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Control of leaf spot disease caused by Cercospora sp on groundnut (*Arachis hypogaea*) using methanolic extracts of yellow oleander (*Thevetia peruviana*) seeds

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Abstract

A study was carried out in Yaounde (Cameroon) on two cultivars of groundnuts (Bafia var. and 55-437 var.) treated with methanolic extracts of *Thevetia peruviana* seeds (METPS), to evaluate the epidemiology of Cercospora leaf spots (CLS) and the antifungal potentials of these extracts under natural conditions. METPS were obtained by maceration of seed powder (0,33 kg/l) in methanol. Two concentrations of METPS (1.8 and 3.7 kg/ha), a benomyl and a control treatments were used in four replicates of a randomised block design. Disease severity, incidence, lesion size and yield were assessed. Two foliar applications of antifungal substances were effected at 15 days interval. The epidemic on both groundnut varieties was low in plots sprayed with METPS and benomyl when compared with the control. Disease incidence, lesion size and CLS severity were reduced to 37.25, 33.10 and 32.61 % respectively in plots sprayed with METPS compared to the control. The Bafia var. was more tolerant to CLS than the 55-437 var. Groundnut yield increased by 32.89 and 41.37 % for the Bafia var following applications of METPS and benomyl respectively. These results suggest that integrating host resistance and METPS efficiently protects groundnut against CLS.

Keywords: Biological control, Cameroon, cercosporosis, plant extract, groundnut, *Thevetia peruviana*
Abbreviations : CLS_*Cercospora* leaf spot; DAP_days after planting; LSD_least significant difference; ME_Methanolic extract; ME1_dose 1 of methanolic extract (1.7 kg/ha); ME2_dose 2 of methanolic extract (3.8 kg/ha); METPS_methanolic extracts from *Thevetia peruviana* seeds; var_variety

Introduction

Groundnut (*Arachis hypogaea* L.) is widely cultivated as a staple food in tropical and sub-tropical developing countries, providing a valuable source of proteins (20-45 %), fats (23.8 %), energy (2800 cal) and minerals (Enwere, 1998; Rakipov, 1987). Most of the world's groundnut is produced and consumed in developing countries. Groundnut is an annual leguminous plant, important both as a subsistence crop and an animal feed. It is used as an after-culture plant in crop rotation in tropical zones (Pavliukov, 1988; Mekoutchou, 1990). Groundnut production in African countries fluctuated greatly, though it never exceeded 8 % of the worlds output over the last decade. Yields per hectare are low, because of a combination of factors: unreliable rains; mostly non-irrigated cultures; small-scale traditional farming with little mechanization; outbursts of pests and diseases; use of low-yielding seed varieties and increased cultivation on marginal land. In Cameroon, groundnut yield is 0.85 t/ha. This low yield is generally due to incidence of diseases such as rust, CLS and rosette (Ambang et al., 2008; Bosc and Bonkoungou, 1990; Ouzounov, 1988). Groundnut CLS diseases are caused by two microscopic fungi (*Cercospora arachidicola* Hori, causal agent of early CLS and *Cercosporidium personatum* (Berk and Curt.) Deighton, which causes late CLS) are the most important foliar diseases of groundnut in the world (Ogwulumba et al., 2008; Fontem et al., 1996; Smith, 1984). Symptoms caused by these fungi are easily distinguishable on the lower leaf surface. Early CLS is characterized by light-brown spots surrounded by yellow halo, while spots of late CLS are black and usually without yellow halo (Suubbra-Rao et al., 1993; Ouzounov, 1988; Subrahmanyam et al., 1982). CLS can affect all the aerial parts of groundnuts and cause losses in yield of over 50 % depending on the method of protection. Varietal resistance and chemical treatments are the two main efficient methods generally used to control CLS. Both however, have their limits. Chemical pesticides can engender harmful consequences on the environment (Ambang et al., 2008). Moreover, these pesticides are expensive and are not always accessible to all farmers. Therefore, the search for alternatives to chemical products such as the use of natural biocides of plant origin is the most promising outlet for a sane and sustainable agriculture. For more than a decade now, researchers have focused their studies on the action of natural substances from yellow oleander (Oji and Okafor, 2000) and other plants on phytopathogenous fungi (Ambang et al., 2010; Ogwulumba et al., 2008; Ngoh Dooh, 2006; Gata-Gonçalves et al., 2003; Kurucheve et al., 1997), bacteria (Obasi and Igboechi., 1991; Saxena and Jain., 1990), pests and rodents (Ambang et al., 2005; Reed and Freedman, 1982). Yellow oleander (*Thevetia peruviana* (Pers.) K. Schum) is a small tree that belongs to the *Apocynaceae* family and is generally used as an ornamental plant in Cameroon. In some Asian, American and African countries, leaves, fruits, seeds and roots of *T. peruviana* are used in traditional medicine (Mantu and Sharma, 1980). In recent years, this plant has been regarded as a source of biocides that are important in crop protection. Up to now, much work on the plant has been in *vitro* and in laboratories. The objective of this field work was to study the combined effect of crude extracts of *T. peruviana* seeds and varietal resistance in the control of CLS epidemiology on two groundnut varieties with different levels of

sensitivity. The results of this study could lead to cheap and efficient methods of plant protection against parasites and contribute to increased crop yield.

Materials and methods

Climatic conditions

The study was realised during the August to December 2005 farming season in an agricultural zone of Yaounde (Cameroon), on a typical ferralitic soil composed of clay, sand and lemon. The soil pH varies from 5.5 to 6.5. Atmospheric temperatures during the experimental period ranged from 23.6 to 26 °C. Recorded precipitation was 187.7 mm in September and 73.7 mm in December with a peak of 251.6 mm in October. The atmospheric humidity of the region was fairly constant (85 %).

This high humidity caused the formation of dew during dry periods, favouring the installation and development of phytopathogenous fungi.

Plant material

Two groundnut varieties (one local: Bafia var. and one exotic: 55-437 var.) were chosen on the basis of their different levels of sensitivity to CLS. The local variety belongs to the *Virginia* type and 55-437 var. is a *Spanish* type. The two groundnut varieties have a vegetative cycle of 90 days. Seeds of *T. peruviana* were also used as plant material. Mature *T. peruviana* fruits were harvested in Yaounde town. Ripe fruits are yellow and fall off when the wind blows or when the plant is shaken.

Experimental set-up

The experimental set-up was a double factorial plan made up of antifungal treatments and groundnut varieties. Methanolic extract (ME) treatments administered in two doses, ME1 (1.7 kg/ha) and ME2 (3.8 kg/ha) were accompanied by a benomyl application and a control (without fungicide application).

The experimental design was constituted of four blocks or replicates. Each block was made up of four treatments containing two elementary plots making a total of 8 plots per block, on which groundnut varieties were randomly planted. Each elementary plot measured 2 m x 1.5 m and was separated from others by 0.8 m. The distance between blocks was 1 m. Planting was done manually with 0.4 m spacing between lines and on lines. The plots were mulched twice during the vegetative cycle.

Obtaining METPS

Fruits of *T. peruviana* were collected, peeled and the nuts cracked. Seeds obtained were dried at laboratory temperatures (25 ± 2 °C) for 14 days and then crushed with a hand machine. The resulting powder was weighed with a 1g "Sartorious" precision scale balance and then added to a saturated quantity of methanol solvent (0.33 kg/l). The mixture was macerated for 48 hr in a 20 l recipient. Maceration was repeated twice. After each maceration, the solvent, now saturated with extractible compounds was filtered using filter paper. To eliminate the solvent, the filtrate was evaporated using a "Buchi-R-200" evaporator at 60 °C under vacuum. Extraction yield was calculated using the formula: Rd (%) = (mass of extract / mass of powder) x 100.

Effect of methanol solvent on fungal growth in vitro

Before using the METPS in the field, the effect of methanol on some fungi in vitro was tested, to attest if this solvent has potentials or not to reduce fungal growth. To attain this goal, PDA medium and four phytopathogenous fungi (*Cercospora arachidicola*, *Phytophthora megakarya*, *Collotetrichum gloeosporioides* and *Helminthosporium turcicum*) were used following usual methods of fungal growth in vitro (Smith and Onions, 1983).

Antifungal treatments

Application of antifungal substances started 45 DAP following the appearance of the first disease symptoms. Two treatments separated by a 15 days interval were made. Methanol extracts were applied at two different doses ME1 (1.7 kg/ha) and ME2 (3.8 kg/ha). Treatments with Benlate 50 WP (50 % of benomyl) were done at a dose of 4 kg/ha. The plants were treated using a sprayer which delivers 1200 l/ha at a maximum pressure of 7 kg/cm^3 with a flat water-spray head. A 1.5 g/l soaking agent (powdered soap) was added to the METPS mixture.

Estimation of disease parameters

The evaluation of early and late symptoms of CLS was done after every 14 days starting from the 44th DAP (at the eve of the first spray). Disease incidence, expressed in percentage was determined by counting the number of infected plants in a given plot while increases in the size of lesion determined by measuring their diameters on the 5th fully developed leaf on the tip of the stem. This operation was repeated thrice with 14 days intervals.

Disease severity was calculated using the Tchoumakov and Zaharova (1990) formula. The degree of infection (proportion of leaf surface infected, estimated in %) was noted on the whole plant according to the Stankevich's scale (1969).

Harvest of groundnuts was done manually at the end of the vegetative cycle (90 DAP). Ripe pods collected after uprooting the plants were dried in sunlight for seven successive days and then shelled. The yield of dry grains was determined by weighing with the aid of a "Sartorious" scale balance.

Data analysis

The evolution of disease on plots was compared with the incidence, growth rate, final disease severity and diameter of lesions. Data obtained was analysed using the statistical analysis system (SAS), a computer programme which uses analysis of variance (ANOVA). The least significant difference of Duncan's test was used to judge the difference between means of treatment and varieties ($p = 0.05$). To compare the different factors (effects of antifungal treatments and varieties) and their interaction, Fishers of Snedecor (F_{cal} and F_{tab}) were used: if $F_{cal} > F_{tab}$, then the effect is significant on the given parameter.

Results

Antifungal potential of methanol solvent

The data obtained from the in vitro test (Table 1) after incubating *C. arachidicola, P. megakarya, C. gloeosporioides and H. turcicum* for seven days showed that

Table 1. Effect of methanol on radial growth of some phytopathogenic fungi in vitro (mean diameter in mm ± SE) after four days of incubation

Solvent	Doses (µl.ml^{-1})	*Cercospora arachidicola*	*Phytophthora megakarya*	*Helminthosporium turcicum*	*Colletotrichum gloeosporioides*
Methanol	0	48.75 ± 3.92 a	56.87 ± 4.33 a	55.86 ± 5.11 a	49.55 ± 4.22 a
	50.0	48.34 ± 3.41 a	57.29 ± 4.47 a	54.86 ± 5.38 a	49.78 ± 4.14 a

Means followed by the same letter in a column are not significantly different according to Duncan test (P= 0.05)

Table 2. Effect of METPS treatments and varieties on lesion sizes of CLS

Treatments	Varieties	Diameter of fungal lesions (mm) 44 DAP	58 DAP	72 DAP	EP (%)
Control	Bafia Var.	3.20 ± 0.51	4.20 ± 1.81	5.33 ± 2.02	39.96
	55-437 Var.	3.73 ± 1.60	5.53 ± 1.07	7.10 ± 0.73	47.46
Benomyl	Bafia Var.	3.10 ± 0.10	4.60 ± 1.17	4.93 ± 0.83	37.12
	55-437 Var.	3.10 ± 0.69	3.73 ± 0.68	5.60 ± 1.21	44.64
ME1	Bafia Var.	3.26 ± 0.23	3.33 ± 1.17	3.40 ± 1.10	4.12
	55-437 Var.	5.43 ± 3.95	5.86 ± 0.70	5.93 ± 1.34	8.43
ME2	Bafia Var.	2.43 ± 0.51	3.10 ± 0.95	3.66 ± 1.15	33.60
	55-437 Var.	4.00 ± 0.40	5.10 ± 1.15	5.53 ± 0.50	27.67

EP (%): Increase of lesion sizes between 44 DAP and 72 DAP

Table 3. Statistical analysis of the effects of treatments and varieties on groundnut yield

Parameters	Treatments Control	Benomyl	ME1	ME2	Varieties Bafia var.	55-437 var.
Yield (t/ha)	0.61±0.21^{a}	0.97±0.21^{b}	0.94±0.02^{b}	0.98±0.16^{b}	0.94±0.17^{b}	0.72±0.15^{c}
LSD		0.04				0.20
F_{cal}		5.00				10.66
F_{tab}		3.24				4.49

the radial growth of these fungi in the presence of 50 µl of methanol solvent was 48.34, 57.29, 49.78 and 54.86 mm respectively. However, there were no significant differences when growth of fungi in methanol was compared with that in control samples (48.75, 56.87, 49.55 and 55.86 mm of radial growth for the fungi *C. arachidicola, P. megakarya, C. gloeosporioides and H. turcicum* respectively). These results show that methanol has no antifungal effect on *Cercospora arachidicola* and other fungi.

Effect of METPS treatments and different varieties on the size of CLS lesions

The sizes of CLS lesions were greater in control plots than in plots treated with METPS and benomyl (Table 2). The diameter of lesions was greater in plots of 55-437 var. than in those of Bafia var. The increase of lesion sizes between 44 DAP and 72 DAP for plots treated with ME1 and for those treated with ME2 was lower than in control and benomyl treatments (Table 2). Statistical analysis show that the antifungal treatments and host resistance on groundnut varieties 72 DAP, significantly reduced the evolution of the diameter of lesions. The interaction between antifungal treatments and varieties was observed.

Effect of METPS treatments and varieties on the evolution of CLS

Observations done 44, 48 and 72 DAP showed that the progression of the CLS incidence was similar on the two groundnut varieties (Fig. 1). Despite reductions observed in treated plots, the spread of the disease was more pronounced on the 55-437 var. (Fig.1b). A highly significant effect of varieties and treatments on the expansion of CLS 58 and 72 DAP (Fcal > $F_{cal)}$ was observed. The incidence of the disease was low on all varieties in treated plots compared to those in untreated plots (Fig.1a and Fig.1b). The final severity of CLS and the rate of increase of the disease in the absolute controls were higher on plots of 55-437 var. (Fig. 2b) compared to the same treatments on Bafia var. (Fig. 2a). During the vegetative cycle, the rate of increase of the epidemic and the severity of CLS were lower in treated plots than in controls. The rate of spread of disease was very low on the varieties treated with benomyl, ME1 and ME2 where -51.47, +5.83 and +2.04 % increase was recorded respectively as compared to controls where the increase rate was +55.11 % (Fig. 3). Symptoms of CLS on groundnut leaves where highly concentrated in control plots when compare to plots treated with METPS and benomyl (Fig. 4).

Effect of treatments and varieties on groundnut yield

In treated plots, yield was 0.94 ± 0.02, 0.98 ± 0.16 and 0.97 ± 0.21 t/ha, for treatments with ME1, ME2 and benomyl respectively, while in the control, the yield of groundnuts was 0.61 ± 0.21 t/ha. This shows that the application of crude extracts and benomyl led to a considerable increase in yield (Table 3). There were no significant differences between yield obtained from METPS and benomyl treated plants. The effects of treatments and varieties was significant on groundnut yield (F_{cal} > F_{tab}). The Bafia var. was more productive than the 55-437 var.

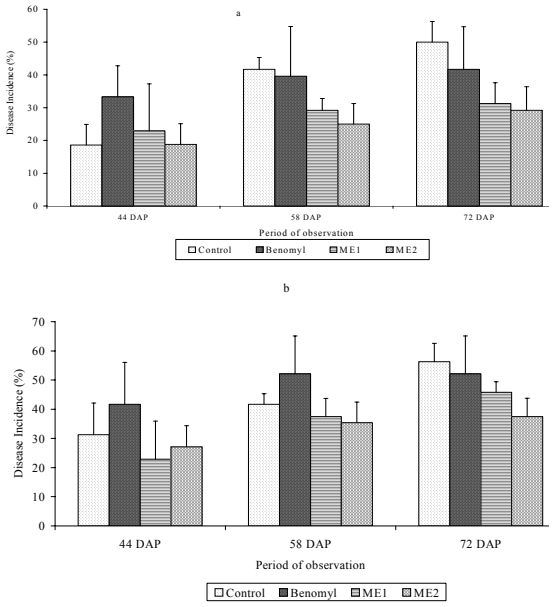


Fig 1. Effect of METPS treatments on CLS incidence on the two groundnut varieties, (a): Bafia variety; (b): 55-437 variety

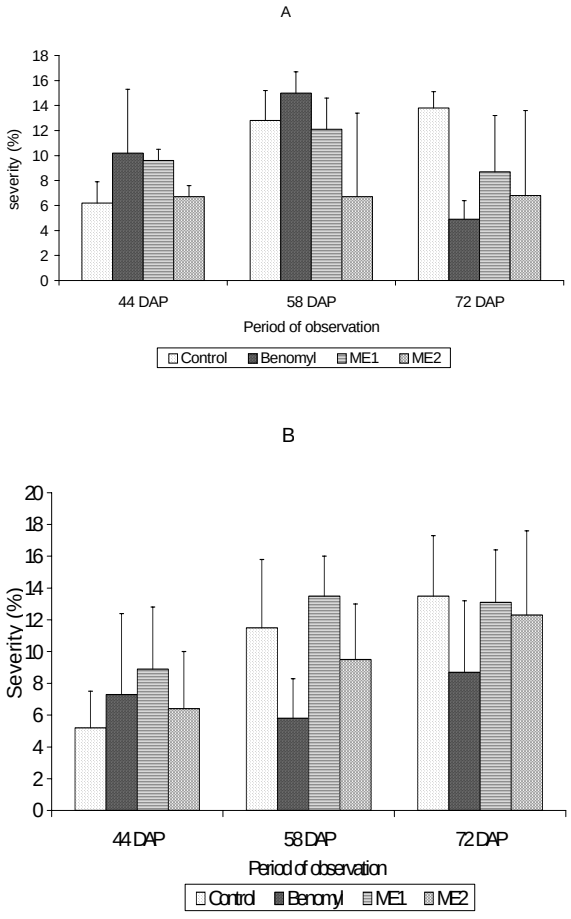


Fig 2. Effect of METPS treatments on CLS severity (%) on the two groundnut varieties
(A): Bafia variety; (B): 55-437 variety

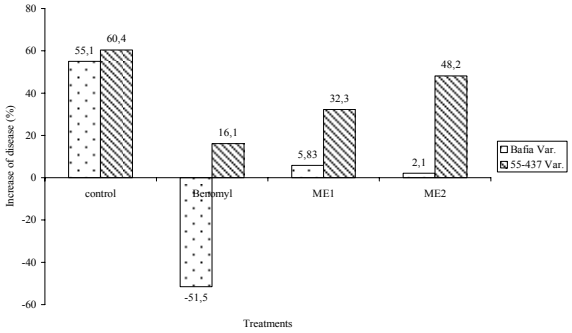
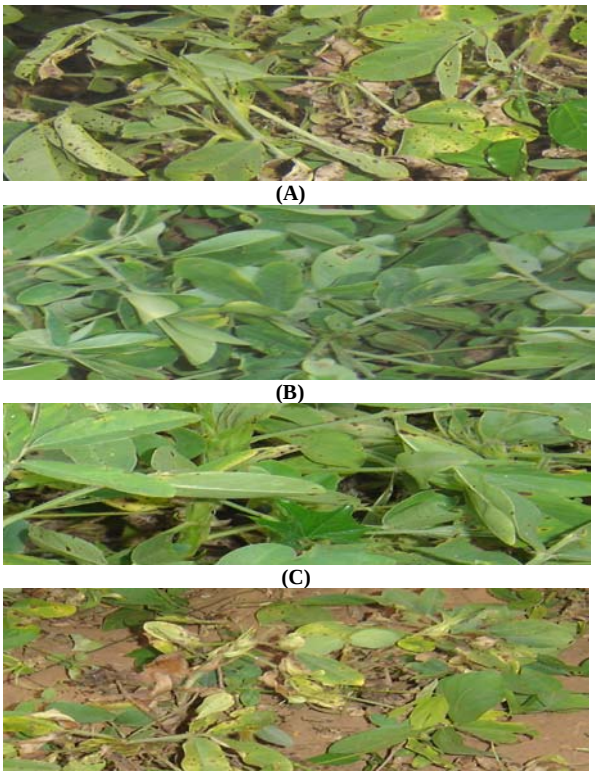


Fig 3. Effect of treatments on the increase of disease on the two groundnut varieties between final severity (72 DAP) and the beginning (44 DAP)



(A)

(B)

(C)

(D)

Fig 4. Effect of METPS treatments on cercosporosis symptoms on groundnut leaves (Var. Bafia) 70 DAP, A - Control ; B - Benomyl treatment ; C - ME2 treatment ; D - ME1 treatment

Discussion

CLS are the most important diseases that seriously reduce groundnut production in Cameroon. The use of biocides from plant origin in crop protection is an important means of promoting biopesticides in crop production. In this field study, attempts were made to control groundnut CLS diseases using METPS treatments and host resistance of two groundnut varieties. The incidence of CLS was low in plots treated with METPS and benomyl. These results show that METPS has a strong capacity to reduce the spread of CLS on groundnut plants. This corroborates the results of Gata-Gonçalves et al. (2003) and Ambang et al. (2010), who, working on similar experiments *in vitro* demonstrated the inhibitory effect of extracts of *T. peruviana* seeds on the development of *Cladosporium curcumerinum* and *Phytophthora megakarya* respectively.The evolution of the size of lesions on leaves of groundnut varieties was

influenced by the application of METPS and benomyl. After two sprays of METPS and benomyl, there was a significant reduction in the evolution of lesion size. Increase in the number of sprays therefore increases efficiency of phytosanitary products. Studies by Bovey et al. (1994), Talukder et al. (2002) and Subrahmanyam et al. (2006) reported similar results. The lowest rate of increase of the lesion sizes recorded on the local cultivar treated with METPS shows that, these extracts are efficient in the inhibition of groundnut *Cercospora* fungi, confirming the results obtained by Ngoh Dooh (2006) and Kurucheve et al. (1997) in similar studies using METPS on *Phytophthora megakarya* and on *Rhizoctonia solani*, respectively. The severity of CLS was influenced by the different antifungal treatments. Statistical transformation of the epidemiological data showed a linear evolution of CLS progression. The disease was more intense on exotic cultivar (55-437 var.) than on the local Bafia var. The low rate of increase of disease recorded between the final and initial severity on groundnut plants treated with METPS and benomyl compared to non-treated plots, show that METPS and the synthetic fungicide have a similar efficiency in the reduction of CLS on the two groundnut varieties. The local cultivar, Bafia var., seems to be more tolerant to CLS than the exotic variety. The fungicide efficacy of METPS observed in this study should be due to the presence of terpene compounds (pulegone, spathulenol, citronellol, carvacrol, nerolidol) and fatty acids (linoleic, palmitic, oleic, caprylic) in these extracts as observed by Gata-Gonçalves et al. (2003). Groundnut plants sprayed with METPS and benomyl produced high yield of dry grains compared to control. This can be explained by the protection assured by the phytosanitary treatments against CLS (Fontem et al., 1996; Sundaresha et al., 2009). The application of METPS associated with host resistance of the tested varieties considerably reduced the progression of CLS on groundnut crops. Consequently, these two methods can be used in an integrated management approach to protect groundnuts against diseases. While waiting for other experiments on the toxicology of METPS on other parasites of groundnut, METPS can be considered as an important biofungicide capable of substituting synthetic fungicides which very much pollute the environment. METPS are easy to obtain, cheap, biodegradable and *T. peruviana* can grow in all tropical regions. As such, vulgarisation of the use of METPS in crop protection would be economically and environmentally rewarding to many crop producers in tropical regions.

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