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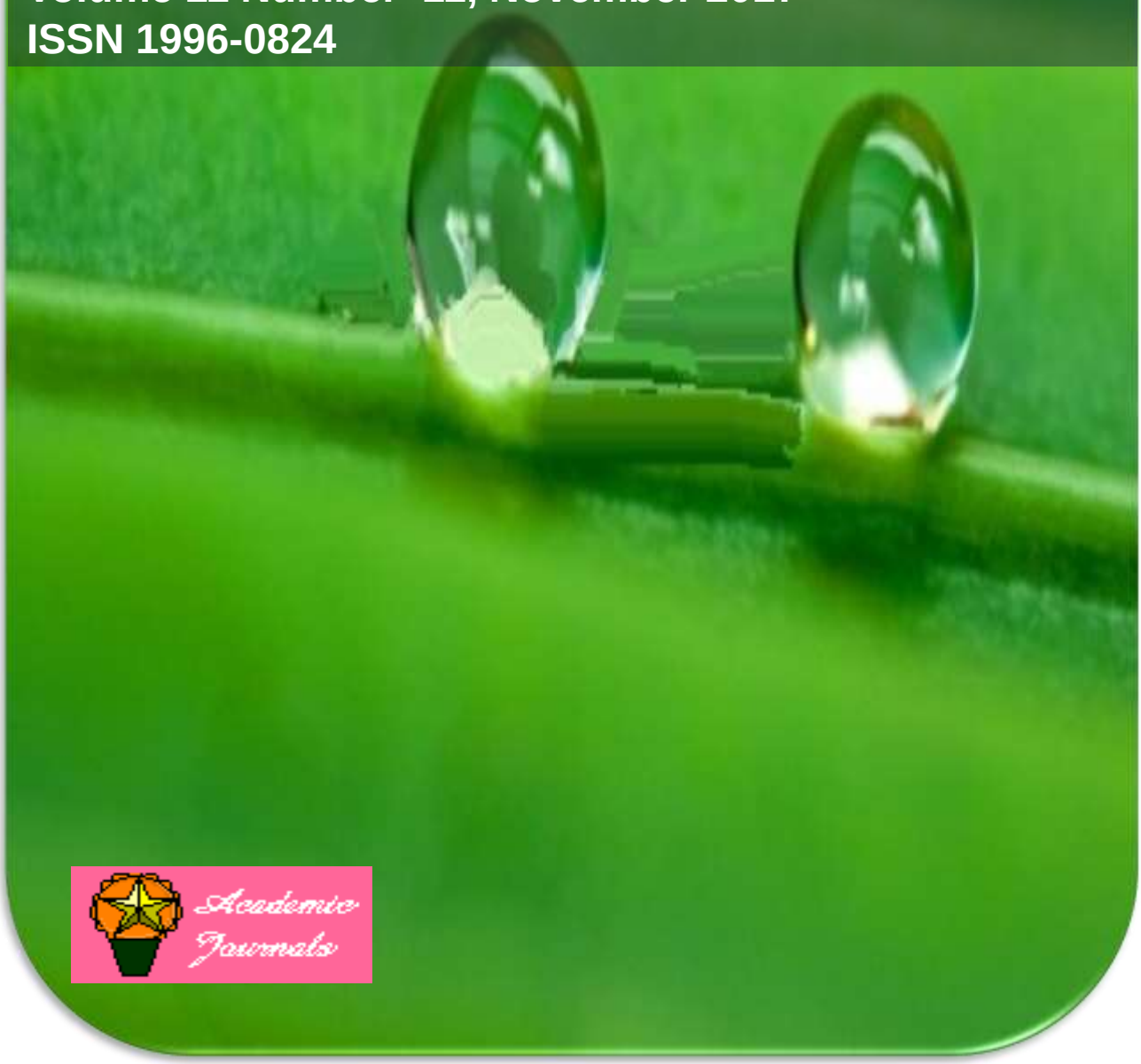

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Full Length Research Paper

Correlation between seed characteristics and biomass production of *Moringa oleifera* provenances grown in Ouagadougou, Burkina Faso

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The correlation between seed size and biomass production of *Moringa oleifera,* was conducted in a field experiment at Ouagadougou (Burkina Faso) in 2014. The experiment included eleven *M. oleifera* provenances from West Africa according to four agro-ecological characteristics (Sahelian, Sub Equatorial, South Sudanian and North Sudanian). Differences among provenances seed traits, leaf morphology, and dry mass (DM) were established by analyses of variance. Significant level was fixed at $P < 0.05$. Pearson's correlation analysis was done to evaluate the relationships between seed traits, leaf morphology, and dry mass. The results showed that significant differences in seed traits were observed among provenances. Provenances were significantly different in leaf morphology (length, width and number of pinnae/seedling). The provenance of National Forest Seed Center (CNSF) from the northern sudanese area of Burkina Faso produced the longest leaf. The mean shoot biomass accumulation per seedling differed significantly among provenances. Significant correlation coefficients were observed among seed traits but no important correlation were found between seed traits and other plant characteristics. No significantly correlation of seed size with either leaf morphology or dry mass were found. Plant grown from large seeds compared to those grown from small seeds was more vigorous and produces greater dry matter.

Key words: Moringa seedling, dry biomass, correlation, seed length, seed weight, seed width.

INTRODUCTION

There are many trees and shrubs of interest in agroforestry systems, and one interesting tree species that has received a great deal of attention recently is *Moringa oleifera* Lam (Mendieta-Araica et al., 2013). The species is planted to produce pods, leaves and seeds for consumers, forage and traditional medicinal purposes. The pods and leaves of *M. oleifera* contains high amount of Ca, Mg, K, Mn, P, Zn, Na, Cu and Fe (Aslam et al., 2005).

In Burkina Faso, *M. oleifera* leaves are commonly used

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to make sauces and as staple food. Cultivations of *M. oleifera* within different environmental conditions throughout the year attract attention of poor smallholders during the last years (Popoola and Obembe, 2013). Biomass productions and seeds yield are of key economic importance. Some smallholders in Burkina Faso formed the associations of women nursery operators engaged in seedlings productions for income generation with the objective of selling the seedlings. They have been found to exchange most of the *M. oleifera* seeds often collected from a few mother isolated trees in their farmlands, and plantations in the communities or outside.

This way of supplying seeds by private producers limited the access to high quality germplasm (Nyoka et al., 2015). *M. oleifera* plants were promoted within smallholders by many non governmental organisations (NGOs) and government, but no varieties with desirable traits for specifics growing conditions (home gardens, farmlands) are available, which makes its cultivation a risky business.

Good seedling establishment is essential for sustainable and profitable crop production, and is therefore recognized as the most critical step of a developing plant. Low seed vigor greatly controls the number of emerging seedlings, the timing and uniformity of seedling growth. This has direct influence on the yield and marketing quality of a crop (Pallo et al., 2009).

Seed weight is an indication of the reserves that seeds contain, and large and heavy seeds reveal that the seed has more reserved food (Wolde-Mieskel and Sinclair, 2000). Many studies have shown that initial seedling size is positively related to seed size, and larger seeds have better seedling survival rate as well as higher competitiveness both within species and among species (Singh et al., 2006). The seed supplies the embryo with sufficient nutrition and energy during germination from the food reserves that the seed acquires during the seed filling phase. Roxas et al. (1994) concluded that higher vigor that occurred in larger seed is due to the larger food reserves in these seeds.

Many studies also indicated a positive linear relationship between seed weight and emergence in the field. Baalbaki and Copeland (1997) reported that in wheat, seed size not only influence emergence and establishment but also affected yield components and ultimately grain yield. A similar observation was made by Arunachalam et al. (2003), while working with the tree species, and this was attributed to the larger food reserves in the larger seeds.

Several studies have been conducted looking for *M. oleifera* growth performance and biomass production (Edward et al., 2014; Förster et al., 2015), biomass production and chemical composition (Mendieta-Araica et al., 2013), seed and germination characteristics. There are however limited studies on the explicit relationship among seed and biomass characteristics.

In addition, the multivariate analyses have been widely used to study the influence of interacting traits on fitness in plant ecology studies (Farris and Lechowicz, 1990), assess plant responses to environmental stress (Hofmann et al., 2013), and examine morphological variation in agricultural crops (Ayana and Bekele, 1999).

The objective of this study was to investigate and compare different growth characters among provenances of *M. oleifera* in smallholders gardening systems. Seed length, width weight, leaf size and shape, and branch number may be useful predictors of potential production in specific environments (Pellis et al., 2004; Weih and Nordh, 2005). The relationships between seed characteristics and biomass production of *M. oleifera* in north sudanian part of Burkina Faso were therefore investigated.

MATERIALS AND METHODS

Location of experimental area

The study was carried out at the women gardening center named “Amicale des Forestières du Burkina Faso (AMIFOB)” located at Ouagadougou, Burkina Faso (12°7’32’’N, 01°40’24’’W). This corresponds to an ecological zone of the north sudanian forest, with average annual rainfall of 800 mm. The soils are sandy clay to clay-sandy Ferruginous leached with very low nutrient content according to French soil classification (Pallo e al., 2009). The common natural vegetation found at Ouagadougou is described as semi-deciduous open woodland. Main genera include, *Eucalyptus, Azadirachta, Mangifera, Vitellaria, Lannea, Piliostigma, Acacia., Ziziphus, Tamarindus,* and *Combretum*.

Seed sources

The experiment included twelve *M. oleifera* provenances which include 1 provenance of from Segou (Mali), 1 from Niangon-Lokoua (Ivory Coast), 1 from Tamale (Ghana) and 9 from Burkina Faso (Ouahigouya, Dano, Gaoua, Ouagadougou, Fada N’Gourma, Dédougou, Bobo-Dioulasso, Koudougou, Centre National de Semences Forestières (CNSF) (Figure 1). In the paper, provenances were referred to four climate areas according to their agro-ecological characteristics (Sahelian, Sub Equatorial, South Sudanian and North Sudanian) (Table 1). Seeds were collected in 2014 in plantation farmland from at least 12 mother trees per provenance.

Experimental design and sampling procedures

Trees were planted in a randomized complete block design (RCBD) with three replications. Each plot represented a provenance planted at 5 x 6 rows in a contiguous arrangement of 20 x 20 cm (Figure 2). Plot measured 10 x 1 m and contained 30 trees, and the distances between blocks were 2 m. Seed samples were pretreated with water for 24 h, and sown in June 1st 2014 in a prepared and cleaned soil using hand hoes. Weeding was done twice during the rainy season. Watering was done once a day in the morning. The seedlings were grown without fertilizers and chemical control.

Data collection and analysis

To investigate the variability in seed parameters (length, thickness,

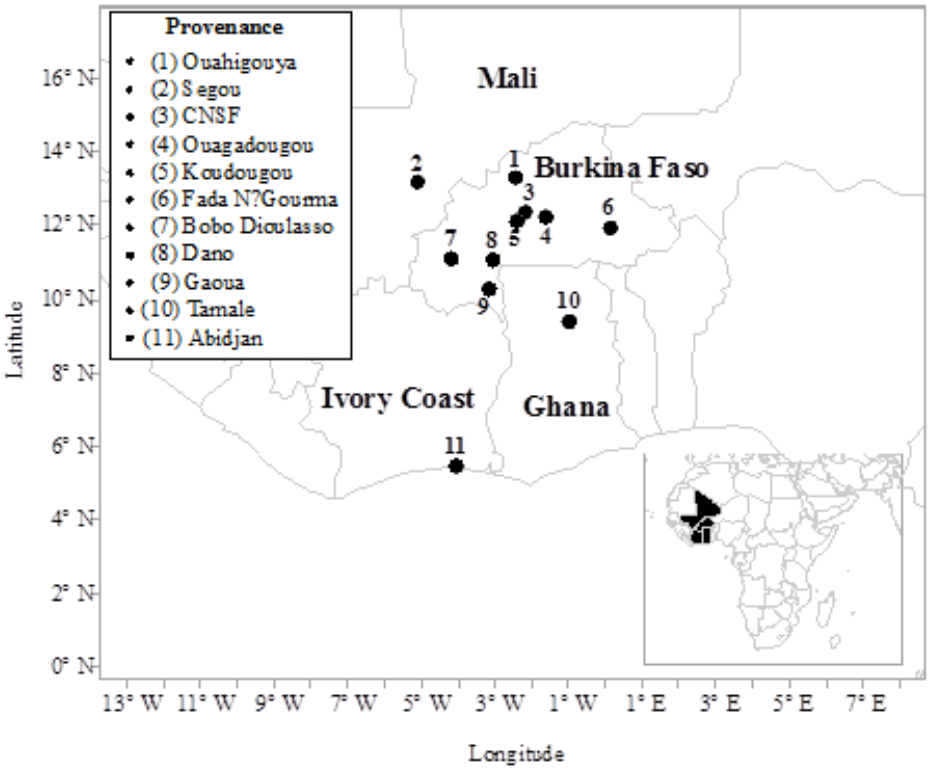

Figure 1. Provenance location.

Table 1. Location and other attributes of the *M. oleifera* provenances.

Prov. code	Ecological Zone	Provenance	Country	Latitude	Longitude	Altitude (m)	Average rainfall (mm/year)	Mother trees number
1	Sahelian	Ouahigouya	Burkina Faso	13°30'4"N	2°24'31"W	306	500	24
2	North Sudanian	Segou	Mali	13°22'05"N	5°16'24"	294	500	35
3	North Sudanian	CNSF	Burkina Faso	12°30'07 N	2°07'34"W	304	800	35
4	North Sudanian	Ouagadougou	Burkina Faso	12°21'58"N	1°31'05"W	315	800	15
5	North Sudanian	Koudougou	Burkina Faso	12°15'04"N	2°22'28"W	308	800	12
6	North Sudanian	Fada N'Gourma	Burkina Faso	12°03'41"N	0°21'30"E	300	900	26
7	Sudanian	Bobo Dioulasso	Burkina Faso	11°11'00"N	4°17'00"W	339	950	20
8	Sudanian	Dano	Burkina Faso	11°9'0"N	3°4'0"W	287	950	30
9	Sudanian	Gaoua	Burkina Faso	10°19'12"N	3°10'12"W	319	1000	17
10	Sudanian	Tamale	Ghana	9°24'27"N	00°51'12"W	169	1100	15
11	Sub-Equatorial	Abidjan	Ivory Coast	5°18'28"N	4°6'19"W	73	2000	18

and weight), each provenance was represented by 240 randomly selected seeds, assessed in four replications of 60 seeds each. Each seed weight was determined by weighing three random samples of 60 seeds each. By the end of 2 weeks; the experiment had been measured twice at effective ages of 5 and 12 days since seed sowing, providing data for the assessment of germination rates of the twelve provenances. Total samples of 20 seedlings per replication for each provenance were assessed. The seedlings were separated into shoot and root components that were oven-dried for 48 h at 70°C, and weighed to determine total dry weight and calculation of root-to-shoot ratios. Height was measured using calibrated height measuring pole. Differences among provenances seed traits, leaf morphology, and dry mass (DM) were established by analyses of variance (ANOVA) using the Linear Model procedure (Standard Least Squares option) of the JMP® Pro 12.0.1 statistical software package (SAS Institute Inc., Cary, NC). Normality and homoscedasticity were graphically verified on residual plots of the linear models (Quinn and Keough, 2002). Where the ANOVA indicated significant treatment effects, treatment means were separated by Tukey's Honestly Significant Difference (HSD) test ($p = 0.05$). Pearson's product- moment correlation analysis was done to evaluate the relationships between the studied traits (Seed weight, distance, thickness; Leaf length, width, pinna number; base, shoot, total, root/shoot dry mass). The provenance relationships

Figure 2. Moringa's plots.

(''similarity'') were examined on the basis of the 10 traits used in the correlation analysis by a hierarchical cluster analysis using the JMP® Pro Cluster Analysis from the Multivariate methods submenu, which applies the ''Ward distance'' (SAS Institute Inc., 2012).

RESULTS

Seed characteristics

Significant differences ($P < 0.05$) in seed traits (length, width and thickness) were observed among provenances (Table 2). Mean seed length ranged from 12 to 16 mm for Koudougou, and the longest provenance Fada N'Gourma, respectively (Table 2). Similarly, mean weight at the same time ranged from 1 to 2 g for provenances Fada N'Gourma -Koudougou and for provenances Gaoua- Ouahigouya respectively (Table 2). Seed thickness ranged from 10 to 11 mm for provenances Koudougou-Bobo Dioulasso and provenances Ouahigouya-Ouagadougou, Segou-Gaoua-Abidjan, respectively.

Leaf morphology and dry biomass production

Provenances were significantly different ($P < 0.05$) in leaf morphology (length, width and number of pinnae/seedling) (Table 2). Among 11 provenances, CNSF produced the longest leaf (45 mm) compared with 17 mm for Bobo Dioulasso. Ouahigouya, Segou, Ouagadougou, Koudougou, Koudougou, Fada N'Gourma, Dano, Gaoua, Tamalé and Abidjan were in the middle of the range. Mean leaf width ranged from 34 to 105 mm for Tamalé and CNSF, respectively (Table 2). Mean number of pinnae per seedling at the same time ranged from 6 to 12 for Segou and CNSF, respectively. At the end of two months, the mean shoot biomass accumulation per seedling differed significantly ($P < 0.05$) among provenances (Table 2). The lowest shoot dry weight of 2.2 g was collected in provenance Bobo Dioulasso, while the highest of 21.4 g was obtained in provenance Gaoua. Provenance differences in root dry biomass per seedling were considerable, with means ranging from 1.2 for 7.4 g for Tamalé and Gaoua, respectively (Table 2). Mean shoot ranged from 1.2 to 14.0 g for Bobo Dioulasso and Gaoua, respectively (Table 2). Similarly, total dry mass produced at the same time ranged from 2.2 to 21.4 g for Bobo Dioulasso and Gaoua, respectively. The ratio root: shoot dry mass ranged from 0.4 to 0.9 g for Dano and Tamalé, respectively.

Interdependence among characters

Significant positive Pearson's product-moment correlation coefficients (r) were observed among seed traits but no important correlation were found between seed traits and other plant characteristics (Table 3). Traits related to seed size (length, width, and weight) were mostly correlated with each other, Seed weight was positively correlated with seed length (r = 0.28), and seed thickness (r = 0.3). Seed length was positively related with seed thickness (r = 0.23). No significantly correlation of seed size with either leaf morphology or dry mass. An exception to the trend was seed thickness with leaf length, shoot dry biomass and total dry mass, which displayed a weak magnitude of the correlation coefficients (r < 0.20). Significant relationships were also detected between traits related to leaves morphology,

Table 2. Mean, range and standard-error of seed traits (length, thickness, weight), leaf morphology (length, width, pinna number) and dry mass (Root, shoot, total, Root/Shoot) of the 11 provenances of *M. oleifera*.

Provenance	Seed			Leaf			Dry mass			
	Length (mm)	Thickness (mm)	Weight (g)	Length (mm)	Width (mm)	Pinna number	Root (g)	Shoot (g)	Total (g)	Root/Shoot (g/g)
Ouahigouya	15^{ab} 12-17	11^{a} 10-12	2^{a} 1.5-2.0	29de 21-38	65de 46-104	9^{c} 7-12	2.2^{cde} 0.5-13.4	4.0^{def} 1.8-10.1	6.2^{def} 2.9-20.4	0.5^{b} 0.2-1.9-
Segou	14^{bc} 12-17	11^{a} 10-13	2^{ab} 1.3-2.0	22^{ef} 12-29	50^{ef} 29-74	6^{d} 5-8	1.8^{cde} 0.1-4.5	3.4^{ef} 0.6-7.3	5.2^{ef} 0.7-9.3	0.5^{ab} 0.2-1.6
CNSF	15^{ab} 12-17	10^{bc} 9-12	2^{bc} 1.4-1.9	45^{a} 28-62	105^{a} 65-178	12^{a} 8-15	5.8^{abc} 2.2-17.6	12.2^{ab} 4.9-21.5	18.0^{abc} 8.1-39.1	0.5^{b} 0.3-1.3
Ouagadougou	13^{cd} 12-15	11^{a} 10-13	2^{ab} 1.5-2.0	43^{a} 34-63	101^{a} 60-150	11^{abc} 6-14	6.6^{abc} 0.8-25.4	11.6^{ab} 5.1-27.9	18.2^{ab} 6.0-51.3	0.6^{ab} 0.2-1.6
Koudougou	12^{d} 11-15	10^{cd} 9-11	1^{d} 1.1-1.6	33^{cd} 26-42	74^{cd} 53-103	10^{c} 8-12	4.5^{abcde} 1.5-23.3	6.3^{cde} 3.0-16.9	10.7^{cde} 4.7-30.4	0.7^{ab} 0.4-3.3
Fada N'Gourma	16^{a} 13-18	11^{ab} 10-12	1^{d} 1.0-1.8	39^{abc} 30-55	86^{abc} 63-122	11^{abc} 7-14	5.3^{abc} 1.9	10.2^{abc} 3.8-25.1	15.5^{abc} 7.0-33.4	0.5^{ab} 0.3-1.4
Bobo Dioulasso	13^{cd} 11-16	10^{cd} 9-11	1^{cd} 1.0-2.0	17^{f} 8-26	35^{f} 16-59	7^{d} 4-9	18.1^{e} 0.1-4.2	1.2^{f} 0.2-3.0	2.2^{f} 0.3-5.6	0.8^{ab} 0.2-3.0
Dano	13^{cd} 11-15	11^{ab} 9-12	2^{bc} 1.4-1.9	41^{a} 19-49	92^{abc} 42-125	11^{abc} 5-13	3.1^{bcde} 0.4-6.3	7.9^{bce} 1.3-13.6	11.0^{bcde} 1.6-19.3	0.4^{b} 0.3-0.6
Gaoua	14^{c} 13-17	11 10-13	2^{a} 1.6-2.0	40^{ab} 27-53	98^{abc} 74-139	11^{abc} 8-13	7.4^{a} 2.7-21.2	14.0^{a} 4.6-32.8	21.4^{a} 7.3-43.8	0.6^{ab} 0.2-1.7
Tamale	15^{ab} 12-18-	11^{ab} 10-12	2^{ab} 1.1-1.9	18^{f} 12-27	34^{f} 20-56	7^{d} 5-11	1.2^{de} 0.4-2.7	1.4^{f} 0.5-2.7	2.6^{f} 0.9-4.7	0.9^{a} 0.4-2.4
Abidjan	15^{ab} 13-17	11^{a} 11-13	2^{ab} 1.1-2.0	34^{bcd} 26-49	80^{bcd} 58-119	10^{bc} 7-13	5.2^{abcde} 1.0-23.5	7.8^{bcde} 3.5-16.7	12.9^{bcd} 4.5-34.4	0.7^{ab} 0.3-2.2
Standard-error	0.3	0.1	0.04	1.0	4.0	0.4 -	0.9 -	1.0 -	1.6 -	0.1 -

Mean followed by the same letter are not significantly different at the 5% *p* level according to Tukey's multiple comparison test.

Table 3. Pearson correlations between seed traits, leaf morphology and dry mass.

Variable		Seed			Leaf morphology			Dry mass			
		Weight	Length	Thickness	Length	Width	Pinna nb.	Root	Shoot	Total	Root/Shoot
Seed	Weight	-	-	-	-	-	-	-	-	-	-
	Length	0.28***	-	-	-	-	-	-	-	-	-
	Thickness	0.3***	0.23**	-	-	-	-	-	-	-	-
Leaf	Length	0.03	0.04	0.16*	-	-	-	-	-	-	-
	Width	0.06	0.02	0.13	0.94***	-	-	-	-	-	-
	Pinna nb.	-0.04	0.06	0.06	0.82***	0.81***	-	-	-	-	-
Dry mass	Root	0.10	0.10	0.11	0.51***	0.49***	0.43***	-	-	-	-
	Shoot	0.09	0.08	0.15*	0.81***	0.82***	0.67***	0.64***	-	-	-
	Total	0.10	0.10	0.15*	0.75***	0.75***	0.63***	0.87***	0.93***	-	-
	Root/Shoot	0.01	<0.01	-0.08	-0.22**	-0.24**	-0.19**	0.49***	-0.17*	0.12	-

***: $p \leq 0.0001$; **: $0.0001 < p \leq 0.01$; *: $0.01 < p \leq 0.05$.

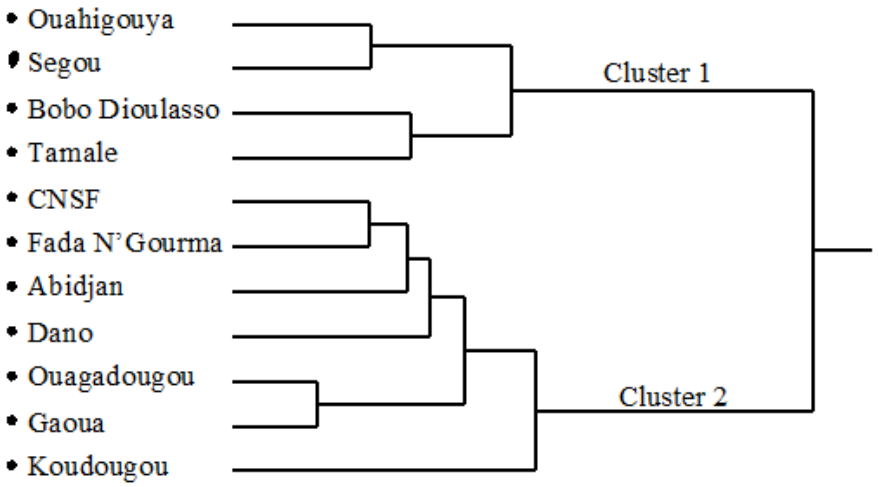

Figure 3. Hierarchical cluster dendrogram of the 11 *M. oleifera* provenances based on seed traits, leaf characteristics, and dry mass.

shoot and root dry biomasses. Leaf length was highly correlated with leaf width and number of pinnae (r > 0.80) (Table 3). Leaf morphology was positively correlated with the aforementioned while root dry mass and total dry mass (r > 0.40) were negatively correlated with root: shoot dry biomass (Table 3).

Cluster analysis

Cluster analysis based on seed sizes, leaf morphology and biomass production revealed two distinct clusters (Figures 3 and 4). Provenances Ouahigouya, Segou, Bobo-Dioulasso and Tamalé located in three different ecological zones formed cluster 1 while the seven other provenances (Dano, Ouagadougou, Fada N'Gourma, Gaoua, Koudougou, CNSF and Abidjan), dispersed also in three different ecological zones formed the second cluster group. Provenances from cluster 1 performed high seed traits, small leaves sizes and lower biomass accumulation than cluster 2. Tamale and Bobo Dioulasso in particular, invested more biomass in root than in shoot (Figure 4). Cluster 2 had large leaves size and excellent dry biomass production.

DISCUSSION

Seed

Seed size is the main determinant of maternal investment in individual seed farmer, and it as been demonstrated

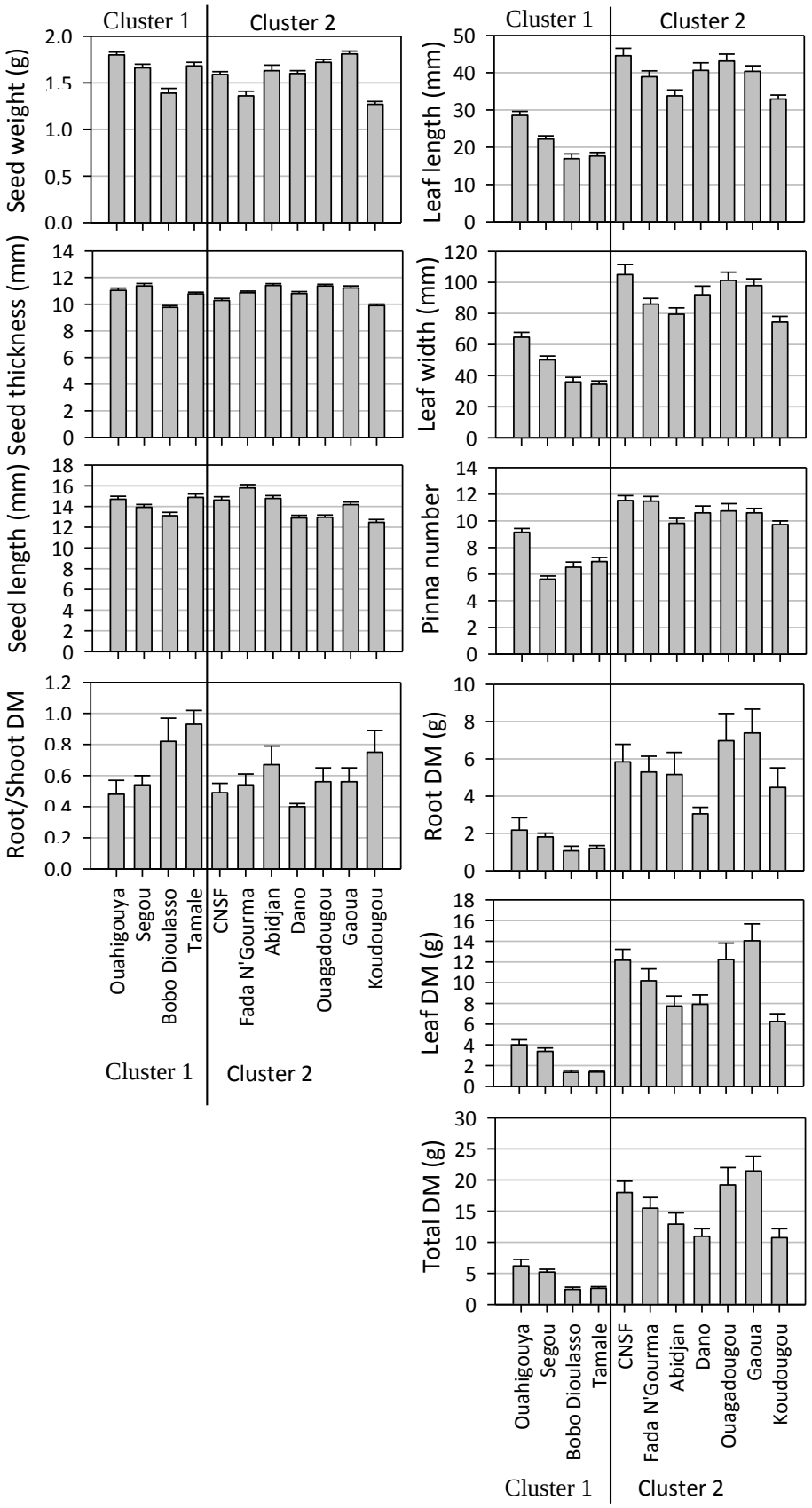

Figure 4. Mean ± standard error of seed traits, leaf characteristics, and dry mass (DM). Provenances are grouped according to the hierarchical cluster analysis.

that tree-to-tree variation in seed size within local populations is under relatively strong genetic. The relationship between seed size and the growth of resulting seedlings can last for about one year or up to 10 years (Farmer, 1997). Investigation of DNA markers have demonstrated that there is a large genetic diversity present in wild collections of Moringa oleifera (Shahzad et al., 2013).

The three seed traits differ significantly between provenance, specially the seed weight. Difference between the heaviest (Gaoua) and the lightest (Koudougo) was up to 43%. No environemental gradient, longitude, latitude, altitude, and annual rainfall explain difference in seed traits between provenance. Grouping provenance in ecological zones did not explain either diffence in seed traits. In the opposite, the regional provenances of *Faidherbia albida* showed a consistent variation in seed length, seed width and seed weight: the southern African provenances had the largest seeds and west African provenances the smallest (Dangasuk et al., 1997).

According to these authors, seed weight and size are adaptive traits, their smaller mean values observed in the west African provenances might suggest strong genetic selection for small seed size which is probably an adaptation to the greater desiccation stress in the Sahelian region. In their study of provenance variation in seed size of Senegal mahogany, Ky-Dembele et al. (2014) indicate that genetic differences exist among families within provenances and provenances in seed traits of *K. senegalensis* in Burkina Faso. In contrast, the high inter- and intra-provenance variation in Moringa seed weight in this study might suggest that this parameter is quite insensitive to environmental factors.

As Moringa seed weight shows significant differences between years (Ayerza, 2011), difference in seed weight could be attributed to the year's harvest. Seed weight has little effect on acacia seedling growth in different provenances of Senegalese (Wolde-Mieskel and Sinclair, 2000), Singh et al. (2006) have found a positive correlation between morphological characters of *Celtis Australis* seeds, family Ulmaceae, including seed weight and elevational gradient. Provenances with heavier seed may provide seedlings that will be able to withstand the adverse climatic conditions.

Growth performance

The study results agree with those of Gamedze et al. (2012) that shows significant differences in growth performances between Moringa provenances in Swaziland. The three provenances had significant differences in the number of leaflets produced.

Conclusion

It may be concluded from this study that significant differences were found among seed traits, leaf morphology, dry diomass and provenances. The research revealed that seed size were not correlated with either leaf morphology or dry mass. However, additional studies are needed to determine factors affecting moringa yields, including potential interactions of genotype×environment on seed per tree production, before making any recommendation about the economic potential of moringa as a new crop for this region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Phytochemical analysis of *Ziziphus mucronata* Willd. extract and screening for antifungal activity against peanut pathogens

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Chemical analysis of aqueous extract of *Ziziphus mucronata* Willd. was determined by liquid chromatography–mass spectrometry (LC-MS) analysis. Among the 11 compounds found, catechin, rutin (quercetin 3-O-rutinoside), delphinidin-3-glucoside, isoquercetin (hyperoside) and quercitrin (quercetine3,7-O-L-dirhanmopyranoside) were identified as the major phenolics components in this aqueous plant extract. To elute the target compounds, the fractionation of crude extract was carried out on solid phase extraction (SPE) columns. The different fractions (from FZ1 to FZ5) obtained after fractionation were evaluated *in vitro* against economically important foliar fungal pathogens of peanut, including *Cercospora arachidicola*, *Phaeoisariopsis personata* and *Puccinia arachidis.* The treatments with *Z. mucronata* fractions were compared with negative control (water) and standard solutions of cathechin and rutin (1 mg/mL). All the fractions recorded an inhibitory effect, firstly on conidial germination and germ tube elongation, secondly on disease evolution on peanut leaves previously inoculated by fungi; the level of efficiency of inhibition varied from 40.55 (FZ1 against *C. arachidicola*) to 57.14% (FZ2 and FZ3 against *P. arachidis*). Then, spores of *P. arachidis* seemed to be more sensitive to the treatment.

Key words: Fungal pathogens, peanut, plant extract, phenolics, *Ziziphus mucronata* Willd.

INTRODUCTION

Peanut (*Arachis hypogaea*) remains a high potential plant whose socio-economic importance is unquestionable throughout the West African sub-region. It is regarded as both food and cash crop, and the processing of products (butter, dough and cake) is an additional source of income for women.

In Burkina Faso, peanut is the second most important oily crops (DGESS, 2015). In 2014, a total production of 335.223 metric tonnes has been recorded throughout the country. However, its cultivation is facing climate changes and an intense parasitic pressure. Foliar diseases constitute a real obstacle to the growth of groundnut

(peanut). The most widespread are the early leaf spot (causal agent *Cercospora arachidicola*), late leaf spot (causal agent *Phaeoisariopsis personata*) and rust (causal agent *Puccinia arachidis*) (Subrahmanyam et al., 1995). Leaf spot are critical yield-limiting diseases of groundnut in West Africa accounting for yield reductions of 50 to 70%, where fungicides are not used (Shokes and Culbreat, 1997).

The need for applications (4 to 5 sprays) of recommended fungicides, such as chrorothalonil, mancozeb, and folicur, discouraged the extensive adoption of peanut by resource-poor farmers of the rain-fed production system (Krishna and Pande, 2005). Thus, the chemical methods are expensive and can affect the beneficial microbial population present in the ecosystem (Kagale et al., 2004). It is therefore imperative to develop and implement research programs in order to achieve better profitability in the sector to make it more attractive.

A major challenge facing crop production is to provide tools for controlling field diseases that would also be able to maintain higher quality crop production (Sarpeleh et al., 2009). Disease management by chemical treatment has shown its limits by the development of resistances in many important pathogens and environmental pollution. The use of plant extracts to control plant pathogens has been suggested as one of the sure alternatives to substitute synthetic chemical products. Natural plant products are an important source of new agrochemicals for the control of plant diseases (Nebie et al., 2002).

Pesticides of plant origin are non-phytotoxic, systemic and easily biodegradable. In the past few decades, many studies have focused on the antifungal activity of plant extracts on several phytopathogenic fungi. For example, the water extract of Neem leaf was found very effective against peanut diseases caused by the fungi *Puccinia arachidis* and *Mycosphaerella berkeleyi* (Ghewande, 1989). It was also successful in preventing fruit rottening in Cucurbitaceae caused by the fungus *Fusarrium equisitifolium* and *Fusarrium semitectum* (Krishna et al., 1986).

Zida et al. (2008) reported that, 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 with inhibition rates of 27 to 72% and 56 to 86%, respectively. Linde et al. (2010) showed the efficiency of *L. rehmannii* essential oil on *Rhizoctonia solani, Fusarium oxysporum* and *Penicillium digitatum* fungi on potato, maize and orange tree pathogens, respectively.

Shakil et al. (2012) conducted experiments in which aqueous extracts of the leaves of *Calotropis procera* were as effective as the fungicide Ridomil in the control of collar decay of groundnut. Concerning the Ziziphus genre, some works have reported the anti microbial activities of certain species. So, different extracts and fractions of the leaves, fruits and seeds of *Ziziphus spina-christi* L. grown in Egypt showed a moderate *in vitro* activity against the fungus *Trichphyton rubrum* (Shahat et al., 2001).

Some recent works have shown the inhibitory effect of *Ziziphus mucronata* Willd. water extracts on rust of groundnut (Koïta et al., 2012). However, no data exists in the nature of the biochemical compounds present in the extract and active against fungi. This calls for further research to identify compounds active in this extract. The objectives of the present study are to realize phytochemical analysis of a crude extract of *Ziziphus mucronata* and, after its fractionation to evaluate the antifungal activity of the crude extract and its different fractions against *Cercospora arachidicola*, *Phaeoisariopsis personata* and *Puccinia arachidis*.

MATERIALS AND METHODS

Plant materials

Z. mucronata, a fruit crop in the Rhamnaceae family native to Asia (Guinko and Assi. 1981), was collected in the Gampêla district (12°25'N, 12°22'E) in Burkina Faso (West Africa). A botanical certification of the species was obtained from the Plant Ecology and Biology Laboratory of University Ouaga I Pr Jospeh KI-ZERBO. The fruits were selected for uniformity of color, absence of mechanical damage and disease symptoms. Using a mortar, the fruits were lightly crushed in order to separate the pulp from the seed. The pulp obtained was ground to a fine powder using an electric grinder (cyclotech sample mill, tecator, Hoganas, Sweden). During the biological test, the leaves from the peanut TS32-1 susceptible variety to late leaf spot and rust, about 30 days after seedling (DAS), were used to carry out artificial contaminations. Fungal spores of *C. arachidicola*, *P. arachidis* and *P. personata* were gathered form the Gampêla district on leaves naturally infected in fields which have not received antifungal treatments.

Extraction procedure

The phytochemistry was realized at the University of Montpellier 2 (France). 100 g of powdered sample was dissolved in 1000 ml of purified water obtained from Milli-Q plus water purification system (Millipore, Bedford, MA, USA) in a 2000 ml Erlenmeyer flask. The mixture was then left at room temperature (15 to 17°C) for at least 3 h. Supernatants obtained by centrifugation at 15,000 rpm for 15 min (centrifuge Sorvall RC 26 Plus) were evaporated completely, under reduced pressure using a rotavapor (Rotavapor R3, BUCHI). The dried material obtained was dissolved in 100 ml of purified water in order to get aqueous extract (1 g/ml), then sterilized using a 0.22 µm filter (Sartorious AG, Göttingen, Germany) before the liquid chromatography-mass spectrometry (LC-MS analysis and fractionations.

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LC-MS analysis

LC-MS analysis was performed using a Waters/Micromass ZQ equipped with an Electro-Spray Ionization source (ESI) that operated in both positive and negative ionization modes. The compounds were detected between 200 and 600 nm on 4 nm step. The mobile phase of HPLC consisted of 0.1% (v/v) formic acid in water (eluent A) and of acetonitrile: 0.1% formic acid (1:1, v/v) (eluent B). The gradient program was as follows: 0 to 8 min, 99 to 90% A; 8 to 17 min, 90 to 83% A; 17 to 26 min, 90 to 83% A; 17 to 26 min, 83 to 73% A; 26 to 55 min, 73 to 0% A and 55 to 60 min, 0 to 95% A. The column (C18 type (XTerra MS (Waters), 2.1 mm × 100 mm, 3.5 µm particle size) was operated at a temperature of 26°C, the flow rate was 1 ml/min, and the injection volume was 5 µl. All eluents used including the acetronitrile, formic acid (Carlo-Erba trademark) or methanol (VWR HiPer Solv Chroma norm trademark) was of HPLC quality. Using LC-MS, the compounds were identified on the basis of their retention time, absorbance spectrum and mass fragmentation compared to data from literature and, when possible, data from chromatography to standard.

Fractionation of *Z. mucronata* crude extract

The fractionation of crude extract was carried out on solid phase extraction (SPE) columns (Bond Elut LR C C_{18} VARIAN, 100 × 4.6 mm) to elute the target compounds. The SPE column was initially washed with 5 ml of methanol and 5 ml of purified water successively, to remove any impurities. Dynamic adsorption and desorption experiments were first carried out to determine the loading volume and the elution conditions. Then, the crude extract (25 ml) was loaded on the column and an elution gradient with increased polarity was applied to the column by adding solvents composed of a water/acetonitrile mixture (100/0; 95/5; 90/10; 85/15; 10/90). Each fraction was collected separately and the process was repeated five times. All the similar fractions were gathered and called FZ1 (100% water), FZ2 (95/5 water/acetonitrile), FZ3 (90/10 water/acetonitrile), FZ4 (85/15 water/acetonitrile) and FZ5 (10/90 water/acetonitrile). Each fraction was concentrated to dryness in a rotavapor (Rotavapor R3, BUCHI) under reduced pressure and controlled temperature (30°C), and then lyophilized. One mg of dried extract was dissolved in 1 ml purified water, then sterilized using a 0.22 µm filter (Sartorious AG, Göttingen, Germany) before assessing for antifungal activity and LC-MS analysis. FZ6 and FZ7 fractions corresponding to the two standard solutions, respectively cathechin and rutin (Karlsruhe, Germany) with a purity ≥ 98.0% at concentration of 1 mg/ml in water were used as positive controls. The use of both compounds as positive controls is explained by numerous studies have confirmed their antifungal efficiency (Gomez-Vasquez et al., 2004; Boligon et al., 2012; Panda et al., 2016).

Spore suspension preparation and antifungal activity

Fungi were isolated from naturally contaminated peanut leaves in Gampêla district. After collection, the leaves were washed with distilled water to be rid of dead spores and incubated for 48 h at 22°C in order to allow sporulation (Subrahmanyam et al., 1982). The spores of each fungal strain were collected by scraping the surface of the leaves suspended in sterile water. The spore suspension was adjusted to a concentration of approximately 100 spore/ml using Malassez counting chamber. Fractions of *Z. mucronata* crude extract (FZ1, FZ2, FZ3, FZ4 and FZ5) were prepared at a concentration of 1 mg of dry extract in 1 ml distilled water and used in the tests. A control (FZ8) consisted in a treatment with sterile distilled water (2 ml). For the spore germination test, 2 ml of each solution were placed in a test tube into which 2 ml of the adjusted spores' suspension was added. This was then incubated in complete darkness at 22°C for 8 h for rust spores and 25°C for 24 h for leaf spot spores. The fungal appressorium was then measured for 100 spores in each treatment with a microscope (Zeiss Primo Star) at ×40 magnification. A spore was considered to have germinated when germ tube length was greater than the width of the spore. The efficiency rate (E) of each extract fraction has been calculated using the formula proposed by Greche and Hajjaji (2000):

$$\mathrm{E}(\%) = 100 \times [\mathrm{MLC} - \mathrm{MLE}] \div \mathrm{MLC}$$

Where MLC is the mean length of the germ tube of the spore with the negative control and MLE is the mean length of the germinal tube of the spore with the tested plant extract.

A foliar stain inhibition test was assessed on the contaminated leaves from a susceptible variety of peanut (TS32-1). The harvested leaves undamaged were placed by ten in glass Petri dishes (90 mm × 20 mm). The infection was carried out by spraying the spore suspension on the abaxial leaf surface. The Petri dishes were placed on a shelf in a culture room at a temperature of 21 ± 2°C with a 12 h photoperiod. From 5 days after inoculation, sprays of *Z. mucronata* fractions or standards (or water for control) were carried out. This treatment was repeated every five days, in all, four treatments were applied to these contaminated leaves. The inhibition of foliar stains of leaf spot was evaluated by using the 9 classes' severity scale of ICRISAT (Subrahmanyam et al., 1982). This operation was repeated five times and observations were made during the 30 days. The treated leaves were compared to the negative control (water) and standard solutions of cathechin and rutin (1 mg/ml).

Statistical analysis

Statistical differences between the treatments and the control were evaluated by ANOVA and students-Newman-Keuls post-hoc tests using XLSTAT, 2010 version 12.5 08. *P* values of < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis of crude extract of *Z. mucronata*

The HPLC profile obtained with crude aqueous extract of *Z. mucronata* fruit pulp and peaks corresponding to compounds 1 to 11 are shown in Figure 1. Most of the compounds were eluted within the first 40 min, and showed a maximum absorption peak at 330 nm.

In this aqueous extract, five compounds were clearly identified (a large polymer (1) and peaks 2, 3, 4, 6). Others peaks are indicated (peak 5, 7, 8, 9, 10 and 11) corresponding to unidentified compounds in the present study. However, during the first 15 min of separation, the presence of a large polymer prevented individualized peaks (1+). This compound exhibited an *m/z* of 289 in negative mode and two maximum UV absorption at 207 and 277nm. With fragments ions at *m/z* 203, 125 and 108, this compound shared characteristics of catechin and / or epicatechin, flavonids monomers of the condensed tannins. The presence of these flavonoids

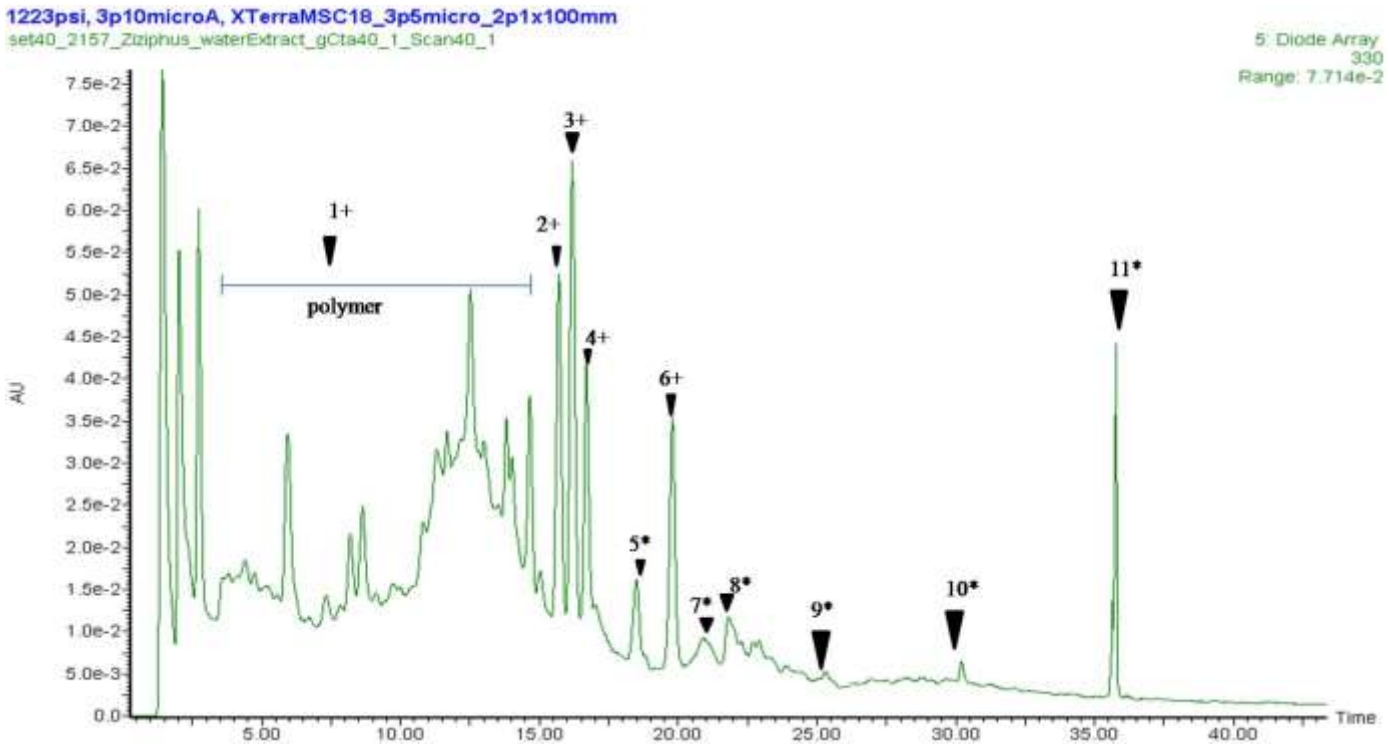

Figure 1. Chromatographic pattern of *Z. mucronata* aqueous extract. Wavelength: 330 nm, injection volume: 5 µl. Caption: The numbers followed by + correspond to identified compound peak and those followed by * are unidentified ones. 1+: catechin and / or epicatechin, 2+: rutin (quercetin 3-O-rutinoside), 3+: the delphinidin-3-glucoside, 4+: isoquercetin (hyperoside), 6+: quercitrin (quercetine3,7-O-L-dirhanmopyranoside), unidentified compounds: 5*, 7*, 8*, 9*, 10* and 11*.

has been reported previously in other species from Rhamnaceae family (Boligon et al., 2012) and particularly in the genus *Ziziphus*, for instance *Z. lotus* L. (Diallo et al., 2004; Borgi et al., 2007; Soumia, 2009). In their study, Berthod et al. (1999) stress the difficulty of precisely identifying those compounds whose polymer chains of different lengths are difficult to separate.

Compound 2 (peak 2+) was detected by UV absorption at 270 and 350 nm at retention time of 15.84 min. Its LC-MS spectrum showed fragments at *m/z* 609 in negative mode and other fragments were detected at *m/z* 301 and 300. This compound was identified as a glycosylated flavonoid namely rutin (quercetin 3-O-rutinoside) based on literature and the LC-MS profile. The presence of this compound in leaves and fruits of *Z. jujuba* and *Z. spina-christa* has been previously reported (Pawlowska et al., 2009; Guo et al., 2011).

Compound 3 corresponding to peak 3+, at a retention time of 16.38 min, included a parent ion *m/z* 464 and fragment ions *m/z* 300, 301, 255 and 299 at high fragmentation in negative mode. The UV absorbance band showed a maximum at 205 and 275 nm. This compound is the delphinidin-3-glucoside, an anthocyanidin, a primary plant pigment which has antioxidant and antimicrobial properties (Dixon et al., 2005; Panjehkeh et al., 2009). The presence of this compound has been reported in the leaves and fruits of the species *Z. jujuba* and *Z. spina-christa* (Guo et al., 2011; Pawlowska et al., 2009).

Fragmentation in negative ion mode of compound 4 (peak 4+) presented parent ion m/z 463 and fragments ion m/z 301 and 300. It showed UV absorption band at 205 and 270 nm at retention time of 16.86 min. All these characteristics corresponded to isoquercetin, also known as hyperoside or quercetin 3-O-glucoside. This compound is a flavonoid whose presence was reported in the leaves and fruits of *Z. spina-christi* (Shahat et al., 2001) and *Z. jujuba* (Pawlowska et al., 2009).

The fifth and last compound identified corresponds to peak 6+. It was recorded at the retention time of 19.88 min. Its absorbance spectrum indicates two absorption peaks at 265 and 345 nm. The mass spectrum of this compound produced by electrospray negative ES- has a parent ion of m/z = 447. The higher energy ionization gives, in addition to the parent ion, fragments ions of m/z = 302, 301 and 300. All these characteristics correspond to quercetin 3,7-O-L-dirhanmopyranoside, one of the synonyms of which is quercitrin. This compound is a flavonoid whose presence was reported by Pawlowska et al. (2009) in the leaves and fruits of *Z. jujuba* and *Z. spina-christi*.

The major compounds isolated and identified in our

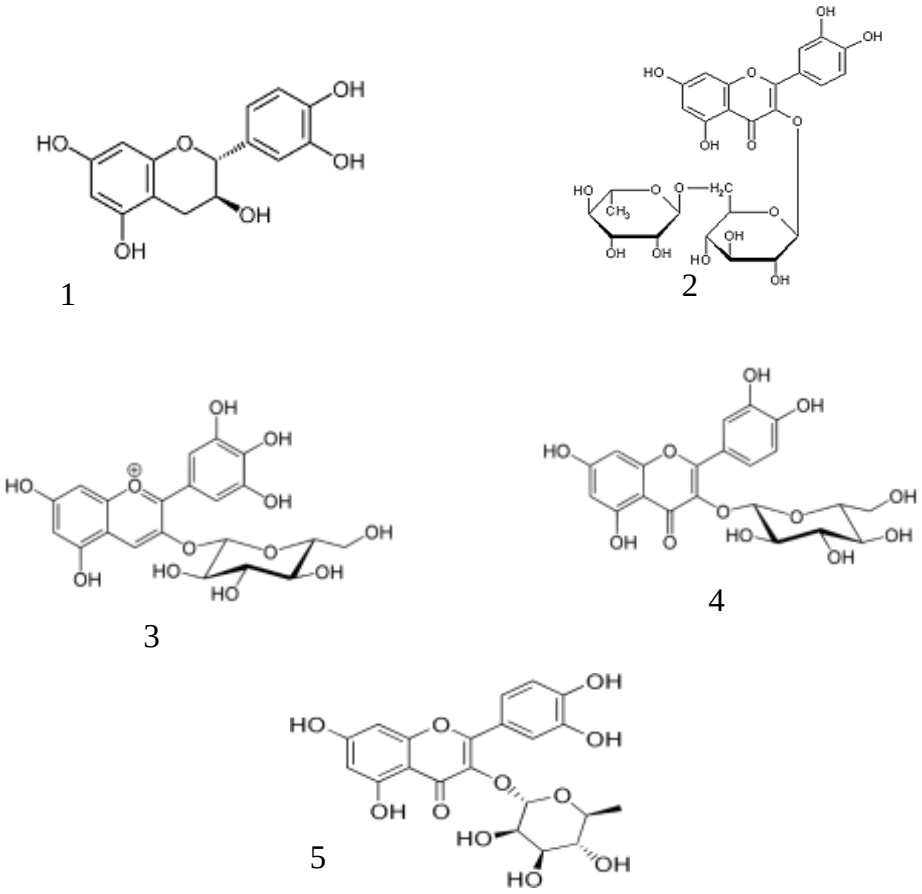

Figure 2. Chemical structures of the major compounds isolated from *Z. mucronata* pulp. Caption. 1: catechin and / or epicatechin, 2: rutin (quercetin 3-O-rutinoside), 3: delphinidin-3- glucoside, 4: isoquercetin (hyperoside /quercetin 3-O-glucoside), 5: quercetin 3,7-O-L-dirhanmopyranoside (quercitrin).

sample are all members of the polyphenol family (flavonoids and tannins). The phenolic compounds found in the sample analyzed are, for 3 of them, quercetin derivatives (Figure 2). Many of this compounds where equally obtained by Panda et al. (2016) and Nemudzivhadi and Masoko (2015) on *Z. mucronata* and other plants species such as *Ricinus communis* L. In addition to this family of secondary metabolites, phytochemical studies by Soumia (2009) on *Z. lotus* reported the presence of unidentified triterpenes, anthraquinones, alkaloids and saponosides. It should be noted that this author used solvents much more apolar such as methanol/water (60/40). This could explain the results he got.

LC-MS analysis of *Z. mucronata* crude extracts fractions

The crude extract obtained by aqueous extraction of *Z. mucronata* pulp was separated into five fractions (FZ1, FZ2, FZ3, FZ4 and FZ5) differing by their polarity. Around 74% of the dry mass of the crude extract was recovered in the five fractions and 80% of this total dry mass was found in FZ1, the first fraction. Then, the yield decreased with the polarity in each fraction, becoming 0.14 and 0.10% in FZ4 and FZ5 fractions, respectively (Table 1). Because of low yields from FZ4 and FZ5, the experiment was repeated 5 times to collect enough dry mass to perform the antifungal tests. The proposed gradient allowed concentrating the delphinidin-3-O-glucopyranoside in the FZ2 fraction and the other two monoglucosylated phenolics, isoquercetin and quercitrin, in the last three fractions. Only catechin polymers and rutin were noticed in all the fractions. Thus, delphinidin-3-O-glycopyranoside is observed only in fraction FZ2 in which the absence of isoquercetin and quercitrin is noted. It also appears that FZ1, the aqueous fraction which represents 80% of the total fraction weight, is essentially composed of catechin derivatives and rutin. The fractions FZ3, FZ4 and FZ5 contain the same compounds (catechin derivatives, rutin, isoquercetin and quercitrin),

Table 1. Phytochemical content of different *Z.mucronata* pulp extracts obtained before (crude extract) and after fractionation (FZ1, FZ2, FZ3, FZ4 and FZ5) on inverse solid phase.

Fraction	Dry mass (mg)	Percentage of total mass (%)	Identified compounds				
			Catechin derivatives (polymers)	Rutin	Delphinidin-3-O-Glucopyranoside	Isoquercetin	Quercitrin
Crude extract	500.0	100	+++	+++	++	++	++
FZ1	299.0	59.80	++	++	nd	nd	nd
FZ2	63.0	12.68	++	++	++	nd	nd
FZ3	7.0	1.40	++	++	nd	++	++
FZ4	0.7	0.14	++	++	nd	++	++
FZ5	0.5	0.10	++	++	nd	++	++

+++: Highly present; ++: present, +- : trace ; nd: not detected. FZ1: 100% water, FZ2: 95/5 water/Acetonitrile, FZ3: 90/10 water/Acetonitrile, FZ4: 85/15 water/Acetonitrile; FZ5: 10/90 water/Acetonitrile. Data present the values obtained after one fractionation.

Table 2. Efficiency rate of *Z. mucronata* pulp fractions (FZ1, FZ2, FZ3, FZ4 and FZ5), standards (FZ6 and FZ7) and water control (FZ8) on inhibition of *C. arachidicola, P. personata* and *P. arachidis* germination.

Treatment		Inhibition of spore germination (%)		
		Cercospora arachidicola	*Phaeoisariopsis personata*	*Puccinia arachidis*
Fractions of *Z. mucronata* crude extract	FZ1	40.55^{c}	44.44^{b}	52.38^{ab}
	FZ2	49.05^{b}	50.00^{ab}	57.14^{ab}
	FZ3	52.00^{ab}	50.00^{ab}	57.14^{ab}
	FZ4	49.35^{b}	44.44^{b}	47.62^{b}
	FZ5	50.08^{ab}	50.00^{ab}	47.62^{b}
Standards control	FZ6	56.24^{b}	55.56^{ab}	61.91^{ab}
	FZ7	68.22^{a}	61.11^{a}	66.67^{a}
	FZ8	0.00^{d}	0.00^{c}	0.00c
Probability		<0.0001**	<0.0001**	<0.0001**

FZ1: 100% water, FZ2: 95/5 water/Acetonitrile, FZ3: 90/10 water/Acetonitrile, FZ4: 85/15 water/Acetonitrile; FZ5: 10/90 water/Acetonitrile; positive controls: FZ6 (catechin) FZ7 (rutin); FZ8: negative control. Values are expressed in percentage and correspond to the mean of 5 measurements. **: highly significant different. Means followed by the same letter were not significantly different between treatment at $p \leq 0.05$ (Newman-Keul's test).

certainly present at varying concentrations.

Antifungal activity of fractions from *Z. mucronata* extract

In vitro antifungal effect of fractions of *Z mucronata* against *C. arachidicola*, *P. personata* and *P. arachidis* was firstly evaluated by the percentage of efficiency of each extract fraction on inhibition of spore germination (Table 2). All the fractions recorded an inhibitory effect on the germ tube elongation of the spores for all the fungi. The level of efficiency of inhibition varied from 40.55 to 57.14% (Table 2), the lowest inhibitory effect being observed with the first fraction against *C. arachidicola* and the highest with second and third fractions (FZ2 and FZ3). Inhibition by solutions of pure catechin (FZ6) or rutin (FZ7) was higher, rutin effect being superior to that of catechin (from 7 to 17.5% more active, according to fungal strain). For the three fungal species, there was a significant difference in the antifungal activity between the fractions and the negative control (FZ8). Except for the fraction FZ4 and FZ5, spores of *P. arachidis* seemed to be more sensitive to treatment. Indeed, with the FZ1 treatment, efficiency rate was 26.6 and 18% higher for *P. arachidis* than for *C. arachidicola* and *P. personata*, respectively. For all the strains, one of the highest inhibition was observed with the FZ3 extract.

Inhibition of fungal growth was also estimated looking to the evolution of fungal diseases on peanut leaves previously inoculated by *C.arachidicola*, *P. personata* and *P. arachidis* in presence or not of fractions of *Z. mucronata*. For the three fungal species (Figure 3), control leaves, which have received a water treatment

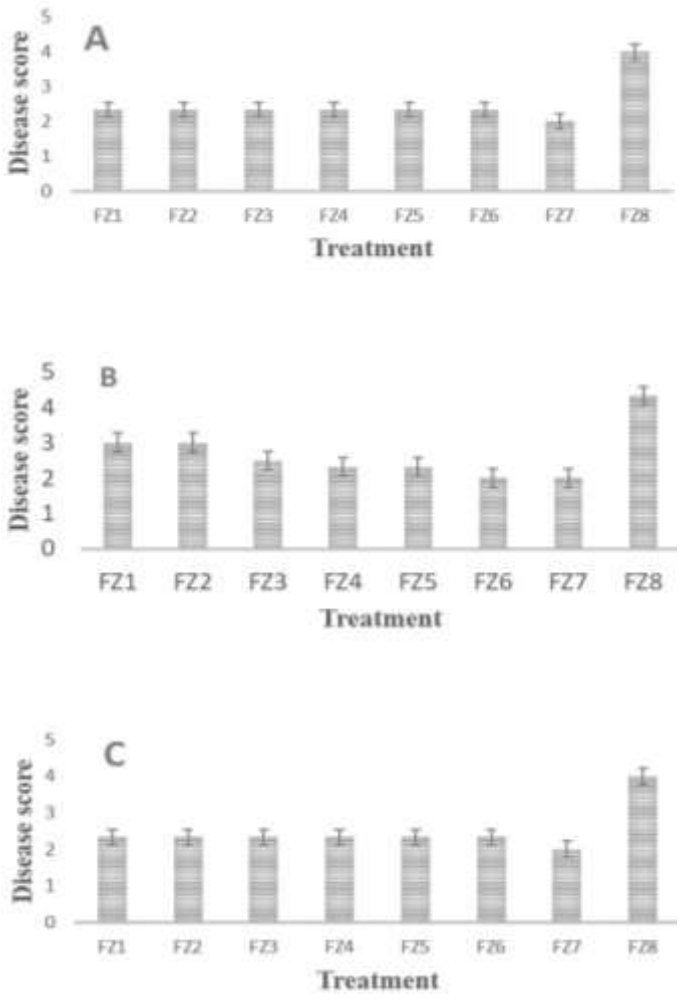

Figure 3. Extension of foliar diseases on peanut leaves artificially contaminated in presence of *Z. mucronata* extracts (FZ1, FZ2, FZ3, FZ4 and FL5), positive control (FZ6 and FZ7) and negative control (FZ8). A: *C. arachidicola*, B: *P. personata* and C: *Puccinia arachidis*. Caption: Error bars represent ± standard deviation of mean (n = 3). FZ1: 100% water, FZ2: 95/5 water/Acetonitrile, FZ3: 90/10 water/Acetonitrile, FZ4: 85/15 water/Acetonitrile; FZ5: 10/90 water/Acetonitrile; positive controls: FZ6 (catechin) FZ7 (rutin); FZ8: negative control. Extension is expressed using the 9 classes' severity scale of ICRISAT.

(FZ8), showed the highest symptom, with a score ranging from 4 to 4.33. On the spores of *C. arachidicola* (Figure 3A), the fractions of extracts (FZ1-5) and the reference solutions showed the same efficiency with a score of 2.5. Only the fraction FZ1 recorded a score of 3.5. On *P. personata* (Figure 3B), FZ6 and FZ7 noted the strongest inhibition with an evolution score of 2.0. Treatments with extract fractions did not show the same sensitivity on this fungus. Thus, the disease evolution scores varied from 2.5 to 3 for fractions FZ1, FZ2, FZ3 and from 2 to 2.33 for fractions FZ4 and FZ5. In the case of rust pustules, no differences in disease severity were observed between crude extract fractions (Figure 3C). The FZ7 fraction had the lowest disease outcome, that is, 2.0.

The results of the screening indicated the presence of compounds which are natural bioactive substances such as tannins and flavonoids. Different biological activities such as antinociceptive and antipyretic (Adzu et al. 2001; Nisar et al. 2007), antioxidant, antilisterial (Al-Reza et al., 2009), larvicidal (De Omena et al., 2007) and antimycobacterial (Suksamrarn et al., 2006) effects have been reported for various constituents of *Ziziphus* species.

Available literature indicates that each compound when taken separately has antimicrobial activity (Boligon et al., 2012; Panda et al., 2016). This may explain the antifungal activities of crude extract fractions, as indicated by the inhibition of the fungal appressorium formation and spot leaves necrosis. The antimicrobial activity of anthocyanidins has been reported (Scalbert, 1991; Snyder et al., 1991). Dixon et al. (2005) suggested that the major function of the anthocyanidins and their derivatives present in the fruits, bark, leaves and seeds of many plants, is to protect them against microbial pathogens, insect pests and large herbivores. Susceptibility to diseases and pests in some plants has been associated with the lack or low concentration of proanthocyanidins oligomers of catechin and epicatechin. For example, susceptibility of coffee varieties to the fungal pathogen *Hemileia vastatrix* was reported to be associated with low proanthocyanidin levels (Gonzalez de Colmenares et al., 1998).

In the present study, all the fractions obtained from aqueous extract of the fruit pulp were enriched in catechin derivatives and rutin and had a relative activity against the different fungi. However, a variation in the level of activity was observable between fractions and may be attributable to the presence of other phenolics, such as quercitrin, isoquercetin or delphinidin-3-O-glycopyranoside. But rutin and catechin derivatives concentrations also varied in the different fractions and this fact could certainly play a part in the variation of the rate of inhibition. Studies have shown a positive correlation between the amount of compounds such as flavonoids and anthocyanidins in plants and their resistance to *Phytophthora* species (Panjehkeh et al., 2009).

Although this study did not determine which of the compounds is involved in inhibiting the growth of the various fungi, it has shown that the phenolic compounds of the pulp of *Z. mucronata* exhibit an antifungal action and that pulp extracts can be used for biological control of peanut fungi. However, further investigations are needed to study the role of each compound in the antifungal activity and understand the detailed mechanism.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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