Evaluation of Fungicide against Taro Leaf Blight Disease Caused by *Phytophthora colocasiae* in Three Agro-Ecological Zones of Cameroon

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MEB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ANT and SC managed the analyses of the study. Author MGA managed study design, statistics and the literature searches. Author FC also designed the study. All authors read and approved the final manuscript.

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ABSTRACT

Globally the taro leaf blight (*Phytophthora colocasiae*) disease causes between 50 to 70% yield loss. Four taro landraces were planted in three agroecological zones of Cameroon; the Western Highlands (Bambui), Mono-Modal Humid Forest (Ekona), and the Bimodal Humid Forest.

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1. INTRODUCTION

The taro leaf blight caused by Phytophthora colocasiae (Raciborski) is a serious disease of (Colocasia esculenta (L) Schott) in Cameroon. The fungus causes foliar and stem blight of taro. It also causes corm rot during the periods of extended leaf wetness, high humidity and optimal temperatures of 24-28°C [1,2]. The taro leaf blight disease (TLBD) is characterized by small, dark brown flecks or light brown spots on the upper leaf surface. These early spots often occur at the tips and edges of leaves where water accumulates [2,3]. The spots enlarge rapidly, becoming circular, zonate, and ranges from purplish-brown to brown colour. As the spots increase in size they coalesce and quickly destroy the leaf [4]. The margins of the lesions are marked by a white powdery band of sporangia with numerous droplets of orange or reddish exudates [5]. The taro leaf blight is considered a major economic disease of taro because it reduces corm yield by 50% [6] and leaf yield by 95% in susceptible genotypes [7]. Phytophthora colocasiae causes corms to rot in the field and during storage, and this has led to heavy storage loss [8]. In most of the taro growing regions in Cameroon, the disease caused a 50-100% yield loss to taro in 2010 [9]. This resulted in a reduction in food, household income, increased poverty, and some farmers abandoned their farms to cultivate other food crops [10,11].

Strategies for managing taro leaf blight disease include major cultural practices such as; removal of infected leaves during the early stages of disease development, selection of sites surrounded by forest as a barrier to disease spread, isolation of new crops from diseased crops, the use of disease-free planting materials and the effects of planting dates [7,12]. These strategies are only effective when the disease is in the endemic phase with a relatively low and restricted disease incidence. When the disease is in an epidemic phase, these strategies are not effective because the enlarged lesions on the leaves rapidly cause almost complete crop defoliation with consequent effects on yield [9]. Foliar application of biological control agents has the potentials to protect taro crops from Phytophthora colocasiae infection. For instance, Trichoderma significantly reduces the number of infected leaves and disease severity in taro [13]. Treatment with bio-pesticides from plant products has significantly improved crop yield and reduced the incidence of crop pests and diseases in farmers’ fields. However, these methods are expensive [9]. A wide range of protectants and systemic fungicides have been shown to effectively control TLBD [7]. Mancozeb, copper, and Metalaxyl are amongst the most commonly recommended fungicides used in the control of taro blight [14]. Mancozeb and copper have protectant activities only while metalaxyl and phosphorus acids are specific for Phytophthora and Pythium diseases [1]. In Cameroon, information on the use of fungicides and biological methods in controlling taro blight is scanty. In the absence of adequate application strategies, fungicide control seems to be the fastest and most effective method of taro blight control [15]. Therefore, the objective of this study was to evaluate the use of fungicide in the control of taro leaf blight disease in three agroecological zones of Cameroon.

Keywords: Taro; leaf blight; copper oxide (600 g)–Metalaxyl (120 g); disease severity, pathogenicity.
2. MATERIALS AND METHODS

2.1 Study Sites

This study was conducted at three field research sites; at the Institute of Agricultural Research for Development (IRAD), Bambui, in North West Region, (IRAD), Ekona, in the South West Region and the International Institute of Tropical Agriculture (IITA) Nkolbisson, Yaounde, Centre Region of Cameroon. The sites chosen were based on the climatic oscillation and rainfall regime of their respective agro-ecological zones. The positions of the experimental sites were recorded using GPS mark Garmin etrex 20. IRAD, Bambui is situated at 32°, 0627’ N latitude, 0659’ E longitude and altitude 1262 m above sea level. IRAD Ekona is located at latitude 32°0537’ N, longitude 0467’ E and altitude 411 m above sea level and IITA is situated at latitude 32°86’ N, longitude 270’ E and altitude 777 m above sea level.

2.2 Application of Fungicide on Taro Leaf Blight Disease

Four landraces of taro viz., dark green petiole with small leaves (L1), red petiole with small leaves (L2), light green petiole with large leaves (L3), and light green petiole with small leaves (L4) were used in the experiment. The corms of the landraces were planted at IITA Yaounde, IRAD Bambui, and IRAD Ekona research farms. In all the field sites, planting was done successively in March and July in 2018 and 2019. Copper oxide (600g)–Metalaxyl (120g), a contact and systemic fungicide was used in the pre and post-control of taro blight. Ninety corms of each landrace were pre-treated with 0.33 % of the fungicide to kill fungi spores before planting. Two hundred and forty taro corms were planted in the control field sites. One taro corm was planted per hole at a depth of 50 cm and intra-row and inter-row spacing of 50 cm by 1m respectively. Each landrace was replicated four times in a Randomized Complete Block Design (RCBD), with four plots per landrace giving a total of 48 plots. Manual weeding was done regularly at monthly intervals and moulding was done after 8 weeks of plant growth. Taro blight was controlled by applying different concentrations of copper oxide (600 g)–Metalaxyl (120 g); 0.4%, 0.33%, 0.27% were applied to the taro landraces; F1= Copper oxide (600 g)–Metalaxyl (120g) at 0.4% on treated landraces, F1A =Copper oxide (600g)–Metalaxyl (120 g) at 0.4% on non-treated landrace, F2 = Copper oxide (600 g)–Metalaxyl (120 g) at 0.33% on treated landraces, F2A = Copper oxide (600 g)–Metalaxyl (120 g) at 0.33% on non-treated landraces, F3 = Copper oxide (600 g)–Metalaxyl (120 g) at 0.27% on treated landrace, F3A = Copper oxide (600 g)–Metalaxyl (120 g) at 0.27% on non-treated landraces. These applications were done at two-week intervals following the onset of the first taro blight symptoms on leaves. Data on the taro blight severity was collected at two weeks interval for six weeks [15]. The data on disease severity was collected only from the middle plants on each ridge because the edges of the ridges served the role of pathogen invasion.

2.3 Evaluation of Disease Severity of Phytophthora colocasiae

The severity of disease on each variety was scored using the 0-9 scale [16];

0 = No symptom.
1 = Presence of lesions less than 10 cm² of leaf area.
2 = Presence of lesions 11- 30 cm² of leaf area.
3 = Presence of lesions 31- 60 cm² of leaf area.
4 = Presence of lesions 61- 90 cm² of leaf area.
5= Presence of lesions more than 90 cm² up to 25% of leaf area.
6= Coalesce of spots more than 25% of the leaf covered.
7= Coalesce of spots more than 50% of the leaf covered.
8= Coalesce of spots more than 75% of the leaf covered.
9= Collapse of petiole accompanied by complete leaf blight

2.4 Screen House Test and Preparation of Spore Suspension of P. colocasiae

The taro blight diseased leaves were collected from all experimental fields, washed under running tap water, and cut from the advancing edges of the lesions into small pieces; surface sterilised with sodium hypochlorite (5%) for 30 seconds and rinsed trice in sterile distilled water for 4 minutes. The leaf fragments were dried on a sterilized filter paper and placed on a solidified cool V8 juice Agar medium in Petri dishes containing ampicillin (250 mg/l), penicillin (250 mg/l), and nystatin (20 mg/l) to inhibit bacteria growth. The dishes were incubated at 26°C in a complete randomized design with ten replicate per cultivar. After four days, leaf fragments were later observed under the microscope for the
presence of the mycelia growth of *Phytophthora colocasiae*. The mycelia fragments were subcultured twice to obtain an axenic culture, which was used in the preparation of the spore suspension after 21 days, by flooding the surface of the growing colonies in each Petri dish with 8 ml of sterile distilled water, and the spores dislodged with a small brush [17]. A drop of tween 80 was added to each spore suspension, centrifuged for two minutes, and the supernatant filtered through a two layered sterile muslin cheesed cloth. The spore concentrations were determined with hemocytometer and adjusted to $3.0 \times 10^6$ spores/ml of distilled water [17].

2.5 Pathogenicity Assessment

The survival of *Phytophthora colocasiae* was confirmed by inoculating 70 days-old healthy potted taro plants in the screen house. A syringe was used to inoculate spore suspension on five spots on two leaves on each plant. The plants in the control experiments were treated with distilled water. The plants were later observed for taro leaf blight symptoms (lesion area) which were recorded daily for 14 days [18].

2.6 Statistical Analysis

The JMP8, (2007) statistical software was used to perform a one-way analysis of variance (ANOVA) for the disease severity of taro in four growing seasons. Their treatment means were separated using student T-test (STT) and the Least Significant Difference (LSD) at a statistical significance of 95% confidence interval. The mean values were used to plot graphs for appropriate representation of the results. The data for the fungi lesion area were analyzed using the JMP11, 2012 statistical package.

3. RESULTS

3.1 Effect of Copper Oxide (600 g)–Metalaxyl (120 g) Application on Taro Disease Severity in the March 2018 Growing Season

Results showed that the control field sites had more damaged plants than the fields sprayed with copper oxide (600 g)–Metalaxyl (120 g) fungicide. The taro blight severity decreased in all sprayed field sites during the rainy season and with the onset of the dry season. In all experimental sites, plants in the controlled fields had fewer leaves and retarded plant growth relative to sprayed fields. In Ekona, some plants of L1 poorly germinated or showed crop failure (Fig. 1).

3.2 Taro Blight Severity in Fields Following Copper Oxide (600 g) – Metalaxyl (120 g) Application at 2 Week Intervals in the March 2018 Growing Season

In the experimental field in Yaounde, no infection was found on L1 in most of the treatments; F1, F1A, F2, F2A F3, F3A except for treatment F2. At Ekona, the control field had the highest mean disease severity of 7.8 while in the 2nd and 4th week, treatments F2, F2A at Bambui and treatments F1, F2 in Yaounde had the lowest disease severity of 0.2 respectively (Fig. 2). For L2 no infection was found in treatments F1, F1A, F2A, F3 in Yaounde, and treatment F3A in Bambiu. In the 6th week, the highest mean disease severity of 3.8 was recorded in Ekona while the lowest mean disease severity of 0.2

Fig. 1. Effect of copper oxide (600 g) – Metalaxyl (120 g) application on taro disease severity in the March 2018 growing season
Different concentrations of copper oxide (600 g) – Metalaxyl (120 g) sprayed on taro landraces

F1 = copper oxide (600 g)–Metalaxyl (120 g) at 0.4% on treated cultivars; F1A = copper oxide (600 g)–Metalaxyl (120 g) at 0.4% on non-treated landraces

F2 = copper oxide (600 g)–Metalaxyl (120 g) at 0.33% on treated landrace; F2A = copper oxide (600 g)–Metalaxyl (120 g) at 0.33% on non-treated landraces

F3 = copper oxide (600 g)–Metalaxyl (120 g) at 0.27% on treated cultivars; F3A = copper oxide (600 g)–Metalaxyl (120 g) at 0.27% on non-treated landraces

was recorded in the treatment; F2, F3A, and control in Yaounde and in treatments F1, F2A, F3 in Bambui, in the 4th and 2nd week respectively (Fig. 2). For L3, no infection was found in most of the treatments except for treatment F1, and in the 6th week, the highest disease severity of 6.5 was recorded in the control fields in Ekona. In the 2nd week, treatments F2, F2A, F3, and F3A in Bambui scored the least mean disease severity of 0.1 (Fig. 2). For L4, no disease was observed in treatments F1, F2, F3, F3A, and control in Yaounde and on treatments F2, F2A, F3, F3A in Bambui. In the 6th week, the highest mean disease severity was recorded in the control field in Ekona while treatments F1, F1A in Bambui and F1A, F2A in Yaounde had the lowest mean disease severity of 0.2 (Fig. 2).
3.3 Taro Blight Severity in Fields  

Following Copper Oxide (600 g) – Metalaxyl (120 g) Application at 2 Week Intervals in July 2018 Growing Season

Results showed that L1 had no disease in the treatment and control fields in Yaounde and the treatment fields in Bambui. The control field in Ekona had the highest disease severity of 3.5 while the treatments F1A, F2 had the lowest disease severity of 0.2 (Fig. 3). L2 had no disease in treatments F1, F2, F2A in Yaounde, and treatment F3A in Bambui. In the 4th week, the control field in Ekona recorded the highest mean disease severity of 3 while in the 2nd and 4th week, treatments F1, F2 in Ekona and treatments F1, F3, in Bambui recorded the lowest disease severity of 0.2 respectively (Fig. 3). L3 showed no disease in treatments F1 in Yaounde and F1, F2, F2A treatments in Bambui. In the 4th week, the highest mean disease severity of 4.2 was obtained in the control field at Ekona while treatments F1A, F3 in Bambui scored the lowest disease severity of 0.2 (Fig. 3). In all field sites, the disease severity trend for L4 was similar to that of L3 with no disease observed in the treatments in Yaounde and treatments F1A, F3 in Bambui. In the 4th week in Ekona, the highest and lowest mean disease severity scores of 3.4 and 0.2 were recorded in the control field and treatment F1A respectively (Fig. 3).

3.4 Taro Blight Severity in Fields  

Following Copper Oxide (600 g) – Metalaxyl (120 g) Application at 2 Week Intervals in March 2019 Growing Season

There was no disease on L1 for the treatments in Yaounde and treatment F1 in Bambui. In the 6th week the control field at Ekona recorded the highest mean disease severity score of 6.8 while in the 2nd, 4th and 6th week in Bambui, treatment; F1A, treatments; F2A, F3, F3A and treatments; F2, F3A scored the lowest disease severity score of 0.1respectively (Fig. 4). Similar trends were obtained for L2 and no disease was present in the treatments in Yaounde and treatments F1A, F2 in Bambui. In the 4th week, L2 in the control field in Ekona had the highest disease severity score of 2.5 while in the 2nd and 6th week in Bambui, treatments; F2A, F3, and F3A and treatment F2A scored the lowest disease severity of 0.1 respectively (Fig. 4). From the 2nd to the 6th week, no disease was observed on L3 in all treatments in Yaounde. In the 4th week, the control field at Ekona scored the highest mean disease severity of 3.7 while treatment F2 in Bambui recorded the lowest mean disease severity of 0.2 (Fig. 4). For L4, treatment F2A in Bambui had no disease and the disease severity for treatment F1A in Yaounde was low. The control field in Ekona scored the highest mean disease severity of 2 while the control field, treatments F1, F2, F3 in Bambui, and treatment F1A in Yaounde scored the lowest mean disease severity of 0.2 (Fig. 4).

3.5 Taro Blight Severity in Fields  

Following Copper Oxide (600g)– Metalaxyl (120g) Application at 2 and 6 Weeks Intervals in July 2019 Growing Season

Except for Bambui, over time, the disease severity of all landraces decreased in all other experimental fields. From the 2nd to the 6th week, No disease was present on L1 in treatments; F1, F1A, F2, F3, F3A in Bambui, treatments F1, F1A, F2A, F3A, and control in Ekona, and treatments F1, F1A, F2, F3, and F3A, in Yaounde. In the 6th week, the control field at Bambui recorded the highest mean disease severity of 2 while in the 2nd and 6th week, treatment F2A and control in Yaounde and treatment F3 in Ekona scored the lowest disease severity of 0.2 respectively (Fig. 5). For L2, the control fields in Bambui recorded the highest mean disease severity of 2.3, while in the 2nd week, treatments F1, F2, F3, and F3A in Ekona recorded the lowest disease severity of 0.2 (Fig. 5). L3 had no disease in most of the treatment fields in Yaounde and Bambui except for treatment F1 in Bambui (Fig. 5). In the 6th week, the Control field at Bambui scored the highest mean disease severity of 2.3 while in the 2nd week treatments F1A, F3, in Ekona, and treatment F2 in Bambui recorded the lowest disease severity of 0.2 (Fig. 5). From the 2nd to the 6th week, the disease severity trend for L4 was similar to those of L2 and L3 in the treatment field in Yaounde. From the 2nd and 6th week diseases were not present on L4 in treatments F1, F2A in Bambui, and treatments F1A, F3 in Ekona respectively. In the 6th week the control field at Bambui recorded the highest disease severity of 1.5 while in the 2nd week, treatment F1A in Bambui and treatments F1, F2A, in Ekona scored the lowest blight severity of 0.3 (Fig. 5).
3.6 Areas of Fungal Lesions on Landraces in the Various Regions Following Inoculation

On all the landraces, fungal lesions appeared on the leaves on the third day following inoculation with fungal spores. The fungal lesions advanced with age and leaves were destroyed over time. The reaction of the taro landraces was broadly identical to the fungus tested. The invasion of wounded leaves by the fungus resulted in severe or slight disease development, depending on the landrace, climate, and region. On the 5th day of lesion area measurement, L1 in Ekona had the
largest fungal lesion area (25 cm³) (Fig. 6). While on the first day of lesion measurement, L2, and L3 in Ekona and L1 in Yaoundé had the smallest fungal lesion area ((1 cm³) (Fig. 6). On the 5th day of lesion measurement of all inoculated landraces in Ekona, L3 was the most severely destroyed compared to other landraces planted in all the regions.

Fig. 4. Effect of copper oxide (600 g) – Metalaxyl (120 g) application on the severity of taro leaf blight disease at 2 week intervals at different field sites on L1. Different concentrations of copper oxide (600 g) – Metalaxyl (120 g) sprayed on taro landraces.

- **F1** = Copper oxide (600 g) – Metalaxyl (120 g) at 0.4% on treated landraces; **F1A** = Copper oxide (600 g) – Metalaxyl (120 g) at 0.4% on non-treated landraces.
- **F2** = Copper oxide (600 g) – Metalaxyl (120 g) at 0.33% on treated landraces; **F2A** = Copper oxide (600 g) – Metalaxyl (120 g) at 0.33% on non-treated landraces.
- **F3** = Copper oxide (600 g) – Metalaxyl (120 g) at 0.27% on treated landraces; **F3A** = Copper oxide (600 g) – Metalaxyl (120 g) at 0.27% on non-treated landraces.

Mean disease severity

Bamnui Ekona Yaounde
Concentration of copper oxide (600 g) – Metalaxyl (120 g) on landrace L1 at different regions

DS 2 WKS = Disease severity at 2 weeks; DS 4 WKS = Disease severity at 4 weeks; DS 6 WKS = Disease severity at 6 weeks; CON = Control field
Mean disease severity

DS 2 WKS = Disease severity at 2 weeks; DS 4 WKS = Disease severity at 4 weeks.
DS 6 WKS = Disease severity at 6 weeks; CON = Control field

Different concentrations of fungiforce sprayed on taro landraces

F1 = Copper oxide (600 g)–Metalaxyl (120 g) at 0.4% on treated landraces; F1A = Copper oxide (600 g)–Metalaxyl (120 g) at 0.4% on non-treated landraces

F2 = Copper oxide (600 g)–Metalaxyl (120 g) at 0.33% on treated landraces; F2A = Copper oxide (600 g)–Metalaxyl (120 g) at 0.33% on non-treated landraces

F3 = Copper oxide (600 g)–Metalaxyl (120 g) at 0.27% on treated landraces; F3A = Copper oxide (600 g)–Metalaxyl (120 g) at 0.27% on non-treated landraces

Fig. 5. Effect of copper oxide (600 g) – Metalaxyl (120 g) application on the severity of taro leaf blight disease at 2 week intervals at different field sites on cultivar L1

Bamnui Ekona Yaounde
Concentration of copper oxide (600 g)–Metalaxyl (120 g) on landrace L1 at different regions
4. DISCUSSION

The current result showed that, in all the field sites and growing seasons, the control fields had more damaged plants than sprayed fields, with the control field at Ekona having the highest number of damaged plants (Fig. 1). This probably suggests that, the climatic and edaphic factors of the Mono-Modal Humid Forest may favour the growth of *Phytophthora colocasiae*. Plants in the control field had retarded growth and fewer leaves than in sprayed fields, indicating the efficacy of copper oxide (600 g)–Metalaxyl (120 g) fungicide in the field control of taro blight severity. Some landraces like L1 at the Ekona field had crop failure or germinated poorly. This may be attributed to the fact that, *Phytophthora colocasiae* infection reduces the quality of planting materials, indicating that Landrace L1 may not be suitable for cultivation in the Mono-Modal Humid Forest zone. The current result is in line with those of some authors who observed significant leaf area damage per plant and an increased corm/cornel yield when 10 *Phytophthora colocasiae* infected taro genotypes by were sprayed with borax (500 ppm) [19]. The fungal lesion area did not increased over time in leaves sprayed with copper oxide (600 g) - Metalaxyl (120 g) at two week intervals, and the lesions dried off to produce new leaves (Fig. 1). This was probably due to the presence of metalaxyl active ingredient in copper oxide (600 g)-Metalaxyl (120 g) fungicide, which inhibited the cellulolytic and pectinolytic activity of the enzymes produced by *P. colocasiae*, to prevent pathogenic spread on the sprayed leaves [20]. Earlier studies reported that, synthetic plant protection products such as potassium iodide and arsenic oxide effectively control mycelia growth, sporangial production and inhibit the production of pectolytic and cellulolytic enzymes produced by *P. colocasiae* on taro [21]. Also, metalaxyl with copper (at 0.3% Ridomi plus 72 WP) effectively controls taro blight when applied at 2 weeks interval [22]. It was interesting to note that, the taro blight severity in all the field sites reduced at the onset of the dry season. This strongly suggests that, field sites with low water levels may reduce taro blight severity. For all landraces, the taro blight severity in sprayed fields decreased below 1.5 and there was no significant difference in the disease severity between sprayed fields. This shows the effectiveness of a broad range of lower concentrations of copper oxide (600 g)-metalaxyl (120 g) fungicide in enhancing taro yield by reducing the growth and rapid proliferation of *P. colocasiae*. Earlier findings by [24] showed that metalaxyl and copper active ingredients in plus 72 WP significantly reduce the severity of potato late blight caused by *P. colocasiae*.

Fungal lesions appeared on all landraces, following inoculation with *Phytophthora colocasiae* and these lesions later became necrotic, enlarged, and coalesced, resulting in the collapse of leaf tissues within a short period (Fig. 6). This confirms the findings that reported that *Phytophthora colocasiae* can cause rapid and complete defoliation of leaves and crop.
The taro leaf blight disease severity reduced below 1.5 in the all experimental field sites sprayed with copper oxide (600 g) -metalaxyl (120g) within two years in four successive growing seasons. In all the field sites, there was no significant difference in the taro blight severity (P ≤ 0.05) between the landraces sprayed with different concentrations of oxide (600 g) -metalaxyl (120 g) fungicides. The severity of taro blight greatly decreased with varying low concentrations of copper oxide (600 g)-metalaxyl (120 g) (0.4%, 0.33%, and 0.27%) applied at two-week intervals. Therefore, for environmental and health reasons, farmers should use the lowest concentration of the fungicide (0.27%) to effectively control taro blight. All the inoculated landraces in the screen house expressed disease symptoms which were exactly similar to taro blight symptoms in the field. In all the agroecological zones, L2 (red petiole small leaves) was highly resistant to P. colocasiae. Therefore, for optimum taro production, L2 should be recommended to farmers for cultivation in the three agro-ecological zones and zones or areas with similar characteristics.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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