Direct Research Journal of Biology and Biotechnology
Vol. 7, Pp. 28-36, 2021
ISSN 2734-2158
DOI: https://doi.org/10.26765/DRJBB092186501
Article Number: DRJBB092186501

http://directresearchpublisher.org/drjbb

Full Length Research Paper

Varietal response and toxicity of aqueous leaf extracts of *Azadirachta Indica* to *Phytophtora Colocasiae* causing Taro leaf blight in Unwana Southeast, Nigeria

Lwanga Azubuike Chukwu[1] and David Nwazuo Enyiukwu[2]*

[1]Department of Agricultural Technology, Faculty of Agriculture, Akanu Ibiam Federal Polytechnic, Uwana, Afikpo, Ebonyi State, Nigeria.
[2]Department of Plant Health Management, Michael Okpara Universty of Agriculture, Umudike, KM 10 Umuahia-Ikot Ekpene Road, PMB 7267 Umuahia, Abia State, Nigeria.
Corresponding Author: enyidave2003@gmail.com

Received 16 May 2021; Accepted 30 May 2021; Published 20 June 2021

ABSTRACT: Taro is one of the major leafy vegetable and tuber staples in the southeast, Nigeria. Production of the crop is, however, seriously constrained by the challenge of taro leaf blight (TLB). Selection of appropriate variety and bio-pesticides are seen as major ways of ameliorating this impediment. This work evaluated the response of 4 varieties of taro to TLB, and effects of 5 levels of *Azadirachta indica* (0, 50, 100, 150, and 200 g/ha), and 200g/ha of *Ageritum conyzoides* and Mancozeb (as positive controls) on the disease initiation and development. The experiment was set up as 4 x 7 factorial experiments in RCBD with 3 replicates. The result showed that NCE-003 which had 56.15% and 2.28 and NCE-002 (60.16% and 2.84) for mean disease and severity scores respectively were the least susceptible varieties, while NCE-005 was the most susceptible to the disease with (82.63% and 7.03) for the respective parameters under consideration. About 50-200g/ha of aqueous extract of *A. indica* inhibited spore germination (77.09-96.02) and radial growth (58.24-68.37) of the fungus compared to (0.00%) obtained from the control experiment. Also these levels of the test extract significantly ($P<0.05$) minimized the mean incidence and severity of TLB from 82.63% and 7.08 on the control plants by (58.24-65.37%) and (1.84-2.61) respectively in the field. Minimization of the disease and crop tissue damage translated to improved mean taro yield by (5.83t-10.90/ha) and mean corm weight (115.50-147.20g) compared with 4.36 and 91.70% recorded on the untreated control experiment; and general performance (leaf area, number of leaves cormels) of the crop. Therefore, integrating the use of NCE-003 and NCE-002 with 150-200g/ha of *A. indica* could effectively improve taro corm and leaf yield for sustainable food sufficiency and security in sub-Saharan Africa.

Keywords: Taro, cocoyam, Taro leaf blight, *Phytophtora colocasiae, Azadirachta indica*, Neem, Mancozeb

INTRODUCTION

Taro (*Colocasia esculenta* L.) Schott (Areacea) an aroid otherwise called cocoyam in Nigeria (*ede* in Igbo) is a corm derived tuber. The crop has heart-shaped leaves borne on long soft petioles (Figure 1, top left and right). Its origin is suspected to be Southeast Asia (Shakywar *et al*., 2004; Pandukur and Amienyo, 2016). Taro is widely grown by smallholder farmers in many humid tropical locations including The Pacific, Asia and Africa. In Africa, taro is a common crop in the farming systems of Ghana, Cameroon and Nigeria (Omeje *et al*., 2015). The carbohydrate rich tuber (Figure 1, bottom right) has a pleasant nutty flavour and can be boiled, fried, roasted or baked while the edible leaves with rich presence of vitamins (riboflavin, thiamine and niacine) and minerals (P, Fe, Zn, K, Cu and Mn) feature prominently as leafy vegetable in many African soups and \porridges (Nielsen *et al*. 1997; Asraku, 2010; Singh *et al*. 2012; Enyiukwu *et al*. 2018). Many factors have been reported to constrain the production of taro including high urbanization rates, general regard of the crop as inferior and poor man's crop, abiotic influences as well as attacks from pest and disease especially fungal disease. Taro leaf blight (TLB) (Figure 1, bottom left) is considered a major fungal disease of the crop; being widespread in taro growing regions including the Pacific islands, Hawaii, Ghana, Cameroun and Nigeria; where in some places 90% of

Figure 1: ***Colocasia esculenta*** L. (Schott) (cocoyam, *ede* in Igbo) growing in the field.
Top left: Health cocoyam; **Top right**: Related species, **Bottom left:** TLB infected leaf; **Bottom right**: healthy cocoyam corms

surveyed households grow the crop. The disease is reportedly caused by diverse fungal agents (*Curvularia* species, *Cladosporium colocasia* and *Phytophytora colocasiae*) (Ooka, 1980; Asraku, 2010; Singh *et al*. 2012). But molecular analyses of the ITS-1 and ITS-4 regions of the gene of the pathogen obtained in Abia Nigeria among other places in Asia-Pacific upheld that *P. colocasiae* (a pseudo-fungus) is responsible for the disease (Bandyopadhyay *et al*. 2011). The pathogen is soil-borne, and overwinters on crop debris, infected planting materials or cysts in soils (Nelson *et al*. 2011). Attacks on the crop could be in the field as blight or as tuber rot in storage (Singh *et al*. 2012). In the field, symptoms appear at points where dew or rain accumulates near leaf margins as small circular water-soaked, dark brown or purple lesions usually surrounded by faint halo (Figure 1). The lesions which coalesced rapidly, may take on a zonate appearance (due to effects of fluctuations in environmental temperature). These lesions keep enlarging with white powdery rings of sporangia around their advancing edges until the entire affected leaf is colonized, dies and collapses in about 7 days. The disease can occur year round, being favoured especially where there is prolonged months of rainfall leading to sustained leaf wetness, high day and night time temperatures, and poor air circulation in the farm due to wind breaks etc. (Ooka 1980; Nelso *et al*. 2011). The disease is widespread and so devastating in monocultures where single, uniform taro cultivars are repeatedly cropped over a long time (Nelson *et al*. 2011). In such severe outbreaks, the disease has been reported to make farmers to abandon their farms or abandon the crop to grow other staples. For instance, out of the 350 varieties of *Colocasia* formerly grown in Hawaii only about 40 cultivars now remain in their farming systems due to TLB (Asraku 2010). In such severe attacks, number of functional leaves of the crop is greatly reduced, culminating in not less than 95% leafy vegetable yield reduction and harvestable root losses ranging between 30-60% in the field, and 70% root rot in traditional tuber storage systems (Ooka 1980; Asraku, 2010; Singh *et al*. 2012; shakywar *et al*. 2012). While losses in the field may be due to reduction in leaf number as well as photosynthetic area and rate of the leaves (Enyiukwu *et al*. 2021), losses in storage are occasioned by nutrient depletion of tuber by the pathogenic oomycete (Amadioha and Kenkwo 2019; Amadioha and Enyiukwu 2019a and b); making the disease a serious threat to

staple food security in tropical third worlds (Singh *et al*. 2012).

Control of TLB has been done by classical cultural strategies (field sanitation, rouging, clean planting stocks), and use of resistant varieties (whose development are still on-going in some endemic locations and where available issues of loss of desirable taste and other agronomic characteristics threatens their successful adoption) (Agarwal and Menthrata, 1987; Nelson *et al*. 2011) others are biological control involving foliar sprays or soil drench of antagonistic agents (such as *Trichoderma* species, *Rhizobacterium* species) (Siram and Misra 2007); and single or compound synthetic fungicides such as mancozeb, diflolatan, and ridomil (metalaxyl) or copper (Agarwal and Menthrata, 1987; Cox and Kasmani 2008; PPP, 2004). However, factors of inefficacies during disease outbreaks, pathogen variability, and lack of simple easy to use formulations; pathogen resistance and mammalian toxicity from chemical residues in treated crops have been reported as serious drawbacks to the respective strategies (Enyiukwu *et al*. 2014). Recently there is upsurge in interests in natural products from higher tropical plants for crop protection (Amadioha *et al*. 2019). For example, extracts of Hy*ptis suaveolus, Moringa oleifera, Azadirachta indica, Ocimum bonilienum* and *Citrus aurantofolia* inhibited formation of sporangia and mycelia elongation of *P. colocasiae* in several trials (Shakywar *et al*. 2012; Severin *et al*. 2018; Okoro and Onaebi, 2020). Generally, however, information on varietal resistance of taro to *P. colocasiae* causal agent of TLB in Nigeria and the control of the disease using natural compounds of plant origin is scanty. Therefore this paper evaluated the response of four cultivars of taro to challenge of TLB (*P. colocasiae*) in typical field conditions and the effects of different concentrations of *Azadirachta indica* in minimizing the disease.

MATERIALS AND METHODS

Experimental site and location information

The experiment was conducted at the Microbiology Laboratory, Faculty of Science and Technology and the Research and Training farm, of the Department of Agriculture, of the Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State, Nigeria in April to August, during the 2020 cropping season. The environmental parameters of the location during the study months were rainfall 159-270.02 mm, temperature range of 25-28^{o}C and relative humidity 72–86.0%. The soil type was sandy clay. The location is a lowland environment with hills rising up to 350 feet above sea level, it has geographic coordinates of latitude 7^{o} 56' 55'' N,, longitude 5^{o} 51'45''N and altitude of 200.00 meters above sea level (GPS Coordinates, 2019).

Preparation of extracts of the plant materials

Leaves of *A. indica* (and *A. conyzoides*) were washed under running tap and rinsed in 500 ml of sterile distilled water, and air-dried on the laboratory bench for 3 weeks. After that, the leaves were separately milled into fine powder (2 kg of each specimen) with Thomas Wiley milling machine (model: ED-500); and stored separately in air-tight bottles. About 50, 100, 150, 200 g of each powdered specimen were weighted out and soaked separately in 1L of sterile distilled water contained in 2L glass wares, and allowed to stand for 6 h. Thereafter they were sieved separately through 4-folds of cheese cloth into different 2L glass wares to obtain respective aqueous extracts which were correspondingly diluted in 100L of water to obtain 50, 100, 150 and 200 g/ha of the test plants (Amadioha, 2003).

Isolation, identification and preparation of spore suspension of the causal agent

Culture medium was prepared using dehydrated potato dextrose (PDA) (Oxoid™ ThermoScientific Product, England, UK). Infected cocoyam leaf with typical symptoms of blight was obtained from a subsistence farm in Uwana, Ebonyi State, Nigeria. The leaf was washed in tap water, cut into bit (6 mm), and sterilized in 10% sodium hypochlorite for 1 minute; rinsed in sterile distilled water, and dried on filter papers (Whatman No 1) before being plated on a moistened filter paper in Petri dishes; and incubated in the incubation chamber for 5 days, to allow the pathogens to grow. Potato dextrose agar (PDA) was prepared by reconstituting 39.5 g of dehydrated PDA in 1000 ml of sterile distilled water and autoclaved at 15 Psi for 15 minutes (Enyiukwu *et al* 2021). Bits of the organisms that grew out of the plated taro leaf tissues were aseptically transferred onto solidified PDA (20 ml) in Petri dishes and repeatedly sub-cultured until pure cultures of the organisms were obtained (Amadioha 2003; 2004).

Pathogenicity test

Ten-day old culture of each of the fungal isolates in Petri dishes was separately scraped into 200 ml sterile distilled water in beakers, and sieved to obtain filtrates of the isolates. The fungal isolates were adjusted to concentration of 1.0 x 10^5 spores/ml of distilled water. Cocoyam corms were planted 2 per pot of 20 kg heat-sterilized topsoil, and watered daily. At 4 weeks after planting (WAP), the seedlings (2 pots each) were separately inoculated by spraying the laminae and petioles of test crop to run-off with different suspensions of each of the fungal isolates. Seven WAP, the isolate which reproduced symptoms typical of taro leaf blight as

on the taro plants at the subsistence farm was re-isolated from the test taro tissues; while those that failed to do the same were regarded as saprophytes and discarded. Slides of the pathogenic isolate were then prepared, mounted on the stage of a compound microscope, observed and compared for morphological and colony similarities or otherwise, with the original pathogen from the infected sample collected from the subsistence farm and the identity of the organism identified with reference to illustrations of imperfect fungi by Barnett and Hunter (1995).

In vitro experiment

Preparation of spore suspension

The spores of the pathogen (*P. colocasiae*) were collected from 8-day old culture-agar stock in Petri dishes by irrigating repeatedly into a beaker. This was sieved through 2-folds of sterile cheese cloth to remove any fragments of agar and mycelia mesh and the filtrate centrifuged for 10 minutes. Then using a haemocytometer counting slide, concentration of the spores' suspension was adjusted to 1.0 x 10^5 spores ml^{-1} sterile distilled water (Alberto, 2013; Amadioha and Kenkwo, 2019).

Evaluation of effects of *Azadirachta indica* on spore germination of *P. colocasiae*

A disc (3mm) of the fungus was placed separately in 2ml of each concentration of aqueous plant extracts or mancozebl (placebo) contained in different test tubes. The test tubes were centrifuged for 10 minutes, and then filtered through 2-folds of cheese cloth. About 0.05 ml of the different preparations was placed separately on 3 sterile slides and incubated at 27^0C for 24 h for spore germination in a humid chamber. The control experiment was set up in like manner with sterile water or mancozeb.Further spore germination was stopped by adding 0.05 ml of lactophenol in cotton blue to each preparation on the slides. Spore germination inhibition effects of the plant tissue extracts on the test fungus was determined by examining 100 randomly selected spores of the pathogen under a microscope field. Records of the number of germinated spores for each treatment and replicate were taken; and were used to determine the percentage inhibition of spore germination of the pathogen compared to the controls using the formula adopted by Enyiukwu *et al*. (2021) as:

$$\% \text{ Inhibition of spore germination} = \frac{(m-n)}{m} \times 100$$

Where m = average number of germinated spores of the test fungus with control

n = average number of germinated spores of the test fungus with treatment.

Evaluation of effects of *Azadirachta indica* on radial growth of *P. colocasiae*

About 1ml of different concentrations of the aqueous plant extracts were smeared separately on the surface of solidified PDA contained in Petri dishes by gentle swirling motion (Amadioha, 2003). A 3mm disc of 10–day old culture of the pathogen was transferred to the center of the solidified PDA-extract medium in the Petri dishes, which had been marked underneath with two perpendicular lines intersecting at the center. The dishes were covered and incubated at 27ºC for 7 days. The controls were set up in the same way but with sterile distilled water or mancozeb mixed with PDA in the dishes.

The radial growth of the pathogen was measured along the perpendicular lines with a meter rule 7 days after incubation. The fungitoxicity of the extract was determined as a percentage of mycelial growth inhibition and calculated by the formula as adopted by Enyiukwu *et al*. (2021):

$$\% \text{ Radial growth inhibition} = \frac{(x-y)}{x} \times 100$$

Where x = average diameter of fungal colony with control
y = average diameter of fungal colony with treatment

Field experiment

Field preparation and layout

The experimental field measuring 70 x 90 meters squared was slashed and tractor-ridged during the 2019 planting season (July-November). The field was laid out in 4 x 7 factorial experiments in randomized complete block design (RCBD) with 3 blocks. The factors were 4 taro varieties (NCE 001, 002, 003 and 005), and 6 rates of plant-based-fungicides and 1 synthetic fungicide.

Crop establishment and treatment

The ridges were planted with 50 g cormels at a spacing of 0.5 x 1.0 m. The distance between plots and across replicates was maintained at 1 meter. Four weeks after planting, the taro seedlings were spray-inoculated at sunset with suspension of P. *colocasiae* (1 x 10^5 spores' ml^{-1} of distilled water) to run-off. They were kept moist for 7 day post inoculation by watering early and late in the evening so as to aid in buildup of dampness which encourages leaf bight disease initiation.

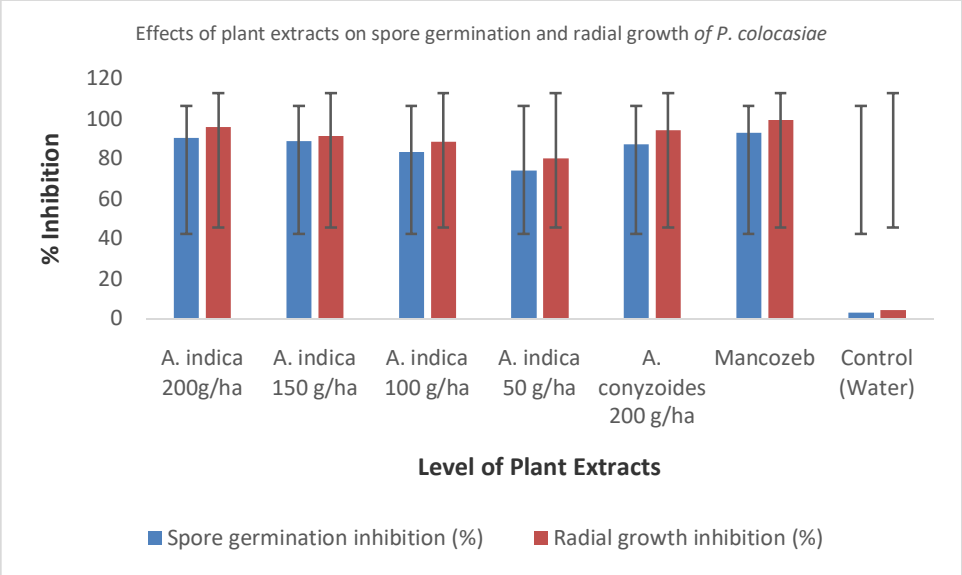

Figure 2: Effects of aqueous extracts of *A. indica* on the spore germination and radial growth of *Phytophtora colocasiae*

One week thereafter at sunset, the plants were separately sprayed with respective test rates of *A. indica* and *the* placeboes and repeated a fortnight afterwards. Records of the number of taro plants that came down with leaf blight were taken per treatment per replicate from 6 WAP and the percentage incidence calculated based on the formula by (Amadioha 2003) as:

% Taro leaf blight incidence = $N/T \times 100$

Where N = number of sweet potato plants with leaf and stem blight
T = total number of sweet potato plants examined

The toxicity of the plant tissue extracts to the fungus was taken as a reduction of leaf blight on the test plant per treatment per replicate compared to the controls; and assessed using a 10-point descriptive key adopted by (Enyiukwu *et al*. 2021) as:

1 = No disease
2 = less than 25% of the tissues
4 = greater than 25% but less than 50% of the lesion present
6 = greater than 50% but less than 75% of the lesion present in the tissues
8 = greater than 75% but less than 100% of the lesion present
10 = Heavy lesion on tissues, defoliation occurs (100%);

The disease severity was calculated based on the formula adopted from (Amadioha *et al*. 2019) as:

$$\text{Disease severity} = \frac{\text{Sum of individual disease ratings}}{\text{Number of plants examined}}$$

Data on plant length, leaf area and number of leaves per plant, weight of cormels and tubers of the taro plants beginning from 10 WAP till harvest were also collected.

Statistical analysis

Analysis of variance (ANOVA) was carried out on all the data collected, using GenStat computer programme 2012 version. Means were separated and compared using Fisher's least significant difference (F-LSD) at 0.05 level of probability.

RESULTS

Effect of plant extracts on spore germination and radial growth of *P. colocasiae*

The results of effect of crude aqueous plant extracts and mancozeb on the spore germination and radial growth of the fungus are presented in (Figure 2). It indicated that both the crude aqueous plant extracts and mancozeb significantly (P≤0.05) inhibited spore germination and radial growth of *P. colocasiae in vitro*. At rate of 200 g/ha, *A. indica* inhibited spore germination and radial growth of pathogen by 90.66% and 96.02% respectively. This was followed by 88.94% and 90.48% obtained for the respective parameters from 150g/ha; whereas 50g/ha

which had 74.09% and 80.21% for spore germination and radial growth inhibition was the least. Similar trends were also observed with reference to *A. conyzoides*. Exposure of the fungus to 200g/ha of the bio-toxicant resulted in 87.18% and 94.34% inhibition for spore germination and radial growth of pathogen respectively. Generally, and numerically speaking mancozeb out-performed all the plant tissue extracts in reducing spore germination (93.32%) and radial growth (99.46%) of the test fungus. However, these effects were statistically at par with results obtained from exposing the pathogen to 200g/ha of *A. indica*. In the same manner, results obtained from 150g/ha of *A. indica* were not significantly different from those recorded from exposure of the fungus to 200g/ha of *A. conyzoides;* suggesting that the organism showed similar sensitivity to both *A. indica* and *A. conyzoides*. Though the plant extracts at 150g/ha and below were not as effective as mancozeb (Figure 2), however, they were all significantly superior to the control experiment at all the test rates in reducing the germination of spores and growth of the pathogen *in vitro.*

Effects of *Azadirachnta indica* on TLB caused by *Colocasiae esculenta* (taro)

The results of incidence and severity of leaf blight on different varieties of the treated taro plant are presented in (Table 1). It indicated that all the varieties though susceptible to the disease, reacted at varying degrees to the fungus. NCE-005 and NCE-001, which had incidences of 66.85% and 66.54%; and severity scores of 2.94 and 3.36 respectively were the most susceptible to TLB; whereas NCE-003 which had 56.18% and 2.28 for the respective parameters was the least sensitive to the pathogen. All the plant tissue extracts used in this study sufficiently minimized the incidence and severity of TLB on the treated taro crop. Plants treated with mancozeb and 200g/ha of *A. indica* had the lowest mean incidences (59.70% and 58.70%) and severity scores (1.76 and 1.87) of TLB, which were significantly higher than effects recorded for these parameters from all other treatments on the test crop; except those exposed to 200g/ha of *A. conizoides* (a second comparative placebo), recording 65.37% and 2.61 for incidence and severity score of TLB respectively. Plant treated with *A. indic*a at 50g/ha had the highest level of TLB expression and tissue damage (Table 1). However, all the extract treatments irrespective of level of application performed significantly ($P<0.05$) better in reducing the incidence and tissue damage on taro than sterile water.

Effects of plant extracts of *Azadirachta indica* on tuber yield and corm weight of taro

Results presented in (Table 2) showed that the treatment had significant effects on tuber yield and corm weight of taro. Taro variety NCE-003 which had 11.18 tonnes per hectare was the highest yielding variety. This was closely followed by NCE-005 and NCE-002 (8.23t/ha and 7.78 t/ha respectively), whereas NCE-001 was the least yielding recording 5.83 tonnes per hectare. Similarly, 162.88g, 146.18g and 72.80 g per corm representing the highest, penultimate highest and least mean corm weight respectively were obtained from NCE-003, NCE-002 and NCE-005 respectively. In terms of concentration effects, taro plants inundated with 200, 150, and 100g/ha of *A. indica* had 10.9t/ha and 147.20g, 9.89t/ha and 135.95g, and 6.52t/ha and 131.23g for corm yield per hectare and mean tuber weight per corm respectively. Similarly *A. conyzoides* at full strength gave (9.07t/ha and 136.44g) for the respective test parameters. These values (with the exception of *A. indica* 100 g/ha) (Table 2) compared well with the results obtained from mancozeb which had 11.21g/ha and 153.03g for corm yield and weight per tuber of corm respectively. However, all the test concentrations of *A. indica* significantly ($P<0.05$) out-performed the control experiment in improving the assayed parameters of taro plant (Table 2).

Effects of tissue extracts of *Azadirachta indica on* yield components of taro

The results of effects of *A. indica* on yield components (leaf area, number of leaves per plant and number of cormels) of the treated taro crop are presented in (Table 3). It indicated that there were significant ($P<0.05$) differences in the treatment effects on the test crop. The observed trends for leaf area, number of leaves and weight of comels indicated that the test varieties of taro reacted differently to the fungus (*P. colocasiae*) while they showed varying degrees of increasing values of these parameters to increasing concentrations of *A. indica*. Mancozeb, out-performed all the other treatments in improving the studied parameters, followed by 100-200g/ha of *A. indica* and 200g/ha of *A. conyzoides*; however taro plants in the control experiment exhibited the least values for these parameters (Table 3).

DISCUSSION

This work studied response of 4 taro varieties to *P. colocaisiae* and effect of different concentrations of *A. indica* to the fungus. The results presented in (Figure 2) showed that all the concentrations of *A. indica* significantly inhibited germination of spores and mycelia elongation of the fungus *in vitro*. The ability of the extracts to effect inhibition of the fungus has been reported to be due to presence of certain fungitoxic phyto-chemical constituents in the extracts. Some workers have reported the presence of terpenoids, alkaloids, glusides, tannins, phenolics and saponins

Table 1: Effects of the test bio-pesticide on incidence and severity of leaf blight (*P. colocasiae*) on taro varieties at 6 WAP.

Variety	Concentration (g/ha) of Plant Extracts, Disease incidence (%) and Severity															
	50g/ha		100g/ha		150g/ha		200g/ha		*A. con* 200 g/ha		Mancozeb		Water (Control)			
	Inc.	Ser.	Inc.	Sev.	Inc.	Sev.	Inc.	Ser.	Inc.	Sev.	Inc.	Sev.	Inc.	Sev.	Mean Inc.	Mean ser.
NCE-001	68.59	2.48	66.21	2.35	64.36	2.26	60.17	1.78	62.17	1.80	61.32	1.74	84.62	7.81	66.85	2.94
NCE-002	65.27	2.41	63.19	2.33	62.51	2.18	58.49	2.01	60.09	2.00	65.59	1.68	81.03	7.69	60.16	2.84
NCE-003	56.38	2.29	54.17	2.21	51.68	2.05	50.69	1.54	58.32	1.65	48.03	1.45	75.67	4.71	56.15	2.28
NCE-005	71.25	3.24	70.05	3.17	67.29	2.56	65.46	2.02	64.05	2.05	61.18	2.18	89.21	8.11	66.54	3.36
Mean	65.37	2.61	63.41	2.52	61.46	2.26	58.71	1.84	61.16	1.88	59.03	1.76	82.63	7.08	62.43	2.86

*Data are means of 3 replicates ** Inc. = incidence, Ser. = severity , *A. con.* = *Ageratum conyzoides*
LSD (0.05) Mean (N) = 0.978
LSD (0.05) Variety (V) = 1.093
LSD (0.05) N x V= 2.186

Table 2: Effects of the test bio-pesticide on yield of on taro varieties infected by TLB (*P. colocasiae*) at 12 WAP.

Treatment	Taro Variety, Yield (g), Corm weight (g) and Concentration (g/ha) of Plant Extracts															
		50g/ha		100g/ha			150g/ha		200g/ha		*A. con* 200 g/ha		Mancozeb		0g/ha	
	Yield	Corm Wt	Yield	Corm Wt	Yield	C/ Wt	Yield	C/Wt	Yield	C/Wt	Yield	C/Wt.	Yield	C/Wt.	Mean Y (t)	Mean C/W (g)
NCE-005	5.29	66.80	6.83	75.70	10.73	74.60	12.24	76.20	8.10	77.30	11.31	78.61	3.07	60.20	8.23	72.80
NCE-002	6.99	133.70	7.45	148.20	8.84	148.10	8.93	168.40	7.98	150.12	9.66	172.09	4.63	75.80	7.78	146.18
NCE-003	7.75	150.80	8.27	151.80	12.01	170.01	15.45	183.30	13.19	162.03	15.03	194.12	6.59	128.10	11.18	162.88
NCE-001	3.29	110.70	3.54	149.20	7.96	151.10	6.99	160.9	7.03	155.09	8.85	167.36	3.17	102.70	5.83	138.60
Mean	5.83	115.50	6.52	131.23	9.89	135.70	10.90	147.20	9.08	136.14	11.21	153.05	4.37	91.70	--	--

*Data are means of 3 replicates ** C/wt. = corm weight, *A. con.* = *Ageratum conyzoides*
LSD (0.05) Mean (N) = 1.543
LSD (0.05) Variety (V) = 2.063
LSD (0.05) N x V = 3.128

Table 3: Effects of test bio-pesticide on leaf area, number and weight of cormels of taro varieties at 10-12 WAP.

Variety	Rate of Application (g/ha) of Plant Extracts, Corm Yield (t), Number and Weight (g) of Cormels																							
	50g/ha			100g/ha			150g/ha			200g/ha			A. C 200g/ha			Mancozeb			0g/ha (Control)			Means		
	LA (Cm2)	NC	WC (g)	LA (Cm2)	NC	WC (g)	LA (Cm2)	NC	WC (g)	.LA (Cm2)	NC	WC (g)	LA (Cm2)	NC	WC (g)	LA (Cm2)	NC	WC (g)	LA (Cm2)	NC	WC	Mean LA cmt)	Mean NC	Mean WC
NCE-001	639	11.97	24.88	671	11.52	26.49	411	14.20	40.59	541	13.79	53.54	640	11.65	43.45	731	10.61	55.01	441	12.42	19.32	582	12.31	37.61
NCE-002	1090	16.47	36.53	1155	11.39	30.29	1402	11.12	31.70	1279	16.86	45.50	1198	10.25	44.26	1412	14.18	52.42	749	10.89	21.13	1183.57	12.31	37.40
NCE-003	1207	13.91	34.51	1211	11.00	35.03	1156	18.60	46.18	1134	14.80	59.61	1250	13.07	48.39	1556	12.86	61.07	1273	14.06	26.47	1255.29	14.04	44.47
NCE-005	911	6.91	31.89	592	4.14	36.15	806	7.55	46.48	699	5.85	41.31	658	8,12	39.56	678	9.23	49.84	690	5.47	19.12	718	6.75	37.69
Mean	961.75	12.33	31.95	907.25	9.51	31.99	944	12.85	41.22	913.00	12.83	50.00	936.50	10.77	43.90	10.94.25	11.72	54.59	788.25	10.71	21.51	--	--	--

*Data are means of 3 replicates; ** LA = leaf area, **NC** = Number of leaves, WC = Weight of cormel , *A. con.* = *Ageratum conyzoides*
LSD (0.05) Mean (N) = Ns
LSD (0.05) Variety (V) = 121.90
LSD (0.05) N x V = 272.70

in extracts of tropical plants (Okwu *et al*., 2007; Okwu and Njoku, 2009; Enyiukwu *et al*., 2013). Indian researchers have isolated azadirachtin and 5 other related triterpenoids from *A, indica*. These compounds may be responsible for the inhibitions noted in this study. And the increase in the

amount of these compounds with level of application explains the increased inhibition with concentration of extract. Findings in this study thus confirm the reports of other workers where extracts of Hy*ptis suaveolus, Moringa oleifera, Azadirachta indica, Ocimum bonilienum* and *Citrus aurantofolia* inhibited spore germination, and formation of sporangia and mycelia elongation of *P. colocasiae* in several trials (Shakywar *et al*. 2012; Severin *et al*. 2018). The range of inhibition of radial growth of the pathogen in this study (Table 1) is also in accord with 81.11-87.21% obtained for *Alternaria alternata* and *Verticilium lateritum* exposed to 10% aqueous extract of *A. indica in vitro* (Padukur and Amienyo, 2016). Results presented in (Table 1) indicated that the test varieties of taro showed differential reaction to *P. colocasiae*, incitant of TLB in this study. NCE-003 and NCE-002 were least susceptible to the disease compared with other varieties. High presence of certain phytochemical such as flavonoids, terpenes, polyphenols and salicylic acids (El-Wakali *et al*., 2013; Enyiukwu and Awurum , 2013) have been reported to prime plant resistance against diseases by stimulating production of higher levels of phytoalexins, hydrogen peroxide and PR-proteins, necessary to ward-off fungal invasion around portal of entry into plant tissues (El-Kazzaz *et al*., 2015; Enyiukwu *et al*., 2016).

The ability of NCE-003 and NCE-002 to show least disease incidence and severity in this study may have stemmed from their higher ability to produce higher levels of these compounds compared to NCE-001 and NCE-005. Aqueous neem extracts used in this study significantly minimized TLB expression on the treated taro plants. This agrees strongly with reports of others workers who found that 10% aqueous extract sprays of neem and tulis effected 72.18 and 72.75% minimization of disease incidence and 26.81 and 25.41% of tissue damage respectively in taro (Shakywar *et al*., 2014). Submissions of Awurum *et al*. (2016) who in a parallel study found that extracts of some tropical flora sufficiently minimized the incidence and severity of *Alternaria porri* and *Colletotrichum* species causing leaf spot and anthracnose in naturally infected onion fields and significantly improved yields and yield attributes of the crop also harmonize with findings from this study. Table 1 also showed that the reduction of disease incidence and severity was dose-wise. Increase in concentration of active principles with concentration may be the reason for this observation. Findings in this study aligns with the views also held by Amadioha and Obi (1998, 1999) and Amadioha (2001) who found extracts of *Cymbopogon citratus, Ocimum gratissimum* and *A. indica* to inhibit the initiation and advancement of anthracnose (*Colletotrichum lindemuthianum*) in cowpea. Many workers have attributed the success of extracts of these tropical plants to stem phyto-fungal diseases to not only directly inhibiting growth and development of the pathogen; but also on their ability to prime the defence systems of the plant against the pathogen (El-Kazzaz *et al*., 2015; Enyiukwu *et al*., 2016). Tables 2 and 3 indicated that the factors of taro variety and concentration of *A. indica* treatment had significant effects on tuber yield and corm weight as well as the yield components of taro. Lower genetic susceptibility of NCE-003 and NCE-002 coupled with the increased inhibition of TLB on the treated taro crop with concentration had translated to the higher tuber yield per hectare, higher corm weight, higher number of cormels, larger leaf area and better performance of these varieties than the others. This view is consistent with the report of Omeje *et al*. (2015) who found from a field trial that different regimes of aqueous extract of *A. indica* amongst other bio-fungicides sufficiently decreased the incidence and severity of attack of TLB on taro, and increased the performance and corm yield of the crop in Nsukka, Nigeria. It is also in tandem with the results obtained from bio-pesticides by Shakywar *et al*. (2012. 2014) where aqueous extracts of neem and tulis significantly minimized development and spread of TLB on taro, and improved yield and yield attributes of the crop in India.

In conclusion, *P. colocasiae* the causal agent of TLB is sensitive to *A. indica* in a dose-wise manner; being most sensitive to application of 150-200g/ha levels of the bio-toxicant. Taro varieties used in this study showed differential susceptibility to attacks of the fungus, NCE-003 and NCE-002 were the least susceptible. Therefore, rural farmers of taro could plant varieties NCE-003 and NCE-002 and treat them with 150-200gkha of aqueous extract of *A. indica* to restrain TLB and improve taro production in Southeast, Nigeria.

REFERENCES

Agarwal A, Menthrata RS (1987). Control of *Phytophtora* leaf light of taro (*Colocasia esculeta*) by fungicides and roguing. *Phytoparasitica* 15: 299-305.

Alberto E (2013). How to calculate the concentration of fumgal spores suspension. www.researchgate.net/post/How_to-calculate_the_concentration_fungal_. Accessed June 13, 2020.

Amadioha A, Enyiukwu DN (2019a). Alterations of biochemical composition of leaf and stem of cowpea (*Vigna unguiculata* L. Walp.) by *Colletotrichum destructivum* O'Gara in Nigeria. *Journal Experimental Agric. Int*. 32(2): 001-007.

Amadioha AC (2001). Fungitoxic effect of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Arch Pytopathol Plant Prot 33: 001-009.

Amadioha AC (2004). Control of black rot of potato caused by *Rhizoctonia bataticola* using some plant leaf extracts. *Arch. Plant Pathol. Plant Protect*. 37:111-117.

Amadioha AC, Obi VI (1999). Control of anthracnose disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimum. Acta Phytopathol Entomol Hungarica* 34 (1-2): 85-89.

Amadioha AC, Enyiukwu DN. (2019b). Biochemical composition of seed and husk of cowpea (*Vigna unguiculata* L. Walp.) infected by *Colletotrichum destructivum* O'Gara in storage. *Annual research and reviews in Biology* 31(1): 001-007.

Amadioha AC, Kenkwo PC, Markson AA (2019). Fungitoxic potentials of extracts of plant origin against fungal root rot of cassava (*Manihot esculenta* Crantz) in storage. *Annual Res. Rev. Biol*. 31(1): Doi: 10.9734/arrb//2019/v31i130036.

Amadioha AC, Obi VI (1998). Fungitoxic activity of extracts from *Azadirachta indica* on *Colletotrichum lindemuthianum* in cowpea. *Journal Herbs Spices Medicinal Plants* 6 (2): 33-40.

Amadioha AC. 2003. Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. *Acta Phytopathol Entomol Hung*arica 38: 259-265.

Asraku JS (2010). Identification of the major foliar fungal diseases of *Colocasiae esculenta* (L.) Schott and its management in the Kumasi metropolis. MSc Thesis Kwame Nkurumah University of Science and Technology, Ghana, August, 2010.

Bandyopadhyay R, S.harma K, Onyeka KJ, Aregbesola A Lava-Kumar P (2011). First report of taro leaf blight caused by *Phytophytora colocasiae* in Nigeria. *Plant Disease* 95(8): 618 Doi: 10.1094/PD15-10-12-10-890.

Barnett HL Hunter BB (1998). Illustration genera of imperfect fungi 4th ed. American Phytopathological Society, Minnesota, USA.

Cox PC, Kasimani C (2008). Control of taro leaf blight using metalaxyl. *Tropical Pest Management* 34(1): Doi: 10.1080/09670878809371214.

El-Kazzaz MK, Salem, EA, Ghonein KE, Elsharkawy MM, Nasr El-Kot GA, Kalboush AE (2015). Integrated control of rice kernel smut disease using plant extracts and salicylic acid. *Arch. Phytopayhol. Plant Protect*. 48(8): 664-675. Doi: 10.1080/03235408.2015.1092202.

El-Wakeil NE (2013). Botanical pesticides and their modes of action. Gesunde Pflanzen 65(4): 125-179.

Enyiukwu DN, Awurum AN, Ononuju CC, Nwaneri JA (2016). Modes of action of potential phyto-pesticides from tropical plants in plant health management. IOSR Journal of Pharmacy 6 (7): 01-17.

Enyiukwu DN, Amadioha AC, Ononuju CC (2018). Significance of cowpea leaves for human consumption. *Greener Trends Food Sci Nutr* 1 (1): 001-010.

Enyiukwu DN, Amadioha AC, Ononuju CC (2021). Evaluation of some pesticides of plant origin for control of anthracnose disease (*Colletotrichum destructivum* O'Gara) in cowpea. *Asian J. Agriculture* 5(1): 004-011.

Enyiukwu DN, Awurum AN (2013). Fungitoxic principles and antifungal activity of extracts from *Carica papaya* and *Piper guineense* on *Colletotrichum destructivum*. Cont J Biol Sci 6 (1): 29-36.

Enyiukwu DN, Awurum AN, Ononuju CC, Nwaneri JA (2014). Biology and management strategies of cowpea anthracnose disease caused by *Colletotrichum* species. *Greener J. Biochem Biotechnol* 1 (2): 052-065.

GPS (Global Positioning Systems) Coordinate (2019). Google Play Store. www.google.com/gps...Accessed June 4, 2021.

Nelson S Brooke F, Teres G (2011). Taro leaf blight in Hawaii. College of Tropical Agriculture and Human Resources University at Manoa. *Plant disease* No PD-71.

Nielsen SS, Ohler TA, Mitchell MC. (1997). Cowpea leaves for human consumption: production, utilization and nutrient composition. In: Singh BB (eds) Advances in Cowpea Research. Ibadan, IITA.

Okoro CA, Onaebi CN (2020). Efficacy of methanolic leaf extracts of *Hyptis suaveolus* and *Moringa oloeifera* in the control of soil-borne fungal pathogens. *American Research Review in Biology* 35(7):56-63. Doi:10.97-34/AJRR/2020/v35i7302.46.

Okwu DE, Awurum AN, Okonkwo JI (2007). Phytochemical Composition and *in vitro* antifungal activity screeninig of extracts from *Citrus* plants against *Fusarium osysporum* of Okra plant (*Hibiscus esculentus*). 8th African Crop Science Confeence, El-minia, Egypt; October 27-31, 2007.

Okwu DE, Njoku EE (2009). Chemical composition and *in vitro* antifungal activity screening of seed and leaf extracts from *Afromonum melenguata* and *Monodora myristica* against *Sclerotium rolfsii* of cowpea plant. Pest Technol 3 (1): 58-67.

Omeje TE, Ugwueke RI, Aha SC, Eze SC, Owulumba SI, Ezema RA (2015). Field management of *Phytophtora* blight disease of cocoyam (*Colocasia esculenta* L.) with spray regimes of selected fungicides in Nsukka, Nigeria. *Agro-Science* 14(2): 36-45. Doi: 10.4314/as.v14i2.6.

Ooka JJ (1980). Taro diseases: A Factsheet. Department of Plant Pathology, University of Hawaii, Kawai Branch Station, Pp 51-60.

Pandukur SG, Amienyo CA (2016). Effects of *Azadirchta indica* on radial growth of some fungi isolated from two varieties of cococyam (*Colocasia esculenta* L.) coms and cormels in some markets in Plateau State, Nigeria. *Journal of Phytopathology and Pest Management* 3(1): 46-59.

PPP (Pacific Pest and Pathogens) (2004). Taro leaf blight caused by *Phytophtora colocasiase*. In: Pacific Pest and Pathogens Factsheet No 014, 2004.

Severin NT, Mbiakeu SN, Sameza ML, Jazet PMD, Tchoumbougnang F (2018). Using *Citrus aurantifolia* essential oil for potential bio-control of *Colocasia esculenta* (taro) leaf blight caused by *Phytophtora esculenta*. *Environ. Sci. Pollution Res*. 25(30): Doi: 10.1007/s11356-07-0506-0.

Shakywar RC, Debashishi S, Toma KS, Pathak M (2014). Eco-friendly approaches for managing leaf blight of taro (*Colocasia esculenta* L. Var.*antiquonum*). *Int. J.ournal of Bioresource Science* 1(1): 31-35.

Shakywar RC, Pathwak SP, Kumar S, Singh AK (2012). Evaluation of fungicides and plant extracts against *Phytophtora colocasiae* Rac. causing leaf blight of Taro. *Journal Plant Dis, Science* 7(2): 197-200.

Singh D, Hunt D, Jackson G, Fullerton RA (2012). Taro leaf blight – A threat to food security. *Agriculture* 2: 182-203. Doi: 10.3390/agriculture2020182.

Siram S, Misra RS (2007). Control of taro leaf blight caused by *Phytophtora colocasiae* (Racibi) and storage losses with *Rhizoacterium*. *Journal Biological Control* 21(2): Doi: 10: 10.18311/jbc/2007/8847.