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In vitro Control of *Phytophthora colocasiae* Causing Leaf Blight of Taro (*Colocasia esculenta* (L.) Schott.) With Selected Botanical Extracts

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ABSTRACT

Background and Objective: Leaf blight incited by *Phytophthora colocasiae* has been a major challenge impacting on yield and commercialization of taro (*Colocasia esculenta* (L.) Schott) in Nigeria since 2009. This study evaluated *in vitro*, the efficacy of selected plant extracts against the causal pathogen. **Materials and Methods:** Four botanical extracts: *Azadirachta indica* A. Juss. *Eucalyptus camaldulensis* Dehn, *Senna alata* L. and *Tithonia diversifolia* (Hemsley) A. Gray were tested *in vitro* for their fungi toxic effects on radial growth and mycelia dry weight of the pathogen at 5, 10 and 20 mg/mL of each extract. Ridomil (0.67 mg/mL) and zero treatment were set along tests and treatments were replicated thrice in a Completely Randomized Design. Data were subjected to analysis of variance at a 95% confidence interval using the SPSS package and significant differences among means were compared using Duncan's Multiple Range Test (DMRT). **Results:** All the extracts exhibited significantly varying degrees of inhibition in the radial growth and mycelia dry weight tests. Percentage inhibition increased as the concentration increased. *Azadirachta indica* and *S. alata* extracts at 20 mg/mL inhibit 100 and 97.13% radial growth, respectively and were significantly ($p<0.05$) superior to 40.00 and 31.81% caused by *T. diversifolia* and *E. camaldulensis*, respectively. Complete (100%) inhibition of mycelia dry weight was obtained with *S. alata* and *T. diversifolia* extracts at all the selected concentrations while *A. indica* and *E. camaldulensis* showed high inhibition at some concentrations. **Conclusion:** These extracts have strong inhibitory effects against *P. colocasiae* and are comparable to the synthetic fungicide Ridomil, therefore, their exploration in the management of TLB is strongly advocated.

KEYWORDS

Oomycetous fungus, *Phytophthora colocasia,* leaf blight, taro leaf, botanical extracts, fungi toxic

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INTRODUCTION

Taro is a tropical herbaceous aroid commonly known as cocoyam[1-3]. It is an ancient traditional tuber crop grown in Nigeria[4,2] and many developing countries of Africa, Asia, Pacific Islands, West Indies, Caribbean and the Mediterranean[5,6] primarily for its underground starchy corms[7]. Amusa *et al.*[4] as well as Temesgen and Retta[8] opined that taro's highly nutritive leaves, petioles and flowers also serve as vegetables in some localities.

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In Nigeria, taro provides an alternative source of carbohydrates to supplement yam and cassava and ranks third in importance after yam and cassava. Corm processed into flour is used for various forms of confectionery and as a composite nutrient in infant meals due to its high (98.8%) digestibility[9,10]. In some localities, they are also used as adjuncts in soup thickening. Besides the nutritional benefits, Alcantara *et al.*[11] noted that taro has great potential as an agro-industrial raw material in pharmaceuticals[12,13] in confectionery, brewery[14] and livestock industries[15]. As a cash crop in Nigeria, surpluses from subsistent production find their way to the market thereby playing a significant role in income generation and poverty alleviation for the local peasant farmers.

Plant disease has remained a constant factor threatening food security and national economic development worldwide. Overwhelming losses of crops to some epiphytotic and their impacts both remote and recent on the course of human history were documented[16,17]. The character of agricultural practices in Nigeria, fraught with subsistence farming, low productivity, high post-harvest losses and inadequate processing and preservation facilities, dictates rising food insecurity. Yet this scenario was worsened by the outbreak in 2009 of *Phytophthora* leaf blight disease which has been devastating taro crops in Nigeria and further reducing food availability.

Phytophthora colocasiae Raciborski is the causal pathogen of the most destructive disease of taro (*Colocasia esculenta* (L. Schott.) commonly referred to as taro leaf blight (TLB)[18-20]. Named at Java in 1900[21], *P. colocasiae* is still the most important pathogen limiting taro production in Nigeria[22,23] and many countries in Sub-Sahara Africa where taro is grown. Yield losses a century later vary from 25-50% in some areas in Oceania and Southeast Asia to more than 90% in others[24]. Besides, the pathogen also causes serious post-harvest decay of corms. Symptoms typical of TLB are initially expressed on the leaf lamina as small, water-soaked, dark brown, round to irregular necrotic lesions on the adaxial leaf lamina[25] which rapidly become enlarged to 2.5-5.0 cm within a few days. As the disease progresses, adjacent lesions coalesce to cover an extensive leaf area showing concentric colour patterns and yellowing. Orange or reddish-brown exudates also ooze out from the spot. The infected leaf dies within 7-14 days causing yield losses of up to 50%[26,19] in severe cases and more than 70% in extremely severe cases[27].

The epidemic of taro leaf blight arrived, in Nigeria in July, 2009[22,24] and almost eliminated commercial and subsistence production of taro. Similar catastrophic events were reported in Cameroon and Ghana[28]. This epidemic, which occurred in South Eastern Nigeria and the South-West and North-West Districts of Cameroon caused serious losses because there was not enough produce to eat or sell. Tremendous losses attributed to this pathogen were earlier reported in Pacific Islands (American Samoa, Western Samoa, Hawaii, Fiji and Papua New Guinea), India and China, Mauritus, Indonesia, Philippines and many other countries[1,5,25,29,30]. Significant reductions in the nutritional and phytochemical constituents of infected corms have also been reported[31].

Control of taro leaf blight has been attempted through cultural practices[32], including removal and destruction of infected leaves, agronomic[33] chemical and breeding methods[1,25,34] in Asia and the Pacific. While these methods have recorded some level of success, each is associated with specific limitations. Removal of leaves would quickly lead to complete defoliation and consequent effects on yield[20].

Current concerns on the accumulation of harmful chemical residues in the environment (soil, water and/or plants and animals)[35] and the associated threats to human health[36,37] in addition to the uneconomic cost of such chemicals have led to an increasing search for alternatives. Moreover, the development of fungicide-resistant biotypes of pathogens has become a major constraint to the control of pathogens with synthetic chemicals. In addition, the choice of taro cultivars by farmers is driven by their tastes and marketability and some of these characteristics are lost during breeding to produce resistant cultivars[20].

In view of the serious threat to food security posed by this disease viz-a-viz the scarcity of produce and lack of planting materials, loss of important genetic resources and the adverse impact on biodiversity caused by synthetic chemicals, the development of environmentally sustainable control measures has become urgently necessary to boost taro production.

Plants are rich sources of bioactive principles (phytochemicals) which occur in different forms and provide viable alternatives to synthetic chemicals when extracted. Only a limited trial with plant extract has been done to control taro leaf blight[38]. Extracts of *A. indica*, *S. alata*, *T. diversifolia* and *E. camaldulensis* have been found effective in reducing the growth and development of many pathogens and have been used in the management of many other plant diseases as safe and eco-friendly alternatives to synthetic chemicals[39-41].

The objective of this study was to evaluate the fungicidal effects of crude extracts of selected plants against the taro leaf blight pathogen, *P. colocasiae*.

MATERIALS AND METHODS

Duration and location of study: The study started during the rainy season on 24 June, 2018 at the National Root Crops Research Institute (NRCRI) Umudike, Nigeria and lasted till December, 2019.

Collection of plant materials: The following plant materials were used for the preparation of plant extracts: Neem (*Azadirachta indica*) leaf, Eucalyptus (*Eucalyptus camaldulensis*) leaf, Bush candle (*Senna alata*) leaf and Mexican sunflower (*Tithonia diversifolia*) leaf. Fresh leaf samples, one kilogram of each of these plants were collected from their natural sources within Abia and Enugu States and their identity was authenticated by Mr. M.I. Nduche of the Department of Plant Science and Biotechnology Michael Okpara University of Agriculture Umudike.

Preparation of plant extracts: The plant materials were washed under running tap water to remove dirt, allowed to drain off the water, cut into small bits and air-dried to brittleness on the laboratory benches at room temperature for two weeks. Each sample was ground into a fine powder using a sieve to remove large particles and stored in airtight plastic containers before soaking following the method of Ofokansi *et al*.[42]. One hundred and fifty grams of each pulverized sample was weighed into a 1000 mL conical flask and soaked in 600 mL of ethanol (1:4 w/v) for 48 hrs after which the content of each flask was filtered into plastic tray through 4 layered cheese cloths. The trays were covered with perforated aluminum foil and placed under a fan in a sterile chamber to evaporate the ethanol. After evaporation of the ethanol, the concentrated crude extracts obtained were transferred into sterile airtight bottles and preserved in the refrigerator at -20°C until usage.

Sterilization of materials and preparation of media: Materials such as Petri dishes, test tubes, flasks, transfer needles, cork-borers, scalpel, cotton wool and filter papers used in the laboratory during the research were sterilized in a hot air Genlab oven (MINI/75/SS/F) supplied by Genlab Ltd., Tanhouse Lane, Widnes, United Kingdom, at 160°C for 1 hr. Potato Dextrose Agar (PDA; 200 g/L Irish potato, 20 g/L dextrose and 20 g/L agar) and water Agar (WA; 20 g/L agar), were prepared according to standard procedure of Mishra *et al*.[1] and autoclaved at 103 KNM^{-2} and 121°C for 15 min.

Isolation of *P. colocasiae*: A modified isolation technique of Chiejina and Ugwuja[24] was used. Leaves of taro plants exhibiting symptoms of blight were collected from the research farm of the National Root Crops Research Institute, Umudike (NRCRI). Thin leaf tissue fragments (2-3 mm) were excised from the periphery of lesions, surface sterilized in 0.1% mercuric chloride for 2 min, rinsed in three changes of sterile distilled water and plated in WA. Following incubation in Petri dishes at room temperature (27±2°C)

for 4 days, mycelia emerging from each tissue fragment were aseptically transferred to PDA amended with100 mg/L ampicillin, 100 mg/L penicillin, 3 mg/L PCNB (Penta-Chloro-Nitro-Benzene) and 3 mg/L nystatin. Transfers of colony growth were aseptically done from PDA cultures to clean PDA plates until pure cultures were obtained. Identification of isolates was based on observed culture growth patterns, mycelia colour and microscopic examinations of vegetative and reproductive structures with reference to relevant manuals[43,44]. Isolates were preserved in McCartney bottles agar slant for subsequent use.

Pathogenicity test: A pathogenicity test was done for each of the organisms isolated from the infected taro leaf according to a modified method of Brooks[30] in order to confirm the pathogen responsible for the initial TLB (Taro leaf blight) disease symptoms observed on the infected taro leaves as was postulated by Koch. The method of Brooks was modified thus: Agar disc inoculums of each isolate were used instead of liquid inoculum. With the aid of sterile cork borer, a 5 mm agar disc was cut from the periphery of a 7 days old actively growing culture of each isolate and placed on previously washed taro leaves laid on sterile plastic trays lined with moist sterile cotton wool. Three replicates of discs of each isolate were made and the setup was incubated at 26°C for 4 days on ICB-30B/BE-125B/125BE incubator supplied by Bioevopeak Inc., Lixa District, Shandong, China.

Inhibitory effect of extracts on radial growth of *P. colocasiae*: Ethanol leaf extracts of *Azadirachta indica*, *Eucalyptus camaldulensis*, *Senna alata* and *Tithonia diversifolia* were tested for their effects on the radial growth of *P. colocasiae*. The effects on radial growth were investigated on PDA. The requisite amount of each extract was weighed with a Mettler Balance (ME403TE/00 Precision) supplied by Chemical Manufacturing Company, China, suspended in sterile distilled water and added to molten PDA at 50°C to achieve a final concentration of 5, 10 and 20 mg/mL. Ridomil (a standard fungicide) at 3 mg/mL was incorporated into PDA and served as positive control while the media to which nothing was added served as negative control. Twenty milliliters of the extract-amended medium of a given concentration was dispensed into 90 mm Petri dishes. The media in the dishes were allowed to cool and solidify. They were centrally inoculated with a 5 mm mycelial disc cut from the periphery of a 7 days old culture of *P. colocasiae* by means of a sterile cork borer. All treatments were replicated thrice and incubated on a laboratory bench for 7 days at room temperature (25±4°C). Radial growth in terms of the diameter of the colony in each culture dish was measured to the nearest millimeter along two equatorial axes from the back side of plates and their averages were recorded for all 3 replicates of a given concentration.

Inhibition of radial growth: The data obtained from radial growth measurements (colony diameter) were used to deduce percentage inhibition of radial growth following the formula stated by Shovan *et al*.[45] as follows:

$$\text{Percentaage inhibition (I)} = \frac{x - y}{x} \times 100$$

Where:

X = Growth of control

Y = Growth of extract-treated plate

Inhibitory effect of extracts on mycelial dry weight of *P. colocasiae*: To determine the effect of the extracts on mycelia dry weight of *P. colocasiae*, potato dextrose broth (PDB) was used. Fresh PDB was prepared using 200 g of peeled sliced potato and 20 g of dextrose in 1000 mL of distilled water. The infusion made from the potato by cooking was mixed with the dextrose-in-water, autoclaved at 121°C and 103 KN M^{-2} for 15 min and allowed to cool. Using the poisoned food technique described by Sinclair and Dhingra[46], requisite quantities of each extract were weighed into separate 250 mL conical flasks and

incorporated into the broth in proper amounts to achieve a final concentration of 5, 10 and 20 mg/mL of the extracts. The content of each flask was stirred thoroughly with a sterile glass rod to ensure uniform distribution of the extract. Two-millimeter agar disc cut from the periphery of a 7 days old culture of *P. colocasiae* was introduced into each flask, shaken and incubated for 10 days at room temperature (27±°C). After the incubation period, the mycelium in each of the flasks amended with extract was harvested, weighed and oven-dried at 50°C for 3 hrs and each was compared with that of the control.

Statistical analysis: The experiment was laid out with 16 treatments replicated thrice in a Completely Randomized Design (CRD). Treatments were formed by the combination of 4 plant extracts and 4 concentrations of extract. Data were subjected to Analysis of Variance (ANOVA) at a 95% confidence interval using the SPSS package (IBM SPSS Statistics 22) and significant differences among means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS

Inhibitory effect of the extracts and concentrations on radial growth of *P. colocasiae*: Results of the effects of the crude ethanol plant extracts and their concentrations on radial growth (colony diameter) (mm) and percentage inhibition (%) of *P. colocasiae* are shown in Table 1. Radial growth was significantly inhibited ($p<0.05$) to various extents by all the extracts in a dosage-dependent manner compared to the control. Crude extract of *A. indica* had significantly ($p<0.05$) the least mycelia growth of 0.00, 0.17 and 1.44 mm at 20, 10 and 5 mg/mL concentrations compared to other extracts and exhibited correspondingly, the highest inhibition of radial growth, 100, 92.17 and 77.46%, respectively. Crude extract of *S. alata* was next in efficacy with 0.18, 1.39 and 1.55 mm radial growths corresponding to 97.13, 78.51 and 75.58% radial growth inhibitions, while *T. diversifolia* and *E. camaldulensis* had lowest inhibition.

Main effect of the extract on radial growth: The main effect of extracts on the radial growth of *P. colocasiae* is presented in Table 2. Radial growth was inhibited at various extents by all the extracts but percentage inhibition differed significantly ($p<0.05$) from one extract to another. *Azadirachta indica* extract was significantly the most superior causing 67.41% inhibition, while *E. camaldulensis* extract was the least efficient with 18.52% inhibition.

Table 1: Effects of plant extracts and concentrations on radial growth of *Phytophthora colocasiae*

	Azadirachta indica		*Eucalyptus camaldulensis*		*Senna alata*		*Tithonia diversifolia*	
Extract rate (mg/mL)	Radial growth (mm)	Percentage inhibition	Radial growth (mm)	Percentage inhibition	Radial growth (mm)	Percentage inhibition	Radial growth (mm)	Percentage inhibition
Control	6.38±0.52[a]	0.00±0.00[a]	6.38±0.52[a]	0.00±0.00[a]	6.38±0.52[a]	0.00±0.00[a]	6.38±0.52[a]	0.00±0.00[a]
5	1.44±1.28[b]	77.46±12.03[b]	5.14±0.03[b]	19.56±0.42[b]	1.55±0.08[b]	75.58±0.83[b]	3.83±0.09[b]	21.23±0.91[b]
10	0.17±0.29[c]	92.17±6.56[c]	4.94±0.10[b]	22.69±1.59[b]	1.39±0.14[b]	78.51±1.96[b]	4.64±0.09[c]	27.33±1.45[c]
20	0.00±0.00[d]	100.00±0.00[d]	4.36±0.74[c]	31.81±11.64[c]	0.18±0.03[c]	97.13±0.45[c]	5.03±0.06[c]	40.00±1.20[d]
Total	2.00±0.55	67.41±8.40	5.21±0.35	18.52±13.81	2.38±0.19	62.86±0.81	4.97±0.19	22.14±0.89

Values presented in the table are means of 3 Replicates±Standard Deviation, Means followed by the same letter within a column are not significantly different and $p<0.05$

Table 2: Main effect of extract on radial growth of *Phytophthora colocasiae*

Extract	Radial growth (mm)	Percentage inhibition
Azadirachta indica	2.00±0.55[a]	67.41±8.40[a]
Senna alata	2.38±0.19[b]	62.86±0.81[b]
Tithonia diversifolia	4.97±0.19[c]	22.14±0.89[c]
Eucalyptus camaldulensis	5.20±0.86[d]	18.52±13.81[d]

Each mean represents the overall performance of a given extract irrespective of concentration, means followed by the same letter within a column are not significantly different and $p<0.05$

Table 3: Main effect of extract concentration on radial growth of *Phytophthora colocasiae*

Extract rates (mg/mL)	Radial growth (mm)	Percentage inhibition
0 (control)	6.38±0.52[d]	0.00±0.00[d]
5	2.99±0.37[c]	48.46±5.55[c]
10	2.79±0.16[b]	55.18±4.46[b]
20	2.39±0.21[a]	67.24±3.32[a]

Each mean represents the overall performance of a given concentration irrespective of extract. Means followed by the same letter within a column are not significantly different and p<0.05

Table 4: Effects of plant extracts and concentrations on mycelial dry weight of *Phytophthora colocasiae*

Extract	*Azadirachta indica*		*Eucalyptus camaldulensis*		*Senna alata*		*Tithonia diversifolia*	
Rates (mg/mL)	Mycelia weight (mg)	Percentage inhibition	Mycelia weight (mg)	Percentage inhibition	Mycelia weight (mg)	Percentage inhibition	Mycelia weight (mg)	Percentage inhibition
0	0.33±0.12	0.00±0.00	0.33±0.12	0.00±0.00	0.33±0.12	0.00±0.00	0.33±0.12	0.00±0.00
5	0.17±0.11	49.49±32.37	0.23±0.07	30.3±15.03	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
10	0.00±0.00	100.00±0.00	0.14±0.05	57.57±0.00	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
20	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
Total	0.13±0.15	62.37±8.09	0.18±0.06	46.96±3.75	0.08±0.03	75.00±0.00	0.08±0.03	75.00±0.00

Values presented in the table are Means of 3 Replicates±Standard Deviation, Means followed by the same letter within a column are not significantly different and p<0.05

Table 5: Main effect of the extract on the mycelial dry weight of *Phytophthora colocasiae*

Extract	Mycelial dry weight (mg)	Percentage inhibition
Azadirachta indica	0.13±0.15[b]	62.37±6.20[b]
Senna alata	0.08±0.19[c]	75.00±0.81[c]
Tithonia diversifolia	0.08±0.19[c]	75.00±0.81[c]
Eucalyptus camaldulensis	0.12±0.86[a]	46.97±13.11[a]

Each mean represents the overall performance of a given extract irrespective of concentration, Means followed by the same letter within a column are not significantly different and p<0.05

Main effect of concentration on radial growth: The main effect of concentrations irrespective of the individual extract on radial growth showed that all the concentrations inhibited radial growth of *P. colocasiae* except the control (0 mg/mL) which showed no inhibition (Table 3). The most efficient concentration was 20 mg/mL causing 67.24% inhibition which differed significantly (p<0.05) from 55.18 and 48.46% inhibitions caused by 10 and 5 mg/mL, respectively.

Inhibitory effects of extracts and concentrations on mycelial dry weight of *P. colocasise*: Results of the effects of crude ethanol extracts of *A. indica*, *E. camaldulensis*, *S. alata* and *T. diversifolia* at 0, 5, 10 and 20 mg/mL concentrations on the mycelial weight of *P. colocasiae* grown on potato dextrose broth are shown in Table 4. The mycelial dry weight of the pathogen was similarly inhibited to varying degrees by all concentrations of the crude extracts. The treatment means were significantly (p<0.05) different from that of the control and percentage inhibition increased as the concentration increased. *Senna alata* and *T. diversifolia* extracts were significantly superior to the other extracts in inhibiting mycelial dry weight by causing complete (100%) inhibitions of mycelia at 5, 10 and 20 mg/mL concentrations respectively, while *A. indica* extract caused 100% inhibition only at 10 and 20 mg/mL. However, *E. camaldulensis* extract gave the least but appreciable inhibition of mycelial dry weight at 5 mg/mL (30.3%), 10 mg/mL (57.57) and complete inhibition at 20 mg/mL.

Main effect of the extract on mycelial dry weight: Mycelial dry weight was significantly inhibited by the various extracts but percentage inhibition differed significantly (p<0.05) from one extract to another. *Azadirachta indica* extract was significantly the most superior causing 62.37% inhibition, while *E. camaldulensis* extract was the least with 46.97% inhibition (Table 5).

Table 6: Main effect of concentration on the mycelial dry weight of *Phytophthora colocasiae*

Concentrations (mg/mL)	Mycelial dry weight (mm)	Percentage inhibition
Control 0	0.33±0.44[d]	0.00±0.00[d]
5	0.10±0.37[c]	69.95±11.85[c]
10	0.04±0.16[b]	89.39±0.00[b]
20	0.00±0.21[a]	100.00±0.00[a]

Each mean represents the overall performance of a given concentration irrespective of extract, Means followed by the same letter within a column are not significantly different and p<0.05

Main effect of concentration on a mycelial dry weight: The main effect of concentrations irrespective of the individual extract on the mycelial dry weight of *P. colocasiae* indicated that all the concentrations inhibited mycelial dry weight except the control (0 mg/mL) and the inhibition increases with higher concentration (Table 6). The most potent concentration was 20 mg/mL causing 100.00% inhibition which significantly ($p<0.05$) differed from 89.39 and 69.95% inhibitions caused by 10 and 5 mg/mL, respectively.

DISCUSSION

Germination of fungal inoculum, growth of mycelium and sporulation are important physiological processes in pathogenesis. Consequently, any botanical extract that affects these aspects of a pathogen's life cycle would invariably affect the survival of such a pathogen on its host given the necessary conditions. Hence the systematic investigation and development of botanical extracts for the control of plant diseases are based on established inhibition of these important physiological processes during disease development.

The results of the present study have shown that the four plant extracts inhibited radial growth and mycelia dry weight of *P. colocasiae* in varying degrees. This was in line with the findings of earlier investigators who observed varying degrees of fungitoxicity with different plant extracts used in the control of fungal pathogens[47-49]. The crude plant extracts may have differed in their activities against the pathogen probably due to the fact that extracts of different plant species and genera vary in their ability to control pathogens largely due to differences in their photochemical constituents. The observed variations in the performance of the extracts could also be attributed to the age of the plants, the method of extraction and the type of solvent used for extraction[50-52]. It is possible that ethanol was either not effective as a solvent for the extraction of the active principles in some of the plants whose extracts exhibited moderate inhibition or could not extract the active principles in large enough quantities.

In the radial growth test, results indicated that *A. indica* extract was the most potent in inhibiting the radial growth of the pathogen while the other extracts showed high to moderate inhibitions. The result supports the observation of other researchers[52,53], who found that neem leaf extract was very efficient in reducing the growth of *Fusarium solani* f.sp. Melongenae, a number of yams rot pathogens (*Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Penicillium digitatum* and *Rhizopus stolonifer*) and *Phytophthora colocasiae* in different experiments.

However, *S. alata* and *T. diversifola* extracts achieved complete inhibition of mycelia weight at all tested concentrations while with *A. indica* and *E. camaldulensis* extracts complete inhibition was not observed in all concentrations.

The observed differences in the performance of these extracts in the solid (PDA) and liquid (PDB) media may be attributed to one or more of such physical and chemical factors that influence the performance of active principles in mixtures[54]. The factors could be atmospheric interaction, nature of the test (*in vivo* or *in vitro*) type of medium (liquid or solid) particle size and redistribution or longevity of active compounds in solution.

The effective performance shown by *A. indica*, *S. alata* and *T. diversifolia* in this study corroborated the findings of other workers who demonstrated the effectiveness of these plant extracts in different trials. Barra *et al.*[39] reported similar results with leaf extract of *A. indica* as a foliar spray against *P. colocasiae*. Similarly, Ashfaq and Yousaf[55] reported the effectiveness of *S. alata* against some fungal pathogens. Chege and Kimaru[56] reported the effectiveness of *T. diversifolia* and *Allium sativum* extracts on *Colletotrichum gloeosporioides* the causal agent of anthracnose in avocado.

Although *E. camaldulensis* extract was the least effective of the four extracts in inhibiting radial growth and mycelia dry weight of *P. colocasiae*, other researchers have lauded its broad-spectrum antimicrobial activities on a number of pathogens[57]. The potency of its leaf extracts and essential oils against *Candida* spp.[58,59] and various dermatophytes including *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum* and *T. Verrucosum* was reported[60]. Barra *et al.*[39] also reported the efficacy of *E. camaldulensis* against *Aspergillus niger* and *Botrytis cinerea*.

The maximum inhibition exhibited by Ridomil which was used as a positive check in this trial supported its usage as a standard foliar chemical in the management of taro leaf blight disease as confirmed by Adomako *et al.*[61].

With respect to the mycelia dry weight test, *S. alata* and *T. diversifolia* recorded no yield in PDB while *A. indica* and *E. camaldulensis* had minimal yields. This result suggested that the infective inoculum of *P. colocasiae* germinated sparingly in the liquid medium amended with the extracts of *A. indica* and *E. camaldulensis* and did not germinate at all in the liquid medium of *S. alata* and *T. diversifolia* extracts. The observed inhibition of spore germination caused by *S. alata* and *T. diversifolia* in the broth and the resultant total (100%) inhibition of mycelia suggests that the activities of these two extracts may be better with a liquid medium which contains ample amounts of water that holds the active principles in solution. It also suggests that these two extracts could be used as biocide-dip in pre-planting treatment for soaking planting materials such as corms and cormels to forestall the emergence of mycelia of the pathogen that might be hidden within them.

However, these observations and suggestions are preliminary in this line of research and require further testing for confirmation. From the present study results, it is evident that various levels of activity were shown by the extracts against the pathogen. This may have a strong correlation with the character of the active ingredient in each crude extract emphasizing, therefore, the need to fractionalize each extract to identify the specific agents responsible for the inhibition and their minimum inhibitory concentrations. This will give us a clue on the actual quantities to be recommended to farmers for use in the control of taro leaf blight disease.

From the results of this experiment, further evaluations of these extracts with other extracting solvents and more trials in the field and screen house to fully ascertain their potential as biocides against the ugly scourge taro leaf blight are recommended. It is hoped that this information will be useful in the selection of plant-derived fungicides for *P. colocasiae* control to recommend the potent ones to farmers as local remedies while further research is in view to characterize their active ingredients for large-scale production.

CONCLUSION

The remarkable inhibition of radial growth and mycelial weight of *P. colocasiae* has been demonstrated by some of the extracts. *Azadirachta indica* proved to be the most potent herbal alternative in the radial growth test while *Senna alata* and *Tithonia diversifolia* were the best and exhibited maximum inhibition in the broth test. These established inhibitions of physiological processes in the pathogen suggest that *A. indica*, *S. alata* and *T. diversifolia* extracts are good candidates for the control of *P. colocasiae* and the blight it causes.

SIGNIFICANCE STATEMENT

This research was undertaken to ascertain the potential toxicity of selected botanicals against *P. colocasiae*, causing TLB disease. *Phytophthora* leaf blight causes heavy loss of taro produce with consequent scarcity and exorbitant prices, lack of planting materials and loss of important genetic resources. These challenges coupled with the adverse effects of chemical fungicides for control of diseases necessitated the research to develop alternative biofriendly measures to boost production. Results established that the crude extracts of *Tithonia*, *Senna* and *Azadirachta* were toxic to the pathogen and produced strong inhibition on growth indicating that they can be employed as local remedies. However, there is a need to fractionalize the crude extracts and determine the active ingredients and the minimum inhibitory concentrations.

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