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COMPARISON OF ISOLATES OF *Phytophthora colocasiae* Raciborski FROM DIVERSE ALTITUDES AND APPRAISAL OF PLANT EXTRACTS FOR ITS MANAGEMENT *IN VITRO*

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ABSTRACT

Leaf blight disease caused by *Phytophthora colocasiae* Raciborski is a serious constraint to taro production. This study was carried out to characterize the isolates of *P. colocasiae* from Makurdi, Nigeria and Befang, Cameroon; and evaluate the potential of aqueous leaf extracts of Eucalyptus, neem and mahogany at 0, 50 and 100% concentrations for management of the pathogen. A synthetic fungicide (Mancozeb) was included as a check. The isolate from Befang had more radial growth than that from Makurdi. The colonies of both isolates were translucent and low on corn meal agar (CMA) but opaque and high on acetate differential agar enriched with dextrose (ADAD). The texture of the Befang isolate was filamentous on CMA but hard and grainy on ADAD; the Makurdi isolate was filamentous on both media. Mancozeb and all the plant extracts inhibited the growth of the pathogen significantly ($P \leq 0.05$). Among the extracts, Eucalyptus at 100% concentration was the most potent (94.4–100.0% inhibition) and it was comparable to Mancozeb (100% inhibition); mahogany at 50% was the least potent (<20%). Both concentrations of neem gave similar levels of growth inhibition (mean=50.0–64.6%) throughout. Other inhibitory levels were 32.0–57.3% for Eucalyptus at 50% and 24.0–53.9% for mahogany at 100%. These results indicated that ADAD and CMA were appropriate culture media for the fungus. The extracts of Eucalyptus, neem and mahogany had fungicidal activity against *P. colocasiae*; therefore they should be incorporated into management programmes for the pathogen.

Keywords: Cultural characterization; Eucalyptus; Leaf blight disease; Mahogany; Neem; Taro.

INTRODUCTION

Cocoyams are tuber crops which are widespread and cultivated throughout the tropics. The two main types are *Colocasia esculenta* and *Xanthosoma* spp. In several countries in West and Central Africa, they are third in importance after cassava (*Manihot esculenta* Crantz) and yam (*Dioscorea* spp) (Onyeka, 2014). They are produced for home consumption and as a source of revenue for the farmers. In terms of nutritional value, they are better than cassava and yam in protein content and they contribute quality carbohydrate and dietary fibre to the nutrition of humans (Nwanekezi *et al*., 2010; Awak *et al*., 2017).

Taro (*Colocasia esculenta* (L.) Schott) is susceptible to many diseases in the field and during storage (Onyeka, 2014). The most destructive among them is leaf blight and the causal organism is *Phytophthora colocasiae* Raciborski (Misra *et al.*, 2008; Lum and Takor, 2021). Leaf blight disease devastates taro in several countries including Cameroon, Ghana and Nigeria (Bandyopadhyay *et al*., 2011; Singh *et al*., 2012; Mbong *et al.*, 2013; Omeje *et al*., 2015; Lum and Takor, 2021). The emergence of this invasive fungus-like oomycetes pathogen in these countries/regions was abrupt. It was first reported in Nigeria in 2009 (Bandyopadhyay *et al*., 2011) and in West Cameroon in 2010 (Mbong *et al.*, 2013). Lum and Takor (2021) reported high incidence of the disease (77.9–96.5%) in taro fields surveyed in several localities in South West Cameroon. Chiejina and Ugwuja (2013) also reported high incidence of the disease (74.2 and 77.2%) in two localities of Nsukka, South East Nigeria during a field survey.

Cultural methods such as intercropping and roguing have given only partial success against leaf blight (Lum and Takor, 2021). The choice and preparation of planting material is critical for guaranteeing healthy crops. Some chemical control measures have given good results but they are not affordable by most farmers and could damage the environment if abused. Manju *et al.* (2017) indicated that Fungiforce® (a synthetic fungicide) reduced the disease incidence in the field. The crop is mostly produced by small-scale farmers who cannot afford the extra resources needed for the synthetic chemicals (Lum and Takor, 2021). The farmers need alternative methods that are cheap and easily available to them. Only a few plant extracts have been tested for management of *P. colocasiae*. Fontem *et al*. (2012) and Lum and Takor (2021)

reported that the aqueous extracts of tropical girdle pod (*Mitracarpus villosus* (Sw.) DC.) and goatweed (*Ageratum conyzoides* L.) have fungicidal activity

against *P. colocasiae*. Based on the importance of taro and the devastating effect of the disease, this study was carried out to characterize the isolates of *P. colocasiae*; and evaluate the aqueous extract of selected indigenous medicinal plants against the pathogen, to determine if they can be used to complement the scarce and expensive exotic fungicidal agents.

MATERIALS AND METHODS

Study area

This study was conducted in the Advanced Plant Pathology Laboratory, College of Agronomy, Federal University of Agriculture, Makurdi, Nigeria in 2016. The fungus was isolated from infected taro leaves sourced from Makurdi (07° 42'N; 08° 31'E; 101 m above sea level) in North Central Nigeria and Befang (05° 53'N; 09° 45' 41″E; 1043 m above sea level) in North West Cameroon. Both locations are at different altitudes and suitable for the cultivation of cocoyams. Makurdi, the capital of Benue State, Nigeria is situated on low land in the sandy Benue River Basin. Befang is situated in Wum, the capital of Menchum Division, North West Region, Cameroon.

Isolation, identification and inoculation of the pathogen

The methods of sample collection, preparation and isolation of the pathogen were as reported by Lum and Takor (2021). In this study, the fungus was isolated using acetate differential agar (Difco, USA) enriched with dextrose (ADAD) and the cultures were purified using potato dextrose agar (Lifesave Biotech, USA). The different media were prepared based on the manufacturers' instructions. Streptomycin sulphate (0.2 g L^{-1} H_2O) was added to the autoclaved media in conical flasks in the laminar airflow hood unit. The contents of the conical flasks were swivelled around to mix without creating bubbles and poured into the Petri dishes. Two lines were ruled under each Petri dish at right angles to each other. The inoculum consisted of suspensions of 9-day old cultures of *P. colocasiae* in sterile distilled water. It was estimated that each inoculum contained 4×10^5 spores based on haemocytometry. The fungus was identified using microscopy (Olympus 100 compound microscope) and fungi identification keys (Barnett and Hunter, 1999).

Preparation of plant extracts

Healthy leaves of Eucalyptus (*Eucalyptus globulus* Labill.), mahogany (*Khaya senegalensis* (Devs.)) and neem (*Azadirachta indica* A. Juss.) harvested from trees growing in Makurdi were washed using tap water and surface sterilized with 1% sodium hypochlorite solution. The leaves were rinsed three times using sterile distilled water, chopped up into

small pieces and blended using an electric blender (Warrington blender; England); the leaf extract of each plant at 100% concentration was prepared using 500 g L^{-1} of sterile distilled water. The plant extracts were allowed to stand for 72 hours after which they were filtered using Whatman No. 1 filter paper into sterile conical flasks.

Evaluation of the cultural characteristics of *P. colocasiae* on two nutrient media

The experiment was setup using a completely randomized design with factorial arrangement. There were two factors (isolates of the pathogen and nutrient media (ADAD and Corn Meal Agar (CMA)). Consequently, the treatments consisted of the following: Makurdi *P. colocasiae* isolate in CMA, Makurdi *P. colocasiae* isolate in ADAD, Befang *P. colocasiae* isolate in CMA and Befang *P. colocasiae* isolate in ADAD; replicated three times. The pathogen was inoculated at the centre of each Petri dish after which it was sealed with masking tape.

Data were collected on the diameter of fungal growth using a translucent rule every 24 hours; the texture, type of edge, opacity and elevation of the growth at 72, 120 and 168 hours after inoculation (HAI). The colour of the growth was recorded based on the Royal Horticultural Society colour charts (2004 version). The experiment was terminated at 168 HAI. The data on diameter of the fungus colony were subjected to analysis of variance and the Student Newmann Keul's test ($P \leq 0.05$) was used to separate the means. Genstat 2nd Edition Discovery was used to process the data.

Assessment of the efficacy of plant extracts on management of *P. colocasiae in vitro*

The experiment was laid out using a completely randomized design. The treatments were extracts of Eucalyptus, mahogany and neem at 0 (control), 50 and 100% concentrations; the synthetic fungicide Mancozeb was included as a check. The treatments were replicated three times. The CMA and extracts were poured in Petri dishes and allowed to set in the laminar air flow system. The pathogen was inoculated at the centre of the Petri dishes; they were incubated on the Laboratory table for 120 hours and data on the growth diameter were collected every 24 hours. The diameter was measured using a translucent rule. The percentage of inhibition was calculated as proposed by Lum *et al*. (2019). The data were subjected to analysis of variance and

Tukey HSD test (P≤0.05) was used to separate the means.

RESULTS AND DISCUSSION

Results of the comparison of *P. colocasiae* isolates from Befang and Makurdi cultured using ADAD and CMA are shown in Table 1. There were significant (*P*≤0.05) differences in the radial growth of the isolates on the culture media. In general, the radial growth of the isolate from Befang (2.63–6.83 cm in CMA; 0.93–6.48 cm in ADAD) was more than that from Makurdi (0.10–3.43 cm in CMA; 0.10–4.90 cm in ADAD). There were no significant (*P*>0.05) differences in the growth of the Makurdi isolate on both media from 24–168 HAI but significant differences (*P*≤0.05) existed for the Befang isolate on both media at 24–96 HAI. At 24 HAI, the Makurdi isolate on both media and the Befang isolate on ADAD had similar radial growth which was less than that of the Befang isolate on CMA. At 48–96 HAI, CMA plates with the Befang isolate had more growth than all the other plates. This was followed by the Befang isolate cultured on ADAD. From 120 to 168 HAI, the Befang isolate had comparable growth on CMA and ADAD.

Table 1. Radial growth of *Phytophthora colocasiae* isolates from Makurdi, Nigeria and Befang, Cameroon cultured on acetate differential agar enriched with dextrose (ADAD) and corn meal agar (CMA)

Treatment (*P. colocasiae* isolate and medium)	Diameter (cm)						
	24	48	72	96	120	144	168
	---------- (Hours after inoculation) ----------						
Makurdi isolate in CMA	0.10a	0.97a	1.40a	2.00a	2.48a	3.10a	3.43a
Makurdi isolate in ADAD	0.10a	0.98a	1.63a	2.50a	3.42a	4.17a	4.90a
Befang isolate in CMA	2.63b	4.28c	5.13c	5.23c	5.67b	6.50b	6.83b
Befang isolate in ADAD	0.93a	2.23b	2.90b	3.80b	4.83b	6.18b	6.48b

Means with the same superscript are statistically similar using Student Newmann Keul's test (P<0.05).

Cultural characteristics of the isolates of *P. colocasiae* on both nutrient media are presented in Table 2. On both media, the texture of the isolates from both locations was filamentous, except the Befang isolate on ADAD which was hard and grainy. The colony colour (back and front sides) for both isolates was basically yellow in ADAD but variable in CMA. With the exception of the Makurdi isolate which had an entire edge when cultured on CMA, the colony edge of the isolates was lobate on both media. Colony opacity and elevation were similar for both isolates in each culture medium. The colonies were translucent and low in CMA but opaque and high in ADAD.

Table 2: Cultural characteristics of isolates of *P. colocasiae* on two nutrient media

Fungus isolate × Medium	Cultural characteristics at periods specified					
	Texture	Colony colour (front)	Colony colour (back)	Edge	Opacity	Elevation
	72 and 144 HAI	---------- 72, 120 and 168 HAI ----------				
Makurdi × CMA	Filamentous	Greyed green (188C fan 4)	Greyed green (188D fan 4)	Entire	Translucent	Low
Makurdi × ADAD	Filamentous	Yellow (13C fan 1)	Yellow (14B fan 1)	Lobate	Opaque	High
Befang × CMA	Filamentous	Red group (48C fan 1)	Red group (48D fan 1)	Lobate	Translucent	Low
Befang × ADAD	Hard, grainy	Yellow orange (13B fan 1)	Yellow orange (14D fan 1)	Lobate	Opaque	High

Colours are based on Royal Horticultural Society Colour Chart Scheme (2004); HAI = Hours after inoculation; ADAD = acetate differential agar enriched with dextrose; CMA = corn meal agar.

The inhibitory effects of the plant extracts on the growth of *P. colocasiae* are presented in Table 3. All the plant extracts and the fungicide (Mancozeb) significantly (P≤0.05) inhibited the growth of the fungus compared to the control. The rate of inhibition varied with the type of plant and concentration of the extract. For each plant extract, there was an increase in the radial growth of the fungus mycelium from 24 to 120 HAI. Also, the radial growth was lower at a concentration of 100% than at 50%; however, this difference was not significant P>0.05) for neem extract. Among the plants, Eucalyptus extract at 100% concentration gave the highest inhibition of fungus growth throughout (94.4–100.0%) and this was comparable to Mancozeb (100.0%). From 24 to 120 HAI, both concentrations of neem extract (mean=50.0–64.6%) and 50% of Eucalyptus (32.0–57.3%) had comparable inhibitory effects on the fungus growth. At 96 HAI, the levels of growth inhibition recorded for neem extract at 50 and 100% (mean=64.6%), Eucalyptus at 50% (57.3%) and mahogany at 100% (53.9%) were comparable. A similar trend was observed for these plant extracts at 120 HAI. Aqueous extracts of neem at both concentrations, Eucalyptus at 50% and mahogany at 100% had similar effect on the fungus.

Among the plant extracts, mahogany at 50% gave the lowest inhibition of the fungus growth (<20%) throughout the study. From 24 to 72 HAI, mahogany at 50% was comparable with the control. At 96 and 120 HAI, mahogany at 50% inhibited the growth of the fungus significantly (P≤0.05) but this was lower than all the other extracts.

Table 3: Effect of aqueous leaf extracts of Eucalyptus, mahogany and neem on mycelial growth of *Phytophthora colocasiae*

	Time (hours after inoculation)									
	24		48		72		96		120	
Treatments	D	PI	D	PI	D	PI	D	PI	D	PI
Mancozeb	0.0^{a}	100.0	0.0^{a}	100.0	0.0^{a}	100.0	0.0^{a}	100.0	0.0^{a}	100.0
Eucalyptus 100%	0.0^{a}	100.0	0.0^{a}	100.0	0.3^{a}	95.6	0.3^{a}	96.6	0.5^{a}	94.4
Eucalyptus 50%	1.7^{bc}	32.0	2.9^{b}	46.3	3.3^{bc}	51.5	3.8^{b}	57.3	4.2^{b}	53.3
Mahogany 100%	1.9^{cd}	24.0	3.5^{b}	35.2	3.9^{c}	42.6	4.1^{b}	53.9	4.3^{b}	52.2
Mahogany 50%	2.3^{cd}	8.0	4.7^{c}	13.0	6.1^{d}	10.3	7.2^{c}	19.1	8.0^{d}	11.1
Neem 100%	0.9^{b}	64.0	2.6^{b}	51.9	2.8^{b}	58.8	3.1^{b}	65.2	3.4^{b}	62.2
Neem 50%	1.2^{b}	52.0	2.8^{b}	48.1	2.9^{b}	57.4	3.2^{b}	64.0	3.7^{b}	58.9
Control 0%	2.5^{d}	0.0	5.4^{d}	0.0	6.8^{d}	0.0	8.9^{d}	0.0	9.0^{e}	0.0

D = Diameter (cm); PI = Percentage inhibition (%); Means with different superscript(s) in a column are statistically different based on Tukey HSD test (P≤0.05).

From the findings obtained in this study, there were variations in the isolates of *P. colocasiae* from Makurdi and Befang when cultured on CMA and ADAD. The isolate from Befang was able to grow more in both media than that from Makurdi. It is likely that the isolate from Befang was more aggressive than that from Makurdi. It is important to note that these locations are at different altitudes. However, both culture media were appropriate for *P. colocasiae*. The growth characteristics of the fungus are important for microscopic identification.

The aqueous extracts of Eucalyptus, neem and mahogany inhibited the growth of the fungus. Among the plants, Eucalyptus extract at a concentration of 100% was the most potent while mahogany at 50% was the least. Both concentrations of neem extract, Eucalyptus at 50% and mahogany at 100% inhibited the fungus growth similarly. The inhibitory effect of both concentrations of all the plant extracts except Mahogany at 50% was >50% at 96 and 120 HAI. These observations indicate that the three plants had fungicidal properties against *P. colocasiae*. The inhibitory effect of Eucalyptus extract at 100% concentration was comparable to that of Mancozeb. These results affirmed the findings of Ndifon and Lum (2021) who reported that aqueous leaf extracts of Eucalyptus and neem possessed fungicidal properties. The authors indicated that these plant extracts inhibited the growth of *Aspergillus niger*, *in* vitro. Ezeonu *et al*. (2018) reported the inhibitory effect of the aqueous extract of neem leaves against the growth of some fungi which cause rot diseases in yam and cocoyam. The extracts of different parts of mahogany have also been reported to possess antifungal activity (Shehu *et al*., 2016). The inhibitory effect of the extracts of Eucalyptus and

Mahogany was higher at 100% concentration than at 50%. Similar observations have also been reported by other researchers who evaluated the effect of plant extracts on some fungi (Javed and Bashir, 2012; Lum *et al*., 2019). Both concentrations of neem extract had similar inhibitory effect on *P. colocasiae*. Both concentrations of neem extract and 50% Eucalyptus inhibited the fungus growth similarly throughout.

CONCLUSION

Acetate differential agar enriched with dextrose and corn meal agar were appropriate culture media for *P. colocasiae*. The aqueous leaf extracts of Eucalyptus, neem and mahogany inhibited the growth of *P. colocasiae*. The inhibitory effect of Eucalyptus extract at 100% concentration was consistently higher than that of the other extracts and comparable to the fungicide, Mancozeb. Although Mancozeb completely inhibited the fungus growth throughout, it is expensive and toxic to humans and the environment. The aqueous leaf extracts of Eucalyptus, neem and mahogany possess fungicidal properties against the pathogen; they are biodegradable and not expensive; and could therefore be used as alternatives to synthetic fungicides for management of *P. colocasiae*. Further work is necessary on the management of this pathogen using these plant extracts in a controlled environment.

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