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Antifungal activities of some plants extract against *Cercospora* Spp causative agent of post-harvest fruits rot

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Abstract
In the present investigation, plant parts of *Ageratum conyzoïdes* and *Chromolaena odorata* were collected to analyse the antifungal activity against *Cercospora* spp. isolated from banana, mango and safou fruits in Dschang, Cameroon. Antifungal activity was determined *in vitro* following Agar-well diffusion method using different concentrations of 1.25 ; 2.5 ; 5 and 10 mg/ml for ethanol extracts and 5 ; 10 ; 15 ; 20 mg/ml for aqueous extracts. Ethanolic extracts of *Ageratum conyzoïdes* completely inhibited the growth of *C. capsici* and *C. mangiferae* at 5 and 10 mg/ml respectively. With the ethanolic extracts of *Chromolaena odorata*, 100% inhibition was observed at 5 mg/ml against *C. mangiferae* and *C. capsici*. Aqueous extracts of *Chromolaena odorata* and *Ageratum conyzoïdes* completely inhibited *C. musae, C. mangiferae* and *C. capsici* at 20 mg/ml. *In vivo* test showed that fruits inoculated with *C. musae* and *C. capsici* and treated with the ethanolic extracts of *A. conyzoïdes* and *C. odorata* did not develop any lesion 5 days after inoculation. Ethanolic extracts possess biofungicidal potential, which can suitably be exploited to control fruit rots.

Keywords: plant extracts, antifungal activity, fungal growth, *in vivo* effect, fruits

Introduction
It has been recognized that fruits are commercially and nutritionally important food product in developing countries. They play an important role in human nutrition by contributing the necessary growth factors such as vitamins and essential minerals in human daily diet maintaining a good and normal health. Tropical fruit production knows more and more increased with fresh bananas which was ranked 1st, with more than 145 million tons produced in 2011 globally [1]. In Cameroon, production of sweet banana in 2010 was 1 333 851 tons. The mango and the safou, despite their low production, also feature prominently after the banana.
Despite their economic and nutritional importance, fruits are subject to several constraints, including losses caused by certain fungi. The fungi are the second most important disease causing organisms which causes severe crop losses all over the world. One third global agriculture production is destroyed each year by different pest and diseases [2]. It has been reported that rot diseases caused by fungal pathogens provoke severe losses of agricultural and horticultural crops every year [3]. Over several decades, various attempts have been accomplished to prevent, control, or eradicate plant diseases, and development of synthetic fungicides was particularly investigated [4]. These pesticides are known to be highly effective in controlling various postharvest diseases of vegetables and fruits. Although effective, their continued or repeated applications may disrupt equilibrium of ecosystems, leading to dramatic disease outbreaks, widespread development of pathogens resistant to one or more chemicals, toxicity to non-target organisms and environmental problems [4]. Sometimes, the application of higher concentrations of chemicals in an attempt to overcome diseases on fruits increases the risk of high levels of toxic residues, which is, particularly serious, since fruit is consumed in arelatively short time after harvest [5].
Natural products of plants are less persistent in the environment and safe for the control of post-harvest diseases than synthetic products [6]. A number of plant species have been reported to possess natural substances that are toxic to many plant pathogenic fungi [7, 8]. Hence, this study was conducted with the objective of assessing the *in vitro* effect of extracts of *Ageratum conyzoïdes* and *Chromolaena odorata* on mycelia growth of *Cercospora* spp isolated from fruits and their *in vivo* efficacy on the development of these postharvest pathogens on some fruits.

Materiels and Methods
Plant extracts
Aerial parts (leaves and stem) of *Chromolaena odorata* and *Ageratum conyzoïdes* L were collected in may 2016 from the locality of Dschang, West region of Cameroon**.** Their identification were confirmed through consultation in the Herbarium of the Department of Plant Biology, University of Dschang. Plant parts collected were washed three times with running tap water and rinsed with sterile distilled water. They were separately air-dried at room temperature and ground in a mortar. One hundred grams of the resulted dried powder were macerated in 500 ml of distilled water or ethanol and mixed thoroughly. For aqueous extract the mixture was allowed to rest for 48 hours and the supernatant passed through whatman's N° 1 filter paper to obtain the extract. For ethanolic extract, after maceration for 4 hours in a warring blender

(Warring International, New Hartford, CT, USA), the macerate was passed through Whatman's N°. 1 filter paper and evaporated using a Rota vapour at 40°C water bath temperature (Heidolph) [9]. Extracts were preserved aseptically in a brown bottle at 4°C until further use [10].

***In vitro* antifungal activities of plant extracts**

The antifungal effect of plant extracts were evaluated on *Cercospora musae*, *Cercospora mangiferae* and *Cercospora capsici* isolated from fruits. The *in vitro* antifungal activity was assessed according to the agar dilution method [11] on PDA (Difco). Plant extracts were dissolved in dimethylsulphoxide (DMSO) and diluted to give serial dilutions that were incorporated into growth medium. Concentrations of 1.25 ; 2.5 ; 5 and 10 mg/ml for ethanol extracts and 5, 10, 15, 20 mg/ml for aqueous extracts were used. PDA medium supplemented with different concentrations of the extracts were inoculated with 6-mm diameter (plugs) of the test pathogen cut from the margin of 7-day-old cultures. The plates were incubated in duplicates over a period of 10 days for *C. gloeosporioides* and *B. theobromae* at 20 ± 2°C. The radial mycelia growth was measured daily and the fungi toxicity was expressed as percentage inhibition of radial mycelia growth.

***In vivo* assay of plant extracts**

Based on the results of *in vitro* test, a concentration of 20 mg/ml for aqueous extract and 10 mg/ml for ethanol extracts were used for this test. Conidial suspension were prepared from pure culture of *Cercospora musae* (on banana) and *Cercospora capsici* (on safou) and adjusted to a concentration of 5 x 10^4 conidia/ml using heamocytometer. Apparently healthy banana and safou fruits collected from the market in Dschang were washed with tap water, dried and surface-sterilized by alcohol. These fruits received simultaneously on the epicarp 50 µl of conidial suspension and 50 µl of each plant extract [12].

Inoculated and treated fruits were arranged in plastic plates containing cotton soaked with sterile distilled water to maintain humidity during the experiment. Five days after inoculation at ambient temperature (22±2°C), the lesion areas developed on the fruits were evaluated using a graph paper. A completely randomized design was used with 4 replications and the experiment was repeated thrice.

Statistical analysis

Data collected on percentage inhibition and lesion area were subjected to analysis of variance (ANOVA) using SPSS software version 17. The mean values were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

Results and discussion

Effect of aqueous extracts

Inhibition percentage of fungal growth by aqueous extracts of *Chromolaena odorata* and *Ageratum conyzoïdes* L. are presented on Table-1. Generaly there are significant differences in the mycelia growth inhibition of plant extract-supplemented samples compared with the negative control (ANOVA and Duncan Multiple Range Test, $P < 0.05$). Aqueous extracts of *Chromolaena odorata*, completely (100%) inhibited the growth of *Cercospora musae, C. mangiferae* and *C. capsici* at the dose of 20 mg/ml. Similarly aqueous extracts of *Ageratum conyzoïdes* completely inhibited the growth of *C. mangiferae* and *C. capsici* at 20 mg/ml, while at this dose, *Cercospora musae* has been inhibited at 81%.

Effect of ethanol extracts

Antifungal effects of ethanol extracts of *Chromolaena odorata* and *Ageratum conyzoïdes* L. on fungal growth are presented on Table-2. There were significant differences in the mycelia growth inhibition of plant extract-supplemented samples compared with the negative control (ANOVA and Duncan Multiple Range Test, $P < 0.05$). The effect of extracts with increasing concentrations showed a gradual inhibition of the growth of C. musae, *C. mangiferae* and *C. capsici*. It was noted that ethanolic extracts of *Ageratum conyzoïdes* completely (100%) inhibited the growth of *C. capsici* and *C. mangiferae* at 5 and 10 mg/ml respectively. With the ethanolic extracts of *Chromolaena odorata*, 100% inhibition was observed for *C. mangiferae* and *C. capsici* at the dose of 5 mg/ml.

In general, we find that *Cercospora musae* is less sensitive to the ethanolic extracts of *Chromolaena odorata* and *Ageratum conyzoïdes* L. than the two other fungal species, because its total inhibition was not obtained with the concentrations used.

The growth inhibition percentages of different fungi by plant extracts proved to be dependent on the concentration, the type of extract and the plant tested. Results obtained from *Ageratum conyzoïdes* extracts are in agreement with previous studies that showed the antifungal activities of this plant against devastating pathogen on variety of economic plants [13, 14, 15]. Similarly, the results achieved with leaves extract of *Ageratum conyzoïdes* are similar to those obtained by [16, 17] which showed that these extracts inhibit the development of *Phytophtora megakarya* (responsible for the brown rot of cocoa) and *P. colocasiae* (causative agent of late blight of taro). A wide range of allelochemicals including alkaloids, flavonoids, chromenes, benzofurans and terpe-noids have been isolated from *A. conyzoides* [18]. According to [19], three phenolic compounds were identified in the leaf, stem and root of *A. conyzoides* including gallic acid, coumallic acid and protocatechuic acid and catechin were found only in the stem. Three additional allelochemicals were also found in the leaf consisting of p-coumaric acid, sinapic acid and benzoic acid. The greater number of allelochemicals found might result in the stronger inhibitory activity.

Also, results obtained with *C. odorata* extract are similar to those reported by [20] which showed that this extract inhibit the development of yeast, filamentous fungi and that of several multicellular dermatophyte fungi [21]. showed the effect of the leaf extract of *C. odorata in vitro* on two isolates of *F. oxysporum*, causing symptoms of *Fusarium* wilt. A qualitative chemical analysis of the extract and fractions showed the presence of biologically active constituents such as some coumarins, flavonoids, phenols, tannins and sterols, this could justify the antifungal activity.

***In vivo* activities of plant extracts**

The results of *in vivo* activity of plant extracts on the development of *Cercospora mangiferae* and *Cercospora capsici* are presented on Table-3. From these results we note that banana and safou fruits treated with ethanolic extracts of

Ageratum conyzoïdes showed no lesions such as those treated by the mancozeb. However the fruits treated with ethanolic extracts of *Chromolaena odorata* presented lesions from15 and 36 mm^2 respectively on banana and safou fruits. Moreover, all the fruits treated with aqueous extracts presented lesions which range from 22 to 25 mm^2. However the fruits treated with extracts showed significantly lower injuries than those of untreated fruit (77 to 92 mm^2).

Conclusion

Results of this study suggest that the ethanolic and aqueous extracts of *A. conyzoides* and *C. odorata* greatly reduced the biomass of *Cercospora musae*, *C. mangiferae* and *C. capsici*. These extracts also inhibited fungal development on artificially inoculated fruits and could be used to extend the life span of banana and safou fruits. It would be very interesting to continue the study against other pathogenic fungi to control the different diseases.

Table 1: Inhibition percentage (%) of radial growth of fungal pathogens by aqueous plant extracts

Aqueous extract		**Inhibition percentage of fungal species**		
	Doses (mg/mL)	***C. musae***	***C. mangiferae***	***C. capsici***
Chromolaena	T-	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
odorata	5	22.16 ± 7.11^{d}	15.69 ± 6.45^{d}	20.39 ± 7.01^{c}
	10	53.92 ± 5.80^{c}	46.86 ± 12.39^{c}	36.67 ± 7.83^{c}
	15	81.96 ± 9.00^{b}	80.59 ± 17.71^{b}	76.47 ± 20.37^{b}
	20	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	T+	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Ageratum	T-	$0,00 \pm 0.00^{f}$	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
conyzoïdes	5	$11,96 \pm 9.70^{e}$	12.35 ± 7.15^{d}	13.33 ± 9.11^{c}
	10	28.23 ± 3.85^{d}	43.72 ± 12.95^{c}	27.25 ± 2.38^{c}
	15	59.41 ± 5.88^{c}	73.53 ± 23.46^{b}	68.03 ± 28.23^{b}
	20	80.19 ± 1.70^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	T+	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Values in the same row followed by different letters are significantly different ($P < 0.05$). T- = negative control (Distilled water) ; T+ = M = mancozeb.

Table 2: Inhibition percentage (%) of radial growth of fungal pathogens by ethanol plant extracts

Ethanolic extract		Inhibition percentage of fungal species		
	Doses (mg/mL)	*C. musae*	*C. mangiferae*	*C. capsici*
Chromolaena	T-	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}
odorata	1.25	25.10 ± 5.71^{d}	57.25 ± 8.93^{b}	32.35 ± 8.17^{c}
	2.5	59.80 ± 8.37^{c}	67.45 ± 12.43^{b}	52.75 ± 1.36^{b}
	5	85.69 ± 4.75^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	10	83.92 ± 0.90^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	T+	100.00 ± 0.00^{a}	100.00 ± 00^{a}	97.06 ± 3.09^{a}
Ageratum	T-	$0.00 \pm 0.00^{d.}$	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
conyzoïdes	1.25	14.31 ± 3.01^{d}	50.78 ± 4.89^{d}	62.54 ± 2.65^{c}
	2.5	35.68 ± 4.75^{c}	64.11 ± 4.44^{c}	77.25 ± 2.06^{b}
	5	68.24 ± 4.44^{b}	78.03 ± 5.59^{b}	100.00 ± 0.00^{a}
	10	81.57 ± 1.48^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	T+	87.25 ± 22.07^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Values in the same row followed by different letters are significantly different ($P < 0.05$). T- = negative control (Distilled water); T+ = M = mancozeb

Table 3: Lesion area developed by different fungal pathogens on banana and safou fruits treated by plant extracts

Pathogens	Ethanolic extract		Aqueous extract	
	Doses (mg/ml)	Lesion area (mm^2)	Doses (mg/ml)	Lesion area (mm^2)
C. musae	*A.c* 10	0.00 ± 0.00^{c}	*A.c* 20	22.33 ± 13.66^{b}
(on Banana)	*C.o* 10	15.66 ± 5.16^{b}	*C.o* 20	23.0 ± 12.33^{b}
	T- 0	92.50 ± 5.43^{a}	T- 0	$92.50 \pm 5,43^{a}$
	T+ 1	0.00 ± 0.00^{c}	T+ 1	0.00 ± 0.00^{c}
C. capsici	*A.c* 10	0.00 ± 0.00^{c}	*A.c* 20	25.50 ± 6.28^{b}
(on Safou)	*C.o* 10	36.66 ± 12.53^{b}	*C.o* 20	25.0 ± 0.00^{b}
	T- 0	77.0 ± 5.24^{a}	T- 0	77.0 ± 5.244^{a}
	T+ 1	0.00 ± 0.00^{c}	T+ 1	0.00 ± 0.00^{c}

Values in the same row followed by different letters are significantly different ($P < 0.05$). T- = negative control (Distilled water); T+ = M = mancozeb ; A. c = *Ageratum conyzoïdes* ; C. o = *Chromolaena odorata*

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