In vitro development of two *Alternaria solani* strains, causal agent of Alternariose in tomato (*Lycopersicon esculentum*) under the influence of *Thevetia peruviana* seeds extracts

Abstract

Alternaria solani is a fungus that causes yield losses of up to 80 % in tomato production in field. Synthetic fungicides are the most widely used for its control, but have harmful consequences. The objective of this work was to test *in vitro* the antifungal potential of *Thevetia peruviana* seed extracts against two *A. solani* strains. Aqueous, methanol, ethyl acetate and acetone extracts, at concentrations 12.5, 25, 50 and 100 µL/mL were used. Two synthetic fungicides Maneb (5.33 µg/mL) and Dimethomorph + Clorothalonil (3.75 µg/mL) and control (0 µL/mL) were also tested on two *A. solani* strains (Mbal and Foum). The investigation was repeated three times. Phytochemical screening, mycelial growth, spore germination and minimum inhibitory concentrations (MIC50 and MIC90) were determined. The results showed that *T. peruviana* extracts are rich in many families of bioactive compounds such as alkaloids, phenolic compounds and sugars. All extracts tested show high inhibition of mycelial growth (100%) and spore germination (100%) of the two strains at highest concentration (100 µL/mL). Acetone extract at a concentration of 50 µL/mL inhibited mycelial growth by 88.45 and 86.55% and spore germination by 88.33 and 80.33%, respectively for the Mbal and Foum strains. The lowest MIC50 (16.63 µL/mL) and MIC90 (54.6 µL/mL) were obtained with the acetone extract on the Mbal strain while the highest MIC50 (27.5 µL/mL) and MIC90 (61.7 µL/mL) were observed with ethyl acetate on the Foum strain. These extracts can therefore be used in the biological control against Alternariose in tomato.

Keywords: *Thevetia peruviana*, extract, strain, *Alternaria solani*, inhibition.

- **INTRODUCTION**

Tomato (*Lycopersicon esculentum* Mill.) is a plant belonging to the Solanaceae family, native to South America and cultivated in more than 177 countries [1]. Tomato is the

most consumed vegetable in the world ahead of watermelon and cabbage [2]. It is consumed as salad, puree, concentrate, condiment and sauce [3]; [4]. Tomato has a high nutritional and economic importance, and its fruit is composed of 95% water and 5 % dry matter.

World production in 2020 is estimated at about 186.8 million tons of fresh fruits on a cultivated area of 5,051.9 thousand hectares with an average yield of 37.1 tons per hectare [5], while total African production is estimated at about 17,175,228 tons. In 2020, Cameroon recorded a production of 1,246,658 tons on 101,350 ha, i.e. about 14 % of African production. This low tomato productivity is due not only to the influence of abiotic factors (temperature, humidity, water stress, etc.) but also to the strong pressure of biotic factors such as diseases and pests [6]. Among the devastating diseases of tomato fields, Alternariosis, a fungal disease caused by *Alternaria solani*, is one of the most important [7]. Alternariosis is manifested by severe and destructive blights, reduced germination capacity of seeds, defoliation of the plant, and deterioration of the products before and after harvest [8]. Several strategies of combating this disease are used, including chemical control, which remains the most used method by producers because it is easy to use. However, these synthetic chemical products have an impact on the health of producers, consumers and the environment, and leads to the development of resistance by certain pathogens [2]. Considerable efforts are directed towards the search for effective alternative solutions that respect the environment and human health [9]. Numerous studies have demonstrated the importance of using plant extracts from *Azadirachta indica*, *Thevetia peruviana* rich in secondary metabolites (phenolic compounds, terpenoids and alkaloids) in the control of crop diseases [10].

Thevetia peruviana (yellow oleander) is an ornamental shrub native to tropical America and is widely cultivated throughout the tropics including tropical Africa [11]. The roots, leaves, seeds and fruits of *T. peruviana* are sources of biological active compounds conferring bactericides [12], virucides [13], insecticides [14], [15] and fungicides [16-17]; [9], [18] potentials.

The present work aims to test *in vitro* the antifungal potential of *Thevetia peruviana* seed

extracts against two *Alternaria solani* strains

- **MATERIAL AND METHODS**

- **Sample collection**

The diseased tomato leaves were collected in two agro-ecological zones of Cameroon, one in the bi-modal rain fall forest zone, more precisely in the locality of Mbamayo (Mbal) belonging to agro-ecological zone 5 with geographical coordinates (N5.2926, E10.3419). The other in the highland or humid savannah zone in the locality of Foumbot (Foum) in agro-ecological zone 3 with geographical coordinates (N3.3109, E11.3010) in Cameroon. These leaves were placed in envelopes and then put in a cooler containing ice cubes and transported directly to the Biotechnology and Environment Laboratory, Plant Pathology and Protection Research Unit of the University of Yaounde 1.

Thevetia peruviana seeds were obtained from fruits collected under trees in Yaoundé and identified in the national herbarium.

The plant material consisted mainly of *T. peruviana* seeds wile fungal material used was two *A. solani* strains (Mbal and Foum) isolated from diseased organs in farmers' fields, in Mbalmayo and Foumbot. Two synthetic fungicides including Plantineb 80 WP (Maneb) and Jumper D (Dimethomorph + Clorothalonil) were used as reference. Laboratory equipment was also used.

- **Obtaining of *Alternaria solani* strains**

Using the identification key of [19] symptomatic leaves were collected and transported directly to the laboratory of Biotechnology and Environment, University of Yaoundé I. These leaves were carefully washed with running tap water, cut into small fragments, immersed in 10 ml of alcohol at 5°C for 2 min and rinsed 3 times with sterile distilled water.

These leaf fragments were dried on sterile filter paper (Whatman N^0 1) and inoculated into Petri dishes containing PDA (Potato Dextrose Agar) then sealed with film and incubated at 23 ± 2 ^{0}C until colonies appeared. After 5 days of incubation, the mycelia were removed from the growth front of the fungus and transferred to new Petri dishes containing PDA.

This last operation was repeated several times until pure strains were obtained, and their microscopic observations were performed following the protocol described by [20].

- **Obtaining of different extracts and their phytochemical screening**

The *T. peruviana* seeds were collected in Yaounde. The obtained kernels were dried in the laboratory at room temperature for two weeks and grinded using manual hand mill grinder. The aqueous extract was obtained by maceration of 100 g of paste in 200 mL of distilled water for 24 hours. The resulting mixture was filtered through a muslin cloth and used directly [16]. For organic extracts, 500 g of pulp were macerated in 2 L of each organic solvent (ethyl acetate, acetone and methanol) for 72 hours. The mixture was filtered using filter paper and the filtrates were concentrated using rotavapor (Büchi R124) at a temperature of 60-70^{0}C. The organic extracts obtained were stored in a refrigerator at 4 ^{0}C until their use.

Phytochemical screening of *T. peruviana* extracts was carried out to determine the composition of the major families of secondary metabolites contained in these plants using classical characterisation methods and specific dyes. Protocol used for phytochemical screening was achieved according to [21] ; [22].

- **Evaluation of the effect of the extracts on the mycelial growth of *A. solani* strains**

- **Preparation of the different concentrations tested**

A stock solution of 500 µL/mL was prepared by mixing 12 mL of extract organic (methanol, ethyl acetate, acetone) with 3 mL of 70°C alcohol added to 9 mL of distilled water. Increasing concentrations of (C=12.5; C2=25; C3=50 and C4=100 µL/mL) were prepared by taking 1.5; 3; 6 and 12 mL of each extract respectively and adding them to 58.5; 57; 54 and 48 ml of the PDA to give a final volume of 60 mL. The antibiotic-amended mixture is aseptically poured into 90 mm diameter Petri dishes at a rate of 10 mL per dish under the laminar flow hood. For the negative controls, 10 mL of medium without additive was poured directly into the Petri dishes. The media preparation was enriched with two synthetic fungicides (Plantineb 80 WP and Jumper D) at concentrations

of C5=5.33 µg/mL and C6=3.75 µg/mL.

- **Evaluation of mycelial growth**

The Explants about 6 mm in diameter of each *Alternaria solani* strains were collected and placed in the centre of each Petri dish containing PDA medium enriched with the different extracts. Each treatment was repeated three times and the whole set was incubated at a temperature of 23±1 ^{0}C under continuous light for one week. Measurements of mycelia growth were taken at approximately the same time each day from the second day until the control (C0=0) plates were completely filled with mycelium. The mycelia growth of both strains was assessed by measuring the two perpendicular diameters plotted on the back of the Petri dish and calculated according to the formula used by [23].

$$D = \frac{D1 + D2}{2} - D0$$

Where D0 is the diameter of the explant; D1 and D2: culture diameters measured in the two perpendicular directions.

The inhibition percentage due to each treatment is assessed in relation to the mycelia growth in the control Petri dishes after 7 days according to the formula developed by [24].

$$IP\ (\%) = \frac{Dt\ (mmm) - Dx\ (mm)}{Dt\ (mmm)} \times 100$$

IP (%): Inhibition percentage; Dt: estimated growth diameter on control medium and Dx: estimated growth diameter in the presence of the tested extract or fungicide.

- **Determination of minimum inhibitory concentrations (MIC)**

The minimum inhibitory concentrations reducing mycelia growth by 50% and 90% were determined by comparing the values of the percentage inhibition (PI) with those of the natural logarithm of the corresponding concentrations [23].

The linear regression line of type Y = ax + b from the function IP = f (ln Ci) was used

where Y = percentage inhibition of mycelia growth or spore germination, a = regression coefficient, b = constant and x = extract concentrations.

- **Evaluation of the effect of *T. peruviana* seed extracts on *Alternaria solani* spore germination**

From a 7-day old culture of the pathogen of both isolates, 20 µl solutions of a spore suspension calibrated at 4,105 spores/ml using a Malassez cell were deposited and spread with a micropipette on the slides. Each treatment was repeated 3 times. All the slides were kept in the dark for 24 hours and the parameters were taken on the basis of counting 100 germinated spores on three different zones of each slide under an ordinary microscope, i.e. a total of 300 spores per slide and 900 spores for each treatment. The calculation of the percentages of inhibition was done using the formula of [25] and [23].

$$IP = \frac{A - B}{A} X\ 100$$

With IP: Inhibition percentage; A: number of spores germinated on the control; B: number of spores germinated in the presence of the test extract.

- **Evaluation of the fungicidal or fungistatic activity of *Thevetia peruviana* seed extracts**

At the end of each trial, the mycelia explants from the Petri dishes where growth was totally inhibited were removed and aseptically placed on the PDA culture medium containing no extract. After 7 days of incubation, depending on whether the fungus had resumed growth or not, the starting extract was classified as fungistatic or fungicide respectively [26].

- **Statistical analysis**

Data on mycelia growth of isolates and spore germination rate were subjected to an analysis of variance (ANOVA) using R software version 3.5.1. Tables and curves were drawn using Microsoft Excel.

- **RESULTS**

- **Characteristics of *Alternaria solani* strains**

Macroscopic observation of the pure strain obtained showed whitish colonies with a downy or cottony subaerial appearance with variations in mycelia growth and regular and irregular borders (Figure 1A). Microscopic observation showed a species with large spore conidia with a porri (Figure 1B) cross-section, with 1-2 filamentous spouts measuring between 12.8 and 121.6 mm; the length of the conidial body varying from 54.4 to 115.2 mm. These different forms identified refer to those of *Alternaria solani*, the causal agent of Alternariose in tomato.

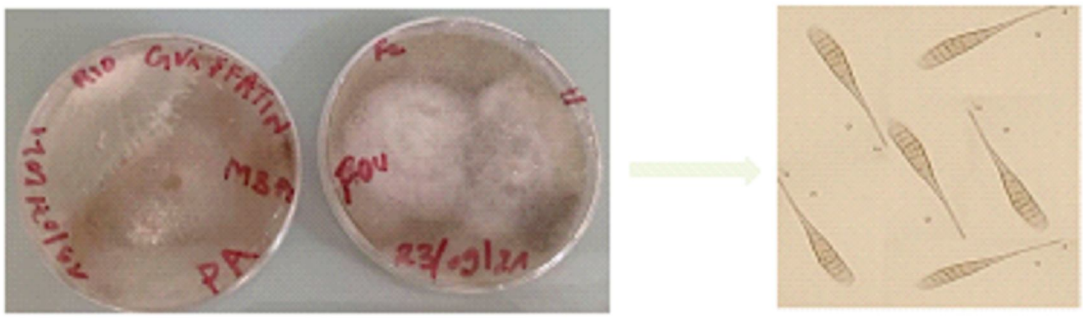


Figure 1. Macroscopic and microscopic characteristics of pure isolates of *Alternaria solani* (A- Macroscopic aspect; B- Conidia seen by photonic microscopy at X 20 magnification).

- **Effect of *Thevetia peruviana* seed extracts on the *in vitro* radial growth of *Alternaria solani***
- **Phytochemical screening of *Thevetia peruviana* seed extracts**

The phytochemical screening carried out revealed the existence of several families of compounds such as: essential and saponified oils, coumarins, sterols, alkaloids, saponins, anthocyanins, steric glycosides and sugars. Coumarins and sterols were present in all extracts and phenols were absent in the aqueous extract, ethyl acetate and in trace

amounts in the methanol extract (Table 1).

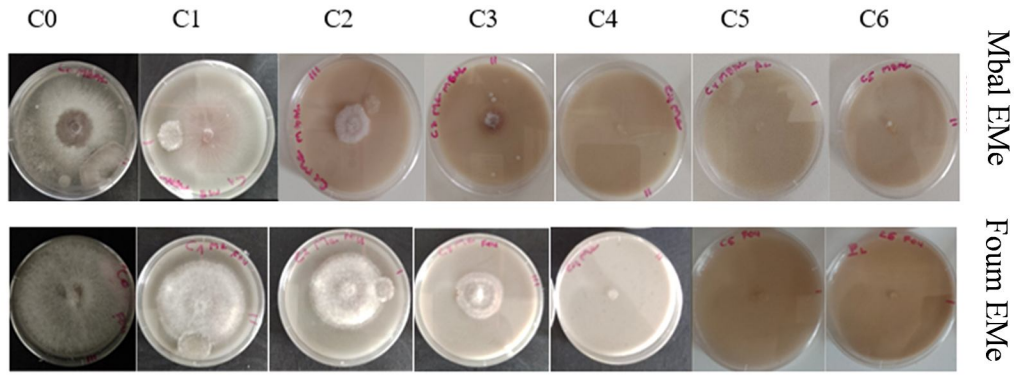
Table 1. Family of compounds in the aqueous and organic extracts of *T. peruviana* seeds.

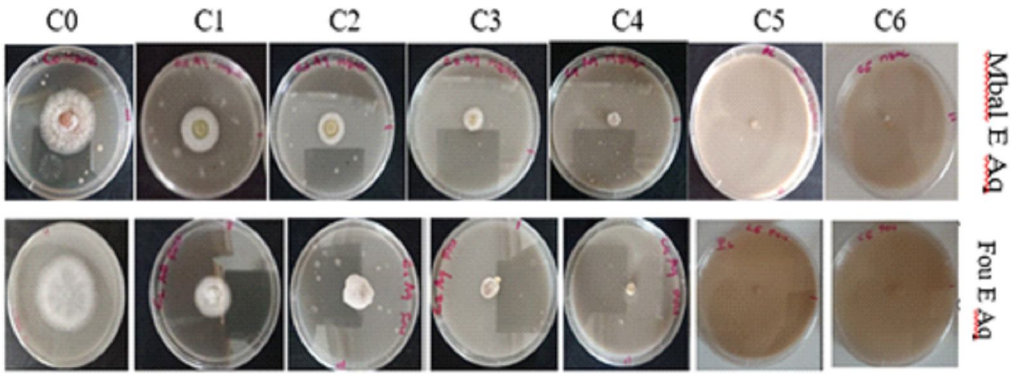
(-): absence of the product; (+): presence of the product; (+++): abundant presence of the product and T: presence in trace form.

Group of compounds	Results of the different extracts			
	Aqueous	**Ethyl acetate**	**Acetone**	**Methanol**
Essential oils	+	-	+	+
Saponifiable oils	+	+	+	-
coumarin	+	+	+	+
alkaloids	+	-	-	+
sterols	+++	+	+	+
Terpenoids	-	-	T	-
Flavonoids	-	-	-	-
Anthraquinones	+	-	-	-
Catechin tannins	-	-	-	+
Gallic tannins	-	-	-	-
Saponins	-+	-	+	+
Anthocyanins	-	+	-	+
Steric glycosides	+	T	+	+
Triterpenoid glycosides	-	-	-	T
Sugars	+++	-	T	T
phenols	-	-	-	T

- **Radial growth of individual strains**

All extracts significantly reduced the radial growth of the different isolates. Mycelial growth decreased with increasing concentration of the different extracts until it was zero with the highest concentration (C4: 100 µL/mL or µg/mL) as well as with the synthetic fungicides (C5 and C6) used based on Maneb and Dimethomorph + Clorothalonil (Figure 2).


C0
C1
C2
C3
C4
C5
C6
Mbal EMe
Foum EMe


C0
C1
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C3
C4
C5
C6
Mbal E Aq
Fou E Aq

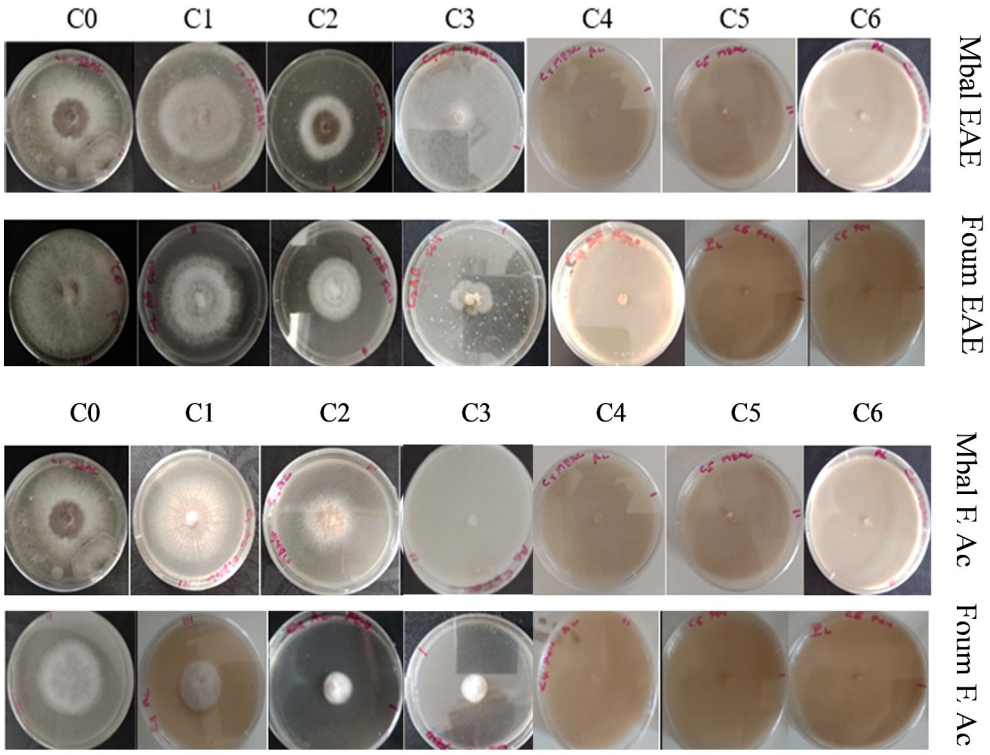

Figure 2. Evolution of mycelia growth of *A. solani* strains as a function of different extract concentrations on the 7th day after incubation (A- Aqueous; B- Methanol; C- Ethyl acetate and D- Acetone).

- **Inhibition percentage of the different extracts of *Thevetia peruviana* seeds**

The inhibition percentage of mycelia growth of *A. solani* varied with the increase of the different extracts. At 7 days post-inhibition (DPI), the acetone extract at concentration C3 (50 µL/mL) showed the highest inhibition percentages compared to the control, respectively 88.45 % for the Mbal strain and 86.55 % for the Foum strain, followed by the aqueous extract (with 85.32 % for the Mbal strain and 83.33 % for that of Foum strain). On the other hand, at the highest concentration C4 (100 µL/mL), total inhibition of mycelia growth (100 %) was observed for the aqueous and organic extracts and the synthetic fungicides used (Figure 3).

* Means in the same column followed by the same letters are not significantly different according to the Tukey test at the 5% probability level;

Figure 3. Variation in the percentage of radial growth inhibition of strains by the different extracts (A: Mbal; B: Foum) 7 JAI

- **Minimum inhibitory concentrations MIC50 and MIC90 of extracts**

The minimum inhibitory concentrations (MICs) for growth of *A. solani* strains varied between extracts. The lowest minimum concentrations that effectively inhibited mycelia growth at 50% and 90% were recorded with the acetone extract where MIC50 and MIC90 were respectively 16.63 L/mL and 54.6 L/mL for the Mbal strain. With the same extract, MIC50 and MIC90 were 18.63 L/mL and 55.6 µL/mL for the Foum strain. The highest minimum concentrations were recorded with the ethyl acetate extract where MIC50 and MIC90 were respectively 26.63L/mL and 60 µL/mL for the Mbal strain. With the same extract, MIC50 and MIC90 were 27.5 L/mL and 60.7 L/mL, respectively for the Foum strain (Table 2).

Table 2. MIC50 and MIC90 values on mycelia growth of *Alternaria solani* according to aqueous and organic extracts and synthetic fungicides

Strains	**Extracts**	**MIC50**	**MICI90**
Mbal	Aqueous (L/mL)	17.6	55.6
	Methanol (L/mL)	22.25	55.7
	Ethyl acetate (L/mL)	26.63	60
	Acetone (L/mL)	16.63	54.6
Foum	Aqueous (L/mL)	20.13	57
	Methanol ((L/mL)	23.5	60
	Ethyl acetate ((L/mL)	27.5	60.7
	Acetone (L/mL)	18.63	55.6

- **Correlation between the concentrations of the different extracts and the percentage of growth inhibition of *Alternaria solani***

The regression equations obtained from the tests with the different extracts showed increasing linear relationships with regression lines all with positive slopes. The correlation coefficient (r) varied from 0.97 to 0.99, i.e. $r > 0.8$ (Table 3). This shows a positive and

perfect correlation between the different concentrations tested and the percentage of radial growth inhibition. The latter is proportional to the different increasing concentrations tested.

Table 3. Correlation between inhibition percentage and the concentrations on the 2 strains tested with the different extracts of *T. peruviana*

Strains	Extracts	Correlation coefficient	Observations
Mbal	Aqueous	0.981	Highly correlated
	Methanol	0.996	Highly correlated
	Ethyl acetate	0.988	Highly correlated
	Acetone	0.953	Highly correlated
Foum	Aqueous	0.986	Highly correlated
	Methanol	0.997	Highly correlated
	Ethyl acetate	0.988	Highly correlated
	Acetone	0.973	Highly correlated

- **Effect of *Thevetia peruviana* seed extracts on spore germination of different strains of *Alternaria solani***

The acetone extract had a very significant influence on the germination of spores of the two isolates compared to the other extracts from the first concentration with inhibition percentages of 56.67; 71.67; 88.33 and 100 % for the Mbal strain, while 50.00; 68.33; 80.00 and 100 % for were recording the Foum strain for C1, C2, C3 and C4 respectively. On the other hand, inhibition percentage of the ethyl acetate extract was lower compared to the other extracts for both strains with 21.6; 41.67; 66.67 and 100 % for Mbal and 18.33; 35.00; 63.33 and 100 % for Foum respectively for the same concentrations (Figure 4).

* Means in the same column followed by the same letters do not differ significantly according to the Tukey test at the 5% probability level;

Figure 4. Inhibition percentage of *Alternaria solani* spore germination according to the different *T. peruviana* extracts (A: Mbal strain and B: Foum strain)

- **Minimum inhibitory concentrations MIC50 and MIC90 for germination of *Alternaria solani* strains**

The minimum germination inhibitory concentrations of *A. solani* strains varied between extracts. The minimum concentrations for spore germination at 50 % and 90 % were lowest with the acetone extracts where MIC50 and MIC90 were respectively 6.38 L/mL and 53.7 L/mL for the Mbal strain. With the same extract MIC50 and MIC90 were 17.6 L/mL and 56.83 L/mL for the Foum strain. In contrast, the highest minimum concentrations were recorded with the ethyl acetate extract where MIC50 and MIC90 were respectively 27.63 L/mL and 62.5 L/mL for the Mbal strain. With the same extract MIC50 and MIC90 were 31.2 L/mL and 66.1 L/mL for the Foum strain (Table 4).

Table 4. Minimum inhibitory concentrations of *Alternaria solani* spore germination in the presence of *Thevetia peruviana* extracts

Strains	Extracts	MIC50	MIC90
Mbal	Aqueous (g/mL)	17.6	57.83
	Methanol (L/mL)	22.9	60.5
	Ethyl acetate (L/mL)	27.63	62.5
	Acetone (L/mL)	6.38	53.7
Foum	Aqueous (g/mL)	22.4	60.7
	Methanol (L/mL)	25.4	62.5
	Ethyl acetate (L/mL)	31.2	66.1
	Acetone (L/mL)	12.13	57.5

- **Correlation between different concentrations and germination percentages of *Alternaria solani* strains**

The regression equations obtained with the different extracts from the germination test of the two strains used showed increasing linear relationships with regression lines with positive slopes. Furthermore, the correlation coefficients r obtained were between 0.96 and 0.99. Thus, the inhibition percentage is proportional with the increase of the

concentrations of the tested extracts (Table 5).

Table 5. Correlation between the germination percentage and the concentrations of the different extracts tested with *Thevetia peruviana* extracts

Strains	Extracts	Correlation coefficient	Observations
Mbal	Aqueous	0.980	Highly correlated
	Methanol	0.993	Highly correlated
	Ethyl acetate	0.986	Highly correlated
	Acetone	0,954	Highly correlated
Foum	Aqueous	0.961	Highly correlated
	Methanol	0.979	Highly correlated
	Ethyl acetate	0.973	Highly correlated
	Acetone	0.990	Highly correlated

- **Fungicide or fungistatic activity of *Thevetia peruviana* seed extracts**

The *Alternaria solani* strains tested were very sensitive to the different concentrations of extracts tested. For all the extracts, only concentration C4 (100 µl/ml) proved to be fungicide on both strains in the same way as the synthetic fungicides used as reference; the other concentrations were fungistatic (Table 6).

Table 6. Fungicide or fungistatic activity of aqueous and organic extracts of *Thevetia peruviana* seeds

Extracts	Concentrations in (g/ml ou (l/ml)	Activities
Aqueous	C1, C2 and C3	Fungistatic
	C4	Fungicide
Methanol	C1, C2 and C3	Fungistatic
	C4	Fungicide
Ethyl acetate	C1, C2 and C3	Fungistatic
	C4	Fungicide
Acetone	C1, C2 and C3	Fungistatic
	C4	Fungicide

- **DISCUSSION**

Phytochemical screening revealed that aqueous and organic extracts of *T. peruviana* contain several families of bioactive compounds such as essential and saponifiable oils, coumarins, sterols, alkaloids, saponins, anthocyanins, steric glycosides and sugars. Plant

extracts of many plants with fungicide properties contain these major groups of bioactive phytochemicals as demonstrated by [9]; [27]; [28].

Spores germination was strongly inhibited by the different extracts with a total inhibition obtained with the highest concentration tested. This highly significant reduction in germination rate could be explained by the effect of secondary metabolites contained in the aqueous and organic extracts of *T. peruviana*. In addition, the present study showed the presence of sterols, saponins and essential oils which could have acted together or independently leading to an effective fungicide activity against the different strains of *A. solani* as demonstrated by [29].

The different extracts tested significantly reduced the mycelia growth of *A. solani* compared to the control with an inhibition percentage of 100 % for the C4 concentration (100 L/mL) and (100 L/mL) in the same way as the synthetic fungicides used. This reduction was more effective with the acetone extract followed by the aqueous extract than with the methanol and ethyl acetate extracts. This would also be due to the presence of different secondary metabolites in these extracts. Indeed, [30] had previously reported that plants are abundant source of various bioactive compounds, many of which are secondary metabolites serving as signal chemicals and conferring resistance to many fungal plant pathogens. [18] previously reported that *Azadirachta indica* extracts had an effective inhibitory effect in the control of *Phakopsora pachyrhizi* responsible for Asian soybean rust. These results are in line with those of [31], who evaluated the effect of aqueous and organic extracts of *T. peruviana* on the *in vitro* development of *Phytophthora colocasiae* and found that they significantly reduced the growth of the fungi at the highest concentrations.

The percentages of inhibition of the plant extracts on the growth of the pathogen varied with increasing concentrations as well as the nature of the extraction solvents. At the highest concentration C4, all extracts showed total inhibition of *Alternaria solani* spore development and germination in the same way as the synthetic fungicides: Maneb and Dimethomorpe + Clorothalonil. This could be explained by the anti-root and antioxidant activity of the anthocyanins present in the said extracts as demonstrated by [32]. In these different tests, the inhibition was more pronounced with the acetone extract followed by the aqueous extract. Acetone showed a high inhibition percentage at the lowest

concentrations on both strains. The effectiveness of this extract on the inhibition of radial growth and spore germination of the two *A. solani* strains can be explained by the fact that it is a highly water miscible compound and by the polarity of this solvent compared to the other three as demonstrated by [33]. This is due to the fact that each compound acts differently on the fungi, i.e. one compound could have a very important action on the pathogen with the acetone extract. However, this result is contrary to that of [11] who in his work on the evaluation of the antifungal activities of *T. peruviana* against *Phytophthora colocasiae* showed that the reduction in growth was more pronounced with the ethyl acetate extracts. The same is true of the work of [10] who showed that it was the methanol extract that appeared to be more effective in inhibiting the mycelia growth of *Phytophthora infestans.*

With regard to MIC50 and MIC90 it was also the acetone extract that was more effective than the other extracts. This efficacy of the acetone extract was evidenced by the lowest MIC values obtained as demonstrated by [34-35] who argued that low MIC values obtained with an extract mark its proven efficacy. This result shows a more pronounced inhibition with the Mbal strain compared to the Foum strain. This would mean that the latter is less sensitive than the Mbal strain and this difference is due to the membrane specificity of each strain. The different antifungal tests carried out revealed that the C1 to C3 concentrations of the aqueous and organic extracts of *T. peruviana* were fungistatic compared to the highest C4 concentration (100 L/mL) which was fungicide. This result confirms that *T. peruviana* extracts would possess both fungistatic and fungicide properties and is in line with that of [36] who obtained fungistatic and fungicide activities with organic extracts (acetone, ethyl acetate, methanol and hexane) of *J. curcas* seeds against *Cercospora malayensis* causal agent of Cercosporiose and insect pests of okra.

CONCLUSION

The present study revealed the significant influence of the aqueous and organic extracts of *Thevetia peruviana*, on spore germination and mycelia development of *Alternaria solani*, and it was the acetone extract that was more effective followed by the aqueous extract compared to the other extracts (ethyl acetate and methanol). For both strains, the inhibition of both parameters was proportional to increasing concentrations of the different

extracts, with total inhibition obtained with the highest concentration (100 L/mL). At this concentration, all extracts were fungicide in the same way as the two synthetic fungicides used as reference. These extracts could therefore be used as a component of a more sustainable integrated control strategy against Alternariosis in tomato. This preliminary study provides a basis for future *in situ* (field) and *in vivo* (greenhouse) trials.

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