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AQUEOUS PLANT EXTRACTS FOR CONTROL OF GROUNDNUT LEAF SPOT IN BURKINA FASO

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ABSTRACT

Early and late leaf spots, the two fungal diseases of groundnut (*Arachis hypogaea* L.) caused by *Cercospora arachidicola* Hori. and *Phaeoisariopsis personata* (Berk and Curt), respectively, cause severe groundnut crop losses in arid zone of West Africa. The objective of this study was to evaluate efficacy of aqueous extracts of four native plants species of Burkina (*Lippia multiflora* Moldenke, *Boscia senegalensis* (Pers.) Lam, *Ziziphus mucronata* Willd. and *Securidaca longepedonculata* L) against these diseases, on a susceptible groundnut variety (TS32-1) in the field during the cropping seasons of 2010 to 2012, in Gampela district in Burkina Faso. The four extracts (50 g dry weight per liter of distilled water) were sprayed by using a manual sprayer (Solo). Disease incidence and severity, defoliation rate, necrotic leaf area, pod yield, and 100 seed weight were evaluated. Disease score which attests to the severity of parasitic attack was done following ICRISAT's disease score scale, which ranges from 1 to 9. Treatments with aqueous extracts of *L. multiflora* and *Z. mucronata* recorded the best disease control. The disease scores of these treatments were less or equal to 4 (corresponding to a resistance of plants), and increased pod yield by 2676.889 kg ha^{-1} (*L. multiflora*) and 2976.926 kg ha^{-1} (*Z. mucronata*), respectively. This field trial has confirmed that plants tested significantly control leaf spot diseases of groundnut.

Key Words: *Arachis hypogaea, Cercospora arachidicola, Phaeoisariopsis personata*

RÉSUMÉ

Les cercosporioses précoce et tardive, deux maladies fongiques de l'arachide causées respectivement par *Cercospora arachidicola* Hori. et *Phaeoisariopsis personata* (Berk and Curt), provoquent d'importantes pertes de production dans la zone aride ouest Africaine. L'objectif de cette étude est d'évaluer l'efficacité antifongique de quatre plantes du Burkina Faso (*Lippia multiflora* Moldenke, *Boscia senegalensis* (Pers.) Lam, *Ziziphus mucronata* Willd. et *Securidaca longepedonculata* L) contre ces maladies sur une variété sensible d'arachide au champ. L'étude conduite pendant les saisons hivernales de 2010 à 2012 à Gampela (Burkina Faso) a permis d'évaluer les paramètres suivants : incidence de la maladie, taux de défoliation, surface foliaire nécrosée, rendement graine et poids de 100 graines. La notation de l'incidence de la maladie a été faite suivant une échelle à 9 classes de l'ICRISAT. Les traitements aux extraits de *L. multiflora* et de *Z. mucronata* ont induit la plus forte réduction de l'incidence parasitaire. Les notes de maladie enregistrées ont été inférieures ou égales à la note 4 dans ces parcelles avec un rendement graine allant de 2676.889 kg ha^{-1} (*L. multiflora*) et de 2976.926 kg.ha^{-1} (*Z. mucronata*). Cette étude confirme l'efficacité *in vitro* des extraits aqueux des plantes testées dans le contrôle des cercosporioses précoce et tardive de l'arachide. Par conséquent, leur utilisation dans le contrôle des cercosporioses pourrait conduire à des augmentations substantielles de rendement pour les producteurs tout en assurant des conditions plus sûres pour les consommateurs, les producteurs et l'environnement.

Mots Clés: Arachide, *Cercospora arachidicola,* extraits de plantes, maladies fongiques, *Phaeoisariopsis personata.*

INTRODUCTION

Groundnut (*Arachis hypogaea L.*) is the sixth most important oilseed crop in the world. Among the fungal diseases of groundnut, leaf spot (early and late leaf spots) is a major constraint in its production in Tropical Africa. During severe disease outbreaks, leaf spot, in addition to rust can result in yield losses up to 50% (Ghewande, 2009).

Diseases arising in the field are not usually controlled; however, they are controlled with synthetic fungicides that often pollute the environment and threaten human health. Thus, finding alternative control measures has become a major research objective. So, far, the antimicrobial properties of plant extracts has shown higher prosperts for becoming an alternative to synthetic fungicides (Deberdt *et al*., 2012; Malkhan *et al*., 2012; Bakeer *et al*., 2015).

Plants contain a wide stock of biochemical substances having antifungal properties. Their utilisation is less detrimental to the environment than synthetic pesticides (Hashim and Devi, 2003). Aqueous extracts of *Cymbopogon citratus*, *Cassia occidentalis*, *Balanites aegyptiaca* and *Portulaca oleracea* inhibited growth of mycelia of *Colletrotrichum graminicola* and *Phoma sorghina*, which are pathogenic fungi of sorghum seeds (Bonzi *et al*., 2012). Likewise, extracts of leaves of papaya tree (*Carica papaya* L.) and vernonia (*Vernonia amygdalina* Delile) were successfully tested against pathogenic fungi of groundnut (Ogwulumba *et al*., 2008). Other research efforts recommended the use butanol extracts of *Carica papaya* and *Azadirachta indica* to control fungal diseases of *Gmelina arborea* Roxb (Johnson *et al.,* 2014).

Recent research revealed that aqueous extracts of *Agave sisalana* contained antifungal properties against fungal pathogens of rice (*Magnaphorte grisae*) (Kassankogno *et al.,* 2015). Another study confirmed a positive effect of extracts of *Azacdiractha indica* on the inhibition of many fungal growths such as in *Alternaria alternata*, *Bipolaris sorokiniana*, *Fusarium oxyporium* and *Helmonthosporium* sp. (Al Hazim, 2013). Extract of *Capsicum frutescens* (10 mg ml^{-1}) was also effective in controlling groundnut seed pathogens such as *Aspergillus niger*, *A. flavus*, *Penicillium* sp. and *Rhizopus* sp. (Soumya and Bindu, 2012). Palm wine was successfully used to protect yam (*Dioscorea* spp.) (Assiri *et al*., 2010) against *Botryodiplodia* sp., *Penicillium* sp. ; *Aspergillus* sp., *Mucor* sp. and *Colletotrichum* sp. fungi causing rots in yam tubes. Similarly, the growth of pathogenic fungi (*Fusarium moniliforme*, *Curvularia lunata*, *Colletotrichum graminicola*, *Exserotrilum rostratum*) of sorghum and millet seeds was inhibited by aqueous extracts of *Acacia gourmaensis* A. Chev. and *Eclipta alba* (L.) Hassk. Inhibition rates of 27-72 and 56-86%, respectively, were observed for the protection of sorghum and millet seeds against the previous pathogens (Zida *et al.,* 2008).

In Burkina Faso, an *in vitro* study proved that extracts of four native plants were efficacious against *Cercospora arachidicola* Hori., *Phaeoisariopsis personata* (Berk et Curt) and *Puccinia arachidis*; which are the respective causal agents of early leaf spot, late leaf spot and rust of groundnut (Koïta *et al.*, 2010; Koïta *et al.,* 2012). Despite their *in vitro* efficacy to inhibit fungal growth or germination, plant extract potential under field conditions is still insufficiently exploited due to deficiency of reliable studies on this subject.

The objective of this study was to investigate the efficacy of aqueous extracts from *Lippia multiflora* Moldenke, *Boscia senegalensis* (Pers.) Lam, *Ziziphus mucronata* Willd. and *Securidaca longepedonculata* L. to control early and late leaf spot of groundnut.

MATERIALS AND METHODS

Study site. This study was conducted in Gampela district, located at longitude 12.22°W and latitude 12.25°N of Ouagadougou in Burkina Faso. The annual temperature ranged between 21.5 and 42.8°C. Treatments involved four aqueous plant extracts, and two controls (negative and positive).

Treatments and design. The test was conducted on the groundnut variety TS32-1 resulting from a cross between varieties spantex and TE3. TS32-1 is the recommended variety by the National Institute of Agricultural Research of Burkina Faso (INERA). TS32-1 is an early maturing (90 days) variety and it is widely cultivated in Burkina Faso. However, TS32-1 is susceptible to early and late leaf spots, rust, rosette and *aspergillus flavus* (Subrahmanyam and Hildebrant, 1992). The fungicidal properties of four native plant species of Burkina Faso was evaluated by applying their aqueous extracts on this susceptible groundnut variety. Plant extract was obtained from leaves of *Boscia senegalensis* Pers. (collected from north of Burkina), root bark of *Securidaca longepedunculata* L.), leaves of *Lippia multiflora* Moldenke and fruits of *Ziziphus mucronata* Will. (collected of middle of Burkina).

These six treatments were laid out in a randomised complete block design, in three replicates. Row to row distance was 1 m; while from plant to plant distance was 15 cm.

The trial was repeated two times (2011 and 2012). No plant extract was applied to the negative control plot. Fungicide, benlate (activer 50%: benomyl) was used in the positive control. A fertiliser composed of 14–23–14, nitrogen- phosphorus – potassium (N-P-K) was applied at a rate of 100 kg ha^{-1}, 21 days after sowing (DAS).

Phytoextracts preparation. The root bark of *Securidaca longepedunculata* was used in its fresh state, by keeping the harvested part at, 8 °C prior to extraction; while leaves and fruits of the other plants species were dried in shade at room temperature (25 to 30 °C). Leaves of *Lippia multiflora* and of *Boscia senegalensis* were ground into powder with a hand-held mill. The powder was preserved in a dry and cool place in the laboratory.

The fruits of *Ziziphus mucronata* were pounded in a manual seed grinder, before the pulp was separated from the seed. It was kept in a dry and cool place in the laboratory in order to preserve the active ingredients of the product. About 50 g of each plant tissue (leaves powder, pulp of the fruit or roots bark) were mixed with 1000 ml of distilled water for the preparation of the aqueous extracts. This quantity was applied to a block of 12 m^2, which was a rate of 4.166 kg per hectare as used in previous studies (Koïta *et al.*, 2010; Koïta *et al.,* 2012). The mixture was shaken for about 20 minutes and then filtered through smooth mesh linen.

Field treatment. Plants were sprayed with aqueous extracts using a hand-held Solo branded sprayer. Spraying was done on the basal leaves which were diseased before the upper ones. Individual plants were sprayed until the quantity of extracts prepared for a treatment was exhausted. Treatments were applied in the mornings between 6 and 9a.m to avoid the heat that could affect the stability of the extract. Spraying was carried out four times at 15 days interval for both years of experimentation starting at 25 days after sowing (DAS)and latest at70DAS.

Data collection and statistical analysis. Disease severity was recorded biweekly, starting at 25^{th} DAS, ending at 85 DAS. Using the ICRISAT scale, which ranges from 1 to 9 (Subrahmanyam *et al.,* 1995), disease scores were recorded depending on visual estimation of necrosis and defoliation. Where 1 = no disease, 2 = lesions present on lower leaves with no defoliation, 3 to 8 associated with increasing levels of defoliation and necrosis and 9 = defoliation of almost all leaves leaving bare stems, with any leaflets present having many leaf spots. From the scores, the percentage of necrotic area was calculated by using the following formula (Narh *et al.,* 2014):

$$\text{Necrosis (\%)} = 1.36 \times \frac{10}{9} \times IS - 1.45$$

Where: IS = ICRISAT visual score

Besides the necrotic area, defoliation was also estimated at 85 DAS, which was the maximum defoliation stage (Subrahmanyam *et al.,* 1995). This record was taken on the main stems of five plants chosen for each treatment. The percentage of defoliation was derived from the ratio between the number of dropped leaves and total number of sprouted leaves.

Pod yield and 100 seed weight were also evaluated after harvesting for each plot. To ensure homogeneity of variances and normality of the distribution of each variable, data recorded as percentages were arcsine-transformed before analysis of variance (ANOVA). Statistical analysis were done with the software XLSTAT-2010. Treatment means were compared using Student Newman-Keul test.

RESULTS

Disease assessment. Data for leaf spot disease progression and percent necrosis are presented in the Table 1. All plant extracts significantly (P<0.05) reduced the severity of *Cercospora arachidicola* and of *Phaeoisariopsis personata* on groundnut leaves. The negative control plot recorded the highest disease severity score of 8.1 on a 9 point scale (ICRISAT scale) corresponding to a high attach level. Aqueous extract of *L. multiflora* recorded the lowest diseases severity score (3.7) among the four plant species. Its score was statistically lower than extracts of the remaining three plants but similar to the score (3.7).

A higher level of necrosis (9.3%) was registered in the negative control plot (Table 1). On the other hand, treated plots showed slower disease progression with a rate of 6%. Among the plots treated, *L. multiflora* recorded the lowest leaf percentage necrotised with a 4.1% score. This value is close to the fungicide control, with a value of 3.3% of necrotic leaf area.

The effects of the fungicide and plants extracts on defoliation are shown in Figure 1. All plant extracts controlled the defoliation to significant level compared to negative control, which recorded the highest defoliation rate (57.05%). This was followed by three plots treated with aqueous extracts of *S. longepedunculata* (47.59%), *B. senegalensis* (44.05%) and *Z. mucronata* (42.22%). Among the plots treated with plant extracts, *L. multiflora* recorded the best control of

TABLE 1. Disease progression (ICRISAT score) and percent necrosis causant by leaf spot regarding treatment applied in experimental field in Gampêla district in Burkina Faso

Treatment	Disease score	Necrotic leaf area (%)
S.longepedunculata	4.9^{b}	6.0^{b}
B.senegalensis	4.8^{b}	5.9^{c}
Z. mucronata	4.3^{b}	5.0^{c}
L. multiflora	3.7^{c}	4.1^{d}
Negative control	8.1^{a}	9.3^{a}
Fungicide	3.4^{c}	3.3^{e}

Mean with similar alphabetical superscript are not significantly different (P = 0.05) by Newman-Keul's test defoliation (37.13%). The fungicide plot recorded 23, 73% which was the lowest defoliation.

Pod yield. The effect of plant extracts on pod yield are shown in Figure 2. The aqueous extracts from all the four plant species, *S. longepedunculata, B. senegalensis, Z. mucronata* and *L. multiflora* increased pod yield over pod yield of the negative control (1226.074 kg ha^{-1}). *Lippia multiflora* and Z. mucronata extracts treated plots recorded the highest pod yield among the four plant extracts, and its pod yield was significantly higher than that of *B. senegalensis* and *S. longepedunculata.* With regards to the control, the extract of *L. multiflora* and *Z. mucronata* had product in excess of more than 900 kg ha^{-1}.

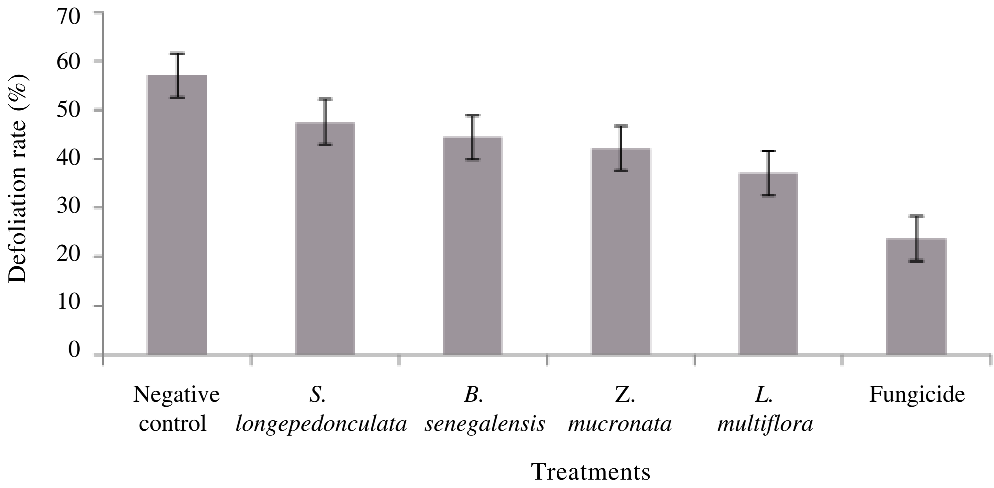

Figure 1. Effect of plant extracts and controls on defoliation rate in experimental field in Gampêla district in Burkina Faso.

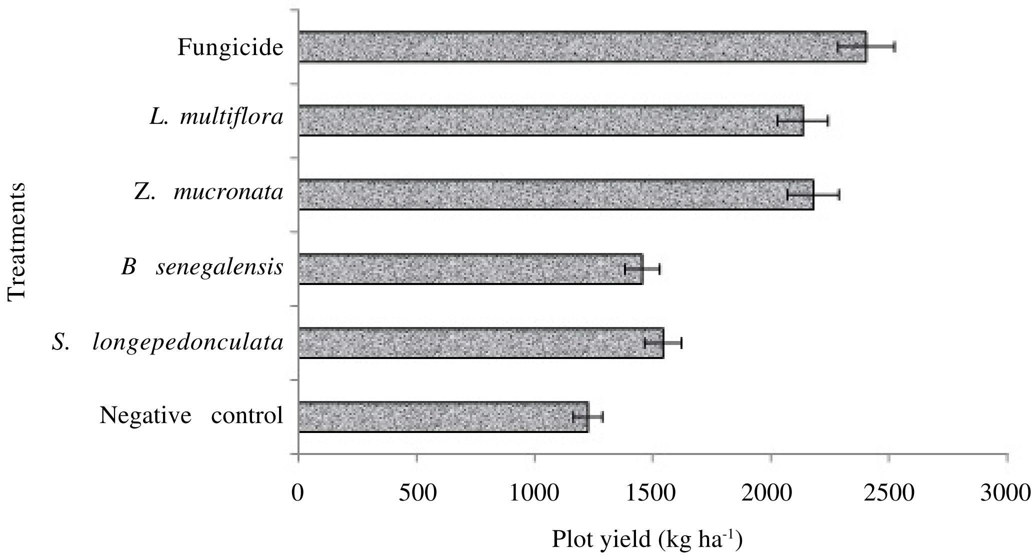

Figure 2. Effect of plant extracts on pod yield regarding treatment applied in experimental fieldin Gampêla district in Burkina Faso.

TABLE 2. Values of 100 seed weight (g) obtained from the six treatmentsin two cropping seasons in Gampêla district in Burkina Faso

Treatment	100 seed weight (g)
Negative control	29.84[c]
S. longepedunculata	33.52[b]
B. senegalensis	34.27[b]
Z. mucronata	35.45[b]
L. multiflora	34.93[b]
Fungicide	37.29[a]

Means with similar alphabetical superscript are not significantly different (P = 0.05) by Newman-Keul's test

100-seed weight. Aqueous extracts of all four plant species significantly increased 100-seed weight (Table 2). Although, the positive control (fungicide) yielded the highest 100-seed weight with 37.29 g. The 100-seed weight from extracts of the four plant species were similar (P>0.05) and varied around 34 g.

DISCUSSION

Disease assessment. The low disease severity scores recorded for plots treated with plant extracts (Table 1) prove the capacity of these extracts to inhibit early and late leaf spot development under field conditions. The negative control plot recorded the highest severity score of and percent necrosis.This confirms *in vitro* antifungal activity of these plants previously observed on *C. arachidicola* and *P. personata*(Koïta *et al.,* 2010) and *Puccinia arachidis* (Koïta *et al.,* 2012). Other studies have been reported the antifungal activity of extracts from other plant species. Insecticidal activity of *Boscia senegalensis* on the groundnut bruchid (*Caryedon serratus*) was reported by Gueye *et al*. (2011). On the other hand, efficacy of *B. senegalensis* extracts in the field against *C. arachidicola* and *P. personata* was confirmed by Karimou (1999). Several studies were carried out on the biological activities of essential oil of *Lippia* genus. Tatsadjieu *et al.* (2009) noted the total inhibition of radical growth of *Aspergillus flavus* by essential oil of *L. rugose*. Besides, efficacy of essential oil of *L. rehmannii* against some fungi including *Rhizoctonia solani, Fusarium oxysporum* and *Penicillium digitatum* pathogens of potato, maize and orange tree respectively have been reported by Linde *et al.* (2010). Owolabi *et al.* (2009) revealed a strong bactericide activity of the essential oils of *L. multiflora* against *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. On the other hand, this report confirms the use of aqueous extracts of *S. longepedunculata*, *L. multiflora* and *Z. mucronata* to succesfully control early and late leaf spots of groundnut under field conditions. The fungi and rust involved in the present study have shown sensitivity toother plant extracts; for instance, successful inhibition of spore germination (greater than 90%) of these fungi was achieved with oils of cinnamon bark (*Cinnamomum cassia*), clove (*Syzygium* aromaticum) and essential oil of citronella (*Cymbopogon citratus*) (Kishore *et al*., 2007). Other studies showed that aqueous and ethanol leaf extracts of *Datura metel*, *Lawsonia inermis* and *Sphaeranthus indicus* at 25% concentration completely inhibited the conidial germination of *P. personata* (Kishore *et al.*, 2001). Chloroform extract of *Hemionitis arifolia* was found to be highly efficacious (inhibition rate > 70%) against *P. arachidis* and *P. personata* (Sahayaraj *et al*., 2009).

Pod yield. Management of leaf spotswith plant extracts in the field has been evaluated by Krishna and Pande (2005). Thus, they reported that foliar application of *Prosopis juliflora* extract effectively reduced groundnut foliar disease severity and increased the pod yield. The present study identified *P. juliflora* extract as a significant component for the integrated management of groundnut foliar diseases. Late leaf spotof groundnut was also controlled in the field using neem seed, black soap and cow dung (Alabi and Olorunju, 2004). Another study revealed that foliar application of panchgavya and neem leaf extract recorded significant improvement in different parameters of chlorophyll content, nitrate reductase activity, root nodule weight, leaf area index, dry matter accumulation, nutrient content and uptake of groundnut and pod yield (Kumawat *et al.*, 2009).

Papaya leaf extracts used against leafy disease of groundnut considerably reduced disease incidence to 2.20% from 74.74% (Ogwulumba *et al.*, 2008) . Shakil *et al.* (2012) conducted experimental tests in which aqueous extracts of the leaves of *Calotropis procera* were as effective as fungicide (Ridomil) in the control of collar decay of groundnut. *Calotropis procera* treatment recorded a pod yield of 1,065 kg ha^{-1}, compared to 1032 kg ha^{-1} pod yield for plot treated with fungicide(Ridomil).

100-seed weight. The weight of 100 seeds, is a value that determines the correct filling of the seeds. This requires a good photosynthetic activity. For this purpose the peanut plant must keep its leaves.For this purpose the peanut plant must keep its leaves. Strong defoliation necessarily results in poor seed filling, low weight of 100 seeds and low yield.

CONCLUSION

The present study confirms the efficacy of aqueous extracts of four local plant species (*S. longepedunculata, B. senegalensis, Z. mucronata* and *L. multiflora)* to control pathogenic fungi involved in early and late leaf spots diseases of groundnut. Among the tested species, aqueous extracts of *L. multiflora* gives the highest pod yields followed by *Z. mucronata* and *S. longepedunculata,* respectively. These results require further investigations to understand the exact mode of reaction of these extracts. A chemical screening study would help to high light the characteristic fungicidal molecules of these species through the isolation and identification of actives molecules.

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