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PHYTOCHEMICAL SCREENING AND IN VITRO ANTIFUNGAL EFFICACY OF MANGO AND MAHOGANY LEAF EXTRACTS AGAINST *PYTHIUM MYRIOTYLUM* INFECTING AMARANTHUS HYBRIDUS

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Abstracts: *The use of plant botanicals have gained a lot of attention in recent times, owing to the health and economic implications associated with their uses. Amaranthus hybridus has numerous nutritional values but it is affected largely by post emergence damping off disease. This research investigated the efficacy of the leaf extracts of three plant Mango (Mangifera indica) and Mahogany (Khaya senegalensis) against Pythium myriotylum the causative pathogen of post emergence damping off disease in Amaranthus hybridus. The plant samples were collected, dried and extraction was done using the soxhlet apparatus. Qualitative and quantitative phytochemical screening was done using standard procedures. The causative pathogen (Pythium myriotylum) was isolated from infected A. hybridus leaves and the rhizosphere on PDA medium. Agar well diffusion methods was used for antifungal studies using two extract concentrations at 500, 800 and 1000 mg/mL. The control was without extract and three replicates of each were made. Incubation was done at 28±2 °C for 7 days. The radial growth of P. myriotylum at day 7 from Mahogany extract at 500, 800 and 1000 mg/ml concentrations were 4.00, 3.99 and 3.99 cm respectively, while Mango extract were 4.00, 3.12 and 2.75 cm. The results obtained showed that the plant extracts have positive potentials towards inhibiting the pathogen (P. myriotylum) with Mango extract having the highest inhibition potential followed by Mahogany extract recorded the lowest. Therefore, this study supports the use of these botanicals in the control of plant damping off disease of A. hybridus as it has proven to be effective.*
Keyword: *Phytochemical, In Vitro, Pythium myriotylum and Amaranthus hybridus*

Introduction

Amarathus hybridus L., popularly called *amaranth* or pigweed is an annual herbaceous plant of 1.6 feet high. The leaves are alternate petiole, 3 - 6 inches long, dull green and with rough hairy ovate or rhombic and wavy margins. The flowers are small, with greenish or red terminal panicles. Taproot is long flashy red or pink. The seeds are long lenticellular in shape, with each seed weighing 0.6 - 1.2 g (CABI, 2021). Most developing countries depend on starch-based foods as the main staple food for supply off both energy and protein. This account in part for protein deficiency which prevails among the populace as recognized by food and agricultural organization (Ladeji *et al.,* 2019). In Nigeria as in most other tropical countries of Africa, where the daily diet is dominated by starch staple foods vegetables are the cheapest and most readily available source of important proteins, vitamin, minerals and essential amino acids (Okafor, 2020).

Damping off is a horticultural disease caused by pathogens that kill or weaken seeds or seedling before or after germination. There are various symptoms association with damping off, these reflect the variety of different pathogenic organisms which can cause the condition, however all symptoms result in the death of at least seedlings in any given population. Fungi species implicated are *Pythium, Sphanomyces* and *Fusarium*. (Abang *et al.,* 2012). Chemical fungicides are commonly used to control plant diseases, but their excessive application can harm food quality, soil health, native microflora, and human health. These fungicides, including captan, folpet, dithiocarbamates, pentachlorophenol, and mercurials, are used to prevent mold on crops (Campanale *et al*., 2023). Hence, other measures or biological control such as the use of plant extracts which are non-toxic, eco-friendly and easy to obtain (Kumar, 2022).

Materials and Methods

Samples collection

Soil samples were collected from the fields at the soil cores/transect to a depth of 15 cm using a soil probe with a diameter of 2.5 cm. The soil cores for each transect were then pooled into a polyethylene bag. At the time of sampling, the soil samples were placed in a cooler with ice and transferred to a cold storage refrigerator (4±2 °C). The seeds and infected leaves of *A. hybridus* were collected alongside fresh and healthy leaves of Mahogany (*Khaya senegalensis*) and Mango (*Mangifera indica L*) were collected and the plants were authenticated in the herbarium of the Department of Plant Biology, Federal University of Technology, Minna, Nigeria

Qualitative and Quantitative Phytochemical Screening of Methanol Extracts

Qualitative Phytochemical

Qualitative phytochemical analysis of methanol extracts indicates the presence of several bioactive compound classes using standard established protocols. Alkaloids were detected by the formation of a yellow creamy precipitate upon addition of Mayer's reagent (Santhi & Sengottuvel, 2016). Flavonoids yielded a bluish-black coloration after treatment with ethyl acetate and diluted ammonia, indicating their presence (Akinyeye *et al.,* 2014). Tannins were identified by the appearance of dark green or blue-black coloration following $FeCl_3$ addition (Sneh *et al.,* 2013). Phenolic compounds were confirmed by the formation of yellow precipitates with lead acetate solution (Manickara & Veerababu, 2014). Saponins were evidenced by persistent frothing in aqueous extracts (Sneh *et al.,* 2013). Steroids were indicated by a color change to blue or green upon treatment with acetic anhydride, chloroform, and concentrated sulfuric acid (Santhi & Sengottuvel, 2016). Terpenoids were detected by the formation of a reddish-brown interface color in a chloroform-sulfuric acid test (Akinyeye *et al.,* 2014). Anthraquinones were confirmed through a pink coloration following boiling with hydrochloric acid, extraction with chloroform, and treatment with ammonia (Krishnaiah *et al.,* 2009). Cardiac glycosides were identified by characteristic brown and green rings formed upon reaction with glacial acetic acid, $FeCl_3$, and sulfuric acid (Akinyeye *et al.,* 2014).

Quantitative Phytochemical

Quantitative analyses were conducted using validated spectrophotometric methods. Total phenolic content was measured using the Folin-Ciocalteu reagent, with absorbance read at 765 nm (Santhi, and Sengottuvel, 2016). Flavonoid content was determined by the aluminum chloride colorimetric method at 415 nm (Chan *et al.,* 2019). Alkaloids quantification involved complexation with sulfuric acid and formaldehyde, monitoring at 565 nm (Oloyede et al., 2010). Saponin content was estimated after appropriate extraction and reaction with ferric sulfate and sulfuric acid, absorbance recorded at 490 nm (Oloyede *et al.,* 2010). Tannins were quantified through Folin-Denis reagent reaction with absorbance at 760 nm (Sneh *et al.,* 2013). Glycosides were measured using the Buljet reagent with colorimetric comparison against standards (Santhi, and Sengottuvel 2016). Terpenoid and steroid contents were determined following standard extraction and chromogenic reagent methods with absorbances read at 700 nm and 550 nm, respectively. Anthraquinone content was quantified based on the reaction with phenol and sulfuric acid, measuring absorbance at 488 nm.

Preparation of Potato Dextrose Agar (PDA)

Thirty-nine (39) grams of PDA (Hi-media) was suspended in 1000ml distilled water and heated to dissolve the powder completely, the medium was sterilized by autoclaving at 121°C for 15minutes (Manufacturer's guide).

Isolation of *Pythium* myriotylum

The infected A. *hybridus* leaves were washed thoroughly under running tap water, then surface sterilized with 70 % sodium hypochlorite solution for 30 to 40 seconds and washed 5 times with sterilized distilled water. Two discs taken from the periphery of necrotic region were placed on Potato Dextrose Agar (PDA), to which streptomycin (1 mlL^{-1}) was added and incubated at 27±2 °C for 3 days. A Single conidium was picked up with a sterile needle under microscopic observation,

transferred individually to PDA plates and incubated at ambient temperature (Subramanian *et al.,* 2013).

In Vitro Antifungal Activities of the Three Plant Extracts on Mycelia Growth of *Pythium myriotylum*

The plant extracts were evaluated for antifungal activities using food poison techniques (Gnannasekaran *et al.,* 2015). 5 ml of the extracts at different concentrations (500, 800 and 1000 mg/mL) was thoroughly mixed with 15 ml of sterile potato dextrose agar (P. D. A.) and 0.5 mL of chloramphenicol contained in the petri-dish with control respectively. After gelling of the medium, seven days old of P. *myriotylum* was inoculated into the centre of the plate using 5mm disk cork borer from their growing edges. The petri-dishes was incubated at 28±2 °C) for 7 days. On each day, the diameter of the extension was measured using a meter rule.

The percentage (%) inhibition was calculated using the formula below;

$$\% \text{ inhibition} = \frac{R1-R2}{R1} \times 100$$

Where R1 is growth in the control and R2 is the growth in the treatment.

Statistical analysis

The data were statistically evaluated using SPSS 21.0 and an ANOVA one-way test, followed by a post-hoc DUNCAN-ALPHA test to determine its importance. Statistically significant P values less than 0.05, but not more than 0.05 are considered statistically significant ($p \leq 0.05$). The data were shown as a medium of a. ± 00 SEM defect.

Results

The percentage yields of methanol crude extracts of *Mangifera indica* L, and *Khaya senegalensis* samples was found to be 22.18 %, and 24.94 %. The qualitative phytochemical composition of plant leaf extract indicates that Saponins, alkaloids, tannins, phenols, tannins and terpenoids were found to be present in the methanol extracts of the plant samples, while glycosides, steroids and anthraquinones were found to be mostly absent (Table 1).

Table 1: Qualitative phytochemical constituents of plant extracts.

S/N	Phytochemicals	Mango Extract	Mahogany Extract
1	Alkaloids	+	++
2	Total phenols	+++	+
3	Saponins	++	+
4	Tannins	+++	++
5	Flavonoids	++	+
6	Glycosides	-	-
7	Steroids	+	-
8	Terpenoids	++	+
9	Anthraquinones	-	-

Present; **+;** Absent **-** .

Quantitative phytochemical constituents of plant extracts

Total phenol (1388.40 ± 46.47^{h} mg/g) and Flavonoids (810.10 ± 29.94^{g} mg/g) were the most abundant in the mahogany extracts, while the least concentration of phytochemical was steroids (0.26 ± 1.23^{ab} mg/g) and glycoside found in mango extract (0.16 ± 0.53^{a} mg/g), and (0.16 ± 0.53^{a} mg/g) in Mahogany extract. Other phytochemicals were found to be significantly present in the plant methanol extracts (Table 2).

Table 4.3: Phytochemical composition of methanolic extract of plant samples.

S/N	Phytochemicals	Mahogany Extract	Mango Extract
1	Alkaloids	9.66 ± 0.20 [b]	165.17 ± 8.10 [f]
2	Total phenols	75.23 ± 0.22 [f]	1388.40 ± 46.47 [h]
3	Saponins	8.48 ± 0.13 [b]	4.02 ± 0.67 [c]
4	Tannins	42.10 ± 0.20 [d]	22.72 ± 1.05 [d]
5	Flavonoids	68.6 ± 0.20 [e]	810.10 ± 29.94 [g]
6	Glycosides	0.16±0.53 [a]	0.92±66.1 [a]
7	Steroids	0.26±1.23 [ab]	3.21 ± 0.41 [bc]
8	Terpenoids	11.24 ± 0.10 [c]	96.33 ± 9.81 [e]
9	Anthraquinones	0.84 ± 0.11 [ab]	0.26±0.35 [a]

Values are in ± mean S.E. (*S.E = Standard error of Mean*).Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5 % level (P < 0.05).

Morphological identification of *Pythium myriotylum*.

All cultures exhibited rapid growth on PDA, developing cotton-like colonies after 7 days of incubation at 25 °C. Microscopic examination revealed hyaline, aseptate hyphae measuring up to 8 µm in width. Finger-shaped appressoria were frequently present either singly or in clusters in aqueous cultures. The sporangia appeared filamentous and swollen, reaching widths of up to 12 µm. All isolates were homothallic, producing abundant sexual structures on both agar and water media. Among them, *Pythium myriotylum* generated the smallest sexual structures, averaging 30.01 µm in diameter. According to conventional definitions, the predominantly observed oospores, averaging 26.06 µm in diameter, were mostly aplerotic, although occasional plerotic oospores were also noted.

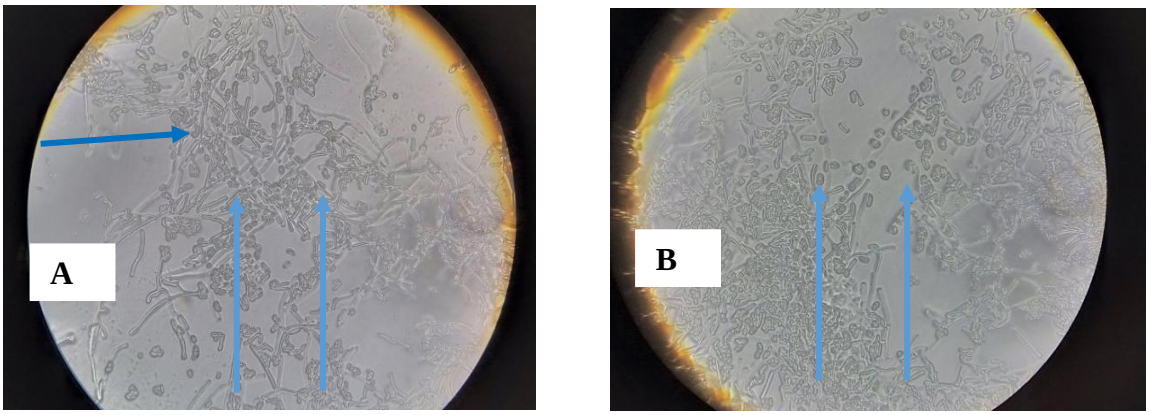

Plate I: *Macro and micro identification of the hyphae and sporangia of Pythium myriotylum.* a. Clustered appressoria under microscope b. Filamentous sporangia under microscope.

In vitro Antifungal Activities of Leave Extracts

The effect of plant extracts on radial mycelia growth of *Pythium myriotylum* (Table 3 – 4) on PDA containing the methanolic extract of Mango and Mahogany leaves which was determined using meter rule. The results shows that after day 3, the 500 mg/ml of the methanolic extracts of mahogany shows the highest radial growth of *Pythium* (1.86 cm) as compared with other concentration of the extracts and with the control groups (1.88 cm). From the table after day 7, 1000 mg/ml of the plant extract still shows a little zone of radial inhibition in growth (3.99 cm) as compared with the control (4.00 cm) as shown table 3. For mango (500 mg/ml) leaves shows the highest radial growth of Pythium (1.74 mm) as compared with other concentration of the extracts and the control groups (1.88 mm). From the table after day 7, 1000 mg/ml of the plant extract still shows a little zone of radial inhibition in growth (3.55 mm) as compared with the control (4.00 mm) (table 4).

Table 3: Mahogany extract Concentration (mg/ml)

Days	500	800	1000	Control
Day 1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Day 2	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Day 3	1.86±0.005[b]	1.63±0.010[b]	1.53±0.005[b]	1.88±0.015[b]
Day 4	2.84±0.015[c]	2.79±0.010[c]	2.64±0.015[c]	2.90±0.010[c]
Day 5	3.86±0.010[d]	3.77±0.010[d]	3.55±0.010[d]	3.94±0.005[d]
Day 6	3.97±0.015[e]	3.95±0.010[e]	3.87±0.005[e]	3.99±0.015[e]
Day 7	4.0±0.020[f]	3.99±0.020[f]	3.99±0.010[f]	4.00±0.00[f]

Values are in ± mean S.E. (*S.E = Standard error of Mean*)Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5 % level ($P < 0.05$).

Table 4: Mango extract Concentration (mg/ml)

Days	500	800	1000	Control
Day 1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Day 2	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Day 3	1.74±0.005[b]	1.23±0.010[b]	0.94±0.005[b]	1.88±0.015[b]
Day 4	2.64±0.015[c]	2.07±0.010[c]	1.75±0.015[c]	2.90±0.010[c]
Day 5	3.56±0.010[d]	2.87±0.010[d]	2.14±0.010[d]	3.94±0.005[d]
Day 6	3.84±0.015[e]	3.03±0.010[e]	2.52±0.005[e]	3.99±0.015[e]
Day 7	4.0±0.020[f]	3.12±0.020[f]	2.75±0.010[f]	4.00±0.00[f]

Values are in ± mean S.E. (*S.E = Standard error of Mean*) Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5 % level ($P < 0.05$).

Discussion

Phytochemical contents of the three plant extracts

The phytochemical analysis of crude methanolic extracts from *Mangifera indica* L., *Azadirachta indica*, and *Khaya senegalensis* revealed the presence of several bioactive constituents. Prominently, alkaloids, saponins, tannins, phenols, and flavonoids were detected in significant quantities, whereas glycosides, steroids, terpenoids, and anthraquinones were present only in trace amounts. These results corroborate previous findings by Imam *et al*. (2019), who similarly reported abundant phytochemical content in *Azadirachta indica* and further suggested the potential biological activity of these extracts due to the abundance of key secondary metabolites.

Quantitative analysis of *Mangifera indica* (mango) leaf extract revealed that total phenols were present at the highest concentration (1388.40 mg/100g), while steroids were found at the lowest level (3.21 mg/100g), consistent with previous findings by Ntengna *et al.* (2019), who identified similar phytoconstituents in methanolic mango leaf extracts. The presence of these phytochemicals, particularly phenols, flavonoids, tannins, saponins, and alkaloids, likely contributes to the observed antifungal activity against *Pythium myriotylum*. These secondary metabolites are recognized for their biological activities and enhance the potential medicinal and bioremediation applications of the plant extract. Specifically, tannins and saponins have documented antifungal properties, while flavonoids and alkaloids may aid in fungal inhibition and potentially support plant tissue repair following fungal damage (Imam *et al.,* 2019). Collectively, these findings highlight the potential of mango leaf extract as a bioactive agent for managing fungal infections and promoting plant health.

In vitro antifungal activities of the three plant extracts

Radial growth of *Pythium myriotylum* assay using *Mangifera indica* L, *Azadirachta indica* and *Khaya senegalensis* methanolic plant extracts from this study reveal that every extract tested decreased mycelial growth of *Pythium myriotylum* by some degree this findings is in agreements with Smith *et al*. (2019) who both reported similar findings and attributes changes to the differing abilities of fungal mycelia to absorb plant extracts. This results also prove the efficacy of the extracts against test fungus

this corroborates with Gnannasekaran *et al*. (2015) who highlighted that the Resistance of fungi growth to methanolic plant extract concentrations.

Conclusion

This study demonstrates the efficacy among different plant extracts in protecting seedlings from infection by *Pythium myriotylum*. Mango leaf extract exhibited the strongest antifungal activity against damping-off disease caused by this pathogen. The study further shows that plant extracts can affect soil microflora, potentially altering the microbial balance essential for soil fertility and crop productivity. While the phytochemicals in these extracts negatively affect certain beneficial soil fungi involved in organic matter decomposition and nutrient cycling, their inhibitory effect on pathogenic *Pythium myriotylum* is significant. Overall, treating soil with methanolic extracts, particularly from mango leaves, shows promise as a natural strategy to control *Pythium* infections in areas where this fungal disease is prevalent.

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