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Effect of aqueous extracts of *Thevetia peruviana* K. seeds on the control of late blight and pest insects of *Solanum tuberosum* L. in Cameroon

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ABSTRACT

In the perspective of effective control of diseases and pests of potatoes (*Solanum tuberosum*), a study carried out in the Highland West Region of Cameroon permitted to evaluate the effect of aqueous extracts of *Thevetia peruviana* seeds (AETPS) on the development of late blight caused by *Phytophthora infestans* and the population of insect pests. Two potato varieties (local (V1) and one improved variety "Cipira" (V2) were used. Five treatments: AETPS (T1), Bravo 720 fungicide (T3), Decis 5 EC insecticide (T4), mixture of the three substances (T2) and the control (T0) were tested using a double factorial system with completely randomized blocks. The evolution of the disease, insect population and tuber yield were evaluated and compared according to different treatments. The results show that a main disease identified in field and laboratory was mildew. 88 pests were collected and 9 pest families were identified. The Coccinelidae family was most represented (23, 86%). The incidence and severity of late blight in the field were respectively reduced to 22.77 and 76.05 % by the AETPS at 50 g/l, as the synthetic fungicide (T3). The number of insects decreased after treatment but remained relatively stable in control. The insecticidal power was maximum 1 h after application of the decis contrary to the AETPS of with maximum was between 24 and 48 h. The commercial yields obtained in the AETPS treatment were greater than 7.5 t/ha compared to 0 t/ha obtained in the controls (T0). Extracts of *T. peruviana* can therefore be used as anti-parasite substances in crop protection.

ABBREVIATIONS:

AETPS: aqueous extracts of *Thevetia peruviana* seeds
DAS: days after sowing
WAS: week after sowing

INTRODUCTION

The potato (*Solanum tuberosum* L.) is a tuberous herbaceous plant native to Latin America. Its production has increased significantly since 1980 up to these days. It rose from 240 million tonnes on 19 million hectares in 1980 to 376 million tonnes in 2013 on 18 million hectares (Anonymous 2013), giving its the fourth place among the cultivated plant after maize, wheat and rice. In agricultural practice, the production cycle of the potato is mainly vegetative, product tubers forming both an asexual reproductive organ, food part of the plant and also a raw material for industrial processing (Ellissèche, 2008). More than 1.5 million hectares are cultivated in Africa (Anonyme, 2007), but in spite of the increase of potato production in the tropics, yields are generally low and are between 3 and 11 t/ha, compared to those of European countries which varied from 25 t/ha to 60 t/ha. Several factors contribute to this decline in production. Some producers continue to use local material or varieties with low potential of production; the prevalence of certain diseases and pests such as mildew, causes almost 60 to 80 % loss of total production (Fontem et *al*., 2003). Several methods are recommended to fight against this scourge. Mildew caused by *Phytophthora infectans* (Mont. De Bary) is one of the most destructive disease of potato worldwide (Fontem et *al*., 2005). Thousands of documents are published each year on *P. infestans* (Fry *et al.,* 2015). Despite all this research, the late blight pathogen continues to cause major losses on potato and tomato in worldwide (Alkher et *al*., 2015). The chemical protection is the mostly used and effective but is expensive and polluting. There are recent researches which report the importance of plant extracts in agriculture as pesticidal used to protect crops (Mullah and Islam, 2007; Ambang et *al*., 2007). Therefore, the search of alternatives to chemicals products such as the use of plant pesticides are promising ways for a reasonable and sustainable agriculture. For a decennia now, researchers have focused their studies on the action of extracts from *Thevetia peruviana* (Oji and Okafor, 2000) on fungi phytopathogens (Ngoh Dooh et *al*., 2014), bacteria (Saxena and Jain, 1990; Obasi & Igboechi, 1991), parasites and rodents (Ambang et *al*., 2002; Chougourou et *al*., 2012). The yellow laurel (*Thevetia peruviana* (Pers.) K. Schum) is a small tree belonging to the Apocynaceae family and is used as an ornamental plant in Cameroon. In recent years have been considered a source of biopesticides in crop protection. The general of this work is to evaluate the effect of aqueous extract of *T. peruviana* seeds on the development of late blight and potato pests.

MATERIAL AND METHOD

Material

The plant material consisted of two varieties of potato (local (V1) and improved variety "Cipira" (V2)) and *Thevetia peruviana* seeds. Chemical material used were Bravo 720 fungicide and Decis 5 EC insecticide. Several other materials were used.

Methods

Obtention of aqueous extracts and plant treatment

The fruits of *Thevetia peruviana* collected in Yaounde have been peeled, the cores obtained were crushed, weighed and the paste were introduced in tap water, kept for about 12 hours and filtered with the muslin tissue. This solution was directly applied in the field by adding soap (Blue) used as wetting. Chemicals pesticides were used at the recommended doses. Their application was done from the 21^{st} week after sowing (WAS) with a weekly frequency, then we made 9 applications.

Experimental apparatus

The experimental design was bifactorial system arranged in 03 completely randomized blocks. Each block comprises 5 plots representing the different treatments tested (T0= control; T1 = Aqueous extracts (50 g/l); T2 = aqueous extract (20 g/l) + Decis 5 CE (0.5 ml/l) + Bravo 720 (0.8 ml/l); T3 = bravo 720 (1.7 ml/l); T4= Decis 5 CE (1.2 ml/l).

Identification of the disease and inventory of different pest families on potato

When symptoms of the suspected disease appeared, the organs attacked were removed and brought back to the laboratory. There, stem and infested leaves were washed several times with tap water and then cut into small fragments of 2 to 4 cm^2 for the sheets and 0.56 to 0.84 cm^3 for the stems. The pieces are soaked in alcohol for 1 minute, rinsed twice with distilled water, and fed in Petri dishes containing paper moistened with water. 04 days after incubation, the mycelium developed was removed and cultured in new petri dishes containing PDA medium (Potato Dextrose Agar). The mycelial strain was then maintained by transplanting to allow purification of the fungus (Djocgoué, 2008). These pure strains are identified by a microscopic observation using an identification key.

The pests were collected in boxes containing alcohol and returned to entomology laboratory where they were identified using dichotomous keys.

Evaluation of the disease

The evaluation of the plant infection by the disease was focused on incidence and severity evaluated the 6^{th}; 8^{th}; 10^{th} weeks after sowing. The incidence or rate of expansion of the disease which expresses the frequency of occurrence of the disease on the plants in a plot was determined according to the formula: $\mathrm{I} = (\mathrm{n}/\mathrm{N})\mathrm{X}100$ (Chumakov and Zaharova, 1990)

Where: I = Incidence of the disease; n = Number of infected plant s in the field and N = Total number of plant s in the parcel

The severity of the disease or the infection intensity of the disease was evaluated on the plants for each plot. It was estimated according to the proportion occupied by the characteristic symptoms of the disease on the aerial part of the plants. It was calculated according to the formula:

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

Where: S= Severity of infection; a= Number of sick plants; b= Degree of infection corresponding to diseased plants; N= Total number of diseased plants; The degree of infection noted according to the standard evaluation scale graded from 0 to 4

Evaluation of the effect of aqueous extracts on the population of insect pests

The insecticidal power of the extracts was evaluated by counting insects before and after spraying at time intervals (1, 24 and 48 hours). Counting is done on each plot (avoiding edge lines) (Ambang et *al*., 2002). The values obtained were compared between the two insecticide treatments (T1 and T4) compared to the absolute control (T0). The insecticidal power was determined according to the formula: $P = [(A - B)/A]$X 100
Or: P = insecticidal power; A = number of insects before treatment; B = number of insects after treatment

Evaluation of the yield

Commercial Tuber yield were determined at 120 days after sowing (DAS). Two parameters were taken into account, the number of tubers per plant for each variety and the weight of the tubers. The number of tubers per plant was evaluated by the exhaustive count of healthy tubers of each plant per plot. Then tubers were weighed using a precision balance 1g and mass estimated per hectare.

Statistical analysis

The results obtained were analysed in the R software that uses the standard of the variance analysis method (ANOVA). For tests (Tukey, Student - Newman- Fischer), the level of significance was evaluated at the 5 % threshold. The Excel 2010 spreadsheet was used for the neck layouts curves, histograms and tables.

RESULTS

Diseases identified in field

The main disease identified in the field from the visual observation method was downy mildew (Fig.1a et b) of which the causative agent *Phytophthora infestans* was demonstrated in the laboratory (Fig. 1c).

a b c

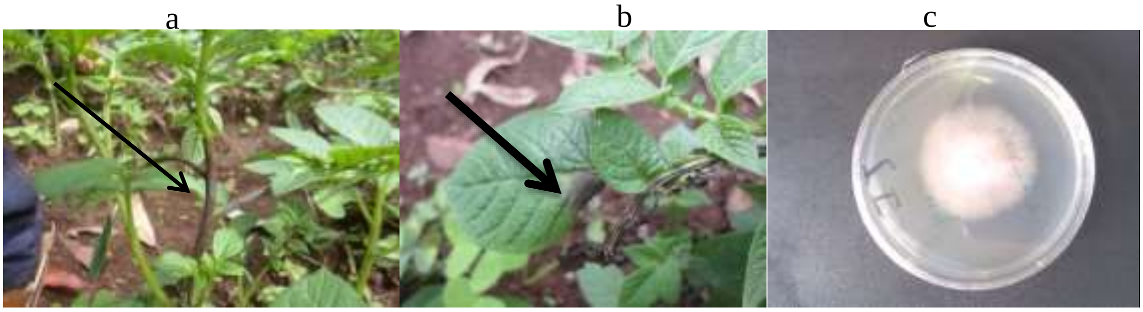

Fig.1. Essential characteristics of potato late blight (a- Symptom on stem; b- Symptoms on the skin; c- Pure strain of *Phytophthora infestans*).

Insects pests

The potato pests inventoried in the study site are very diverse. The dichotomous keys used made it possible to inventory individuals belonging to four orders (Coleoptera, Hemiptera, Orthoptera and Homoptera) and nine families (Chrysomelidae , Coccinelidae , Pentatomidae , Tettigonidae , Scarabae idae , Pigomorphidae , Acrididae , Ciccadellidae and Oedemeridae). (Table I). The family most represented is *Coccinelidae* (21) followed by that of *Pentatomidae* (14). Beetles and Orthoptera are the orders of the most abundant insect pests.

Table I. Diversity of insect pests encountered in the field

Ordre	Famille	Abondance
Beetle	Chrysomelidae	13 (14.77)
Beetle	Coccinelidae	21 (23.86)
Hemiptera	Pentatomidae	14 (15.90)
Orthoptera	Tettigonidae	10 (11,36)
Beetle	Scarabaeidae	6 (6,81)
Orthoptera	Pigomorphidae	4 (4,54)
Orthoptera	Acrididae	8 (9.09)
homopteran	Ciccadellidae	7 (7,95)
Beetle	Oedemeridae	5 (5.68)

Individuals of some families of insect pests encountered in the field of potato plants, because damage to the aerial parts of the plants (Fig.2a). Some of these families preferentially cause damage to the leaves (Fig.2b and 2c) and on the stems (Fig.2d).

Fig. 2. Some pests on *Solanum tuberosum* in the field: *acrididae* (a); *Tettigonidae* (b); *Coccinelidae* (c and d); *Ciccadellidae* (e); *Scarabaeidae* (f).

Incidence of the disease

The different rates of disease expansion determined for each variety in different treatments varied with time (Table II). T1 treatment reduced the incidence of late blight in the same way as synthetic fungicides. At 6th WAS, the incidence of the disease in the T0 plots was 80.00 ± 17.00 and 80.33 ± 9.23% respectively for V1 and V2, very different from those of the other treatments (T1, T2, T3 and T4) which tees lower impact to 60 %. The observations made 8th WAS show that the fungal infection has spread on more than half of the plants (60%). At 10th WAS, the applied products (T1, T2, T3 and T4) reduced the incidence of the disease contrary to T0 where the rate was 100 %. The lowest rates are recorded in variety V2 compared to variety V1.

Table II. Evolution of the incidence of the disease (%) on the seed potatoes according to the treatments.

treatments	variety	Time (weeks) 6 WAS	8 WAS	10 WAS
T0	V1	83, 00 ± 17.00 a	100, 00 ± 0.00 a	100.00 ± 0.00 a
	V2	80.33 ± 9.23 a	100.00 ± 0.00 a	100.00 ± 0.00 a
T1	V1	35.66 ± 21.12 b	88.66 ± 12.66 b	91.33 ± 8.50 a
	V2	38.66 ± 12.66 b	74.66 ± 14.43 bc	77.33 ± 9.81 b
T2	V1	49.66 ± 21.73 b	83, 00 ± 8.00 bc	97.00 ± 5.19 a
	V2	41.66 ± 14.43 b	69.33 ± 9.81 c	74.66 ± 8.50 b
T3	V1	46.66 ± 9.81 b	85.66 ± 4.61 b	94.00 ± 5.19 a
	V2	41.33 ± 8.50 b	69.00 ± 5.19 c	80.33 ± 4.61 b
T4	V1	55.00 ± 12.76 b	88.33 ± 4.61 b	97.00 ± 5.19 a
	V2	36.00 ± 1.76 b	83.00 ± 17.00 bc	91.66 ± 14.43 a

The averages followed by the same letter in the same column are not significantly different at the 5% threshold according to the Student- Newman-keuls test.

Severity of the disease

The intensity of the disease infection varied with the time depending on the treatments and varieties. The aqueous extracts (T1) greatly reduced the severity of the disease compared to the control T0. At 6th WAS, T0 presented the highest severities (30.25 ± 12.06 and 30.55 ± 14.61 % respectively for V1 and V2) unlike other treatments where the severity was less than 13 %. At 8 WAS, the intensity increases from 30 to 100 % tested in the control plots of the two varieties, showing that late blight reappears very rapidly in the absence of treatment. Similarly, in the other treatments (T1, T2, T3 and T4), severity rates have almost doubled. Variety V1 in T1 plots increased from 6.88 to 13.45 %; the same variety in T3 increased from 7.69 to 11.95 % (Table III). At 10th WAS, disease intensity doubled in all treatments except T0 where intensity was already highest at week 8. Overall, T3 plots had the lowest severity rates followed by T1. The variety V2 seems more tolerant than V1 to the different antifungal treatments.

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

Treatments	Variety	Time (weeks)		
		6th WAS	8th WAS	10th WAS
T0	V1	30.25 ± 12.06 a	100.00 ± 0.00 a	100.00 ± 0.00 a
	V2	30.55 ± 14.61 a	100.00 ± 0.00 a	100.00 ± 0.00 a
T1	V1	6.88 ± 5.06 b	13.45 ± 2.87 c	30.22 ± 3.82 c
	V2	7.13 ± 1.52 b	9.05 ± 2.01 d	23.95 ± 3.81 de
T2	V1	12.38 ± 2.48 b	17.61 ± 4.02 b	36.42 ± 2.50 b
	V2	9.54 ± 1.50 b	11.08 ± 3.19 cd	26.03 ± 4.58 d
T3	V1	7.69 ± 2.03 b	11.95 ± 1.5 cd	24.49 ± 3.33 de
	V2	5.78 ± 1.57 b	8.54 ± 1.17 c	20.71 ± 1.98 e
T4	V1	12.03 ± 1.05 b	16.17 ± 0.84 bc	39.05 ± 2.36 b
	V2	13.41 ± 1.43 b	15.63 ± 3.40 bc	31.97 ± 2.66 c

Means followed by the same letter in the same column are not significantly different at the 5% according to the Student test -Newman- Keuls.

In fields from 6th WAS all leaves and stems of plants in the control plots (T0) were severely infected with downy mildew (Fig.3a) in contrast with those of other plots (Fig. 3b, 3c, 3d and 3e). From 10th WAS, all plants of plots T0 were decimated by *Phytophthora infestans* (fig 3f).

Fig.3. Plants attacked by mildew on different plots 10th WAS: a- witness (T0); b- aqueous extract (T1); c- aqueous extract + bravo + decis (T2); d- bravo 720 (T3); e- Dec 5 EC (T4); f: control.

Effect of aqueous extracts on the pest population

The number of insects on potato plants varied after application of the insecticidal substances. After spraying, the average number of insects on the plants was 10; 3 and 0.5 individuals in T1 respectively 1; 24 and 48 h on the V1 variety compared to 15 individuals recorded prior to treatment (Table IV). Overall, the number of insects decreases after treatment and remains relatively stable in the controls. This decrease was significantly between treatments but not between the varieties. The number of insects was reduced at 100 % 1 hour after treatment with decis 5 EC (T4). At the same time, the insect population does not decrease almost in T1 as in T4 treatment. During the following 24 and 48 h, the number of insects on the plants was almost totally decreased in T1 and T4 plots while this number remains relatively high in the T0 plots.

Table IV. Variation of the number of pests by variety and treatments.

Treatments	Varieties	Before treatment	Duration after treatment		
			1 hour	24 hours	48h
T0	V1	19.50 ± 3.53 a	17.50 ± 0.70 c	17.00± 2.82 b	17.00± 4.24 b
	V2	15.50 ± 2.12 a	20.50 ± 0.70 c	22.50± 4.94 b	16.50± 4.94 b
T1	V1	15.00 ± 1.41 a	10.00 ± 1.41 b	3.00 ± 1.41 a	0.50 ± 0.70 a
	V2	13.00 ± 2.82 a	11.00 ± 1.41 b	0.00 ± 0.00 a	2.00 ± 1.41 a
T4	V1	12.50 ± 7.77 a	0.00 ± 0.00 a	1.00 ± 0.00 a	0.00 ± 0.00 a
	V2	12.50 ± 0.70 a	0.00 ± 0.00 a	0.00 ± 0.00 a	1.00 ± 0.07 a

Means followed by the same letter in the same column are not significantly different at the 5% according to the Student test -Newman- Keuls.

Insecticidal power varied with treatments in both varieties (Fig. 4). 1 h after treatment, it was 33.33 and 100 % respectively in T1 and T4 of the variety V1. 24 and 48 h after treatment, insecticidal activity increased from 33.33 % to over 80 % in T1 plots and remained relatively stable in T4 plots (Fig.4a). Similar results are obtained with the variety V2 where the insecticidal power ranged from 15.38 to 84.61 % and from 100 to 92 % respectively in the T1 and T4 treatments (Fig.4b).

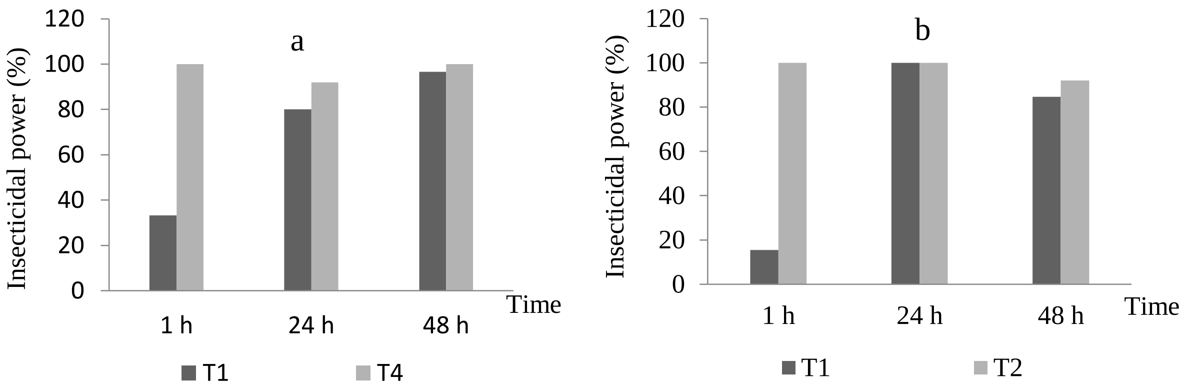

Fig.4. Evolution of the insecticidal power with time (1; 24 and 48 hours): a- Local variety; b- Improved variety; T1- Aqueous extract; T2- Decis 5 EC.

Yield

Significant differences were observed between plots and between varieties. Yields of aqueous extracts treatment (T1) significantly increased comparatively to the control (Table V). The number of tubers per plant and yields in t/ha of variety V1 were the same in all treatments according to student test. Variety V2 presented higher tuber numbers and yields in t/ha than V1 in all treatments except in T0 plots where both were nil. The highest yield in t/ha was registered in T3 treatment with 7.85 ± 0.89 for the variety V1 and 12.68 ± 0.06 for the variety V2, followed by treatment T2 (Table V).

Table V. Yield of potato in treatments and varieties.

Treatments	Varieties	Number of tubers per plant	Yield in tonnes per hectare (t/ha)
T0	V1	0.00 ± 0.00 a	0.00 ± 0.00 a
	V2	0.00 ± 0.00 a	0.00 ± 0.00 a
T1	V1	6.94 ± 0.12 b	0 7.6 ± 0.19 b
	V2	8.41 ± 0.79 c	10.29 ± 0.01 c
T2	V1	7.52 ± 0.80 b	7.36 ± 1.11 b
	V2	10.52 ± 0.78 d	12.60 ± 0.99 d
T3	V1	7.72 ± 0.54 b c	7.85 ± 0.89 b
	V2	10.16 ± 0.38 d	12.68 ± 0.06 d
T4	V1	6.85 ± 0.25 b	7.11 ± 0.22 b
	V2	7.33 ± 0.08 b	9.25 ± 0.53 c

Means followed by the same letter in the same column are not significantly different at the 5% according to the Student test -Newman- Keuls.

DISCUSSION

The appearance of late blight on young potato stalks 23 days after sowing, as well as the nine identified pest families, would indicate that climatic conditions during the experimental period were favourable for disease and insect pest development. In addition, the previous crop being an associated crop of beans and potatoes, the experimental site would be more favourable to the development of pests. Schaafsma et al. (2001) showed that the previous crop (source of the inoculum) and the climate respectively explained 21 and 48 % of the variation in deoxynivalenol (DON) levels produced by *Fusarium graminearum* in their similar study done on the corn.

The results obtained on the rates of disease expansion and on the intensity of the disease infection show that the aqueous extracts strongly reduced the development of the disease compared to the control; thus, showing the antifungal potential of this natural substance. Similar results were obtained by Ambang et *al.* (2007) who demonstrated that this extract reduced the incidence and severity of *Cercosporia* in peanuts from 32.61 % to 37.25 % compared with the control. Globally, all the phytosanitary products tested reduced the infection of the disease confirming the work of Habtamu *et al.* (2012) who found that synthetic pesticides reduced the incidence of late blight in potatoes from 38.66 to 59.52 %. Binyam *et al.* (2014) equally showed that synthetic pesticides combined with the genetic capabilities of varieties reduced the severity of late blight by 68-80 % in similar potato trials. However, at the last observation, high rates were recorded in all plots. The importance of plant contamination in the trial would be due not only to wind and high rainfall, but also to the vicinity of diseased plants as demonstrated by Williams *et al*. (1994b) in similar research on the epidemiology of late blight. Control plots showed 100 % severity in both varieties. This level of infestation including the tolerant variety (Cipira) could thus confirm the emergence of new strains of late blight in recent years as reported by George and Preston (2004) when searching ways to control downy mildew.

Both varieties were relatively susceptible to insect pests. Variance analyses showed that there was no significant difference ($P < 0.05$) between varieties. The number of insects was totally reduced (100%) one hour after treatment with Decis 5 EC (T4). This confirms the efficacy of this insecticide reported in other plants of the tropics and subtropics like the work of Ambang *et al*. (2005) on corn weevils. At the same time, the number of insects decreases very little in the plots sprayed with aqueous extracts (T1) and after 24 and 48 hours, the plots T4 and T1 presented similar results showing an almost total reduction of insects. This could be explained by the fact that the toxins are released very slowly by the aqueous extracts and will act after a more evolved time unlike the synthetic insecticide (Decis 5 CE) as demonstrated by Ambang *et al.* (2002) in similar tests on larvae and imagos of *Andrector ruficornis* on *Solanum tuberosum* plants in Cameroon. The high insecticidal potential of the extracts could be due to the presence of toxic molecules such as terpenes and palmitic acids contained in the seeds as shown by Berhaut (1971), Gata-Gonçalves et *al.* (2003), Chougourou et *al.* (2012) in similar studies on yellow laurel.

The phytosanitary products tested greatly increased the yield compared to the control. These high yields in the different treatments are certainly related to the reduction of the diseases ensured by the aqueous extracts of the laurel seeds and the synthetic pesticides combined with the genetic capacities of the tested varieties. These results confirm those of Fontem *et al.* (2007), Muchiri *et al.* (2009), Girma *et al.* (2013) in similar research on the formulation of fungicides effective in the control of late blight. Cipira variety presented a high yield than the local variety. This would be due to the improved genetic capabilities of this variety to produce more tuber unlike the local varieties as described by the breeder. The best yields were observed in the T3 treatment (Bravo 720) and in the T2 treatment (mixture of extract + Bravo + Decis). This would result in the proven efficacy of synthetic fungicides registered for the control of late blight and increase the yields. The nil yields observed in both varieties in the control plots confirm the devastating effect of downy mildew, which can lead to a total yield loss, as stated by George and Preston (2004) in similar research on apple blight.

CONCLUSION

The aqueous extract of the yellow laurel seeds (*Thevetia peruviana*) were tested on two potato varieties (*Solanum tuberosum L.*), with the aim of determining its impact on the control of downy mildew (*Phytophthora infestans*) and insect pests of potato in the field. The aqueous extracts of *T. peruviana* significantly reduced the course of the disease and the population of insect pests compared to the control (To) which contained no application of phytosanitary product. Likewise, this extract increased the yield relative to T0 thus demonstrating its potential against cultivated plants nuisances. The nil yields observed even in Cipira variety which is an improved variety, could show the emergence and the virulence of new strains of late blight during recent years. This represents a serious challenge for potato growers and breeders. It is therefore necessary to intensify biological options to control this disease, including resistant varieties and plant pesticides such as laurel.

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