Research Article

Field management of Taro (*Colocasia esculenta* (L.) Schott) leaf blight via fungicidal spray of foliage

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Abstract

Taro leaf blight (TLB) epidemic hit Cameroon for the first time in 2009. Since then, the disease is persistent and its typical devastating legacy is threatening Taro **(***Colocasia esculenta***)** in the North and South West Regions of Cameroon. This study was initiated with the objective to determine the potentials of some fungicides to control TLB. The experimental design was completely randomized with a 3x15x2 factorial, including 3 treatments: T1 (Callomil plus72WP), T2 (Mancoxyl plus 720WP) and T3, 1:1 ratio T1 + T2 all applied at concentrations of 4g/L; 15 repetitions and 2 planting seasons (dry season i.e. October 2014 – March 2015 and rainy season i.e. April-October 2015). Disease incidence and disease severity were used to evaluate the disease progression while corm yield was used to appraise the economic injury. The results revealed disease incidence of 0% during the dry season and 18.2%, 27.3% and 100%, for T1, T2 and T3 and control during rainy season respectively. Disease severity was 75% in control and only 1% for the different treatments. Corm yield in the rainy season was 17.4kg, 15.08kg, 14.27kg and 5.89kg for T1, T2, T3 and control respectively. This study suggests that TLB epidemic can effectively be managed by foliage spray with Metalaxyl containing fungicides at a weekly dosage of 4g/L.

Key words: Chemical control, *Colocasia esculenta*, Epidemic, Phytopathology, *Phytophthora colocasiae*

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Résumé

L'épidémie de la maladie foliaire du taro (MFT) a frappé le Cameroun sévèrement en 2009. Depuis lors, cette maladie est persistante et sa nature typiquement dévastatrice est une menace pour le Taro (*Colocasia esculenta*) dans les régions du Nord-Ouest et du Sud-Ouest du Cameroun. Cette étude a été lancée dans le but de déterminer le potentiel de certains fongicides pour lutter contre la MFT. Le dispositif expérimental était complètement randomisé de 3x15x2 facteurs, comprenant 3 traitements: T1 (Callomil plus 72WP), T2 (Mancoxyl plus 720WP) et T3, rapport T1: T2 = 1: 1), tous appliqués à des concentrations de 4g / L; 15 répétitions et 2 saisons de plantation (saison sèche d'octobre 2014 à mars 2015 et saison des pluies d'avril à octobre 2015). L'incidence et la sévérité de la maladie ont été utilisées pour évaluer la progression de la maladie, tandis que le rendement en cormes a été utilisé pour évaluer le dommage économique. Les résultats ont révélé une incidence de la maladie de 0% pendant la saison sèche et 18,2%, 27,3% et 100% pendant la saison des pluies pour T1, T2, T3 et le traitement témoin (contrôle), respectivement. La sévérité de la maladie était de 75% chez les témoins et de seulement 1% pour les différents traitements. Les rendements de cormes étaient de 17,4 kg, 15,08 kg, 14,27 kg et 5,89 kg pour T1, T2, T3 et le contrôle, respectivement. Cette étude suggère que l'épidémie de la MFT peut être efficacement contrôlée par la pulvérisation foliaire avec des fongicides contenant du Metalaxyl à raison de 4 g / L chaque semaine.

Mots Clés: Contrôle chimique, *Colocasia esculenta*, Epidémie, Phytopathologie, *Phytophthora colocasiae*

Introduction

Taro (*Colocasia esculenta* (L.) Schott), a tropical aroid with nearly 1000 known cultivars, is an important subsistence crop for millions of people in Africa, Pacific islands, Caribbean islands and South East Asia (Chandra and Sivan, 1984). The plant is a herbaceous perennial, made up of a collection of long-petioled 30-150 cm leaves emerged from the top of a swollen underground stem or corm. The heart-shaped leaves vary in size from 20-30 x 30-60 cm. The inflorescence is a spadix of closely packed, small male and female flowers surrounded by a yellowish-green spathe (Sunell and Arditti, 1983). In Cameroon, the three main varieties of taro cultivated year round are Ibo coco, atangana and country (Mbong et al. 2013). Some peasants in taro growing hubs in the North West and South west Regions of Cameroon, explained that, corms, as well as fresh and dry leaves of taro appear at least four times in their weekly menu during the peak season. With the advent of the *Colocasia* leaf blight in Cameroon in 2009 and its outbreak in 2010 (Mbong et al. 2013), an alarming yield loss of up to 90% was reported (Guarino 2010). Among recently reported repeated outbreaks of taro epidemics in other intense *Colocasia* growing hubs in West Africa are the Easten Region of Ghana (Omane et al. 2012) and the Abia State of Nigeria (Banddyopadhyay et al. 2011) and there are fears that the disease will spread further. Given that the disease symptoms are mainly foliar, it was described as Taro Leaf blight (TLB) (Jackson et al. 1980). TLB is caused by *Phytophthora colocasiae* Racib., a fungus reported to be the most destructive of *Colocasia* (Thankappan 1985). TLB symptoms appear as small, water soaked spots, which increase in size to form dark brown lesions, often with a yellow margin and red droplets along the margin (Mishra et al. 2008). The normal lifespan of a healthy taro leaf is about 40 days but once attacked, this can last only 15-25 days (Jackson et al. 1980). Its inoculum in the form of spores is spread by wind-driven rain and dew to adjacent *Colocasia* plants (Jackson 1999). The use of planting material from infected corms increases TLB incidence in subsequent *Colocasia* crops (Ooka 1990). Density of plants, temperature and humidity appear to be the primary factors influencing infection and spread of the disease (Ivancic et al. 1996).

The rapid decline in *Colocasia* production in Cameroon due to TLB is threatening the crop (Mbong et al. 2013) and extinction is eminent in the country if controlling the outbreak is not considered a matter of urgency. To the best of our appreciation, rational chemical control is the fastest method of managing diseases and pests already at an epidemic stage. Phenylamide and copper-based fungicides have been reported to be effective in controlling diseases caused by fungi of the Oomycetes phylum (Mishra et al. 2008) like *Phytophthora colocasiae* Racib. However, the disease is new in Cameroon and very scanty literature exists on its management under specific agro ecological zones. Hence, the general objective of this study was to settle on fungicides that have the potential to reduce the TLB progression to a less economically injurious level, based on the hypothesis that foliar spray with Phenylamide fungicides interrupts disease cycle of *P. colocasiae.*

MATERIALS AND METHOD

Study site

The study was carried out in the Musang neighborhood of Bamenda town, Cameroon located at 6°2'N, 10°7'E, at 1239 m asl. The area is made up of 8 months rainy season (mid-March to October) and 4 months dry season (late October to mid-March) with average day length of 12.25h and 11.75h in the rainy and dry seasons respectively. In this site, the mean annual temperature range is 19.5-20.3°C, average humidity is 86%, average days of rainfall/month is 24 and 8 days respectively during the rainy and dry seasons. Mean rainfall is 270-300 mm and

25.8-30 mm during the rainy and dry seasons respectively (Anonymous 2014). The plot on which the experiment was carried out had *Colocasia* grown on it for over years and was reputed to be a hotspot for TLB reoccurrence for 5 years, hence the soil was considered to be highly infested with *P. colocasiae* spores.

Land preparation and sowing

An initially infested plot of 15m x 10m was cleared and ploughed into mound rows using a hoe. The grass was not burnt prior to ploughing in order to maintain the population of the pathogen. Each row was separated from the adjacent one by 1 m. The planting materials were collected from a field with a repeated history of epidemics for 5 years. For the sake of homogeneity, corms of relatively the same size were selected and sown in rows at an intra-distance of 75 cm.

Experimental design

Between 7-12 days after sowing all the corms had germinated and after two weeks, robust plants were randomly tagged using red, yellow, blue and white strings representing the different treatments and the control. The three treatments (T) were Callomil Plus 72WP (T1) (1kg of Callomil Plus contains 120g of Metalaxyl + 600g of copper oxide), Mancoxyl Plus 720 WP (T2) (80g/kg of Metalaxyl +640g/kg of Mancozeb), and1:1volume ratio of Callomil + Mancoxyl (T3). The controlled plants did not receive any treatments. Fifteen randomly selected plants constituted the sample size of each treatment. The composition of the spray solution was 4g/L of water. The spraying was done such that the laminar of all the leaves per plant stand were in contact with the spray solution hence no specific volume was used per plant because the plants were not homogenous in agronomic characteristics. For the rainy season experiment (15^{th} April – 5^{th} September) of this study, spraying was done at an interval of 14 days between April to June and 7 days interval from July-September. Close observations for the first symptoms as described by Mishra et al (2008) were made on daily bases in the course of the treatments. The experiment was completely randomized with 3 treatments and 15 repetitions. Given that the leaves of *Colocasia* are slanting and highly waxy, adherence of spray solution was difficult and performance was estimated to be low. To resolve this problem, an adjuvant called DOSTH (manufactured by SDS Ramcides Crop Science Pvt. Ltd, India) with role to reduce the surface tension, enhance stickiness, coverage and penetration of active ingredients into the target sites was added to the spray solution at a dosage of 2 ml/L. Besides, spraying between 6-7 AM before sunrise optimized adherence of the spray solution on the waxy leaf surfaces (Fig 1).

Fig. 1. Enhancement of spray solution adherence on taro waxy leaf surface due to the presence of an adjuvant

The experiment was repeated in the dry season (26^{th} October-10^{th} March) with irrigation done by watering can. Each plant received 1 L of water at about 9:00 -10:00 AM between 15^{th} November to 10^{th} of December at an interval of 3 days and then at a 2 days interval from mid-December till the end of the study. Water was gently applied at the base of each plant and not on the leaves to minimize any splashing of soil particles to leaves. Treatments for the dry season experiment were to start only when the first symptoms appeared as described by Mishra et al. (2008).

Identification of pathogen

During the early hours of the morning, before sun rise, infected leaves with fresh flecks water soaked lesions as symptoms were identified. While still attached to the plant, a transparent sticky tape of 1 cm wide was positioned at the margin of a lesion and cut with a razor blade to a length of 1cm. The 1 cm^2 sticky tape was pulled off the leaf and placed on a clean microscope slide and observations made under a binocular photon microscope at 100X. The number of sporangia was counted at three different areas and average taken. 24 hours after treatments of infected area, samples were collected once more at the margins of water soaked lesions and observed again under the microscope.

Parameters assessed

Two epidemic parameters studied were disease incidence (I) and disease severity (S).

Disease incidence (I)

For this parameter, 11 established plants (3-5 leaf stage) per treatment were selected randomly, tagged and scrupulously monitored thrice a week within a period of 12 weeks. A plant was considered infected if a water soaked lesion with associated characteristics described by Mishra et al (2008) appeared on any of its leaves. The total number of plants infected per treatment was noted and cumulatively assessed at the end of the study period. Disease incidence (I) was computed using the following formula

$$I = \frac{number\ of\ infected\ plants\ for\ a\ given\ treatment}{Total\ number of plants\ in\ the\ treatment}\ x\ 100$$

Disease severity (S)

Four plants out of the 11 tagged for incidence served as the sample size. S was defined as percentage of the leaf surface affected by blight; either lesions or lesions plus lesion-related chlorosis (James 1971). Three leaves were tagged and examined every 3 days for symptom initiation and subsequent progression of symptoms, using the syndrome scale of Horsfall and Cowling (1978) within a period of 27 days. According to this syndrome scale,

0 =No disease, 1= necrotic area less than 10 cm^2 of leaf area, 2 = necrotic area 11 – 30 cm^2 of leaf area, 3 = necrotic area 31 – 60 cm^2 of leaf area, 4 = necrotic area 61 – 90 cm^2 of leaf area, 5= necrotic area more than 90 cm^2 or up to 25% of leaf area, 6 = Coalesce of spot more than 25% of the leaf covered, 7= Coalesce of spot more than 50% of the leaf covered, 8 = Coalesce of spot more than 75% of the leaf covered, 9 = Collapse of petiole accompanied by complete leaf blight.

Besides, the impact of disease factors (I and S), other growth parameters like leaf area, number of suckers and corm yields were also evaluated.

Leaf surface area

Leaf surface area (Sf) of plants infected by the disease was assessed using the maximum length and breadth of a specific leaf on plants under the various treatments. Data for Sf assessment was collected from the 5th leaf in randomly selected plants per treatment. Sf was calculated using the equation:

$Sf = 7.9012 + 0.8437(L_2 \times W)$, derived by linear measurement of leaf blade (Carolina et al. 2011).

Number of suckers

Mean number of suckers growing from the mother plant was counted at the 10th week.

Evaluation of corm yields

Ten randomly selected samples per treatment were uprooted after 6 months and the remaining leaves and all the adventitious roots cut off. The corms were washed to get rid of soil particles. The fresh weight of corms from each treatment was measured using a balance.

Data for all parameters examined, was analyzed using SPSS version 22. To determine the level of significance, mean values for the treatments were compared using the student t-test.

Results

Influence of cultivation time on disease incidence

No disease symptoms were observed throughout the study period in the dry season (October 26th to March 10th). On the other hand, symptoms of the blight (Figs 2B and 3) were observed when the same experiment was done in the rainy season. Initial symptoms observed on infected plants were small dark spots which enlarged rapidly over time and turned purplish brown with yellowish margins. The lesions expanded, formed concentric zones and exuded drops of a yellowish liquid. The lesions appeared to increase in circumference mainly during the night while during the day they appeared dry. Whitish fuzz representing sporangia could be seen covering the diseased tissues during the morning hours before sun light. Advanced necrosis led to the disintegration of leaf tissues forming holes of irregular sizes and shape on the affected leaf lamina.

Identification of parasite

Microscopic observation of tissues bordering the necrosis revealed spherical sporangia, each containing 1-2 spores (Fig. 4). Between 350 and 400 sporangia were counted within 6 mm^2 of the necrosed tissues observed. On the other hand, no spores were observed from samples collected 24 hours after the three fungicide treatments were applied.

Influence of fungicide treatment on disease incidence (I) and severity (S)

Disease incidence was appreciably higher in controlled plants (100%) compared to plants under the different treatments where scores of 18.8% in T1 and 27.27% for T2 and T3 were observed (Fig.5). When samples under the three treatments presented 6-7 leaves per plant, only 3-4 leaves could be counted on the controlled plants where disease incidence was 100%.

The disease was more severe in the controlled compared to plants subjected to T1, T2 and T3. The water-soaked lesions were observed both on the lamina and the petioles of controlled experiment while in treated plants; the few necroses observed were limited only on the lamina. Even though disease symptoms occurred in leaves of plants treated with T1, T2 and T3, no further progression of water-soaked lesions was observed once the treatment was administered on the infected area. Rather, the soggy lesions became dry and within 5 days, the dead tissues fell off leaving a hole on the lamina (Fig. 2c). Having been freed from the pathogen, tagged leaves under treatments remained healthy on the plant until their natural senescence that ranged between 37-43 days. The progression of the disease was very rapid on lamina of controlled plants with over 50 % of the lamina surface infected with rust-like look within 10 days of incubation. This high severity caused the lamina to wither (Fig. 3, stage 9) between 16-22 days after attack.

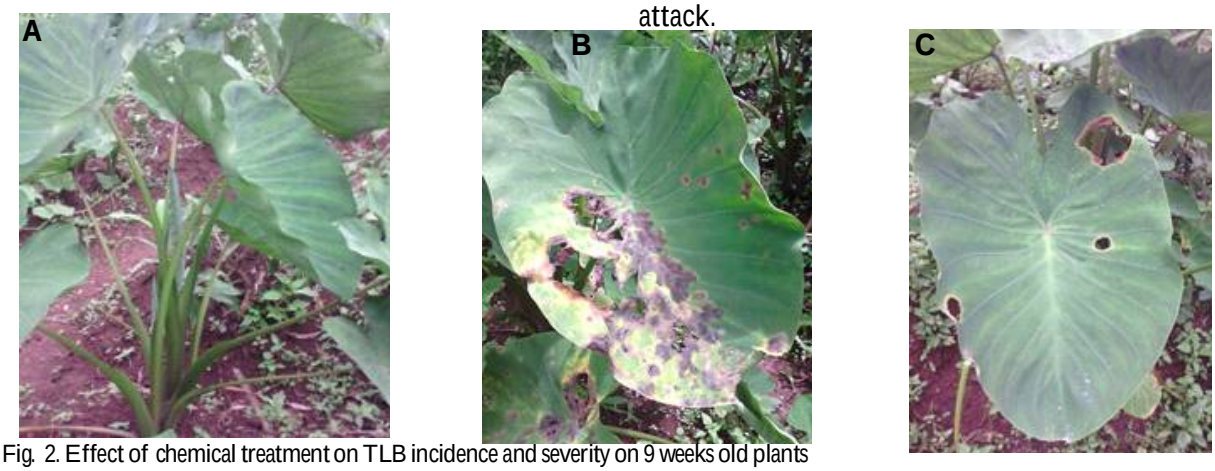

Fig. 2. Effect of chemical treatment on TLB incidence and severity on 9 weeks old plants
A. plant under T1, B. controlled plant, C. Progression of necrosis on lamina discontinued after application of T1

Fig.. 3. Syndrome scale used to grade disease progression on tagged leaves over time
1= necrotic area less than 10 cm^2 of leaf area, 2 = necrotic area 11 – 30cm^2 of leaf area, 5= necrotic spots more than 90 $cm^2$and up to 25% of leaf area, 7= Coalesce of spot more than 50% of the leaf covered, 9 = Collapse of petiole accompanied by complete leaf blight

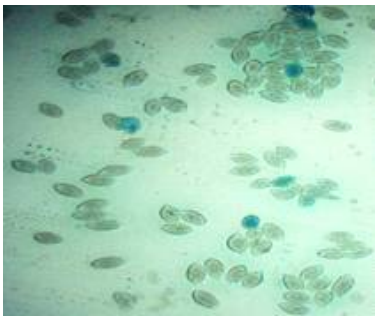

Fig.. 4. Sporangia of *P. colocasiae* (Mg: 100X) Bar, 0.2mm

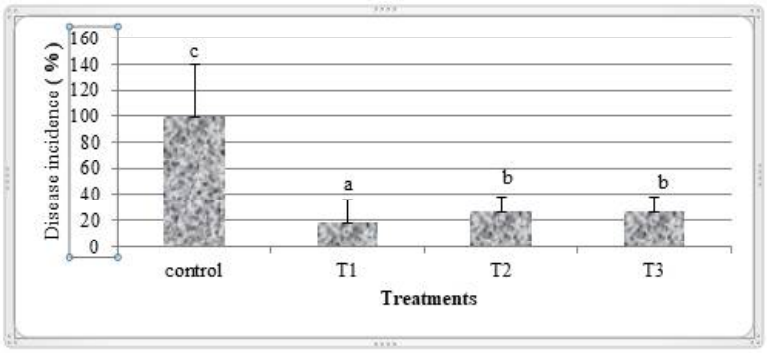

Fig. .5. Effect of chemical treatment on taro leaf blight incidence
*Different letters on histograms indicate significant differences (P<0.05)

Leaf area

Fungicide-treated plants (T1, T2 and T3) presented a larger leaf surface area compared to the controlled (Fig. 6). The differences in leaf area were not significant between samples under the three treatments but all treatments were significantly different from those of the controlled plants. It was noticed that the higher the disease severity, the smaller the leaf area of subsequent leaves.

Effects of fungicide treatment on number of suckers and corm yield

The controlled samples presented the least mean number of suckers which differed significantly at P<0.05, with those of the three fungicide treatments (Fig. 7). Even though T1 presented a higher number of suckers than T2 and T3, the difference was not significant.

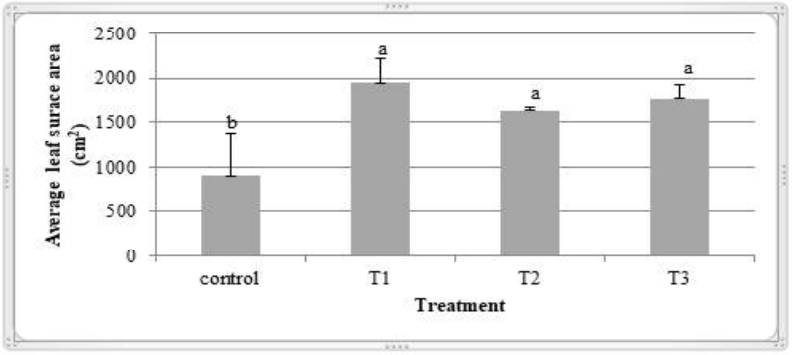

Fig. 6. Effect of fungicide treatment on average leaf surface area of the 5th leaf
*Different letters on histograms indicate significant differences (P<0.05)

The average fresh weight of corms obtained from ten random plants under the various treatments stood at 17.40 kg, 15.08 kg and 14.27 kg for T1, T2, and T3 respectively while merely 5.98 kg was obtained for control samples (Fig. 8). Significant differences were noticed in corm yields between the treatments and the control. However, no significant differences were found between the T1, T2 and T3 in terms of corm yield.

Discussion

Plant diseases continue to pose serious threat to food security on national economies world wide. Given that Taro leaf blight (TLB) is a polycyclic epidemic, the three major tactics that must be put in place to better manage new infections include reduction of initial inoculums, infection rate and the duration of the epidemic. The total absence of disease symptoms in the dry season (October to March) as observed in this study may perhaps be an indication that a drop in humidity on leaf surface is a limiting factor for growth and reproduction of the *P. colocasiae*. On the other hand, the reappearance of symptoms with the onset of rains could imply that the pathogen was present in the soil but remained in a dormant spore stage due to adverse environmental conditions. These results corroborate with those reported by Misra and Chowdhury (1995) who concluded that shifting planting time in such a way that the crucial stage of plant growth and optimum climatic conditions for disease development do not concur with each other. This is an effective cultural control technique of *P. colocasiae*. Fullerton and Tyson (2001) reported that the primary reproductive unit of the pathogen is the sporangium which requires

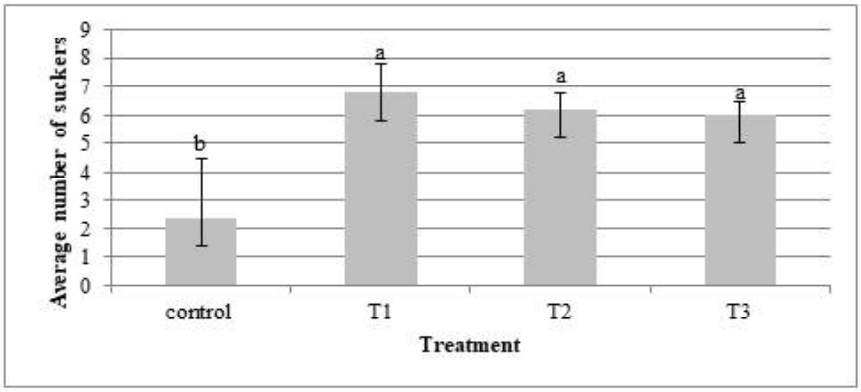

Fig.7. Effect of fungicide treatment on number of sucker outgrowth
*Different letters on histograms indicate significant differences at (P<0.05)

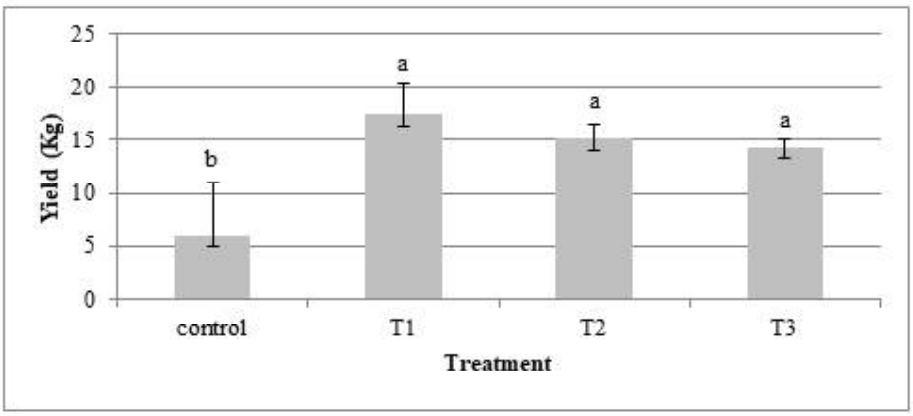

Fig.8. Effect of fungicide treatment on average *Colocasia* yields of ten plants/ treatment
*Different letters on histograms indicate significant differences

free water to germinate. Although taro leaves have a waxy surface, minute droplets of water that accumulate on leaves provide sufficient moisture for sporangia to loose, letting zoospores to germinate, penetrate and infect the plant (Quitugua and Trujillo 1998). The high spore density observed under the microscope in this study could explain the rapidity of the progression of the necrosis of lamina to cause new infections on the lamina given the large propagule population. Once zoospores settle onto the leaf surface, they lose their flagella within 10 min and form a rounded cyst which soon germinates to form a germ tube (Fullerton and Tyson 2001). This indirect mode of germination provides a strong ecological advantage to the pathogen as it generates up to a 15-fold increase in inoculum within 10 min, compared to the direct mode which takes a longer time (5-6 h). In the direct mode, the sporangia first germinate into germ tubes that infect the leaves, mature before producing new inoculum (Davinder et al. 2012).

The reduction in disease incidence for plants under T1, T2 and T3 could imply that the different treatments contained active ingredients (Metalaxyl and copper oxide) that were able to reduce the virulence of the pathogen by creating unfavorable conditions for its development. These active ingredients, probably act by: inactivating the inoculum; preventing zoospores germination or destroying spores once in contact with the fungicides, thus interrupting polycyclic nature of the pathogen. Corroboratory results have been reported by Bergquist (1974) in which a substantial TLB control was achieved in Hawaii by applying Mancozeb at 7-day intervals. Aggarwal et al. (1987) also reported good disease control with Metalaxyl. Similarly, Cox and Kasimani (1990) obtained a 50% increase in corm yield in Papua New Guinea upon five applications of Metalaxyl at 3-week intervals. The fact that necrotic lesions dried off leaving a hole on the lamina after foliar spray of T1, T2 and T3 and senescence between 37-43 days as observed in this study, could imply that the pathogen was sensitive to the fungicides, hence its growth cycle was effectively interrupted.

A common characteristic among the two fungicides used in this study is the presence of Metalaxyl, the active ingredient. It has been reported that Metalaxyl based fungicides show excellent protective, curative and eradicative antifungal activity and exclusively control diseases caused by the Order Peronosporales of Oomycetes (Schwinn and Staub 1995). Similar studies had reported that fungicides containing Metalaxyl are efficient in destroying several pathologies caused by *Phytophthora*. This is the case with potato late blight caused by *P. infestans* (Runno and Koppel 2006; Muchiri et al. 2009), wax gourd and cucumber blight caused by *P. melonis* (Wu et al. 2011), Brown pod disease of cocoa caused by *P. megakarya* (Deberdt et al. 2008). In general, Metalaxyl is effective for controlling diseases incited by Oomycetes because it is absorbed by the leaves and roots under various environmental conditions (Easton and Nagle 1985). Namanda et al. (2004) showed that Mancozeb applied as a protectant can be effective in reducing the impact of late blight under tropical conditions. The mode of action of Metalaxyl in retarding the progression of the epidemic is probably associated to processes that impair the biosynthesis of RNA hence mitosis of the pathogen is inhibited (Fisher and Heyes 1982). Generally, fungicides kill fungi by damaging their cell membranes, inactivating critical enzymes or proteins, or by interfering with key processes such as respiration. However some fungicides impact specific metabolic pathways such as the production of sterols or chitin. For example, phenylamide fungicides like Metalaxyl selectively inhibit ribosomal RNA synthesis of sensitive Oomycetes fungi by interference with the activity of the RNA polymerase I-template complex (Davidse et al. 1988).

The plant's efficiency in converting solar energy into dry matter is dependent on the photosynthetic activity of the individual leaves. The **physiological and economic implications of leaves on vegetative growth and corm yield of Cocoyam had been demonstrated by Asumandu et al. (2011). It is therefore eminent that pre-mature death of leaves due to foliar disease will affect yields directly as observed in this study.** Higher corm yields obtained in T1, T2 and T3 compared to the control can be attributed to the fact that treated plants were healthier than the controlled plants during the vegetative growth phase, hence they efficiently carried out their vital photosynthetic functions and the assimilates were stored in the corms which constitute the main sink in *Colocasia*.

Conclusion

In order to limit the risks associated with outbreak of TLB, there is need to use a number of different approaches in an integrated manner. As observed, off season cultivation of taro (October – March) is a cultural practice that prevents TLB outbreak. This study also showed that TLB epidemic can effectively be managed by Metalaxyl containing fungicides like Callomil and Mancoxyl. Foliage spraying of these fungicides at 4g/l fortnightly between April - June and at 7 days interval from July–September due to frequent rains and lower temperatures could significantly reduce the progression of the epidemic to a level that is not injurious socioeconomically. However, starting with disease-free planting material, site preparation and establishing good drainage will not only limit TLB incidence and severity, but also guarantees improved soil health.

Authors and their contribution

Tabi KM, conceived the project, executed methodology and drafted manuscript

Ntsomboh GN, conceived project, built up methodology, shaped and proof read manuscript

Tonfack LB, built up methodology, shaped manuscript and proof read manuscript

Youmbi E: modified methodology, proof read manuscript

Conflict of interest

The authors declare no conflict of interest.

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