

*Full Length Research Paper*

# **Assessment of leaf spot disease on water leaf (*Talinum triangulare* (jacq.) Willd and *in vitro* effect of three medicinal plant extracts on pathogen(s) in the Northwest Region of Cameroon**

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Received 18 March, 2022; Accepted 17 August, 2022

**Cultivation of waterleaf improves the life quality of families and provides additional income for farmers. However, waterleaf production in Cameroon has suffered a major blow from leaf spot disease. Ten farms with dimensions of 4 m by 3 m each in Nkwen, Bamenda, Cameroon were surveyed for leaf spot disease of *Talinum triangulare*. Disease incidence and severity were assessed by surveying the farms after every fourteen days for three months. Small pieces of infected leaf parts were inoculated on prepared plates of potato dextrose agar. After 7 days of incubation, pure cultures were made, and isolated fungi were identified according to recommended references. Aqueous extracts from *Moringa oleifera* (Leaves), *Ocimum gratissimum* (Leaves) and *Zingiber officinale* (Rhizomes) were evaluated for *in vitro* antifungal activities on *Alternaria* and *Cercospora*. Data collected for disease incidence and severity showed varying intensities at the different locations. Two fungi species were isolated from the diseased leaf samples collected from 5 farms and were identified as *Alternaria* spp. and *Cercospora* spp. All the extracts had inhibitory activities on the test fungi with the highest inhibition (79.6%) obtained from *Zingiber officinale* (Rhizomes) extract and the lowest inhibition (4%) obtained from *Ocimum gratissimum* extract.**

**Key words:** Identification, incidence, severity, leaf spot, plant extracts, waterleaf.

## **INTRODUCTION**

Increase in world population has led to increasing demands for more enhanced vegetable consumption and meat-based diets over the second half of the last century (Rasadhika et al., 2018; Ismail et al., 2020). This has led to a large increase in the urban population in Africa

(Holdsworth and Landais, 2019). There are projections that the urban population will reach 63% by 2050, in West and Central Africa. Therefore, there is the need for effective urban and peri urban agricultural production systems to complement rural systems (Billa et al., 2017).

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Water leaf (*Talinum triangulare* (Jacq.) is a plant belonging to the family, Portulacaceae and it is commonly found in humid tropics. It is believed to be of South American origin but an African origin may not be doubted (Oluwole et al., 2019). Waterleaf cultivation like other leafy vegetable cultivation in home gardens improves the life quality of families and may provide additional income for female farmers (Mwadzingeni et al., 2021). Waterleaf plays a major role in eradicating malnutrition in Africa (Ikeyi et al., 2020).

Leaf spot diseases are very common in ornamental crops and are usually caused by micro-organisms such as fungi and bacteria (Dicklow, 2019) but the most common are fungal leaf spot (Douglas, 2020). The causal agents of a great deal of crops include fungi species such as *Alternaria*, *Cercospora*, *Colletotrichum* and *Myrothecium species*, as well as bacterial species such as *Xanthomonas* and *Pseudomonas* species (Dicklow, 2019). Some indicators of leaf spot diseases include the appearance of yellow, orange, red or brown spores that appear to be powdery (Grabowski, 2018). The spots occur in various sizes, ranging from pinhead to those that may occupy the whole plant leaf (Douglas, 2020). Also, there is usually the appearance of dead lesions where the edges appear black or dark, surrounding a brownish or reddish center (Douglas, 2020). Leaf spot disease can be controlled by ensuring proper sanitary measures and minimizing the moisture content around the crops (Dicklow, 2019). Another measure is to space plants during planting (Grabowski, 2018).

Various production practices and variation in climatic conditions are thought to influence disease incidence, severity and damage to the crops, under field conditions (Stenglein et al., 2003; Alleorent and Savary, 2005; Mwangombe et al., 2007). Leaf spot diseases are mostly identified based on cultural, morphological, biochemical and physiological tests for rapid differentiation of the causative pathogen (Kaur et al., 2012). Current crop protection efforts focus on developing biopesticides that are environmental friendly and effective in managing plant pests and diseases. Natural plant products have been shown to be good sources of agrochemicals since they are easily biodegradable and do not pollute the environment (Goussous et al., 2010). Various natural products of plant origin have been proven to be effective in controlling various foliar pests and pathogens in a similar way as the synthetic pesticides (Goussous et al., 2010, Kagale et al., 2004). The antimicrobial activities of several plant products against fungal pathogens have been studied under both *in vitro* and *in vivo* conditions (Kagale et al., 2004). Therefore, the study was carried out to identify the pathogen responsible for leaf spot disease of waterleaf and to evaluate the *in vitro* effect of three medicinal plant extracts, *Moringa oleifera*, *Ocimum gratissimum* and *Zingiber officinale* on the pathogen. The botanical extracts are considered less costly, readily available, and more ecologically friendly as they do not

cause pollution.

## MATERIALS AND METHODS

### Description of the study site

This study was carried out in ten different farms in ten quarters of Nkwen village in Bamenda 3 subdivision of Mezam division in the North West Region of Cameroon. It is located between latitude 5°56' and 5°93' North, longitude 10°10' and 10°16' (Ngoran and Ngoran, 2015). Bamenda 3 is situated in the North east of the Bamenda Station Highland which rises to an average height of 1700 m. It constitutes part of the main town (Down Town) which is in a semi basin situated at the foot of the Bamenda fault scarp, lying at an altitude of 1200 m above sea level (Chombeng, 2014). The climatic variation of this area fits into two seasons being the dry season and the rainy season. It experiences the tropical rain forest climate. The rainy season begins in March and ends in October while the dry season begins in October and ends in February. The temperature of the rainy season ranges between 23 to 30°C (Kinge et al., 2016). People in this area carry out subsistence agriculture, cultivating crops like maize, beans, sweet potatoes, banana, plantain, cocoyam just to name a few. Some engage in water leaf cultivation. Figure 1 shows the map of the study farms.

### Determination of disease incidence and severity

Survey was conducted to determine the percentage disease incidence and severity of leaf spot disease on waterleaf in 10 farms with dimensions of 4 m by 3 m for each of the farm in 10 different quarters of Nkwen for 3 months, February to April 2021. Leaf spot disease on *Talinum triangulare* was recorded from all the ten farms during the three months but with varying intensities. The symptoms observed in the different farms were light brown to dark brown, roundish-oval to irregular spots of 1 to 2 mm in diameter at the initial stage, which later expanded, often coalesced and produced 'Shot hole' during severe infection. The plants were examined every fourteen days and quantitative assessment (number of plants /leaves infected) was made. Assessment of the number of infected plants and leaves was done in two randomly selected quadrates (1 m by 1 m) per farm and scored for leaf spot severity on a scale of 0-6 as shown in Table 1 according to the method of Annih et al. (2020). The following formulae were used to determine the percentage of disease incidence and severity;

$$\text{Percentage of disease incidence (PDI)} = \frac{\text{Total Number of Infected Plants}}{\text{Total Number of Plants Assessed}} \times 100$$

$$\text{Percentage of disease severity (PDS)} = \frac{\text{Number of Individual ratings}}{\text{Number of plants Assessed}} \times \frac{100}{\text{maximumscale}}$$

### Isolation and identification of the causative pathogen

The farms were visited, and pictures of the diseased plants (Figure 2) were taken. 23 samples of the diseased leaves were collected from 5 different farms in 5 quarters [Below Foncha (farm 1), Ntasen (farm 2), Muyeb (farm 3), Ndamukong (farm 4) and Banja (farm 5) of Nkwen village]. 5 samples were collected from farms 1, 2 and 3 each and four samples from farms 4 and 5 each. The samples were separately put in zip lock plastic bags and transported by public transport to the University of Buea Life Science Laboratory for isolation and identification of the causative pathogen(s) of leaf spot disease of *Talinum triangulare*.

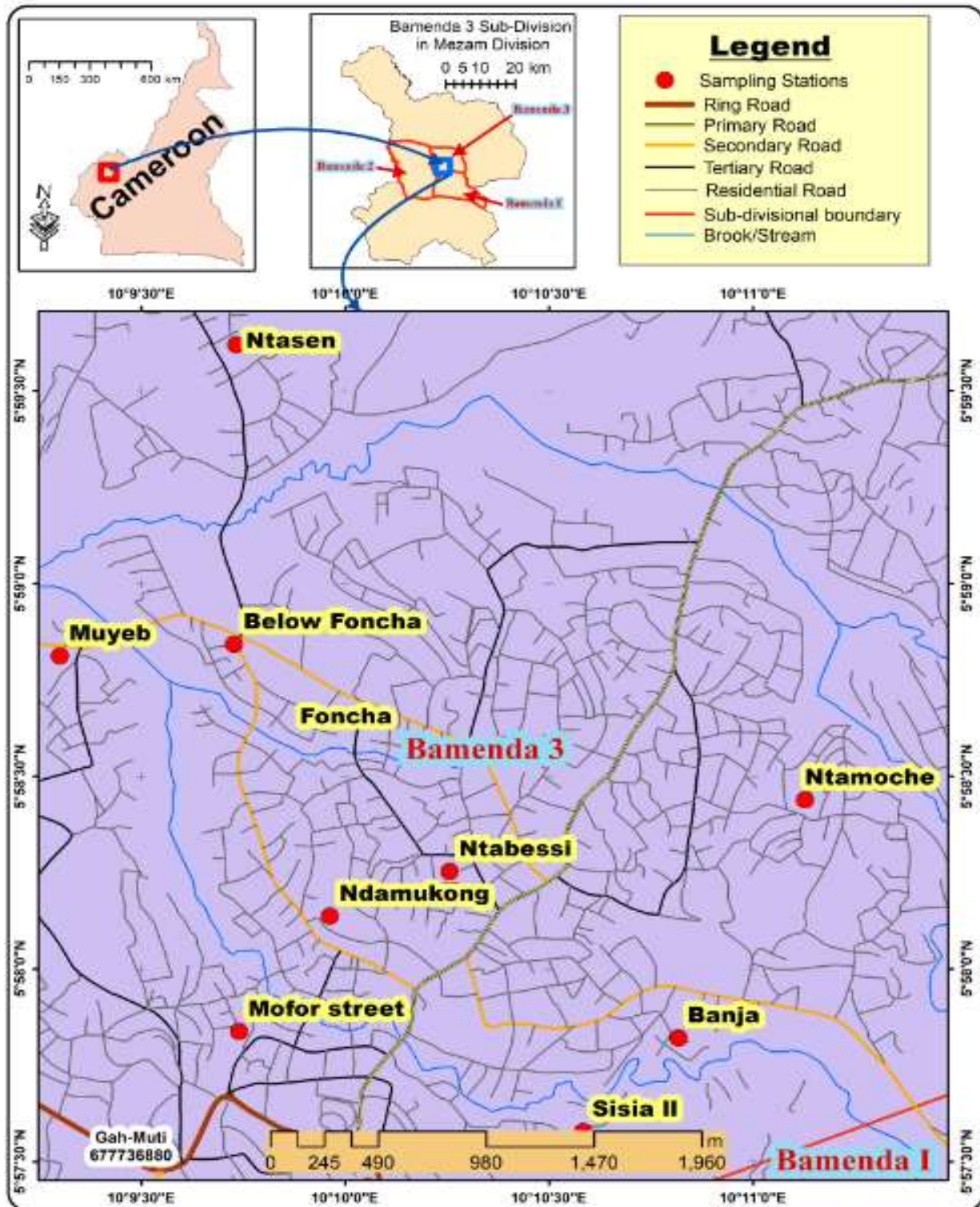


Figure 1. Location map of study area.  
Source: Author

**Media preparation**

Potato dextrose agar (PDA) was used for the isolation of fungi, and

was prepared following the manufacturer's instructions (Beever and Bollard, 1970), in which 42 g of potato dextrose agar (PDA) was weighed and distilled water added to make 1000 ml. The

**Table 1.** Disease severity scale.

Index	Percent affected (%)	Description
0	0	No disease infection
1	5 - 15	Few lesions of leaf spot disease infection dotted on fewer leaves
2	15 - 25	Moderate lesions of leaf spot infection on fewer leaves
3	25 - 50	Leaf spot infection on at least half of the water leaf plants
4	50 - 75	Leaf spot disease on the surface of the leaves and showing holes
5	75 - 85	Leaf spot infection on almost all the leaves with spots coalescing
6	Above 85	About 90% of the leaves showing leaf spot disease with leaves tearing off

Source: Author



**Figure 2.** Picture of diseased water leaf plants (*Talinum triangulare*).

Source: Author

medium was autoclaved at 121°C for 15 min, allowed to cool to about 50°C before the addition of 0.1 g/L of gentamycin and penicillin to prevent growth of bacteria. The mixture was stirred by gently swirling the flask, before pouring into sterile Petri dishes. The medium was allowed to solidify before inoculation.

### Isolation of fungi

Isolation of the fungi was based on the method described by Hallman et al. (2007). Three diseased areas including some healthy part of about 3 mm were cut under aseptic conditions from each diseased leaf sample using a sterile scalpel. The small pieces of the diseased parts were washed with tap water to remove dust and debris adhering to them. They were then immersed in 10% sodium hypochlorite solution for 3 min and were removed using a sterile forceps and rinsed in distilled water. This was followed by rinsing with 70% alcohol for 1 min. They were finally rinsed in three changes of sterile distilled water and blotted dry on sterile tissue paper. Each plate was inoculated with 3 pieces of the surface sterilized diseased area of the diseased leaf samples. Inoculation was done such that there were 23 plates; 15 from samples of farms 1, 2 and 3 (5 Petri plates for each farm), 8 from samples of farms 4 and 5 (4 Petri plates for each farm). Plates were sealed with parafilm wax and incubated at room temperature (25°C) in the dark for 7 days. Fungi growing out of the inoculants were sub-cultured on fresh PDA plates at room temperature. Fresh plates of PDA were prepared. The distinct fungi colonies from the primary cultures

were cut out using a sterile scalpel, and then transferred to the fresh PDA plates to obtain pure cultures. Inoculated plates were sealed with parafilm wax and incubated at room temperature (25°C), in the dark for 7 days.

### Morphological and cultural characterization

For macroscopic observation, the cultural appearances (colony diameter, colony colour, texture, margin, form, elevation and aerial hyphae) were noted on PDA. Colony colours were determined using Methuen handbook of colour chart (Kornerup and Wanscher, 1978). A laboratory manual and a pictorial atlas for identification of fungi by Watanabe (2002) were equally used in the description of colony morphology.

For micro-morphological characterization, all isolates sub-cultured were observed under a microscope for the presence of hyphae, presence or absence of septa on their hyphae, clamp connections, the kind of asexual reproductive structures in order to determine their taxonomic classes. Identification of the fungi was done according to Fawole and Oso (1995). A drop of methylene blue stain was placed on a clean slide and with the aid of a mounted needle, a small portion of the mycelium from the fungal cultures was removed and placed in the drop of stain. The mycelium was spread very well on the slide with the aid of the two mounted needles and a cover slip was gently lowered on it. The slide was then examined under the microscope. The observation was done at high power objective (×40) of the microscope.



Morphological characteristics of the fungi such as type of hyphae and asexual reproductive structure were observed. Pictures of fungi isolates were taken for further characterization and comparison with a document book of fungi (Sarah et al., 2017) and other representative pictures of fungi species.

#### **In vitro control of fungi that cause leaf spot disease on waterleaf**

The extracts of the three plants used were leaves of *Moringa oleifera* and *Ocimum gratissimum* and the rhizomes of *Zingiber officinale*. These plants were selected because of their availability, accessibility, affordability or low cost, environmental friendliness, high versatile anti-microbial spectrum (Okoi and Afuo, 2009; Mohammed et al., 2012) and because they cause no health issues on humans when consumed. The rhizomes of *Z. officinale* were bought from Nkwen market, Bamenda, Cameroon and the leaves of *M. oleifera* and *O. gratissimum* were harvested from Ndamukong quarter Nkwen, Bamenda, Cameroon. They were sealed in zip lock plastic bags and transported to the University of Buea Life Science Laboratory for extraction.

Fungi inhibitory effect was checked by food poisoning technique using different plants extract in the laboratory. For this purpose, three plants were selected; *M. oleifera* (Leaves), *O. gratissimum* (Leaves), *Z. officinale* (Rhizomes). 10 grams of plants' relevant part was macerated with the help of pistol and mortar by adding equal amount of distilled water (1: 1 w/v). At last extracts were filtered with the help of muslin cloth and poured into sterile test tubes. An aqueous solution (100%) was obtained. Further, the extracts were diluted by adding sterilized water to get 10% concentration. Plant extracts were heated at 50°C to avoid contamination. 1 and 0.5 mL of the plant extract measured with a micro pipette was poured in 10 mL PDA Petri plates and gently swirled for mixing of plants extract in the media. When PDA and plant extracts solidified, then 8 mm disc of 10 days old fungi (*Alternaria* and *Cercospora*) were separately placed in the center of the Petri plates. Two Petri plates were prepared as control for each fungus and no plant extract was introduced into them. Only sterile distilled water was used. All Petri plates were sealed with parafilm wax and incubated at 23°C for 10 days. Growth inhibition of fungi inoculated and uninoculated was calculated according to the formula given by Vincent (2012):

$$I (\% \text{ Inhibition}) = \frac{C-T}{C} \times 100$$

Where; I=Percent inhibition; C=Growth in control and T=Growth in treatments.

#### **Data analysis**

All data collected on disease incidence and severity were subjected to analysis of variance (ANOVA) at the end of the study by using GenStat Release 10.3 (2011) software application and statistical software (J.M.P.B). Data for percentage disease incidence and severity were presented in tables.

## **RESULTS**

### **Disease incidence**

The data collected for disease incidence (Table 2) show that the percentage of disease incidence ranged between 5.1 and 92.4% at different locations. The highest mean percentage (63.9%) was observed in the Below Foncha

farm and the lowest mean percentage (25.8%) is observed in the Muyeb farm. As for the months, the highest mean percentage (69.5%) was observed in the first half of March and the lowest mean percentage (12.5%) was observed in the first half of February. The highest monthly mean percentage (56.2%) was observed in the month of March and the lowest monthly mean percentage (27.4%) was observed in the month of February. The difference in the monthly mean percentage between the months of February and April was 2.6%.

### **Disease severity**

After conducting survey on the ten farms using the dimensions 4 m by 3 m on each of the farm for three months, the data collected for disease severity (Table 3) show that the percentage disease severity ranged between 2.5 and 73% at different locations. The highest mean percentage disease severity (30.5%) was observed in Below Foncha farm, and the lowest mean percentage disease severity (10.5%) was observed in Muyeb farm. As for the months, the highest mean percentage disease severity (33%) was observed in the first half of March and the lowest mean percentage (4.8%) was observed in the first half of February. The highest monthly mean percentage (27.3%) was observed in the month of March and the lowest monthly mean percentage (12%) was observed in the month of February.

### **Isolation and Identification of pathogens**

After seven days of inoculation of diseased area samples on PDA, two types of fungi culture were found growing on the culture media: A white fungi culture and a black fungi culture. These two fungi cultures were sub-cultured and after seven days, pure fungi culture of the black and white fungi isolates were obtained as shown in Figures 3 to 7.

The description of some cultural and morphological characteristics of the fungi isolates such as colony diameter, colony color, texture, margin, form, elevation and aerial hyphae is shown in Table 4.

### **Micro-morphological identification**

When the sub-cultured isolates were observed under the microscope, it was seen that both the black and white fungi isolates had branched septate hyphae with no clamp connections. Conidia, conidiophores and conidiospores were also seen (Figures 8 and 9).

Based on cultural, macro-morphological and micro-morphological characters, the two fungi isolates were believed to belong to Phylum Ascomycota, since they all had septate and irregularly branched hyphae which lacked clamp connections. Both isolates also had conidia,

**Table 2.** Data for percentage disease incidence of leaf spot disease on waterleaf collected every 14 days from ten farms for three months.

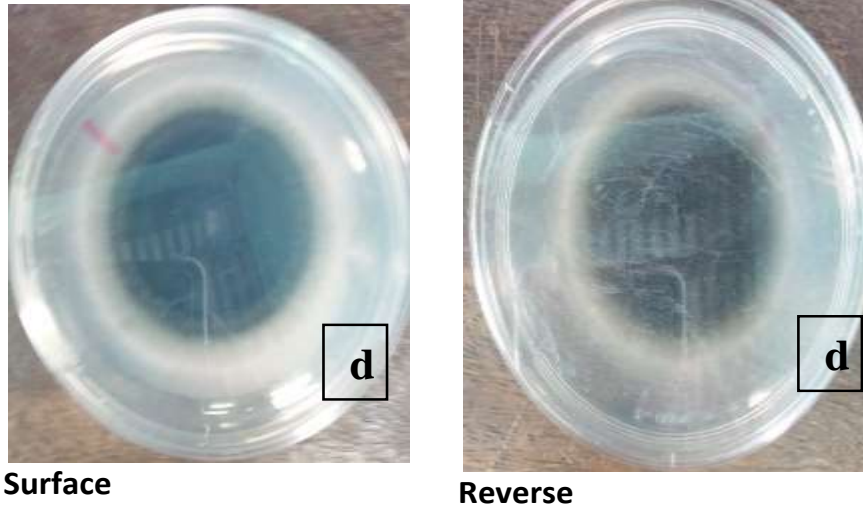
Quarters farms	February				March				April			
	1 <sup>st</sup> half	2 <sup>nd</sup> half	1 <sup>st</sup> half	2 <sup>nd</sup> half	1 <sup>st</sup> half	2 <sup>nd</sup> half	1 <sup>st</sup> half	2 <sup>nd</sup> half	1 <sup>st</sup> half	2 <sup>nd</sup> half	1 <sup>st</sup> half	2 <sup>nd</sup> half
	Infected plants	Percentage	Infected plants	Percentage	Infected plants	Percentage	Infected plants	Percentage	Infected plants	Percentage	Infected plants	Percentage
Below Foncha	86	16.7	280	54.4	476	92.4	432	83.8	381	73.9	321	62.3
Ntasen	67	15.4	200	45.9	321	73.8	269	61.8	206	47.4	132	30.3
Muyeb	50	10.8	150	32.4	239	51.7	108	23.4	100	21.6	71	15.4
Foncha	25	7.8	70	21.8	186	58.1	119	37.2	112	35	71	22.2
Ndamukong	45	10.9	130	31.7	362	88.3	139	33.9	67	16.4	27	6.6
Ntabessi	38	13.1	125	42.9	193	66.3	71	24.4	30	10.3	28	9.6
Sisia II	43	11.4	139	36.8	182	48.3	152	40.3	171	45.4	26	33.4
Banja	73	14.5	237	47.2	371	73.9	342	68.1	305	60.7	257	51.2
Mofor	33	8.5	176	45.4	252	64.9	109	28.1	53	13.7	20	5.1
Ntamoche	55	15.7	227	64.8	269	76.8	121	34.6	89	25.4	46	13.1
	12.5		42.3		69.5		43.6		35		24.9	
	27.4				56.2				30			

\*All data collected on disease incidence and severity were subjected to analysis of variance (ANOVA) at the end of the study by using GenStat Release 10.3 (2011) software application and statistical software (J.M.P.B). Data for percentage disease incidence and severity was presented on tables.  
Source: Author

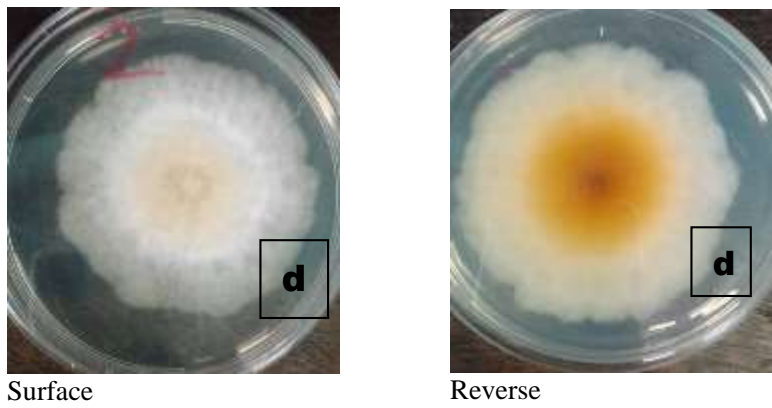
**Table 3.** Data for percentage disease severity of leaf spot disease on waterleaf collected every 14 days from ten farms for three months

Quarters/ farms	February		March		April		Mean (%)
	1 <sup>st</sup> half	2 <sup>nd</sup> half	1 <sup>st</sup> half	2 <sup>nd</sup> half	1 <sup>st</sup> half	2 <sup>nd</sup> half	
Below Foncha	8	40	73	35	15	12	30.5
Ntasen	6	18	38.4	24	17.8	25	21.5
Muyeb	3	15.3	18.3	12.6	8	6	10.5
Foncha	2.5	13.2	20	18	12.3	9.8	12.6
Ndamukong	4	24.3	49	27	15	8.2	21.2
Ntabessi	5	17	21	14	16.2	10.5	14
Sisiall	3.5	9.6	19.8	12.8	13.4	11	11.7
Banja	6	17	30	25	42	39.4	26.6
Mofor	3	13.5	25.5	23	16.2	13.1	15.7
Ntamoche	7.3	29	35	24.7	19.7	14	21.6
Mean (%) for each half	4.8	19.6	33	21.6	17.3	14.9	
Monthly mean (%)		12		27.3		16.1	

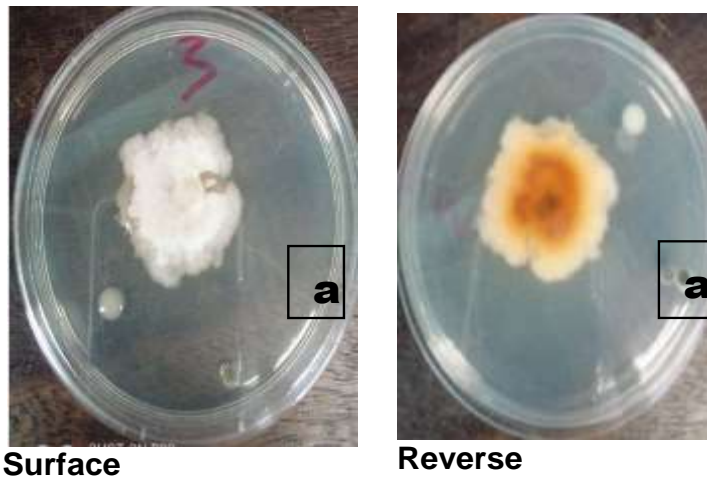
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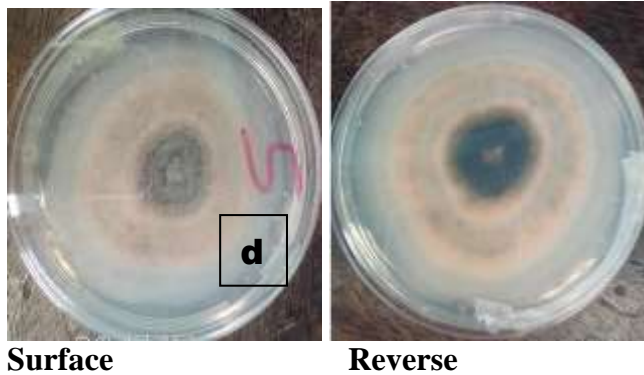
**Figure 3.** Culture of fungi isolate from farm 1, sample (d).  
Source: Author



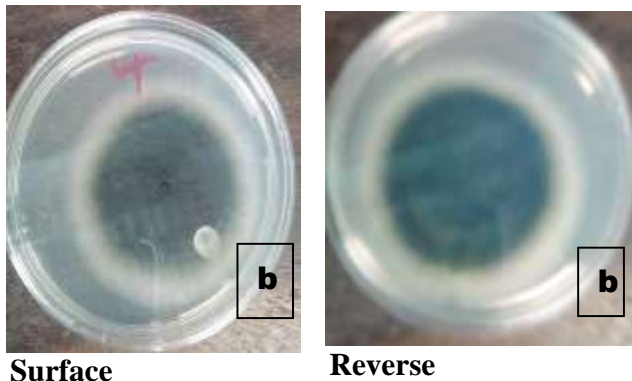
**Figure 4.** Culture of fungi isolate from farm 2, sample (d).  
Source: Author



**Figure 5.** Culture of fungi isolate from farm 3, sample (a).  
Source: Author



**Figure 6.** Culture of fungi isolate from farm 5, sample (d).  
Source: Author



**Figure 7.** Culture of fungi isolate from farm 4, sample (b).  
Source: Author

conidiophores and conidiospores. The white fungus isolate was identified as *Alternaria* while the black fungus isolate was identified as *Cercospora*. The *Alternaria* culture was white to grey-brownish, suede-like to floccose in nature, and based on micro-morphological features, the *Alternaria* culture was made up of multicelled irregularly branched septate chains (hyphae) with dark brown conidiophores. Obclavate to obpyriform conidia bearing large dark spores were seen on the conidiophores. The conidia also had short beaks and fine long septae. The *Cercospora* culture has a dark velvet surface texture, round margin and raised at the centre and based on micro-morphological features, the *Cercospora* culture had branched septate hyphae with conidiophores and conidia which were obtuse at the apex and truncated at the base, bearing obclavate (tail shaped) spores.

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

Three plant extracts (*M. oleifera*, *O. gratissimum* and *Z. officinale*) were tested against the black and white fungi

isolates. According to the result shown in Figures 10 to 13, fungal growth inhibition ranged between 4 and 64% against the black fungus and between 18.9 and 79.6% against the white fungus. The maximum growth inhibition against the black fungus (64%) was recorded by *Z. officinale*, followed by *M. oleifera* (20%) and the least growth inhibition (4%) by *O. gratissimum*. The maximum growth inhibition against the white fungus (79.6%) was recorded by *Z. officinale*, followed by *M. oleifera* (22.2%) and the least growth inhibition (18.9%) by *O. gratissimum*. Generally, it is observed that growth inhibition by plant extracts increased with increase in the concentration of extract from 0.5 to 1 mL.

The percentage inhibition of fungi growth by plant extracts as explained above is represented in Figures 14 and 15.

## **DISCUSSION**

### **Disease Incidence and Severity**

The survey revealed that the disease incidence and severity had varying intensities in the different farms. This could have been due to different geographical and environmental conditions prevailing in each locality, the farming practices adopted by smallholder farmers in the area; for instance, the use of poor-quality seed and cuttings, lack of crop rotation due to land scarcity and poor management practices exacerbated by conducive environment conditions for disease development. This is in line with the findings of Njingulula et al. (2014). The highest mean percentage incidence (63.5%) and severity (30.5%) of the disease in Below Foncha quarters may be associated with the presence of shade trees and shrubs around the locality of the farms.

These conditions reduced light intensity and increased humidity that favors the distribution of the pathogen (Durand-Bessart et al., 2020). This corresponds with the findings of Steinglein et al. (2003) who reported that moderate temperatures and high relative humidity were the most favourable conditions for fungal disease epidemic development. On the contrary, the sampling site of Muyebe is characterized by higher altitude, low temperature, and low humidity that did not favor the spread of the pathogen. This is in line with the findings of Srivastava and Tewari (2002).

The season of the year might have also had an effect on disease incidence and severity as it has a direct relationship with the water content (Jayashree and Wesely, 2019). This research was carried out in the dry season and a lot of irrigation was needed. In the farms surveyed, the source of irrigation was underground water, mainly the surface wells. The season of the year during which cultivation is carried out has an effect on disease development. Sahile et al. (2008) observed that altitude alone had no effect on the development of leaf spot; the interaction of altitude and season had an effect on the



**Table 4.** Data of cultural and morphological characteristics of fungi isolates after seven days of subculture

Sample code	Average colony diameter (cm)	Surface texture	Margin	Elevation	Colony tint		Zonation	Growth pattern	Aerial hyphae
					Surface tint	Reverse tint			
F1(c)	10	Velvet	Round	Flat	Cream white	Light brown	None	Radiate	None
F1(d)	20	Velvet	Round	Raised at centre	Dark centre, white margin	Dark centre, white margin	Two zones	Zonate	Present at centre
F2(b)	13	Velvet	Round	Raised at centre	Black centre, grey margin	Black centre, white margin	Two zones	Zonate	None
F2(d)	23	Cottony centre, velvet margin	Irregular	Raised at centre	Light brown centre, white middle, Cream white margin	Light brown centre, cream white margin	Three zones	Zonate	None
F3(a)	9	Cottony	Irregular	Raised	White	Light brown centre, white margin	None	Radiate	Present
F3(b)	21.5	Velvet	Round	Flat	Black	Black	None	Radiate	None
F4(b)	21	Velvet	Round	Flat	Black centre, white margin	Black centre, white margin	Two zones	Zonate	None
F4(d)	23	Cottony	Round	Raised	Cream white centre, Grey margin	Light brown centre and cream white margin	Two zones	Radiate	Present
F5(c)	20	Cottony and velvet	Round	Flat and raised	Grey centre, cream middle, brown margin	Black centre, cream middle, brown margin	Three zones	Radiate	Present
F5(d)	25	Cottony and velvet	Round	Flat and raised	Dark centre, grey margin	Black centre, cream white middle, brown margin	Three zones	Radiate	Present

Source: Author

disease's development. This points to the need to undertake multiyear/multi-season studies of leaf spot disease of waterleaf across different altitudinal ranges in Nkwen, Northwest Region of Cameroon. Fluctuating weather conditions, especially relative humidity, temperature, and rainfall have been reported to favour diseases development under field conditions (Jayashree and Wesely 2019). This is similar with the results of Correa-Victoria et al. (1989), Anderson et al. (2004) and Scheuemann et al. (2012), who suggested that disease incidence and severity may vary by year, location and even within field depending on environmental conditions and crop management practices.

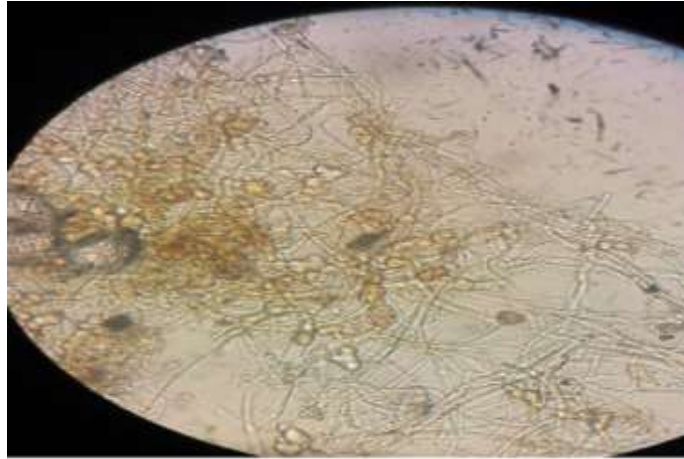
Synthetic chemicals used by farmers to control diseases could have also been the cause of the varying intensities of the disease incidence and severity. These chemicals are not easily available in the market and if available, they are very expensive and this increased the production cost. This is in conformity with the results of Reis et al. (2005) who indicated that production cost increased by 20% when farmers used chemicals to control diseases. This made it difficult for some farmers to control the disease. At times, farmers do not respect the correct dosage of the pesticides to administer to the crops leading to a further increase in the disease incidence and severity. This is in line with the findings of Jallow

et al. (2017).

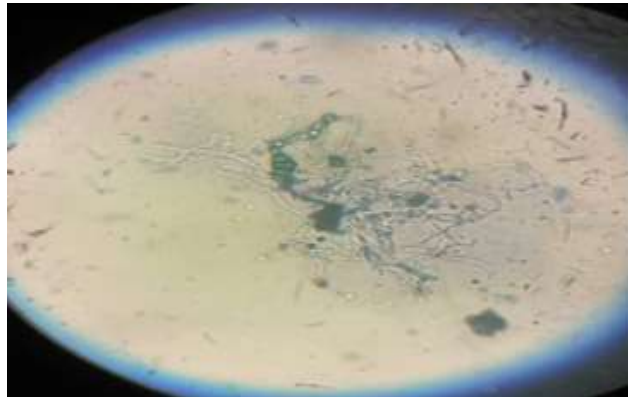
#### Identification of fungi isolates

The symptoms on the leaves of the plant appeared as small, circular to irregular spots of 2 to 4 mm in size. Further, light brown to dark brown patches with characteristic concentric zonnations inside the spots were conspicuous and in severe cases, the spots enlarged in size with complete drying and blighting of leaves. Similar results were reported by Nagrale et al. (2007) and Farhood and Hadian (2012).

The two fungi isolates identified were believed t



**Figure 8.** White fungus (*Alternaria* sp.).  
Source: Author



**Figure 9.** Black fungus (*Cercospora* sp.).  
Source: Author



**Black fungus (*Cercospora*)**



**White fungus (*Alternaria*)**

**Figure 10.** Effect of equal concentrations of *Ocimum gratissimum* on the growth of the black and white fungi.  
Source: Author

to belong to the phylum Ascomycota because, based on cultural, macro-morphological and micro-morphological characters, the two fungi isolates had septate and

branched hyphae which lacked clamp connections. Both isolates also had conidia, conidiophores and conidiospores. The two fungi isolates were believed to be



**Black fungus (*Cercospora*)**



**White fungus (*Alternaria*)**

**Figure 11.** Effect of equal concentrations of *Moringa oleifera* on the growth of the black and white fungi.  
Source: Author



**Black fungus (*Cercospora*)**



**White fungus (*Alternaria*)**

**Figure 12.** Effect of equal concentrations of *Zingiber officinale* on the growth of the black and white fungi.  
Source: Author



**White fungus (*Alternaria*)**

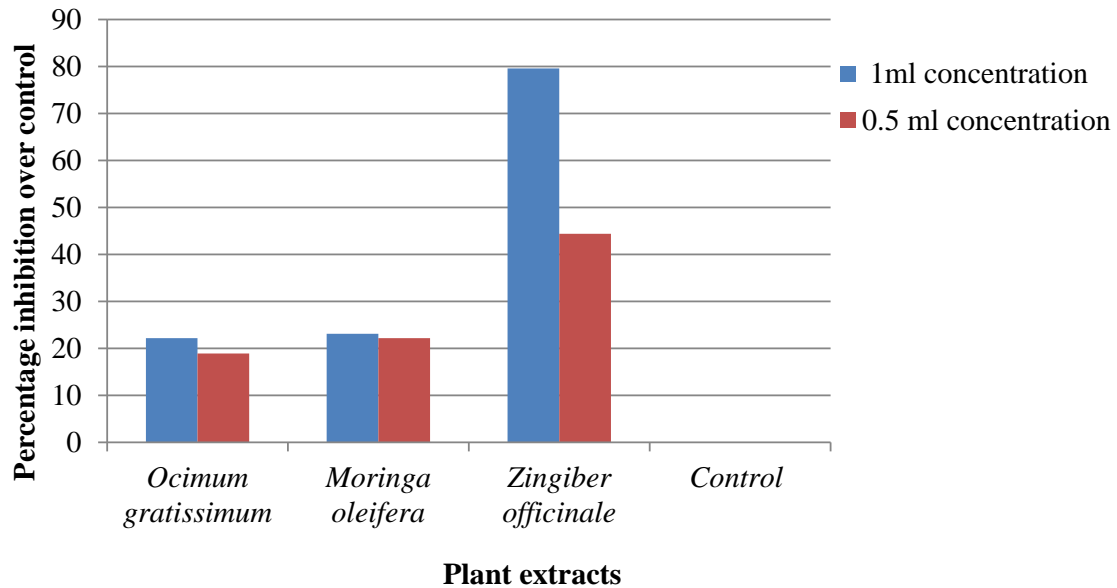


**Black fungus (*Cercospora*)**

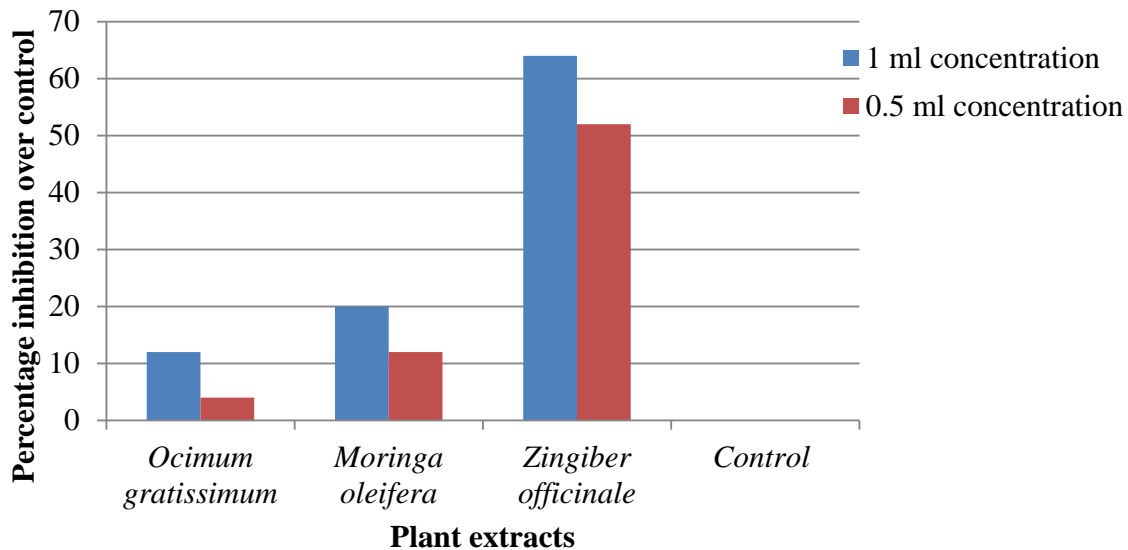
**Figure 13.** Fungi culture (control) with no plant extract.  
Source: Author

*Alternaria* (the white fungus isolate) and *Cercospora* (black fungus isolate) based on the pictures of the fungi isolates taken for further characterization and comparison with a documents book on fungi (Sarah et al., 2017) and other representative pictures of fungi species. The *Alternaria* culture was white to grey-brownish, suede-like to floccose in nature and based on micro-morphological features, the *Alternaria* culture was made up of multicelled irregularly branched septate chains (hyphae) with dark brown conidiophores. The characters like

irregular branching of mycelium and septation are in conformity with the results of Shinde (2003) who observed similar kind of mycelium of *Alternaria* spp. infecting gerbera, pomegranate, water melon, soybean and chilli, respectively. Obclavate to obpyriform Conidia bearing large dark spores were seen on the conidiophores. The conidia also had short beaks and fine long septae. The present findings about shape and septation of conidia are in conformity with the reports of Shinde (2003).



**Figure 14.** Growth inhibition of *Cercospora* by plant extracts.  
Source: Author



**Figure 15.** Growth inhibition of *Alternaria* by plant extracts.  
Source: Author

The *Cercospora* culture had a dark velvet surface texture, round margin and was raised at the centre. Based on the micro-morphological features, *Cercospora* culture had branched septate hyphae with conidiophores and conidia which were obtuse at the apex, truncated at the base, had 3 to 20 septae, bearing obclavate (tail shaped) spores. These morphological characteristics were consistent with those of *C.amalayensis* (Sung-Hee et al., 2017).

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

Natural plant products have been shown to be good sources of agrochemicals since they are easily biodegradable and do not pollute the environment (Goussous et al., 2010). Various natural products of plant origin have been proven to be effective in controlling various foliar pests and pathogens in a similar way as the synthetic pesticides (Kagale et al., 2004; Goussous et al.,



2010). The antimicrobial activities 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, we investigated the antifungal activities of extract of *M. oleifera* (leaves), *O. gratissimum* (Leaves) and *Z. officinale* (Rhizomes) against *Alternaria* and *Cercospora in vitro*. From the result of this study, there was a great similarity in the actions of *M. oleifera* and *O. gratissimum* extracts. Similar studies have been carried out by different researchers on the antifungal activities of extracts of many plants (Anwar and Rashid 2007; Satish et al., 2007; Jamil et al., 2010).

The results clearly showed that the extract of *M. oleifera* leaves tested at different concentrations had some antifungal activity against the fungi isolates *in vitro*. The inhibition of the fungal growth was observed from the decreased growth of the fungal colony compared to that of the control. These results are consistent with those obtained by other investigators such as Adandonon et al. (2006), Al-Asker and Rashad (2010), Talreja (2010), Abdulmoneim and Zaid (2011), Seint and Masara (2011), and Moyo et al. (2012), who found an antifungal activity of *Moringa* plant extracts against several pathogens. The fungicidal effect of *Moringa* extracts on some soil-borne fungi such as *Rhizoctonia*, *Pythium* and *Fusarium* was recorded by many investigators. Dwivedi and Enespa (2012) indicate that *Moringa oleifera* extracts (leaves, bark and seeds) 75% (v/v) showed significant inhibition in the mycelial growth of *Fusarium solani* and *Fusarium oxysporum* f. sp. *Lycopersici*. *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitosterol, caffeoylquinic acid and kaempferol which have antifungal and antibacterial activities as confirmed by the findings of Anjorin et al. (2010). The results of this work showed that the antifungal activity of *M. oleifera* was greater than that of *O. gratissimum* but lower than that of *Z. officinale*. This is probably because the antifungal active principles of *M. oleifera* are more efficient in inhibiting fungal growth than those of *O. gratissimum*. The difference observed in fungitoxic activity of the extracts is likely to be due to the solubility of the active compound(s) in water or the presence of inhibitors to the fungitoxic principle. This also agrees with the report of Qasem and Abu-Blan (1996), Amadioha (2001), Phiri and Mbewe (2010)

The results obtained from this study also indicated that the extract of *O. gratissimum* leaves is able to inhibit the growth of both fungi *in vitro* to an extent. The findings of this study also showed that the inhibitory activity of the plant extract of *O. gratissimum* against both fungi was affected by the concentrations of the extract. The antifungal activity of the plant extract increased with increased concentration of the extract. This finding agrees with the earlier report of Bansa et al. (1999) that indicated that antifungal compounds found in the plant extracts were fungistatic at lower concentrations, while becoming fungicidal at higher concentrations of the extracts. Amadioha (2001) was able to show that *O.*

*gratissimum* leaf extract was able to control spore germination and mycelia growth of *Rhizopus oryzae*. The active principles present in plants are influenced by many factors which include the age of plant, extracting solvent, method of extraction and time of harvesting plant materials as indicated by Qasem and AbuBlan (1996), Amadioha (2001), Okigbo and Ajalie (2005). The phytochemical evaluation of *O. gratissimum* by Sofowora (1993) shows that it is rich in alkaloid, tannins, phytates, flavonoids, steroids and oligosaccharides in the coastal area of Nigeria. The results of this study show that *O. gratissimum* had the least antifungal activity probably because the active principles are not very efficient in inhibiting fungal growth.

The results obtained from this study also indicated that extract of *Z. officinale* was very efficient in inhibiting the growth of both fungi *in vitro* compared to *O. gratissimum* and *M. oleifera* extracts. The findings of this study also showed that the inhibitory activity of the plant extract of *Z. officinale* against both fungi was greatly affected by the concentrations of the extract. The greater the concentration of the plant extract, the more efficient the anti-fungal effect. This finding agrees with the earlier report of Bansa et al. (1999) who indicated that antifungal compounds found in the plant extracts were fungistatic at lower concentrations, while becoming fungicidal at higher concentrations of the extracts. In addition, growth inhibitory activity of the crude plant extracts might be due to the synergistic effect of various active compounds that are in higher or small proportion in the crude extract but not due to the action of a single active compound. This agrees with the findings of Bansa et al. (1999), Díaz Dellavalle et al. (2011), Pinto et al. (2015), and Rashid et al. (2016). The chief active constituents of ginger are volatile oil (zingiberene, zingiberol, D-camphor), Shogaols, Diarylheptanoids, Gingerols, Paradol, Zerumbone, 1-Dehydro-(10) gingerdione, Terpenoids and Ginger flavonoids (Baliga et al. 2012). Ginger has wide range of biological activities that are attributed to its active constituents (Shukla and Singh, 2007). The most prevalent compound identified in the rhizome of *Z. officinale* is the phenolic compound gingerol (in natura), which can be potentially active and converted into compounds such as shogaol and zingerone (dehydrated). These bioactive compounds play an important role in disease prevention. This is in accordance with the findings of Bansa et al. (1999), Díaz Dellavalle et al. (2011), Pinto et al. (2015), Rashid et al. (2016), but the mechanism of biological action is still not well understood. Various biological functions are altered by ginger, including antioxidant, anti-inflammatory, and antimicrobial activities (Suhad et al., 2012; Rahmani et al., 2014; Liu et al., 2017; Sharifi-Rad et al., 2017). The gingerol produced by *Z. officinale* may be responsible for this antimicrobial activity, as when in contact with the cell wall, it ruptures the cell wall and consequently inhibits cellular permeability leading to cell damage and death of

the microorganism. This is in line with the results of Oonmetta-Aree et al. (2006), Poeloengan (2011), Hasan et al. (2012), Suhad et al. (2012) and Rahmani et al. (2014). The antimicrobial activity of extracts (methanol and hexane) from the rhizome of *Z. officinale* is related to the previously mentioned bioactive compounds. They can attack cell wall and cell membrane, affecting permeability and interfering in cellular functions such as electron transportation, nutrients, synthesis of proteins and nucleic acids, and enzymatic activity. Thus, such bioactive compounds may have several invasive targets that may lead to inhibition of microbial growth. This is in conformity with the results of Oonmetta-Aree et al. (2006), Poeloengan (2011) and Hasan et al. (2012).

## Conclusion

This survey revealed that the disease incidence and severity had varying intensities in the different farms. Based on micro-morphological characters and other representative pictures of fungi species, the two fungi species were identified as *Alternaria* and *Cercospora*. These two fungal species identified in this study could be regarded as the most common causes of leaf spot disease of waterleaf. *In vitro* antifungal control using extracts of *M. oleifera*, *O. gratissimum* and *Z. officinale* carried out in this study showed that *Z. officinale* was very efficient in inhibiting the growth of *Alternaria* and *Cercospora*. Ginger is easy to obtain, cheap and grows in all regions. Using ginger to inhibit the growth of fungi and protect crops would be economically and environmentally rewarding to many leafy vegetable producers.

## CONFLICT OF INTERESTS

The authors declared no competing interests.

## ACKNOWLEDGEMENTS

The authors are grateful to the Life Science Laboratory of the University of Buea for providing laboratory space to carry out the experiment.

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