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Insecticidal activity of *Vitex doniana* (Black plum) on *Spodoptera littoralis* (moth)

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ABSTRACT

Vitex doniana is a medium-sized deciduous tree of 8–18 m high, with a heavy rounded crown and a clear pole up to 5 m in height. The study evaluated the insecticidal efficacy of *V. doniana* extract on *Spodoptera littoralis* using bark and leaves. The plant parts were collected from Abubakar Tafawa Balewa University, Bauchi. Samples were left to dry by air under normal conditions in the laboratory for 2 weeks. The dried samples were ground into powder using a blender. The powder was then extracted using 350% ethanol by cold maceration. The culture of *S. littoralis* was maintained in the laboratory on cabbage and corn leaves for the insect to acclimatize to the environment. The phytochemicals found in the extract were alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and terpenoids. The study revealed that the crude ethanolic extract of leaves at 400 mg concentration was more effective against the seventh larva stage than that of the bark at the same concentration with average mortality rate of 6.08%, compared to 4.08% for the bark. Hence, the use of *V. doniana* leaves as an insecticide for the control of *S. littoralis* is safe and environmental friendly compared to the synthetic insecticides, which are hazardous, and its residual effect is persistent in the environment.

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1. Introduction

Many diseases have been treated using medicinal plants on a daily basis all over the world (Okoli et al., 2007). The use of medicinal plants in Nigeria and other part of the black African countries started many centuries ago and more than 60% of the rural population are still dependent on traditional medicine for their healthcare needs and agricultural purposes, especially in Nigeria (Edeoga et al., 2005). *Vitex doniana* is known by different names in different languages, dinya (Hausa), uchakoro (Igbo), oori-nla (Yoruba), and black plum in (English).

The reckless use of chemical pesticides has given rise to numerous problems, including genetic

resistance of pest species, residual effect, and increase in cost of application and pollution of the environment (Hamad, 2017). This has brought about worldwide interest in the development of alternative strategies, including the search for new types of insecticides that are safe and reliable such as used traditional medicines for pest control. Plant crude extracts mostly consist of many different compounds or metabolites which may act synergistically (Berenbaum, 1985). The concept of using nontoxic antifeedant to protect crops without directly killing the pest is a good alternative and has received remarkable attention (Dodia et al., 2010). Pesticides from medicinal plants have proven to be very effective, safe, and environmentally friendly (Nathan et al., 2006).

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Spodoptera littoralis infests a wide range of cultivated food plants numbering around 112, belonging to 44 families (Baskar et al., 2012). Pest control requires a large number of alternative products that meet food safety, sustainability, and environmental care requirements. One of the strategies used to obtain new natural agents for protecting crops and domestic animals is the exploration of a diversity of plants and their metabolites (Hüter, 2011).

Natural products with pesticide properties have been described to be an important alternative source of compounds either in their natural form or as semi-synthetic derivatives showing better effects at controlling pest. Moreover, the chemical structures of the active components of natural products have guided the synthesis of other active compounds (Hüter, 2011). The understanding of the benefits and the use of natural products are increasing rapidly, focusing on identifying metabolites for use in the treatment of human and plant diseases, as well as pest control in the agricultural sector (Spiegler et al., 2017).

The importance of using medicinal plants in many different sector and their effect, safety, (Nathan et al., 2006), and biodegradable effect (Abdel El-Aziz, 2011; Khedr and El-Kawas, 2013) has resulted in the increased study of many different plant derivatives to control human diseases and agricultural pests as well. Knowledge on the insecticidal activity of *V. doniana* is lacking but it is only known to be effective in the treatment of human diseases and as a sweetener. Therefore, this study aimed at assessing the effectiveness of *V. doniana* leaves and bark extracts on *S. littoralis*.

2. Materials and Methods

2.1. Collection and extraction of plant material

Plant parts were collected from Abubakar Tafawa Balewa University, Bauchi, in September 2019. The samples were thoroughly washed to remove dust and dirt and were then left to dry by air under normal conditions in the laboratory for 2 weeks. The dried samples were ground and extracted accordingly. Each extract was allowed to evaporate under vacuum at 40°C–50°C where the obtained residue was weighted. About 100 g of powdered leaves and bark plant material was separately extracted by cold maceration with 350 ml of ethanol at room temperature for 72 hours with occasional shaking. The extract was filtered and the filtrate was reduced under vacuum using a rotary

evaporator and dried in an oven at 40°C. They were stored in a refrigerator at about 4°C.

2.2. Insect maintenance

A standard laboratory culture of *S. littoralis* was maintained on cabbage and corn leaves for the insect to acclimatize to the environment.

2.3. Phytochemical tests

The extract was tested for the presence of phytochemical constituents: saponin, tannin, flavonoids, glycoside, steroids, alkaloids, and terpenoids using standard methods as described by Trease and Evans (2002), Khandelwal (2008), and Evans (2002).

2.3.1. Test for tannins

1 g of *V. doniana* stem bark powder was boiled in 10 ml of distilled water and filtered. 2 ml of the filtrate was added to three drops of ferric chloride solution. While to another 2 ml portion, 1 ml of lead acetate was added. The appearance of a brown-green precipitate with ferric chloride and a creamy brown precipitate with lead subacetate suggested the presence of tannins.

2.3.2. Test for alkaloids

1 g of *V. doniana* stem bark powder was warmed in 5 ml of 10% H_2SO_4 for 2 minutes and filtered. Two drops of Mayer's reagent was added to 1 ml of the filtrate. The rest of filtrate was alkalized using dilute ammonia and extracted with 2 ml of chloroform. Chloroform was evaporated and left a solid residue which was dissolved in 0.2 ml of 10% H_2SO_4 and separated into two portions. A drop of Mayer's reagent was added to one portion while a drop of Dragendorff's reagent was added to the other portion. The appearance of white to buffy precipitate with Mayer's reagent and an orange red precipitate with Dragendorff's reagent suggested the presence of alkaloids.

2.3.3. Test for glycosides

1 g of *V. doniana* stem bark powder was suspended in 10 ml of 70% alcohol and heated in a water bath at 70°C for 20 minutes and was then filtered. 10 ml of distilled water was added to the filtrate, and then a solution of lead subacetate. The solution was then filtered and 10% H_2SO_4 was added until no further precipitation occurred. The resulting solution was filtered and extracted with 5 ml portions of chloroform added. The chloroform extracts were combined, washed with distilled water, filtered, and

evaporated to dryness. The dry extracts were subjected to Keller-Killian and Kedde's tests.

2.3.4. Kedde's test

Two drops of Kedde's reagent was added to one of the portion of the dry extracts. Purple color indicated the presence of cardiac glycosides.

2.3.5. Keller-Killian test

To the second extract, 0.4 ml of acetic acid containing trace ferric chloride was added; it was shaken gently to dissolve and 0.5 ml of concentrated H_2SO_4 was added; a reddish brown color at the inter phase, which later turned blue green, indicated the presence of deoxy sugars.

2.3.6. Test for saponins frothing test

A small amount of *V. doniana* stem bark powder was placed in a test tube and distilled water was added. The mixture was shaken and left to stand. Persistent frothing suggested the presence of saponins.

2.3.7. Test for flavonoids (Shibata's reaction)

Approximately 3 ml of extract was warmed with three pieces of magnesium turnings and mixed with three drops of concentrated HCl . An orange pink coloration was an indication of presence of flavonoids.

2.3.7.1. Antifeedant activity (choice test). The antifeedant activity of the tested plant extracts was experimented against the seventh instar larvae of *S.*

littoralis as described by Sammour et al. (2011). Five replicates were used for the tested extract.

3. Results

3.1. Phytochemical investigation

Phytochemical groups present in *V. doniana* were alkaloids, glycosides, saponins, steroids, flavonoid, terpenoids, and tannins detected in both bark and leaves of *V. doniana*, as shown in Table 1.

3.2. Antifeedant activity (choice test)

The data in Table 1 show varying degrees of the anti-feeding activities of the ethanolic extract of both leaves and bark of *V. doniana* against *S. littoralis*. Five insects (seventh larva stage) were fed with 10 g of corn first without applying the extract of *V. doniana* and they consumed (M_0) = 5.02 g in 3 days = $5.02/3 = 1.67$ g/day, which represent their normal feeding rate.

Similarly, five insects (seventh larva stage) were fed with 10 g of corn and the extract of *V. doniana* (bark and leaves) were also applied in 400, 200, 100, 50, and 25 mg concentrations, respectively, and results are shown in Tables 2 and 3.

M_0 (g/day) = Normal feeding rate;

M_1 (g) = Initial mass of corn feeds;

M_2 (g) = Mass of corn feeds remnant;

M_2 (g) = Mass of corn feeds remnant;

M_3 (g) = Mass of corn feeds consumed = $M_1 - M_2$

Percentage feeding (%) = $M_3/M_0 \times 100\%$

Feeding rate (g/day) = $M_3/\text{No. of days}$

Table 1. Phytochemical groups present in *V. doniana* leaves and bark.

Chemical class	Phytochemical test	Bark relative abundance	Leaves relative abundance
Alkaloid	Mayer's	++	+
Glycosides	Kedde	+	+
Saponins	Frothing test	+++	++
Steroids	Buchard's test	++	+
Flavonoids	Shibata's reactions	+++	++
Terpenoids	Gelatin test	+	++
Tannins	Dragendroff	+	+

+, Trace amounts; ++, moderate; +++, Abundant.

Table 2. Percentage feeding (%) for bark extract of *V. doniana*.

Concentration	M_1 (g)	M_2 (g)	M_3 (g)	No. of days	Feeding rate (g/day)	Percentage feeding (%)
400.00	10.00	9.76	0.24	2.00	0.12	7.17
200.00	10.00	8.64	1.36	2.20	0.62	36.94
100.00	10.00	7.50	2.50	3.00	0.83	49.80
50.00	10.00	6.26	3.74	3.00	1.25	74.50
25.00	10.00	5.30	4.70	3.00	1.57	93.63

Table 3. Percentage feeding (%) for leaves extract of *V. doniana*.

Concentration	M_1 (g)	M_2 (g)	M_3 (g)	Day(s)	Feeding rate (g/day)	Percentage feeding (%)
400.00	10.00	9.92	0.08	1.00	0.08	4.78
200.00	10.00	9.27	0.73	1.25	0.58	34.90
100.00	10.00	8.11	1.89	3.00	0.63	37.65
50.00	10.00	6.86	3.14	3.00	1.05	62.55
25.00	10.00	6.10	3.90	3.13	1.25	74.46

Table 4. Antifeeding effect of *V. doniana* bark extract on *S. littoralis*.

Concentration (mg)	Mortality	Day(s)	Time (hours)	Antifeedant activity	Mortality rate (No./Hr)	Average mortality rate (No./Hr)
400	1	1	24	No feeding	0.04	0.08
400	2	2	48	No feeding	0.04	
200	2	1	24	Very low feeding	0.08	0.12
200	2	2	48	Very low feeding	0.04	
100	3	2	48	Low feeding	0.06	0.09
100	2	3	72	Low feeding	0.03	
50	1	2	48	Moderate feeding	0.02	0.08
50	4	3	72	Moderate feeding	0.06	

Table 5. Antifeeding effect of *V. doniana* leaves extract on *S. littoralis*.

Concentration (mg)	Mortality	Day(s)	Time (hours)	Antifeedant activity	Mortality rate (No./Hr)	Average mortality rate (No./Hr)
400	3	1	0.5	No feeding	6.00	6.08
400	2	1	24	No feeding	0.08	
200	2	1	24	Very low feeding	0.08	0.18
200	3	2	30	Very low feeding	0.10	
100	3	2	48	Low feeding	0.06	0.09
100	2	3	72	Low feeding	0.03	
50	2	2	48	Moderate feeding	0.04	0.08
50	3	3	72	Moderate feeding	0.04	

4. Discussion and Conclusion

The phytochemical constituents, especially flavonoids, found in these plants extract agrees with the findings of Sharma et al. (2009) and Krishnarao et al. (1997). The findings of these researches revealed that an increase in the concentration of *V. doniana* leaves and bark extracts resulted in a corresponding decrease in feeding rate (Tables 3 and 4) and increase in mortality rate (Tables 5 and 6). Similarly, Nathan et al. (2006) also used the plants extract against rice leaf folder (*Cnaphalocrocis medinalis*), and it was effective by acting directly as a strong enzyme inhibitor.

Contrary to the findings of Egbekum et al. (1996), which revealed *V. doniana* as a nutritive sweetener and

found only vitamin C in the plant, Ladeji et al. (2005) also revealed these plant as antidiarrheal, while in this study the ethanol extract of leaves was found to be effective against *S. littoralis* with many different phytochemicals as showed in Table 1. Saponins, flavonoids, and terpenoids were found to be moderate in the leaf extract while saponins and flavonoids were found to be in abundance in the bark extract in the present study. This disagrees with the findings of Sharaf et al. (2000) as phenol appears to be the most abundant in the leaf extract, and agrees with the findings of the bark extract as alkaloids and saponins appear to be the most abundant.

The insecticidal activity of the leaf extract of these study may be due to the presence of phytochemicals (Table 1) present in the plant as it was reported by Isman (1997) that defense of plants against herbivores could be a result of mixtures of closely related compounds, instead of a single toxicant alone. He further pointed out that isoflavonoids, glycosides, and tannins could be the cause of the death of insects. Similarly, a research conducted by Dambolena et al. (2016) used terpenes natural products for controlling insects. Moreover, Harmatha (2000) worked on the role of spirostanol saponins in the interaction between plants and insects.

The commercial saponins extracted from *Quillaja saponaria* have shown a larvicidal activity against two species of mosquito larvae (*Aedes aegypti* and *Culex pipiens*) with 100% mortality using 1,000 mg/l within 5 days (Pelah et al., 2002). Antifeeding activity of the insect may be a result of the saponins found in both leave and bark extracts, saponin extract of *Ilex apocea* in a research conducted by Barbosa et al. (1990), inhibited the food uptake of *Limantria dispar*. Similarly, saponins were also an antifeedant for a mite species *Oligonichus illicis*.

Flavonoids, on the other hand, which appears to be in abundance in the bark extract, is mostly known for its medicinal activity and in food industry with little information on its insecticidal effect (Mierziak et al., 2014). Finally, from these findings, it was revealed that the leaves extract resulted in higher mortality and lower feeding rate compared to the results obtained from bark extract at the same concentration (Tables 4–6). Both bark and leaves extract of *V. doniana* showed insecticidal activity against the seventh instar larva of *S. littoralis*, with the leaves having the highest activity in the seventh instar larva. Therefore, *V. doniana* has the potential to be used as an insecticides that is safe and environmentally friendly. So, we recommend that further research be conducted to determine the most active compound that was effective against the study insect.

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