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Antifungal Activity of Garlic (*Allium sativum*) Essential Oil and Wood Ash against Post-harvest Fruit Rot of Banana (*Musa acuminata* L.) in Yola, Adamawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FKC, IBC, BB and BGZ managed the analyses of the study. Authors KUT, KMF and KBS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Studies on antifungal effect of garlic (*Allium sativum*) essential oil and wood ash were determined on causative agents of post-harvest fruit rot of banana. Different concentrations of essential oil of garlic (0.15, 0.50, and 1.0%) and quantities of wood ash (0.1, 0.3 and 0.5g) were used. The treatments were laid out in Completely Randomized Design (CRD) with three replications. Four (4) fungal pathogens associated with rots of banana fruits were isolated. The fungi isolated and their incidence of occurrence included *Colletotrichum musae* (18%), *Pyricularia grisea* (18%),

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Rhizoctonia solani (38%) and *Rhizopus stolonifer* (26%). *Rhizoctonia solani* and *Rhizopus stolonifer* were the most pathogenic with rot covering more than 75% of the fruit surface. All the tested concentrations (0.15, 0.5 and 1.0%) of essential oil of garlic significantly ($p < 0.05$) suppressed mycelial growth of the fungi *in-vitro*. Also all tested quantities of wood ash (0.1, 0.3 and 0.5g) significantly ($p < 0.05$) reduced the rots of the fungi *in-vivo*. The effect was proportional to the concentrations of essential oil of garlic and quantities of wood ash used and reduction was highest at 1.0% of garlic oil and 0.5g of wood ash. Both garlic (*Allium sativum*) essential oil and wood ash proved effective in the control of disease severity and these natural plant materials are recommended as an alternative to pesticides which are often harmful and costly.

Keywords: Antifungal activities; garlic essential oil; wood ash; Musa acuminate; Yola.

1. INTRODUCTION

Banana and plantain are important dietary sources of carbohydrate in the humid tropical zones of Africa, Asia and South America [1]. The fruit is rich in vitamins A, B6, C, minerals and dietary fibre [2,3,4]. Nutritionally, *Musa* spp. constitutes a rich energy source, with carbohydrates accounting for 22 and 32% of fruit weight for banana and plantain, respectively.

Despite the importance of this fruits to our daily diets, *Musa* spp. faces serious post-harvest losses. The reasons for the post-harvest losses have been attributed to poor handling, transportation, storage methods and post-harvest diseases. Fruit rot and crown rot which are complex of diseases incited by *Colletotrichum musae, Verticillium theobromae*, *Fusarium roseum*, *Fusarium moniliforae*, *Botryodiplodia theobromae*, *Deighthoniella torulosa* and *Ceratocystis paradoxa* are the most common post-harvest diseases [5]. Other post-harvest diseases includes pitting (*Pyricularia grisea*), Fusarium wilt (*Fusarium oxysporum*) and Ceratocystis fruit rot (*Ceratocystis paradoxa*). The most important is the fruit rot whose control must start early under field conditions through proper harvesting, transporting and handling practices as mechanical injuries incite the development of the disease [6,7].

Essential oils are complex volatile compounds produced in different plant parts, which are known to have various functions in plants including conferring pest and disease resistance [8]. The complexity in essential oils is due to terpene hydrocarbons as well as their oxygenated derivatives, such as alcohols, aldehydes, ketones, acids and esters [9]. Previous reports showed that garlic has antifungal, antioxidant and antiviral activity [10,11].

Channya and Chimbekujwo [12] reported that ash treatment significantly reduced disease severity of *Aspergillus niger, Aspergillus flavus, Fusarium moniliforae* and *Botryodiplodia theobromae.* The wood ash used was found to have a pH of 7 which is unfavorable for fungal growth and might have been responsible for reduction in disease severity.

Banana being a highly perishable fruit suffers severe post-harvest losses both in terms of quality and quantity [13]. The susceptibility of freshly harvested product to diseases increases during prolonged storage as a result of physiological changes and senescence which enable pathogen to develop in the fruit [14].

Many studies have been conducted regarding the effectiveness of garlic essential oil and wood ash in controlling fungal rot of banana in Egypt, Brazil, Malaysia and Southern/Eastern Nigeria where the fruit are grown commercially but in northern Nigeria, especially Yola, not much work has been done on banana fruits. [15] Worked on fungi associated with market fruit of *Musa* spp. in South Western Nigeria.

Application of synthetic fungicides is the most common practice for commercial control of banana rots [16,17,18] and environmental politics worldwide have been questioning the excessive use of agrochemicals/synthetic fungicides [19]. Attempt by researchers to look for an alternative to these agrochemicals proved successful as consistent responses from alternative methods have suggested applying essential oil or crude plant extract because they have fungi toxic action and they induce fruit resistance through proper elicitor compound [20,21].

There is little or no reports indicating that control of post-harvest rot of banana has been carried out using these natural plant products in the study area and these natural plant protectants have wide public acceptance in comparison with the synthetic fungicides which are harmful to human health. Hence the objective of the work is to determine the effectiveness of garlic essential oil and wood ash on fungi rot of banana which would serve as an alternative to synthetic fungicides.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the Department of Plant Sciences, Modibbo Adama University of Technology Yola, Adamawa State in 2015. Modibbo Adama University of Technology Yola is located in Girei Local Government Area of Adamawa State. Girei lies between Latitudes 8ºN and 11ºN and Longitude 11.5ºE and 13.5ºE. Girei has a distinct tropical climate marked by dry and wet seasons with temperature and humidity varying with seasons [22]. The people there are mostly farmers.

2.2 Source of Samples

Banana fruits (both healthy and diseased) were collected from five major markets in Yola which are Shinko markets, Jimeta Modern Markets, Jambutu Market, Jimeta Old Market, and Jimeta Shopping Complex. Samples of *Musa acuminata* (L) fruit showing spoilage and rotting were collected from different selling points randomly in the markets. Both the fresh and the diseased banana fruits were packed into sterilized polythene bags and taken to the Plant Science Laboratory at Modibbo Adama University of Technology (MAUTECH), Yola for isolation, pathogenicity test, virulence test and control trials.

2.3 Isolation and Identification of Fungi from Banana Fruit

The banana fruits showing deterioration and rot symptoms were aseptically cut into sections of approximately 5mm square with a heat sterilize knife. The sectioned fruits were surface sterilized with 1% sodium hypochlorite for 30 seconds and rinsed with three (3) changes of sterile distilled water to remove surface contaminants. The sterilized sectioned fruits were dried between filter papers and then plated on 9cm diameter already prepared PDA media. The plates were incubated at room temperature of 25 ± 2^0C for 3 days before sub-culturing on fresh sterile PDA plates. The sub-culturing on fresh sterile media was carried out adopting the method of [23] until pure cultures were obtained.

Identification of the isolated fungi was carried out based on their cultural characteristics on growing media. Hyphae containing spores were picked using a sterile needle and placed on a sterile glass slide with a few drops of Lactophenol cotton blue and were examined under photographic microscope using the method of [24]. The cultural and morphological features observed were compared with structures in the identification guides of the International Mycological Institute Kew and [25].

2.4 Determination of the Frequency of Occurrence (FC) of the Isolated Fungi

The frequency of occurrence of the isolated fungi was determined by using the percentage incidence of occurrence as follows:

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

2.5 Pathogenicity Test

Healthy banana fruit (semi ripe) were surfaced sterilized with 0.5% sodium hypochlorite for 30 seconds and rinsed in three changes of sterile distilled water adopting the approach of [23]. A 5 mm diameter flame-sterilized cork borer was used to puncture and remove a cylindrical core from the healthy. The healthy fruits were then aseptically inoculated with seven (7) day old fungal culture of the isolates in three replicates. The cylindrical core removed was then replaced and Vaseline jelly was smeared to completely seal the hole to prevent external infection or contamination. It was then incubated at room temperature of 25±2ºC. The control was inoculated in the same manner except that sterile agar was used instead of the isolates. For each fungal isolates, three (3) banana fruit (*Musa acuminata* L.) were inoculated and three controls were also set up. All experiment was carried out in the laboratory using completely randomized design and was incubated for seven (7) days. Symptoms of rots observed from different fugal isolates were compared to the original natural rot.

2.6 Determination of Rot Severity

Observation for level of virulence of fungal isolates on the fruit of *Musa acuminata* .L

(Cavendish) was made daily for 7 days and results was determined adopting the method of [23].

Percentage rot severity was determined using the formula below;

$$\text{Percentage rot severity} = \frac{\text{Total Diameter of rot covered}}{\text{Total Diameter of fruit surface}} \times 100$$

2.7 Collection of Garlic Essential Oil (*Allium sativum)* and Wood Ash

Readily available essential oil of garlic (*Allium sativum)* was purchased from Jimeta modern market; Wood ash used for the experiment was obtained and prepared from the wood of *Balanites aegytiaca* plants from MAUTECH,Yola campus and Sangere village.

2.8 Effect of Garlic Essential Oil on Fungal Mycelial Growth

In-vitro antifungal effect of garlic (*Allium sativum*) essential oil was determined using poisoned food method adopting the approach of [26]. Aseptically, high concentrations of garlic essential oil were prepared by dissolving in sterilized distilled water and a few drops of the emulsifier "Tween 80" (Sigma Co.) was added to obtained essential oil volumes. Different volumes of garlic essential oil emulsion were tested [0ml (Control), 1.5ml, 5ml and 10ml].These were added aseptically to sterile conical flasks containing cool sterile PDA medium before its solidification to obtain the proposed concentrations. The tested concentrations were 0.15, 0.50 and 1.0 %. The agitated media was poured into Petri-dishes (9 cm) about 10 ml each. A disc of 5mm diameter of seven (7) day old pure culture was inoculated on each of the media. PDA plates free of garlic essential oils were also prepared as control. The petri plates was completely sealed with masking tape and were incubated at 25±2ºC, until fungal growth in the control filled the whole Petri-plates, and then all treatments were examined and assessed. All the treatments were laid out in a completely randomized design in three (3) replicates.

2.9 Effect of Wood Ash on Fungal Mycelial Growth

A little modification of the approach of [27] was used to evaluate the *in-vivo* effect of ash treatment on fungal growth. 5mm diameter flamed sterilized cork-borer was used to puncture and make a cylindrical core on a healthy semi ripe fruit of *Musa acuminata* L. The cylindrical core was inoculated with pure fungal cultures of the isolates, 0.5g, 0.3g and 0.1g of ash was used to completely seal the cylindrical cores of the banana. Inoculated fruits were put in transparent sterile polythene bags in three (3) replicates and incubated for 7 days at room temperature 25±2ºC after which disease severity was determined. The set up was a completely randomized design. The control (set up by inoculating the cylindrical core with fungal culture of the isolates) was also incubated. Rot diameter of each fungal isolates was measured using ruler, caliper and with the help of hand lens.

Mean radial mycelial growth (*in-vitro* control) and mean radial rot (*in-vivo* control) of each isolate was recorded and data were transformed into inhibition percentage by using the following formula [28].

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

Where DC - Average Diameter of fungal spores germinated in control
DT - Average diameter of fungal spores germinated with treatment.

2.10 Experimental Design and Statistical Analysis

Results and data collected were analyzed statistically using Statistical Analysis System version 7 and means that were significantly different were separated using protected Fisher's Least Significance Difference test (LSD) at $p < 0.05$.

3. RESULTS

The fungi isolated from rotted banana fruits were *Rhizoctonia solani, Colletotrichum musae, Rhizopus stolonifer* and *Pyricularia grisea*. *Rhizoctonia solani* occurred more frequently with a total of 38% of which 52% of this was recorded in Shinko Markets, followed by Jimeta Shopping Complex (46%) and the least incidence of *Rhizoctonia solani* was recorded in Jimeta Modern Market (19%). *Rhizopus stolonifer* had 26% incidence of which the highest incidence was recorded in Jimeta Modern Market (47%), followed by Jambutu Market (31%), and the least was recorded in Jimeta Old Market (7%).The least occurring fungi were *Colletotrichum musae* and *Pyricularia grisea* with 18% each (Table 1).

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 rotted fruits; hence they are the causal agent of the fruits rot observed. All the fungal isolates exhibited different degrees of pathogenic effect on the banana fruits. They were not only able to grow on the fruits but also able to induce some level of fruit rot indicating their virulence (Table 2). No growth evidence or rot formation was observed within the first 24 hours after inoculation in all cases. *Rhizoctonia solani* and *Rhizopus stolonifer* showed very high growth of mycelia on the 10th day of inoculation (rots covered 100% of the fruits surface) while *Pyricularia grisea* and *Colletotrichum musae* showed only a medium pathogenic effect on the fruits. Growth and rots covered less than 50% of the fruit surface.

Test carried out on the effectiveness of garlic essential oil (*Allium sativum)* showed a significant difference with the control at $p < 0.05$. The essential oil tested significantly ($p < 0.05$) reduced the mycelial growth of the fungi *in-vitro* at all concentrations (Table 3). The effectiveness of garlic essential oil significantly ($p < 0.05$) increased with increase in concentration. All quantity of wood ash significantly ($p < 0.05$) reduced the rot caused by all the isolated fungi (Table 4). The effectiveness of wood ash also significantly ($p < 0.05$) increased with increase in quantity of ash.

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

Fungi isolated	Incidence of fungi (%)					
	SHM	**JMM**	**JM**	**JOM**	**JSC**	**Total****
Colletotrichum musae	16	10	24	36	16	18
Pyricularia Grisea	16	24	7	21	15	18
Rhizopus stolonifer	16	47	31	7	23	26
Rhizoctonia Solani	52	19	31	36	46	38
Total*	100	100	100	100	100	100

*Key: * = Percentage of Fungi Isolated Per Market, ** = Percentage of Isolate Per study Area,SHM- Shinko Markets, JMM - Jimeta Modern Market, JM - Jambutu Market, JOM -Jimeta Old Market, JSC -Jimeta Shopping Complex*

Table 2. Virulence of fungal isolates on fruits of *Musa acuminata* L. (Cavendish)

Fungal isolates	No. of fruits	Days after inoculation						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Colletotrichum musae	1	-	-	-	+	+	++	++
	2	-	-	-	+	+	++	++
	3	-	-	-	+	+	++	++
Pyricularia Grisea	1	-	-	-	+	++	++	++
	2	-	-	-	+	++	++	++
	3	-	-	-	+	+	++	++
Rhizopus stolonifer	1	-	-	-	-	++	++	+++
	2	-	-	-	+	++	++	+++
	3	-	-	-	-	++	++	+++
Rhizoctonia solani	1	-	+	+	+	++	+++	++++
	2	-	+	+	+	++	+++	++++
	3	-	+	+	+	++	+++	++++

Key: -No visible growth/fruit rot, + Low growth/fruit rot (less than 25% of fruit surface covered)
++ Medium growth/fruit rot (26-50% of the surface covered)
+++ High growth/fruit rot (51-74% of the surface covered)
++++ Very high growth/fruit rot (75% and above of fruit surface covered

Table 3. Inhibition effect of different concentrations of essential oil of Garlic (*Allium sativum*) on isolated organism

Concentration (%)	Inhibition (%)			
	Fungal pathogens			
	P. grisea	*R. solani*	*R. stolonifer*	*C. musae*
0.15	37.96	13.70	19.25	38.82
0.50	55.05	53.33	35.55	74.90
1.0	90.50	94.44	93.33	86.27
Control	0.00	0.00	0.00	0.00
Mean	45.88	40.36	37.03	49.99
LSD ($p < 0.05$)	7.29	12.47	8.75	11.95

Table 4. Inhibition effect of different quantities of wood ash of *Balanites aegyptiaca* on isolated organisms

Quantity (grams)	Inhibition (%)			
	Fungal pathogens			
	P. grisea	*R. solani*	*R. stolonifer*	*C. musae*
0.1	54.09	6.42	44.31	58.65
0.3	58.19	35.63	50.14	71.01
0.5	65.56	75.73	66.47	79.85
Control	0.00	0.00	0.00	0.00
Mean	44.46	29.44	40.23	52.37
LSD ($p < 0.05$)	11.93	11.30	17.32	4.57

4. DISCUSSION

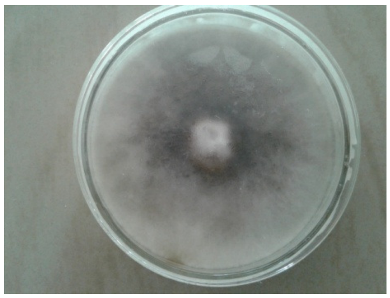
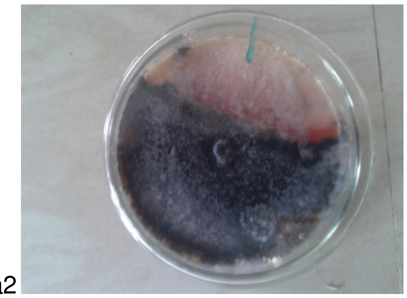
The study showed that a number of fungi are associated with post-harvest rot disease of banana fruit in the study area. These fungi include *Pyricularia grisea, Rhizoctonia solani, Colletotrichum musae* and *Rhizopus stolonifer*. They have been previously reported as fruit rot pathogens of banana fruit [29,30,31,26].

The result from the pathogenicity test of *Rhizoctonia solani, Rhizopus stolonifer*, *Colletotrichum musae* and *Pyricularia grisea* on fresh partially ripe banana fruits revealed that 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 [31] who reported that *Rhizoctonia solani* was responsible for the fruit spoilage of banana. *Rhizopus stolonifer* was also rated as high (mycelia and/rots covered more than 50% of the fruit surface) and this agrees with [32] who found *Rhizopus* spp high on *Daucus* spp and *Carica papaya. Pyricularia grisea* and *Colletotrichum musae* were rated as having only a medium pathogenic effect (rots covered less than 50% of the fruit surface).

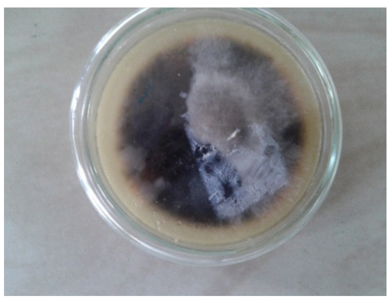
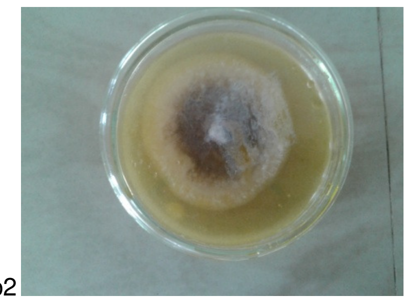
The effect of different concentrations of essential oil of garlic on mycelial growth of *Pyricularia grisea*, *Colletotrichum musae, Rhizoctonia solani* and *Rhizopus stolonifer* showed that there was a highly significant reduction in the mycelial growth of *Pyricularia grisea* and *Colletotrichum musae* at the low concentration of 0.15%, but *Rhizoctonia solani* and *Rhizopus stolonifer* showed a slight reduction in the mycelial growth. This reduction increased gradually by increasing the concentration of the essential oil to reach complete fungal growth inhibition at the concentration of 1.0%.

Antifungal effect of garlic essential oil was reported by Anjili et al. [33] who studied *in-vitro* and *in-vivo* control using garlic essential oil on date palm fruit. Studies on the biochemical reaction of onion, garlic, eucalyptus, caraway, fennel, blackcumin, mustard, carnation, neemix and trilogy essential oils against mycelial growth of *Rhizoctonia solani* and *Pythium debaryanumin vitro* was also reported by El-Toony et al. [34].

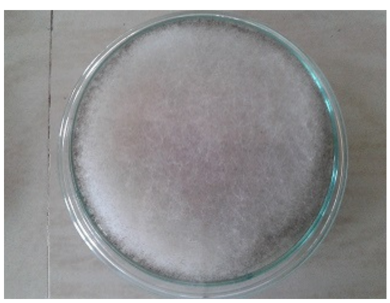
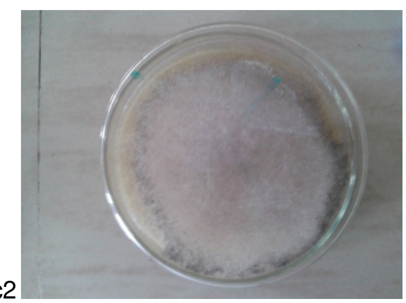
The response of the test organisms to the treatment with wood ash of *Balanites aegyptiaca* indicated that *Rhizopus stolonifer*, *Colletotrichum musae* and *Pyricularia grisea* showed significant reduction in fruit rot than *Rhizoctonia solani* at 0.1- 0.3grams. But significant reduction in fruit rot was recorded from all the isolates at 0.5grams. This is in agreement with the findings of [35] who reported that wood ash exhibit insecticidal and antifungal properties. Furthermore ash treatment at 0.5grams suppressed the rots of all the isolates, the highest inhibition value was on *Colletotrichum* spp followed by *Rhizoctonia solani* while the lowest inhibition value was observed for *Pyricularia grisea* and this agrees with [36] who reported that palm oil and wood ash effectively controlled sweet potato tuber rot caused by *Rhizopus stolonifer* and *Penicillium expansum.* Apart from the general response of the isolates to ash treatment, *Rhizoctonia solani* which showed little response to ash at 0.1gram and 0.3gram, showed remarkable fall in disease severity with 0.5gram of ash treatment.

a1  a2 

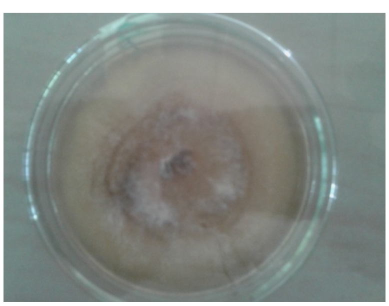
a*: Rhizoctonia solani*

b1  b2 

b: *Pyricularia grisea*

c1  c2 

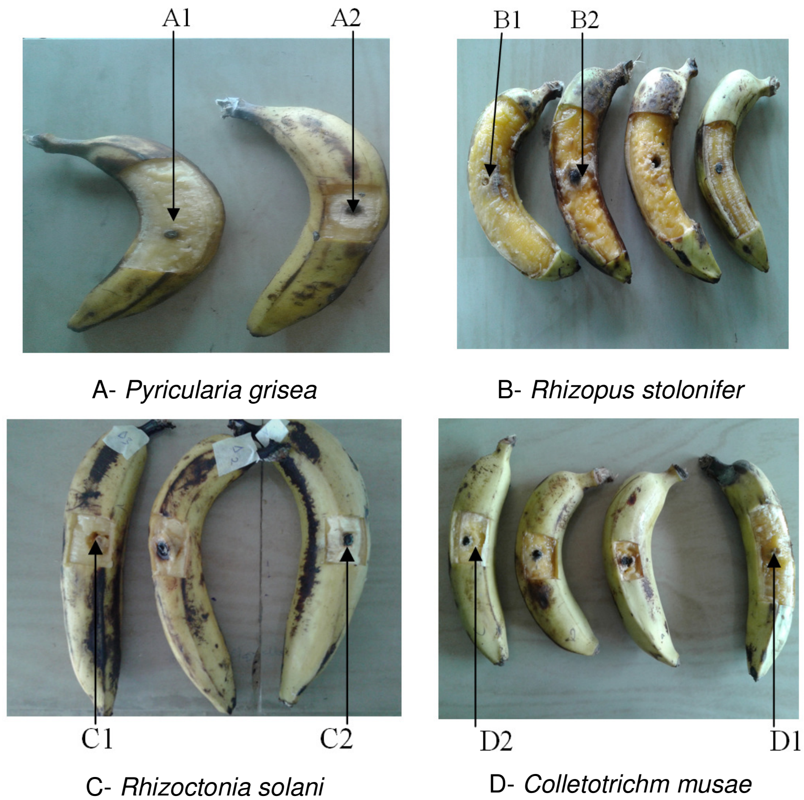
c: *Rhizopus stolonifer*

d1  d2 

d: *Colletotrichum musae*

Plate 1. *In-vitro* suppressed mycelial growth of the various fungal isolates under the impact of garlic essential oil (*Allium sativum*)

a1,b1,c1,d1: Control petri plates; a2,b2,c2,d2: Suppressed mycelial growth of the various fungal isolates



A- *Pyricularia grisea*

B- *Rhizopus stolonifer*

C- *Rhizoctonia solani*

D- *Colletotrichm musae*

Plate 2. *In-vivo* suppressed rot of the various fungal isolates under the impact of wood ash (*Balanites aegyptiaca*)

A1,B1,C1,D1: Control treatment; A2,B2,C2,D2: Suppressed rots of the various fungal isolates

Channya and Chimbekujwo [12] also reported that ash treatment proved effective in the control of fungal pathogens responsible for the rots of banana. Comparing among the control, both proved effective in the control and reduction of disease severity.

5. CONCLUSION

The findings from this study revealed that *Rhizoctonia solani, Rhizopus stolonifer, Colletotrichum musae* and *Pyricularia grisea* were responsible for the post-harvest rot of banana and that essential oil garlic and wood ash which possess fungitoxic properties were highly effective against the four (4) isolated organisms and serve as an alternative means of control which do not possess dangerous health hazards to humans and animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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