Effects of the Insecticide, Lambda-cyhalothrin on the Growth, Productivity and Foliage Anatomical Characteristics of *Vigna unguiculata* (L) Walp

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Abstract

The effects of the pyrethroid insecticide, Lambda-cyhalothrin on the vegetative, reproductive and foliage anatomical characteristics of cowpea (Vigna unguiculata) were investigated. Seedlings of V. unguiculata were sprayed with 1 ml L^{-1}, 2 ml L^{-1}, 3 ml L^{-1}, and 4 ml L^{-1}, concentrations of the insecticide, but the control was not sprayed. The maximum plant height, stem circumference, leaf area, number of leaves, flowers, pods, pod dimensions, lesions, leaf cuts, thickness of the leaf, upper and lower epidermises, number of stomata, length and width of stomatal pores and the number of mesophyll cells per unit area were determined. The results showed that the treatments increased significantly ($P < 0.01$), the vegetative parameters when compared with the control. The control had significantly ($P < 0.05$) more lesions and leaf cuts than all the treatments. All the treatments significantly ($P < 0.05$) increased the flowers, pods, pod dimensions leaf thickness, number of spongy cells and stomata on the abaxial leaf surface compared with the control, especially 2 ml to 4 ml treatments, while the stomatal pore width decreased. The result indicates that Lambda-cyhalothrin can control insect attack, may improve crop growth, development, yield, reduce transpiration and perhaps induce drought avoidance mechanism in V. unguiculata.

Keywords: Lambda-cyhalothrin, Productivity, Anatomy, Cowpea

Introduction

An insecticide is a pesticide used against insects in all developmental stages. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20th century. Nearly all insecticides have the potential to significantly alter the ecosystems; many are toxic to humans; and others are concentrated in the food chain. It is necessary to balance agricultural needs with environmental and health issues when using insecticides (Wikipedia, 2008). Lambda-cyhalothrin is a pyrethroid insecticide registered by the United States Environmental Protection Agency (USEPA, 1988). Pyrethroids are synthetic chemicals that are structurally similar to the natural insecticides, pyrethrins. Pyrethroid insecticides were developed to enhance biological activity and desired physical and chemical properties relative to pyrethrins. Lambda-cyhalothrin is similar to the pyrethroid, cyhalothrin, which is a mixture of four isomers, and two of these isomers compose Lambda-cyhalothrin. Pyrethroids affect the nervous system of an organism and act by disrupting the gating mechanism of sodium channels that are involved in the generation and conduction of nerve impulses. They disrupt the sodium channel activation gate by keeping it in an open position and delayed closing of the gate results in prolonged excitation of nerve fibres (WHO, 1990). Lambda-cyhalothrin causes rapid paralysis and death to an insect when ingested or exposed externally and it has repellent properties (Toth and Sparks, 1990). When insects are repelled or killed, the harvested or growing crops are protected from insect attacks, thereby enhancing greater productivity.

Cowpeas (*Vigna unguiculata* (L.) Walp. Leguminosae-Papilionoideae) are leguminous pulse crops grown mostly for their seeds which contain about 23% protein (Janick *et al.,* 1981, Vickery and Vickery, 1979). They may be ground into flour and also used as a coffee substitute. The fresh seeds and young pods are eaten as vegetable and the young shoots and leaves as spinach. The plant has trifoliate leaves and may be climbing or erect. The flowers may be white, pink, purple or yellow and the pods are long and narrow. Cowpeas mature quickly, taking about 60 – 80 days from seed sowing to harvest, but yields are not high and could be improved by better farming methods (Vickery and Vickery, 1979), including the application of insecticides.

Insects, in their feeding habit may riddle crops with lesions, cuts, holes and tunnels thereby reducing their productivity. An entire plant may be devoured by insects (Janick *et al.,* 1981). Allen and Amechebe (1981) reported that at least one major insect pest affects each stage of cowpea growth. They reported that *Aphis craccivora* may attack the seedling stage of cowpea, *Megalurothrips sjostedt* (flower thrips) at flowering, *Maruca vitrata* (pod borer) at pod formation and *Callosobrochus malculatus* (weevil) during seed storage. Cowpea is also susceptible to a number of fungal, bacterial and viral diseases. Pesticide usage can lead to toxicity issues, which may adversely affect plant growth and development. Application of foliar insecticides could adversely affect photosynthesis by clogging, or at least partially blocking stomata, which allow gaseous exchange to take place in plants. Reduced photosynthetic rates can delay production times or reduce plant productivity and quality, in addition to any visible damage due to chemical toxicity. Subtle negative effects of insecticides on individual crops can add up and lead to economic loss when multiple crops are grown. While visible phytotoxicity and toxicity to humans and animals are tested prior to insecticide registration, subtle impacts such as effects on flower production, stunted growth or longer production times are not often tested (Spiers *et al.,* 2008). The structure and ontogeny of stomata in different plants vary with the application of different growth regulators (Kishorekumar *et al.,* 2006) and some fungicides and insecticides may act as plant growth regulators, in addition to their function as fungicides and insecticides. Leaf anatomy is an important feature for internal water balance of plants and the anatomical characteristics may change due to the application of growth regulators (Kishorekumar *et al.,* 2006). There is therefore, the need to determine whether a pesticide can positively or negatively affect plant growth, anatomical characteristics and productivity of plants. The objective of this work is to investigate the effects of the insecticide, Lambda-cyhalothrin, on the growth, productivity and foliar anatomical characteristics of *Vigna unguiculata*.

Materials and Methods

Two seeds each, of *Vigna unguiculata* (L.) Walp. (Leguminosae-Papilionoideae) were sown in four perforated, black polythene bags (39,744 cm^3 average volume, each) containing an average of 14 kg of topsoil, dug out from the Botanic Garden in the University of Nigeria, Nsukka. The insecticide, Lambda-cyhalothrin and the seeds were obtained from the Crop Science Department, University of Nigeria, Nsukka. One ml L^{-1}, 2ml L^{-1}, 3 ml L^{-1}, and 4 ml L^{-1} concentrations of the insecticide were prepared and sprayed on the seedlings at two weeks interval, until flower production (The recommended dose is 3 ml L^{-1}, at 2 weeks interval according to the manufacturer's product label). Four bags containing two seedlings each, were sprayed with 1 ml L^{-1}, insecticide preparation, 40 ml for the two seedlings. This was repeated using 2ml L^{-1}, 3 ml L^{-1}, and 4 ml L^{-1}, respectively, instead of 1 ml L^{-1}, and labeled accordingly. The control was not sprayed with the insecticide. The experiment was carried out in three replicates in a completely randomized design. The maximum plant height, stem circumference, leaf area, number of leaves, number of flowers, number of pods, pod length, pod circumference, lesions and leaf cuts were determined. The leaf area was determined using the method of Francis *et al*. (1969), based on length x maximum width of leaf x 0.75. The following foliage anatomical characteristics were determined under x 400 magnification, using stage and eye piece micrometer calibrations: leaf thickness, thickness of upper and lower epidermises, number of stomata in the abaxial and adaxial leaf surfaces, length and width of stomatal pores and number of palisade and spongy cells per unit area (x 400 magnification). The data obtained were subjected to Analysis of Variance (ANOVA). Multiple comparisons were made between treatment means using Duncan's multiple range tests at $P < 0.05$ confidence level (Edafiogho, 2006).

Results

The result of treating *Vigna unguiculata* with different concentrations of the insecticide, Lambda-cyhalothrin, showed that the various treatments increased significantly ($P < 0.01$) the height, stem circumference, leaf area and number of leaves of the crop when compared with the control (Table 1). The treatments with 2 ml and 3 ml significantly ($P < 0.05$), produced the highest plant height and leaf area when compared with all the other treatments. This means that the insecticide might have caused the plant to grow in height, stem circumference, leaf area and number of leaves. The control had significantly ($P < 0.01$) more lesions and leaf cuts than all the treatments (Table 1). The lesions and leaf cuts decreased consistently as the concentration of the insecticide applied increased, to the extent that 4 ml had no lesions and leaf cuts, yet the plant height and leaf area significantly decreased, compared with these treated with 2ml and 3 ml. Therefore, Lambda-cyhalothrin might have effectively controlled insect attacks as evidenced by lesions and cuts. However, 3 ml and 4 ml concentrations gave temporary leaf shocks to the plants and bleached some leaves. These were not observed on the plants treated with 1 ml, 2ml and the control.

The results of reproductive parameters showed that significantly ($P < 0.01$) more flowers and pods were produced by the treatments than by the control (Table 2). This showed that the insecticide enhanced flower and pod production. The higher the concentration, the more the flowers and pods were produced. This was significant. However for the number of flowers, pods and pod circumference, the control was not significantly different from 1 ml, but for pod length, the control was significantly ($P < 0.01$) different from 1 ml. This showed that the insecticide might have produced more flowers and pods, as well as fatter and longer pods on *V .unguiculata*.

The result of foliage anatomical characteristics showed that the leaf thickness and the number of spongy cells per unit area significantly ($P < 0.05$) increased in the treated plants when compared with the control (Table 3). The higher the concentration of the insecticide, the more they increased. So the insecticide might have made the crop to grow in terms of leaf thickness and number of spongy cells. On the contrary, the lower epidermis and the number of palisade cells significantly ($P < 0.05$) decreased when compared with the control. The control and 1ml treatments were not significantly different from one another. There were no significant differences between all the treatments and the control, as regards the thickness of the upper epidermis. Therefore, the insecticide did not increase the upper and lower epidermises and palisade cells.

As regards the number of stomata in the adaxial surface, there was no significant difference between the control and all the treatments (Table 4). However, 1 ml treatment significantly ($P < 0.05$) differed from 3 ml and there were no significant differences between 2 ml, 3 ml and 4 ml treatments. There were variations in the number of stomata on the abaxial surface. There were no significant differences between the control and all the treatments, as regards the stomatal pore length. However, the stomatal pore width decreased significantly ($P < 0.05$) in the treatments when

Table 1: Vegetative parameters, lesions and leaf cuts on *Vigna unguiculata* treated with different concentrations of Lambda-cyhalothrin.

Treatment	Plant height	Stem circum	Leaf area	Leaf no.	Insect marks	Leaf cuts
Control	44.3 ± 3.2	2.4 ±0.04	72.9± 1.5	20.9± 0.7^{f}	13.9± 2.0	6.6 ±0.9
1 ml L^{-1}	127.6± 2.9^{a}	2.7± 0.04^{c}	102.9± 1.9^{d}	28.1± 1.0^{f,g}	5.4 ±1.5^{h}	2.3± 0.5^{k}
2 ml L^{-1}	130.7± 2.7^{a,b}	2.8 ±0.05^{c}	112.1± 1.6^{e}	32.8± 0.8^{g}	2.3 ±0.5^{h,j}	2.1±0.8^{k}
3 ml L^{-1}	142.8 ± 3.7^{b}	2.8 ±0.04^{c}	110.0± 1.6^{e}	33.4± 0.3^{g}	0.17 ±0.17^{j}	0.08 ±0.08^{l}
4 ml L^{-1}	111.6 ± 10.1	2.8 ±0.06^{c}	104.1 ±1.7^{d}	34.2± 5.7^{g}	0.0± 0.0^{j}	0.0 ± 0.0^{l}

Values represent means ± standard error, Values followed by the same letters in the same column are not significantly different at P < 0.05, ***key:*** *Circum = circumference, No. = number*

Table 2: Reproductive parameters of *Vigna unguiculata* treated with different concentrations of Lambda-cyhalothrin

Treatment	No. of flowers	No. of pods	Pod length	Pod circumference
Control	2.7 ± 0.2^{a}	2.5 ± 0.2^{h}	10.7 ± 1.0	2.2 ± 0.09^{f}
1 ml L^{-1}	3.3 ± 0.3^{a}	2.4 ± 0.3^{h}	16.0 ± 0.7^{c,d}	2.2 ± 0.06^{f}
2 ml L^{-1}	4.3 ± 0.4	3.6 ± 0.2	17.9 ± 0.7^{c,e}	2.3 ± 0.05^{f,g}
3 ml L^{-1}	7.3 ± 0.3^{b}	6.7 ± 0.3^{j}	18.6 ± 0.7^{e}	2.6 ± 0.06
4 ml L^{-1}	6.9 ± 0.3^{b}	6.5 ± 0.3^{j}	18.1 ± 0.7^{d,e}	2.4 ± 0.1^{g}

Values represent means ± standard error, Values followed by the same letters in the same column are not significantly different at P < 0.05. No. = number

Table 3: Thickness of leaf, epidermis and number of spongy and palisade cells of *Vigna unguiculata* treated with different concentrations of Lambda-cyhalothrin

Treatment	Thickness of leaf	Thickness of upper epidermis	Thickness of lower epidermis	Number of spongy cells	Number of palisade cells
Control	0.67±0.01^{a}	0.14±0.09^{c}	0.06±0.00^{d}	20.0±0.4^{f}	23.5±1.2^{g}
1ml L^{-1}	0.78±0.04^{a,b}	0.04±0.00^{c}	0.05±0.00^{d,e}	24.0±0.7	24.5±1.6^{g}
2ml L^{-1}	0.79±0.03^{a,b}	0.04±0.00^{c}	0.04±0.01^{e}	23.1±2.2^{f}	18.0±1.5^{h}
3ml L^{-1}	0.80±0.05^{b}	0.04±0.00^{c}	0.03±0.00	23.8±1.3	18.5±0.6^{h}
4ml L^{-1}	0.84±0.07^{b}	0.04±0.00^{c}	0.04±0.00^{e}	25.0±0.6	18.5±1.5^{h}

Values represent means ± standard error, Values followed by the same letters in the same column are not significantly different at P < 0.05.

Table 4: Stomatal number and stomatal pore length and width of *Vigna unguiculata* treated with different concentrations of Lambda-cyhalothrin

Treatment	No. of stomata in adaxial surface	No. of stomata in abaxial surface	Stomatal pore length	Stomatal pore width
Control	17.0±1.1^{a,b}	30.8±1.1^{c,d}	0.0520±0.0020^{f}	0.0125±0.0026^{g}
1 ml L^{-1}	24.0±1.5^{a}	33.3±1.3^{c}	0.1625±0.1125^{f}	0.0080±0.0000^{g,h}
2 ml L^{-1}	23.3±4.0^{a,b}	38.5±0.3	0.0500±0.0033^{f}	0.0040±0.0023^{h}
3 ml L^{-1}	15.5±1.3^{b}	31.0±1.4^{c,e}	0.0480±0.0020^{f}	0.0060±0.0020^{h}
4 ml L^{-1}	16.8±4.2^{a,b}	27.5±1.5^{d,e}	0.0460±0.0023^{f}	0.000±0.0010^{h}

Values represent means ± standard error, Values followed by the same letters in the same column are not significantly different at P < 0.05, No. = number

compared with the control, but the 1 ml treatment was not significantly different from the control.

Discussion

Judging from the control, Lambda-cyhalothrin increased the plant height, stem circumference, number of leaves, leaf area, leaf thickness, number of spongy cells and number of stomata on the abaxial surface (for 2ml L^{-1} treatment only) of *Vigna unguiculata*, in the present investigation. This is similar to the work of Kishorekumar *et al.* (2006) who found that triazole fungicides (hexaconazole and paclobutrazol) increased the leaf thickness, the number of palisade, spongy cells and chloroplasts of *Solenostemon rotundifolius*. Asami *et al.* (2000) also reported that triadimefon (triazole fungicide) treatments increased the thickness of leaf in plants. The present results showed that Lambda-cyhalothrin may possess growth regulating properties, as well as act as an insecticide, since the lesions and leaf cuts decreased as the concentration of the treatment increased. Also the number of stomata varies in the present investigation. Gupta *et al.* (2004) observed that the structure and ontogeny of stomata in different plants vary with the application of different growth regulators. The foliage anatomical characteristics were changed by the application of Lambda-cyhalothrin in the present work. Kishorekumar *et al*. (2006) reported that the anatomical characteristics were found changed due to the application of growth regulators. Fletcher and Hofstra (1990) also reported that triazole compounds (systemic fungicides) have plant growth regulating properties. Fletcher *et al.* (2000) further explained that plant growth regulating properties of triazoles are mediated by their interference with isoprenoid pathway and shift in the balance of plant hormones.

In the present investigation, the stomatal pore width significantly decreased with the treatments but the decrease in the stomatal pore length was not significant. Kishorekumar *et al.* (2006) observed several variations in the stomatal pore length, width and unequal accessory cells in the treated leaves of *Solenostemon rotundifolius.* These workers observed that in the untreated leaves, all stomata were open with large stomatal pore length, but the width of the stomata gradually decreased in the leaves of treated plants. Fletcher and Hofstra (1988) also observed that triazole treatments caused the closure of stomata in bean. Since stomatal pore width was decreased in the present work, it means that transpiration might have been decreased. Therefore, the judicious application of Lambda-cyhalothrin on *Vigna unguiculata* may be a useful tool for decreasing transpiration and in turn inducing drought avoidance mechanisms in the plant. Kishorekumar *et al.* (2006) made similar observations on *S. rotundifolius* after treating the plant with hexaconazole and paclobutrazol.

Lambda-cyhalothrin did not cause plant damage in all the treatments, except for temporary leaf shocks and bleaches observed in 3ml and 4ml concentrations. This is similar to the work of Spiers *et al.* (2008) who found that the insecticides: abamectin, Bifenthrin (pyrethroid) and Spinosad, did not cause plant damage on gerbera, even when applied at 4x the recommended rate. In the present work, Lambda-cyhalothrin improved the plant growth and development by increasing the plant height, stem circumference, number of leaves, leaf area, flowers, pods, pod length and pod circumference. So the insecticide improved the plants growth and development and so may be used as a plant growth regulator. The increased growth and development observed in the treated plants might not have been entirely due to the absence or reduction of insect attacks because 4ml L^{-1} had no lesions, or cuts, yet there were significant decreases in plant height, leaf area, number of flowers pod circumference, number of stomata on the abaxial surface and stomatal length, compared with 3 ml L^{-1}. This indicates that the insecticide acted as a growth regulator on *V. unguiculata.* This is contrary to the work of Spiers *et a.,* (2008), who reported that Triact 70 (neem oil extract) reduced photosynthesis, growth, development and flower production of gerbera. Triact 70 was labeled as both a fungicide and an insecticide.

Evidence from the present work showed that Lambda-cyhalothrin may help to reduce transpiration, induce drought avoidance mechanism, improve crop growth, development and yield as well as control insects that attack *V. unguiculata.* The low water solubility and high binding affinity of Lambda-cyhalothrin indicates a low potential to contaminate ground water. However, the insecticide may be toxic to fish, aquatic invertebrates, birds, bees and human beings (USEPA, 2001). More work needs to be carried out to determine the toxicity of the insecticide to plants, animals and man.

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