Wild poinsettia, African ebony and Ashanti pepper: Potential Biofungicides Against *Phytophthora colocasiae* Raciborski

ABSTRACT

Plant extracts could be used as substitutes to synthetic pesticides in the management of fungi and other plant pathogens. The biofungicidal potential of aqueous extracts of wild poinsettia (*Euphorbia heterophylla* L., a weed) and African ebony (*Diospyros crassiflora* Hiern, a tree) leaves, and Ashanti pepper (*Piper guineense* Schumach., a spice) seeds was assessed against *Phythophthora colocasiae in vitro*. The plants were screened at the rates of 0 (control), 45 and 90%. The synthetic fungicide, mancozeb was included. Results indicated that all the plant extracts were fungitoxic and there was a variation in their potency. Also, the level of inhibition was higher at 90% concentration than at 45%. At 24–120 hours after exposure (HAE), a concentration of 90% Ashanti pepper consistently gave complete inhibition of the fungus similar to mancozeb. Complete inhibition of the fungus was also recorded for 45% Ashanti pepper at 24–48 HAE. At 45% concentration, the inhibitory levels of the extracts were Ashanti pepper: 89.6–100.0%, African ebony: 29.6–33.0 and wild poinsettia: <20.0%. At a concentration of 90%, the levels were Ashanti pepper: 100%, African ebony: 36.7–39.0% and wild poinsettia: 17.4–26.9%. The plant extracts showed promising activity against *P. colocasiae* and should be exploited for incorporation in management programmes for the fungus.

Keywords: Antifungal potency, leaf blight, *Phytophthora colocasiae*, plant extracts

1. INTRODUCTION

Taro (*Colocasia esculenta* (L) Schott) is an important perennial crop which is widely cultivated in West and Central Africa. The crop is a staple root and tuber crop, as well as a source of revenue for small-holder farmers in Cameroon. It is highly cherished for its leaves, petioles, flowers, corms and cormels which are prepared in various forms for consumption in Cameroon. It is an important source of minerals, proteins, carbohydrates and vitamins [1, 2]; and it possesses medicinal properties [3].

The pathogenic fungus, *Phytophthora colocasiae* Raciborski causes leaf blight disease which severely threatens taro production [4, 5]. When the disease infects taro, the symptoms occur on the crop's leaves as small, brown lesions which rapidly enlarge and cause defoliation and rotting [5, 6]. High levels of the disease incidence (49–100%) have been reported in some taro growing regions in West and Central Africa (5–9].

Synthetic fungicides have been used successfully to manage plant diseases caused by fungi and other phytopathogens. These chemicals are expensive for small-holder farmers who form the bulk of taro producers. Most farmers are usually unable to meet up with the schedule for the application of these fungicides due to the high cost [5). In addition, they are toxic to humans and the environment, and their indiscriminate use has caused pest resistance. Residues of fungicides in foods and agricultural products have been reported [10]. It is therefore necessary to identify other methods that are not expensive for these farmers, and non-hazardous to humans and the environment.

Studies have revealed that the aqueous extracts of wild poinsettia (*Euphorbia heterophylla* L.), African ebony (*Diospyros crassiflora* Hiern) and Ashanti pepper (*Piper guineense* Schumach.), are active against pathogenic microorganisms [11–13]. However, there is limited information on the management of *P. colocasiae* using these plants. Previous experiments revealed that the aqueous leaf extracts of billy goatweed (*Ageratum conyzoides* L.), tropical girdlepod (*Mitracarpus villosus* (Sw.) Cham. & Schltdl. ex DC), Eucalyptus (*Eucalyptus globulus* Labill.), mahogany (*Khaya senegalensis* (Devs.)) and neem (*Azadirachta indica* A. Juss.) had the potential to inhibit the growth of *P. colocasiae* [5, 14, 15]. Aqueous plant extracts are easy to prepare and environment-friendly. In this study, aqueous extracts of wild poinsettia, African ebony and Ashanti pepper, medicinal plants which grow in the tropics, were assessed for their potency against the pathogen.

2. MATERIALS AND METHODS

2.1. *Phythophthora colocasiae*

The isolation of *P. colocasiae* and screening of plant extracts were done in the Life Sciences Laboratories, University of Buea, South West Region, Cameroon in 2015. Buea is in the monomodal humid zone and it is one of the taro growing areas in Cameroon. Taro leaves with blight disease were obtained from farms near the University campus and washed with running tap water. Tiny pieces of the taro leaves consisting of infected and healthy

sections were sterilized in NaClO and rinsed in sterile distilled water. After drying the leaf pieces on blotting paper they were placed on potato dextrose agar (PDA) (prepared according to the manufacturer's instructions) augmented with streptomycin sulphate (0.2 g L^{-1}); the plates were incubated at room temperature. The fungus was subcultured to obtain pure cultures six days after incubation; it was identified using its cultural and morphological characteristics. Wet mounts were prepared from the pure cultures obtained and the mycelial characteristics observed using an optical microscope were compared to standard identification guides [16, 17].

2.2. Collection of plant samples and preparation of extracts

The plants screened for antifungal activity consisted of fresh and disease-free samples of wild poinsettia, African ebony and Ashanti pepper. The leaves of wild poinsettia were harvested from farmlands and gardens in the Buea Municipality; the leaves of African ebony were harvested from forests close to Buea; seeds of Ashanti pepper were purchased from the Buea Central Market. The plants were identified in the Limbe Botanical Garden, South West Region. Additional information about the plants are stated on Table 1.

Table 1. Plants assessed for antifungal activity

No.	Common name	Scientific name	Family name	Type	Part used
1	Wild poinsettia	*Euphorbia heterophylla* L.	Euphorbiaceae	Weed	Leaves
2	African ebony	*Diospyros crassiflora* Hiern	Ebenaceae	Tree	Leaves
3	Ashanti pepper	*Piper guineense* Schum. & Thonn.	Piperaceae	Spice	Seeds

The plant materials (90 g) were cleaned using tap water and sterilized in NaClO solution. Each sterilized plant material was properly rinsed with distilled water after which, the different plant leaves were chopped up in preparation for extraction. All the plant materials were ground with distilled water using an electric blender to obtain an extract of 90% (w/v). Each plant extract was allowed to stand overnight, after which, it was passed through a fine sieve into a sterile beaker. Half of the filtrate was further diluted to a concentration of 45%.

2.3. Screening plant extracts for antifungal activity

The treatments were 0 (negative control), 45 and 90% concentrations of aqueous extracts of the three plants; and a positive control with the synthetic fungicide Mancozeb. Blank agar plates containing PDA with no extract were used to set up the negative treatment. The completely randomized design was used and treatments were replicated three times.

The extracts were screened for fungicidal activity as outlined by Lum *et al*. [12]. For each extract, 1 ml was dispensed per Petri dish and 9 ml of autoclaved PDA amended with streptomycin sulphate were added. The plates were gently rotated to enable the extracts disperse evenly, then the PDA-extract mixture was allowed to set. They were inoculated with *P. colocasiae* from a six-day old culture, incubated at room temperature and examined daily for fungal growth.

Fungal growth was obtained using a transparent ruler from 24 to 120 hours after exposure (HAE). The inhibitory level of the plant extracts was determined as stated by Lum *et al*. [12].

Inhibition (%) = ((a–b)/a) × 100, where a = radial growth of the fungus (control);

b = radial growth of the fungus (test).

2.4. Data analyses

The data were analysed using the SPSS version 21 software; the Tukey HSD test ($P \leq 0.05$) was used to compare the means of the various treatments.

3. RESULTS AND DISCUSSION

The aqueous extracts of the medicinal plants tested exhibited significantly different ($P \leq 0.05$) levels of fungal growth inhibition throughout the period of incubation (Figures 1–3). In general, all the plant extracts at both concentrations and Mancozeb inhibited the fungal growth significantly ($P \leq 0.05$) compared to the control. The inhibitory effect of the plant extracts was higher at a concentration of 90% than at 45%. Overall, the extract of Ashanti pepper seeds at 90% concentration, and the synthetic fungicide Mancozeb recorded complete inhibition (100%) throughout the period of incubation. The extract of Ashanti pepper seeds at both concentrations had higher inhibitory effects against the pathogen than those of African ebony and wild poinsettia leaves. The leaf extracts of wild poinsettia and African ebony at both concentrations inhibited the fungal growth by <50%. Among the plant extracts, wild poinsettia at both concentrations provided the lowest level of fungal growth inhibition throughout.

At 24 HAE, the extract of Ashanti pepper seeds at both concentrations and Mancozeb fungicide gave complete inhibition (100%) of the fungal growth (Figure 1). This was followed by 90% concentration of the leaf extract of African ebony (36.7%); then 45% of African ebony (31.3%). At each concentration of the plant extracts, wild poinsettia gave the lowest inhibition of fungal growth (26.9% inhibition at 90% concentration and 18.1% inhibition at 45% concentration).

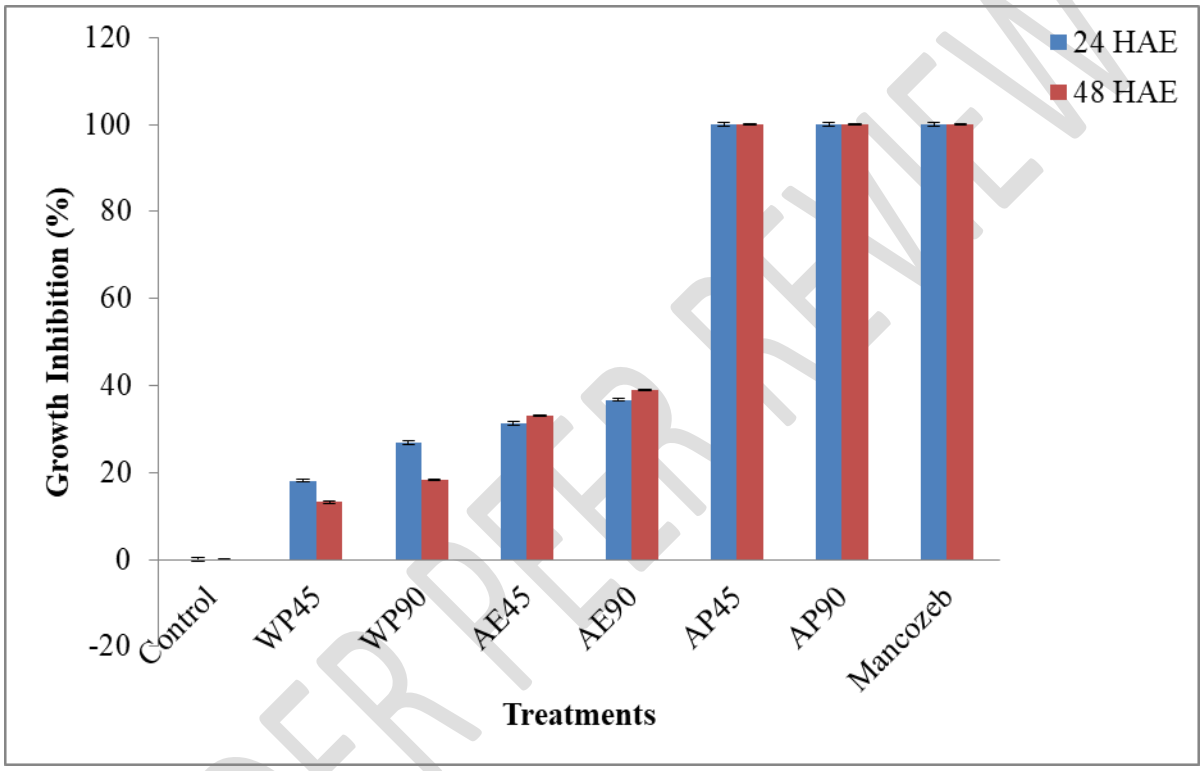

Figure 1. Percentage Growth inhibition of *Phythophthora colocasiae* exposed to aqueous extracts of selected medicinal plants for 24 and 48 hours (WP45=45% wild poinsettia; WP90=90% wild poinsettia; AE45=45% African ebony; AE90=90% African ebony; AP45=45% Ashanti pepper; AP90=90% Ashanti pepper; HAE=Hours after exposure)

At 48 HAE, both concentrations of the extracts of Ashanti pepper seeds were highly effective (100%) against the fungal growth, similar to Mancozeb (Figure 1). At both concentrations, the leaf extract of African ebony inhibited the growth of the pathogen by 33.0–39.0%. Both concentrations of wild poinsettia leaf extract inhibited the fungal growth by <20% from 48 to 120 HAE.

At 72 HAE, Mancozeb inhibited the mycelial growth of the fungus by 100% and this was similar to the inhibition level recorded for the extract of Ashanti pepper seeds at both concentrations (Figure 2). The leaf extracts of African ebony and wild poinsettia inhibited the fungal growth more at 90% concentration than at 45% concentration. Overall, wild poinsettia gave the lowest level of fungal inhibition at each concentration tested.

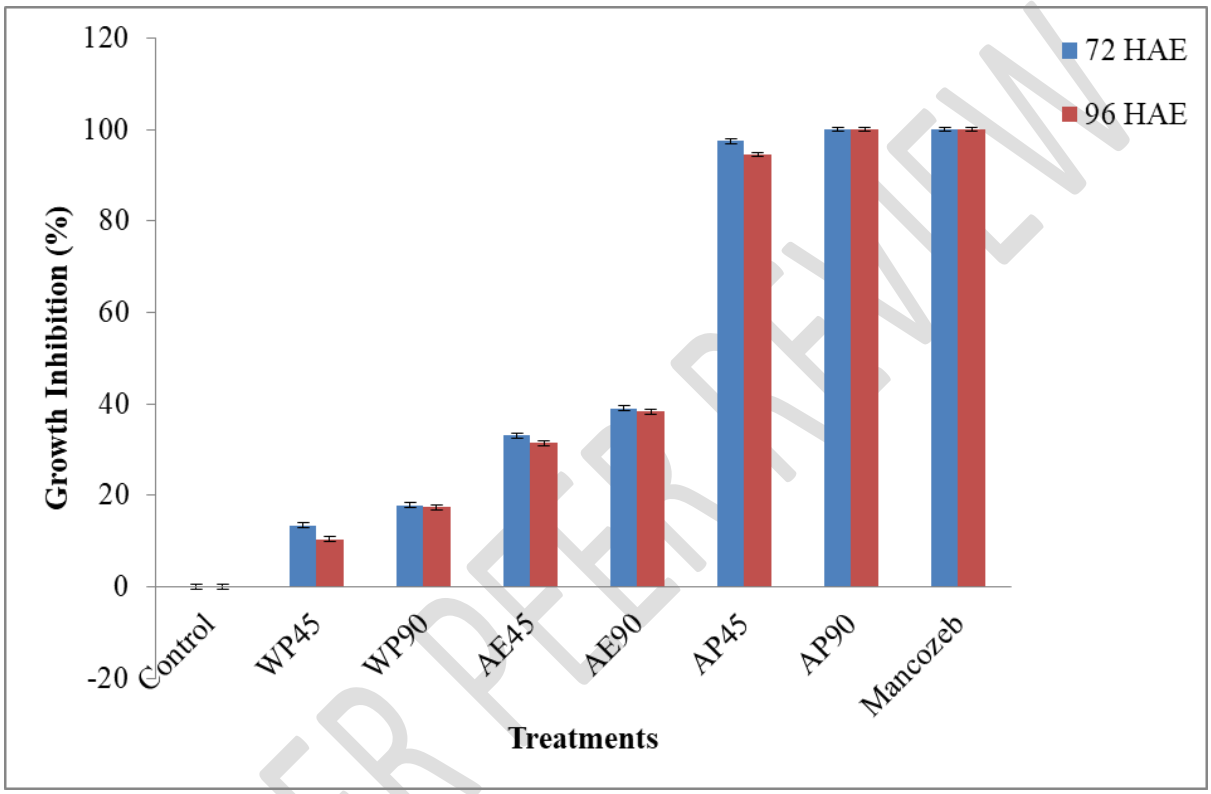

Figure 2. Percentage Growth inhibition of *Phythophthora colocasiae* exposed to aqueous extracts of selected medicinal plants for 72 and 96 hours (WP45=45% wild poinsettia; WP90=90% wild poinsettia; AE45=45% African ebony; AE90=90% African ebony; AP45=45% Ashanti pepper; AP90=90% Ashanti pepper; HAE=Hours after exposure)

At 96 HAE, 90% concentration of Ashanti pepper and Mancozeb completely inhibited the fungal growth (100%) (Figure 2). The order of inhibition was Mancozeb = 90% Ashanti pepper > 45% Ashanti pepper > 90% African ebony > 45% African ebony > 90% wild poinsettia > 45% wild poinsettia.

At 120 HAE, 90% Ashanti pepper and Mancozeb were highly effective, with an inhibitory effect of 100% (Figure 3). This was followed by the extract of Ashanti pepper at 45% concentration which inhibited the fungal growth by 89.6%. The extract of African ebony

inhibited the fungal growth by 38.6% at a concentration of 90%, and by 29.6% at a concentration of 45%. Both concentrations of wild poinsettia extract inhibited the fungal growth by 10.4–17.1%.

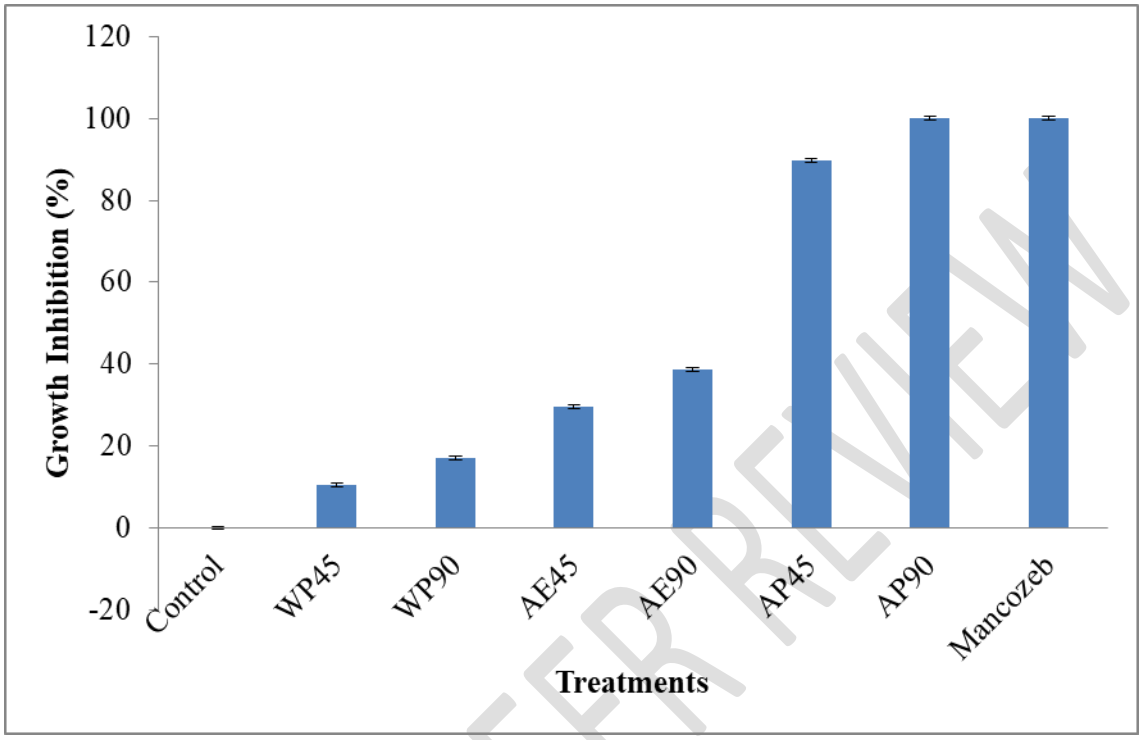

Figure 3. Percentage Growth inhibition of *Phythophthora colocasiae* exposed to aqueous extracts of selected medicinal plants for 96 hours (WP45=45% wild poinsettia; WP90=90% wild poinsettia; AE45=45% African ebony; AE90=90% African ebony; AP45=45% Ashanti pepper; AP90=90% Ashanti pepper; HAE=Hours after exposure)

These results indicated that the medicinal plant extracts and the fungicide Mancozeb were able to inhibit the fungal growth. This could be attributed to the availability of fungicidal properties in the plants which were sufficiently potent against the pathogen. Although all the plant extracts had fungicidal activity, there was a variation in the potency levels. Throughout the study period, the extract of Ashanti pepper seeds at 90% concentration resulted in complete inhibition (100%) of the fungal growth similar to Mancozeb. Among the plant extracts tested, this treatment was highly effective in inhibiting the fungal growth throughout the incubation period. Another treatment which performed similarly though at different times was 45% concentration of Ashanti pepper seeds at 24–72 HAE. Although the

other plant extracts did not completely inhibit the fungal growth, they had significant ($P<0.05$) effect.

The antifungal activity recorded for the different medicinal plant extracts may be due to the bioactive compounds in them. Phytochemicals such as saponins, tannins, flavonoids and terpenoids have been reported in aqueous extracts of Ashanti pepper [11, 18] and wild poinsettia [19, 20]. Also, the plant extracts provided higher inhibitory effect at 90% than at 45%. It is possible that the plant extracts were more potent at a higher concentration than a lower one. Similar results have been reported by earlier researchers who tested the potency of different concentrations of plant extracts [12, 15, 21, 22]. The superior activity of some of the treatments against the fungus reported in this study may be attributed to the existence of high concentrations of active compounds. The findings also suggest that the inhibitory effect of the aqueous plant extracts on the fungus depends on the type of plant and the concentration. Similar reports were made by earlier researchers who evaluated the potency of plant extracts against other pathogenic fungi [5, 12, 15, 22–25].

4. CONCLUSION

The aqueous extracts of Ashanti pepper seeds and leaves of African ebony and wild poinsettia possess fungicidal properties against *P. colocasiae*. The aqueous extract of Ashanti pepper seeds at 90% concentration was highly effective in managing the fungus and consistently gave complete inhibition (100%) similar to the synthetic fungicide Mancozeb throughout the exposure period. The extract of Ashanti pepper seeds at 45% concentration was also effective against the fungus although this treatment did not always completely inhibit its growth (45%-Ashanti pepper seeds=89.6–100% inhibition). The extract of African ebony leaves at both concentrations was moderately effective against the fungus (45%-African ebony=29.6–33.1%; 90%-African ebony=36.7–39.0%). Among the plant extracts tested, the leaf extract of wild poinsettia at 45% concentration was the least effective (10.4–18.1% fungal inhibition). At 90% concentration, the leaf extract of the weed provided 17.1–26.9% inhibition of the pathogen. These plant extracts showed promising activity against *P. colocasiae*. Complete inhibition of fungal growth was obtained for the synthetic fungicide; however, it is hazardous to humans and the environment. Given that the plant extracts are easy to prepare and eco-friendly, they could be considered in management programmes for the pathogen.

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