In vitro Antifungal Activity of Various Local Plant Extracts in the Control of *Phoma sorghina* (Sacc.) Boerema *et al.* and *Colletotrichum graminicola* (Ces.) Wilson, as Sorghum Seed Mold Pathogen in Burkina Faso

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Summary

The use of phytosanitary products is becoming increasingly restricted, due to their high cost, as well as the unavailability of certain products on the local market and their damaging effects on the environment and health. These different reasons motivated the search for alternative solutions to the use of synthetic pesticides. On this basis, we took on the task of evaluating the anti-fungal effectiveness of aqueous plant extracts from Balanites aegyptiaca*,* Cymbopogon citratus*,* Cassia occidentalis and Portulaca oleracea*. These extracts, at 30% concentration, were obtained after different maceration periods (6, 12, 24 and 48 hours) and tested* in vitro *against* Colletotrichum graminicola *and* Phoma sorghina*. The effect of the extracts on the mycelium growth of various fungi, when evaluated 10 days after incubation (DAI), shows that extracts of* C. citratus*,* B. aegyptiaca*,* P. oleracea *and* C. occidentalis *inhibit the growth of* C. graminicola *by 100%, 65%, 43% and 38%, respectively. The extract of* C. citratus *prevents the development of* C. graminicola*, regardless of the maceration period. This extract produces a greater inhibitory effect than the fungicide Calthio DS (20% Lindane and 25% Thirame). Compared to* P. sorghina*, inhibition percentages of 100, 72 and 16 were recorded for extracts of* C. citratus*,* P. oleracea *and* C. occidentalis*. However, extracts of* C. citratus *(macerated for 24 and 48 hours) and extract of* P. oleracea *(macerated for 48 hours) were more effective than the other extracts.*

Résumé

Efficacité *in vitro* de quelques extraits de plantes locales dans la lutte contre *Phoma sorghina* (Sacc.) Boerema *et al.* et *Colletotrichum graminicola* (Ces.) Wilson, agents de moisissure des semences de sorgho au Burkina Faso

Les produits phytosanitaires connaissent de plus en plus des limites d'emploi du fait de leur coût élevé, de la non disponibilité de certains produits sur le marché local et aussi de leurs conséquences néfastes sur l'environnement et la santé. Ces diverses raisons ont motivé la recherche de solutions alternatives à l'usage des pesticides synthétiques. C'est ainsi que nous avons entrepris d'évaluer l'efficacité antifongique des extraits aqueux de plantes de Balanites aegyptiaca*, de* Cymbopogon citratus*, de* Cassia occidentalis *et de* Portulaca oleracea*. Ces extraits concentrés à 30% ont été obtenus après différentes durées de macération (6, 12, 24 et 48 heures) et testés* in vitro *contre* Colletotrichum graminicola *et* Phoma sorghina*. L'effet des extraits sur la croissance mycélienne des différents champignons, évalué 10 jours après incubation (JAI), montre que les extraits de* C. citratus*,* B. aegyptiaca*,* P. oleracea *et* C. occidentalis *inhibent la croissance de* C. graminicola *respectivement de 100%, 65%, 43% et 38%. L'extrait de* C. citratus *empêche le développement de* C. graminicola *quelle que soit la durée de macération appliquée. Cet extrait induit un effet inhibiteur plus important que le fongicide calthio DS (20% de Lindane et 25% de Thirame). Contre* P. sorghina*, les pourcentages d'inhibition de 100, 72 et 16 sont notés pour les extraits de* C. citratus*,* P. oleracea *et* C. occidentalis*. Toutefois, les extraits de* C. citratus *macérés pendant 24 et 48 heures et celui de* P. oleracea *macéré pendant 48 heures sont plus efficaces que les autres extraits.*

Introduction

Fungi are responsible for agricultural product losses, both while crops are growing and when they are later stored. In fact, during storage, fungi can make food crops unfit for consumption, by changing the nutritional value of the seeds or producing mycotoxins that are harmful for human and animal health. At global

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level, over 25% of cereals are contaminated by known mycotoxins and more than 300 of the metabolites produced by fungi are toxic for human beings and animals (9). *Phoma sorghina* is a fungus frequently found on sorghum seeds. Studies were conducted in 2006, with the aim of evaluating fungal populations, on 50 sorghum samples from different regions of Burkina Faso and again in 2008 on 67 sorghum samples collected from different regions of the same country. These studies revealed that this fungus was present in all the samples collected. Somda *et al*., (10) show that, out of 37 samples of the different species that were cultivated and evaluated, 33 were infected with *Phoma sorghina*. Studies by Boiron (2) have shown that *Phoma sorghina* secretes a toxin called tenuazonic acid, which is dangerous to human health. *Phoma sorghina* also contributes to the pre-emergent and post-emergent mortality of cultivated plants (8).
Colletotrichum gramincola is known to be one of the most damaging fungal agents affecting sorghum in Burkina Faso. It is responsible for stunted growth, seed rot, leaf necrosis, red rot on the stems and seed discoloration. Losses caused by *C. graminicola* can range from 30-70%, depending on which organ is infected (11).
The use of synthetic products makes it possible to effectively control fungi transmitted by seeds. However, the use of these synthetic pesticides is limited as they are not available on the local market. In addition, if they were available, their cost would often be considered excessive for most growers. At the same time, the use of pesticides has damaging effects on the environment and, because of their toxicity, they cannot be used for protecting stored seeds (4, 5).
For these reasons, it is necessary to search for alternatives to the use of synthetic products. Laboratory studies have shown that aqueous plant extracts have anti-bacterial, insecticide and anti-fungal properties (5, 7). Natural pesticides represent a sound alternative to the use of synthetic fungicides, as they generally have less damaging effects on the environment and human health (12). This study aims to test the *in vitro* effectiveness of several local plants against *Colletotrichum graminicola* (Ces.) Wilson and *Phoma sorghina* (Sacc.) Boerema *et al*.

Materials

Plant species tested

Cassia occidentalis L., *Portulaca oleracea* L., *Balanites aegyptiaca* (L.) Del and *Cymbopogon citratus* (D.C.) Stapf were tested. Most of these species have medicinal properties.

The fungi tested

The fungus species tested were *C. graminicola* and *P. sorghina*. These fungi were isolated from the following samples: 751So06 (from Diebougou) and So32-06 (from the country's central region) in potato dextrose agar medium (PDA).

Methods

Plant organ powder macerations

Preparation of macerations

An aqueous extract concentrated at 30% was prepared for each plant species. The extract was obtained by leaving 30 g of the plant, in its ground state, to macerate for 6, 12, 24 and 48 hours, respectively, in 100 ml sterile water at 25 °C.

Preparation of PDA medium base aqueous extract and fungicide

The plant extract-based medium was obtained, by adding 39 g PDA to 1 litre of the 30% extract. The mixture was sterilised in the autoclave for 30 minutes at 120 °C. The medium base containing Calthio DS (20% Lindane and 25% Thirame) was obtained by adding 2.5 g/litre of this product, after cooling, to the agar medium.

Inoculation and incubation of media

The fungi to be tested were cultivated in a PDA medium. Using a 5-day old colony, four mycelium explants of identical size (5 mm) were placed in the centre of Petri dishes and incubated in the heat chamber at 28 °C and keep in the dark for 10 days.

Evaluation, data analysis and results exposition

Evaluating radial growth involved tracing two perpendicular lines on the Petri dish lid, which pass through the centre of the explant. The diameters of the mycelium colonies (in cm) were measured at 10 DAI (days after incubation). An average diameter was calculated for each of the three Petri dishes.
A variance analysis was then conducted using SAS INC software and the average diameters for each fungus were compared, using the Newman-Keuls multiple comparison test based on a 5% threshold.

Results

Effectiveness of aqueous extracts on the mycelium growth of *Colletotrichum graminicola*

All the plant species tested saw significantly lower radial growth of *C. graminicola* compared to the water control (Table 1). Comparison with the fungicide control indicated that only the citronella extract produces a total inhibitory effect, regardless of the maceration period (Table 1). It fully controls the development of *C. graminicola* for all the maceration periods applied and produces a significantly different inhibitory effect when compared tested with the fungicide control (Table 1). Extracts of *B. aegyptiaca*, *Cassia occidentalis* and *Portulaca oleracea* were the least effective in terms of inhibiting radial growth of the fungus, compared to the fungicide control (Table 1).

Effectiveness of aqueous extracts on mycelium growth of *P. sorghina*

The variance analysis shows significant differences

Table 1
Effect of plant extracts macerated for different lengths of time on radial growth of *Colletotrichum graminicola*

Treatments	Radial growth (in cm)			
	C. occidentalis	*C. citratus*	*B. aegyptiaca*	*P. oleracea*
T.E.	8.13a	7.30a	6.33a	9.00a
T.F.	1.00d	1.06b	0.83c	1.20c
M6	5.26bc	0c	2.53b	5.26b
M12	5.63b	0c	2.20b	5.46b
M24	5.40bc	0c	2.30b	5.73b
M48	5.03c	0c	2.50b	5.13b

T.E.: Water control, W.C.: Fungicide control, M6, M12, M24, M48: Macerated for 6, 12, 24, 48 hours. Averages followed by the same letter, in the same column, are not significantly different to the 5% threshold, according to the Newman-Keuls multiple comparison test.

between treatments for all the plant species used (Table 2).

Extracts of *C. citratus* that have been macerated for 6 and 12 hours stimulate the mycelium growth of *P. sorghina*. However, after longer maceration periods (24 and 48 hours), extracts of *C. citratus* completely inhibit the mycelium development of *P. sorghina*. After these maceration periods, citronella significantly reduces the mycelium growth of *P. sorghina* compared to the fungicide control (Table 2).

The inhibitory effect of the extracts of *P. oleracea*, which were macerated for 6 and 12 hours, on the mycelium growth of *P. sorghina* is weak and does not differ significantly from the water control. The toxicity of the extracts increases with the duration of maceration. For example, after 24 hours of maceration, the extract of *P. oleracea* significantly reduces the mycelium growth rate of *P. sorghina* compared to the water control. When macerated for 48 hours, the extract of *P. oleracea* produces a significantly different inhibitory effect from the water and fungicide controls (Table 2).

When it came to extracts of *C. occidentalis*, only the extract that was macerated for 48 hours significantly reduced the mycelium growth of *P. sorghina,* compared to the water control. Regardless of the *C. occidentalis* extract used, the reducing effects observed were significantly weaker than those produced by the fungicide control (Table 2).

The macerated extracts of *B. aegyptiaca* do not in any way inhibit the mycelium growth of *P. sorghina* (Table 2).

Comparative effectiveness of plant extracts, after different maceration periods, on the mycelium growth of *Colletotrichum graminicola* and *Phoma sorghina*

The comparative effectiveness of extracts of the different plants tested shows that the extract of *C. citratus* significantly reduces radial growth of *C. graminicola,* compared to the other plant species tested, regardless of the maceration period applied. It is followed by *B. aegyptiaca*. No difference could be observed between *C. occidentalis* and *P. oleracea*. However, the extract of *C. occidentalis* causes a slight reduction in mycelium growth, after being macerated for longer periods (24 and 48 hours).

Comparison of the effectiveness of the plant extracts tested with *P. sorghina* indicates that extracts of *C. citratus* macerated, or 24 and 48 hours, are the most effective, followed by extracts of *P. oleracea* that were macerated for the same periods (Table 3). Extracts of *C. citratus* produce slightly greater growth than other plant extracts after the shortest maceration periods (6 and 12 hours). After six hours of maceration, the extract of *P. oleracea* was more effective than the other extracts tested. The extracts of *C. occidentalis*, *B. aegyptiaca* and *P. oleracea* differed significantly from that of *C. citratus* (Tables 1 and 2).

Table 2
Effect of plant extracts macerated for different periods on the radial growth of *Phoma sorghina*

Treatments	Radial growth (in cm)			
	C. occidentalis	*C. citratus*	*B. aegyptiaca*	*P. oleracea*
T.E.	4.63a	5.26b	5.03a	4.43a
T.F.	0.90c	1.00c	0.96b	2.56b
M6	4.60a	6.20a	5.06a	3.16ab
M12	4.53a	6.70a	4.40a	4.36a
M24	4.06ab	0d	5.00a	2.16b
M48	3.86b	0d	4.4a	1.23c

T.E.: Water control, T.F.: Fungicide control, M6, M12, M24, M48: Macerated for 6, 12, 24, 48 hours. Averages followed by the same letter, in the same column, are not significantly different from the 5% threshold, according to the Newman-Keuls multiple comparison test.

Discussion

The effectiveness of the plant extracts varies according to the fungus species. In fact, the results obtained show that *Colletotrichum graminicola* is more sensitive to the toxicity of the plant extracts tested, unlike *Phoma sorghina*. These results are in line with those obtained by Adekunle and Ikumapayi (1), which showed that *Aspergillus flavus* Link ex Fries, *Candida albicans* Brown, *Microsporium audouinii* TER, *Penicillium* sp., *Trichophyton mentagrophytes* Ger., C. Takashio M., *Trichoderma* sp. and *Trichosporon cutaneum* Has. present different levels of sensitivity, when using aqueous extracts of *Funtumia elastica* Fresen. and *Mallotus oppositifolius* (Geiss) Muell. Arg. Fresen.

Depending on the fungus and maceration period, plant extracts can produce contrasting effects. Extracts of

Cymbopogon citratus that were macerated for 6 and 12 hours stimulate the radial growth of *P. sorghina,* while those macerated for 24 and 48 hours completely inhibited development of the fungus. At weak doses, extract of *C. citratus* behaves like a nutritive substance for *P. sorghina*. It then enriches the culture medium and produces greater mycelium growth than the water control. Increasing the maceration period increases the active substance concentration in extracts that were macerated for 24 and 48 hours, which then become more effective in terms of inhibiting the mycelium growth of the fungus. Similar results were obtained by Coventry and Allan (3), who showed that a weak dose of neem extract contains substances, which promote radial growth of *Aspergillus flavus* Link ex Fries. Similarly, studies by Nwachukwa and Umechuruba (6) reveal that diluting extracts of *Carica papaya* L., *Azadiractha indica* A. Juss., *Ocimum basilicum* L. and *Vernonia amigdalina* L. reduces the inhibitory effect of these extracts on *Aspergillus flavus, A. niger* Van Tieghem and *Botryodiplodia theobromae* Pat.

The study also shows that the sensitivity of *C. graminicola* varies, according to which plant species is tested. The fungus is more sensitive to the extract of *C. citratus,* followed by extract of *B. aegyptiaca*. Extracts of *C. occidentalis* and *Portulaca oleracea* show lower levels of effectiveness. The difference in effectiveness observed between the plant species could be explained by a variation in the concentration of the active substance(s), depending on the plant species. Similar results were obtained by Wilson *et al.* (12) who show that *Allium* and *Capsicum* species are more effective in terms of inhibiting fungal conidial germination, compared to other plant species, such as *Adenocalyma* sp*., Tulbaghia* sp., *Amaranthus* sp., etc., by evaluating the anti-fungal properties of 345 plant species compared to *Botrytis cinerea* Pers. ex Fr.

Our studies show that the toxicity of certain plant species only targets a very limited number of fungi. This seems to be true of *B. aegyptiaca,* the macerated extracts of which significantly reduce radial growth of *C. graminicola,* but have no effect on mycelium growth of *Phoma sorghina*. However, others are effective against several fungi at the same time. This difference can be explained by the nature of the compounds found in the different extracts. The diverse variety of extracts identified in this case is due to the specific synthesis capacity of each plant species.

If we consider *P. sorghina,* it emerges that increasing the maceration period would increase the toxicity of extracts of *C. occidentalis*, *P. oleracea* and *C. citratus*. When it comes to these plants, the liberation of the active substance(s) therefore depends on the maceration period. With *B. aegyptiaca,* increasing the maceration period does not seem to have any effect on the active substance concentration(s) in the extract. The solubility of the active substance(s) would therefore be high for *B. aegyptiaca*, whereas the solubility would gradually increase in the other species, depending on the maceration period.

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