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Phytochemical and Antifungal Activity of Leaf Extracts of *Corchorus olitorius* and *Gongronema latifolium* on Fungi Associated with Post-Harvest Deterioration of Maize (*Zea mays*) Seeds in Oban Community, Nigeria

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ABSTRACT

This study was carried out to investigate the fungal pathogens associated with post-harvest deterioration of maize seeds in storage in Oban community, Cross River State, Nigeria, determine the effect of the pathogens on the nutritional content of the maize seeds through biochemical analysis as well as evaluate the phytochemical contents and antifungal activity of ethanolic leaf extracts of *Corchorus olitorius* and *Gongronema latifolium* in controlling the isolated fungi *in vitro*. The fungal pathogens isolated as the causative agents of post-harvest deterioration of maize seeds in this study were *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*., and *Rhizopus stolonifer*. The result of biochemical analysis of fungal infected and non-infected maize seeds carried out showed a significant increase ($P \leq 0.05$) in the moisture content of the fungal infected maize seeds as compared to healthy ones (control), while there was a significant decrease ($P \leq 0.05$) in the crude fibre, fat, ash, protein and carbohydrate contents of the fungal infected maize seeds as compared to the healthy ones (control). For the *in vitro* antifungal assay, various concentrations of the extracts ranging from 20g/200ml, 40g/200 ml, 60g/200 ml, 80g/200 ml and 100g/200 ml were separately added to PDA media. Results shows that at 60g/200 ml and 80g/200 ml and 100g/200 ml concentrations, ethanolic leaf extracts of *C. olitorius* completely inhibited the radial growth of *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*., and *R. stolonifer* after seven days observation period while those of ethanolic extracts of *G. latifolium* completely inhibited the redial growth of the fungal pathogens at 40g/200 ml, 60g/200 ml, 80g/200 ml and 100g/200 ml concentrations. Results, however, shows that the extracts had a significant effect ($P \leq 0.05$) on the

radial growth of the fungal pathogens at all the different concentrations tested as compared with the control. Phytochemical screening of the extracts revealed the presence of cardiac glycosides, reducing sugars, flavonoids, saponnins, tannins, hydocyanin, and anthraquinones with traces of alkaloids.

***Keywords*:** Phytochemical; antifungal; extracts; maize; fungi

1. INTRODUCTION

Maize (*Zea mays* L.) commonly known as corn is the third most important cereal grain worldwide after wheat and rice [1]. It is referred to as the cereal of the future for its nutritional value and utilization of its products and by-products [2]. The demand for maize has been estimated to increase by 50%, from 558 million metric tons in 1995 to 837 million metric tons in 2020 [3], fuelled by diverse uses, from food processing, animal feed, to ethanol production [4]. It is a basic staple food grain for large parts of world including Africa, Latin America, and Asia [5]. In tropical and subtropical countries, a large proportion of the grain (such as maize) is harvested and stored under hot and humid conditions, and most farmers lack proper knowledge, equipment and methods of drying grains [6].

Maize is among a few crops grown on almost every continent. According to [4], global maize production has increased by nearly 50 percent over the pasts ten years. The total global production for 2011/12 decreased due to severe drought in some part of the US, which is the biggest producer of maize [7]. The total world production for 2011/12 was 0.8 billion metric tons; the US contributed 36.19 % of the overall world's total. Other major producers of maize were China (22.1 %), EU-27 (7.44 %), Brazil (7.15 %), Argentina (2.54 %), India and Mexico (2.48 % and 2.36 % respectively), Ukraine (2.59 %), South Africa (1.38 %), and others (15.77 %) [8,9].

Maize has been in the diet of Nigerians for centuries. it is the most important cereal crop after sorghum and millet [10]. The cultivation of maize was formerly for subsistence purpose but it has gradually become an important commercial crop in which many agro-allied industries depend on as the raw material [11]. The total land area planted to maize in Nigeria in 2003 was about 4.7 hectares with an estimated output of about 5.2 million metric ton (FAO, 2006). According to [12] until recently, the bulk of maize grain produced in Nigeria was from South-Western zone whereas 50% is shared between Northern and Eastern zones.

The kernel, or seed, of a maize plant consists of three main parts the pericarp, endosperm and embryo [13]. Maize grain is subdivided into distinct types based on endosperm and kernel composition, kernel color, environment in which it is grown, maturity, and its use [14]. [15], reported six major varieties that are commercially grown specifically for human consumption, including *Zea mays* var. dent (*Indurate Sturt*), flint (*Indurate sturt*), popcorn (*Everta Sturt*), waxy, and sweet (*Saccharata Sturt*). According to [4] the importance of cereal grains in human nutrition is widely recognized, as they provide substantial amounts of energy and protein to millions people, especially in developing countries. [15], stated that cereal provides an estimated 10 % and 15 % of the world's calories and protein, respectively. Maize is a multipurpose grain; it can be used directly as a human food, but provides even greater nutritional values when used as an ingredient in the food processing industry and the animal feeding industry [16]. Maize is important as a food security crop. It meets the consumption and income needs of households and as an important component of livestock

feeds. Typical proximate compositions of the main parts of the maize kernel (yellow dent corn). Chemically, dried maize kernel contains about 10.4 % moisture, 6.8 % to 12% protein, 4% lipid, 1.2 % ash, 2.0 % fibre and 72 % to 74 % carbohydrate [17]. It also contains macro and micronutrients such as calcium, phosphorus, iron, sodium, potassium, zinc, copper, magnesium, and manganese, with 7 mg/100 g, 210 mg/100 g, 2.7 mg/100 g, 35 mg/100 g, 287 mg/100 g, 2.2 mg/100 g, 0.3 mg/100 g, 127 mg/100 g, and 0.45 mg/100 g each, respectively in dry matter basis (db) [15].

Maize also contains important vitamins such as thiamine 0.38 mg/100 g, riboflavin 0.20 mg/100 g and niacin 3.63 mg/100 g, pantothenic acid 0.42 mg/100 g and folate 19 mg/100 g [15]. These will vary due to variety, hybrid, growing seasons, and soil. The production of maize has always been proven to be insufficient in meeting the need of the population especially during the dry season [18].

The biodegradation of maize grains by fungi in the field according to survey shows that fungi may enter the maize seed through wounds created by rodent and insects. Some workers [19,20], have revealed that *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *R. stolonifer* and F*usarium oxysporum* were the causal agents of maize seed rots in the field and storage in Nigeria and the infection starts in the field and manifest during storage.

In view of complaints by farmers and the observed adverse effect of fungi deterioration of maize seeds in storage in Oban, a major maize producing community in Cross River State, Nigeria, it became necessary to isolate and identify the fungal pathogens associated with the deterioration of the maize seeds in storage, determine the effect of the pathogens on the nutritional content of the maize seeds through proximate analysis as well as evaluate the phytochemical and antifungal activity of leaf extracts of *Corchorus olitorius* and *Gongronema latifolium* in controlling the isolated fungi *in vitro*.

2. MATERIALS AND METHODS

2. 1. Collection of Samples

Healthy and matured leaves of *Corchorus olitorius* and *Gongronema latifolium* were obtained from the Botanic Garden of the Department of Botany, University of Calabar, Calabar, Cross Rivers State, Nigeria. Biochemical composition (nutrient) analysis of infected and non-infected maize seeds was carried out in the Department of Biochemistry, University of Calabar, Calabar, Nigeria. The experiment was carried out in the Laboratory of the Department of Botany, University of Calabar, Calabar, Cross Rivers State, Nigeria.

2. 2. Pathogenicity Test

Pathogenicity test was ascertained using Koch's postulates. To test pathogenicity of the isolated fungal pathogens, some pieces of spores were re-inoculated into matured healthy maize seeds. The healthy maize seeds were washed with distilled water and surface sterilized with 99% ethanol. For the spore inoculation, a spore suspension was produced using the method of [23]. With a sterilized hypodermic needle, approximately 2 x 10^{-5} spore was inoculated on the maize seeds by spraying to run-off level. The green bean seeds was put into transparent polyethylene bags and allowed to stay for 24hours. Spores measurement was done with a haemocytometer. The control experiment was carried out with sterile distilled water

without spores. All the experimental setup was observed for symptom development. The experiment was carried out in three replicates.

2. 3. Effect fungal infection on biochemical composition of maize seeds

To ascertain the effects of fungal infection on biochemical (nutrient) composition of maize seeds, matured maize seeds showing signs of fungal infection were obtained and the moisture content of the maize seeds were determined. The maize seeds were then oven dried at 60 °C for 24 hrs and grounded into fine powder using mortar and pestle. The powdered samples were stored in plastic container for laboratory analysis. Most of the methods adopted in this research work were those recommended by Association of Official Analytical Chemist [24].

2. 3. 1. Biochemical Analysis of Moisture Content

A clean 100 ml beaker was dried in an oven to constant weight (a). A known amount of the 5 g sample was introduced in the beaker and weighed (b). The samples were then fried in a ventilated electrically heated atmosphere oven at 75 °C for about 24hrs and cooled in desiccators until constant weight was obtained (c). The Percentage Moisture content was calculated from the formula:

$$\% \text{ Moisture content} = \frac{\text{Weight loss of sample} \times 100}{\text{Weight of the original sample 1}}$$

The experiment was carried out in triplicates.

2. 3. 2. Ash content

5 kg sample were accurately weighed into the crucible. This was ignited at 55°C for about 24 hrs in desiccators and weighted. This step was repeated until a constant weight was obtained. The percentage ash content was calculated from:

$$\% \text{ Ash content} = \frac{\text{Weight of ash x 100}}{\text{Weight of sample 1}}$$

Determination was made in triplicates.

2. 3. 3. Crude fat or ether extract

5 g samples were accurately weighed into a thimble. About 120 ml petroleum ether was poured into a previously dried and weighted round bottom flask. The Soxhlet extractor into which the thimble with content had been introduced was then filled into the round bottom flask and the condenser and extraction apparatus set up with a cramp and stand. Gentle heat had been applied then the heater evaporated and as it condensed, it dropped into the thimble where it extracted ether soluble constituents into the round bottomed flask. The extraction then continued for about 8 hours. The thimble was then removed and air-dried (later far free extract was used for fibre determination). The petroleum ether in the flask was distilled off and collected in the Soxhlet extractor tube. The flask was then fried in an air circulating desiccators for 8 hours. The round bottom flask and the lipid extract were then weighted.

The flask and content was again dried and weighed till a constant weight was obtained. The amount of lipid extracted was obtained from the difference between the weight of the flask before and after extraction.

$$\% \text{ Fat} = \frac{\text{Weight loss of sample (extracted fat) x 100}}{\text{Weight of sample 1}}$$

2. 3. 4. Crude fibre

5 g far free material was weighted and quantitatively transferred into 400 ml beaker, which had been previously marked at 200 ml level. 50 ml of 1.25% sulphuric acid were added and the mixture was made up to 200 ml mark with distilled water. The contents of the beaker were heated to boiling point for 30 minutes.

2. 3. 5. Crude Protein (Micro Kjedahl Method)

40% Sodium hydroxide (NaOH) pellets (40 g pellets carbonates free were dissolved in 100 ml distilled water). Concentrated sulphuric acid (H_2SO_4), Selenium Kjedahl Catalyst (each tablet containing 1g Sodium sulphate, and 0.05 g Copper sulphate ($CuSO_4$) was dissolved in 0.1% hydrochloric acid (HCl). Methyl red-ethylene blue indicator was prepared by mixing the equal volume of 0.2% twice recrystallized methyl red and 0.0% methylene blue made up in absolute ethanol. This sample was then stored in a dark brown bottle in a refrigerator.

2. 3. 6. Digestion (Micro Kjedahl)

1 g sample was weighed out into a 50 ml Kjedahl digestion flask. 20 ml of antidumping chips were added. The mixture was incinerated to gentle boiling on a digestion rack and then heated strongly until the digest became clear. The digest was removed, cooled and quantitatively transferred to a 100 ml volumetric flask and made up to mark. An Erlenmeyer flask containing 10 ml of boric acid indicator solution was placed at the tip of the condenser extended below the surface of the solution. 10 ml of the sample digest was introduced into quick fit micro Kjedahl flask and steam heated. 10 ml of 40% Sodium hydroxide (NaOH) solution was added to the digest and the digested steam distilled into the Erlenmeyer's flask until the contents become more than double of its original volume as the ammonia (NH_3) changed to green. A blank determination was carried out in a similar manner as described above except 1 g digestion sample was replaced by 1 ml of distilled water.

2. 3. 7. Titration

The content of the Erlenmeyer flask was titrated with 0.1% hydrochloric acid to a pink end point.

Calculation

1 ml of HCl (Test) – ml of HCl (Blank) × NX × 100 % Protein = 1000 × 10 × 1
N = Normality of the acid
10 = Ml of digest use
I = Gram of sample used

2. 4. Preparation of plant materials

Leaves of *Corchorus olitorius* and *Gongronema latifolium* used in the study were separately washed thoroughly using distilled water and surface sterilized with 99% ethanol and sun-dried for 6 days. The dried plant leaves were blended separately using a sterile electric blender to obtain 200 grams of fine powder of each sample.

2. 4. 1. Ethanolic extracts

Ethanolic extracts of plant materials were obtained by adding the powdered sample at different concentrations, 20 g, 40 g, 60 g, 80 g and 100 g to 200 mls of ethanol. This was stirred vigorously and allowed for 24 hours at room temperature 28 ±1 °C. The solution was then filtered through four-folds of sterile cheese cloth for all the plant materials. The filtrates obtained were used as ethanolic extracts of the test plants and stored in reagent bottles for further use.

2. 5. Susceptibility test

The extracts percentage concentrations were prepared at 20 g/200 ml, 40 g/200 ml, 60 g/200 ml, 80 g/200 ml and 100 g/200 ml with ethanol as solvent.

2. 6. *In vitro* antifungal assay

5 ml of each concentration of the ethanolic extracts was first poured into different Petri-dishes using sterile syringe. The sterile potato dextrose agar (PDA) was also poured into the plates containing the solvent extracts after which the plates containing the solvent extracts were gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized cork borer (5mm in diameter), a disc of the matured culture was punched out from advancing margin of a four- day old pure culture and inoculated at the centre of plates and incubated at room temperature (28 ±10c) for 7 days. The experiment was replicated thrice. Area of inhibition was measured daily for 7days using a meter rule and recorded.

2. 7. Statistical analysis

Data obtained in this study were analysed using Student T-test and a one way Analysis of Variance (ANOVA) at 5% probability level ($P \leq 0.05$).

2. 8. Phytochemical Screening

Phychemical screening of the ethanolic extracts of leaves of *Corchorus olitorius* and *Gongronema latifolium* was carried out using the methods of [25-27]. Phytochemical screening was carried out in the Department of Biochemistry, University of Calabar, Calabar, Cross River State, Nigeria.

2. 8. 1. Test for Tannin

Plant extract (about 1.0 g) was stirred with sterile distilled water (10 ml) and filtered (using Whatman's no. 1 filter paper). A blue colouration resulting from the addition of 2 drops of 10% $FeCl_3$ reagent to the filtrate indicated the presence of tannins.

2. 8. 2. Test for Alkaloids

One half of a gram of the plant extract was dissolved in 1% HCl (5 ml) on a steam bath. The filtrate (1 ml) was treated with few drops of Dragendorff's reagent (Potassium iodide 0.11 M, Bismuth nitrate 0.6 M in Acetic acid 3.5 M). Turbidity or precipitation was taken as indicative of the presence of alkaloids.

2. 8. 3. Test for Tannin

Plant extract (about 1.0 g) was stirred with sterile distilled water (10 ml) and filtered (using Whatman's no. 1 filter paper). A blue colouration resulting from the addition of 2 drops of 10% $FeCl_3$ reagent to the filtrate indicated the presence of tannins.

2. 8. 4. Test for Flavonoids

An aliquot of the extract (0.2 g) was dissolved in ethanol and methanol (2 ml) and heated. A chip of magnesium metal strip was added to the mixture followed by the addition of a few drops of concentrated Hcl. The occurrence of a red or orange colouration was indicative of the presence of flavonoids.

2. 8. 5. Test for Saponins

Powdered sample (about 2 g) was boiled in distilled water (20 ml) in a water bath and then filtered. The filtrate (10 ml) was mixed with distilled water (5 ml) and shaken vigorously to form a stable persistent froth. The froth was mixed with 3 drops of olive oil, shaken vigorously, and then observed for the formation of emulsions.

2. 8. 6. Test for Steroids

The extract (about 0.5 g) was dissolved in $CHCl_3$ (3 ml) and then filtered. To the filtrate was added concentrated H_2SO_4 to form a lower layer. A reddish brown colour was taken as positive for steroids.

2. 8. 7. Test for Reducing Sugars

One millilitre each of Fehling's solutions I and II was added to an aqueous solution of the extract (2 ml). The mixture was heated in a boiling water bath for about 2–5 min. The production of a brick red precipitate indicated the presence of reducing sugars.

2. 8. 8. Test for Anthraquinones

A sample of plant extract (0.5 g) was boiled with 0.01M Hcl and filtered while still hot. The filtrate was shaken with benzene (10 mL). The benzene layer was removed, and ammonium hydroxide (5 ml, 10%) was added. A violet, red, or pink colouration in the ammonia phase is positive for anthraquinones.

2. 8. 9. Test for Cardiac Glycosides

The extract (about 0.5 g) was dissolved in glacial acetic acid (2 ml) containing 1 drop of 1% $FeCl_3$. This was under laid with concentrated H_2SO_4. A brown ring at the interface

indicated the presence of a deoxygenated sugar, characteristic of cardiac glycosides. A violet ring may form just above ring and gradually spreads through this layer.

3. RESULTS

3. 1. Isolated fungal pathogens

The fungal pathogens isolated and identified as the causative agents of post-harvest deterioration of maize seeds from this study were: *Penicillium sp*. (Figure 1), *Fusarium sp*. (Figure 2), *Aspergillus sp*. (Figure 3) and *Rhizopus stolonifer* (Figure 4).

Fig. 1. Photomicrograph of *Penicillium sp*. × 200

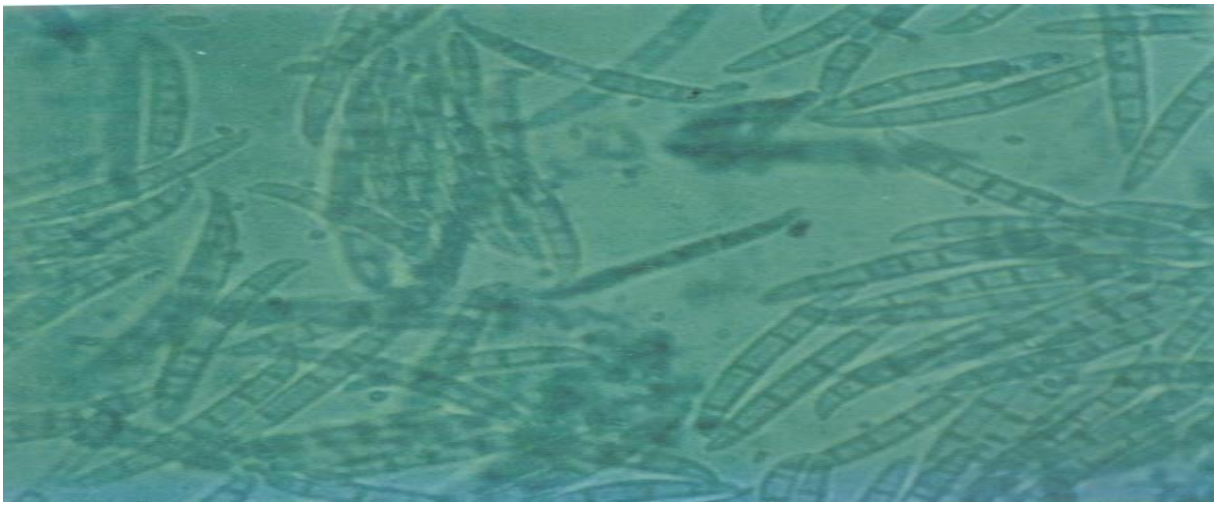

Fig. 2. Photomicrograph of *Fusarium sp*. × 200

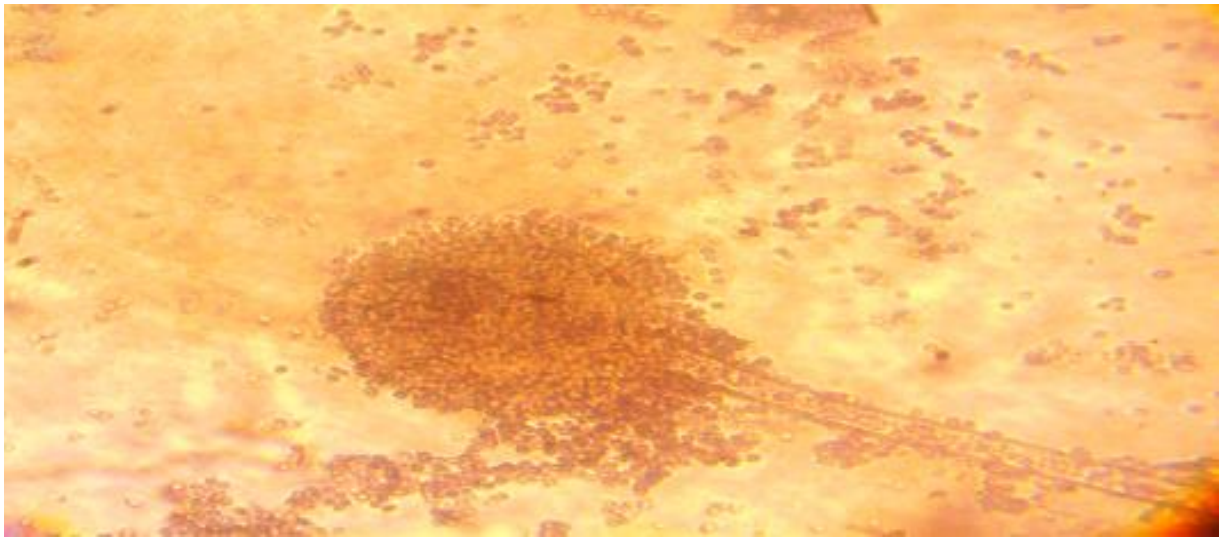

Fig. 3. Photomicrograph of *Aspergillus niger* × 200

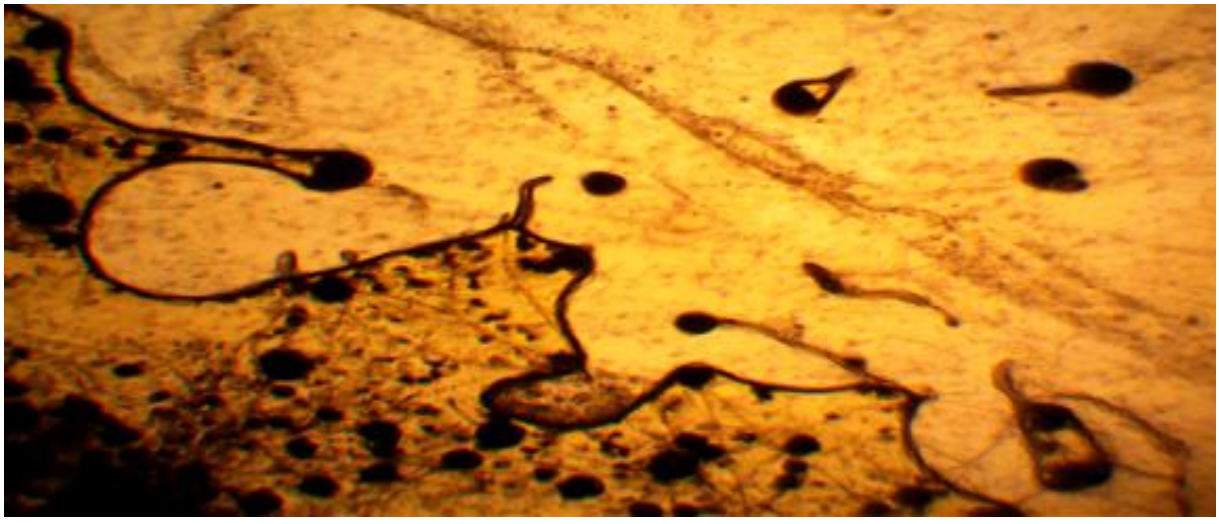

Fig. 4. Photomicrograph of *Rhizopus stolonifer* × 200

3. 2. Pathogenicity Test

Symptoms observed on the maize seeds inoculated with *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*. and *Rhizopus stolonifer* were similar to those observed on the deteriorating maize seeds obtained from storage. Symptoms such as soft rots and dry rots were observed on the maize seeds.

3. 3. Effect of fungi infection on biochemical composition of the maize seeds

The results of biochemical analysis in mg/100 g of fungal infected maize seeds showed that there was an increase in the moisture contents. The moisture content increased from

52.04 ±0.20 in the non-infected maize to 60.02 ±1.04 in the maize grain infected with *Penicillium sp*. while the following parameters were found to decrease in the infected than in the non-infected maize seeds. viz protein content (0.06 ±0.41), fat (0.12 ±0.03), crude fibre (0.40 ±0.11), ash content (0.10 ±0.25) and carbohydrate (2.15 ±1.02) in the *Penicillium sp*. infected maize whereas the uninfected maize grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ±2.30 respectively (Table 1).

Table 1. Biochemical composition of *Penicillium sp*. infected and non-infected maize grain (mg/100g) (dry matter).

Maize	**Composition of maize Grain (mg/100g)**					
Sample	**Moisture**	**C/F**	**Fat**	**Protein**	**Ash**	**CHO**
Psp-infected	60.02 ±1.04[a]	0.40 ±0.11[a]	0.12 ±0.03[a]	0.06 ±0.41[a]	0.10 ±0.25[a]	2.05 ±1.02[a]
Non-infected	52.04 ±0.20[b]	3.10 ±1.04[b]	1.70 ±0.90[b]	2.60 ±2.03[b]	0.50 ±0.11[b]	7.50 ±2.30[b]
T-value	3.26					

Note. C/F = Crude fibre, CHO = Carbohydrate, Psp = *Penicillium sp*.

The results of biochemical analysis in mg/100 g of fungal infected maize seeds showed that there was an increase in the moisture contents. The moisture content increased from 52.04 ±0.20 in the non-infected maize to 60.05 ±0.04 in the maize grain infected with *Fusarium sp*. while the following parameters were found to decrease in the infected than in the non-infected maize seeds. viz protein content (0.06 ±0.21), fat (0.12 ±0.01), crude fibre (0.40 ±0.13), ash content (0.10 ±0.21) and carbohydrate (2.25 ±1.01) in the *Fusarium sp*. infected maize whereas the uninfected maize grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ±2.30 respectively (Table 2).

Table 2. Biochemical composition of *Fusarium sp*. infected and non-infected maize grain (mg/100g) (dry matter).

Maize	**Composition of maize Grain (mg/100g)**					
Sample	**Moisture**	**C/F**	**Fat**	**Protein**	**Ash**	**CHO**
Fsp-infected	60.05 ± 0.04[a]	0.40 ± 0.13[a]	0.12± 0.01[a]	0.06 ± 0.21[a]	0.01 ± 0.21[a]	2.25± 1.01[a]
Non-infected	52.04 ± 0.20[b]	3.10 ± 1.04[b]	1.70± 0.90[b]	2.60± 2.03[b]	0.50± 0.11[b]	7.50± 2.30[b]
T-value	3.26					

Note. C/F = Crude fibre, CHO = Carbohydrate, Fsp = *Fusarium sp*.

The results of biochemical analysis in mg /100g of fungal infected maize seeds showed that there was an increase in the moisture contents. The moisture content increased from 52.04 ±0.20 in the non-infected maize to 59.21 ±0.28 in the maize grain infected with *Aspergillus sp*. while the following parameters were found to decrease in the infected than in the non-infected maize seeds. viz protein content (0.02 ±0.04), fat (0.20 ±1.01), crude fibre (0.09 ±0.06), ash content (0.10 ±0.12) and carbohydrate (2.02 ±0.05) in the *Aspergillus sp*. infected maize whereas the uninfected maize grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ±2.30 respectively (Table 3).

Table 3. Biochemical composition of *Aspergillus sp.* infected and non-infected maize grain (mg /100g) (dry matter).

Maize	**Composition of maize Grain (mg/100g)**					
Sample	**Moisture**	**C/F**	**Fat**	**Protein**	**Ash**	**CHO**
Asp-infected	59.21 ± 0.28[a]	0.09 ± 0.06[a]	0.20± 1.01[a]	0.02 ± 0.04[a]	0.10 ± 0.12[a]	2.02± 0.05[a]
Non-infected	52.04 ± 0.20[b]	3.10 ± 1.04[b]	1.70± 0.90[b]	2.60± 2.03[b]	0.50± 0.11[b]	7.50± 2.30[b]
T-value	3.26					

Note. C/F = Crude fibre, CHO = Carbohydrate, Asp = *Aspergillus sp*.

The results of biochemical analysis in mg/100g of fungal infected maize seeds showed that there was an increase in the moisture contents. The moisture content increased from 52.04 ±0.20 in the non-infected maize to 68.03 ±0.04 in the maize grain infected with *Rhizopus stolonifer* while the following parameters were found to decrease in the infected than in the non-infected maize seeds. viz protein content (0.06 ± 0.31), fat (0.01 ±0.03), crude fibre (0.40 ±0.01), ash content (0.01 ±0.21) and carbohydrate (2.15 ± 1.01) in the *Rhizopus stolonifer* infected maize whereas the uninfected maize grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ±2.30 respectively (Table 4).

Table 4. Biochemical composition of *Rhizopus stolonifer* infected and non-infected maize grain (mg /100g) (dry matter).

Maize	**Composition of maize Grain (mg/100g)**					
Sample	**Moisture**	**C/F**	**Fat**	**Protein**	**Ash**	**CHO**
Rs-infected	68.03 ± 0.04[a]	0.40 ± 0.01[a]	0.01± 0.30[a]	0.06 ± 0.31[a]	0.01 ± 0.21[a]	2.15± 1.01[a]
Non-infected	52.04 ± 0.20[b]	3.10 ± 1.04[b]	1.70± 0.90[b]	2.60± 2.03[b]	0.50± 0.11[b]	7.50± 2.30[b]
T-value	3.26					

Note. C/F = Crude fibre, CHO = Carbohydrate, Rs = *Rhizopus stolonifer*

3. 4. Phytochemical screening

Phytochemical screening of the ethanolic leaf extracts of *Corchorus olitorius* and *Gongronema latifolium* showed that cardiac glycosides, hydrocyanin and anthraquinones were strongly present in ethanolic leaf extracts of *C. olitorius* while saponins and tannins were strongly present in *G. latifolium* extracts. Saponins were highly present in ethanolic *C. olitorius* extract while flavonoids, hydrocyanin, cardiac glycosides, Anthraquinones and reducing sugars were highly present in *G. latifolium* extract. Flavonoids and reducing sugars were present in *C. olitorius* while polyphenols were slightly present but absent in *G. latifolium* extract. Alkaloid was slightly present in *C. olitorius* extract while traces of alkaloids were observed in *G. latifolium* extract as presented in (Table 5).

Table 5. Phytochemical screening of ethanolic leaf extracts of *C. olitorius* and *G. latifolium*.

Phytochemical contents	*C. olitorius*	*G. latifolium*
Alkaloids	+	±±
Flavonoids	++	+++
Saponins	+++	++++
Tannins	++	++++
Hydrocyanin	++++	+++
Cardiac glycosides	++++	+++
Anthraquinones	++++	+++
Polyphenols	+	-
Reducing sugars	++	+++

Note. (++++) = strongly present, (+++) = highly present, (++) = present, (+) = slightly present, (±±) = trace, (-) = absent

3. 5. *In vitro* effect of ethanolic leaf extracts of *Corchorus olitorius* on *Penicillium sp.*, *Fusarium sp.*, *Aspergillus sp.*, and *Rhizopus stolonifer* at the different concentrations.

The *in vitro* effect of the ethanolic leaf extracts of *Corchorus olitorius* at the different concentrations on the radial growth of *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*. and *Rhizopus stolonifer* is presented in (Tables 6-9). Results from the study showed that ethanolic leaf extracts of *C. olitorius* had a significant effect on the isolated fungal pathogens at all levels of concentration (20 g/200 ml, 40 g/200 ml and 60 g/200 ml, 80 g/200 ml and 100 g/200 ml) tested as compared with the control. Results (Table 6-9) showed that ethanolic leaf extracts of *C. olitorius* at 60 g/200 ml, 80 g/200ml and 100 g/200 ml concentrations completely inhibited the radial growth of *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*. and *Rhizopus stolonifer* in the first to seventh day of incubation and at 20g/200ml concentration on the first to third day of incubation respectively while at 40g/200ml concentration, the radial growth of the fungal pathogens was mostly inhibited in the first to fourth day respectively as compared with the control.

Table 6. *In vitro* effect of ethanolic *Corchorus olitorius* leaf extracts on the radial growth of *Penicillium sp*. (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.00	0.00	0.90	1.20	1.25	1.31
40g/200ml	0.00	0.00	0.00	0.00	0.80	1.00	1.10
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.60	0.80	2.40	2.90	3.20	3.60	3.80
LSD	0.68*						

Note. Values are means of three replicates

Table 7. *In vitro* effect of ethanolic *Corchorus olitorius* leaf extracts on the radial growth of *Fusarium sp*. (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.00	0.70	0.80	0.92	1.10	1.25
40g/200ml	0.00	0.00	0.00	0.70	1.00	1.05	1.10
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.90	1.80	2.20	2.70	3.00	3.20	3.50
LSD	0.51*						

Note. Values are means of three replicates

Table 8. *In vitro* effect of ethanolic *Corchorus olitorius* leaf extracts on the radial growth of *Aspergillus sp*. (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.00	0.00	0.00	1.00	1.10	1.16
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.90	1.10
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.90	1.70	1.90	2.20	2.80	3.10	3.30
LSD	0.51*						

Note. Values are means of three replicates

Table 9. *In vitro* effect of ethanolic *Corchorus olitorius* leaf extracts on the radial growth of *Rhizopus stolonifer*. (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.00	0.00	0.70	0.80	1.10	1.27
40g/200ml	0.00	0.00	0.00	0.00	0.90	1.00	1.14
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.70	1.60	2.90	3.70	4.00	4.50	4.50
LSD	0.74*						

Note. Values are means of three replicates

3. 6. *In vitro* effect of ethanolic *Gongronema latifolium* leaf extracts of on *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*., and *Rhizopus stolonifer* at the different concentrations.

The *in vitro* effect of the ethanolic leaf extracts of Gongronema latifolium at the different concentrations on the radial growth of *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*. and *Rhizopus stolonifer* is presented in (Tables 10-13). Results shows that at 20 g/200 ml, 40 g/200 ml, 60 g/200 ml, 80 g/200 ml and 100 g/200 ml ethanolic leaf extracts of *G. latifolium* inhibited the radial growth of *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*. and *Rhizopus stolonifer* at the different concentrations tested when compared with the control. Complete inhibition was observed at 40 g/200 ml, 60 g/200 ml, 80 g/200 ml and 100 g/200 ml concentrations of ethanolic leaf extracts of *G. latifolium* in the first to seventh day of incubation respectively and at 20 g/200 ml concentration on the first to second day of incubation and *Rhizopus stolonifer* only on the first day of incubation respectively as compared with the control. It is noteworthy to state that results shows that the ethanolic leaf extracts of *G. latifolium* was more effective in inhibiting the redial growth of the fungal isolates than those of the ethanolic leaf extracts of *C. olitorius* at the different concentrations tested.

Table 10. *In vitro* effect of ethanolic *Gongronema latifolium* leaf extracts on the radial growth of *Penicillium sp*. (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.00	0.92	1.15	1.20	1.26	1.35
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.90	1.20	1.90	2.30	2.80	3.40	3.60
LSD	0.53*						

Note. Values are means of three replicates

Table 11. *In vitro* effect of ethanolic *Gongronema latifolium* leaf extracts on the radial growth of *Fusarium sp*. (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.00	0.80	1.22	1.30	1.37	1.50
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.70	1.50	1.90	2.40	2.70	3.10	3.50
LSD	0.51*						

Note. Values are means of three replicates

Table 12. *In vitro* effect of ethanolic *Gongronema latifolium* leaf extracts on the radial growth of *Aspergillus sp*. (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.40	0.70	0.90	1.10	1.20	1.60
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.90	1.90	2.50	2.80	3.20	3.50	3.70
LSD	0.50*						

Note. Values are means of three replicates

Table 13. *In vitro* effect of ethanolic *Gongronema latifolium* leaf extracts on the radial growth of *Rhizopus stolonifer* (cm).

Concentration	Days of incubation and radial growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20g/200ml	0.00	0.00	0.63	0.99	1.20	1.50	1.90
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.60	1.20	2.90	3.80	4.00	4.50	4.50
LSD	0.77*						

Note. Values are means of three replicates

4. DISCUSSION

Fungal rot of maize seed is worldwide in occurrence and have been reported in almost all parts of the world. Post-harvest deterioration of maize seeds during storage by fungi is a major limiting factor in the production and storage of maize worldwide. Seed decay due to fungal invasion during storage and cost of controlling this infection constitute a significant burden in agricultural enterprise and has serious socio-economic and environmental consequences. Consequently, there is an urgent need for effective and sustainable control of this disease. The current trend in the effective and sustainable management of plant diseases requires the use of botanical pesticides which are non-phytoxic to human and are environmentally friendly. In this study, the fungal pathogens isolated as the causative agents of post-harvest deterioration of maize seeds in storage were *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*., and *Rhizopus stolonifer*. Results of this study revealed that these fungi were responsible for the post-harvest deterioration of maize seeds in storage obtained from Oban community in Akamkpa Local Government Area of Cross River State as evidenced by the pathogenicity test. The degradation of Maize grains by fungi in storage according to survey shows that fungi may enter the maize seed through wounds created by rodent and insects. Some workers [19,20], have revealed that *Aspergillus niger, A. flavus, A. fumigatus, Rhizopus stolonifer* and *Fusarium oxysporum* were the causal agents of maize seed rots in the field and storage in Nigeria and the infection starts in the field and manifest during storage. [28], reported *Aspergillus niger, Fusarium solani, Rhizopus stolonifer* and *Penicillium sp.* to be responsible for postharvest spoilage of maize grains in India and that the infection of maize starts in the field and manifest during storage, transit and marketing.

The result of biochemical analysis (mg/100g) of non-infected and infected maize seeds (Tables 1-4) revealed that the moisture content increased from 52.04 ±0.20 in the non-infected maize to 60.02 ±1.04 in the maize grain infected with *Penicillium sp*., 52.04 ±0.20 in the non-infected maize to 60.05 ±0.04 in the maize grain infected with *Fusarium sp*., from 52.04 ±0.20 in the non-infected maize to 59.21±0.28 in the maize grain infected with *Aspergillus sp*. and 52.04 ±0.20 in the non-infected maize to 68.03 ±0.04 in the maize grain infected with *Rhizopus stolonifer*. This result is in agreement with the findings of [29] who reported an increase in moisture content from 5.09 in the non-infected to 6.13 in the infected of groundnut (*Arachis hypogeae*) infected with *R. stolonifer* and other moulds. Similarly, [30] reported an increase in the moisture and ash content of (*Anonia muricata*) fruits infected by *Colletotrichum gloeosporoides* and *R. stolonifer*. However, [31] reported that moisture content (5.81%) of non-infected cocoa beans were higher, but were seriously depleted when infected with *P. Capsici* and *P. Megarkaya* (43.97 and 3.13) respectively. [32], have also found that carbohydrate content of non-infected pods (91.0) have decreased to (13.2) in infected cocoa pods. [33], reported that moisture decreased from 36.49 to 10.4 g/100 g in infected samples. Results (Tables 1-4) also revealed that the fungal pathogens had a significant effect ($P \leq 0.05$) on the maize seeds as the following parameters were found to decrease in the infected than in the non-infected maize seeds. viz protein content (0.06 ± 0.41), fat (0.12 ±0.03), crude fibre (0.40±0.11), ash content (0.10 ±0.25) and carbohydrate (2.15 ±1.02) in the *Penicillium sp*. infected maize whereas the uninfected maize grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ± 2.30 respectively, protein content (0.06 ±0.21), fat (0.12 ±0.01), crude fibre (0.40 ±0.13), ash content (0.10 ±0.21) and carbohydrate (2.25 ±1.01) in the *Fusarium sp*. infected maize whereas the uninfected maize

grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ±2.30 respectively, protein content (0.02 ±0.04), fat (0.20 ±1.01), crude fibre (0.09 ±0.06), ash content (0.10 ±0.12) and carbohydrate (2.02 ±0.05) in the *Aspergillus sp*. infected maize whereas the uninfected maize grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ±2.30 respectively and protein content (0.06 ±0.31), fat (0.01 ±0.03), crude fibre (0.40 ±0.01), ash content (0.01 ±0.21) and carbohydrate (2.15 ±1.01) in the *Rhizopus stolonifer* infected maize whereas the uninfected maize grain recorded 2.60 ±2.03, 1.70 ±0.90, 3.19 ±1.04, 0.50 ±0.11 and 7.50 ±2.30 respectively.

The decrease in protein content, fat, crude fibre, carbohydrate and ash content in the infected sample is in agreement with the findings of [31] who reported that protein (81.14%), fibre (3.59), ash (5.81) and fat (5.23%) of non-infected cocoa beans were higher, but were seriously depleted when infected with *P. capsici* and *P. megakarya* to 4.80, 1.80, 2.13 and 4.02%, respectively. [34], have also reported that infected onion leaf showed a significant decrease in the quantity of the carbohydrate, crude protein, fat, fibre and ash content. [35], reported that ash content of non-infected pods (10.7) and beans (8.0) were depleted when infected with *P. palmivora* to 9.3 and 7.8 in cocoa pods and beans, respectively. It could therefore be deduced that the relative increase of moisture and decrease in carbohydrate in the infected beans may be caused by the digestion, degradation and dissolution of the beans tissue into a mush (water rot) by the pathogens. These degradation activities by pathogens might have also resulted to the relative reduction in the carbohydrate, protein, fat, fibre, and ash contents of the infected maize seeds. The carbohydrate, protein, fat, fibre and ash might have been broken down by the fungi into smaller molecules that they absorbed [30,36], reported that complex molecules such as polysaccharide and protein are required by fungi to build the hyphal wall (chitin, glucan and cellulose) and for respiration to obtain energy. This suggests that these pathogens might have denied the man of these essential nutrients upon consumption through their degradation activities, thereby causing some great damaging effects to human health. [37], stated that the deficiency of fibre in our diet leads to diverticular diseases and intestinal cancer. However [38] who also reported a decrease in fat, ash and protein content of cocoa infected by fungi, stated that the nutrient depletion in entire test plant sample might have been as a result of the internal defence system of the host tissue.

Phytochemical screening of the test plants *Corchorus olitorius* and *Gongronema latifolium* leaf extracts used in this study was investigated to determine their exact phytochemical contents. Phytochemical screening of the ethanolic leaf extracts of *Corchorus olitorius* and *Gongronema latifolium* showed that cardiac glycosides, hydrocyanin and anthraquinones were strongly present in ethanolic leaf extracts of *C. olitorius* while saponins and tannins were strongly present in *G. latifolium* extracts. Saponins were highly present in ethanolic *C. olitorius* extract while flavonoids, hydrocyanin, cardiac glycosides, Anthraquinones and reducing sugars were highly present in *G. latifolium* extract. Flavonoids and reducing sugars were present in *C. olitorius* while polyphenols were slightly present but absent in *G. latifolium* extract. Alkaloid was slightly present in *C. olitorius* extract while traces of alkaloids were observed in *G. latifolium* extract as presented in (Table 5). Phytochemicals, as compounds which occur naturally in plants, form part of plants defense mechanisms against diseases [39]. They are classified into primary and secondary, based on their activity in plant metabolism. The primary ones comprise of sugars, amino acids, proteins and chlorophyll [40], while secondary ones include the phenolic compounds such as tannins, flavonoids, alkaloids, saponins, anthraquinones, phlobatannins, proanthocyanidins, etc. [41].

These phenolic compounds have been reported to possess considerable antimicrobial properties, which is attributed to their redox properties [42,43]. Thus the antimicrobial properties of plants have been attributed to the presence of these secondary metabolites [44]. It is noteworthy to state that a strong presence of saponins and tannins were observed in *G. latifolium* leaf extracts, the presence of saponins and tannins in the leaves of *G. latifolium* may account for the bitter and astringent taste of the leaves as reported by [45]. The presence of these phytochemicals also gives an insight on the medicinal properties of *Gongronema latifolium* leaves.

In this study, the antifungal activity of ethanolic leaf extracts of *Corchorus olitorius* and *Gongronema latifolium* were tested *in vitro* on fungi isolated from deteriorating maize seeds obtained from storage. Results showed that the ethanolic leaf extracts of *C. olitorius* and *G. latifolium* investigated, exhibited various antifungal activities against the species of fungi isolated. The antifungal activity of the ethanolic leaf extracts of *C. olitorius* and *G. latifolium* on the isolated fungal pathogens is presented in (Tables 6-9) and (Tables 10-13) respectively. The results showed that, the ethanolic extracts had a significant ($P < 0.05$) effect on the radial growth of the fungal pathogens and the rate of antifungal activity differed from one concentration to the other. The differences in the fungitoxic potentials between these plant extracts may be attributed to the susceptibility of each of the fungal pathogens to the different plant extracts. This agrees with the results of some workers like [46] and [47]. [48], reported that some plants contain phenolic substances and essential oils, which are inhibitory to micro-organisms. The presence of these compounds in these extracts has been reported to be responsible for their antifungal properties [49]. It is noteworthy that of all the tested plant extracts ethanolic leaf extracts of *G. latifolium* had a more significant effect in inhibiting the redial growth of the fungal pathogens as complete inhibition was observed at (40 g/200 ml, 60 g/200 ml, 80 g/200 ml and 100 g/200 ml) concentrations while that of *C. olitorius* was observed at (60 g/200 ml, 80 g/200 ml and 100g/200 ml) concentrations. Results also showed that the level of inhibition increased with a corresponding increase in the concentration of the extracts. The inhibitory potency of the plant extracts may be attributed to the phytochemical compounds like tannins, alkaloids, flavonoids, cardiac glycosides and saponins in them and the extraction solvents as reported by [49]. This is also in agreement with the works of [50] and [51,52] who reported that the high potency of plant extracts containing the same bio-active compounds could be used for the control of fungal pathogens of plants. The antifungal efficacy of the leaf extracts of *C. olitorius* and *G. latifolium* were evaluated in order to develop the cheaper methods of controlling the post-harvest fungal deterioration of maize seeds in storage.

5. CONCLUSIONS

The fungal pathogens isolated and identified from this study as the causative agents of post-harvest deterioration of maize seeds in storage were *Penicillium sp*., *Fusarium sp*., *Aspergillus sp*., and *Rhizopus stolonifer*. The results of biochemical analysis of fungal infected and non-infected maize seeds showed that there was an increase in the moisture content of the fungal infected maize seeds relative to the healthy ones (control), while there was a decrease in the carbohydrate, fat, fibre, protein and ash contents of the fungal infected maize seeds relative to the healthy ones (control). Results of the *in vitro* antifungal assay

carried out showed that the ethanolic leaf extracts of *Corchorus olitorius* and *Gongronema latifolium* were effective against the seed rot fungal pathogens of maize at the different concentrations tested. To this effect, timely spraying of maize seeds with leaf extracts of *C. olitorius* and *G. lafolium* prepared at higher concentrations before and during storage will reduce the damaging activities of the fungal pathogens and contamination with mycotoxins and other related fungal metabolites that might be hazardous to human health.

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