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Evaluation of the insecticidal activity of the extracts of *Trichilia emetica*, *Trichilia capitata,* and *Azadirachta indica* against the *Spodoptera frugiperda* (Fall Armyworm) on maize crop

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Abstract: Plant extracts contain many active compounds called secondary metabolites, which are tremendously fruitful for plant defense against several insect pests. The main objective of the present study was to evaluate the aqueous and ethanolic plant extracts of *Trichilia emetic*, *T. capitata,* and *Azadirachta indica* for their insecticidal activity against *Spodoptera frugiperda* known as fall armyworm on maize crop as well as the preliminary phytochemical evaluation. All extracts were prepared using a Soxhlet apparatus, the insecticidal activity was performed on the disc immersion diffusion method, and the preliminary phytochemical analysis was done using the classical methods. The concentrations of the extracts for the insecticidal activities ranged from 0.01 to 1% (w/v). The third instar larvae of *S. frugiperda* were fed with corn leaves treated with these extracts, separately. Ethanol and distilled water were used as controls. The results showed a strong insecticidal activity of the extracts of the three plants tested, with LC_{50} of 0.212 for the aqueous extract of the leaves of *T. emetica*; 0.155 for aqueous extract of the stem bark of *T. emetic*a; 0.12 for aqueous extract the leaves of *T. capitata*; 0.051 for aqueous extract of the stem bark of *T. capitata*, and 0.13 for aqueous extract of the leaves of *A. indica*. These activities were dependent on the concentration, in which the stem barks aqueous extract of *T. capitata*, with LD_{50} of 0.051% was the most active. The preliminary phytochemical analysis was carried out on all extracts to identify the secondary metabolites that could be related to the observed insecticidal activities. All the extracts showed the presence of flavonoids, anthraquinones, and condensed tannins. Alkaloids and glycosides were detected in all extracts except for the leaves aqueous extract of *A. indica*. However, *T. capitata* was evaluated for the first time for the biological activity as well as the phytochemical analysis. It could be concluded that all the plant extracts possessed significant insecticidal properties and could be introduced as botanical insecticides after field evaluations.

Keywords: *Spodoptera frugiperda; biopesticides; Trichilia emetica; Trichilia capitata; Azadirachta indica.*

I. Introduction

Spodoptera frugiperda (J. E. Smith, 1797) (Lepidoptera: Noctuidae), also known as the fall armyworm, is a polyphagous insect of enormous agricultural importance, not only by the capacity to damage crops, also due to difficulties of its control. The specie is a migratory pest endemic to the Western Hemisphere that occurs from southern Canada to Argentina and causes considerable economic losses in several important crops such as maize, sorghum, rice, cotton, alfalfa, forage grasses, and occasionally other crops in most of the countries of its range [1], including Mozambique.

Mozambique is located in the Southern region and on the East coast of Africa, with more than 2,000 km long coastline extending from Tanzania to South Africa. The dominant vegetation types of the country are savannah and secondary forests covering up to 70% of the total area. The population is primarily working in agriculture. The sector of fishing also plays an important role and its main area is the Mozambique Channel, a branch of the Indian Ocean between Mozambique and Madagascar [2]. With 28 million inhabitants, the majority of the population is engaged in agricultural activity. More than 90% constitute the family farmers, who live in rural areas, depending mainly on rain-fed agriculture as a means of subsistence and income. Pests and crop diseases are the main problems in the agricultural sector.

Synthetic chemical insecticides are available sources for controlling this destructive pest, which a key success for modern agricultural practices and enhances crop yield. However, indiscriminate use of synthetic pesticides for crop production and protection poses poisonous effects through contact, exposure, and has become a cause of cancerogenesis, fertility problems, and dietary humans [3].

These circumstances led to searching for effective and eco-friendly pest control alternatives, especially from natural plant resources. Many insecticides derived from botanical sources are available and easily affordable and accessible to the farming community; they are safer for human beings and the environment with minimal residual effect, they are target-specific and less toxic to vertebrates, pollinators, and fish [3]. Studies have demonstrated that plant-based products possess promising insecticidal properties against *S. frugiperda* [1,4,5]. Therefore, the leaves and stem barks of *T. emetica* and *T. capitata* and the leaves of *A. indica* species belonging to the Meliaceae family were selected for the present study. The selected plants possess a natural ability to deter insect attack.

As medicinal plants, *T. emetica*, *T. capitata*, and *A. indica* are found within the country and are known in the traditional medicine for having many pharmacological properties [6–8] and repellent effects [9] The phytochemical analysis of *T.* emetic has been shown the presence of limonoids with selective inhibitory activity toward DNA repair-deficient yeast mutants [10], Kurubasch aldehyde benzoate with cytotoxicity activity [11], while from *A. indica*, the azadirachtin derivatives [12], triterpenoids with insecticidal activities against *Anopheles stephensi* [13,14], and limonoids with cytotoxicity and apoptosis-inducing activities [15] have been isolated. Up to date, no pharmacological and phytochemical evaluations have been done on *T. capitata*.

Due to issues concerning the use of synthetic pesticides and increasing resistance in pest species, pests can be managed by introducing botanical insecticides, especially against *S. frugiperda*. To our knowledge, no previous studies have been conducted regarding the insecticidal activity of the extracts of *T. emetica*, *T. capitata*, and *A. indica* against *S. frugiperda*. To investigate safe alternatives for the management of this pest, the present study was conducted to evaluate the insecticidal effects of crude aqueous and ethanolic extracts of the leaves and stem barks of T*. emetica* and *T. capitata*, and the crude aqueous and ethanolic extract of the leaves of *A. indica* under laboratory conditions, as well as the preliminary phytochemical investigations of the crude extracts.

II. Experimental Section

II.1 Plant collection

The leaves and stem barks of *T. emetica* were collected in July 2019 in Namaacha District, Maputo Province, located in the Southern part of Mozambique, while the leaves and stem barks of *T. capitata* in the same month in Cheringoma District, Sofala Province in the central part of the country. The leaves of *A. indica* were collected in August 2019 in the Herbarium of the Botanical Garden at Eduardo Mondlane University, located in Maputo City. After collection, the samples were authenticated in the Herbarium of the Biological Sciences Department of Eduardo Mondlane University by comparing these with the species found in the herbarium with voucher Numbers: 2781 (*T. emetica*), 1122 (*T. capitata*), and 345 (*A. indica*), respectively. The samples were dried separately

in an oven at 40°C for 48h at the Natural Products Laboratory of the Chemistry Department, Science Faculty, Eduardo Mondlane University. The dried samples were ground into a fine powder and stored in a freezer until use.

II.2 Extraction of plant materials

Each powdered material (15 g) was subjected to extraction by Soxhlet with 120 ml of water for 8 h to obtain crude aqueous extracts. The same amount of plant material was subjected to the same procedure using ethanol 96% to obtain the ethanolic crude extracts. The obtained extracts were concentrated in the rotary evaporator at 50 ºC for aqueous extracts and 40º C for ethanolic extracts to yield crude extracts. The obtained extracts were kept in a freezer for further analysis.

II.3 Phytochemical analysis

The phytochemical analysis aimed to identify the metabolites present in the plants under analysis and which are responsible for the insecticidal activity. In general, the analysis followed the procedures proposed by Grover and co-workers [16] for the presence of alkaloids, flavonoids, glycosides, steroids and terpenoids, tannins, amino acids, saponins, reducing sugars, coumarins, and anthraquinones were investigated.

II.4 Rearing of *Spodoptera frugiperda* under laboratory conditions

For the rearing *S. frugiperda*, the corn leaves were used as hosts. For this purpose, the maize/corn seeds were sown in pots and placed in the greenhouse of the Faculty of Agronomy and Forest Engineering of Eduardo Mondlane University, until the plant reached the optimum stage to be used in bioassays (25 to 40 days). The first colony that consisted of larvae of the 5th instar of *S. frugiperda* was obtained from the farmers of Paulo Samuel Kankomba, in the municipal Boane District, which is located 30 km outside of Maputo Center. After the acquisition, the larvae were individualized in flasks with approximately 4 cm in diameter and 5 cm in height. The larvae were fed with corn leaves until they reached the pupal stage. The lids of the flasks were punctured with a needle to allow the larvae to breathe and the corn leaves were changed every two days. When the insects reached the pupal stage, they were removed from the flasks and placed in a cage, where they remained until reaching adulthood. The adult insects remained in the cage, in an amount of six butterflies per cage and were fed with a 10% honey solution and water. Three corn plants were placed in each cage for laying. After laying, the corn leaves that contained the eggs were cut with a chopper and placed in plastic bowls covered with cloth where that remained until the larvae hatched. The newly hatched larvae remained in the bowls, being fed with leaves of young corn. To face the cannibal behavior that characterizes the Fall Armyworm larvae, as soon as the larvae reached the 2nd instar, they were individualized in flasks, where they remained until reaching the 3rd instar, which is the stage in which the larvae were used for the bioassays.

II.5 Larvicidal activity test

The bioassays for determining the larvicidal activity of plant extracts were carried out in the entomology laboratory, located at the Faculty of Agronomy and Forestry Engineering, at 30 ± 5 ºC of temperature, 70 ± 5% of relative humidity, and natural photophase, using the immersion method. Third instar caterpillars from breeding were individualized in breeding tubes, into which a small number of corn leaves were placed. Before the caterpillars were offered, the corn leaves were immersed in extracts of different concentrations for 3 minutes and left outdoors for 10 minutes for evaporation of excess moisture.

Every two days old leaves were removed and new leaf portions were offered to caterpillars. The trial consisted of seven repetitions, per treatment, in a completely randomized design.

Assessments were made daily until pupae emerged, checking larval mortality and larval periods (days). Caterpillars were considered dead when touched with a brush, they showed no movement. Lethal concentrations (LC_{50}) were also estimated, first carrying out preliminary tests to determine the upper and lower limits, that is, a concentration that caused mortality close to 100% and another with mortality close to the control. For the calculation of the LC_{50}, the mortality and concentration data were submitted to the probity analysis.

II.6 Statistical analysis

Larval mortality data were subjected to analysis of variance (ANOVA) and in cases where there were significant treatment effects; the average was compared using the Turkey test at 5% probability using the SPSS version 15 statistical package.

III. Results and Discussion

III.1 Preliminary phytochemical analysis

Preliminary qualitative tests are useful in the detection of bioactive compounds and subsequently may lead to drug discovery and development of novel drugs. Herbal standardization is an essential step to assess the quality of drugs, based on the concentration of their active principle, physical, and chemical standards. Moreover, a plant extract may contain several thousands of different secondary metabolites; however, the phytochemical analysis will reveal only a narrow spectrum of its constituents. Successive isolation of bioactive compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [16,17].

The results of the preliminary phytochemical analysis of various extracts of *T. emetica*, T*. capitata*, and *A. indica* are presented in Table 1. All the extracts revealed the presence of flavonoids, anthraquinones, and condensed tannins. According to Table 1, all extracts showed the presence of alkaloids and glycosides, except for the aqueous extract of the leaves of *A. indica, which* was the only sample that indicated the presence of hydrolyzed tannins.

Table 1. The phytochemical analysis of the extracts of T. emetica, T. capitata, and A. indica

Plant material	Extract	Phytochemicals										
		Alc	Fla	Gly	S/T	CT	HT	Prot	Sap	Cou	AQ	RS
T. emetica leaves	Aqueous	+	+	+	+	+	-	+	+	-	+	+
	Ethanol	+	+	+	-	+	-	-	-	+	+	-
T. emetica stem bark	Aqueous	+	+	+	+	+	-	+	+	-	+	+
	Ethanol	+	+	+	+	+	-	+	-	-	+	+
T. capitata leaves	Aqueous	+	+	+	+	+	-	+	+	-	+	+
	Ethanol	+	+	+	-	+	-	-	-	+	+	-
T. capitata stem bark	Aqueous	+	+	+	+	+	-	+	+	-	+	+
	Ethanol	+	+	+	-	+	-	+	-	-	+	+
A. indica leaves	Aqueous	-	+	-	+	+	+	-	+	+	+	+
	Ethanol	+	+	+	-	+	-	+	-	-	+	+

Legend: *+: Detected; -: Not detected; Alk: Alkaloids; Fla: Flavonoids; Gly: Glycosides; S/T: Steroids/Terpenoids; CT: Condensed Tannins; HT: Hydrolysable Tannins; Prot: Proteins; Sap: Saponins; Cou: Coumarins; AQ: Anthraquinones; RS: Reducing sugar.*

III.2 Larvicidal activity

All larvae fed with corn leaves treated with ethanol extracts from the three plants, including control (larvae fed with corn leaves previously immersed in ethanol) died within 24 hours after treatment. The

mortality rate among the larvae treated with the aqueous extracts varied depending on the concentration, the plant species, and the part of the plant used in the bioassay (Tables 2 and Table 3). Regarding the aqueous extract of *T. capitata* stem bark, which we will take as an example of the treatments, the average mortalities caused by these extracts in concentrations of 0.01, and 0.03% did not differ significantly with those of the control by the Turkey test at 95% significance. 100% mortality was achieved at concentrations of 1%, 0.8%, 0.8%, 0.6, and 0.6% of aqueous extracts of leaves, stem bark of T. emetica, leaves, and stem bark of *T. capitata* and *A. indica* leaves, respectively. For the comparison between the different extracts, LD_{50} (concentration capable of causing mortality in 50% of the tested individuals) was calculated (Table 4). According to these values, greater insecticidal activity was observed in the aqueous extract of *T. capitata* stem bark with LD_{50} = 0.051 $g.ml^{-1}$, while the lower larvicidal activity was observed in aqueous extracts of *T. emetica* leaves with LD_{50} = 0.212 $g.ml^{-1}$. Figures 1 and 2 show the graphs through which the LD_{50} values were calculated for the extracts *of T. capitata*. The same procedure was used to estimate the LD_{50} of other tested extracts.

The results of biological tests with aqueous extracts showed a strong larvicidal activity of these extracts depending on its concentrations. These results are not surprising, since many other plants of the genus *Trichilia* have already shown insecticidal activity against *S. frugiperda* larvae. On the other hand, the insecticidal and antifeedant properties of *A. indica* are well established, particularly against pests of the *lepidopteran* order [18]. The higher concentrations of the extracts caused mortality of the larvae in a very short period, while lower concentrations; either caused slow mortality of the larvae or lengthened the larval stage of the insects in comparison with the control. This act may be because high dozen of these insecticides cause intoxication of the larvae, causing them to die quickly, and low dozen cause a reduction in the feeding of the larvae, causing them to die slowly, or even lengthening their larval period.

The effectiveness of the extracts *Trichilia* species and *A. indica* on the larvae of *S. frugiperda* have been confirmed by the other authors [19], which could be suggested that the plants investigated may be used in the future as natural pesticides in controlling *S. frugiperda* in maize crop.

Table 2. Mortality percentage of 3rd instar larvae of S. frugiperda exposed in different concentrations of aqueous extract of T. capitata leaves (n = 100)

Concentration (% w/v)	Mortality (n)	% Mortality
0,8	10	100 ± 0,000 a
0,6	9	90 ± 0,316 ab
0,4	7	70 ± 0,483 abc
0,2	6	60 ± 0,516 abcd
0,1	4	40 ± 0,516 bcde
0,05	2	20 ± 0,422 cde
0,01	1	10 ± 0,316 de
0	0	0 ± 0,000 e

The averages followed by the same letter do not differ, according to the Turkey test (p≤ 0.05)

Table 3. Mortality percentage of 3rd instar larvae of S. frugiperda exposed in different concentrations of aqueous extract of T. capitata stem bark (n = 100)

Concentration (% m/v)	Mortality (n)	% Mortality
0,6	10	100 ± 0,000 a
0,4	9	90 ± 0,316 a
0,2	8	80 ± 0,422 ab
0,1	7	70 ± 0,483 abc
0,05	5	50 ± 0,527 abcd
0,03	3	30 ± 0,483 bcd
0,01	2	20 ± 0,422 cd
0	0	0 ± 0,000 d

The averages followed by the same letter do not differ, according to the Turkey test (p≤ 0.05)

Table 4. LD_{50} estimates of aqueous extracts of species tested for third instar larvae of S. frugiperda

Plant specie	Part of the plant	DL_{50}
Trichilia emetica	leaves	0,212
	Stem bark	0,155
Trichilia capitata	leaves	0,12
	Stem bark	0,051
Azadirachta indica	leaves	0,13

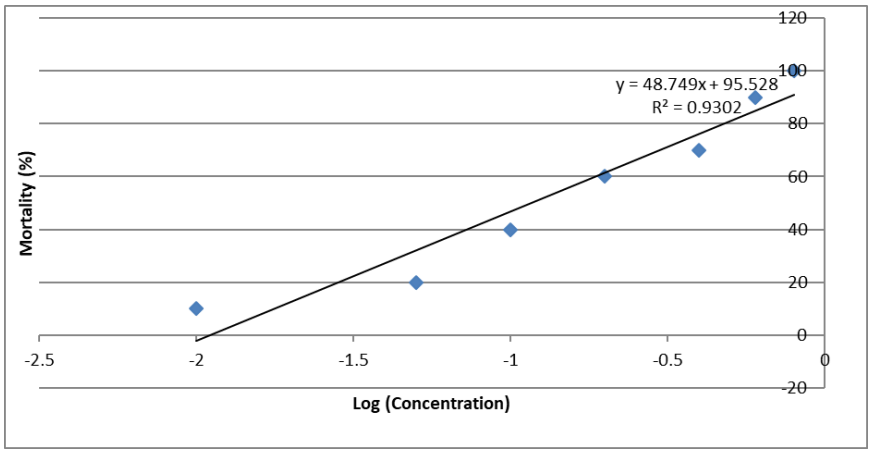

Figure 1. Mortality of S. frugiperda third instar larvae as a function of the log of the aqueous extract concentration of T. capitata leaves

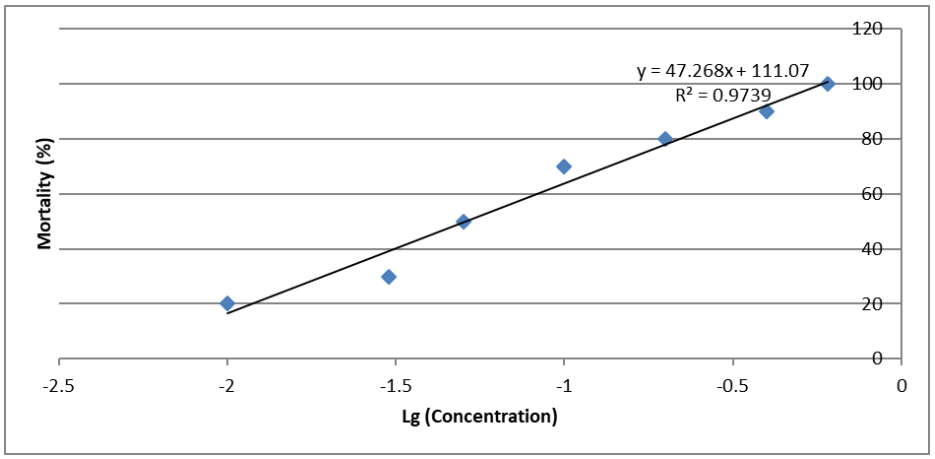

Figure 2 Mortality of S. frugiperda third instar larvae as a function of the log of the aqueous stem bark extract concentration of T. capitata

IV. Conclusion

The results of biological tests suggest that, as *A. indica*, *T. emetica* and *T. capitata* constitute a valuable source of botanical insecticides, which are at the disposal of corn producers, especially in countries like Mozambique where most corn producers are family farmers who have few financial resources to purchase synthetic insecticides. On the other hand, these biopesticides are easy to prepare and offer no danger during their handling, in addition to not being aggressive to the environment.

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Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

V. References

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