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Evaluation of larvicidal activity of selected plant extracts against *Plutella xylostella* (Lepidoptera: Plutellidae) larvae on cabbage

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ABSTRACT

Plutella xylostella (Lepidoptera: Plutellidae) popularly known as the moth-of-crucifers is a major pest of brassica worldwide which can cause damage to brassica crops up to 100% loss of total production. The use of synthetic pesticides for its control has harmful effects on public health and the environment, besides the development of resistance to these synthetic insecticides. One of the alternative control methods developed in recent years has been the use of plant extracts. In the present study, was evaluated the insecticidal potential of the leaves, root bark and stem bark extracts of three Mozambican medicinal plants: *Trichilia emetica*, *Anacardium occidentale* and *Cymbopogon citratus* to control the development of diamondback moth (L.) on cabbage. The plant extracts were obtained by maceration in organic solvents (n-hexane, ethyl acetate and methanol). The preliminary phytochemical tests were conducted to identify classes of chemical constituents present in the extracts. To assess insecticidal activity, the extracts were tested on the second instar larvae of diamondback moth using the leaf disc immersion methodology, where the larvae were fed with cabbage leaves dipped in each extract, being observed larval mortality in function of extract concentration. The evaluation of the insecticidal activity showed that the methanol crude extract from the root bark of *Trichilia emetica* caused the highest larval mortality (LC_{50} = 0.94 $mg.ml^{-1}$). The LC_{50} values obtained for all the extracts tested indicate that the crude methanol extracts have a higher larvicidal potential than those obtained in the sequential extraction. The results suggest that the larvicidal activity of the extracts under study can be related to the presence of the identified metabolites that act synergistically or individually in producing larval mortality.

Keywords: *Plutella xylostella*, larvicidal, *Trichilia emetica*, *Anacardium occidentale*, *Cymbopogon citratus.*

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INTRODUCTION

The phytochemical study of plants has awakened throughout history the interest of scientists of different sectors (pharmacists, chemists, doctors, agronomists) with the aim of discovering or justifying the activity of those used as medicines (Matos, 1997). All this interest is due to the great diversity of bioactive natural products obtained from plant species, which have an immense potential and several fields of application.

Torres et al. (2006) reported that one of the most notable aspects in the reduction of the production of vegetables in different regions of the world has been the occurrence of pests such as *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), which is held as the main pest of crucifers. This pest causes serious damage by depreciating the product, interfering with the growth of the plant and even causing its death or total loss.

Cabbage is grown almost everywhere in Mozambique and at all times of the year, particularly in the cold season and is a great source of nutritional and income for low-income families, especially those living in suburban regions. However, it is increasingly more prone to serious damage by the diamondback moth *P. xylostella*, which

causes reduction of production, and contributes to the reduction of its nutritional and commercial value.

Synthetic pesticides such as organophosphates, carbamates and pyrethroids have been the main strategy used by the farmers to control this pest. Studies have shown that the use of these agrochemicals has harmful effects on crops and public health as well as on the environment (Boiça Júnior et al., 2005). One of the alternative control methods developed in recent years has been the use of plant extracts.

Studies involving plant extracts with medicinal properties have reported insecticidal properties such as mortality (Boiça Júnior et al., 2005; Bandeira et al., 2013; Mishra and Singh, 2014) and feeding deterrence (Zhang et al., 2003; Liu et al., 2007; Chandrashekharaiah et al., 2015; Couto et al., 2016) in *P. xylostella* larvae as well as repellence (Hou et al., 2002) and infertility in adults (Gu et al., 2004).

Plants with insecticidal properties, such as neem (*Azadirachta indica* A. juss.) in the form of powders, extracts and oils have been extensively studied and suggested as an alternative for the control of *P. xylostella* (L.) (Lepidoptera: Plutellidae) (Medeiros et al., 2005).

Several researchers highlighted some advantages of botanical extracts over synthetic pesticides: they offer new compounds that pests cannot yet inactivate; are less concentrated and therefore are potentially less toxic than pure compounds; they undergo rapid biodegradation and exhibit multiple modes of action, making possible a wide spectrum of use while retaining a selective action within each class of pests; they are derived from renewable resources and maintain the environmental balance without leaving chemical residues with toxic action to the animals and the man (Quarles, 1992; Almeida et al., 2004; Torres et al., 2006; Medeiros et al., 2005, b; Dong et al., 2013) and they are relatively inexpensive and easy to prepare locally (Kudom et al., 2011).

The search for insecticidal properties in medicinal plants has increased significantly in recent years and has become a promising field of research. The increasing demand of production and the emergence of populations of *P. xylostella* resistant to synthetic insecticides employed, justifies the search for novel active molecules and new control strategies. This has led researchers world-wide to search for botanical insecticides in different genera of plant species for the control of *P. xylostella* (Bandeira et al., 2013).

The plants *Anacardium occidentale*, *Trichilia emetica* and *Cymbopogon citratus* are used in Mozambican traditional medicine for treating various ailments and their biological properties including insecticidal activity are well documented in the literature (Leite et al., 2016; Komane et al., 2011; Olorunnisola et al., 2014; Pinto et al., 2015; Oparaeke and Bunmi, 2006; Mavundza et al., 2013),

T. emetica belongs to the Meliaceae family which has attracted in the last decades a great interest among phytochemists interested in bioproduction because of its complex and diverse chemical structures and its biological activity including insecticidal activity (Vieira et al., 2013). Phytochemical studies of *Trichilia* genus have revealed that the genus is rich in terpenoids, incluiding triterpenes, limonoids, steroids and other terpenes derivatives (Vieira et al., 2014; Rodrigues et al., 2010; Ramírez et al., 2000) and biological studies conducted on plant extracts of *Trichilia* genus demonstrated insecticidal activity on different insect pests (Xie et al., 1994; Freitas et al., 2014; Ayo et al., 2013).

Several studies on *A. occidentale* have shown that possesses insecticidal properties against a wide range of insects. Adedire et al. (2011) and Ileke and Olotuah (2012) reported that both powder and oil extracts of *A. occidentale* possess strong insecticidal activity against storage pests. Nwaogu et al. (2013) reported the insecticidal efficacy of oil extracts of cashew nuts against *Callosobruchus maculatus Fabr*. (Coleoptera: Bruchidae). Guissoni et al. (2013) reported that the cashew nut shell liquid (CNSL) and its fractions showed larvicidal potential against *Aedes aegypti*. Ileke et al. (2014) found in his studies that *A. occidentale* oil extract showed insecticidal effect on both the larvae and pupae of the mosquito, *Anopheles gambiae* Giles.

Insecticidal activity is one of the biological effects of most plants of the *Cymbopogon* genus, it is either applied as pest control for stored crops or mosquito repellent insecticide (Avoseh et al., 2015). Recent studies revealed that *C. citratus* essential oil and their main components (citral and 1,8-cineole) are important repellent and insecticide against housefly (Pinto et al., 2015; Kumar et al., 2013); maize weevil (*Sitophilus zeamais*) (Kabera et al., 2011); *Anopheles gambiae* (Nonviho et al., 2010).

Studies on insecticidal activity of the three species *T. emetica*, *A. occidentale* and *C. citratus* involved mainly essential oil (*C. citratus*), fruits and its derivatives including oil (*T. emetica* and *A. occidentale*); few studies were related to extracts of leaves, stem and roots particularly against *P. xylostella*. Therefore, the aim of the present study was to evaluate the insecticidal activity of the root bark, stem bark and leaves extracts of *T. emetica*, stem bark and leaves extracts of *A. occidentale* and leaves extracts of *C. citratus* for controlling the development of *P. xylostella* (L.) on cabbage. Such plant extracts can provide an easy, natural and economical method of managing *P. xylostella* using the tools of the flora itself.

MATERIALS AND METHODS

Plant material collection

Samples of root bark, stem bark and leaves of *T. emetica*; stem bark and leaves of *A. occidentale* as well as leaves of *C. citratus* were collected in July 2014 in the municipality of Manhiça, District of the same name, Maputo Province. The species were authenticated at the Herbarium Unit, Department of Biological Sciences, Faculty of Science of Eduardo Mondlane University, by comparison with existing specimen with Voucher No. 2228 (*Trichilia emetica*), No. 532 (*Anacardium occidentale*) and No. 12

(*Cymbopogon citratus*). The roots, stem barks and leaves of the three species were dried separately at room temperature for a period of three months in the Laboratory of Natural products, Department of Chemistry, Faculty of Science – Eduardo Mondlane University. The dried samples were pulverized in fine powders and stored separately in closed plastic containers at room temperature prior to extraction.

Rearing of *Plutella xylostella* in the laboratory conditions

Cabbage was used as host plant for rearing the diamondback moth and for all laboratory bioassays. For growing cabbage, seeds were sown in suitable trays containing Plantmax substrate in the Greenhouse of the Faculty of Agronomy and Forestry Engineering, and after 35 days, were transplanted into the definitive vessels in the number of 30. Subsequently, irrigation was carried out by sprinkling whenever necessary. Standard techniques for the cultivation of brassicas have been adopted for their production.

The insects were created in the Laboratory of Plant Physiology of the Faculty of Agronomy and Forest Engineering. To begin the rearing, the pupae were collected in the cabbage plantations of the Farmers Association of Mahotas in the outskirts of Maputo city, and after the emergence of the adults, were released in cages containing an 8 cm disc of cabbage leaf placed on a filter paper disc of equal size, slightly moistened with distilled water.

This paper was laid over a transparent plastic cup with the opening facing down, the cabbage leaf being raised into the transparent cage where oviposition occurred. At the apex of the container was made a 2.3 cm opening, used for fixing a cotton sponge soaked in aqueous solution of honey, which was fastened as a small "bundle" in that opening.

In each cage was made a circular lateral opening covered with A4 paper. The cabbage leaf discs where were carried out the postures, were removed from the cages and transferred to Petri dishes until the hatching of the larvae which were transferred to plastic bowls with cabbage leaves, replaced when necessary, until the larvae reached the second instar of development.

The larvae were collected using a spatula and placed in plastic bowls sealed with plastic film (PVC) with small holes made with the aid of a stylus to allow air circulation.

Preparation of plant extracts

Crude methanol extracts

To obtain the crude methanol extracts, each 20 g of the root bark, stem bark and leaves powder of the three plants under study were separately submitted to maceration with 150 ml of methanol in Erlenmeyer flasks covered with aluminum foil to avoid contamination, for a period of 24 h and with occasional stirring. After 24 h, each sample was filtered and the filtrate was concentrated in a rotary evaporator.

Sequential extraction in increasing order of polarity

The root bark, stem bark and leaves powder of the plants under study was subjected to maceration in organic solvents in increasing order of polarity: hexane, ethyl acetate and methanol. 20 g of each sample was macerated in 150 ml of organic solvent for 24 h to give hexane extract, ethyl acetate extract and methanol fraction.

Preliminary phytochemical analysis

In order to identify the metabolites present in the plant extracts under study, were used standard procedures as described by Ahmad et al. (2013), Tiwari et al. 2011 and Khan et al. (2011) with minor changes. For the present study, phytochemical tests were carried out to investigate hydrolysable and condensed tannins, flavonoids, alkaloids, free anthraquinones, coumarins, free amino acids, proteins, terpenes/steroids, reducing sugars and saponins.

Larval mortality bioassay

The leaf disc immersion method was used to determine the insecticidal activity. The concentrations (m/v) of the extracts were determined according to the methodology proposed by Torres et al. (2006) and Medeiros et al. (2005) where suitable quantities of extracts in milligrams (mg) were dissolved separately in a volume of 25 ml of distilled water until reaching the desired concentration.

To allow the subsequent calculation of the LC_{50}, preliminary tests were initially carried out to define the limit concentrations: a concentration that causes 95% to 100% mortality of the larvae and a second that causes mortality close or equal to the negative test (Torres et al., 2006; Medeiros et al., 2005). The used concentrations for the different extracts are summarized in Table 1.

Cabbage discs approximately 8 cm in diameter were immersed in each extract over a period of 10 minutes and to allow the extract to adhere to the entire surface of the cabbage leaf, was used an adherent (liquid soap). The same was done for the insecticide HALT 5% WP used as positive control and distilled water used as negative control.

After immersion of the cabbage leaf discs, they have been left outdoors for drying and thereafter transferred to the Petri dishes containing a filter paper slightly moistened with distilled water. Twelve larvae of *Plutella xylostella* of the second instar were confined to each treatment for a period of 72 h. Three replicates were made in each treatment. The cabbage discs were changed daily and the Petri dishes were covered to prevent larvae from escaping. After the period of 72 h, the mortality of the larvae in function of the concentration was recorded for each treatment.

For the determination of the lethal concentration for 50% of the population, a graphical illustration of the dose-response type (concentration versus% of mortality) was done, where a logarithmic dependence between concentration and percentage of mortality was observed using Microsoft Excel 2013. Extrapolating the concentration values to 50% of the population or from the respective curve equation ($y = a\ln x + b$) the respective LC_{50} values were obtained for each extract.

Statistical analysis

The statistical evaluation of the results regarding the study of insecticidal activity of the extracts of the three plants in study was made to determine the mean values of larval mortality in three replicates of each treatment. Significant statistical differences at $p < 0.05$ were made using the analysis of variance (ANOVA) using the Tukey test at 5% probability, from the statistical package SPSS version 20.

RESULTS

Phytochemical screening

The results of the phytochemical screening of the extracts of the three plants studied are summarized in Table 2. The phytochemical screening revealed the presence of Alkaloids, flavonoids, hydrolyzed and

Table 1. Concentrations of crude methanol extracts and extracts from the sequential extraction used in the different treatments for bioassays.

Plant material	Crude methanol extract conc. ($mg.ml^{-1}$)	Hexane extract conc. ($mg.ml^{-1}$)	Ethyl acetate extract conc. ($mg.ml^{-1}$)	Methanol fraction conc. ($mg.ml^{-1}$)
Trichilia emetica – root bark	0.80; 0.90; 0.96; 1.04; 1.12; 1.20; 1.28	2.40; 2.64; 2.88; 3.12; 3.36; 3.60; 3.84	1.60; 1.76; 1.92; 2.08; 2.24; 2.40; 2.56	1.20; 1.40; 1.60; 1.80; 2.00; 2.20; 2.40
Trichilia emetica – Stem bark	0.90; 1.00; 1.10; 1.20; 1.30; 1.40; 1.50	2.54;2.84; 3.14; 3.44; 3.74; 4.04; 4.34	1.40; 1.60; 1.80; 2.00; 2.20; 2.40; 2.60	1.30; 1.58; 1.86; 2.14; 2.42; 2.70; 2.98
Trichilia emetica - Leaves	1.00; 1.12; 1.24; 1.36; 1.48; 1.60; 1.72	2.56; 2.92; 3.28: 3.36; 4.00; 4.36; 4.72	1.20; 1.60; 2.00; 2.40; 2.80; 3.20; 3.40	1.12; 1.40; 1.80; 2.00; 2.60; 2.80; 3.20
Anacardium occidentale – Stem bark	1.80; 2.00; 2.20; 2.40; 2.60; 2.80; 3.00	3.36; 3.52; 3.68; 3.84; 4.0; 4.16; 4.32	2.52; 2.64; 2.76; 2.88; 3.00; 3.12; 3.28	2.00; 2.20; 2.40; 2.60; 2.80; 3.00; 3.24
Anacardium occidentale - Leaves	2.00; 2.40; 2.80; 3.20; 3.60; 4.00; 4.40	3.60; 3.80; 4.00; 4.20; 4.40; 4.80; 5.20	2.40; 2.80; 3.20; 3.60; 4.00; 4.40; 4.80	2.20; 2.40; 3.00; 3.20; 3.80; 4.00; 4.60
Cymbopogon citratus - Leaves	1.60; 2.00; 2.40; 2.80; 3.20; 3.60; 4.00	3.20; 3.40; 3.60; 3.80; 4.0; 4.20; 4.40	3.00; 3.20; 3.40; 3.60; 3.80; 4.00; 4.20	2.92; 3.12; 3.32; 3.52; 3.72; 3.92; 4.22

Table 2. Results of qualitative phytochemical tests of extracts of *Trichilia emetica* (root bark, stem bark and leaves), *Anacardium occidentale* (stem bark and leaves) and *Cymbopogon citratus* (leaves).

Plant material	Extract	Phytoconstituents										
		AD	FV	CT	HT	SP	CR	TS	AQ	AA	RS	PT
Root bark of *T. emetica*	crude-MeOH	+	+	-	+	+	-	+	+	+	+	+
	Hexane	-	-	-	-	-	-	+	-	-	-	-
	AcOEt	-	-	-	-	-	-	-	-	-	-	-
	Fn MeOH	+	+	-	+	+	-	+	+	+	+	+
Stem bark of *T. emetica*	crude-MeOH	+	+	-	+	+	-	+	+	+	+	+
	Hexane	-	-	-	-	-	-	+	-	-	-	-
	AcOEt	-	-	-	-	-	-	-	-	-	-	-
	Fn MeOH	+	+	-	+	+	-	+	+	+	+	+
Leaves of *T. emetica*	crude-MeOH	-	+	+	-	+	+	-	+	+	-	-
	Hexane	-	-	-	-	-	-	-	-	-	-	-
	AcOEt	-	+	-	-	-	-	-	-	-	-	-
	Fn MeOH	-	+	+	-	+	+	-	+	+	-	-
Stem bark of *A.occidentale*	Crude-MeOH	+	-	+	+	+	-	-	-	-	+	+
	Hexane	-	-	-	-	-	-	-	-	-	-	-
	AcOEt	-	-	+	+	-	-	-	-	-	-	-
	MeOH	+	-	+	+	+	-	-	-	-	+	+
Leaves of *A. occidentale*	crude-MeOH	+	+	+	-	+	-	-	-	-	+	+
	Hexane	-	-	-	-	-	-	-	-	-	-	-
	AcOEt	-	+	+	-	-	-	-	-	-	-	-
	Fn MeOH	+	+	+	-	+	-	-	-	-	+	+
Leaves of *C.citratus*	crude-MeOH	-	+	-	-	+	+	+	-	+	-	-
	Hexane	-	-	-	-	-	-	+	-	-	-	-
	AcOEt	-	+	-	-	-	-	-	-	-	-	-
	Fn MeOH	-	+	-	-	+	+	-	-	+	-	-

Legend: +: detected; -: not detected; MeOH: methanol; AcOEt: ethyl acetate; Fn MeOH: methanol fraction (from sequential extraction); T. emetica: Trichilia emetica; A. occidentale: Anacardium occidentale; C. citratus: Cymbopogon citratus; AD: Alkaloids; FV: Flavonoids; CT: Condensed Tannins; HT: HydrolysableTannins; SP: Saponins; CR: Coumarins; TS: Terpenoids/steroids; AQ: Anthraquinones; AA : Aminoacids; RS: reducing sugars; PT: Proteins.

condensed tannins, saponins, coumarins, terpenes/steroids, anthraquinones, amino acids, reducing sugars and proteins. Most of the metabolites were detected in crude methanol extract and methanol fraction from sequential extraction.

Larvicidal activity

The results of larvicidal activity of different concentrations of plant extracts tested against the second instar larvae of *P. xylostella* showed that the larval mortality after 72 h varied depending on concentration and extract. For illustration, was reported in Tables 3, 4 and 5, the variation of larval mortality in case of crude methanolic extracts of *T. emetica* (root bark, stem bark and leaves). The same procedure has been followed for all extracts tested (crude methanol extracts of *A. occidentale* and *C. citratus* as well as all hexane, ethyl acetate and methanolic extracts from sequential extraction), but herein all results have not been reported in tables as done for crude methanol extracts of *Trichilia emetica* for illustration of the methodology used. In Table 1 were summarized all concentrations used and the high concentration for each extract produced between 94 and 100% of larval mortality. For comparing the extracts, LC_{50} value (concentration causing 50% of larval mortality) has been used. LC_{50} values for all extracts were summarized in Table 6. For their determination, the graphs in Figures 1, 2 and 3 which led to the determination of LC_{50} in case of crude methanol extracts of *T. emetica* (LC_{50} = 0.94 $mg.ml^{-1}$, 1.16 $mg.ml^{-1}$, 1.23 $mg.ml^{-1}$ for root bark, stem bark and leaves respectively) were an illustration of the methodology used for all extracts.

Table 3. Mortality of *Plutella xylostella* larvae (n = 12) after 72 h of exposure at various concentrations of the crude methanol extract of the root bark of *Trichilia emetica*.

Treatment	Concentration ($mg.ml^{-1}$)	Mortality	% Mortality
T1	1.28	12.000 ± 0.000[a]	100.00
T2	1.20	10.333 ± 0.577	86.11
T3	1.12	9.333 ± 0.577	77.77
T4	1.04	7.333 ± 0.577	61.11
T5	0.96	5.333 ± 0.577	44.44
T6	0.88	2.667 ± 0.577	22.23
T7	0.80	0.333 ± 0.577	2.78
T8	0.75	12.000 ± 0.000[a]	100.00
T9	-	0.000 ± 0.000[a]	0.00

Legend: T1 = Treatment 1; T2 = Treatment 2; T3 = Treatment 3; T4 = Treatment 4; T5 = Treatment 5; T6 = Treatment 6; T7 = Treatment 7; T8 = corresponds to positive control, and T9 = corresponds to negative control. a = t cannot be calculated since the standard deviation is null.

Table 4. Mortality of *Plutella xylostella* larvae (n = 12) after 72 h of exposure at various concentrations of the crude methanolic extract of *Trichilia emetica* stem bark.

Treatment	Concentration ($mg.ml^{-1}$)	Mortality	%Mortality
T1	1.50	11.333 ± 0.577	94.44
T2	1.40	10.000 ± 0.000[a]	83.33
T3	1.30	8.667± 0.577	72.23
T4	1.20	7.333 ± 0.577	61.11
T5	1.10	5.667 ± 0.577	47.23
T6	1.00	2.333± 0.577	19.44
T7	0.90	0.667 ± 0.577	5.56
T8	0.75	12.000 ± 0.000[a]	100.00
T9	-	0.000± 0.000[a]	0.00

Legend: T1 = Treatment 1; T2 = Treatment 2; T3 = Treatment 3; T4 = Treatment 4; T5 = Treatment 5; T6 = Treatment 6 T7 = Treatment 7; T8 = corresponds to positive control; and T9 = corresponds to negative control. a = t cannot be calculated since the standard deviation is null.

Table 5. Mortality of *Plutella xylostella* larvae (n = 12) after 72 h of exposure at various concentrations of the crude methanolic extract of leaves of *Trichilia emetica.*

Treatment	Concentration (mg.ml^{-1})	Mortality	% Mortality
T1	1.72	11.667 ± 0.577	97.23
T2	1.60	10.667 ± 0.577	88.89
T3	1.48	10.000± 1.000	83.33
T4	1.36	8.000 ± 1.000	66.67
T5	1.24	7.000 ± 1.000	58.33
T6	1.12	4.667 ± 0.577	38.89
T7	1.00	1.000 ± 1.000	8.33
T8	0.75	12.000 ± 0.000[a]	100.00
T9	-	0.000 ± 0.000[a]	0.00

Legend: T1 = Treatment 1; T2 = Treatment 2; T3 = Treatment 3; T4 = Treatment 4; T5 = Treatment 5; T6 = Treatment 6 T7 = Treatment 7; T8 = corresponds to positive control, and T9 = corresponds to negative control. a = t cannot be calculated since the standard deviation is null.

Table 6. LC_{50} values (concentrations causing 50% larval mortality) obtained for the different crude methanol extracts and extracts from sequential extraction.

Plant material	LC_{50} (mg.ml^{-1})			
	Crude MeOH extract	Hexane extract	Ethyl acetate extract	MeOH fraction
Root bark of *Trichília emetica*	0.94	3.06	1.94	1.64
Stem bark of *Trichília emetica*	1.16	3.52	1.98	2.09
Leaves of *Trichília emetica*	1.23	3.78	2.81	2.50
Stem bark of *Anacardium occidentale*	2.23	3.41	2.80	2.53
Leaves of *Anacardium occidentale*	2.67	4.20	3.54	3.47
Leaves of *Cymbopogon citratus*	2.54	3.60	3.36	3.14

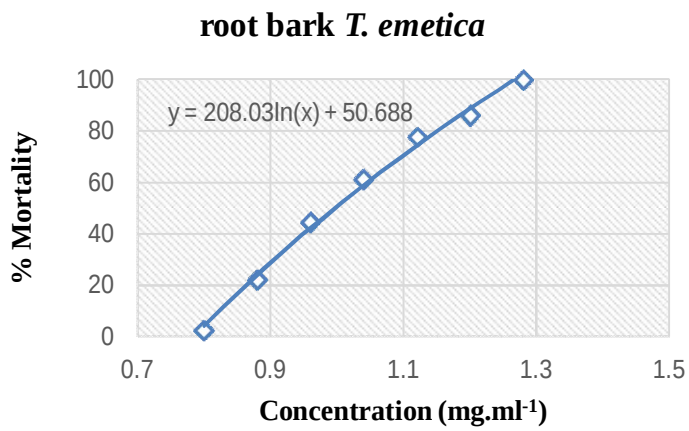

Figure 1. Mortality of *Plutella xylostella* larvae submitted to different concentrations of crude methanol extract from the root bark of *Trichilia emetica.*

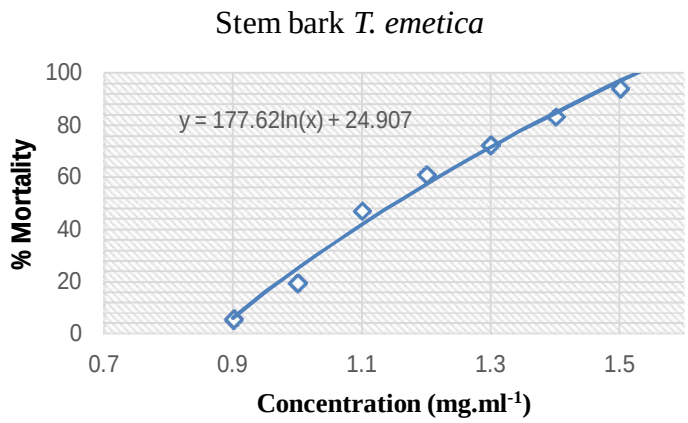

Figure 2. Mortality of *Plutella xylostella* larvae submitted to different concentrations of methanol crude extract from stem bark of *Trichilia emetica.*

DISCUSSION

Phytochemical screening

The results of phytochemical screening for the extracts of *T. emetica* as shown in Table 2, revealed the presence of alkaloids, flavonoids, hydrolysable tannins, saponins, terpenes and steroids, anthraquinones, amino acids, reducing sugars and proteins in extracts of root bark and stem bark while condensed tannins, saponins, coumarins, anthraquinones and amino acids were found in leaf extracts. In extracts of root bark and stem bark, the

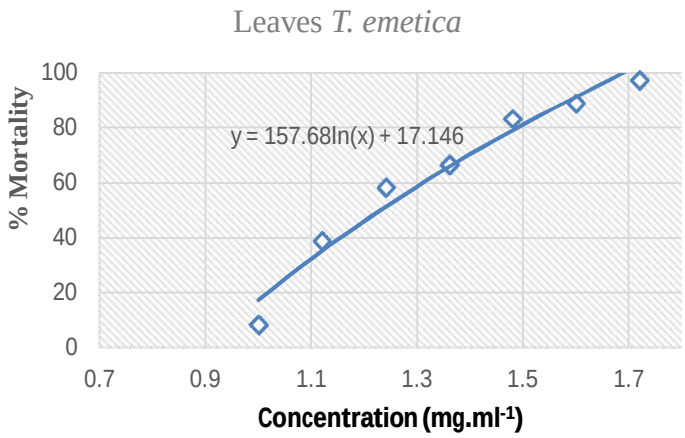

Figure 3. Mortality of *Plutella xylostella* larvae submitted to different concentrations of methanol crude extract from leaves of *Trichilia emetica.*

presence of the secondary metabolites: alkaloids, flavonoids, hydrolysable tannins, saponins, terpenes / steroids and anthraquinones are found mainly in the crude methanol extract and in the methanol fraction (resulting from sequential extraction). These results are in agreement with those described in the literature where they report the occurrence of the same secondary metabolites in plants of the genus *Trichilia* (Figueiredo, 2010). Ayo et al. (2013) reported the presence of carbohydrates, reducing sugars, alkaloids, cardiac glycosides, saponins, flavonoids and tannins in methanol extracts of the leaves of *Trichilia roka*, while in the petroleum ether extracts of the leaves, were found alkaloids, cardiac glycosides, flavonoids and tannins. Germano et al. (2006) reported, in addition to the isolation of limonoids, the presence of tannins in the roots and stem bark of *T. emetica*.

For the extracts of *A. occidentale*, was noted the presence of alkaloids, condensed and hydrolyzable tannins, saponins, reducing sugars and proteins in stem bark, and alkaloids, flavonoids, condensed tannins, saponins, reducing sugars and proteins in leaf extracts. These results are consistent with literature data: Santos et al. (2013) reported the presence of hydrolysable tannins, phenols, flavones, flavonols, xanthones, chalcones, aurones, flavonoids, catechins and alkaloids in the ethanolic extract of the leaves of *A. occidentale*, whereas in the stem bark, reported the presence of condensed tannins, phenols, catechins and alkaloids. Bouzada et al. (2009) reported the presence of alkaloids, triterpenes, tannins, flavonoids and anthraquinones in the methanol extracts of bark. Paes et al. (2006) also confirmed the presence of condensed tannins in the bark.

In the leaves of *C. citratus*, the presence of flavonoids, saponins, coumarins, terpenes / steroids and amino acids in the crude methanolic extract was observed in this work whereas in the methanol fraction resulting from the sequential extraction, the same metabolites are noted except for terpenes / steroids which were identified in the hexane extract. Their absence in the methanol fraction from sequential extraction can be explained by the fact that during sequential extraction, the terpenes / steroids were probably removed (extracted) by hexane. These results corroborate with several studies in which the chemical constituents in the extracts of *C. citratus* were determined. The studies performed by Ekpenyong et al. (2014) on the chemical composition of *C. citratus* revealed the presence of tannins, saponins, flavonoids, phenols, anthraquinones and various constituents of essential oils. Several studies on chemical composition of the essential oil of *C. citratus* leaves revealed that although the chemical composition of the essential oil of *C. citratus* varies according to the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered and among the fixed constituents of the aerial part were: flavonoids, alkaloid substances, a sterol saponin, β-sitosterol, hexacosanol, triacontanol, triterpenoids, cymbopogonol and cymbopogone (Negrelle and Gomes, 2005).

Although different metabolites were identified in the extracts of the three plants studied, their presence varied from extract to extract. Most of the results from phytochemical screening were similar to those of the literature and even where found any difference, that can be explained by the fact that in general, the chemical composition of the extracts may vary according to different parameters including the geographical origin, the genetic variations, the part of the plant used, the extraction method, the age/maturation stage, the harvesting season, among others.

Larvicidal activity

All extracts tested were found to have some insecticidal activity against *P. xylostella* larvae. Particularly, the crude methanolic extracts from root bark, stem bark and leaves of *T. emetica* were highly lethal for second instar larvae of *P. xylostella*, causing mortalities in the order of 100%, 94.41 and 97.25% respectively at concentrations of 1.28 $mg.ml^{-1}$, 1.50 $mg.ml^{-1}$ and 1.72 $mg.ml^{-1}$ (Tables 3, 4 and 5). As shown in Tables 3, 4 and 5, the range of concentrations for crude methanol extracts varied from 0.80 to 1.72 $mg.ml^{-1}$ and the percentage of mortality between 2.78 and 100% after 72 h of exposure. In all tests the insecticide HALT 5% WP used as positive control gave 100% of larval mortality at the same concentration of 0.75 $mg.ml^{-1}$. Distilled water used as negative control gave 0% of larval mortality.

The crude methanol extracts of root bark, stem bark and leaves of *T. emetica*, which are the most active fractions against larvae of *P. xylostella*, contain mainly as secondary metabolites: alkaloids, flavonoids, tannins,

saponins, terpenes / steroids and anthraquinones. Each of these secondary metabolites has been reported in the literature to possess insecticidal properties and in some cases even against the insect-pest *P. xylostella*. Trindade et al. (2008) reported that the alkaloids present in the ethanolic extracts of *Aspidosperma pyrifolium* had excellent insecticidal properties against *P. xylostella*. In the literature have been reported the correlation between the antifeedent activity of the root extracts of *T. emetica* and the presence of seco limonoids (Komane et al., 2011; Guanatilaka et al., 1998; Traore et al., 2007). The root and leaf extracts of *Trichilia roka* yielded a series of "Trichilins" as antifeedants against *Spodoptera eridania* and *Epilachna* (Kubo and Klocke, 1982); and the same compounds have been isolated and identified also in *T. emetica* (Guanatilaka et al., 1998). The presence of Limonoids which are tetra-nor-triterpenes, in extracts of *T. emetica* is reported to be mainly responsible for the bioactivities including insecticidal activity.

Viegas Júnior (2003) reported that in the organic compounds, mostly alkaloids and terpenoids were good candidates for insecticidal compounds that could be an alternative for insects control with a low toxicity to human and household animals and ecologically friendly. He reported various examples of compounds belonging to different classes of terpenes (from monoterpenes to triterpenes and limonoids) which possess insecticidal properties. A diversity of limonoids derived from plants of the Meliaceae family have been reported to exhibit larvicidal activity to *P. xylostella* (Park et al., 2014; Yan et al., 2015),

The insecticidal activity of saponins is well documented in the literature (Chaieb, 2010; D'Incao et al., 2012). Their insecticidal activity may be associated to the ability of producing alterations in the feeding behaviour, in the molting process, of interacting with hormones that regulate the growth and causing death in the different stages of development (D'Incao et al., 2012). Chaieb (2010) reported that besides *P. xylostella* being a phytophagous specific insect consuming plants belonging to brassicaceae family, its larvae are unable to attack one Brassicaceae species: *Barbarea vulgaris*, because of the involvement of a triterpenic saponin with two sugars in C_3 position, in the important inhibition of the food uptake activity.

Alkaloids have been reported to constitute part of the plant defenses against phytophagous animals together with terpenoids, phenols, flavonoids, steroids, etc. and are insecticidal at low concentrations but with varied mode of action(Acheuk and Doumandji-Mitiche, 2013).

In Figures 1, 2 and 3 was shown the percentages of larval mortality as a function of extracts concentrations in seven treatments and the curve shows significant exponential growth. The three figures are an illustration only for the crude methanol extracts of root bark, stem bark and leaves of *T. emetica*; but the same procedure has been done for all extracts (crude methanol extracts of *A. occidentale* and *C. citratus*, and all extracts from sequential extraction) for the determination of LC_{50}. All LC_{50} values were presented in Table 6 but herein were not given all graphs. Figures 1, 2 and 3 were only an illustration of the methodology used for all extracts.

As shown in table 6, crude methanol extracts and methanolic fractions resulting from sequential extraction had a greater insecticidal effect against *P. xylostella* (low LC_{50} values) in relation to the other extracts; This can be explained by the fact that methanol extracts a large number of bioactive secondary metabolites.

On the other hand, fractionation or sequential extraction in the order n-Hexane, ethyl acetate and methanol allowed separation of the active principles from the extracts according to their polarity. This may positively or negatively influence the insecticidal activity of the extracts depending on the concentration of the active ingredient in the fraction, since the metabolites act on the larvae independently or synergistically (Torres et al., 2006).

The leaves extracts of *A. occidentale* and *C. citratus* exhibited the highest values of $LC_{50,}$ that is, the lowest larval mortality independently of the extraction solvent, while the crude methanol extract of the root bark of *T. emetica* exhibited the highest larvicidal activity against *P. xylostella* larvae with LC_{50} of 0.94 $mg.ml^{-1}$ (Table 6).

Several authors described the relationship between the biological activity of plant extracts and their chemical composition. This relationship often suggests that the biological activity of an extract can be attributed to both its major components and components present in lower concentration. It is possible for them to act together synergistically, contributing to the total toxicity of the test extract (Dias and Moraes, 2014).

In the Mahotas farmers' horticultural plantations, they spray 10 to 20 g of insecticide Halt 5% WP in each treatment in 20 L of water, corresponding to a concentration of 0.5 to 1.0 $mg.ml^{-1}$. Thus the LC_{50} obtained for the crude methanol extract of the root bark of *T. emetica* (0.94 $mg.ml^{-1}$) is within this range, making it the most efficient against *P. xylostella* larvae of all extracts evaluated.

CONCLUSION

The results indicated that the extracts of the three plants studied show a potential insecticide on the second instar larvae of *P. xylostella*.

In view of the results achieved and the increasing need to find new substances capable of combating the development of pests causing large damages in crucifers while providing greater safety for humans and the environment, the crude methanol extract of the roots bark of *T. emetica* has the potential to be used as larvicide against *P. xylostella*.

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