Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 18(4): 1224-1244, August 2024

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Assessment of leaf blight on *Colocasia esculenta* and *in vitro* effect of three medicinal plants extracts on leaf blight disease pathogen in Oshie-Njikwa, North West Region-Cameroon

Eugene NJEH ASONGWE, Alphonse ERVE NOUCK and Rosemary KINGE TONJOCK*

Department of Plant Sciences, Faculty of Science, The University of Bamenda, P.O. Box 39, Bambili, Northwest Region, Cameroon.

** Corresponding author; E-mail: rosemary32us@yahoo.com*

Received: 10-04-2024 Accepted: 15-07-2024 Published: 31-08-2024

ABSTRACT

Taro (*Colocasia esculenta*) is a major staple food in Cameroon. This work assessed leaf blight disease on *Colocasia esculenta* and *in vitro* effect of three medicinal plants extracts on the pathogen in Oshie. Questionnaires were administered to 200 randomly selected farmers to assess the impact of the disease. Eight farms in Oshie were surveyed for leaf blight disease during a period of six weeks for disease incidence and severity. Twenty diseased leaf samples were collected, inoculated on Potato Dextrose Agar and after 7 days of incubation, pure cultures were identified. Crude extracts of hexane and methanol from leaves of *Clematis hirsuta*, *Clematopsis scabiosifolia* and *Telferia occidentalis* were evaluated for *in vitro* antifungal activities on *Phytophthora colocasiae.* Leaf blight disease of taro had a huge impact on farmers as it reduced yield of the tubers. Disease incidence ranged between 15% and 99%. Disease severity ranged from 46.24% to 61.82%. All three plants extract at 0.5 g/ml concentration had inhibitory activities on the test fungus. The three plants extracts obtained using methanol inhibited the growth of *Phytopthora colocasiae* more than the same three plants extracts obtained using hexane. The three medicinal plants can be used to control taro leaf blight *in vitro*.

Keywords: Taro Leaf blight, Incidence, Severity, Plant extracts, Bioactivity.

INTRODUCTION

Taro is viewed by many agricultural traditions as a staple food. This ties with the view of Ooka and Brennan (2000) who stipulated that Taro (*Colocasia esculenta* (L.) Schott) is a major staple food and remains an important crop to many cultural and agricultural traditions worldwide. Joshua (2010) supported this claim and indicated that, it is consumed as a staple crop in West Africa, particularly in Ghana, Nigeria and Cameroon. All parts of the plant including corm, cormels, rhizome, stalk, leaves and flowers are edible and contain ample starch (Bose et al., 2003). Taro leaves are nutritious and contain higher levels of protein, carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fibres. According to FAO (2013) statistics on high producers of taro worldwide, Nigeria, China, Ghana and Cameroon were ranked the top producers of taro. Cameroon produces 1.5

DOI : https://dx.doi.org/10.4314/ijbcs.v18i4.3

million tons as compared to Nigeria whose total production was estimated at 3.2 million tons (FAO, 2012).

Despite the advent of improved varieties and increased trade, an increase in crop diseases has been observed. Monoculture, ignorance of the modes of contamination, the conditions conducive to the appearance of the diseases, the biology of pathogens, and their rapid development would cause significant economic losses (Yaya et al., 2023). Virus related diseases have been a dilemma to taro producers as these diseases decrease the crop yield. This is in line with the research of Yusop et al. (2019) that taro is affected by viral diseases which decrease the yield. Mbong et al. (2013) posits that the constraints to taro production are diseases and pests. The crop is susceptible to fungal, bacterial, viral and nematode infections (Gadre and Joshi, 2003). Singh et al. (2006) theorized that among infectious diseases that affect crops, taro leaf blight disease is one of the most economically important diseases of taro because it reduces corm yield up to 50 and leaf yield of up to 95% in susceptible genotypes. *Phytopthora colocasiae* causes corms to rot both in the field and in storage, and this has led to heavy storing lost (Brunt et al., 2001). This fungal disease in taro known as leaf blight was first observed in Cameroon in 2010, and resulted in 50 to 100% taro yields loss in most of the crop growing regions (Guarion, 2010; Fontem and Mbong, 2011).

Leaf blight disease of taro has led to a reduction in planting materials, food, household income, increase poverty and some farmers have abandoned their farms and are now growing other crops (Guarion, 2010; Fontem and Mbong, 2011). Diseased plants have small, brown, water-soaked lesions that enlarge and coalesce into large lesions with yellow exudate, ultimately leading to the defoliation and decay of the plants within few weeks (Omane et al., 2012). Misra et al. (2007) devised a farmer-friendly integrated disease management bundle to managed taro blight which includes growing short-duration crop with early planting i.e., in March, one protective spray with mancozeb (0.2%) at 45 days after planting followed by one spray with metalaxyl (0.05%) at 60 days after planting, intercropping with non-host crops like okra, use of disease free seed tubers and seed tuber treatment with *Trichoderma viride*.

Pests and disease infestation are among the greatest challenge faced by taro farmers. Fungi infestation commonly called taro leaf blight is the predominant taro disease and has negatively affected the yield of taro for the past years (Guarion, 2010). If these pests and diseases remain untreated, the future is uncertain, as it is not clear if alternative food crops can fill the gap left by the demise of taro. Fortunately, several treatments approaches are adopted with the use of chemically synthesized antimicrobial compounds being the most applied (Fullerton and Tyson, 2003). The use of chemically synthesized fungicides has registered much limits such as environmental hazards, health related problems and worse being the development of resistance by the fungi. Plants are well known sources of bioactive compounds (Wu et al., 2023). These bioactive compounds have shown to exert antimicrobial effects on some bacteria and fungi. It is estimated that, only about 1/3 of the plant's biodiversity potentials have been assessed. *Clematopsis scabiosifolia, Clematis hirsuta* and *Telferia occidentalis* are plant species well known by the Oshie population as it is used in fork medicine in infusions or decoctions to treat health related problems of microbial origin. The three plants selected are from two botanical families. *Clematis hirsuta* and *Clematopsis scabiosifiolia* are from the family Ranunculaceae while *Telferia occidentalis* is from the Cucurbitaceae family. In view of these, the present investigation was undertaken to assess the indigenous knowledge, incidence and severity of Taro leaf blight caused by *Phytophthora colocasiae* and determine the in-vitro effect of three medicinal plants on the pathogen in Oshie.

MATERIALS AND METHODS

Study area

This study was carried in Oshie village (Figure 1), located in Njikwa sub-division, Momo Division of the Northwest region of

Cameroon. Oshie experiences the tropical climate which has two distinct seasons; the wet and dry seasons. The rainy season starts in mid-March and extends up to mid-October. During the rainy season, the south west trade winds which bring moisture from the Atlantic Ocean bring orographic and convectional rainfall because of the relief features that surrounds the region. The average yearly rainfall here is about 2500 mm. The average temperature during this season is about 18°C and the humidity is quite high due to the presence of moisture in the atmosphere (Binda, 2008). Oshie has different soil types mostly the fertile alluvial soils which have favoured agriculture as the majority of the inhabitance depends on subsistence farming. The Oshie people are mostly peasant farmers who engage in agriculture for their livelihood. Almost 90% of the population is involved in agriculture.

Indigenous knowledge and impact of the Disease on the Farmers / Population of Oshie

To investigate the traditional knowledge and effects of taro leaf blight on the livelihood of the population of Oshie, semi structured questionnaires, consisting of closed and open-ended questions were administered to farmers. The questionnaires were administered at random in all the ten quarters of Oshie. The questionnaires were administered per quarter as follows: Fum = 10, Fumeibei = 12, Beiban = 16, Edom = 14, Fun = 08, Nyebai = 28, Ekeh = 20, Togobei = 24, Bereje = 32 and Barimbong = 36. The number of questionnaires administered in some quarters were relatively small due to the crisis that has forced some farmers to leave. A total of 200 persons were administered the questionnaire amongst which were 71 men, 98 women and 31 youths.

Disease Incidence and Severity Assessment

Disease incidence

Eight (08) farms of equal dimensions (25 m x 25 m) were selected for survey for disease incidence and severity. The farms were selected based on the relief and vegetation of the quarters. The farms in Ekeh, Edom, Fun, Fum and Nyebai were located on hills with open savannah vegetation, while the farms in Bereje and Togobei were located in the valleys with patches of gallery forest. The farm in Barimbong was located on a hill with patches of natural forest. A total of 1600 plants, taking two hundred plants per farm were assessed for disease incidence. The sampling for disease incidence was carried out during the rainy season for a period of six weeks from 15/08/2022 to 25/09/2022. Disease incidence was assessed on the entire plants in each quarter. The percentage incidence of the blight disease was computed using the formula as the ratio of the number of *Colocasia esculenta* plants with the disease symptoms to the total number of *Colocasia esculenta* plants assessed multiplied by 100 (Chaube et al., 2005).

$$\textbf{Disease Incidence (\%)} = \frac{\text{Number of infected } \textit{Colocasia esculenta} \text{ plants}}{\text{Total number of taro plants assessed } 1} \times 100$$

Disease severity

The sampling for disease severity was carried out for a period of six weeks from 15/08/2022 to 25/09/2022. The severity of the blight on the *Colocasia esculenta* plants observed in the various quarters in Oshie was calculated and scored as described by (Chaube et al., 2005) below, and defined as the ratio of the area of *Colocasia esculenta* leaves infected over total area of *Colocasia esculenta* leaves multiplied by 100. In each farm, ten (10) plants were randomly assessed and mean scored for severity indices.

$$\textbf{Disease Severity (\%)} = \frac{\text{Area of } \textit{Colocasia esculenta} \text{ Leaves Infected}}{\text{Total area of } \textit{Colocasia esculenta} \text{ Leaves}} \times 100$$

The disease severity measurement was based on a 5-point score (0 – 4 SCALE), as shown in Table 1.

Sample Collection, Isolation and Identification of the Pathogen

Sample collection

Sampling was done through purposive sampling; where collection of diseased leaves of *Colocasia esculenta* was done by judging from symptoms. Portions of infected leaves showing typical symptoms of taro blight of

Colocasia esculenta were collected from 10 taro farms in Oshie. Twenty samples were collected, taking two samples per farm in each quarter. These samples were placed in separate zip lock bags, labelled with a code representing the quarter from which the samples were collected. These samples were placed in separate zip lock bags.

Isolation and identification

Isolation of the fungus was done as described by Zhu et al. (2001). Leaf fragments showing young lesions were washed under running tap and cut into approximately 1 cm^2. These samples were surface sterilized in tap water, 10% sodium hypochlorite, then distilled water, 70% ethanol and sterile distilled water for two minutes. They were rinsed twice with sterile distilled water, blotted dry on sterilized filter paper and plated in 20 petri dishes containing Potato Dextrose Agar (PDA), as seen in Figure 2.

The plates were labelled with codes representing the quarters from which the samples were collected and incubated at room temperature for 6 days. After 6 days, a sterilized inoculation loop was used to pick up samples from cultured plates, mounted on slides stained with methylene blue for clarity and mounted on the stage of a light microscope for observation. The fungus was observed and identified under a light microscope based on cultural and morphological characteristics, followed by web-based identification guides. After identification, these samples were sub cultured by taking a portion of the mycelium using a 5 mm cork borer and inoculating it on a fresh plate of PDA. Hyphal tips were transferred to freshly prepared Potato Dextrose Agar in 20 petri dishes. All collected samples were processed within 72 hours of collection. The pathogen isolated was purified and identified on the basis of morphological characteristics (Waterhouse, 1963). Cultural characteristics observed from the pure cultures in the laboratory were recorded and analysed using descriptive statistics and results presented in tables.

In-vitro* control of fungi that cause leaf blight disease on *Colocasia esculenta

Source of Plant Material and Extraction

Three medicinal plants; *Clematis hirsuta, Clematopsis scabiosifolia* and *Telferia occidentalis* were collected from Oshie (Figure 1). These three plant species are well known by the Oshie population and are highly used in fork medicine as either infusion or decoction to treat health related problems of microbial origin. The researcher has been observing these three plants for long and notice that their leaves are hardly attack by blight especially the young ones.

The extraction process took place in the Natural Products and Medicinal Chemistry Laboratory of the University of Buea. The extracts from these medicinal plants were prepared from the leaves of these plants (Figure 3). The collected plant leaves were washed thoroughly with running tap water and distilled water, the samples were shade dried and finely ground. The powdered plant material was weighed to 300 g and poured in a 1000 ml conical flask containing hexane, stirred homogenously and then allowed to soak for 2 days. It was then filtered using Whatman filter paper to clarity into round bottom flask and dried with a water bath and rotary evaporator respectively. This separated the solvent from the plant extract. The plant materials were first extracted with hexane, then with methanol.

The Food Poison Technique

Half a gram (0.5) of the pure extract of each plant was added to 1ml of methylene chloride to ensure it was in solution form for easy diffusion in the culture media. A drop of each plant extract was added to 20 ml of sterile PDA in Petri plates. The plates immediately upon addition of the plant extract were shaken to ensure the plant extracts spread uniformly in the culture media. The plates were allowed to cool and the methylene chloride to evaporate. A 5 mm diameter of the actively growing mycelium disc of the pathogen of 6-7 days old culture was placed in the centre of the petri dishes. Plates without plant extract served as controls. In all, ten plates for each plant extract and each plant had two extracts obtained with

two separate solvents were cultured. Upon inoculation, the plates were incubated at room temperature and kept for one week. After one week, the plates were observed and the colony diameter (in mm) of the plates were recorded. The results were compared with the control. The experiment was repeated twice and means of the two readings was taken for calculations as seen on Tables 2 and 3 respectively.

X= colony diameter of plates with *Clematis hirsuta* extract

Y=colony diameter of plates with *Clematopsis scabiosifolia* extract

Z=colony diameter of plates with *Telferia occidentalis* extract

Percent growth inhibition of the fungus was calculated using the formula;

I= [(C-T)/C] × 100

Where

I= Percent Growth Inhibition (%)

C= Colony diameter in control (mm)

T= Colony diameter in the presence of plant extracts (mm) (Vincent, 1947).

Data Analysis

Data collected to assess the impact of the disease on the farmers/population was analyzed using descriptive statistics. The data was analysed using the statistical formula;

$$\textbf{\% response} = \frac{\textit{frequency of a particular response option } (f)}{\textit{Total sample } (N)} \times 100$$

Data on disease incidence and severity was analysed by computing means first per week and secondly for the entire collection period (6weeks). Cultural characteristics observed from the pure cultures in the laboratory were analysed using descriptive statistics. The data was analysed using the statistical formula;

$$\% \text{ per given character} = \frac{\textit{frequency of a given cultural characteristic } (f)}{\textit{Total sample } (N)} \times 100$$

The effect of the three medicinal plants extract on the pathogen *in vitro* were analysed by calculating percentage inhibition. The above analysis was done using the statistical package statgraphics centurion version 18.0.0.1 for windows.

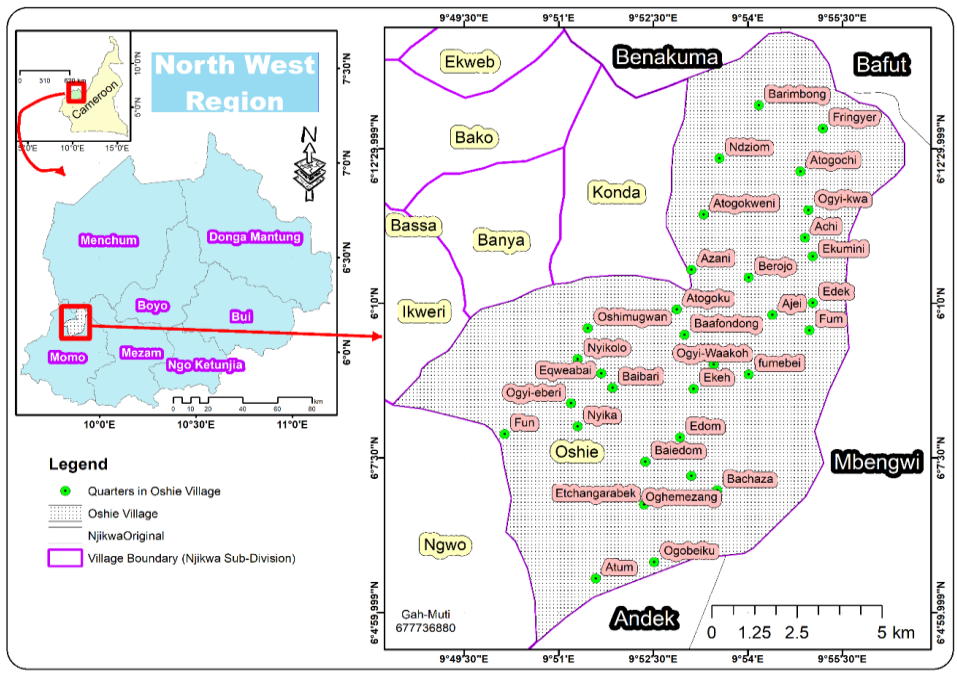


Figure 1: Map of Oshie showing the study area sites.

Table 1: A 5-point score for disease severity measurement.

Scale	Severity score		Interpretation
0	< 1	=	No infection
1	1 – 25	=	Low infection
2	26 – 50	=	Moderate infection
3	51 -75	=	High infection
4	>75	=	Very high infection

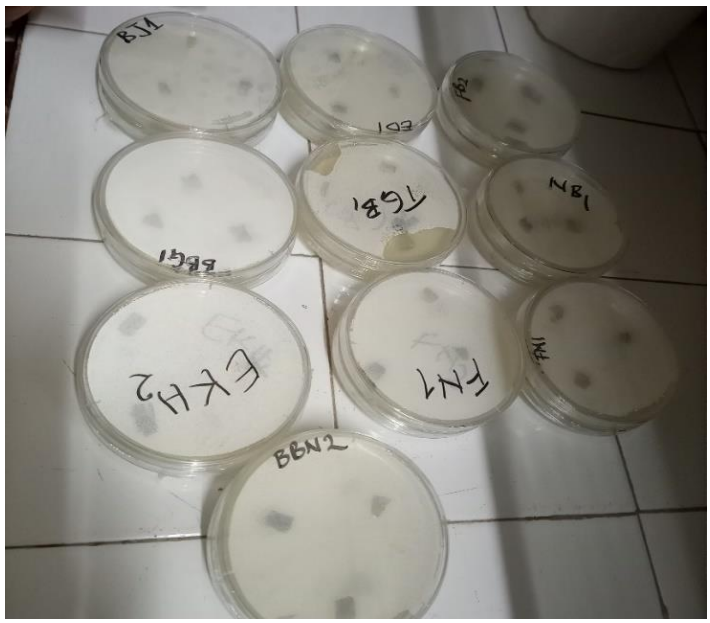


Figure 2: Labelled inoculated media in petri dishes ready for incubation.

Figure 3: Images of A-*Clematis hirsuta*, B-*Clematopsis scabiosifolia* and C-*Telferia occidentalis.*

Table 2: Colony diameters of plates with the three plants extracts obtained with hexane (non polar solvent).

S/N	CODE	X(mm)	Y(mm)	Z(mm)	CONTROL (mm)
1	**BBG_2**	47	47	49	71
2	**BBN_1**	78	63	59	75
3	**BJ_1**	64	64	57	65
4	**ED_2**	60	56	69	50
5	**EKH_2**	58	59	64	69
6	**FB_1**	58	65	54	69
7	**FM_2**	49	54	40	61
8	**FN_1**	64	59	53	57
9	**NB_1**	75	64	68	65
10	**TGB_2**	74	61	72	75

BBG =isolate obtain from sample collected from Barimbong, BBN= **B**eiban**,** BJ= Bereje**,**
ED = Edom, EKH = Ekeh, FB= Fumeibei, FM= Fum, FN = Fun, NB = Nyebai and TGB= Togobei. 1 and 2 represent sample one and two since two samples were collected per farm in each quarter. The codes apply same for all tables.

Table 3: Colony diameters of plates with the three plants extracts obtained with methanol (polar solvent).

S/N	CODE	X(mm)	Y(mm)	Z(mm)	CONTROL (mm)
1	**BBG_2**	24	01	51	71
2	**BBN_1**	38	16	73	75
3	**BJ_1**	32	03	71	65
4	**ED_2**	46	19	66	50
5	**EKH_2**	53	21	56	69
6	**FB_1**	47	04	36	69
7	**FM_2**	60	16	60	61
8	**FN_1**	43	14	61	57
9	**NB_1**	42	06	67	65
10	**TGB_2**	50	13	67	75

RESULTS

Indigenous knowledge and impact of taro blight on *Colocasia esculenta*

Data obtained from the questionnaires revealed that all the farmers administered questionnaires are aware of the disease (taro leaf blight of *Colocasia esculenta*) and their farms had been attacked by the disease for varying seasons (Figure 4). Ninety-point five percent (90.5%) of the farmers could identify and differentiate healthy *Colocasia esculenta* plants from the ones infected by taro leaf blight

Seventy-seven of the farmers indicated that visible symptoms were seen on the plants after five months from the date of planting, 46 farmers said visible symptoms could be seen on the plant after four months from the date of planting meanwhile 39 said visible symptoms were seen after three months from the date of planting as shown in Figure 5.

Seventy-five (75%) of the farmers indicated that the most affected part of the plant due to leaf blight were the leaves, 17 stated that the leaves and tubers were the most affected parts. Eleven farmers were of the opinion that the leaves and stems were the most affected parts whereas 16 farmers indicated that all plant parts were seriously affected by the disease as seen in Figure 6.

One hundred and eighty-six farmers said no form of treatment were administered whenever their farms were attacked by taro leaf blight of *Colocasia esculenta* (Figure 7).

Ninety-five-point-five percent (95.5%) of the farmers indicated that the yield of *Colocasia esculenta* had dropped and even supply to the local market due to taro leaf blight while 186 farmers stated that the storage life of the tubers was highly affected by the disease and decay after a few days in storage as shown in Figure 8.

Disease Incidence and Severity

Disease incidence

Data from the survey of eight farms of equal dimension 25mx25m revealed that the percentage incidence ranged between 15% and 99% in the different farms for the six weeks of observation as show in Figure 9.

Togobei recorded the highest incidence of 59% while Edom had the lowest incidence of 15% for week one of the survey. Barimbong had the highest incidence of 67.5% and Ekeh scored the lowest incidence of 18% during week two of the survey. Togobei scored the highest incidence of 78.5%, 94%, 98% and 99% for week three, four, five and six of the survey respectively. Nyebai recorded the lowest incidence of 26.5% and 38% for week three and four of the survey respectively whereas Ekeh recorded the lowest incidence of 61.5% and 68% for week five and six of the survey respectively as seen in Figure 10. The highest mean percentage incidence (81.58%) was observed in Togobei and the lowest (40.08%) was observed in Ekeh as seen in Figure 10.

Disease severity

The mean percentage severity for eight (08) farms for each week were calculated and presented in Table 4 for a period of six (06) weeks. The mean percentage severity for the six weeks ranged between 08.35% and 89.44% in the different farms. Togobei had the highest mean percentage severity of 35.45%, 63.36% and 89.44% for the 1st, 2nd and 3rd weeks of the survey respectively. Nyebai scored the lowest mean percentage severity of 19.23% for the 1st week meanwhile Bereje recorded the lowest mean percentage severity of 27.40% and 08.35% for the 2nd and 3rd week respectively. Barimbong had the highest mean percentage severity of 89.44% during the 4th week while Bereje recorded the highest mean percentage severity of 72.19% and 82.73% for the 5th and 6th weeks respectively. Togobei was observed to have the lowest mean percentage severity of 44.0% and 33.88% for the 4th and 5th weeks of the survey and Ekeh scored the lowest mean percentage severity of 53.70% during the 6th week of the survey (Table 4).

From Table 4, the mean (severity score) for each farm after six weeks of the survey were computed, scaled and presented in a Table 5. Data from the survey revealed that, the mean percentage severity for the six weeks (severity score) ranged from 46.25% in Fum to 61.82% in Barimbong (Table 5). The farms in Barimbong, Ekeh, Fun and Togobei had very

high infection rates whereas those in Bereje, Edom, Fum and Nyebai had moderate infection rates after the six weeks of the survey (Table 5).

It should be noted that ten farms were chosen to be surveyed for disease incidence and severity but the data above is for 8 farms. One field assistance failed to collect complete data. Therefore, the incomplete data for the 2 farms was not analyse and the two farms were not considered as part of the study for disease incidence and severity

Characteristics of strains of *Phytophthora colocasiae*

After subculturing twice, 16 out of 20 of the infected samples gave pure cultures of *Phytophthora colocasiae*. The cultural characteristics of the 16 pure cultures were observed and recorded in Table 6. The cultural characteristics observed were; colony form, colony elevation, surface colony colour, reverse colony colour. The colony diameters of the pure cultures were measured using a transparent ruler and recorded too.

NB$_1$: when measurements of colony diameter were made, 5 mm was subtracted from each of the diameter measured. This 5mm was the initial inoculant taken from the initial culture and introduced into each petri dish with the use of a 5 mm cork borer during the sub-culturing process.

NB$_2$:10% glycerol was prepared by dissolving 10ml of glycerol in 90 ml of distilled water. Samples of the pure cultures were taken and preserved in 10% glycerol and sterile distilled water in eppendorf and screw cap tubes for future studies.

The results for the cultural characters are presented in Table 7. Thirteen (81.25%) out of the 16 isolates had a regular colony form whereas 3 (18.75%) had an irregular colony form. Twelve (75%) of the isolates had umbonate colony elevation meanwhile 04 (25%) had raised colony elevation. Seven (43.75%) out of the 16 isolates were observed to have an ash surface colony colour, 4 (25%) had a creamy surface colony colour and 5 (31.25) were observed to have a fluffy surface colony colour. Reverse colony colour was observed to be violet in 6 (37.5%) out of the 16 isolates meanwhile 4 (25%) were whitish yellow and 6 (37.5%) of the isolates had a creamy reverse colony colour as summarised in Table 7.

Micro-morphological identification

Subcultured isolates were observed under a light microscope at 40x and 10x. The sporangia seen under the microscope were compared with web-based images of *Phytopthora colocasiae* and were observed to be semi papillate, caducaous and ovoid in shape, confirming the isolates to be *Phytopthora colocasiae* (Figure 11A).

Based on cultural, and micro-morphological characters, the 16 fungi isolates were believed to *Phytopthora colocasiae.* Colonies produced by *P. colocasiae* are white with cottony growth pattern (Figure 11B). Mycelium is hyaline, coenocytic with less than 1 µm diameter. Sporangia are formed terminally on aseptate sporangiophore and are semi-papillate, caducous, ovoid in shape with mean diameter ranging from 77×43.2 µm with short pedicel (3.7-5.9 µm).

***In vitro* activity of plant extracts**

Three plant extracts (*Clematis hirsuta, Clematopsis scabiosifolia* and *Telferia occidentalis)* were tested against ten isolates of *Phytopthora colocasiae*. The ten isolates represented the ten farms from the ten quarters in Oshie from which the infected samples initially came from. The percentage growth inhibition of the various isolate plates with the three plants extracts obtained using hexane and methanol at the same concentration of 0.5 g/ml were calculated and are presented in Table 8.

Going by the results shown in Table 8, the fungus growth inhibition ranged between 1.33% and 34.43% against *Phytopthora colocasiae* for the three plants extracts obtained using hexane as the solvent of extraction. Six out of the 10 isolates were inhibited by *Clematis hirsuta* extract obtained using hexane, eight out of the 10 isolates were inhibited by *Clematopsis scabiosifolia* and *Telferia occidentalis* extracts obtained using

hexane as solvent of extraction at a concentration of 0.5 g/ml (Figure 12).

The fungus growth inhibition ranged between 1.64% and 98.59% against *Phytopthora colocasiae* for the three plants extracts obtained using methanol as the solvent of extraction. All 10 isolates were inhibited by *Clematis hirsuta* and *Clematopsis scabiosifolia* extracts while 6 out of 10 isolates were inhibited by *Telferia occidentalis* extract obtained using methanol as solvent of extraction at a concentration of 0.5 g/ml (Figure 13).

The maximum growth inhibition against *Phytophthora colocasiae* (all 10 isolates inhibited) were recorded by *Clematis hirsuta*, and *Clematopsis scabiosifiolia* and the least growth inhibition (6 out of 10 isolates) by *Telferia occidentalis* at a concentration of 0.5 g/ml. The three plants extracts obtained using methanol as the solvent of extraction inhibited the growth of *Phytopthora colocasiae* more than the same three plants extracts obtained using hexane as the extraction solvent. Some isolates were not inhibited by the three plants extracts irrespective of whether the extracts were obtained using methanol or hexane. These isolates had negative values of percentage growth inhibition but their absolute values were taken and are represented in Table 8 in red. The growth of the isolates in this case were rather promoted by the extracts.

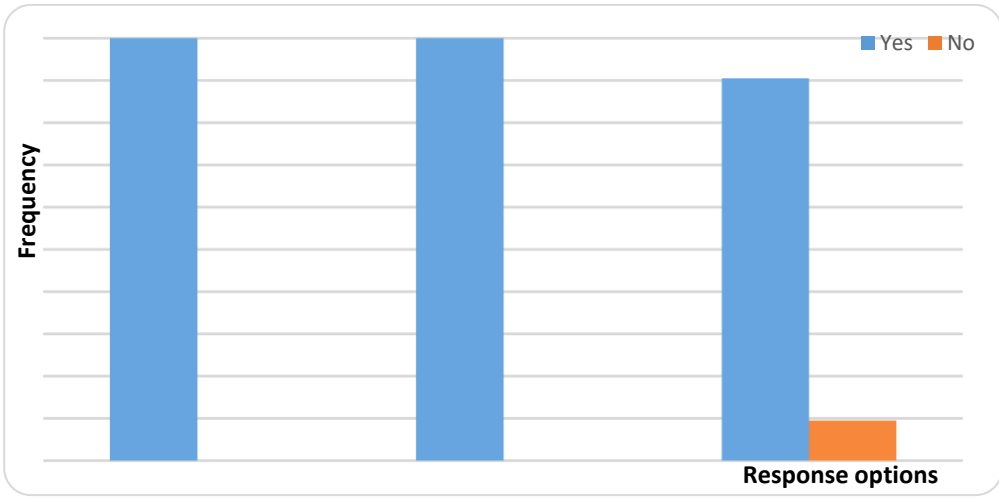


Figure 4: Awareness, infestation and identification of blight.

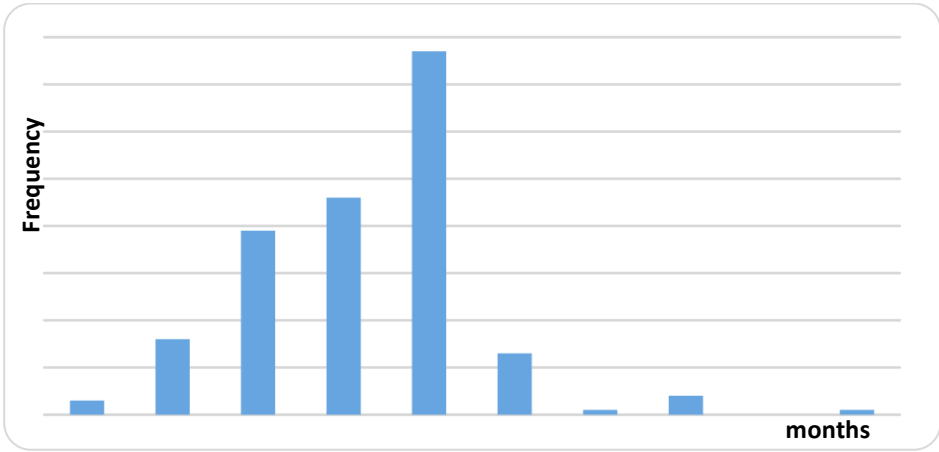


Figure 5: Visible symptoms of leaf blight in months.

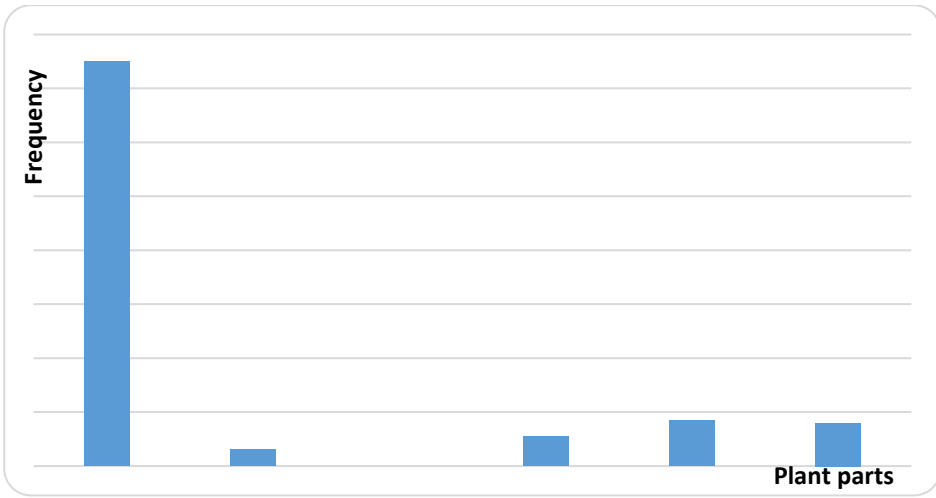


Figure 6: Taro leaf blight infested plant part(s).

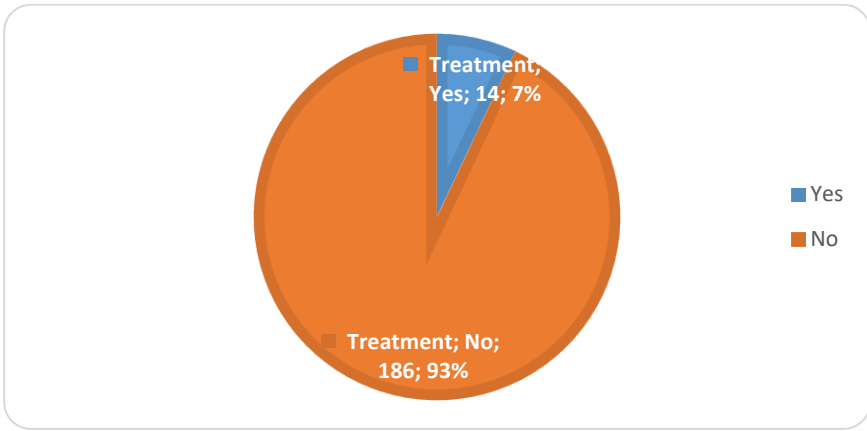


Figure 7: Administering treatment of taro leaf blight of *Colocasia esculenta.*

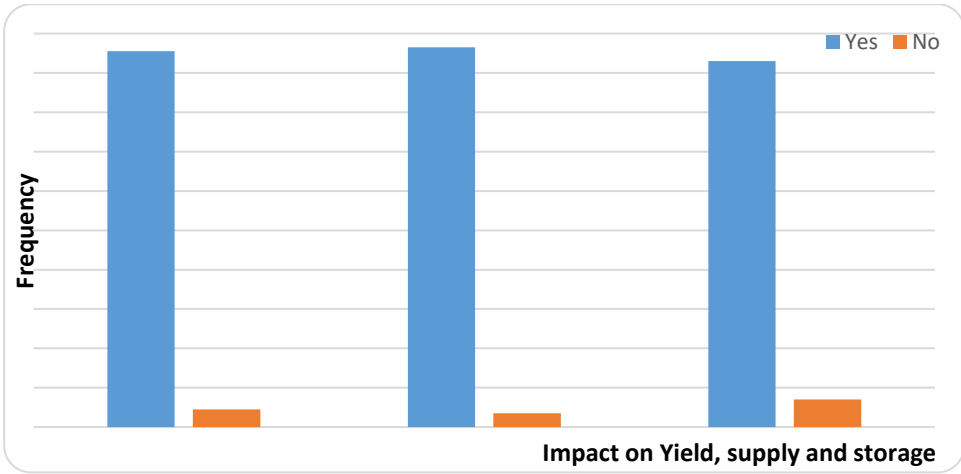


Figure 8: Impact of taro leaf blight on yield, supply and storage of *Colocasia esculenta.*

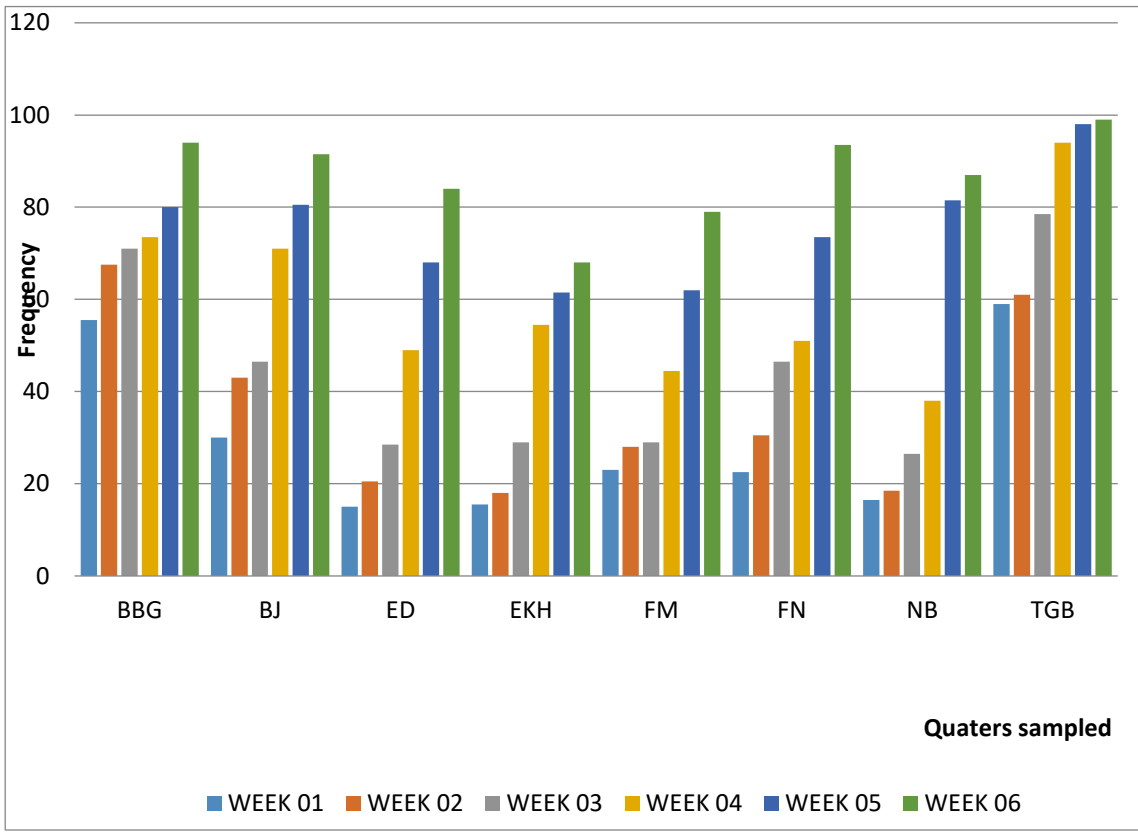


Figure 9: Percentage incidence of leaf blight of *Colocasia esculenta* of eight farms for a period of 6 weeks.

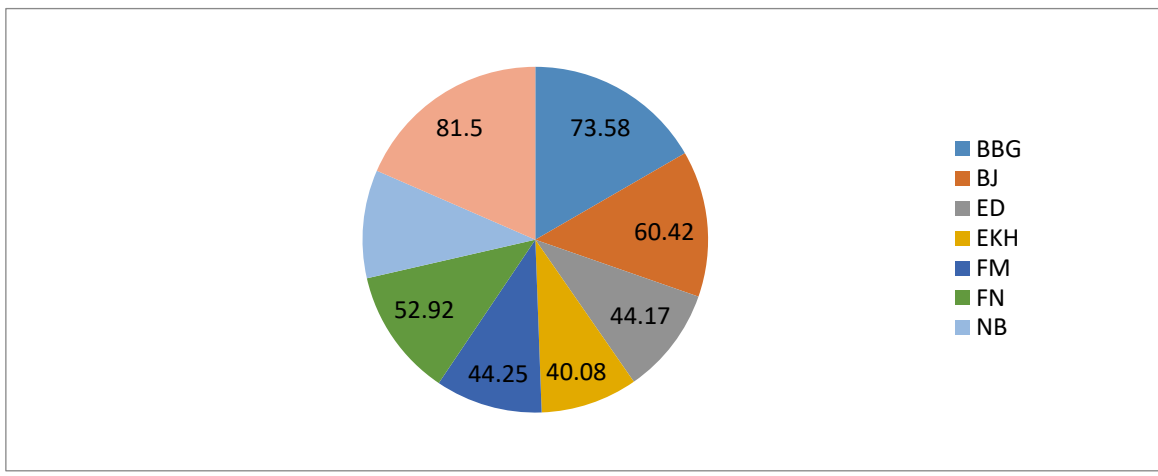


Figure 10: Mean percentage incidence of leaf blight of *Colocasia esculenta* of eight farms for a period of 6weeks.

Table 4: Mean percentage severity for eight (08) farms for six weeks.

CODE	WEEK 01	WEEK 02	WEEK 03	WEEK 04	WEEK 05	WEEK 06
BBG	32.16	54.59	76.91	87.58	63.61	56.05
BJ	34.67	27.40	08.35	66.37	72.19	82.73
ED	28.33	50.03	18.33	44.98	66.54	75.69
EKH	31.70	55.14	66.72	73.17	51.31	53.70
FM	26.47	50.58	33.73	63.73	43.29	59.69
FN	32.41	55.57	73.96	53.83	71.23	59.73
NB	19.23	45.43	44.55	65.31	69.23	59.11
TGB	35.45	63.36	89.44	44.00	33.88	65.78

Table 5: The mean (severity score) for each farm after six weeks.

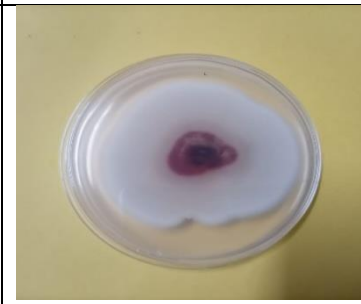
CODE	Mean (severity score)	scale	Interpretation
BBG	61.822	03	High infection
BJ	48.62	02	Moderate infection
ED	47.32	02	Moderate infection
EKH	55.29	03	High infection
FM	46.25	02	Moderate infection
FN	57.79	03	High infection
NB	50.48	02	Moderate infection
TGB	55.32	03	High infection

Table 6: Cultural characteristics of the 16 isolates.

Sample code	Colony diameter (mm)	Colony form	Colony elevation	Surface colony colour	Reverse colony colour
BBG_1	64	Circular	Raised	Ash	Creamy
BBG_2	56	Irregular	Raised	Fluffy white	Creamy
BBN_1	75	Circular	Raised	Fluffy white	Creamy
BJ_1	62	Circular	Umbonate	Creamy	Whitish yellow
ED_1	69	Circular	Raised	Ash	Violet
ED_2	50	Circular	Raised	Ash	Violet
EKH_2	69	Irregular	Raised	Fluffy white	Creamy
FB_1	69	Circular	Umbonate	Fluffy white	Creamy
FB_2	25	Irregular	Umbonate	Fluffy white	Creamy
FM_2	61	Circular	Raised	Ash	Violet
FN_1	57	Circular	Raised	Ash	Violet
FN_2	61	Circular	Raised	Ash	Violet
NB_1	65	Circular	Umbonate	Creamy	Whitish yellow
NB_2	68	Circular	Raised	Ash	Violet
TGB_1	75	Circular	Raised	Creamy	Whitish yellow
TGB_2	75	Circular	Raised	Creamy	Whitish yellow

Table 7: Cultural characters observed.

Cultural Characteristics	**Particular characteristic**	**Images**	**Frequency**	**Percentage**
Colony form	circular		13	**81.25**
	irregular		03	**18.75**
Colony elevation	raised		04	**25**
	umbonate		12	**75**

Surface colony colour	ash		07	**43.75**
	creamy		04	**25**
	Fluffy white		05	**31.25**
Reverse colony colour	creamy		06	**37.5**
	violet		06	**37.5**

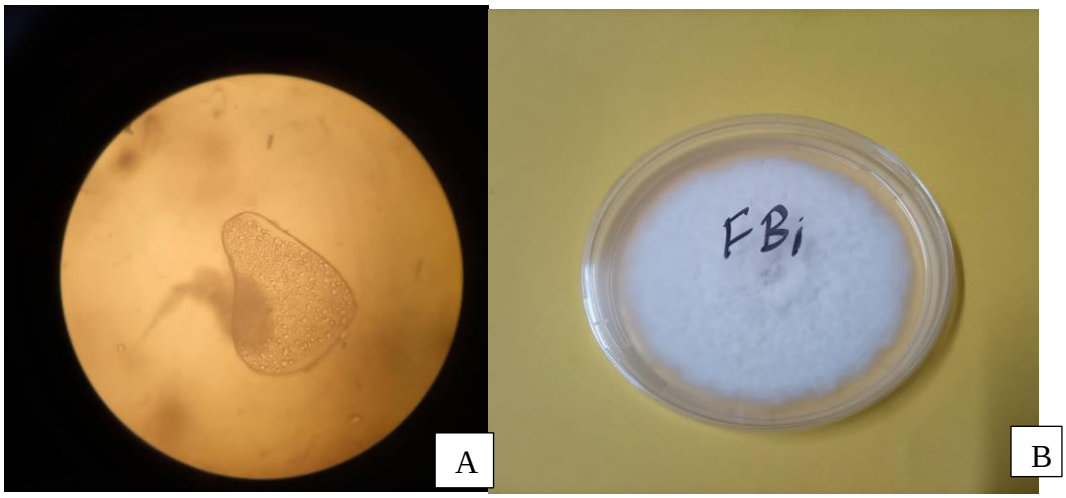


Figure 11: A-Micro-morphological presentation of fungus sporangium, B-white cottony colony growth pattern of *Phytopthora colocasiae.*

Table 8: Percentage growth inhibition of the three plants extracts obtained using hexane and methanol.

Code of isolates	Extracts obtained with hexane (non polar solvent)				Extracts obtained with methanol (polar solvent)			
	% inhibition for *Clematis hirsuta* extract	% inhibition for *clematopsisscabiosifolia* extract	% inhibition for *Telferia occidentalis* extract	Control	% inhibition for *Clematis hirsuta* extract	% inhibition for *clematopsisscabiosifolia* extract	% inhibition for *Telferia occidentalis* extract	Control
BBG_2	33.80	33.80	30.98	100%	66.20	98.59	28.17	100%
BBN_1	04.00	16.00	21.33	100%	49.33	78.67	02.67	100%
BJ_1	01.54	01.54	12.31	100%	50.77	95.38	09.23	100%
ED_2	20.00	12.00	38.00	100%	08.00	62.00	32.00	100%
EKH_2	15.94	14.49	07.25	100%	23.19	69.56	18.84	100%
FB_1	15.94	05.80	21.74	100%	31.88	94.20	47.83	100%
FM_2	19.69	11.47	34.43	100%	01.64	73.77	01.64	100%
FN_1	12.28	03.51	07.02	100%	24.56	75.44	07.02	100%
NB_1	15.38	01.54	04.61	100%	35.38	90.77	03.08	100%
TGB_2	01.33	18.67	04.00	100%	33.33	82.67	10.67	100%

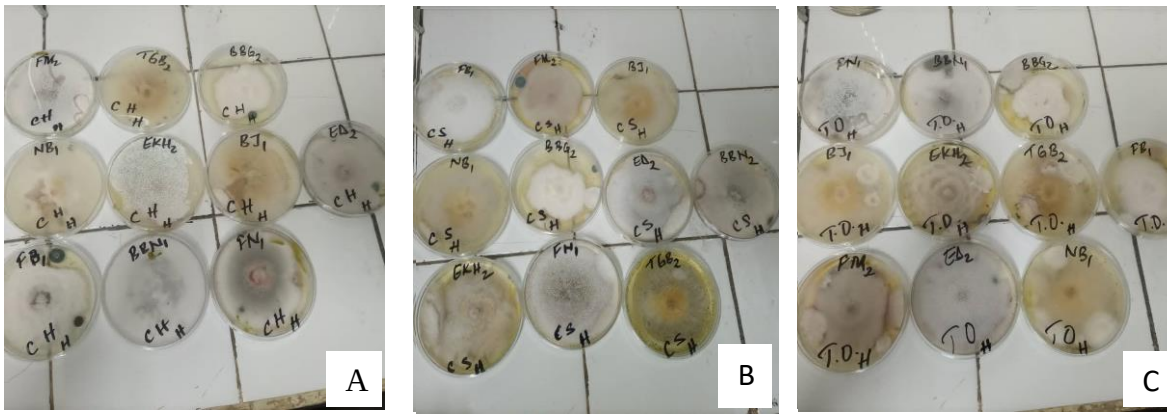


Figure 12: Effect of A-*Clematis hirsuta,* B-*Clematopsis scabiosifolia* and C-*Telferia occidentalis* extracts obtained using hexane as solvent of extraction, on the growth of *Phytopthora colocasiae.*

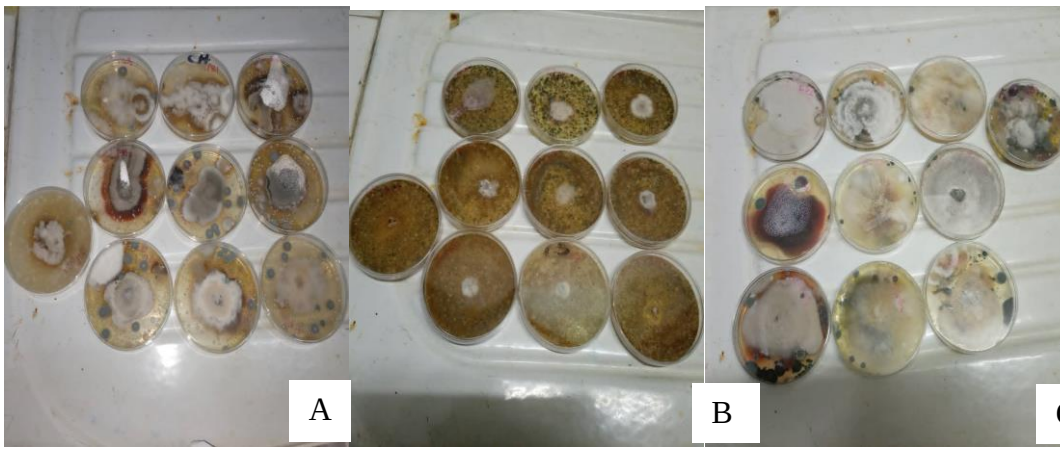


Figure 13: Effect of A-*Clematis hirsuta,* B-*Clematopsis scabiosifolia* and C-*Telferia occidentalis* extracts obtained using methanol as solvent of extraction, on the growth of *Phytopthora colocasiae.*

DISCUSSION

Indigenous knowledge and impact of taro blight of *Colocasia esculenta* on the farmers of Oshie

All the farmers administered questionnaires were aware of the disease (taro leaf blight of *Colocasia esculenta*) and their farms had been attacked by the disease for varying seasons. The survey also revealed that, despite the numerous socioeconomic benefits of *Colocasia esculenta* plant, the yield of this crop continues to decline yearly due to a number of constraints and among them is the problem of taro leaf blight disease caused by the fungus-like Oomycete *Phytophthora colocasiae*. One hundred and ninety-one out of the 200 farmers administered questionnaires in Oshie, indicated that the yield of *Colocasia esculenta* has dropped due leaf blight of *Colocasia esculenta.* Ninety-five-point five percent (95.5%) of the farmers stated that the supply of taro in the local market has drop drastically due to poor harvest as a result of the disease. Eight percent (8 %) farmers stated that all part of the crop is affected by the disease. This is in line with Maheshwari et al. (2007) who reported that the disease affects all parts of the crop including the leaves, corms, petioles and cormel, resulting in extensive damage of the foliage and reduced yield. Most of the

farmers were of the opinion that the price of a 20 L bucket has increased due to scarcity of taro because of taro leaf blight severity. This is in line with the work of Muntala et al. (2020), who stated that the taro leaf blight epidemics have the potential to reduce food availability with a corresponding increase in food prices, thus posing a serious threat to the rural dwellers and regional food security. One hundred and eighty-six farmers indicated that the storage life of the infected tubers have been greatly reduced by taro leaf blight. This is in conformity with Brunt et al. (2001), who reported that *Phytophthora colocasiae* causes corms to rot both in the field and in storage, and this has led to heavy storage lost.

Disease Incidence and Severity

Findings revealed that the disease incidence and severity had varying intensity in the different quarters in Oshie. This could be as a result of slight differences in environmental conditions prevailing in each quarter, the farming practice, for example; used of untreated seeds. The highest mean percentage incidence (81.58%) of the disease was observed in Togobei. This probably may be because the farms are located in the valleys which are often flooded in the rainy seasons. Also, presence of raffia palms around the farms too aid in pulling up water to the surface and this increases the humidity. The raffia also reduces the light intensity and these two conditions favours the spread of the pathogen. This corresponds with the findings of Stenglein et al. (2003) who reported that moderate temperatures and high relative humidity were the most favourable conditions for fungal disease epidemic development. Ekeh (40.08%), Edom (44.17%), Fum (44.25%) and Nyebai (44.67%) had low mean percentage incidence. This is due to the fact that the farms in this quarters are located on hills (high altitude) characterized by low temperatures and low humidity that do not favour the spread of the pathogen. The results move in line with the works of Alvarez and Cho (1978), who reported that black rot of cabbage occurred with greater frequency on farms located between 488-640 m (low altitude) than on those located between 731-1,006 m (high altitude).

The highest mean percentage severity (61.82%) of the disease in Barimbong may be due to shade trees and shrubs around the locality of the farms. Also, the slightly warm moist climate of Barimbong also favours rapid decay of infected leaves of *Colocasia esculenta*. Farms located on the windward sites of hills also had high incidence and severity as seen in the case of the farm in Fun that was selected for the survey. The season of the year during which cultivation is carried out has an effect on disease development and this survey was carried out in the middle of the rainy season and this could have accounted for the varying incidence and severity in the various quarters in Oshie and it matches with the work of Misra et al. (2008) who reported similar results. Fluctuating weather conditions especially relative humidity, temperature and rainfall have been reported to favour disease development under field conditions. No use of treated seeds, chemical fungicides together with slightly varying environmental conditions could have also accounted for the varying intensity of the disease incidence and severity in the different quarters in Oshie.

***In vitro* activity of plant extract**

Plants are well known sources of bioactive compounds. These bioactive compounds have shown to exert antimicrobial effects on some bacteria and fungi. It is estimated that, only about 1/3 of the plant's biodiversity potentials have been assessed. The antimicrobial activity of several plant products against fungal pathogens have been studied under both *in vitro* and *in vivo* conditions (Kagale et al., 2004). In this study, the antifungal activities of extracts of *Clematis hirsuta* (leaves), *Clematopsis scabiosifolia* (Leaves) and *Telferia occidentalis* (leaves) was investigated on *Phytophthora colocasiae in vitro*. From the result of this study, there was a great similarity in the actions of the extracts of the three plants (*Clematis hirsuta, Clematopsis scabiosifolia* and *Telferia occidentalis*) obtained using hexane. Similar studies have been carried out by different researchers on

antifungal activity of extracts of many plants (Satish et al., 2007; Jamil et al., 2010). The results clearly showed that the three plants (*Clematis hirsuta, Clematopsis scabiosifolia* and *Telferia occidentalis*) leaves extracts obtained using hexane and methanol tested at same concentration (0.5 g/ml) had some antifungal activity against *Phytophthora colocasiae* isolates *in vitro*. The inhibition of the fungal growth was observed from the decrease growth of the fungal colony compared to that of the control.

The three plants (*Clematis hirsuta, Clematopsis scabiosifolia* and *Telferia occidentalis*) extracts had maximum inhibition on the isolates obtained from the farm in Barimbong and Fum while *Clematis hirsuta* had the least inhibition on two isolates from Togobei and Bereje. *Clematopsis scabiosifolia* had the least inhibition on two of the isolates obtained from the farms in Nyebai and Bereje. The isolates from the farms of Fun and Togobei were least inhibited by *Telferia occidentalis* extract. The isolate obtained from the farm in Edom quarter was not inhibited by any of the three plant extracts obtained using hexane as solvent of extraction. Four isolates were not inhibited by *Clematis hirsuta* extract, two by *Clematopsis scabiosifolia* extract and two by *Telferia occidentalis* extract, suggesting that all the ten isolates were not genetically the same.

The great similarity observed in the inhibitory activity of *Clematis hirsuta* and *Clematopsis scabiosifolia* extracts obtained using methanol as both extracts inhibited all the 10 isolates form different farms though to different extend whereas the maximum inhibition for *Telferia occidentalis* extract was observed on the isolates from just one the farm in Fun. The extract of *Clematopsis scabiosifolia* obtained using methanol as solvent of extraction recorded the best inhibition as all ten isolates. This result might be due to the variation in the inhibitory activities of the various plant extract obtain with the two solvent. The solvents used for extraction (methanol and hexane) as well as methylene chloride used for dissolution of the extracts may have influenced the antifungal properties of these plants extracts.

Conclusion

Leaf blight disease of taro has a huge impact on the farmers and the population of Oshie as it reduces yield, storage and shelf life of the tubers and supply to the local market. Disease incidence and severity had variable intensities in the different quarters in Oshie. Varying altitude and slight differences in environmental conditions, use of low-quality seeds (not treated), no use of chemical fungicide application could account for the high incidence and severity in the various quarters in Oshie. Crude extracts of the three plants (*Clematis hirsuta, Clematopsis scabiosifolia* and *Telferia occidentalis*) extracted with methanol gave better inhibition than extracts of the same three plants obtained using hexane at the same concentration (0.5 g/ml). However crude extract of *Clematopsis scabiosifiolia* obtained using methanol gave the best inhibition, given that all ten isolates were inhibited above 60%. Therefore, extracts of *Clematis hirsuta* and *Clematopsis scabiosifolia* extracted with methanol can be the base for the formulation of biofungicides. Therefore, extracts of *Clematis hirsuta* and *Clematopsis scabiosifolia* can be used to inhibit the growth of *Phytophthora colocasiae* and protect crops and would be economically and environmentally rewarding to taro producers.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

ENA, AEN, RKT designed the study. ENA conducted the experiments and performed data analysis under the supervision of AEN and RKT. ENA wrote the first draft of the manuscript. All authors contributed to and agreed on the final version of the manuscript.

ACKNOWLEDGEMENTS

The authors thank Mr. Mbaabe Felix and Miss Moforcha Lilian Zemenjuh for their assistance during laboratory work in the Life Science Laboratory of the University of Buea and all the taro farmers in Oshie for their

support and help during field sampling. Also, we are thankful to Dr. Tacham Walter Ndam and Ndong Fabrice for field assistance during sample collection.

REFERENCES

Alvarez AM, Cho JJ. 1978. Black rot of cabbage in Hawaii: inoculum source and disease incidence, *Phytopathology*, **68**: 1456-1459.

Binda KN. 2008. *Tilly Light in Oshie.* Maryland Printers.

Bose TK, Kabir J, Maity TK, Parthasarathy VA, Som MG. 2003. *Vegetable Crops*. (Vol. 1). Prokash Kolkata Publishers: Naya; 413-442.

Brunt J, Hunter D, Delp C. 2001. A Bibliography of Taro Leaf Blight; Secretariat of the Pacific Community: New Caledonia 1-10.

Chaube HS, Pundhir VS. 2005. Crop diseases and their management. PHI Learning Pvt. Ltd. disease incidence. *Phytopathology*, **68**: 1456-1459.

FAO. 2012. FAOSTAT. FAO Statistics Division. http://www.fao.org/faostat/en/

FAO. 2013. Food and Agriculture Organization, statistical yearbooks. FAO.

Fontem DA, Mbong G. 2011. A novel epidemic of taro *(Colocasiae esculenta)* blight caused by *Phytophthora colocasiae* hits Cameroon (Abstract). Third Life Science Days held at the University of Dschang, Cameroon. 26-28 Mai 2011.

Fullerton R, Tyson J. 2003. The biology of *Phytophthora colocasiae* and implication for its management and control. In Proceedings of the Third Taro Symposium, Nadi, Fiji Islands, Secretariat of the Pacific Community: Noumea, New Caledonia 107– 111.

Gadre UA, Joshi MS. 2003. Influence of weather factors on the incidence of leaf blight of Colocasia. *Annual of Plant Protection Science*, **11** (1): 168-170.

Guarion L. 2010. Taro leaf blight in Cameroon. Agricultural Biodiversity Weblog. Available on line: http://agro.Biodiver.se/2010/07/taro-leafblight-in-Cameroon/ (accessed on 15 May 2012).

Jamil A, Shahid M, Khan M, Ashraf M. 2010. Screening of some medicinal plants for isolation of antifungal proteins and peptides. *Pakistan Journal of Botany*, **39**: 211-221.

Joshua SA. 2010. Identification of the major foliar fungal disease of *Colocasia esculenta* (L.) schott and its management in the Kumasi metropolis. M.Sc Thesis. Kwame Nkrumah University of Science and Technology 20-21.

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R. 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Daturametel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. Oryzae. *Physiological and Molecular Plant Pathology*, **65**(2): 91–100. DOI: https://doi.org/10.1016/j.pmpp.2004.11.008

Maheshwari SK, Misra RS, Sriram S, Sahu AK. 2007. Effect of dates of planting on *Phytophthora* leaf blight and yield of *Colocasia*. *Annals of Plant Protection Science*, **15** (1): 255-256.

Mbong GA, Fokunang CN, Fontem LA, Bambot MB, Tembe EA. 2013. An overview of *Phytophthora colocasiae* of cocoyams: A potential economic disease of food security in Cameroon. *Discourse Journal of the Science of Food and Agriculture*, **1**(9): 140-145.

Misra RS, Maheshwari SK, Sriram S, Sharma K, Sahu AK. 2007. Integrated Management of *Phytophthora* Leaf Blight Disease of Taro (*Colocasia esculenta* (L.) Schott). *Journal of Root Crops*, **33** (2): 144-146.

Misra RS, Sharma K, Mishra AK. 2008. Phytophthora leaf blight of Taro (*Colocasia esculenta*) a review. *Asian Australas Plant Biotechnology Journal of Science*, **2**: 55-63.

Muntala A, Patrick MN, Kwadwo G. 2020. Incidence and severity of taro (*Colocasia esculenta* L.) blight disease caused by *Phytophthora colocasiae* in the Bono

Region of Ghana. SSRG-*International Journal of Agriculture and Environmental Science* (SSRG-IJAES), **7**(2): 56-63.

Omane E, Oduro KA, Cornelius EW, Opoku IY, Akrofi AY, Sharma K, Lava Kumar P, Bandyopadhyay R. 2012. First report of late blight of Taro (*Colocasia esculenta*) caused by *Phytophthora colocasiae* in Ghana. *Disease Notes*, **96**(2): 292. DOI: https://doi.org/10.1094/PDIS-09-11-0789

Ooka J, Brennan BM. 2000. Crop profile for taro in Hawaii. College of Tropical Agriculture and Human resources, University of Hawaii Manoa, 1-2.

Satish S, Mohana DC, Raghavendra MP, Raveesha KA. 2007. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus sp*. *Journal of Agricultural Technology*, **3**(1): 109-119.

Singh D, Guaf J, Okpul, T, Wiles G, Hunter D. 2006. Taro (*Colocasia esculenta*) variety release recommendations for Papua New Guinea based on multi-location trials. *New Zealand Journal of Crop and Horticultural Science*, **34**: 163-171. DOI: https://doi.org/10.1080/01140671.2006.9514402

Stenglein S, Ploper LD, Vizgarra O, Balatti P. 2003. Angular Leaf Spot: A disease caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris on *Phaseolus vulgaris* L. *Advances in Applied Microbiology*, **52**: 209-243.

Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 1**59**: 850-850.

Waterhouse GM. 1963. Key to the species of *Phytophthora* de Bary. UK: Common Wealth Agricultural Bureaux. *Mycological Papers*, **92**. 1-22.

Wu PH, Chang HX, Shen YM. 2023. Effects of synthetic and environmentally friendly fungicides on powdery mildew management and the phyllosphere microbiome of cucumber. *PLoS* **8**: 18. DOI: 10.1371/journal.pone.0282809.

Yusop MSM, Saad MFM, Talip N, Baharum SN, Bunawan H. 2019. A Review on Viruses Infecting Taro (*Colocasia esculenta* (L.) Schott). *Pathogens*, **8**(2): 56. DOI: 10.3390/pathogens8020056.

Yaya K, Modibo BS, Karim D, Amakéné N, Salif D, Hamadoun A, Mamadou K. 2023. Efficacy of several species of fungi bioagents and fungicides against *Magnaporthe oryzae in vitro*. *International Journal of Biological and Chemical Sciences*, **17**(3): 935-949 DOI: https://dx.doi.org/10.4314/ijbcs.v17i3.14

Zhu J, Zhang Z, Yang Z. 2001. General research methods on pathogen of potato late blight (*Phytophthora infestans*). *Journal of Agricultural Sciences*, **24**: 112-114.