Journal of Advances in Microbiology

Volume 24, Issue 7, Page 46-59, 2024; Article no.JAMB.119026
ISSN: 2456-7116

In vitro Development of Two *Alternaria solani* Strains, Causal Agent of Alternariose in Tomato (*Lycopersicon esculentum*) under the Influence of *Thevetia peruviana* Seeds Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jamb/2024/v24i7838

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119026

Original Research Article

Received: 01/05/2024
Accepted: 05/07/2024
Published: 10/07/2024

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Cite as: *Ngata, L. N., E. L. M. Ngonkeu, A. P. Ndogho, C. K. Ntongo, F. C.G. Gbaporo, P. Z. Ngatsi, S. L. L. Dida, L. T. V. Dongmo, T. S. Atindo, W. N. T. Kuate, and Z. Ambang. 2024. "In Vitro Development of Two Alternaria Solani Strains, Causal Agent of Alternariose in Tomato (Lycopersicon Esculentum) under the Influence of Thevetia Peruviana Seeds Extracts". Journal of Advances in Microbiology 24 (7):36-45. https://doi.org/10.9734/jamb/2024/v24i7838.*

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 controbl, 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* isolates. 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* isolates (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 isolates. The lowest MIC50 (16.63 µL/mL) and MIC90 (54.6 µL/mL) were obtained with the acetone extract on the Mbal isolate while the highest MIC50 (27.5 µL/mL) and MIC90 (61.7 µL/mL) were observed with ethyl acetate on the Foum isolate These extracts can therefore be used in the biological control against Alternariose in tomato.

Keywords: Thevetia peruviana; extract; isolates; Alternaria solani; inhibition.

1. 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 [5-8].

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 [9], 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 [10]. Among the devastating diseases of tomato fields, Alternariosis, a fungal disease caused by *Alternaria solani*, is one of the most important [11]. 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, resulting in yield losses of up to 80 % in field tomato production [12]. 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 [13]. 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 [14].

Thevetia peruviana (yellow oleander) which belongs to the Apocynaceae family, is an ornamental shrub native to tropical America and is widely cultivated throughout the tropics including tropical Africa [15]. The roots, leaves, seeds and fruits of T. peruviana are sources of biological active compounds conferring bactericides [16], virucides [17], insecticides [18,19] and fungicides [20,21,13,22] potentials.

The present work aims to test *in vitro* the antifungal potential of *Thevetia peruviana* seed extracts against two *Alternaria solani* isolates.

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves showing typical symptoms of the disease 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* isolates (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.

2.2 Obtaining of *Alternaria solani* Strains

Using the identification key of [23] 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 [24].

2.3 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 [20]. For organic extracts, 500 g of powder 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 [25,26].

2.4 Evaluation of the Effect of the Extracts on the Mycelial Growth of *A. solani* Strains

2.4.1 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.

2.5 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 isolates was assessed by measuring the two perpendicular diameters plotted on the back of the Petri dish and calculated according to the formula used by [27].

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

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 [28].

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

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

2.6 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 [27].

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.

2.7 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 [29,28].

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

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

2.8 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 [30].

2.9 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.

3. RESULTS

3.1 Characteristics of *Alternaria solani* Isolates

Macroscopic observation of the pure isolate obtained showed whitish colonies with a downy or cottony subaerial appearance with variations

in mycelia growth and regular and irregular borders (Fig. 1A). Microscopic observation showed a species with large spore conidia with a porri (Fig. 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.

3.2 Effect of *Thevetia peruviana* Seed Extracts on the *In vitro* Radial Growth of *Alternaria solani*

3.2.1 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).

3.2.2 Radial growth of individual isolates

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 (Fig. 2).

3.2.3 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 isolate, followed by the aqueous extract (with 85.32 % for the Mbal isolate and 83.33 % for that of Foum isolate). 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 (Fig. 3).

3.3 Minimum Inhibitory Concentrations MIC50 and MIC90 of Extracts

The minimum inhibitory concentrations (MICs) for growth of *A. solani* isolates 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 µL/mL and 54.6 µL/mL for the Mbal isolate. With the same extract, MIC50 and MIC90 were 18.63 µL/mL and 55.6 µL/mL for the Foum isolate. The highest minimum concentrations were recorded with the ethyl acetate extract where MIC50 and MIC90 were respectively 26.63µL/mL and 60 µL/mL for the Mbal isolate. With the same extract, MIC50 and MIC90 were 27.5 µL/mL and 60.7 µL/mL, respectively for the Foum isolate (Table 2).

3.4 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.

3.5 Effect of *Thevetia peruviana* Seed Extracts on Spore Germination of Different Isolates 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 isolate, while 50.00; 68.33; 80.00 and 100 % for were recording the Foum isolate 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 isolates 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 (Fig. 4).

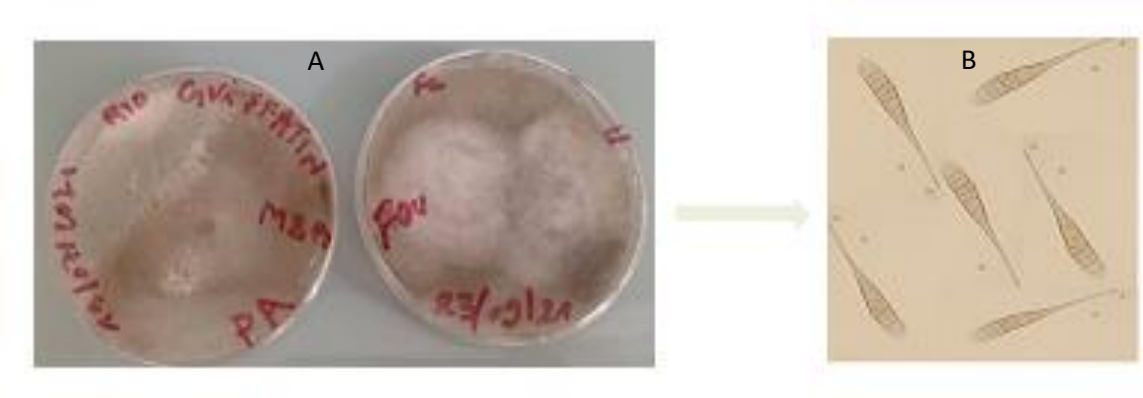

Fig. 1. Macroscopic and microscopic characteristics of pure isolates of *Alternaria solani* (A-Macroscopic aspect; B- Conidia seen by photonic microscopy at X 20 magnification)

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

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

Isolates	Extracts	MIC50	MICl90
Mbal	Aqueous (µL/mL)	17.6	55.6
	Methanol (µL/mL)	22.25	55.7
	Ethyl acetate (µL/mL)	26.63	60
	Acetone (µL/mL)	16.63	54.6
Foum	Aqueous (µL/mL)	20.13	57
	Methanol ((µL/mL)	23.5	60
	Ethyl acetate ((µL/mL)	27.5	60.7
	Acetone (µL/mL)	18.63	55.6

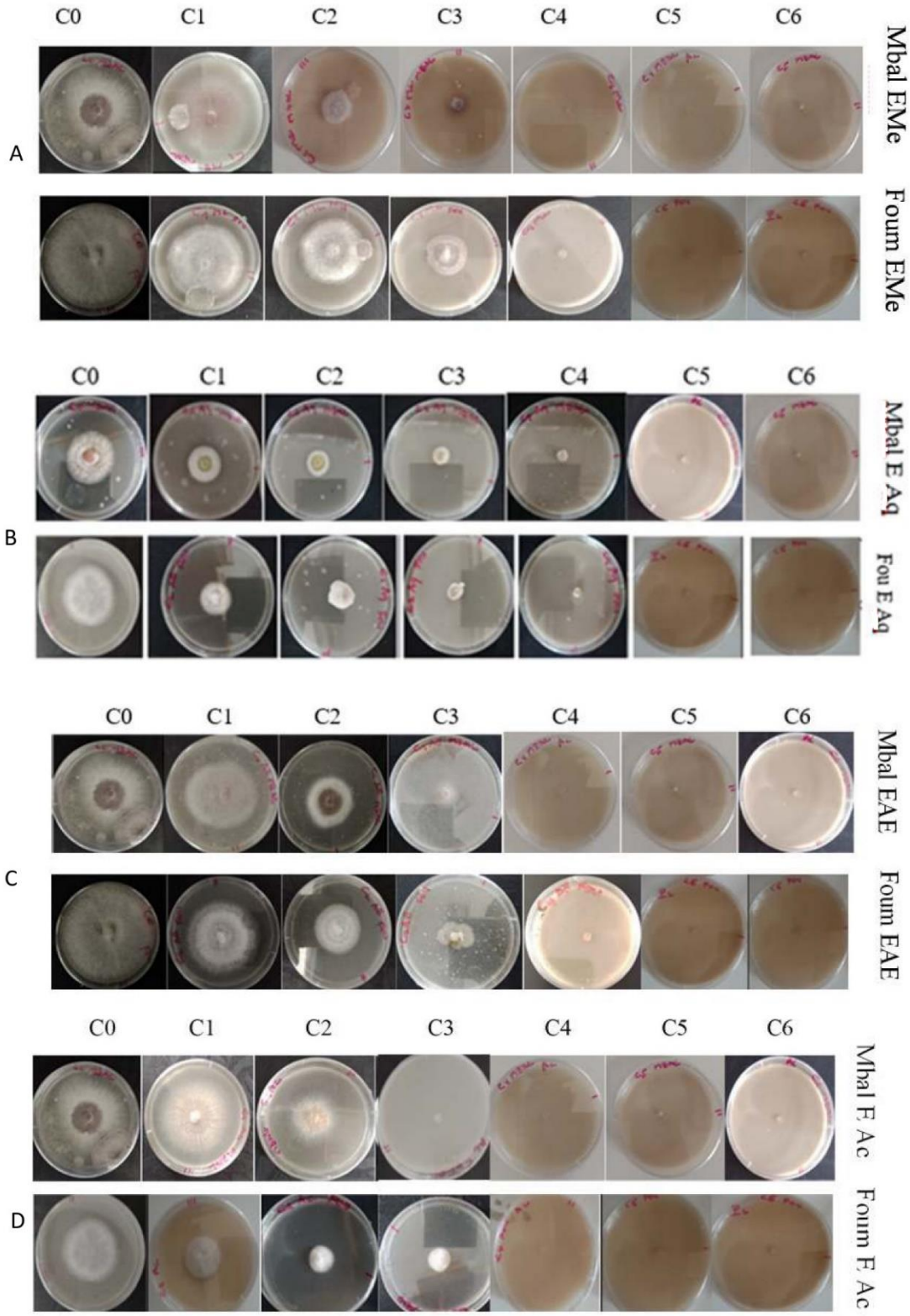

Fig. 2. Evolution of mycelia growth of *A. solani* isolates as a function of different extract concentrations on the 7th day after incubation (A- Aqueous; B- Methanol; C- Ethyl acetate and D- Acetone)

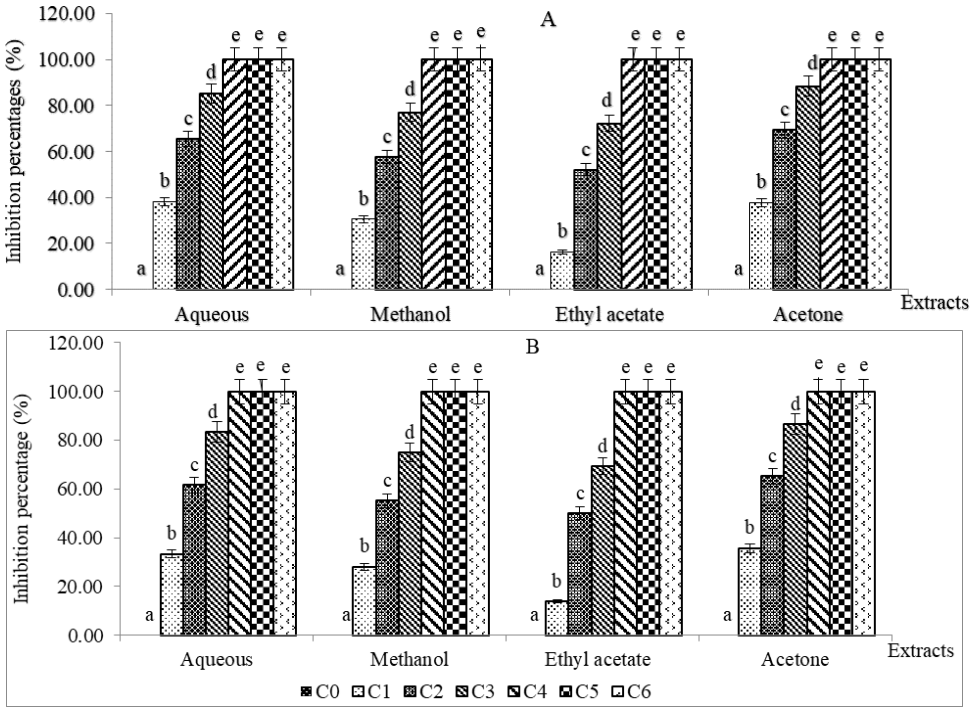

Fig. 3. Variation in the percentage of radial growth inhibition of isolates by the different extracts (A: Mbal; B: Foum) 7 JAI

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

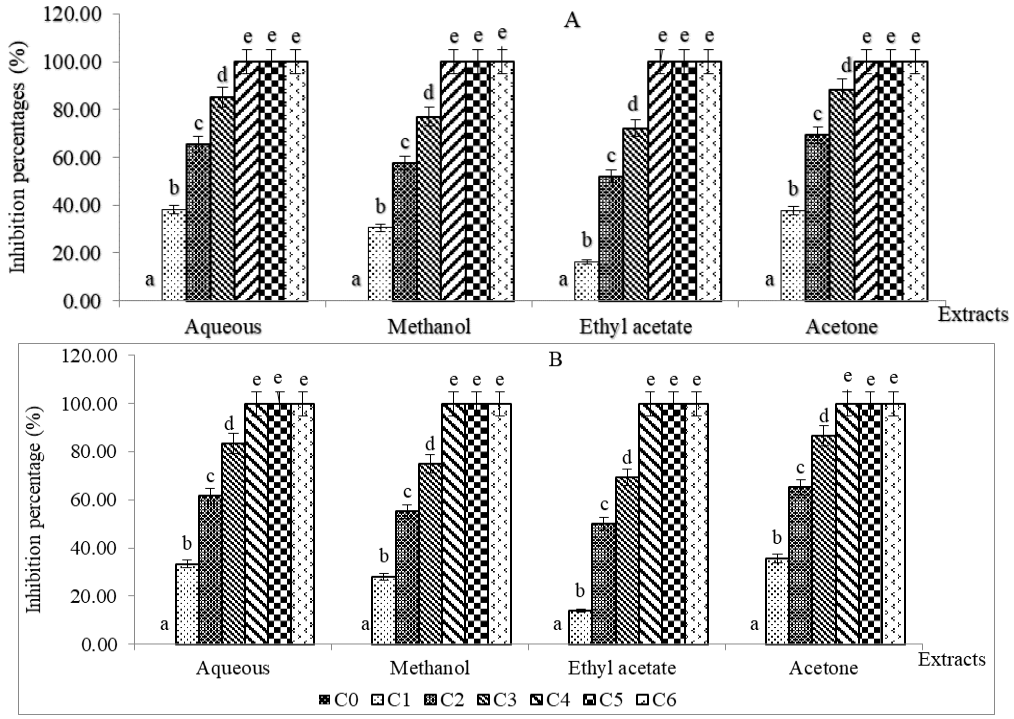

Fig. 4. Inhibition percentage of *Alternaria solani* spore germination according to the different *T. peruviana* extracts (A: Mbal isolate and B: Foum isolate)

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

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

Isolates	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

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

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

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

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

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

Extracts	Concentrations in (µg/ml ou (µl/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

3.6 Minimum Inhibitory Concentrations MIC50 and MIC90 for Germination of *Alternaria solani* Isolates

The minimum germination inhibitory concentrations of *A. solani* isolates 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 µL/mL and 53.7 µL/mL for the Mbal isolate. With the same extract MIC50 and MIC90 were 17.6 µL/mL and 56.83 µL/mL for the Foum isolate. In contrast, the highest minimum concentrations were recorded with the ethyl acetate extract where MIC50 and MIC90 were respectively 27.63 µL/mL and 62.5 µL/mL for the Mbal isolate. With the same extract MIC50 and MIC90 were 31.2 µL/mL and 66.1 µL/mL for the Foum isolate (Table 4).

3.7 Correlation between Different Concentrations and Germination Percentages of *Alternaria solani* Isolates

The regression equations obtained with the different extracts from the germination test of the two isolates 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).

3.8 Fungicide or Fungistatic Activity of *Thevetia peruviana* Seed Extracts

The *Alternaria solani* isolates 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 isolates in the same way as the synthetic fungicides used as reference; the other concentrations were fungistatic (Table 6).

4. 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 [13,31,32].

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 isolates of *A. solani* as demonstrated by [33].

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 μL/mL) and (100 μL/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, [34] 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. Ndogho [22] 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 [35], 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 [36]. 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 [37]. 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 [15] 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 [14] 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 [38,39] who argued that low MIC values obtained with an extract mark its proven efficacy. This result shows a more pronounced inhibition with the Mbal isolate compared to the Foum isolate. This would mean that the latter is less sensitive than the Mbal isolate and this difference is due to the membrane specificity of each isolate. 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 μL/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 [40] 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.

5. 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 μL/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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

Our thanks to Professor MKOUNGA Pierre and his collaborators (Dr PAGNA MAWOUMA Issah Julio and Mr. LITCHANG Martial Lamer) of the laboratory of natural products and application of organic synthesis in the Department of Organic Chemistry of the University of Yaounde I who kindly put at our disposal the necessary equipment for the realization of a pact of this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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