

Research Article

# Causal agents of Post-harvest Rot of Pumpkin (*Cucurbita pepo* L.) and their control using Indigenous Practices in Hong, Adamawa State

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## Abstract

Pumpkins (*Cucurbita pepo*) are grown all around the world for a variety of reasons ranging from agricultural purposes to commercial and ornamental sales. The pathogens causing the rot of pumpkin in the world include fungi, bacteria, and viruses. The study was aimed to identify fungal pathogens of pumpkin rot during storage, as well as control measures of the diseases using wood ash, mango leaf and rice chaff. Three hundred and sixty-six (366) fruits of pumpkins were studied in Pela, Gaya and Kulinyi districts of Hong Local Government Area of Adamawa State. The diseased samples (fruits) were randomly purchased. Of all the districts visited, Kulinyi has the highest percentage of disease samples (43.82%) while the least is Gaya district with 21.35%. Potato Dextrose Agar (PDA) was used for the isolation of pathogens and these gave *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, and *Phytophthora capsici*. All the fungal isolates exhibited different degree of pathogenic effect on the pumpkin fruits. The pathogens are susceptible to treatment both *In-vitro* and *In-vivo* control trials with wood ash and mango leaf at  $p \leq 0.05$ . Inhibition improved with increased concentration of the wood ash and mango leaf. Rice chaff treatment equally proved worthwhile with significant inhibition compared to the control at  $p \leq 0.05$ .

## Introduction

Pumpkin, one of the common names for flowering plants that belong to the *Cucurbitaceae* family with four genera which include *Curcubita maxima*, *Curcubita pepo*, *Curcubita moschata* and *Cucurbita mixta*. They are characterized by spreading vines with showy yellow-orange flowers, large lobed leaves, long twisting tendrils and thick shell which contain the seeds and pulp [1]. They are grown all around the world for a variety of reasons ranging from agricultural purposes (such as animal feed) to commercial and ornamental purposes [2].

In Nigeria, it is a traditional vegetable crop, grown mainly for its leaves, fruits, and seeds and, consumed either by boiling the leaves and fruits or by baking the seed [3]. In Hong local government of Adamawa state, there was a huge production of pumpkins, but the crops suffer from many diseases which destroy both the crops and fruits during harvest and storage resulting to economy loss.

## More Information

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Keywords: Pumpkin fruit pathogens; Ash; Mango leaf; Rice chaff



crops and in some perishable crops especially in developing countries; they destroy more than 30% of the crop yield [4,5]. The marketability and the nutritional value of infected pumpkin are highly reduced and they are usually being thrown away as useless [6]. Fungi are the most important and prevalent pathogens, infecting a wide range of host plants and causing destructive and economically important losses of most fresh fruits and vegetables during storage and transportation [7]. Crop losses due to the soil borne fungus like oomycete *Phytophthora capsici* (Leonian) have been well documented [8,9]. *Phytophthora capsici* affects a wide range of solanaceous and cucurbit hosts worldwide [9]. *Fusarium* crown and foot rot of squash and pumpkin is caused by *Fusarium solani* f. sp. *cucurbitae*. The pathogen exhibits host specificity for all cucurbits [10]. Bacterial wilt can be a serious disease in pumpkin plantings if striped or spotted cucumber beetles are present when plants first emerge from the soil. Mosaic viruses can cause problems on pumpkin plantings in the home garden.

Postharvest diseases destroy 10-30% of the total yield of

Pumpkin disease management begins with cultural and



preventative controls such as proper site selection, field preparation and the use of resistant varieties, and by remembering that excess water is the enemy of your pumpkin planting. Ijato, [11] reported that plant parts, powder of plant, ash, aqueous extracts which are environmentally non-hazardous, locally available and can be cheaply maintained are suitable alternatives to the expensive synthetic fungicide. Bristone, et al. [12] reported that when tubers of sweet potatoes were treated with wood ash, rot caused by *Rhizopus stolonifer* and *Penicillium expansum* was reduced to minimal level. Extract of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails Bonaldo, et al. Rodrigues, et al. [13,14]. Aisha, et al. [15] reported the use of guinea corn chaff on *Solenoslemon rotundifolius* [17]. Muhammed, et al. [16], observed that rice product like rice husks composted soil reduced the incidence of wilting of *Parkia biglobosa* caused by *Fusarium solani* in the range of 31.4% to 70.3%. This study will be useful in providing knowledge on the fungal pathogens of pumpkin diseases during storage, as well as on control measures of pumpkin diseases using wood ash, mango leaf and rice chaff, thereby helping in reducing losses of the crops during storage.

## Materials and Methods

### Study areas and samples collection

The study of pathogens associated with pumpkins fruit was conducted in three districts (Kulinyi, Gaya and Pella) in Hong local government of Adamawa state. In each district, 122 fruits were inspected and collected from market for further studies. The experimental design was Complete Randomized Design (CRD) arranged in three replicates.

### Survey of rot pumpkin

Pumpkin that had been harvested from three out of the six districts of Hong local government which has been stored for commercial purposes were observed and every fruit with a rot was recorded. The incidence of fruit spoilage/rot was expressed in percentage using the adopted method of Anjili, et al. [17], Plants to

$$\frac{\text{Number of pumpkin fruits with rot}}{\text{Total number of pumpkin fruits}} \times 100$$

### Isolation and identification of isolates

The isolation and identification from diseased pumpkin fruit was carried out using the methods of Fawole and Oso, [18] and Robert, et al. [19]. Under aseptic conditions the disease sample from spoilt pumpkin fruit was cut into approximately 2cm with a neat sterile scalpel. The portion was immersed into 1% sodium hypochlorite contain for surface sterilization for 30 seconds. The sterilize portion was rinsed in three changes of sterile distilled water and then air dried between sterile filter papers. It was then plated out on steile

solidified Potatoes Dextrose Agar (PDA) and incubated at room temperature of  $31 \pm 2$  °C.

### Determination of severity during the pathogenicity analysis

The pathogenicity test was carried using the method of Chukwuka, et al. [20], and the severity of rot was measured and recorded after seven days of incubation. Fifteen healthy pumpkins fruit were randomly selected and surfaced sterilized, then rinsed in three (3) changes of sterile distilled water. A sterile cork borer with 5mm diameter was used to puncture pumpkin fruits and spores of the isolated fungi were inoculated into the fruit using sterile needle. A control set up was set with sterile distilled water to replace the fungal inocula. Then it was incubated for 7 days after which each fruit was collected and the extent of rot (severity of infection) was measured and recorded. The pathogens were re-isolated from the new host to show whether they are same with the originally inoculated pathogen.

### In-vitro effect of mango leaf extracts and ash on fungi Growth

The approach of Ijato, [11] and Nene and Thapilyal, [21] were used to evaluate the effects of the extract on fungal growth *In-vitro* by introducing 0.5, 1.0, 1.5ml of plant extract into the Petri-dish containing equal amount of PDA media (food poisoned method) and swirled to mix. Each plate was inoculated with 2mm diameter plug of the isolate and incubated at  $31 \pm 2$  °C and the control plates contained only the PDA media. Mycelia growth diameter of each isolates was measured and recorded when the growths of the isolates were completed in the control treatment. Similarly, different grams of sterilize ash were tested (0g for control, 5g, 10g and 40g). The set up was completely randomised design in three replicates, incubated at  $31 \pm 2$  °C for 7 days. The data recorded was analysed using SAS version 7.

### In-vivo effect of mango leaf extracts, ash and rice chaff on fungi growth

The methods of Bristone, [22] and Anjili, et al. [23] were used for *In-vivo* control trail by inoculating the fruits with spores' suspension of the isolated pathogens then 20g, 30g and 40g of ash, mango leaf respectively were sprinkled separately on the bore samples in three replicates. A control was set with ash free. It was incubated at  $31 \pm 2$  °C for 7 days.

Similarly, healthy pumpkins were sterilized, punctured with sterilized 5mm diameter cork-borer and inoculated with the isolated fungi. It was placed in-between rice chaff, and the control set up was without rice chaff. The set up was replicated thrice (3). It was kept for seven (7) days and after which the level of rot was measured with thread and ruler. The data recorded was analysed using SAS version 7.

Data collected were analysed using statistical tool 'Statisti-

cal Analysis System (SAS) for analysis of variance (ANOVA) and the means were separated using the least significant difference (LSD) at 95% probability level ( $P \leq 0.05$ ).

## Result and Discussion

Pumpkin fruits were observed to show different types of rot symptoms. A total number of 366 harvested pumpkin fruit were inspected in the three districts from the studied area, 89 diseased pumpkins were recorded during the survey (Table 1). Kulinyi district had the highest disease fruits with 39, followed by Pella district with 31 and Gaya district had the least with 19. The major infection was black rot and water-soaked lesions. They were well spread and extensively prevalent in all the three districts of the studied area. Tsado, et al. [24] also reported fruit rots of pumpkin in markets and farms in Minna, Niger State of Nigeria. The highest incidence of disease fruit was however, more prominent in Kulinyi areas than in the other areas. This may not be unconnected with the number of farming activities. Tsado, et al. [24] also suggested that the presence of many pathogens in soils may be major sources of microorganisms present on many vegetables. Mapanda et al. [25] also shared the same view that pathogens existing in soils or water can be the source of both pre- and post-harvest contamination of several vegetables. Amadioha and Obi, [26] have shown how most of the vegetables consumed are not produced by highly knowledgeable people therefore, liable to be unsafe for consumption.

The isolated fungi from the pumpkin fruit in Hong Adamawa State were identified as *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, *Phytophthora capsici*.

*Rhizopus stolonifer* was the most prevalent in Kulinyi compared to the other districts, while *Phytophthora capsici* was the lowest isolated pathogen and it was observed only in Pella district (Table 2).

All the fungal isolates exhibited different degree of pathogenic effect on the pumpkin fruits. They were able not only to grow on the fruits but also were able to induce

some level of rot indicating their virulence. However, there is no growth evident or rot formation within the first 24 hours after inoculation in all cases. Rots caused by this fungus occurred on all pumpkin fruits. The lesions started as water-soaked, sometimes sunken, and light to dark spots which often exuded watery ooze. Older lesions were dried and cracked, and fruiting bodies of the fungus were common seen on the surface and deep when it was cut. The rots were characterized by a water-soaked appearance and infected fruit often collapsed completely in the time between inspections. Internally, the tissue was spongy and sometimes dried out, with the mycelium of the fungus clearly visible. The analysis of variance to determine the level of severity at  $p < 0.05$  showed to be highly significant among all the pathogens with control. *Rhizopus stolonifer* had the highest severity mean of 121.67mm, followed by *Aspergillus niger* with 19.85mm, *Aspergillus flavus* (8.71mm), while *Phytophthora capsici* had the least mean of 2.71mm (Table 3). Zakari, et al. [27] reported similar observation on the degree of pathogenicity though on pepper fruits a different vegetable where he also found *Aspergillus* species more virulence than *Phytophthora capsici*. The differences in severity of the fungi isolated might be due to their ability to overcome the natural defense mechanism of the fruit or their ability to induce resistance in the fruit when infected [28]. On the *Phytophthora* rot, Babadoost, [29] further maintained that *Phytophthora capsici* can infect pumpkin fruit at any stage of development (during transit, and in storage). Hausbeck, et al. [30] also reported *P. capsici* has been found to associate with postharvest rot of some vegetables viz., taro (*Colocasia esculenta* (L.) Schott), bottle gourd (*Lagenaria siceraria* (Molina) Standley), eggplant (*Solanum melongena* L.), common bean (*Phaseolus vulgaris* L.), sponge gourd (*Luffa aegyptiaca* Mill.) and tomato (*Lycopersicon esculentum* Mill.).

The phytochemical characteristics of mango leaves investigated revealed the presence of medicinally active constituents and secondary metabolites, these includes tannins, flavanoids, saponins, terpenoids, alkaloid, glycoside, steroids and phenols (Table 4) and the phytoconstituents in the leaf extracts may be responsible for the antifungal [31] and antibacterial activity of the plant [32].

### The effects of mango leaves, ash and rice chaff on isolated organism

Mango leave extracts both *in-vivo* and *in-vitro* produced a significant difference between the mango leave extract and the control at  $p \leq 0.05$  with retardation of vegetative growth of the fungi when compared with the control. Treatment with mango

**Table 1:** Percentage of Disease Fruits (Pumpkin) from the Selected Districts of the Area of Study.

Districts	Number of diseased fruits	% diseased fruits
Gaya	19	21.35
Kulinyi	39	43.82
Pella	31	34.83
Total	89	27.48

**Table 2:** Percent Incidence of Postharvest Fungi of Pumpkin fruits from three districts.

Fungi	Districts		
	Gaya (%)	Kulinyi (%)	Pella (%)
<i>Rhizopus stolonifer</i>	45.00	50.00	35.00
<i>Aspergillus niger</i>	25.00	30.00	30.00
<i>Aspergillus flavus</i>	30.00	20.00	25.00
<i>Phytophthora capsici</i>	0.00	0.00	5.00

**Table 3:** Severity Mean of the Organisms on the fruits.

Source	Lesion size (mm)	Control (mm)
<i>R. stolonifer</i>	121.67	0
<i>A. niger</i>	19.85	0
<i>A. flavus</i>	8.71	0
<i>P. capsici</i>	2.71	0

**Table 4:** Qualitative and Quantitative Determination of Phytochemical Properties of the Mango Leave Extracts.

Phytochemicals	Mango leaf	Mango leaf in %
Tannin	+	11.3
Saponin	+	7.8
Terpenoid	+	4.6
Flavonoid	+	16.9
Alkaloid	+	7.1
Glycoside	+	21.7
Steroid	+	3.1
Phenols	+	18.3
Anthraflanol	-	-
Protein	-	-
Carbohydrate	-	-

leaf extract on organisms differs significantly with the quantity of the extracts applied and among the isolates treated. It has been documented that different solvents and quantities have diverse solubility capacities for different phytoconstituents. Jigna and Sumitra [33], report the difference in activities may be associated with the presence of oils, wax, resins, fatty acids or pigments, which had been reported capable of blocking the active ingredients in the plant extracts, thus, preventing the plant extract from accessing the fungal cell wall. Of all the fungi tested the *Phytophthora capsici* was slightly more susceptible to the extracts than the *Aspergillus* species and *Rhizopus stolonifer*. The finding agreed with earlier reports that *Phytophthora capsici* are more susceptible to extracts from *Tridax procumbens*, *Vernonia amygdalina*, and *Azadirachta indica* than other *Aspergillus* species among other fungi isolated from pepper in Yola [27] (Tables 5 and 7).

Ash treatment in both *in-vitro* and *in-vivo* produced a significant difference between ash treatment and the control at  $p \leq 0.05$ . Treatment also differs significantly with the quantity of the ash applied and this was also reported by Anjili, et al. [23] and [34] who worked on fungi isolated from date palm fruits and cowpea blotch respectively. Channya and Chimbekujwo [35], reported that wood ash effectively controlled fungal rot of plantains (*Musa parasidiaca* L.). Bristone, et al. [12] reported that when tubers of sweet potatoes were treated with wood ash, rot caused by *Rhizopus stolonifer* and *Penicillium expansum* was reduced to minimal level. Oguntade and Adekunle, [36], also reported that wood ashes proved effective in preserving stored crops against pest and microbes (Tables 6 and 8).

The control using rice chaff produced a significant difference between rice chaff and the control at  $p \leq 0.05$ . Muhammed, et al. [16] in their studies observed that rice product like rice husks composted soil reduced the incidence of wilting of *Parkia biglobosa* caused by *Fusarium solani*. According to Aliyu, et al. [37] amending cowpea with rice husk showed considerable less susceptibility to the virus pathogen infecting cowpea compared to the non-amended plants. He further showed that the rate and time of application of the

Rice-husk powder was a key factor in the ameliorative effect of this organic amendment in the suppression of the viral inoculums. Muhammed, et al. [38] reported a significant effect of guinea corn chaff on *Solenoslemon rotundifolius* microbes. These clearly show why fungi isolated from pumpkin responded differently with different amendment (Table 9).

## Conclusion

*Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, *Phytophthora capsici* are some of the pathogenic fungi which cause rots of pumpkin in the studied area. The pathogenicity test result indicated that the isolated fungi are pathogenic and attributed to rots of pumpkin in Hong L.G.A. The activities of these fungi can affect the market value of fruits. Of all the treatments, wood ash which is alkaline shows to be more promising than other treatments. Therefore, there is need to assess the phytochemical properties of the fruits in order to determine the level of nutritional loss as a result of the activities of pathogens.

**Table 5:** The Efficacy of different Concentration of Mango Leaf Extract on Isolated Organisms *In-vivo*

Conc. (ml)	<i>R. stolonifer</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>
5	66.61	19.85	4.33	1.71
10	22.29	8.50	4.05	0.62
15	13.33	5.90	2.30	0.43
0	121.67	34.80	8.71	2.71
LSD (0.05)	20.62	9.12	1.21	0.70

**Table 6:** The Efficacy of Wood Ash on Isolated Organisms *In-vivo*.

Conc. (g)	<i>R. stolonifer</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>
20	95.76	18.81	8.52	2.71
30	48.05	12.10	5.57	2.52
40	24.38	5.52	2.81	2.11
0	121.67	19.85	8.71	7.05
LSD (0.05)	17.42	3.73	1.21	1.50

**Table 7:** The Efficacy of Different Concentration of Mango Leaf Extracts on Isolated Organisms *In-vitro*.

Conc. (ml)	<i>R. stolonifer</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>
5	30.90	11.30	11.50	15.74
10	27.32	9.40	9.33	14.73
15	23.32	3.31	6.82	4.05
0	40.10	17.28	16.25	31.81
LSD (0.05)	4.11	1.78	1.90	3.61

**Table 8:** The Efficacy of Wood Ash on Isolated Organisms *In-vitro*.

Conc. (g)	<i>R. stolonifer</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>
1	30.07	8.15	13.71	13.00
3	28.50	7.70	12.00	12.52
5	16.62	6.70	9.20	2.20
0	40.06	17.28	16.26	31.81
LSD (0.05)	4.50	1.22	1.41	3.63

**Table 9:** Mean Efficacy of Rice Chaff *In-vivo* on Isolated Organisms.

Conc.	<i>R. stolonifer</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>
Treatment	9.33	5.81	3.05	1.48
Control	121.67	19.85	8.71	7.05
LSD (0.05)	1.50	1.55	1.29	0.65



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