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In vitro control of *Mycosphaerella arachidis* Deighton, the early leaf spot disease pathogen of groundnut, by extracts from six medicinal plants

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Abstract: Ground nut (*Arachis hypogaea*) is one of the most popular commercial crops in Nigeria. Its successful production has been drastically affected by early leaf spot disease caused by *Mycosphaerella arachidis* Deighton. *In vitro* control of the pathogen by six medicinal plants (*Entada africana, Vitex doniana, Lawsonia inermis, Azadirachta indica, Acalypha hispida* and *Nuaclea latifolia*) was assessed in this study. The extracts of the plants were prepared using cold and hot water and alcohol. The pathogen was isolated from ground nut infected with early leaf spot disease. The results revealed a significant difference ($P < 0.05$) in yield of extracts between cold water, hot water and alcohol extracts. A significant difference ($P < 0.05$) was observed in percentage concentrations of the various phytochemical constituents present in the extracts. Flavonoids percentage concentration was the highest (0.68-1.95%) followed by saponin (0.09-1.53%) in *N. latifolia* extracts. Steroiods had the lowest percentage concentrations (0.00-0.09%) followed by terpenoids (0.02-0.71%) and proanthocyannin (0.05-0.86%). *N. latifolia* extracts produced the highest percentage concentrations (0.07-1.95%) of all the phytochemicals followed by *A. indica* (0.05-1.64%) and lowest concentrations were obtained in *A. hispidia* (0.09-0.87%) and *V. doniana* (0.00-0.88%). The extracts inhibited spore germination and growth of *M. arachidis*. The inhibition by alcohol extracts was high and significantly different ($P > 0.05$) from cold and hot water extracts. Alcohol extract of *L. inermis* gave 100% spore germination inhibition followed by *N. latifolia* and *A.indica* with 97.75% and 85.60% inhibition, respectively. Therefore, field trials with these six medicinal plants on the control of early leaf spot disease of ground nut are recommended.

Key words: plant extract, phytochemicals, inhibition

Introduction

Groundnut *Arachis hypogaea* is one of the most popular commercial crop in Nigeria which accounted for 70% of the total Nigeria export earning between 1956 and 1967 and in 2002 the production was 23,390,000 mt. Groundnut is an important legume crop used as a food, oil and cash source, for making margarine, candy, salted groundnut, crackers/cookies, salad oils and soaps. The production started to decline from peak productions of the 1960s due to severe biotic constraints, which included diseases of fungi and viruses (Godfrey & Olorunju, 2009). Early leaf spot disease caused by the fungus *Cercospora arachidis* S. Hori teleomorph *Mycosphaerella arachidis* Deighton) is one of the major destructive diseases of groundnuts worldwide (Ogwulumba *et al*., 2008). Leaf spot diseases cause nearly complete defoliation,

30-70% loss in pod yield and reduction in the kernel quality (Reddy *et al.*, 1997). Control of *Cercospora* leaf spot (CLS) diseases in Nigeria has depended on some cultural practices, multiple applications of fungicides and development of cultivars tolerant to this disease. Effective and long-term control of leaf spot disease can be achieved by applying recommended fungicides at their recommended time intervals. However, repeated application of fungicides could lead to reduced efficacy of the fungicides due to a gradual loss of sensitivity in the target pathogen population. It could also contribute to higher production costs and environmental pollution (Ambang *et al*., 2008). Therefore, the search for alternatives to chemical products such as the use of natural biocides of plant origin is the most promising option for a safe and sustainable agriculture. Plants produce several secondary metabolite compounds with antimicrobial activity that are specific against a particular pathogen or may have a broad spectrum and can be used for control of fungal diseases in crops (Ambang *et al*., 2010; Ogwulumba *et al*., 2008; Kishore *et al*, 2001).

Therefore, the objective of this work was to study the potential of crude extracts of *E. africana, V. doniana, L. inermis, A. indica, A. hispida* and *N. latifolia* in the control of CLS epidemiology on groundnut 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.

Material and methods

Collection of plant materials

Wild and locally cultivated plants of *E. africana, V. doniana, L. inermis, A. indica, A. hispida* and *N. latifolia* were collected in Lapai, Lapai Local Government in Niger State, Nigeria.

Preparation of plant extracts

The fresh leaves of the plants were washed using sterile distilled water (SDW) and were air dried at room temperature (28 ± 2 °C) for four months. The materials were separately ground in porcelain mortal and later with a blender (model MC – BL1242), sieved and stored in air tight bottles. Aqueous and ethanol extraction were made.

Isolation of fungi strain (Mycosphaerella arachidis)

Groundnut plants infected with early leaf spot were carefully identified and collected during the cropping season of 2011 from experimental farm of Agricultural Science Department, Ibrahim Badamasi Babangida University, Lapai Niger State Nigeria. The pathogen was isolated and identified using both morphological and microscopic characteristics. The pathogen was stored in slants on PDA at -4 °C.

Pathogenicity

Pathogenicity test was conducted to confirm the authenticity of the pathogen as causative organism (*M. arachidis*) of early leaf spot disease of groundnut.

Phytochemical screening

Phytochemical test was conducted to qualitatively verify the presence or absence of secondary metabolites in the extract of the plants earlier collected.

In vitro antifungal screening tests
Spore germination assays were conducted according to Kishore *et al*. (2001). Agar well diffusion assay were conducted accoirding to Okigbo & Ogbbonnaya (2006).

Results and discussion

Pathogenicity test
The result of the pathogenicity test confirmed *M. arachidis* as the causative organism.

Phytochemicals
The results of the phytochemical screening showed the presence of secondary metabolites such as alkaloids, saponins, proanthocyanins, steroids, flavonoids, tannins and terpenoids in the plants sampled. The result was in agreement with earlier work of Abiodun *et al*. (2011) who reported on some of these metabolites in different plants extracts.

In vitro antifungal screening
Ethanol extracts of the medicinal plants (Figures 1-3) gave the highest spore germination and radial growth inhibition followed by hot water extracts. Overall, the potency of the extracts on the radial growth of *M. arachidis* was significantly different ($P < 0.005$). The fungitoxic actions of these leaf extracts were due to the presence of metabolites. They also have been reported to inhibit spore germination and radial growth of different pathogens (Soetan, 2008; Ishida *et al*., 2009; Obasi *et al*., 2010).

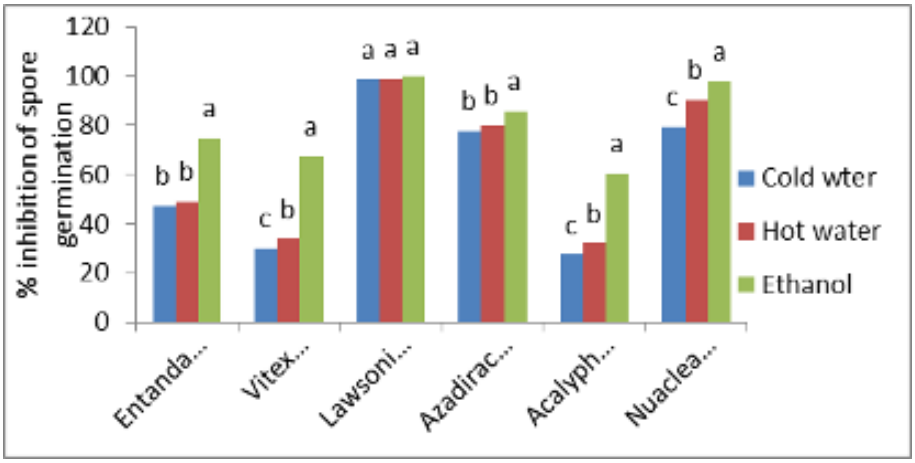

Figure 1. Percentage inhibition of spore germination of *Mycosphaerella arachidis* D. by leaf extracts of six medicinal plants. Bars with the same letter are not significantly different ($P < 0.05$).

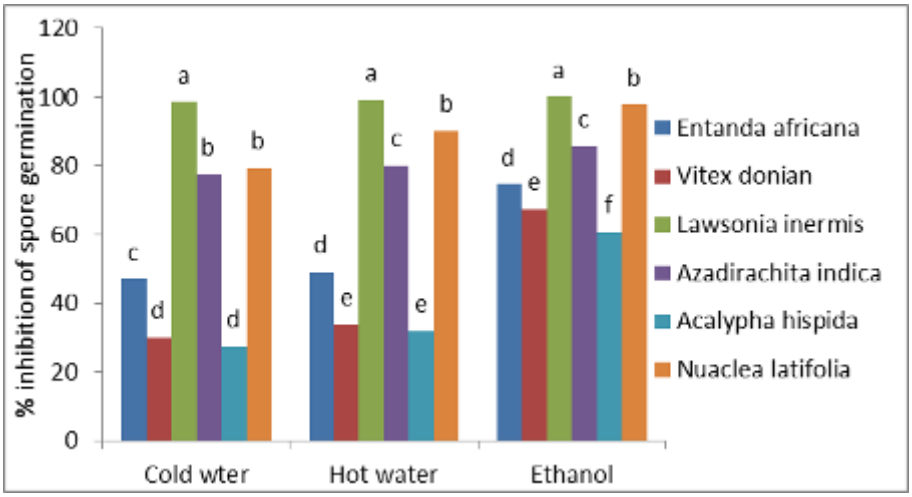

Figure 2. Effect of different extracts of six medicinal plants on *Mycosphaerella arachidis* D. Bars with the same letter are not significantly different ($P < 0.05$).

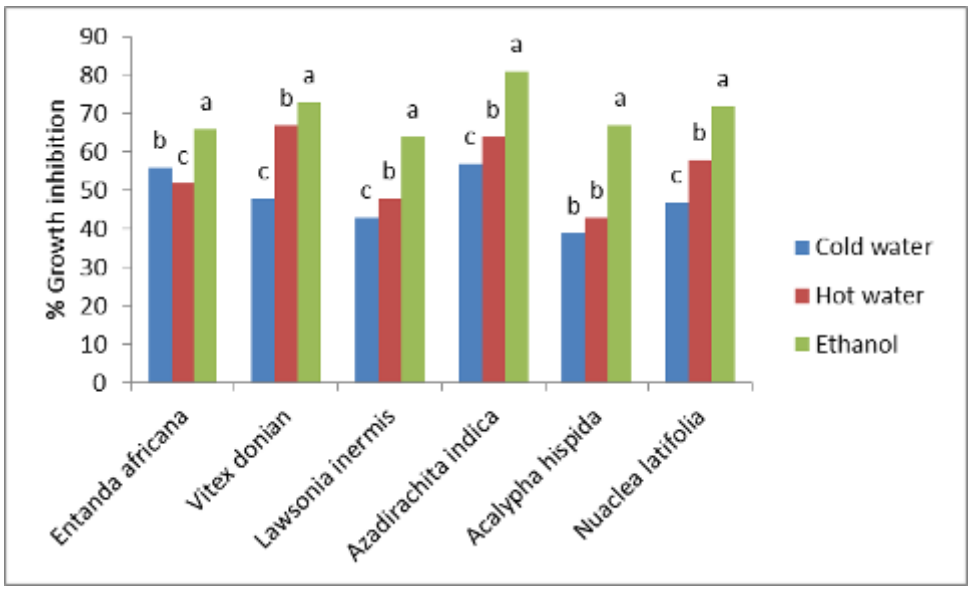

Figure 3. Percentage radial growth inhibition of *Mycosphaerella arachidis* D. by leaf extracts of six medicinal plants.

Conclusion

This research has shown spectra of antifungal activities of extracts from six medicinal plants and provides support to some traditional uses of these medicinal plants. Since these plants are easy to obtain and the extracts could easily be made via a simple process of maceration or infusion, they could therefore be cheaper substitutes for conventional fungicides in controlling various plant diseases.

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