

## Original Research Article

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## Effectiveness of *Moringa oleifera* (Lam) Extracts against *Sclerotinia sclerotiorum* (Lib) De Bary, the Causative Agent of White Mold of Common Bean (*Phaseolus vulgaris* L.)

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### ABSTRACT

Common bean (*Phaseolus vulgaris* L.) plays an important role in human and animal nutrition. However, its cultivation in Cameroon is affected by diseases, especially the white bean mold caused by *Sclerotinia sclerotiorum*. Methods of protection against this pathogen are the use of chemical fungicides which are very expensive and degrade the environment. The search for alternative solutions is necessary. The objective of this work is to evaluate the antifungal activity of aqueous and organic extracts of *Moringa oleifera* seeds on the development of two strains of *S. sclerotiorum*. The experiment was conducted in the laboratory using the Potato Dextrose Agar culture medium and three doses of extracts from organic solvent (methanol, ethanol and acetone) and water extracts were used. These doses were 12.5 (C1); 25 (C2) and 50 (C3) µl/ml. The results showed that, at the highest concentration of 50 (C3) µl/ml, methanol, aqueous, acetone and ethanol extracts of *M. oleifera* showed a percentage inhibition of 52.56; 60; 97.18 and 100 % respectively for strain 1, and 45.13; 13.85; 56.02 and 97.44 % respectively for strain 2. No significant difference ( $P < 0.05$ ) was observed between the percentages inhibition of extracts with ethanol and acetone for strain 1 (100 and 97.18% respectively) and that obtained with the synthetic fungicide Plantineb 80WP (100%). Minimal inhibitory concentration which reduced growth up to 50% ranged from 0.87 to 1.70 µl/ml ethanolic extract of *M. oleifera* for strains 1 and 2 respectively compared to 2 and 11.13 µl/ml aqueous extract respectively. The percentage inhibition of growth of strains in C3 dose showed that aqueous and organic extracts of *M. oleifera* seeds compared to synthetic fungicide can be an alternative control method of *S. sclerotiorum*.

#### Keywords

*Moringa oleifera*,  
*Sclerotinia sclerotiorum*,  
*Phaseolus vulgaris*,  
Radial growth, Bio-fungicide

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### Introduction

Common bean (*Phaseolus vulgaris* L.) is a leguminous food originating from Central and

South America (Chacón *et al.*, 2005). Rich in starch and protein, it plays an important role in human and animal nutrition. Its high protein content makes it one of the most

important food crops for the populations of the different Southern countries (Broughton *et al.*, 2003; Blair *et al.*, 2006). Globally, bean is the first leguminous consumed dry, with an estimated production of 30.4 million tons per 34.5 million hectares in 2018 (Anonymous, 2020). In Cameroon, it is the second most cultivated legume after groundnuts because of its important nutritional value, with an estimated national production of 402,054 tons from 298,795 hectares. The main production comes from the Western Highlands with a total production of 284.676 tons from 183.592 hectares in 2016 (Anonymous, 2017). Beans belong to the group of crops capable of fixing and using atmospheric nitrogen, thanks to the *rhizobium* located in the nodules (Doucet, 1992). In nitrogen-poor soils, it can act as an alternative to soil fertility, especially in developing countries (Roland, 2002). Although bean is highly valued in almost all of Cameroon and internationally, the cultivation of common beans in Cameroon is hindered by an epidemic: white mold of bean caused by *Sclerotinia sclerotiorum*. This disease affects the leaves, stems and pods of common beans and causes significant losses of 30 to 100% in the field in the absence of appropriate control measures (Buruchara *et al.*, 2010).

In order to ensure high food and nutrition security, many countries have opted for the use of synthetic fungicides (Carmichael *et al.*, 2008). Although effective and easy to use, their intensive and uncontrolled use still presents many disadvantages (Salim, 2011) the residues on surface and ground water, phytotoxicity, the appearance of new forms of resistance in the targeted pests and insects, imbalance in the food chain, high cost and danger to human health and the environment (Camara, 2009; Gueye *et al.*, 2011). Faced with these difficulties, alternative control methods less harmful to human health and the environment are increasingly being used.

Therefore, plant extracts are advantageous not only because of their low cost to farmers, but because they are non-toxic and easily biodegradable and therefore environmentally sound (Okigbo and Omdamiro, 2006). Research works have shown the antifungal effects of plant extracts on the growth plant pathogens (Ambang *et al.*, 2010).

However, no information is available on the effect of *M. oleifera* seed extracts on *Sclerotinia* of bean in Cameroon. The present study aims to evaluate the *in vitro* effectiveness of aqueous and organic extracts of *M. oleifera* on the growth of *S. sclerotiorum*.

## **Materials and Methods**

Plant material included *M. oleifera* fruits. These fruits were harvested in Yaoundé (Cameroon). Fungal material included pure strains of *S. sclerotiorum* obtained from strains collected from leaves of two varieties of common beans (NITU G16187 and GLP 190S), collected from an experimental plot in Akonolinga (N 03°48.136' and E 012°15.518', altitude 671 m) in the Central region of Cameroon that showed a high intensity of white mold. The collected leaves were immediately taken to the plant pathology Laboratory of the University of Yaoundé I.

## **Obtention of crude extracts**

The mature *Moringa oleifera* seeds obtained from fruits, were previously dried at room temperature for seven days in the laboratory. These seeds were ground using a manual "Victoria" mill to obtain its powder. Subsequently, 1200g of seed powder was weighed using a "Sortorios" balance with a precision of 0.01 g and macerated 300g each for 72 hours in 1 liter of organic solvent (Acetone, Methanol and Ethanol) and in distilled water for 24 hours (Stoll, 1994). The

mixtures were filtered separately on filter paper. The filtrates from the organic solvents were concentrated using a rotary evaporator (Ciulei, 1980). The concentrated extracts obtained were weighed and the extraction yield calculated according to the formula used by Ngoh dooh *et al.*, (2014). The organic solvent extracts were stored in the refrigerator at 4°C until they were used.

### Preparation of culture medium

The Potato Dextrose Agar growing medium is prepared from two hundred grams of potato cut into small pieces and boiled; the juice collected was made up to one liter with distilled water. Fifteen grams of Agar and twenty grams of D-glucose were added to the potato juice obtained.

The mixture was homogenised and then sterilised in an autoclave at a temperature of 121 °C for 15 minutes at a pressure of 1 bar. Streptomycin sulphate was added to the medium (400 ppm) and was poured into 9cm diameter Petri dishes under controlled conditions in a laminar flux.

### Isolation and purification of strains of *S. sclerotiorum*

Leaves infected with *Sclerotinia sclerotiorum* from both cultivars were disinfected in 5% sodium hypochlorite solution for 2 minutes and cut into fragments of about 5mm<sup>2</sup> from the growing area of the pathogen. The resulting fragments (4) were deposited in a Petri dish containing PDA culture medium.

After three days of incubation in the laboratory at 23 ± 1°C, the visible filaments around the fragments were removed and transferred to new Petri dishes containing PDA culture medium. This process was repeated several times until pure cultures of *S. sclerotiorum* were obtained.

### Obtention of different extract concentrations

A sample solution of 500µl ml<sup>-1</sup> was prepared by mixing 1ml of the extract to 0.7ml of the solvent and 0.3 ml of distilled water. From this solution, 0.75, 1.5 and 3 ml were obtained and added to 59.25, 58.5 and 57ml from the culture medium to obtain 12.5, 25 and 50 µl. ml<sup>-1</sup> concentrations respectively which gave a final volume of 60ml.

### In vitro evaluation of the antifungal activity of *Moringa oleifera* extracts

The *in vitro* evaluation of the antifungal activity of *M. oleifera* seed extracts was carried out using 12.5 (C1); 25 (C2) and 50 (C3) µl /ml concentrations for organic extracts and aqueous extract. 12.5µl.ml<sup>-1</sup> of PLANTINEB 80 WP commonly used in the control of fungal diseases in plant crops was used as a positive control (T+) and PDA was used as a negative control (T-). Mycelial fragments of *S. sclerotiorum* 7mm in diameter were removed from a seven-day old pure culture and placed in the centre of the Petri dish containing treatments with three repetitions each. Incubation was carried out at 23±1°C for one week. A daily measurement of the radial growth diameter of each cultured fragment was taken and this continued until the mycelium filled at least one Petri dish. The radial growth of the pathogen as well as the inhibition percentage was calculated according to the formula used by Singh *et al.*, (1993)

$$I(\%) = \frac{D_{to}(\text{mm}) - D_{xi}(\text{mm})}{D_{to}(\text{mm})}$$

I (%) is inhibition percentage; D<sub>to</sub> is the average diameter of the control batch and D<sub>xi</sub> is the average diameter of the batches in the presence of the extracts (Dohou *et al.*, 2004).

$$D = \frac{D_1 + D_2}{2} - D_0$$

D0 is the fragment's diameter; D1 and D2 are the culture diameters measured in the two perpendicular directions.

From the linear curve between the concentrations (abscissa) and the growth inhibition percentages of strain (ordinate), the concentration reducing the growth of the fungus by 50% and 90% was determined according to the method used by Dohou *et al.*, (2004).

The growth inhibition percentages of mycelia were transformed into probit values (Finney, 1971). The linear curves were established:  $y = a \log x + b$ , where a is the regression coefficient, b is a constant, x is the fungicide concentration, y is the probit, log is the decimal logarithm.

Using these linear curves, the minimum concentrations of the extract that reduce the mycelia growth of the fungus by 50% (MIC50) and 90% (MIC90) were determined by simple projection.

### Statistical analysis

The R computer software was used. Extract activity modalities were compared on the basis of growth diameter of different strains with one dimensional analysis of variance test (ANOVA). Duncan's multiple range tests

permitted the constitution of homogeneous sub-units at a threshold of 5%.

## Results and Discussion

### Extraction yield

Yields of *M. oleifera* seed extracts with methanol, acetone, ethanol and water were 15.5, 19.1, 16.6 and 14.7% respectively. Water extracts showed a lower yield compared to methanol, ethanol and acetone extracts.

### Effect of *Moringa oleifera* extracts on the mycelial growth of *S. sclerotiorum*

The seed extracts significantly reduced the radial growth of *S. sclerotiorum*. The radial growth of the different strains of the fungus was reduced with increasing concentration of the extracts and varies with the type of extract used (Fig. 1). Indeed, ethanol extract of *M. oleifera* completely inhibited the growth of strains 1 and 2 at C2 and C3 doses, whereas only a little inhibition of growth of strains 1 and 2 was observed in the aqueous, methanolic and acetone extracts for C2 and C3 concentrations. In the control treatments, the growth of *S. sclerotiorum* was significantly higher compared to the different concentrations of the tested extracts (Fig. 2).

**Table.1** Extract yields and characteristics

Extraction Solvent	Organ used	Yield (%)	physical aspect	colour
Methanol	Seeds	15,5	Creamy	Yellowish
Acetone	Seeds	19,1	Creamy	Yellowish
Ethanol	Seeds	16,6	Creamy	Yellowish
Water	Seeds	14,7	Milky	Whitish

**Table.2** Inhibition percentage of *M. oleifera* extracts on mycelial growth strain 1 and 2 of *S. sclerotiorum*

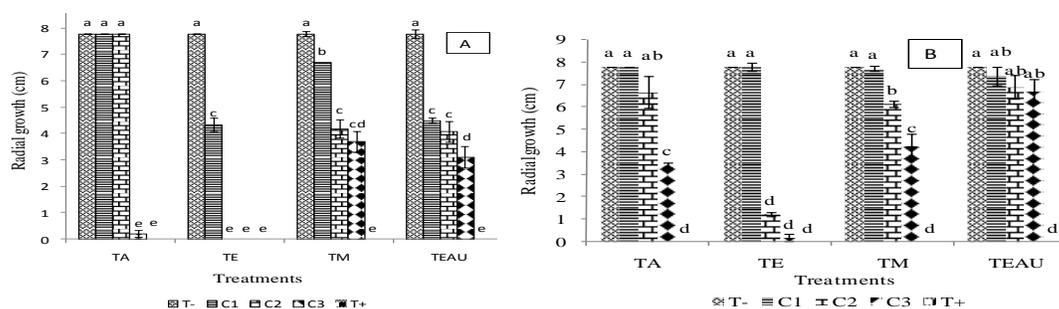
Treatments	Concentrations ( $\mu\text{l.ml}^{-1}$ )	Strain 1	Strain 2
Control	0	0.0a	0.0a
Plantineb 80 WP	12.5	100.0e	100.0d
Methanol extract	C1: 12.5	15b	0,89a
	C2: 25	46,15c	21,15b
	C3: 50	52,56cd	45,13c
Ethanol extract	C1: 12.5	44,10c	7,94ab
	C2: 25	100e	84,36d
	C3: 50	100e	97,44d
Acetone extract	C1: 12.5	0a	0a
	C2: 25	0a	14,62ab
	C3: 50	97,18e	56,02c
Aqueous extract	C1: 12.5	42,31c	5,13a
	C2: 25	47,69c	11,54ab
	C3: 50	60d	13,85ab

For each strain, the values followed by the same letter in the same column are not significantly different according to Duncan's 5% test

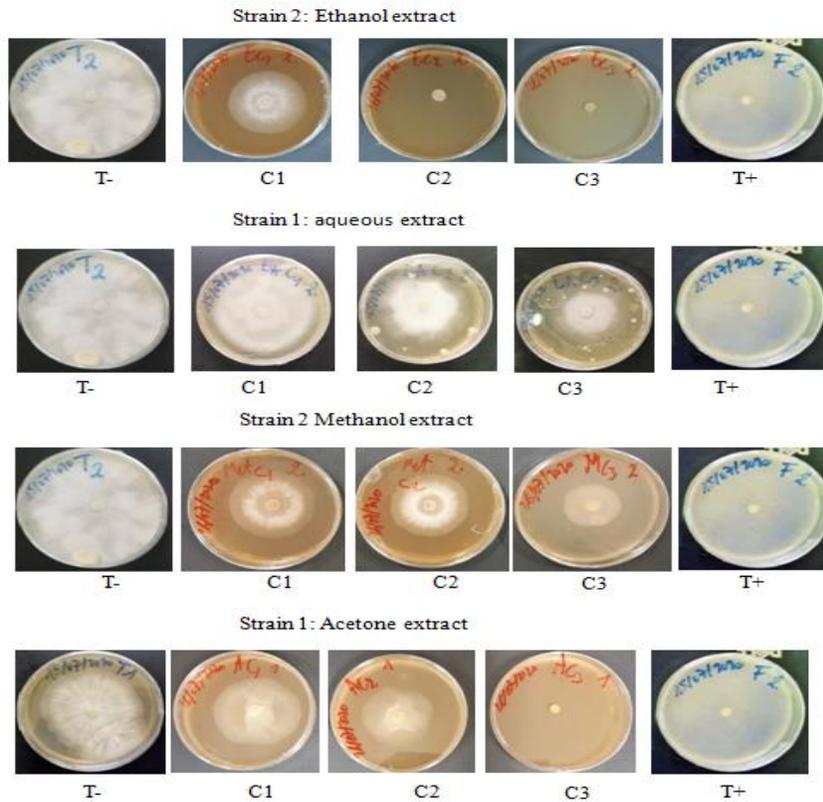
**Table.3** Correlation between inhibition percentages and concentrations of different extracts on *S. sclerotinia* strains

Concentration ( $\mu\text{l.ml}^{-1}$ )	Type of extracts	Strain 1	Strain 2
CMI 50	Aqueous extract	2	11,13
	Acetone extract	2,36	2,94
	Methanol extract	2,64	3,24
	Ethanol extract	0,87	1,70
CMI 90	Aqueous extract	6,52	20,31
	Acetone extract	3,18	4,37
	Methanol extract	4,77	5,05
	Ethanol extract	2,30	2,59

**Fig.1** Effect of treatments and concentrations on the growth of *S. sclerotiorum* strains (C1: 12.5  $\mu\text{l.ml}^{-1}$ ; C2: 25  $\mu\text{l.ml}^{-1}$  and C: 50  $\mu\text{l.ml}^{-1}$ ) (A: Strain 1 and B: Strain 2)



**Fig.2** *In vitro* inhibitory activity of organic and aqueous extracts of *M. oleifera* on radial growth of *S. sclerotiorum* after 6 days of incubation on PDA medium (T-: control; C1: 12.5  $\mu\text{l.ml}^{-1}$ ; C2: 25  $\mu\text{l.ml}^{-1}$ ; C3: 50  $\mu\text{l.ml}^{-1}$  and T+: plantineb 80WP)



### Inhibition percentage of *Moringa oleifera* extracts

The methanolic, aqueous, acetone, and ethanol extracts of *M. oleifera* at the concentration (C3) showed an inhibition percentage of; 52.56; 60; 97.18 and 100% respectively for strain 1 and 45.13; 13.85; 56.02 and 97.44% respectively for strain 2 (Table 2). However, no significant difference ( $P < 0.05$ ) was obtained between the radial growth inhibition percentages of *S. sclerotinia* with ethanol extract for both strains and acetone extract for strain 2 compared to the synthetic fungicide (Plantineb 80WP). No growth inhibition of the two strains of *S. sclerotiorum* was observed in the negative control treatment.

### Minimal concentrations of the extract reduced mycelial growth of the fungus by 50% and 90%

From the linear curves obtained after the correlation tests, the concentrations of the different extracts inhibiting the growth of *S. sclerotiorum* strains by 50% and 90% (MIC50; MIC90) were determined. The lowest minimal inhibitory concentrations (MIC50) were obtained with ethanol extract, i.e. 0.87 and 1.70  $\mu\text{l.ml}^{-1}$  for strains 1 and 2 respectively. For MIC 90, low minimal inhibitory concentrations of 2.30 and 2.59  $\mu\text{l.ml}^{-1}$  were obtained for strains 1 and 2 respectively. The highest MIC50 and MIC90 were obtained with the aqueous extract of 11.13 and 20.31  $\mu\text{l.ml}^{-1}$  for strain 2 and strain 1 of *S. sclerotiorum* respectively. The highest

minimal concentration was obtained only with MIC 90 ( $6.52\mu\text{l.ml}^{-1}$ ) (Table 3).

Extraction yields varied from one solvent to another. The difference in yield observed between aqueous and organic solvents could be explained by the fact that organic solvents extract more compounds compared to water and therefore increase in yield (Ciulei, 1980). Furthermore, the solubility of a compound in a solvent comes from the properties of the later, namely its polarity or its capacity to form hydrogen bonds. Thus, the high polarity of organic solvents (methanol, ethanol and acetone) allows them to be more efficient in the extraction of many compounds (Muhammad *et al.*, 2013).

The different extracts tested, significantly reduced the radial growth of *S. sclerotiorum* compared to the negative control. This reduction was more pronounced with the ethanol extract independent of *S. sclerotiorum* strains. These extracts may contain substances that would inhibit or retard the growth of the fungus. Ling *et al.*, (2003) reported that extracts from plant parts contain compounds such as tannins, flavonoids and alkaloids that have fungicidal properties. Different concentrations of extracts significantly influenced the radial growth of the fungus; high concentrations were more inhibitory. Similar results on the antifungal activity of organic and aqueous extracts of *Jatropha curcas* on *Cercospora malayensis*, the causative agent of okra cercosporiosis (kone *et al.*, 2018) and Djeugap *et al.*, (2011) using acetone extracts of *Callistemon viminalis* and methanol extracts of *Eucalyptus saligna* on *Phytophthora infestans*, the causative agent of late blight in nightshade and potato was demonstrated.

The inhibition percentages of the extracts on the growth of the pathogen also varied with increasing concentrations. At C3

concentrations, ethanol and acetone extracts from *M. oleifera* seeds showed complete suppression of fungal growth, similar to that obtained with synthetic fungicides. Similar results of the antifungal activity of the extracts were reported by kone *et al.*, (2018) against *C. malayensis*, the causative agent of okra cercosporiosis, and Djeugap *et al.*, (2011) on *P. infestans*, the causative agent of black nightshade. Furthermore, the organic extracts were more active at the concentration (C3) than the aqueous extract at the same concentration. This difference could be attributed to a difference in the concentration of chemical compounds during the extraction process. According to Bougandoura and Bendimerad (2012), ethanol followed by acetone and methanol would allow a better extraction of compounds such as flavonoids and terpenoids which are molecules known for their antifungal activity. However, Akhilesh *et al.*, (2010) reported that extraction with methanol was more effective on antimicrobial activity than that with water. Furthermore, Tsopmbeng *et al.*, (2014) reported that methanolic extracts of *Cupressus lusitanica* and *Callistemon viminalis* at a dose of 5 mg/ml and *Eucalyptus saligna* at a concentration of 15 mg/ml completely inhibited the radial growth of *Phytophthora colocasiae in vitro* compared to water extracts.

The inhibition percentages obtained at high concentration with the ethanol and acetone extracts compared to the fungicide (PLANTINEB 80 WP) did not show any significant difference. These extracts at high doses could be more effective than the chemical fungicide. From the works of Mboussi *et al.*, (2016) *in vitro*, the effect of *Thevetia peruviana* extracts and Ridomil Gold Plus on *Phytophthora megakarya* strain showed that high doses of extracts can completely inhibit the growth of the pathogen in the same way as the synthetic fungicide.

Minimal inhibitory concentrations (MIC50 and MIC90) reducing mycelial growth of *S. sclerotiorum* strains by 50% and 90% were determined. The low values of MIC50 and MIC90 were obtained with ethanol and acetone extract for strains 1 and 2, demonstrating the effectiveness of these different extracts on the mycelial growth of the pathogen. According to Doumbouya *et al.*, (2012) low values of MIC show the effectiveness of an extract. The authors showed that high inhibition of the development of phytopathogenic fungi with *Ocimum gratissimum* extracts is observed with low MIC values.

In conclusion the study showed that *M. oleifera* extracts inhibited the radial growth of *S. sclerotiorum* *in vitro*. These extracts were found to be active on *S. sclerotiorum* and may therefore be an alternative in the fight against white mold of common bean. Although their activity was comparable to that of the reference fungicide, Plantineb 80 WP, the fact remains that these crude extracts contain a large number of different compounds which once purified, would have a higher activity than fungicides.

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