kuta et al (2013) who asserted that variations in the values of MIC and MBC could be attributed to low amount of active compound contained in the extracts. Gottlieb et al (2002) reported that plant extracts are antagonistic against bacterial pathogens, as plant extracts play important role in crop production, this will have prominent role in the development of future commercial pesticide for crop production strategy in the management of plant diseases.

Conclusion and Recommendation

Aqueous extracts of *V. amygdalina* exhibited significant inhibitory effects on bacteria associated with cocoyam rot. The extracts are not known to possess any harm to man and the environment. *V. amygdalina* plants are readily available. Hence, they can be used as bio protectant on cocoyam by rural dwellers that may not be able to afford the cost of chemicals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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