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Identification of some volatile antifungal compounds from *Thevetia peruviana* seed extracts and their efficacy in controlling late blight on potatoes

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Abstract: To enhance potato production through sustainable management of potato late blight caused by *Phytophthora infestans*, this study aims to control the development of late blight on potatoes field using *Thevetia peruviana* seeds extracts of analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The experimental design was a split-plot with main plots potato varieties (Manate and Cipira) and in sub-plots treatments (aqueous extract; synthetic fungicide Bravo 720 combined with an insecticide Decis 5CE; synthetic fungicide Bravo 720; methanol extract and control) was used during two cropping seasons (rainy season (RS) and dry season (DS)). Disease evolution and tuber yield were evaluated. As results, the most prevailing biochemical active compounds identified by GC-MS analysis were beta-Sitosterol (86.22%) and n-Hexadecanoic acid (19.08%) in aqueous and methanol extract, respectively. The treatments reduced late blight severity of 90% during the DS and 75% during the RS. Globally, commercial yields ranged from 0 to 13.93 t. ha^{-1} during the RS compared with 4.98 to 16.97 t/ha during the DS. Control recorded the lowest commercial yields (0 t. ha^{-1} during RS and 4.98; 5.50 t. ha^{-1} during DS, respectively for local and improved varieties. Principal Component Analysis reveals that for both seasons, aqueous and methanol extract, synthetic fungicide and combination of fungicide and insecticide were closer to yields compared to the control treatments, which were very close to disease incidence and severity. The local variety Manate was more susceptible to late blight than the improved variety Cipira. *Thevetia peruviana* extract can be considered a good source of natural antimicrobials for disease management strategies.

Keywords: potato; Late blight; *Thevetia peruviana* extracts; GC-MS analyses; inhibitory effect.

Introduction

Potato (*Solanum tuberosum* L.) is the world's most widely grown tuber crop and the global potato production is about 400 million tons annually (Zhao et al., 2020). Potato ranks as the fourth most important food crop after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.), respectively, in the world (Islam et al., 2018). In addition, potatoes produce more food per unit area than any cereal crop within short periods and various consumer products are prepared from potato (Mishra et al., 2020; Tadesse et al., 2021). Nearly two million hectares were cultivated in Africa, producing 26,534,489 tons in 2019, compared with 24,932,066 tons in 2010 (FAOSTAT, 2022). But despite the increase in potato production in the tropics, Africa only accounts for 7% of global production. This remains below its real potential, as yields are generally low, ranging from 3 to 16 $t.ha^{-1}$, while those in European countries average 25 $t.ha^{-1}$and reach 60 $t.ha^{-1}$in some countries (FAOSTAT, 2022). Potatoes have been grown in certain agro-ecological zones of Cameroon since the 19th century. With a production of 363,556 tons on an area of 21,471 ha in 2019, the potato occupies a very important place among the tubers marketed in Cameroon. Several so-called local and improved potato varieties (Cipira, Dosa, Desire, Panamera, Tubira, Cardinal, Jacop, Bambui Wonder, Mondial, Spunta, Mafor, IRAD 2005, Jacob 2006…) are grown, and numerous potato seed promotion and extension programs have been set up (Ousmane et al., 2025). The North-West and West regions are Cameroon's biggest producers, accounting for over 80% of national output. However, yields in these regions remain low (average 14 $t.ha^{-1}$) and vary according to production zone (Agri-Stat, 2017). So, despite the importance of potatoes in the national economy, total production remains below actual potential. Several factors contribute to this drop in production, such as the use of local varieties with low production potential, and the predominance of certain diseases, the most dreaded of which is late blight, which remains the real scourge. Late blight is one of the main potato diseases caused by the Oomycete *Phytophthora infestans* (Mont.) De Bary. All parts of the plant are susceptible to this oomycete. This pathogen affects leaves,

stems, and tubers, which can destroy a potato field within a few days if no control measure has been taken (Hijmans et al., 2000; Fry et al., 2015). It develops most rapidly at low temperatures and high humidity. Temperatures between 15 and 25°C and relative humidity above 90% are particularly conducive to disease development (Grewal and Panag, 2015). Control measures for this disease involve frequent applications of fungicides. However, field management of late blight with these fungicides is generally limited due to the development of resistance by *P. infestans* populations (Lal et al., 2019). The widespread use of synthetic fungicides has led to the selection of isolates resistant to these active ingredients, which mainly belong to the phenylamide group (metalaxyl and its enantiomer mefenoxam, benalaxyl, oxadixyl) (Gisi and Cohen, 1996; Chaudhary et al., 2020). The reduced efficacy of metalaxyl and maneb against late blight, for example, has been demonstrated in Cameroon (Fontem et al., 2005; Dorn et al., 2007). To find effective alternatives to this toxic and environmentally costly chemical control, many researchers have shown the importance of using plant species, not only for human health (Omolara et al., 2007) but also for agriculture, as a rich source of biopesticides that can be used for crop protection (Rabach et al., 2022). Plant extracts as biofungicide have received increased attention in recent years (Meena et al., 2021). In Cameroon, numerous studies showed the importance of controlling pathogen through the use of biofungicides to reduce the intensification of these chemical fungicides (Djeugap et al., 2023; Toka et al., 2023; Tize et al., 2024; Ndja'a et al., 2025). Among these plants, *Thevetia peruviana* (Pers.) K. Schum which belongs to the Apocynaceae family has been considered as a source of bioactive compounds which are important to control crop diseases and pests (Meena et al., 2021). Most of the research work on the bio-efficacy of botanicals against plant diseases has been limited to in vitro assays (Dida et al., 2024). Therefore, it is important to understand the performance of two potatoes varieties in different treatments based on *Thevetia peruviana* seed extracts to reduce the impact of late blight in field. The current study was conducted to control the development of late blight on potatoes field using *Thevetia peruviana* extracts analyzed by Gas Chromatography Mass Spectophotometry (GC-MS)

Results

Gas Chromatography Mass Spectrometry (GC-MS) analysis

GC-MS of aqueous extract and methanol extract of *Thevetia peruviana* confirmed the presence of various volatiles compounds with different retention time (Table 1). A total of 25 phytochemical compounds were identified in both extracts. The identification was based on the molecular weight, peak area, mass spectrometry fragmentation and retention time. The most prevailing bioactive compounds in AqE were beta-Sitosterol (86.22%) followed by; n-Hexadecanoic (5.95%); Thietane, 2,4-Dimethyl- acid (3.14%) and Hexadecanoic acid, methyl ester (3.02%). While, the least prevailing compound was alpha.-L-Galactopyranoside, methyl 6-deoxy- (1.66%). For ME the most prevailing compound were (E)-9,12-Octadecadienoic acid (Z,Z)- (33.23%) followed by n-Hexadecanoic acid (19.08%) ; (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (12.76%) and 9-Octadecenoic acid, methyl ester (9.05%) while the least prevailing compound were Docosanoic acid (0.39%); 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (0.46%); 2,3-dihydroxypropyl ester (0.56%); cis-13-Eicosenoic acid; 9-Octadecenal (0.58%); 9-Octadecenoic acid (Z)-, (1%).

The most reported and identified major compounds with their chemical structures and their biological properties in AqE and ME of *T. peruviana* seeds were presented in table 1. The phytoconstituents present in different extracts of *T. peruviana* seeds were from the families of terpenoids and fatty acids esters

Table 1. The most reported and identified major compounds in aqueous and methanol extracts of *Thevetia peruviana* seeds with biological activities

Extracts	Peak	RT(min)	Area (%)	Name of the compound	Biological activity	References
Aqueous	1	1.619	1.66	alpha.-L-Galactopyranoside, methyl 6-deoxy-	Undetermined	-
	2	2.311	3.02	Thietane, 2,4-dimethyl-	Undetermined	-
	3	4.217	3.14	Hexadecanoic acid, methyl ester	Undetermined	-
	4	4.302	5.95	n-Hexadecanoic acid	Antifungal, Antioxidant, Antibacterial and antiviral	Agoramoorthy *et al*. (2007) Ghaidaa et al. (2016)
	5	4.755	86.22	beta.-Sitosterol	Anti-inflammatory pesticide, antibacterial And fungicidal	Mbambo *et al*. (2012) Njinga et al. (2016) Khan & Javaid (2020). Alawode et al. (2021)

Methanol	1	2.163	0.91	5-Hydroxymethylfurfural	Antifungal	Ojinnaka *et al*. (2015)
	2	2.328	3.60	5-Hydroxymethylfurfural	Antifungal	Ojinnaka *et al*. (2015)
	3	3.072	2.21	Ethanedithioamide, N,N'-diethyl-	Undetermined	-
	4	2.328	3.36	.alpha.-D-Glucopyranoside, methyl	Undetermined	-
	5	4.211	4.15	Hexadecanoic acid, methyl ester	Antibacterial, Antioxidant and antifungal	Chandrasekaran *et al*. (2011)
	6	4.371	19.08	n-Hexadecanoic acid	Antifungal, Antioxidant, Antibacterial and antiviral	Agoramoorthy *et al*. (2007) Ghaidaa *et al*. (2016)
	7	4.709	9.05	9-Octadecenoic acid, methyl ester, (E)-	Antimicrobial, Nematicidal	Chandrasekharan *et al.* (2008); Lima *et al*. (2011) Ali *et al*. (2016)
	8	4.755	0.97	Methyl stearate	Undetermined	-
	9	4.875	33.23	9,12-Octadecadienoic acid (Z,Z)-	Undetermined	-
	10	4.983	3.01	Octadecanoic acid	Undetermined	-
	11	5.109	0.39	9,12-Octadecadienoic acid (Z,Z)-	Antibacterial and antifungal	Hong-Wei *et al*. (2014)
	12	5.533	0.89	Eicosanoic acid	Undetermined	-
	13	6.185	2.19	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Undetermined	-
	14	6.385	0.39	Docosanoic acid	Undetermined	-
	15	6.494	0.58	cis-13-Eicosenoic acid	Undetermined	-
	16	7.038	1	9-Octadecenal, (Z)-	Undetermined	-
	17	7.301	12.76	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Undetermined	-
	18	7.329	1.20	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antimicrobial	Agoramoorthy *et al*. (2007)
	19	7.381	0.56	Octadecanoic acid, 2,3-dihydroxypropyl ester	Undetermined	-
	20	7.575	0.46	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Undetermined	-

Isolation and Identification of Phytophthora infestans

The macroscopic and microscopic (Fig. 1) characters of pure strains revealed that the *P. infestans* isolates were aerial aseptate mycelium, which were sometimes dense and whitish in color (Fig 1a). These mycelium present sporangia characteristic of *P. infestans* (Fig 1b).

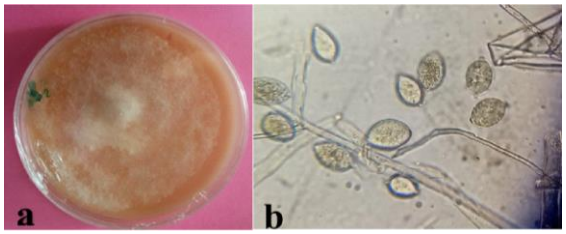

Fig 1. Macroscopic and microscopic characters of a pure 14-day-old HPMN02 isolate of *P. infestans* from Ngui locality on V8 medium (a) and sporangia (b) seen with a photonic microscope (magnification X 100).

Effect of Thevetia peruviana extracts on late blight development in the field during two growing seasons
Incidence of late blight in plots treated with Thevetia peruviana extracts

The different rates of disease expansion determined for each variety in each treatment varied significantly ($P < 0.05$) between treatments over time (Weeks After Planting (WAP)) and over the two cropping seasons (Table 2). These rates were very high in the plots during the campaign from March to June 2018 (Rainy Season (RS)).

No significant difference ($P > 0.05$) was observed for interaction variety*treatment in both cropping seasons. A significant difference ($P < 0.05$) was registered between treatments eat 6, 8 and 10 WAP, while the variety effect was significant ($P < 0.05$) at 10 WAS during the rainy season. The incidence of late blight was higher in the rainy season (RS) than in the dry season (DS). Aqueous (AqE) and methanol (ME) extracts of *T. peruviana* reduced late blight incidence to the same extent as the synthetic fungicide treatment (F) compared with the control. The incidence of late blight was identical in both varieties.

Table 2. Evolution of late blight incidence (%) on potato plants.

Varieties	Treatments	6 WAP		8 WAP		10 WAP	
		RS	DS	RS	DS	RS	DS
V1	C	53.00 ± 12.48 c	28.33 ± 2.88 d	76.66 ± 7.09 c	65.00 ± 13.22 b	100.00 ± 0.00 f	100.00 ± 0.00 d
	AqE	14.66 ± 2.08 a	15.00 ± 5.00 bc	34.00 ± 5.00 ab	21.66 ± 7.63 a	79.66 ± 3.51 cde	68.33 ± 7.63 ab
	FI	11.66 ± 2.51 a	1.66 ± 2.88 a	24.66 ± 5.77 a	13.33 ± 5.77 a	62.00 ± 5.56 ab	51.66 ± 2.88 a
	F	18.66 ± 4.61 a	5.00 ± 5.00 ab	33.33 ± 5.50 ab	16.66 ± 2.88 a	76.66 ± 6.65 bd	66.66 ± 10.40 ab
	ME	18.66 ± 5.03 a	15.00 ± 5.00 bc	45.66 ± 5.13 b	21.66 ± 2.88 a	94.00 ± 5.29 ef	76.66 ± 5.77 bc
V2	C	38.33 ± 3.21 bc	20.00 ± 5.00 cd	78.33 ± 2.88 c	63.33 ± 7.63 b	100.00 ± 0.00 f	91.66 ± 7.63 cd
	AqE	20.00 ± 1.00 a	6.66 ± 2.88 ab	32.33 ± 2.30 ab	23.33 ± 2.88 a	71.33 ± 5.50 ad	68.33 ± 7.63 ab
	FI	10.00 ± 2.64 a	3.33 ± 5.77 ab	21.66 ± 4.5 a	10.00 ± 5.00 a	57.33 ± 6.65 a	53.33 ± 10.40 a
	F	16.33 ± 5.03 a	3.33 ± 5.77 ab	37.33 ± 4.16 ab	13.33 ± 5.77 a	69.33 ± 5.50 abc	65.00 ± 5.00 ab
	ME	22.33 ± 8.62 ab	6.66 ± 2.88 ab	44.66 ± 14.84 b	20.00 ± 5.00 a	84.66 ± 6.42 de	70.00 ± 8.66 ab
Interactions		0.05447 *	0.197097 ns	1.0000 ns	0.9635 ns	0.537693 ns	0.7196 ns
Varieties		0.38504 ns	0.006154 **	0.8933 ns	0.4960 ns	0.004765 **	0.2739 ns
Treatments		<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

ns: not significant; WAP = weeks after planting; RS = rainy season and DS = dry season.

Disease severity in plots treated with Thevetia peruviana extracts

The intensity of disease infection varied according to observation time, cropping seasons, treatments and varieties (Table. 3). All interactions between varieties and treatments are non-significant ($P > 0.05$) during all observation periods (6; 8 and 10 WAP). The treatment and variety effects were significant in both growing seasons. Aqueous and methanol extracts greatly reduced disease severity, as did chemical fungicide treatments compared with the control, in all observation periods (6; 8 and 10 WAP) and in both growing seasons (RS and DS). Improved variety Cipira appears to be more tolerant than local variety Manate to the various antifungal treatments.

The physical aspect of the plots in the field 10 weeks after planting (WAP) and treatments shows plants totally destroyed by late blight in some plots (Fig 2). Young potato plants are totally destroyed by late blight in the control plots during the rainy season (Fig 2A), while during the dry season, the control plots are not totally destroyed (Fig 2C). On the other hand, plants treated with extracts (Fig 2B and 2D) were apparently healthy.

Effect of Thevetia peruviana extracts on potato tuber yields

The yield in number of tubers per plant (Table 4) and the yield in weight of commercial and non-commercial tubers in tons per hectare (Table 5) were determined in order to assess the effect of aqueous seed extracts on yield gain compared with synthetic fungicides and the absolute control.

Table 3. Evolution of late blight severity on potato plants according to treatments and varieties.

Varieties	Treatments	6 WAP		8 WAP		10 WAP	
		RS	DS	RS	DS	RS	DS
V1	C	29.37 ± 4.64 c	6.11 ± 1.92 a	78.53 ± 6.39 b	29.65 ± 4.68 d	100.00 ± 0.00 d	58.16 ± 6.00 d
	AqE	8.58 ± 1.29 ab	5.83 ± 0.72 a	12.11 ± 1.74 a	8.88 ± 1.73 ac	24.36 ± 3.40 ab	14.47 ± 1.18 ab
	FI	6.47 ± 0.97 ab	1.66 ± 2 .88 a	8.21 ± 0.66 a	5.83 ± 1.44 ab	20.39 ± 2.15 a	8.21 ± 0.36 a
	F	8.60 ± 0.63 ab	5.41 ± 0.72 a	12.09 ± 2.55 a	8.05 ± 0.47 ac	23.00 ± 2.38 ab	9.94 ± 2.02 ab
	ME	12.34 ± 1.38 b	7.52 ± 2.14 a	15.39 ± 3.60 a	12.66 ± 0.28 c	30.77 ± 2.83 c	16.90 ± 1.73 b
V2	C	24.91 ± 4.28 c	5.66 ± 1.15 a	68.43 ± 2.83 c	25.08 ± 3.13 d	100.00 ± 0.00 d	50.48 ± 3.92 c
	AqE	9.08 ± 1.10 ab	2.50 ± 2.50 a	11.30 ± 1.57 a	8.16 ± 0.76 ac	23.52 ± 0.83 ab	12.52 ± 0.46 ab
	FI	5.36 ± 1.50 a	1.66 ± 2.88 a	8.04 ± 1.34 a	3.33 ± 2.88 a	18.22 ± 2.50 a	7.39 ± 0.43 a
	F	6.84 ± 1.57 ab	1.66 ± 2.88 a	10.55 ± 0.65 a	6.25 ± 1.25 ac	21.82 ± 2.59 a	8.98 ± 1.56 a
	ME	9.77 ± 0.48 ab	3.33 ± 5.77 a	15.26 ± 0.28 a	11.55 ± 1.50 bc	28.50 ± 2.06 bc	14.49 ± 0.44 ab
Interactions		0.4131 ns	0.278275 ns	0.02419 *	0.60463 ns	0.8852ns	0.149427 ns
Varieties		0.0320 *	0.005355 **	0.02032 *	0.01622 *	0.1213 ns	0.006894**
Treatments		<0.001***	0.015622 *	<0.001***	<0.001***	<0.001***	<0.001***

ns: not significant; WAP = week after planting; RS = rainy season and DS = dry season.

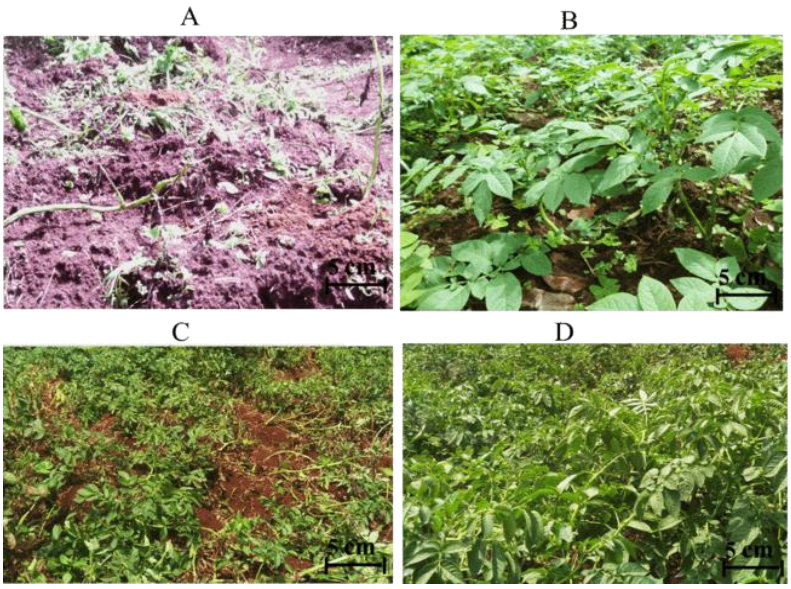

Figure 2. Phytosanitary aspect of plots control and FI ten weeks after planting during the two growing seasons. A: control plot during the rainy season; B: plot treated with aqueous extracts (AqE) during the rainy season; C: control plot during the dry season; B: plot treated with aqueous extracts AqE during the dry season.

As for the results obtained for the number of tubers per plant parameter, the control plots recorded the lowest numbers of apparently healthy tubers per plant, with zero during the rainy season for both varieties (Table 4). Plots that received phytosanitary treatments AqE; FI; F and ME had much higher numbers of tubers than the control plots. The number of apparently healthy tubers per plant was relatively low during RS, ranging from 0 to 12.73, compared with 4.55 to 13.99 during DS. Only the treatment effect was significant ($P < 0.05$) for the number of tubers per plant parameter in both cropping seasons and all observation periods. As for yields in tons per hectare, no significant difference ($P > 0.05$) was recorded with interactions varieties and treatments during all observation periods (6; 8 and 10 WAS). The treatment effect was significant ($P < 0.05$) in both growing seasons, while the variety effect was significant on commercial yields only in the rainy season. Commercial yields were low overall in the plots during the rainy season compared with the dry season (Table 5). Commercial yields ranged from 0 to 13.93 $t.ha^{-1}$ during the RS compared with 4.98 to 16.97 $t.ha^{-1}$ during the DS. Control plots recorded the lowest commercial yields (0 $t.ha^{-1}$ during RS and 4.98; 5.50 $t.ha^{-1}$ during DS respectively for local variety Manate and improved variety Cipira) compared with plots AqE; FI; F and ME. Extracts significantly increased commercial yield compared with controls in both growing seasons. Aqueous extracts recorded relatively higher commercial yields than methanol extracts. In all cases, both extracts (AqE and FI) significantly increased commercial yields (by up to 100%), as did treatments with chemical fungicides compared with the control.

Table 4. Yield as number of tubers per plant (NTP) according to treatments and varieties. RS = rainy season and DS = dry season; ns: not significant.

Varieties	Treatments	Number of tubers per plant	
		RS	DS
V1	C	0.00 ± 0.00 a	3.11 ± 0.84 a
	AqE	9.22 ± 1.29 b	10.11 ± 1.16 b
	FI	12.19 ± 1.24 c	13.44 ± 1.01 b
	F	9.38 ± 1.30 b	13.99 ± 2.96 b
	ME	9.43 ± 0.55 b	11.33 ± 1.45 b
Means	V1	8.04 ± 4.40 A	10.39 ± 4.28 A
V2	C	0.00 ± 0.00 a	3.21 ± 1.28 a
	AqE	10.20 ± 0.35 bc	12.66 ± 0.57 b
	FI	12.73 ± 0.42 c	12.33 ± 4.48 b
	F	11.16 ± 0.38 bc	13.33 ± 2.02 b
	ME	9.15 ± 1.43 b	11.77 ± 0.50 b
Means	V2	8.65 ± 4.67 A	10.76 ± 4.34 A
Interactions		0.30532 ns	0.5705 ns
Varieties		0.07433 ns	0.7209 ns
Treatments		<0.001***	<0.001***

Non-commercial yields were recorded in all plots. These yields were low in the RS plots, ranging from 0.51 to 1.97 $t.ha^{-1}$, compared with 1.31 to 3.26 $t.ha^{-1}$ in the DS plots.

Table 5. Potato yields according to treatments and varieties. ns : not significant; RS = rainy season and DS = dry season.

Varieties	Treatments	Yields in t/ha			
		RS		DS	
		Commercial	Non-commercial	commercial	Non-commercial
V1	C	0.00 ± 0.00 a	0.51 ± 0.14 a	4.98 ± 1.78 a	2.10 ± 0.67 ab
	AqE	10.99 ± 0.40 bd	0.97 ± 0.25 ad	12.73 ± 0.41 bc	2.67 ± 0.83 ab
	FI	12.47 ± 0.49 de	0.81 ± 0.22 ac	15.08 ± 0.35 bd	1.31 ± 0.54 a
	F	9.52 ± 1.25 b	1.36 ± 0.17 d	13.39 ± 1.25 bc	2.34 ± 0.43 ab
	ME	9.49 ± 0.72 b	1.45 ± 0.17 d	12.23 ± 0.53 b	2.45 ± 0.10 ab
Means V1		8.49 ±4.58 A	1.02 ± 0.39 A	11.68 ± 3.71 A	2.17 ± 0.68 A
V2	C	0.00 ± 0.00 a	0.66 ± 0.05 ab	5.50 ± 1.11 a	3.04 ± 0.88 ab
	AqE	11.81 ± 0.55 d	1.05 ± 0.07 bcd	15.57 ± 1.77 cd	2.11 ± 0.35 ab
	FI	13.93 ± 0.70 e	0.77 ± 0.16 ac	16.97 ± 0.80 d	1.39 ± 0.16 a
	F	11.37 ± 0.65 cd	1.19 ± 0.21 cd	14.43 ± 0.46 bd	2.85 ± 0.90 ab
	ME	9.97 ± 0.50 bc	1.97 ± 0.17 e	12.98 ± 0.75 bc	3.26 ± 0.65 b
Means V2		9.41 ± 5.07 A	1.13 ± 0.49 A	13.09 ± 4.25 A	2.53 ± 0.90 A
Interactions		0.1220550 ns	0.03301 *	0.32981 ns	0.251114 ns
Varieties		0.0007184 ***	0.10143 ns	0.00158 **	0.130328 ns
Treatments		<0.001 ***	<0.001 ***	<0.001 ***	0.004266 **

Principal component analysis and classification of treatments according to the different parameters studied in the field

Principal component analysis (PCA) was used to group the different treatments for each potato variety (CV1. AqEV1. FV1. FIV1. MEV1; CV2. AqEV2. FV2. FIV2. MEV2) on the basis of three variables, namely incidence (I), severity (S) and yield (Y) at three observation periods (6, 8 and 10 weeks after planting) (Figure 3). During both seasons (RS and DS), the system returned fairly reliable information, with a good rate of return of information on total variability on axes 1 and 2 (93.15%). FV1 and FV2 treatments and AqEV1, FIV1, MEV1. AqEV2. FIV2 and MEV2 are closer to yields (CY RS and CY DS) with a trend very close to treatments AqEV1. FIV1. MEV1. AqEV2. FIV2 and MEV2 for non-commercial yields (NCY RS and NCY DS). On the other hand, the CV1 and CV2 control treatments are very close to the epidemiological parameters studied during the three observation periods (I 6 RS. I 6 DS. I 8 RS. I 8 DS. I 10 RS. I 10 DS. S 6 RS. S 6 DS. S 8 RS. S 8 DS. S 10 RS and S 10 DS). This analysis also reveals that the V1 variety is closer to severity than the V2.

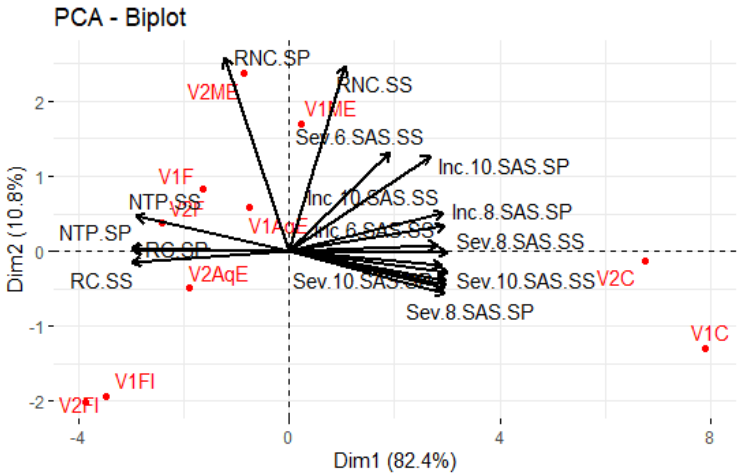

Figure 3. Principal component analysis of the various field parameters studied.

I 10 RS = incidence 10 weeks after planting in rainy season; I 10 DS = incidence 10 weeks after planting in dry season; S 10 RS = severity 10 weeks after planting in rainy season; S 10 DS = severity 10 weeks after planting in dry season; NTP RS = number of tubers per plant in rainy season ; NTP DS = number of tubers per plant in dry season; CY RS = commercial yield in rainy season; NCY RS = non-commercial yield in rainy season; CY DS = commercial yield in dry season; NCY DS = non-commercial yield in dry season; CV1, AqEV1, FV1, FIV1, MEV1, CV2, AqEV2, FV2, FIV2 and MEV2 = treatments (C, AqE, F, FI, ME) for each variety (V1 and V2)

The classification of these different treatments according to these different parameters studied using a dendrogram (5% dissimilarity) reveals three groups of treatments (Figure 4). The first group comprises the control treatments CV1 and CV2. The second group comprises treatments AqV1, FIV1, MEV1 and MEV2 and the third group is made up of the control treatments AqEV2, FIV2, FV1 and FV2.

In general, these analyses reveal that treatments with *Thevetia peruviana* extracts (AqE and ME) reduced the incidence and severity of late blight and increased yields in the same way as the chemical fungicide treatment (FI) during both growing seasons.

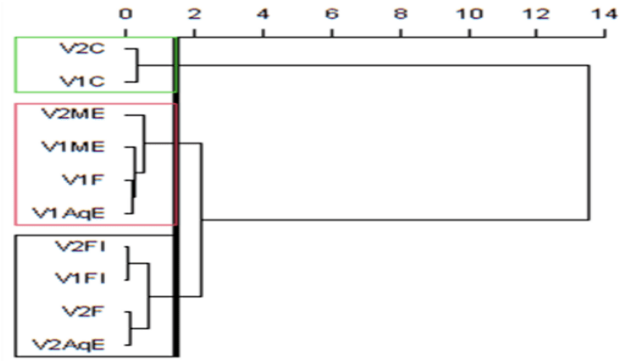

Figure 4. Cluster classification of different treatments according to their effects. FIV1, MEV1, CV2, AqEV2, FV2, FIV2 and MEV2 = treatments (C, AqE, FI, F, ME) for each variety (V1 and V2)

Discussion

A simple method for identification of *Phytophthora infestans*, permit us to confirm the potato late blight as a major disease on fields experimentation. According to Hussain et al. (2015), appropriate diagnostic of potato late blight was considered to be the most important issue in designing the proper management plans for potato diseases. The macroscopic and microscopic characters of pure isolates obtained *in vitro* revealed that the *P. infestans* isolates were aerial aseptate mycelium, which were sometimes dense and whitish in color. These mycelium present sporangia characteristic of *P. infestans* as described by Dida et al. (2020). Gas chromatography-mass spectrometry (GC-MS) analysis of aqueous and methanol extracts of *Thevetia peruviana* seeds revealed the presence of 25 several volatile compounds from the families of terpenoids and fatty acids esters. Some of these compounds showed to possess biopesticide potential. These include beta-sitosterol (Mbambo et al., 2012; Njinga et al., 2016; Khan and Javaid, 2020; Alawode et al., 2021); n-Hexadecanoic acid (Agoramoorthy et al., 2007; Ghaidaa et al., 2016); Exadecanoic acid, methyl ester (Chandrasekaran et al., 2011); (E)-9,12-Octadecadienoic acid (Z,Z)- (Hong-Wei et al., 2014); 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (Agoramoorthy et al., 2007); 9-Octadecenoic acid, methyl ester (Chandrasekharan et al., 2008; Lima et al., 2011); 5-Hydroxymethylfurfural (Ojinnaka et al., 2015). The most prevailing biochemical active compounds was beta.-sitosterol, (86.22%) and 9,12-Octadecadienoic (33.23%) from AqE and ME, respectively. This difference can be explained by the fact that solvents dissolve compounds based on their polarity. Differences in solvent polarities have been reported to account

for differences in solubility of active plant principles (Ngo et al., 2017). Priya et al. (2014) identified eighteen phytochemical constituents from the ethanolic extract of the leaves of *Desmodium gyrans* by Gas chromatography Mass spectrometry (GC-MS). Aja et al. (2014), working on GC/MS analysis of *Moringa oleifera* leaf and seed revealed that 9-octadecenoic acid (20.89%) was the major constituent while oleic acid (84%) was the major biocomponent. In the field, late blight appeared very early in the different plots (18 days after planting during the rainy season and 27 days after planting during the dry season) on young potato plants. This could be explained that climatic conditions influence the development of the disease. Field results on disease expansion rates (incidence) and infection intensity (severity) show that the aqueous (AqE) and methanol (ME) extracts significantly reduced disease severity on a par with chemical fungicide treatments compared with the control in all observation periods (6, 8 and 10 WAP) and during both cropping seasons (rainy and dry). The antifungal power of this natural bioproduct was thus similar to the results of Ambang et al. (2007), who showed that *T. peruviana* extracts reduced the epidemic parameters of Cercosporiosis in field peanuts compared with the control. In general, all the phytosanitary products tested reduced the expansion and intensity of the disease. Mekonen and Tadesse (2018) showed that synthetic pesticides combined with the genetic capacities of varieties significantly reduced the severity of late blight in field. However, at 10 WAP, high rates of incidence were recorded in all plots. The control plots showed 100% incidence in both varieties over the two growing seasons. The extent of plant contamination in the trial is thought to be due to wind and high rainfall, which favors the spread of sporangia and spores during the rainy season. Severity rates were low in the control plots (below 55%) during the dry season, compared with the rates recorded in the control plots (100%) during the rainy season. Similarly, the products applied (AqE, FI, F and ME) reduced late blight severity by an average of 90% during the dry season, compared with an average 75% reduction in severity during the rainy season. These differences could be explained by the fact that unfavorable climatic conditions during the dry season increased the efficacy of the phytosanitary products tested, as well as plant resistance to *P. infestans* in the control plots. The aerial parts of the plants (leaves and stems) in the control plots were completely destroyed by late blight 10 WAP (100% severity) during RS, in contrast to dry season. Shrestha et al. (2019) obtained similar results 58 days after planting on the potato variety Kufri Jyoti in their work on evaluating the resistance of 07 local potato varieties against late blight in Nepal. Indeed. Harrison (1992) has shown that humid conditions favor the disease (late blight) and high humidity (over 90%) accelerates the development of sporangia, which germinate rapidly on wet leaves; whole plants can be killed in a very short space of time. However, this level of severity, including the so-called tolerant improved variety (Cipira), could confirm the emergence of new *P. infestans* strains as revealed by phenotypic characterization (presence of both sexual types). The efficacy of the different extracts on fields control of late blight would be due to the presence of the bioactive compounds revealed by GC-MS analysis. GC-MS of extracts (AqE and ME) of *Thevetia peruviana* seeds revealed the presence of some major antifungal compounds like beta-sitosterol, Hexadecanoic acid, methyl ester (Dida et al., 2024; Mbega et al., 2024). Morever, GC-MS chromatogram realized by Meena et al. (2021) showed the presence of benzoic acid and oxo-benzoate in active fraction of *T. peruviana* leaf extract which is already known chemical among the phytochemicals described for antifungal activity. This phytochemicals compounds in plant extracts could inhibit potato late blight by acting on metabolic functions such as biosynthesis of pathogen cell wall components and inhibiting cell wall synthesis (Da et al., 2019). The *T. peruviana* extracts tested greatly increased yield in terms of number of tubers per plant, as well as commercial yield in tons per hectare, as did Bravo 720 compared with the control, in both cropping seasons. These high yields in the different plots was certainly due to the disease reduction provided by the aqueous extracts of *T. peruviana* seeds and the synthetic pesticides combined with the genetic capacities of the varieties tested, thus coming closer to the results of Siddique et al. (2016) in similar research work on the fungicides effective in controlling late blight. Jang and Kuk (2019) reported that plant extracts had growth stimulating effects on various crops. Djeugap et al. (2023) showed that field application at 134 g/L concentration of aqueous extract of *Euphorbia hirta* could be used as a biofungicide for rice brown spot management. The same outcomes research work with *Tephrosia vogelii* and *Tithonia diversifolia* extract applied as foliar sprays on beans at 10% increased the chlorophyll content, the number of pods per plant bean and the overall seed yield (Mkindi et al., 2020). No yields were obtained in the control plots, and low yields (less than 11 $t.ha^{-1}$) in the plots treated with plant protection products during the rainy season, in contrast to the control plots during the dry season (4.98 ± 1.78 to 5.50 ± 1.11 $t.ha^{-1}$for V1 and V2 respectively). The high aggressiveness of the pathogen and the favorable climatic conditions for the pathogen completely destroyed the potato plants before the end of the vegetative cycle of both potato varieties during the rainy season. This was not the case during the dry season, when the favorable climatic conditions and the plant's resistance capacities limited the aggressiveness of the pathogen. No yields observed for both varieties in the control plots confirm the devastating effect of late blight, which can go as far as total yield loss, as Fontem et al. (2005) stated in similar research on potato late blight. According to Dida et al. 2020, This result can be explained by the presence of new population of A2 mating type in the *P. infestans* population in Cameroun which are more competent and aggressive in attacking the crop and causing the damage to potato tuber in field. These yields are very low compared with those of Habtamu et al. (2012) who, despite the high severity recorded in the plots during the rainy season (up to 71%), obtained much higher commercial yields in the control plots which ranged from 16.7 to 19.8 $t.ha^{-1}$with an average of 21 $t.ha^{-1}$in the plots that received phytosanitary products in Ethiopia at the Hawassa site. This difference could be explained by the difference in the study sites, the varieties used, the fungicide active ingredients used and the frequency of spraying, as well as the different levels of aggressiveness of the pathogen. The Cipira variety performed better than the local variety during both seasons. This difference may be due to the genetic capacity of these varieties.

Materials and methods

Preparation of Thevetia peruviana extracts

Thevetia peruviana fruits were collected and identified from the National herbarium in Cameroon. To collect the seeds, fruits were harvested and the nuts broken. The seeds were dried at laboratory temperature (25 ± 2 °C) for 14 days and then grinded using a hand mill grinder. The powder obtained was weighed and then added to methanol (organic extract) or water (aqueous extract) at concentration of 500 g/L. The mixture was macerated for 48 hours in a 5 L container (Kumar, 2003). After maceration, the mixture was filtered through filter paper. To remove the solvent for organic extract, the filtrate is evaporated using a Rotavapor at 60°C under vacuum and stored at 4°C for use in the laboratory. The filtrate of aqueous extracts was used directly in fields

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

After preparation of the various extracts, 1.5 g of anhydrous sodium acetate and 6 g of magnesium sulphate previously autoclaved and cooled in a desiccator were added to 10 mL of solvent (Acetonitrile) and 10 mL of aqueous extract. The mixture was vortexed and centrifuged at 4000 rpm to obtain the supernatant. Then 8 mL of supernatant were transferred to a flask to perform GC-MS analysis. On the other hand, 6 g of anhydrous magnesium sulphate were added to 8 mL of methanol extract and introduced directly into a bottle for GC-MS. GC-MS analysis was carried out on Agilent GC 7890A gas chromatograph interfaced to a mass spectrometer (Agilent 5975 C TAD VL MSD) employing the following conditions: Column Elite-1 fused silica capillary column (30 m x 0.25 mm with 0.25 µm film thickness, composed of 5% phenyl methyl silox), operating in Electron multiplier volts1329.412 eV; Helium was used as the carrier gas (1.5 mL/min) and an injection volume of 1 µL was employed (split ratio of 10:1); Injector temperature of 150 °C; Ion-source temperature of 250 °C. The oven temperature was programmed from 35 °C (isothermal for 5 min.), with an increase of 4 °C/min, to 150°C, for 2min, then 20 °C/min to 250 °C, for 5 min. Mass spectra were taken at average velocity of 44.297 cm/sec; a hold up time of 1.1287 min, pressure of 11.604 psi and frequency of 50 Hz (Oyekunle, 2017). Total GC running time was 13.44 min. The biochemical active compounds from different extracts were identified by comparing their spectra with those of the database of National Institute Standards and Technology (NIST) having more than 62,000 patterns.

Evaluation of the antifungal potential of Thevetia peruviana seed extracts in the field

Study site

The study was carried out in the locality of Tola (623307 N and 628303 E) located in the Babadjou in the West region of Cameroon during two cropping seasons. One campaign during the rainy season (RS) which runs from March to June 2018 and another campaign during the dry season (DS) which runs from November to February 2019. The preparation of the area to be used consisted in delimiting it using a decameter, and after clearing the site using a machete, the grasses were piled up in a fallow plot. Then, using hoes, the experimental designs was put in place.

Experimental design and culture conditions

The experimental design was a split-splot into completely randomized blocks with three repetitions. The varieties constituting the main plots randomly in blocks with two variants (V1 = local variety ''Manate'' and V2 = improved variety Cipira). Five treatments (Control, AqE: aqueous extract, FI: fungicide + insecticide, F: fungicide and ME: methanol extract) represented the sub-plots randomized in main plots. The blocks are spaced 1.5 m apart and the plots by 1 m apart. Planting was carried out manually, with an equivalent seeding rate of 1.2 $t.ha^{-1}$ at a spacing of 50 cm x 25 cm (50,000 $plants.ha^{-1}$), and planting depths of 7 to 10 cm. As the previous crop was potatoes, a mixture of fertilizers (urea 130 kg/ha, triple superphosphate 400 kg/ha and potassium sulphate 200 kg/ha) was spread on the soil 2 days before planting to restore its fertility. Manual weeding was carried out every two weeks starting 21 days after planting. The aim of manual weeding was to avoid weed competition for light, water and nutrients. In potato cultivation, quality weeding also eliminates possible reservoirs of pathogens (nematodes, bacteria, viruses, insects), and facilitates harvesting.

Soil sample, bating and Isolation

Soil samples were collected from main potato production basins of Cameroun. Five individual samples were taken arbitrarily to a depth of 15 cm and mixed to represent a single composite, with a final volume of 100 cm^3 (Hussain et al., 2015). *P. infestans* isolation from soil was made different from as described by Lacey et al. (1965). Mycelial cultures were grown on V8 medium agar supplemented with antibiotics (nystatin: 19 mg/mL, rifampin: 20 mg/mL and ampicillin: 200 mg/mL) and maintained with regular transfers (Gamboa et al., 2019). Isolates were scored for appearance of hyphae, size, and shape of sporangia and chlamydospores. Identification was based of microscopic descriptions of *Phytophthora* species (Gallegly and Hong, 2008).

Application of phytosanitary treatment

From potato sowing, a total of 12 applications were made throughout the study, at weekly intervals during the main growing season (rainy season). Before application, 20 g of powdered soap were added to 15 l of *T. peruviana* extracts. Additional spraying was performed when it rained within 24 hours of treatments. Four phytosanitary solutions, each corresponding to treatments AqE, FI, F and ME, were applied in potato field using a 15 L flat-bed knapsack sprayer according to the minimal inhibition concentrations (MIC50 and MIC90) of *Thevetia peruviana* seed extracts on *Phytophthora infestans* growth (Dida et al., 2024). These treatments were: i) a solution of aqueous seed extract (AqE) at concentration of 16.66 g/L (which

represented 0,25 kg of aqueous extract/15L, ie 5 kg/ha) ii) a solution of contact fungicide (Bravo 720) + insecticide (Décis 5 CE) at 100 ml and 20 mL in 15 L of water, respectively, i.e. 2.5 l/ha; iii) a contact fungicide solution (F) (Bravo 720) at 100 ml in 15 l of water and iv) a solution of methanol extract (ME) of *T. peruviana* seeds at 16.66 ml/L concentration (which represented 250 ml of methanol extract/15L, ie 5 L/ha). The same doses were applied during experimentation in the second campaign (dry season), but only on a weekly basis, for a total of 12 applications.

Evaluation of disease incidence and severity in experimental fields

Incidence (I) or disease expansion rate is the frequency of disease occurrence on plants in a plot; it was determined at 6, 8 and 10 weeks after planting (WAP) during the two cropping periods and expressed as a percentage according to the formula: I=n/N X100 (Chowdhury et al., 2013).

Where I = Disease Incidence; n = Number of infected plants in plot; N = Total number of plants in plot

Disease severity (S) or intensity of disease infection on plants for each plot was also determined at 6, 8 and 9 WAP and then calculated as a percentage (%) using the formula:

$S= (\sum(ab))/N$ X100.

Where S = Severity of infection; $\sum ab$ = Sum of multiplications of number of diseased plants (a) corresponding to degree of infection (b); N = Total number of diseased plants; The degree of infection (b) (proportion of infected leaf area, estimated in %) was scored for the whole plant using the Horsfall-Barratt rating scale from 1 to 12 (1 = 0%, 12 = 100% disease severity) (Berger, 1980).

Evaluation of yield

To assess the effect of the plant protection products tested on yield, commercial and non-commercial yields in tons per hectare and the tubers number of each plant were determined at 120 days after planting (DAP) for both cropping periods. The tuber number per plant was assessed by exhaustive counting of apparently healthy tubers from each plant per plot, and an average was assigned for each treatment. These apparently healthy tubers were then weighed using a balance, and the average mass estimated per hectare according to the rule of three represented the commercial yield. The non-commercial yield was determined by weighing tubers showing signs of late blight infection, using the procedure described above (Habtamu et al., 2012).

Statistical analysis

Data collected on epidemiological parameters (incidence and severity) and yields were entered and processed with Excel spreadsheet 2013 for each treatment and variety. The data obtained were then imported and analyzed with R software version 3.6.2, which uses the standard analysis of variance (ANOVA) method through the R Studio interface. Tukey's multiple comparison test was performed at the 5%. The results obtained from the analysis in the form of mean ± standard deviation to performed tables. Pearson correlations between the various epidemiological parameters studied and yields were performed using IBM SPSS version 20.0 software. Principal component analysis (PCA) and cluster were performed using R software version 3.6.2 between varieties, treatments, epidemiological parameters (incidence and severity), and yield, with a view to detecting the correlation between varieties and treatments less susceptible to late blight.

Conclusion

The products applied (AqE, FI, F and ME) reduced late blight severity by an average of 90% during the dry season, compared with an average 75% reduction in severity during the rainy season. The extracts increased yield compared with control, demonstrating their fungicidal potential against crop pests. No commercial yields observed during the two cropping seasons showed the emergence of new, extremely virulent strains of late blight in Cameroon. This situation represents a serious challenge for potato growers and breeders alike. Biological options to combat this disease, including tolerant varieties and plant-based pesticides such as yellow laurel, need to be intensified. Results suggest that appropriate management programs are necessary to limit tuber losses due to late blight in the crop season in Cameroon. *Thevetia peruviana* extracts can be used as part of an integrated approach or as part of a biological control program to better control this dreaded disease and avoid harmful consequences for the environment.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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