Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2017, 9 (1):1-6
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Effect of aqueous and ethanol extract of Citrus paradisi on fungi rots of banana (M. acuminata L) in Jalingo, Taraba State

Muazu SA*, Channya FK, Chimbekujwo IB, Tukur KU, Fauziya KM, and KB Samuel

[1]Departmentof Biological Sciences, Taraba State University, Jalingo, Nigeria
[2]Departmentof Plant Science, Modibbo Adama University of Technology, Yola, Nigeria

ABSTRACT

Studies on isolation and identification of fungi associated with banana rots were carried out. The effect of aqueous and ethanol extract of Citrus paradisi were determined in vitro on causative agents of post-harvest rot of banana. Concentrations of 20, 40 and 60% of both aqueous and ethanol extract of Citrus paradisi were used. The treatments were laid out in Completely Randomized Design (CRD) with three replications. Rhizoctonia solani, Rhizopus stolonifer, Colletotrichum musae and Pyricularia grisea were isolated and identified to be associated with the banana rots. Rhizoctonia solani has the highest frequency of occurrence with 37.50% followed by Rhizopus stolonifer with 26.25% and the least was Pyricularia grisea with 17.50%. Concentrations of both aqueous and ethanol extract (20, 40 60%), significantly ($p< 0.05$) inhibited radial mycelial growth of the fungi compared with the control. All citric extracts at varying concentrations were effective in reducing the mycelial growth and the effect was proportional to the concentration of the extract. The inhibition was highest at 60% concentration and lowest at 20% concentration. Both Aqueous and ethanol extract suppressed the growth of all the fungal isolates, but the ethanolic extract showed higher inhibition on fungal isolates. The study revealed that both aqueous and ethanol extract have antifungal properties to control banana rot caused by Rhizoctonia solani, Rhizopus stolonifer, Colletotrichum musae and Pyricularia grisea which serves as good option to synthetic fungicide which are hazardous and often costly.

Keywords: Antifungal activity, Musa acuminata, Citrus paradisi, Aqueous and ethanol extract, Jalingo

INTRODUCTION

Natural plant protectant with antimicrobial and antifungal properties have been advocated as an alternative to synthetic fungicides in the control of fungi associated with post-harvest rots of most vegetables and edible fruits, especially banana fruits (Musa spp). Banana being a highly perishable fruit suffers severe post-harvest losses both in terms of quality and quantity [1], and the major limiting diseases of most bananas in the world are phytopathogenic fungi, bacteria, and viruses. For example, 50 to 90% losses of marketable fruit have been reported from virus infection [2]. Bacterial spot caused by a seed-borne bacterial pathogen (*Xanthomonas campestris pv. vesicatoria*) is also capable of causing severe defoliation of plants, resulting in reduced yield and loss of quality of harvested fruit when severe damage occurs on enlarging fruits.

Medicinal plant materials have been successfully used for the treatment of fungal and bacterial infections in humans [3] suggesting that some plant materials may also possess antifungal and antibacterial constituents that are useful in controlling plant diseases [4]. Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails [5-7].

Grapefruit extract for example contain different classes of polyphenolic flavonoids that have been shown to exert antifungal activities [8]. Citric extract of grapefruit is a liquid extract derived from the seeds, pulp, and white membranes of grapefruit [9]. This extract has been stated by some practitioners of alternative medicine to possess antibacterial, antiviral, and antifungal properties [10]. Previous report [11,12] shows that grapefruit seed and pulp extract prepared or contaminated with ethanol, known as ethyl alcohol, is effective in inhibiting bacteria.

Application of synthetic fungicides is the most common practice for commercial control of banana rots [13-15]. Although synthetic fungicides proved effective in the control of major postharvest diseases, their application may be

harmful to human health and the environment and they become ineffective after prolonged use. Attempts have been made towards banana rot control through cultural, physical and biological [15] methods as an alternative to synthetic fungicides [16].

As researchers are looking for alternative to these agrochemicals [17], natural plant products derived from plants effectively meet this criterion and have enormous potential to influence modern agrochemical research [18]. The use of natural plant pesticides is now emerging as one of the prime means to protect crops and their products and the environment from pesticides [19]. Plant pesticides degrade more rapidly than most chemical pesticides, and therefore are considered to be ecofriendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention [18]. Most of the plant pesticides generally degrade within a few days, and sometimes even within a few hours [20].

Despite the alarming rate of spoilage caused by fungi on banana fruit, little or no researched has been carried out to test the fungitoxic properties of these plants pesticides in the study area which serves as an alternative to synthetic fungicide to small scale farmers and therefore, the objective of the study was to isolate and identify fungi causing post-harvest rot of banana fruit. In addition, test on the efficacy of aqueous and ethanol extract of grapefruit on the isolated organism was also evaluated.

MATERIALS AND METHODS

Study Area

The study was conducted in Biological Science Laboratory, Taraba State University, Jalingo. Taraba State. The University is located between latitudes 8°47′ to 9°01′N of the equator and longitudes 11°09′ to 11°30′ E of the Greenwich Meridian. The city of Jalingo within which the University is located in the administrative headquarters of Taraba State with climatic condition typical of the tropics having raining and dry seasons. The raining seasons starts from May to October while the dry season commences from November to April. It has an annual mean rainfall of 125 mm and temperature of 30ºC to 40ºC.

Collection of banana fruits

Crown of banana cultivar "Cavendish" showing deterioration and rotting were collected from Jalingo Main markets located in Jalingo, Taraba State, Nigeria. Mature green apparently healthy banana fruit (*Musa acuminata L.)* cultivar "Cavendish" of uniform sizes were also collected and packed into a sterilized polythene bag and were taken to the Biological Science Laboratory at Taraba State University (TSU), Jalingo for isolation and other studies.

Isolation and identification of fungi from diseased fruits

The medium used for the study was Potato Dextrose Agar (PDA) [21] and fungi associated with diseased banana fruits were isolated using Blotter method as recommended by the International Seed Health Testing Association (1976). The banana fruits were cut into sections with a sterilized knife. The sectioned fruits were surface sterilized with 1% sodium hypochlorite for 8 seconds and were rinsed with three changes of sterile distilled water to remove surface contaminants. The sterilized sectioned fruits were dried between filter papers and then plated on already prepared PDA media. The plates were incubated at room temperature for 3 days before sub-culturing on new set of PDA plates. The sub-cultures were incubated for 5-7 days and sub-cultured repeatedly on new sets of PDA plates until pure cultures of the isolates were obtained.

Identification of the isolated fungi were carried out based on their cultural characteristics on growing media, under binocular microscope and with the help of identification scheme of Snowdon [22] manual. The name of each fungus and dates of inoculation were labeled on the plates.

Determination of the frequency of occurrence of the isolated fungi

The frequencies of isolations of the different types of fungi associated with banana fruit rot diseases were determined adopting the method of Zakari et al. [23] using the percentage frequency of occurrence as follows:

$$\% FC = \frac{\text{Number of times a fungus was encoutered}}{\text{Total fungal isolations}} \times 100$$

Pathogenicity test

To ascertain the pathogenicity of the various fungi that were isolated, the approach of Balogun et al. [24] was employed.

Apparently Fresh and healthy matured banana fruits were surfaced sterilized with 0.5% sodium hypochlorite for 3 second and then rinsed in sterile distilled water. With a 5 mm diameter flame sterilized corkborer, cylindrical cores were removed from each fruit which were then inoculated aseptically with 5 mm diameter disc from the advancing edge of 7-day old fungal cultures of any one isolate. Vaseline jelly was smeared to completely seal the surface of the inoculated banana fruit to prevent external infection before incubating for 10 days. The controls were inoculated with disc of solidified potato dextrose agar medium. Fruit were inoculated in three replicates in a complete randomized design. Rot symptoms developed with different fungal isolates were compared to the natural original rot.

Collection and extraction of plant materials

Plant materials used were seed and juiceless pulp of *Citrus paradisi*. The seed and juiceless pulp of the grape fruit were collected, washed and air-dried for two weeks to prevent loss of active component. Then were ground into powder with electric blender, water and ethanol were used for extraction [25].

Forty grams of dry seed and juiceless pulp powder were placed in 160 ml of sterile distilled water and left to stand at room temperature for 24 hours. The mixture was filtered with 3 layer cheese cloths and filtrate extract of 60%, 40% and 20% dilution was prepared using distilled water.

Ethanol extraction

The same method was used for the ethanol extract. Except that in this, the extract was placed into a wide tray to evaporate ethanol and equal quantities of distilled water were added to make citric extract.

Effect of citric extract on fungal mycelial growth

The approach of Amadioha and Obi [26] and Ijato [27] was used to evaluate the allelopathic effect of the citric extract on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicated the center of the plates. This was done before dispensing PDA into each of the plates. About 60 ml, 40 ml and 20 ml of the citric extracts of *Citrus paradisi* was separately introduced into the conical flask containing the same quantity of media (250 ml). The amended media were plugged with cotton wool and heated for about 10 minutes to avoid contamination [28] before dispensing 10 ml each into the Petri-dishes (poisoned food method)) Nene and Thalpiyal [29]. Each Petri dish was inoculated with 5 mm plug of pure isolate taken from margins of actively growing culture of pathogen. PDA plates free of the extracts were also prepared as control. Three (3) plates were used as replicates for each particular treatment as well as control. Then Petri plates were incubated at 25° ± 2°C until fungal growth in the control filled the whole petri dish, and then all treatments were examined.

Mean radial mycelial growth of each isolate was recorded and data were transformed into inhibition percentage by using the following formula [30].

$$\textit{Inhibition percentage}\ (\%) = \frac{DC - DT}{DC} \times 100$$

Where DC - Average Diameter of fungal spores germinated in control

DT - Average diameter of fungal spores germinated with treatment.

Experimental design and statistical analysis

Completely Randomized Design (CRD) as described by Gomez and Gomez [31] were used with three replicates. Data collected were analyzed statistically using SAS program and the inhibitions of radial mycelial growth were examined using Analysis of Variance (ANOVA) and means that were significant were separated using protected Fisher's Least Significance Difference test (LSD) at $p<0.05$.

RESULTS

The isolated fungi from the banana fruit were *Colletotrichum musae, Pyricularia grisea, Rhizoctonia solani* and *Rhizopus stolonifer. R. solani* occurred more frequently with 37.50%, followed by *R. stolonifer* with 26.25%, *Colletotrichum musae* with 18.75% and the least was *Pyricularia grisea* with 17.50% (Table 1). All isolated fungi were pathogenic on banana fruits, from physical observation the symptoms were similar to those exhibited by the rotten banana fruits from which the organism were isolated. *Rhizoctonia solani* and *Rhizopus stolonifer* completely disintegrated the affected tissue (100% infection) with extensive rots covering the fruit and mycelial growth on PDA within 3-4 days. The rot induced by *Colletotrichum musae* and *Pyricularia grisea* shows moderate to severe infection.

All concentration of aqueous citric extract significantly ($p<0.05$) suppressed the mycelial growth of the four tested pathogens (Table 2), the effect was proportional to the concentration of the extract and this was also statistically significant ($p<0.05$). The inhibition was highest at 60% concentration and lowest at 20% concentration. However, aqueous extract inhibited the growth of *Colletotrichum musae* more than *R. solani, R. stolonifer*, and *Pyricularia grisea*. All concentration of ethanol extract also significantly ($p<0.05$) suppressed the mycelial growth of the isolated organism and the effect was also proportional to the concentration of the extract. The inhibition was highest at 60% concentration and lowest at 20%. However, the toxicity of ethanol extract was higher than that of aqueous extract in the percentage growth inhibition of the four tested pathogens (Table 3).

Table 1: Percentage frequency of occurrence of fungi from rotted banana fruits.

Fungi isolated	Numbers of times isolated	Percentage frequency (%)
P. grisea	14	17.50%
R. solani	30	37.50%
R. stolonifer	21	26.25%
C. musae	15	18.75%

Table 2: Inhibition effect of aqueous extract of *Citrus paradisi* on the mycelial growth of pathogen.

Concentration (%)	Inhibition (%)Fungal pathogens			
	P. grisea	*R. solani*	*R. stolonifer*	*C. musae*
20	33.55	1.25	7.08	60.85
40	42.27	6.58	9.32	79.99
60	51.66	13.9	15.29	85.45
Control	0.00	0.00	0.00	0.00
Mean	31.87	5.43	7.93	56.57
LSD ($p< 0.05$)	6.37	4.74	3.54	9.25

Table 3: Inhibition effect of ethanol extract of *Citrus paradisi* on the Mycelial growth of pathogen.

Concentration (%)	Inhibition (%)Fungal pathogens			
	P. grisea	*R. solani*	*R. stolonifer*	*C. musae*
20	51.91	40.51	9.25	37.64
40	84.60	59.83	87.40	85.10
60	85.57	93.97	94.07	85.03
Control	0.00	0.00	0.00	0.00
Mean	55.52	48.58	47.68	60.29
LSD ($p<0.05$)	13.98	4.26	4.60	3.21

DISCUSSION

The study showed that a number of fungi are associated with post-harvest rot diseases of banana fruits in the study area. These fungi include *Pyricularia grisea, Rhizoctonia solani, Colletotrichum musae* and *Rhizopus stolonifer*. The result of the pathogenicity test carried out showed that the fungal organisms re-isolated had the same characteristics with those isolated originally from the rotten banana fruits; hence they are the causal agent of the fruits rot observed. This agrees with the report of Meredith [32], Adisa [33], Hansen et al. [34], Oyewole [35], and Abd-Alla et al. [36] that *Pyricularia grisea*, *Rhizoctonia solani*, *Colletotrichum musae* and *Rhizopus stolonifer* were among the fungi associated with the post-harvest fruits rot of banana (Musa spp).

All the fungal isolates are pathogenic on the banana fruits used in the study, although they differ to some extent. The results further revealed that *Rhizoctonia solani* is the most pathogenic owing to the size of rot caused and this was in agreement with the findings of Oyewole [35] who reported that *Rhizoctonia solani* was responsible for the fruit spoilage of banana. Ewekeye et al. [37] also reported on the pathogenic effect of *Rhizopus stolonifer* on Daucus spp and *Carica papaya*. *Colletotrichum musae* and *Pyricularia grisea* shows moderate to severe effect on the banana fruits.

The aqueous and ethanol extract of *Citrus paradisi* tested for antifungal activity against four fungi isolated from banana fruits showed that both aqueous and ethanol extract tested showed antifungal activity against all organism isolated. The antifungal activity of citric extracts was reported by Sim [10], Krauss and Johanson [38] who studied extracts of citric seeds under the post-harvest period. All concentration of aqueous extract of *Citrus paradisi* suppressed the mycelial growth of the four tested pathogens, the effect was proportional to the concentration and

inhibition value at 60% (higher concentarion), was higher for *Colletotrichum musae* followed by *Pyricularia grisea*. However, lowest inhibition value was observed from *Rhizoctonia solani*. All concentration of ethanol extract of *Citrus paradisi* suppressed the mycelial growth of the four tested pathogens, and this was also reported by Shivpuri et al. [39] who noticed that ethanol extracts of *A. indica* showed fungitoxic properties against 5 pathogenic fungi (*Alternaria brassicola, Colletotrichum capsici, F .oxysporum, R. solani* and *Sclerotinia sclerotiorum*) when tested under laboratory conditions at 500 and 1000 µg/ml. Previous report by Kampf and Kramer [11] Cvetric and Vladimir-Knezevic [12] shows that grapefruit seed and pulp extract prepared or contaminated with ethanol, known as ethyl alcohol, is effective in inhibiting bacteria.

The effect of ethanol extract of Citrus paradisi was also proportional to the concentration and inhibition value at 60% concentration (highest concentration), was higher for *Rhizopus stolonifer*, followed by *Rhizoctonia solani* and the lowest inhibition value was observed from *Colletotrichum musae*. Comparing Citric extracts of aqueous with ethanol, the inhibition percentage in each increased gradually with the extract concentration, both suppressed the growth of all the fungal isolates but the ethanolic extract showed higher inhibition on the growth of all the fungal isolates. Further investigation is therefore suggested on testing the fungicidal effect of the extract *in-vivo* on the fruit and to fractionate the extract to identify, isolate, purify and then characterize the principles responsible for the reported control. The study shows that the used of aqueous and ethanol citric extract against banana rot pathogens would have better result as they are biologically based and environmentally safe.

CONCLUSION

The findings from this study revealed that most rots of banana in Yola are caused by these four isolated fungi; they are *Colletotrichum musae, Pyricularia grisea, Rhizoctonia solani* and *Rhizopus stolonifer*. All were found to be highly pathogenic on banana fruit. The inhibitory effect of the citric extracts against fungal isolates shows that the extract proved effective in the control of all the pathogens and this could be due to the presence of antifungal substances present in the extract. Higher inhibition of fungal growth was observed at higher concentrations of the aqueous and ethanol extracts. The result also indicated that ethanol is better solvent than water for the extraction of the active ingredients of the extract.

ACKNOWLEDGEMENT

The author wish to acknowledge the contribution and support of Dr. F.K. Channya, Prof. I.B. Chimbekujwo and Dr. Delphine.L.David for their assistance towards the success of this work.

REFERENCES

[1] De-Costa, D.M., and Erabadupitiya, H.R.U.T., *Postharvest Biological Technology*, **2005.** 36 (1): p. 31- 39

[2] FAO *Statistics Division of Crops production,* **2008.**

[3] Akinyosoye, F.A., and Oladunmoye, M.K., *Nigerian Journal of Microbiology,* **2000.** 14: p. 91- 94.

[4] Amadioha, A C., *Journal of Herbs, Spices and Medicinal Plants,* **1998**. 6(2): p. 41-47.

[5] Bonaldo, S.M.,et al., *Fitopatologia Brasileira*. **2004.** 29: p. 128-134.

[6] Bonaldo, S.M., et al., *Summa Phytopathologica*. **2007.** 33: p. 383-387.

[7] Rodrigues, E., et al., *Summa Phytopathologica*, **2007.** 33: p. 20-24.

[8] Dembinski, A.,et al., *Journal of Physiological Pharmacology*. **2004.** 55(4): p. 811 – 821.

[9] Von Woedtke, et al., *Pharmazie,* **1999.** 54: p. 452 – 456.

[10] Sims, J., *Gale Encyclopedia of Alternative Medicine.* **2001.**

[11] Kampf, G., and Kramer, A., *Clinical Microbiology. Rev,* **2004.** 17(4): p. 863–893.

[12] Cvetnić, Z. and Vladimir-Knezević, S., *Acta Pharmacy,* **2004.** 54 (3): p. 243–250.

[13] Shillingford, C.A., *Plant Disease Reporter*, **1976.** 66(9): p. 788–793.

[14] Cox, J.R., *Pests and Diseases*, **1996.** 1: p. 373–378.

[15] Lassois, L., De Lapeyre de Bellaire, L., and Jijakli, M.H., *Biological Control,* **2008.** 45(3): p. 410- 418.

[16] Dionisio, G.A., and Natsuaki, K.T., *Crop Protection,* **2007.** 26(11): p. 1667–1673.

[17] Mari, M., and Guizzardi, M., *Phytoparasitica*, **1998.** 26(1): p. 59-66.

[18] Al-Samarrai, G., Singh, H., and Syarhabil, M., *Annals of Agricultural and Environmental Medicine,* **2012.** 19*(4): p. 673-676.*

[19] Sanjay, G., and Tiku, A.K., *Biomedical Life Science,* **2009.** p. 317.
[20] Siddiqui, F.A. and Gulzar, T., *Chemical Pharmerceutical Bulletin*. **2003.** 51: p. 415-417.
[21] Smith, D., and Onion, S.A., *The preservation and maintenance of living fungi*. **1983.** p. 63.
[22] Snowdon, A.L., *A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables*, **1990.** 1: p.672.
[23] Zakari, B.G., et al., *Global Journal of Biology, Agriculture and Health Sciences*, **2015.** 4(1): p. 142-148.
[24] Balogun, O.S., Odeyemi, G.A., and Fawole, O.B., *Journal of Agricultural Research and Development*, **2005.** 4(2): p. 159-169.
[25] Adedeji, G.B., Fagade, O.E., and Oyelade, A.A., *African Journal of Biological Research,* **2007.** 10: p. 183- 187.
[26] Amadioha, A.C., and Obi, V.I., *Actaphytopathol*ogy *Entomology*. *Hungerica*, **1999.** 34(92): p. 85-89.
[27] Ijato, J.Y., *Academia Arena*, **2011.** 3(1): p. 115-119.
[28] Madari, S., and Singh, R.P., *Indian Phytopathology*. **2005.** 58: p. 189-193.
[29] Nene, Y., and Thapilyal, L., *Poisoned food technique of fungicides in plant disease control*. **2000.**
[30] Naz, F., et al., *Pakistan Journal of Phytopathology*, **2006.** 18(1): p. 36-43.
[31] Gomez, K.A., and Gomez, A. A., *Statistical Procedures for Agricultural Research,* **1984.**
[32] Meredith, D.S., *Annals of Applied Biology,* **1963.** 52: p. 453-463.
[33] Adisa,V.A., *Fitopatologia Brasileira,* **1983.** 8: p. 29-36.
[34] Hansen, L.E., et al., **2003.** p. 1-5.
[35] Oyewole, O.A., *Frontiers in Science*, **2012.** 2(5): p. 86-91.
[36] Abd-Alla, MA., et al., *Plant Pathology and Quarantine*, **2014.** 4(1): p. 1-12.
[37] Ewekeye, T.S., et al., *American Journal of Research Communication*, **2013.** 1(10): p. 209-223.
[38] Krauss, U., and Johanson, A., *Crop Protection*, 2002. 19: p. 151-160.
[39] Shivpuri, A.O.P., Sharma and Jhamaria, S.L., *Journal of Mycology and Plant Pathology*, **1997**. **27**(1): p. 29-31.