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Research Paper

In-vitro effect of some plant extracts and synthetic fungicide in the control of cocoyam leaf necrotic fungi in Aguata LGA, Anambra State

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Abstract. Cocoyam leaves of two tropical crops, taro (old cocoyam, *Colocasia esculenta* (L.) Schott) and blue or purple taro (new cocoyam tanier, *Xanthosoma sagittifolium* (L.) Schott) obtained from the farmers' field in two villages in Ekwulobia of Aguata local government area of Anambra State. The villages included Ula and Okpo. Cocoyam leaves and petioles showing small dark-brown or black lesions were collected for isolation of the causal pathogen. The organism recovered from the infected cocoyam leaves was *Aspergillus niger*. This organism may have had access into these cocoyam leaves through wounds created by working equipment (for harvesting) and pests. The antifungal effects of ethanol, methanol and petroleum ether extracts of lemon grass leaves and stalks (*Cymbopogon citratus*) and fruits of pepper fruit *(Dennettia tripetala*) on the growth of *Aspergillus niger* were investigated *in-vitro* at concentrations of 1, 1.5, and 2 g/ml, while the synthetic fungicide (Apron plus) was used as a standard control. The data collected were analyzed using ANOVA. The experimental design used was split – split – plots in completely randomized design with three replications and means were separated using least significant difference (LSD) at 5% probability level. All plant extracts and Apron plus inhibited the fungus. Apron plus caused the highest level of inhibition (75%) followed by lemon grass (49.04%) while the least was pepper fruit (39.48%). The study also showed that the higher the concentration, the higher the inhibition with 2g/ml having the highest inhibition effect (72.58%) for synthetic fungicide The extract of lemon grass (*Cymbopogon citratus*) had a higher percentage inhibition value in the days of the culture, while Soxhlet extraction using Pet ether as the extracting solvent had the highest inhibition level (53.03%). Thus, the plant extracts used in this research could be suggested as an alternative to synthetic fungicides. More studies should be carried out on these plant extracts to identify their active ingredients to facilitate their commercial production and availability to farmers as these extracts are expected to be biodegradable and may reduce the rate of application of synthetic fungicides which are detrimental to human health and are more persist in the environment.

Keywords: *In-vitro* experiments, plant extracts, synthetic fungicide, cocoyam, fungi, Anambra State.

INTRODUCTION

Cocoyams are herbaceous perennial plants belonging to the family Araceae and are grown primarily for their edible roots, although all parts of the plant are edible. Cocoyams that are cultivated as food crops belong to either the genus *Colocasia* or the genus *Xanthosoma* and are generally comprised of a large spherical corm (swollen underground storage stem), from which a few large leaves emerge. The petioles of the leaves stand erect and can reach lengths of 1 m (3.3 ft). *Colocasia esculenta* (L.) Schott (taro, elephant ear or cocoyam) is an emergent perennial aquatic and semi-aquatic herbaceous species of the Araceae family native to Asia. Nowadays, C*. esculenta* is considered the fifth most consumed root worldwide. It is also used as an ornamental

plant.

Cocoyam grows best in fertile, well-drained, sandy loam soil with a pH 4.2 to 7.5. It can be grown in a wide variety of conditions including paddies in wetland areas using a system similar to that of rice. *Xanthosoma* sp. (blue taro, purple taro, or new cocoyam tanier) requires temperature above 21°C (69.8°F) to grow properly. Unlike *Colocasia* sp., it will not tolerate waterlogging and grow best in deep, well-drained loams with a pH between 5.5 and 6.5 in partial shade. Cocoyam will thrive when planted in full sunlight or partial shade. The plants can survive for short periods at temperature of 10°C (50°F) but will be damaged or killed by lower temperatures (Wilson, 1987).

Diseases of cocoyam include Phytophthora leaf blight caused by *Phytophthora colocasiae*, Phyllosticta leaf spot caused by *Phyllosticta colocasiophila*. Phyllosticta spots general1y resemble those caused by *P. colocasiae* except for the absence of sporangia produced on *P. colocasiae* lesions. Also, Cladosporium leaf spot is caused by *Cladosporium colocasiae*. *C. colocasiae* causes a relatively innocuous disease common on dryland taro (Parris, 1941). The disease attacks both wetland and upland taro and occurs mainly on the older leaves. The disease results in loss of leaves which eventually reduces yield. All over the world, current agricultural practices are moving away from the use of synthetic chemicals due to their adverse effects on ecosystem. The adoption of practices and use of plant materials that are environmental friendly constitutes a vital component of sustainable agriculture. Nigeria is endowed with abundant plant materials, which may be employed to fight plant pests and diseases; this approach has recently been the pre-occupation of many crop protectionists in Nigeria. Shukla *et al.* (2012) reported that numerous *in-vitro* studies have validated the efficacy of plant-derived pesticides in many branches of agriculture. These investigators however inferred that plant materials were fungitoxic and prophylactic against rots organisms (Dwivedi and Shukla, 2000).

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as menstruum (Amita and Shalini, 2013).

Synthetic fungicides have an established reputation in agriculture. Their uses have been credited with enhancing yield of agricultural crops and increasing per capital returns on farm investments (Saifullah and Masahiro, 2013).

MATERIALS AND METHODS

The study was carried out at the laboratory of the Department of Crop Science and Horticulture, Faculty of Agriculture as well as its screen house. Plant extraction was carried out at the Biotechnology Research Institute, Nnamdi Azikiwe University Awka, Anambra State.

Infected leaves of two varieties of Cocoyam (*Colocasia* sp.) were obtained by random sampling in farmers' field from two villages in Ekwulobia of Aguata local government area in Anambra State, namely: Ula and Okpo. The two varieties selected from the two villages include: *Colocasia esculenta* and *Xanthosoma sagittifolium*. The botanicals (lemon grass and pepper fruit) used were gotten from Eke Awka market in Awka south local government area, Anambra State. Systemic fungicide used in this study was Apron plus served as standard control.

Preparation of potato dextrose agar (PDA) medium

Twenty grams of PDA powder (Lab M Limited 1Quest Park, Moss Road, Heywood, Lancashire BL9 JJ, United Kingdom) was dissolved in 500 ml distilled water conical flask which was then corked with cotton wool which was wrapped in foil. The PDA was then autoclaved at a temperature of 120°C, pressure of 15 ± 1 psi, for 20 to 25 min.

Isolation of the causal organism from infected cocoyam leaves

Infected cocoyam leaves were washed in tap water, and cut into sections with sterilized scalpel. They were surface sterilized in 10% ethanol for about two minutes and then rinsed twice in sterile distilled water (SDW). Ten pieces of the surface-sterilized leaves were plated on Petri dishes containing 20 ml of PDA supplemented with 2 drops of lactic acid to prevent bacterial growth. The plates were incubated at room temperature (28 ± 2°C) for 7 days and observed daily for fungal development. When fungal growth has established, subcultures were prepared using inoculum from the different organisms in the mixed cultures to obtain mono cultures. Pure culture was obtained by transferring hyphal tips from the colony edge of the mixed cultures to fresh plates of PDA using flame sterilized inoculating needle. The plates were incubated at 27°C. This was done three times until a pure culture was obtained. The pure culture plates of the test fungi were then sealed with masking tape to prevent contamination.

Microscopic identification of recovered fungi

The resulting pure cultures were identified with the aid of a compound microscope. Temporary slides were prepared by placing one drop of water on the surface of the slide and a sample from the interface of continuous growth of the culture was released in it with the aid of a sterile needle) and then covered with a cover slip (Sulton, 1980). The fungi were identified using illustrated pictures of fungi by Barnett and Hunter (1999). Pure cultures were stored in refrigerators for further use. Micrographs of the identified pathogen were taken.

Preparation of plant extracts

Fruits of pepper fruit (*Dennettia tripetala*) and leaves and stalks of lemon grass (*Cymbopogon citratus*) collected from Eke Awka market in Awka south local government area, Anambra State were used for extractions. Plant materials were washed, air dried, and then oven dried at 110°C for 30 min using a laboratory oven. Oven-dried materials were grinded using Qlink electric blender into powder (130 g) for use in the study.

Extraction with ethanol and methanol

Using cold solvent extraction method (Junaid *et al*., 2006), 50g of each plant material (pepper fruit and lemon grass) was soaked in 500 ml of ethanol and methanol, separately in white plastic containers for 3 days with vigorous shaking by hand at intervals during this period. The extracts where then filtered with white cheese cloth first and then filtered again with the use of a buckner funnel. Then ethanol and methanol were separated from the plant extract by the use of a rotary evaporator.

Continuous extraction (Soxhlet)

Thirty gram of each plant material (lemon grass and pepper fruit) was placed into the thimble. Paper thimble was used in the soxhlet apparatus using petroleum ether as the extracting solvent. After extraction the solvent was evaporated from the plant extract using a water bath at 60°C.

Effect of plant extracts on fungal growth

Effect of plant extract on mycelia growth of the isolated fungi was studied using the food poisoning technique (Sangoyomi, 2004). The plant extracts were dissolved using dimethyl sulfoxide, one milliliter of each plant extract at concentration (1, 1.5 and 2 g/ml) was dispensed per petri dishes and 15 ml of the medium (molten PDA) was added to each of the petri dishes containing extract and carefully spread evenly over the plate, this gave rise to PDA–extract mixture. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the center with a 4-mm-diameter disk of 7-day-old pure cultures of the test fungus using a cork borer. Three replicates (Petri plates) were made for each treatment. PDA plates with no extract served as the negative control. All plates were inoculated with the test fungus as described above. Petri-dishes of PDA supplemented with one ml Apron plus (commercial fungicide) at 1, 1.5 and 2 g/ml concentrations were also inoculated with the test fungus and served as the positive control. All plates were then incubated at 28 ± 2°C for 4 days and examined daily for fungal growth. Colony diameter was measured and the effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Whipps (1987), as follows:

$$\text{Percentage inhibition} = \frac{Do - Dt}{Do} \times 100$$

Where: Do is the mean colony diameter of pathogen in control plates while Dt is the mean colony diameter of pathogen in extract-incorporated agar plates.

Experimental design

The experimental design used was split-split-plot design laid in a complete randomized design (CRD). The data collected were subjected to analysis of variance (ANOVA) and means were separated using least significant difference (LSD) at 0.05 probability level. The Genstat release 7.2 version was used for all the statistical analysis. The major factor used in this work is the plant extract, the sub factor is the different concentration levels and the sub-sub factor is the method of extraction (solvent used in extraction).

RESULTS

Necrotic fungi isolated from cocoyam leaves in Ekwulobia, Anambra State

Fungal organism isolated was identified as *Aspergillus niger* (Figure 1) based on the growth characteristics and fruiting bodies and confirmed using illustrated pictures of fungi by Barnett and Hunter (1999).

Effect of plant extracts, synthetic fungicide, concentration and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture

Table 1 shows that there was significant effect (P = 0.05)

Figure 1. Pure culture of *Aspergillus niger* isolated from cocoyam leaves on potato dextrose agar medium.

Table 1. Effect of plant extracts, synthetic fungicide, concentration and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture.

Treatment	Incubation period (days), growth inhibition (%)			
	1	2	3	4
Lemon grass (LG)	49.04	38.27	32.32	22.87
Pepper fruit (PF)	39.48	32.30	27.18	21.79
Apron	75.00	75.00	72.62	72.92
$LSD_{0.05}$	7.11	1.59	5.41	5.07
Conc.				
0.0	0.00	0.00	0.00	0.00
1.0	56.47	45.49	39.27	32.19
1.5	65.56	55.42	46.95	38.57
2.0	72.58	62.93	57.27	47.48
$LSD_{0.05}$	6.00	4.45	4.01	2.90
Extraction method (EM)				
CME	45.12	37.52	32.78	22.61
CMM	47.80	38.41	32.96	28.03
SPE	53.03	46.94	41.87	38.04
$LSD_{0.05}$	5.19	3.44	2.99	2.28

Key: CME= cold maceration with ethanol, CMM =cold maceration with methanol, SPE= soxhlet extraction with pet ether, PE = plant extract, CONC = concentration, EM = extraction method.

Table 2. Interaction effect of plant extract, synthetic fungicide and concentration levels on percentage inhibition of the radial growth of *Aspergillus niger* in culture.

Treatment	Incubation period (days), concentration (g/ml) and growth inhibition (%)															
	1				2				3				4			
	0.0	1.0	1.5	2.0	0.0	1.0	1.5	2.0	0.0	1.0	1.5	2.0	0.0	1.0	1.5	2.0
LG	0.00	55.91	67.26	72.98	0.00	43.26	52.08	57.76	0.00	33.66	43.80	51.80	0.00	21.99	30.75	38.74
PF	0.00	42.51	52.37	63.04	0.00	29.56	43.89	55.74	0.00	24.63	35.58	48.49	0.00	19.77	28.69	38.71
AP	0.00	100.00	100.00	100.00	0.00	100.00	100.00	100.00	0.00	100.00	90.48	100.00	0.00	100.00	91.67	100.00
$LSD_{0.05}$			12.29				8.39				8.45				6.62	

Key: CONC = concentration, LG = Lemon grass, PF = pepper fruit, AP= apron plus, PE = plant extracts.

of plant extracts and synthetic fungicide on percentage growth inhibition of *A. niger* in culture, the synthetic fungicide (Apron) had the highest percentage growth inhibition (75.00) than the plant extracts, which was significantly higher (P = 0.05) than Lemon grass (*Cymbopogon citratus*) (49.04%) and Pepper fruit *Dennettia tripetala* (39.48%). This was followed by lemon grass (49.04%) which was significantly (P = 0.05) higher than pepper fruit (39.48%). This trend also occurred in all the days of the culture, where synthetic fungicide (Apron) did significantly (P = 0.05) better than the plant extracts.

Table 1 also shows that there was significant (P = 0.05) difference in the effects of the various concentration levels. The result shows that the higher the concentration, the higher the value of inhibition percentage, this trend occurred in all the days of the culture. The concentration of 2.0g/ml had the highest inhibition value (72.58%) for day 1, 62.95% for day 2, 57.27% for day 3 and 47.48% for day 4. This was followed by concentration level 1.5 g/ml which consecutively produced higher inhibition than 1.0 g/ml in all the days of the culture. Concentration of 1.0 g/ml had the least inhibition values in the days in culture but all the concentrations performed better than the control.

Table 1 also shows that there was significant difference (P = 0.05) in the effect of extraction method on the inhibition of *Aspergillus* sp. in culture. Soxhlet extraction with pet ether consistently gave significantly higher (P=0.05) growth inhibition values than the rest of the extraction methods in the days of the culture ((53.03%), (46.94%), (41.87%), (38.04%)) in days 1 to 4, respectively.

This was followed by cold maceration with methanol which also consistently produced higher growth inhibition values than cold maceration with ethanol. There was no significant difference (P = 0.05) between cold maceration with methanol and cold maceration with ethanol in days 1, 2, 3 except in day 4 in culture where cold maceration with methanol (28.03%) significantly (P = 0.05) differed from 22.61% in cold maceration with ethanol. It was also observed that the effects of extraction methods was reducing with time in culture, the inhibition kept on reducing from day 1 till the last day in culture.

Interaction effect of plant extract, synthetic fungicide and concentration levels on percentage inhibition of the radial growth of *Aspergillus niger* in culture

Table 2 shows that there was significant effect (P = 0.05) between interaction of plant extracts and concentration levels on the percentage growth inhibition of *A. niger* in culture. The synthetic fungicide (apron plus) had significantly higher (0.05) percentage growth inhibition (100.00) at the different concentration levels than the plant extracts in the days of the culture.

Lemon grass (*Cymbopogon citratus*) ×2.0g/ml was also consistently proved higher (72.98) percentage growth inhibition followed by lemon grass×1.5g/ml which had a higher percentage growth inhibition (67.26%) than lemon grass×1.0g/ml which had the least percentage growth inhibition (55.91%) in day 1, this trend also occurred in all the days of the culture with inhibition values 57.76% at day 2, 51.80% at day 3, 38.74% at day 4 for Lemon grass ×2.0 g/ml.
Pepper fruit ×2.0/ml consistently gave significantly (P = 0.05) higher growth inhibition values than the other concentration levels with inhibition values of ((63.04%), (55.74%), (48.49%), (38.71%)) in days 1 to 4, respectively. This was followed by pepper fruit ×1.5 g/ml concentration in day 1 this trend also continued in day 2, 3 and 4 with inhibition values 55.74% at day 2, 35.58% at day 3, 28.69% at day 4, with pepper fruit ×1.0g/ml concentration level (42.51%), had the least percentage growth inhibition. the result also showed that all the plant extracts at the different concentration levels did

Table 3. Interaction effect of between plant extract and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture.

Treatment	Incubation period (days) and growth inhibition (%)											
	1			2			3			4		
Extraction method	CME	CMM	SPE	CME	CMM	SPE	CME	CMM	SPE	CME	CMM	SPE
LG	54.79	50.39	41.92	36.33	36.33	36.18	35.50	30.41	31.04	16.48	24.29	27.84
PF	26.67	36.42	55.36	29.39	29.15	46.36	17.81	23.26	40.46	14.27	17.31	33.79
$LSD_{0.05}$		7.07			2.57			5.29			4.96	

Key: CME = cold maceration with ethanol, CMM = cold maceration with methanol, SPE = Soxhlet extraction with pet ether, LG = Lemon grass, PF = Pepper fruit.

Table 4. Interaction effect of concentration and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture.

Conc. (g/ml)	Incubation period (days), Extraction method and growth inhibition (%)											
	1			2			3			4		
	CME	CMM	SPE	CME	CMM	SPE	CME	CMM	SPE	CME	CMM	SPE
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.0	51.01	55.03	63.36	39.54	42.65	54.29	35.65	35.28	46.87	22.03	30.41	44.12
1.5	62.85	65.04	68.81	52.77	51.39	62.09	45.19	41.63	54.02	31.23	34.75	49.73
2.0	66.65	71.12	79.97	57.78	59.61	71.40	50.28	54.93	66.60	37.16	46.95	58.32
$LSD_{0.05}$		NS			7.45			6.55			4.91	

Key: CME = cold maceration with ethanol, CMM = cold maceration with methanol, SPE = soxhlet extraction with pet ether, CONC = concentration, EM = extraction method.

better than control.

Interaction effect of plant extract and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture

Table 3 shows the interaction between plant extracts and extraction methods which also had a significant (0.05) effect on percentage growth inhibition on *A. niger* in culture. Lemon grass×cold maceration with ethanol (LG×CME) had a higher percentage growth inhibition (54.79%) than pepper fruit × cold maceration with ethanol (PF×CME) in day 1. This trend was consistent for all the days of the culture.

Lemon grass × cold maceration with methanol (LG×CMM) had a higher percentage growth inhibition (50.39%) than pepper fruit × cold maceration with methanol (PF×CMM) in day 1 (36.42%), this was also consistent for all the days in the culture with inhibition values LG×CMM (36.33% for day 2, 30.41% for day 3, 24.29% for day 4) and PF×CMM (29.15% for day 2, 23.26% for day 3, 17.31% for day 4). Pepper fruit×soxhlet pet ether (PF×SPE) had significantly higher growth inhibition value (55.36%) than lemon grass×soxhlet pet ether (LG×SPE) (41.92%) in day 1, this trend remained consistent for day 2 (46.36%), day 3 (40.46%) and 4 (33.79%).

Pepper fruit×soxhlet pet ether (PF×SPE) had the highest inhibition value followed by Lemon grass×cold maceration with ethanol (LG×CME) while the least was pepper fruit× cold maceration with methanol (PF×CMM).

Interaction effect of concentration and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture

Table 4 shows the effect that the interaction between concentration and extraction methods had significant (P=0.05) effect on the percentage growth inhibition of *Aspergillus niger* in culture. There was no significant difference in interaction

Table 5. Interaction effect of plants extracts, concentration and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture.

Treatment	Incubation period (days), Concentration (g/ml) and growth inhibition (%)												
		1			2			3			4		
Extraction method		CME	CMM	SPE	CME	CMM	SPE	CME	CMM	SPE	CME	CMM	SPE
LG	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	60.83	58.89	48.01	45.19	41.73	42.85	38.59	31.68	30.71	12.89	24.89	28.29
	1.5	80.83	69.26	51.68	58.52	51.49	46.23	49.13	42.81	39.47	24.11	32.56	35.59
	2.0	77.50	73.43	68.00	65.56	52.09	55.64	54.27	18.63	53.98	28.94	39.73	47.56
PF	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	26.67	36.67	64.20	15.73	25.40	47.56	12.47	18.63	42.79	8.56	13.33	37.42
	1.5	33.33	49.33	74.45	32.17	36.43	63.08	26.74	25.64	54.07	20.65	19.25	46.17
	2.0	46.67	56.67	82.79	37.65	54.76	74.81	32.05	48.46	64.97	27.88	36.67	51.57
Apron plus		100.00	100.00	100.00	100.00	100.00	100.00	100.00	90.48	100.00	100.00	91.67	100.00
$LSD_{0.05}$			13.85			9.38			9.20			7.17	

Key: CME = cold maceration with ethanol, CMM = cold maceration with methanol, SPE = soxhlet extraction with pet ether, CONC = concentration, EM = extraction method, LG = Lemon grass, PF = pepper fruit.

between concentration and extraction methods in day 1 of the culture. Soxhlet extraction with pet ether×2.0 g/ml consistently gave significantly (P=0.05) higher growth inhibition values than the rest of the extraction methods with the inhibition values ((71.40%), (66.60%), (58.32%)) in days 2 to 4, respectively. This was followed by cold maceration with methanol×2.0g/ml concentration which also consistently produced higher growth inhibition values than cold maceration with ethanol×2.0 g/ml inhibition values ((59.61%), (54.93%), (46.95%)) in days 2 to 4, respectively.

Soxhlet extraction with pet ether(SPE)×1.5 g/ml and SPE×1.0g/ml concentration also showed higher percentage growth inhibition than cold maceration with methanol and cold maceration with ethanol at these levels of concentration (1.0 and 1.5 g/ml) in days 2 to 4.

This was followed by cold maceration with methanol×1.0 g/ml concentration which consistently produced higher growth inhibition values than cold maceration with ethanol×1.0g/ml concentration in days 2 (42.65%) and 4 (30.41%). All the extraction methods at the different concentration levels also produced significantly (P = 0.05) higher percentage growth inhibition values than the control (0 g/ml) which showed no growth inhibition.

It was also observed that the effects of concentration by extraction methods reduced with time in the culture with day 4 having the lowest percentage growth inhibition value.

Interaction effect of plants extracts, concentration and extraction method on percentage inhibition of the radial growth of *Aspergillus niger* in culture

Table 5 shows that there was significant (P = 0.05) effect in interaction between plant extract, concentration and extraction method has on percentage growth inhibition of *Aspergillus niger* in culture. The synthetic fungicide (apron plus) had significantly higher (P=0.05) percentage growth inhibition (100.00) at the different concentration levels than the plant extracts in the days of the culture.

Lemon grass (LG)×cold maceration with methanol (CME)×1.5 g/ml concentration gave the highest growth inhibition value than the rest of the extraction methods at the different levels of concentration(80.83%), this was followed by LG×CME×2.0 g/ml this trend continued in days 2 (65.56%) and 3 (54.27%) but in day 4 LG×soxhlet extraction with pet ether (SPE)×2.0 g/ml gave significantly (P=0.05) higher growth inhibition value (47.56%) than the rest of the extraction method at the different levels of concentration with this plant extract (lemon grass).

Pepper fruit (PF)×SPE×2.0g/ml consistently gave significantly (0.05) higher growth inhibition values than the rest of the extraction methods at the different concentration levels with the use of this plant extract (pepper fruit) in all the days of the culture ((82.79%), (74.81%), (64.97%), (51.57%)). This was followed by PF×CMM×1.5 g/ml and PF×cold maceration with methanol (CMM)×1.0 g/ml which also consistently produced higher growth

Figure 2. Micrograph of *Aspergillus niger* (X) isolated from cocoyam leaves.

inhibition values than PF×CMM×1.5 g/ml and PF×CMM×1.0 g/ml in days 1and 2, in days 3and 4 in the culture where there was no significant difference between PF×CME×1.5 g/ml and PF×CMM×1.5 g/ml.

All the extraction methods at the different concentration levels with the different plant extracts also produced significantly (P=0.05) higher percentage growth inhibition values than the control which showed no growth inhibition.

It was also observed that the effects of plant extracts by concentration by extraction methods was reducing with time in the culture with day 4 having the lowest percentage growth inhibition value in all the days of the culture.

DISCUSSION

Isolation of fungal pathogen

The fungal organism isolated from cocoyam leaves obtained from the local farmer's field from two villages namely: Ula and Okpo in Ekwulobia local government area in Anambra State, was identified as *Aspergillus niger* (Figure 2) which is in line with works by Aidoo, 2015, it causes at first small dark brown or black lesions on the leaves, petioles. The lesion is often surrounded by chlorotic halo enlarged and coalesced, resulting in extensive necrosis of the leaves (Amusa, 1997). After which the affected leaves dried up and died.

Effect of plant extracts, synthetic fungicide, concentration and extraction method and their interactions on percentage inhibition on the radial growth of *Aspergillus niger* in culture

The results of effects of plant extracts, synthetic fungicide (Apron plus), concentration and extraction method and their interactions on percentage inhibition on the radial growth of *Aspergillus niger* isolated from two varieties of *Colocasia esculenta*, showed that synthetic fungicide (Apron plus) had the highest percentage growth inhibition effect which was significantly (0.05) higher than *C. citratus* and *D. tripetala.* This is in agreement with the findings of Ibiam *et al.* (2000), who stated that seed dressings with Apron plus controlled seed-borne fungi of rice.

The plant extract *C. citratus* gave significantly (P=0.05) higher percentage growth inhibition than *D. tripetala* this is in agreement with Baratta *et al.* (1998) who reported that *C. citratus* gave 91% inhibition of the growth of *A. niger* in liquid culture media. Okwu and Morah (2007) also reported that, the fruit extract of *D. tripetala* inhibited *A. niger.*

The result of this research showed that the antifungal activity of the phytochemicals increase with increase in concentration where 2.0 g/ml had the highest percentage growth inhibition value in day 1, this trend occurred in all the days of the culture. This is in agreement with Derbaiah *et al*. (2011), who reported in their experiment that the efficacy and safety of some plant extracts against

tomato early blight disease caused by *Alternaria solani,* that increasing concentration of the botanic extract led to increase in toxicity of the active bio-compounds.

The result of this research showed that Soxhlet extraction with pet ether consistently gave significantly higher (P=0.05) growth inhibition values than the rest of the extraction methods in the days of the culture. This is in agreement with Sukhdev *et al*. (2008), who reported that Soxhlet advantageous, when compared to cold maceration with methanol and ethanol, as large amounts of plant extract can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs.

CONCLUSION

The plant extracts of *C. citratus* was more effective in inhibiting the radial growth of *A. niger in vitro* than *D. tripetala.* The phytochemicals likely to be responsible for the fungitoxicity are alkaloids, flavonoids, glycosides, saponins and tannins. Concentration level of 2.0 g/ml had the highest percentage growth inhibition value in the days of the culture. Soxhlet extraction with Pet ether gave the highest inhibition values than the rest of the extraction methods in the days of the culture. From this research it was discovered that the major organisms isolated were fungi and therefore, it has consequently been implicated as the major cause of necrosis of cocoyam leaves in the area studied.

RECOMMENDATIONS

Based on the present results the plant extracts could be suggested as an alternative to synthetic fungicides. More studies should be carried out on these plant extracts to study their bio-compounds or the phytochemicals present to be able to commercialize their production and make them available for farmers as these extracts are biodegradable and also to reduce the rate of application of synthetic fungicides which are detrimental to human health and the environment as they persist in the environment.

More research is needed to determine standard extractants for extracting the active properties from the plants either based on family or genera of plants or based on the type of compound(s) being sought.

Biotechnology and genetic engineering technologies should be strongly emphasized and adopted in the study of these fungitoxic phyto-metabolites to explore and exploit possibilities of encoding rapidly growing weeds with the genetic ability to produce these metabolites.

REFERENCES

Aidoo KA (20157). Improving Pit Storage Systems to Reduce Rots of White yam (*Dioscorea rotundata*) In Ghana. Sci. J. Agric. Res. Manage. pp. 1-7.

Amita P, Shalini T (2013). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J. Pharmacogn. Phytochem. 2(5):115-119.

Amusa NA (1997). Fungi associated with anthracnose symptoms of yam in south-west Nigeria and their roles. Crop Res. 13:177-183.

Baratta MT, Dorman HJD, Deans SD, Figueieredo AC, Barroso JG, Ruberto G (1998). Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and Fragrance J. 13:235-244.

Barnett HL, Hunter BB (1999). Illustrated Genera of Imperfect Fungi (4th Edition). The *American Phytopathological Society* St. Paul, Minnesota. p. 218.

Derbaiah AS, El-Mahrouk MS, El-Sayed AO (2011). Efficacy and safety of some plant extracts against tomato early blight disease caused by *Alternaria solani*. Plant Pathol. J. 10:115-121.

Dwivedi BP, Shukla DN (2000). Effect of leaf extract some medicinal plants on spore germination of some fusarium species, Karmataka. J. Agric. Sci. 13(1):153-154.

Ibiam OF, Umechuruba CI, Arinze AE (2000). Field Evaluation of Seed-Dressing Fungicides, Bavistin, Benlate Fernasan-D and Apron Plus 50 DS associated with three rice varieties Faro 12, Faro 15, and Faro 29. J. Health Visual Sci. 2:96-106.

Junaid SA, Olabode AO, Owuliri FC, Okworiu AEJ, Agina SE (2009). The antimicrobial properties of *Occimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. Afr. J. Biotechonol. 5(22):2315-2321.

Okwu DE, Morah FNI (2007). Antimicrobial and phytochemical evaluation of seed of *Gracinia kola* and *Dennettia tripetala* fruits. J. Med. Aromat. Plants 29:20-25.

Parris GK (1941). Diseases of taro in Hawaii and their control; with notes on field production. Honolulu (HI): University of Hawaii, 18:29.

Saifullah S, Masahiro M (2013). Promoting investment in agriculture for increased production and productivity. Japan Trust Fund project "Support to study on appropriate policy measures to increase investment in agriculture and to stimulate food production'', pp. 1-104.

Sangoyomi TE (2004). Post-harvest fungal deterioration of yam (*Dioscorea rotundata Poir*) and its control. PhD Thesis IITA, Ibadan Nigeria. pp. I79.

Sukhdev SH, Suman PSK, Gennaro L, Dev DR (2008). Extraction technologies for medicinal and aromatic plants, Int. Centre Sci. high technol. p. 260.

Sulton BC (1980). The coelomycetes fungi Imperfecti with *Pycinidia acervuli* and stromata. Common wealth mycological institute Kew, surrey, England. p. 696.

Whipps JM (1987). Effects of media on growth and interactions between a range of soil borne glass pathogens and antagonistic fungi. New Pathol. 107:127-142.

Wilson JW (1987). Taro planting material. USP Institute for Research, Extension and Training in Agriculture (IRETA).