

Full Length Research Paper

Effects of neem leaf extracts on Lepidopteran pest species attacking *Solanum macrocarpon* L. (Solanaceae) in southern Togo

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Lepidopteran pests cause considerable damage to *Solanum macrocarpon* Linnaeus (Solanaceae). Their control by the use of botanical extracts is a promising alternative to improper use of chemical insecticides. The objective of this study was to evaluate the effects of three doses of leaf extract of the neem tree, *Azadirachta indica* Adrien-Henri de Jussieu (Meliaceae) against Lepidopteran pest species that attack *S. macrocarpon* L. in southern Togo. The experimental design used for the study was randomized complete blocks with three replicates and five treatments: three doses (N1: 300, N2: 600 and N3: 900 L/ha) of neem leaf aqueous extract, a synthetic insecticide "Cydim Super" (C.S.) and a Control (C) in field. Botanical extract and synthetic insecticide were applied after Lepidopteran pest species frequency and number collected once a week for 8 weeks. The yield data were obtained by weighing the aerial parts (leaves and stems) of *S. macrocarpon* harvested. Three species of Lepidoptera (*Selepa docilis* Butler (Noctuidae), *Spoladea recurvalis* Fabricius (Crambidae) and *Scrobipalpa ergasima* Meyrick (Gelechiidae)) were recorded. The neem leaf extract reduced frequency and numbers of all the three species found on *S. macrocarpon* than Control. *S. recurvalis* and *S. ergasima* were not recorded on plots treated with N3: 900 L/ha. No Lepidopteran pest species was recorded on plots treated with synthetic insecticide. *S. macrocarpon* yields obtained on plots treated with neem leaf extract N1, N2 and N3 were higher (5.42 ± 1.80 t/ha, 7.39 ± 1.88 t/ha and 6.97 ± 0.96 t/ha, respectively) than that of synthetic insecticide which was 3.51 ± 0.72 t/ha.

Key words: Biopesticide, Lepidopteran pests, *Solanum macrocarpon*, southern Togo.

INTRODUCTION

Solanum macrocarpon Linnaeus commonly known as « gboma » is an important native African vegetable, especially in West and East Africa where both the leaves and fruits are eaten for fiber and mineral nutrients. Regular consumption of leaves especially is

recommended as this vegetable contains high levels of proteins (Dougnon et al., 2012). The saponins present in the leaves also act as cholesterol-lowering agents by binding with cholesterol in the intestinal lumen (Ghule et al., 2006) which lowers circulating cholesterol. Both the

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leaves and fruits of this vegetable, have a cholesterol lowering effect (Sodipo et al., 2012; Dougnon et al., 2014). In Togo, Kanda et al. (2014) showed that the most represented vegetable families grown by market gardeners are Solanaceae (10 species), Alliaceae (4), Amaranthaceae, Asteraceae, Cucurbitaceae, Lamiaceae and Poaceae (3 species each). Apiaceae, Brassicaceae, Fabaceae and Malvaceae are represented by two species each. All other families are represented by a single species. Among leafy vegetables, *S. macrocarpum* (46%), *Lactuca sativa* Linnaeus (39%), *Corchorus olitorius* Linnaeus (36%) and *Hibiscus sabdariffa* Linnaeus (10%) predominate.

However, vegetable production is constrained by the damage caused by several insect pests (Koba et al., 2007; Agboyi, 2009; Oso and Borisade, 2017). Application of synthetic insecticides remains the most common control strategy against pest damage, even though this practice causes health and environmental problems (Toé et al., 2002; PAN-Africa, 2004; PAN-UK, 2005). Insecticidal properties of neem (*Azadirachta indica* A. Juss, Meliaceae) have been traditionally used in cultural practices for several thousand years (Philogène et al., 2003; Philogène et al., 2008). Neem compounds cause effects ranging from repellency to toxicity against a wide spectrum of insect pests including Orthoptera, Lepidoptera, Coleoptera, Diptera and Hemiptera (Schmutterer, 1990; Isman, 2006; Siddiqui et al., 2009; Degri et al., 2013; Shannag et al., 2014; Mondéjji et al., 2016). These biological properties are mediated by different groups of compounds among which limonoids and particularly azadirachtin mainly present in the neem seeds. Those compounds are considered the most active components responsible of both antifeedant and insecticidal effects (Isman, 2006). Meliaceae-based insecticides have low environmental impact because of a rapid degradation in plants and in the soil (Isman, 2006) and low effects on beneficial insects (Charleston et al., 2005a; Defago et al., 2011).

Neem originating from Southeast Asia grows in many countries around the world including Togo (Klu, 2008). Despite two fruiting periods per year by the neem tree, their unavailability throughout the year limits the use of seed-based preparations. Interestingly, numerous active compounds including limonoids have also been found in neem leaves (Siddiqui et al., 2000) and leaf extracts had been shown to exert insecticidal effects against several insect pest species (Brunherotto et al., 2010; Egwurube et al., 2010). The choice of neem was made from literature but more importantly from the traditional practices of local gardeners in Togo. Under this scenario, extract based on neem preparation could be an important new compound for Lepidopteran pest species management on *S. macrocarpon*.

Owing to the high insect pest damage to vegetable crop grown in Togo and the potential of neem leaf-based preparation to control insect populations, our hypothesis

was that neem leaf extract could affect the frequency and number of three Lepidopteran pests which attack *S. macrocarpon* and increase the yield of the vegetable. The objective of this study was therefore to evaluate the effects of *A. indica* leaf extract compared to a chemical insecticide "Cydim Super 388 EC" on the frequency and the number of Lepidopteran pests attacking *S. macrocarpon* and on the yield of this vegetable.

MATERIALS AND METHODS

Site and experimental conditions

The study was carried out in Lomé (southern Togo) with a tropical Guinean climate marked by two rainy seasons (April-July and September-October) separated by two dry seasons (August and November-March). Average monthly temperatures range from 25 to 29°C during the year and the average annual rainfall is around 932 mm. The mean annual relative humidity is about 82% and the photoperiod of (12: 12) h LD.

The study was conducted on Agronomic Experiments Station located at University of Lomé campus (6° 17'N and 1° 21'E) during the rainy season from May to July 2017. This site is dominated by a man-made savanna with exotic plant species such as *A. indica*, *Carica papaya* Linnaeus (Caricaceae), *Hibiscus lunarifolius* Willd. (Malvaceae), *Senna siamea* Lamarck Irwin Barneby (Fabaceae), *Leucaena leucocephala* Lamarck de Wit (Mimosaceae), *Manguifera indica* Linnaeus (Anacardiaceae) and annual and seasonal crops (cassava, maize, cowpea, vegetables).

Experimental design and agronomic practices

The *S. macrocarpon* was grown on plots using randomized balanced complete blocks. Three blocks (B1, B2 and B3) were made (Figure 1). Each block consisted of five elementary plots: one untreated elementary plot served as control (C); one plot treated with chemical insecticide named Cydim Super (C.S.) and three elementary plots treated with different doses of neem leaf extract (N1, N2 and N3). In order to avoid or minimize insecticide drift during the treatments, a distance of 1 m separated elementary plots. Each elementary plot (1.6 m × 6.8 m) carried four rows of plants with 17 *S. macrocarpon* plants per row. The spacing of the plants was 0.4 m within rows and 0.4 m between the rows (Figure 2). The maintenance of the plots was essentially watering, weeding and hoeing. The watering of the plots was done with pipes fitted with a finely drilled piece (head) every day. Weeding and hoeing were done with a hoe and a forked hoe respectively every two weeks.

Preparation of botanical extract

Fresh leaves of neem were collected on the domain of the University of Lomé. Extract was obtained by soaking 1 kg of crushed fresh leaves in 1.5 L of water overnight at 25-30°C. After maceration for 12 h under ambient conditions, the solution was filtered. The filtrate was then applied to the plots.

Preparation of chemical insecticide

The chemical insecticide was prepared by diluting 3.5 ml of Cydim Super in water to obtain 1500 ml of solution. Cydim Super is a

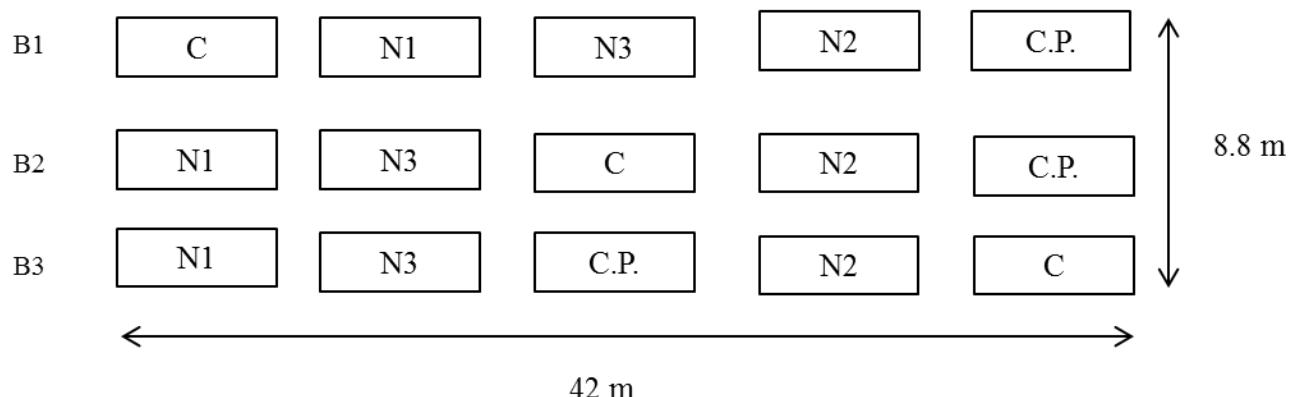


Figure 1. Experimental plots arrangement. B: Block; C: Plot untreated (Control); C.S.: plot treated with chemical insecticide (Cydim Super); N1: plot treated with the low dose of the aqueous neem leaf extract; N2: plot treated with the medium dose of the aqueous neem leaf extract; N3: plot treated with the high dose of the aqueous neem leaf extract.

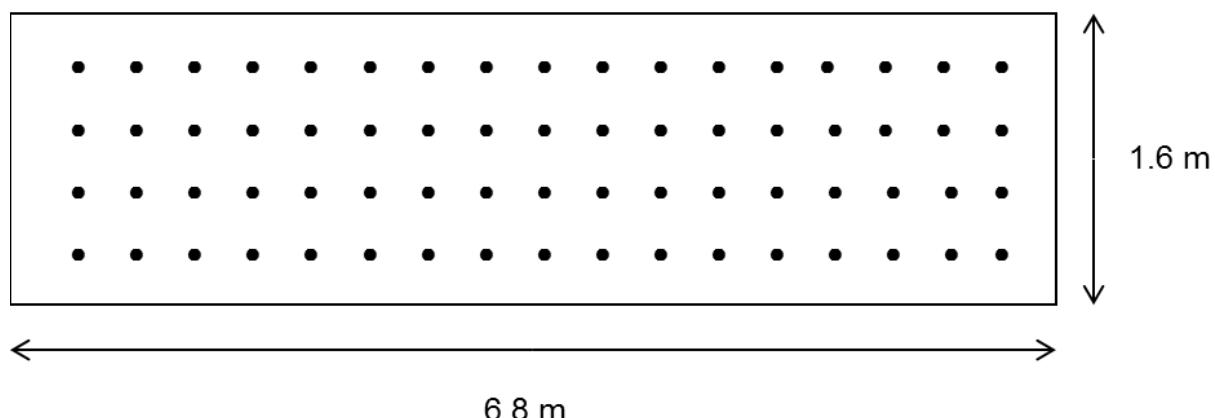


Figure 2. Arrangement of *S. macrocarpon* plants on plots (aligned points).

binary insecticide composed of 400 g/L Cypermethrin and 36 g/L Dimethoate.

Treatment of plots

Application of treatments began two weeks after transplanting. The treatments were carried out using ALTIMATE PRO 16 model with maintained pressure backpack sprayer. The treatments of the elementary plots were performed once a week during six weeks period (6 applications in total). The dose of chemical insecticide applied was 1 L of Emulsifiable Concentrate per hectare. The three doses of neem leaf extract (N1: 300, N2: 600 and N3: 900 L/ha) were applied. The control plots were untreated (Table 1).

Evaluation of treatments effects on Lepidopteran pests of *S. macrocarpon* plants

Observations were made the day before each application of treatment in the various *S. macrocarpon* plots (every seven days). The evaluation of a treatment effects was based on 30 plants in the middle of each elementary plot to avoid the bias associated with the

edge effect. The presence or absence of each species of Lepidopteran was recorded during each observation on plots. This made it possible to calculate the frequency of species for each treatment. Results were expressed in terms of frequency F = (Number of observations in which the species was present / Total number of observations) × 100.

The number of larvae of each of the Lepidopteran pests found on *S. macrocarpon* plants per plot for each treatment, was recorded to determine the numbers of each species by treatment.

Evaluation of treatments effects on *S. macrocarpon* yield

The yield data were obtained by weighing the aerial parts (leaves and stems) of the 30 plants of *S. macrocarpon* harvested from the two central lines of each plot two weeks after the last application of treatment. Yields were then estimated per hectare.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0. The comparisons of mean frequencies, numbers and yield were made

Table 1. Doses of extract and applied synthetic pesticide.

Treatment	Phytosanitary products used	Doses (L/ha)
C	No products used (Control)	0
C.S.	Cydim Super	1 (E.C.)
N1	Aqueous neem leaf extract (low dose)	300
N2	Aqueous neem leaf extract (medium dose)	600
N3	Aqueous neem leaf extract (high dose)	900

E.C. : Emulsifiable Concentrate.

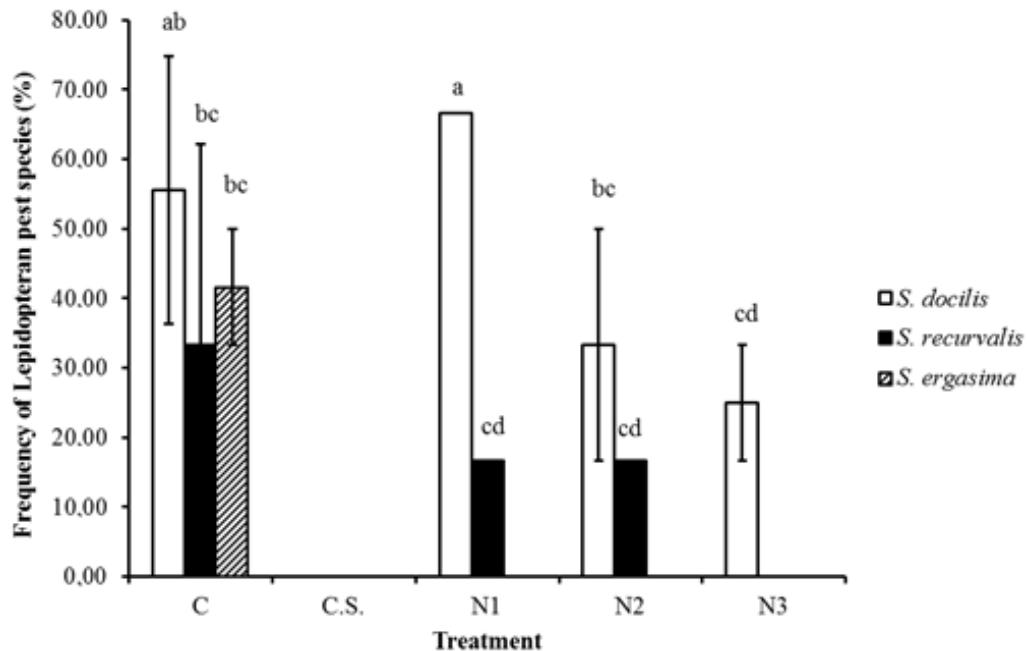


Figure 3. Mean frequency ($X \pm SD$) of Lepidopteran larvae (*S. docilis* Butler (Noctuidae), *S. recurvalis* F. (Crambidae) and *S. ergasima* Meyrick (Gelechiidae)) following treatment. Different letters over the columns indicate statistically significant differences ($F_{(14, 44)} = 14.096$; $df = 14$; $p = 0.000$), B: Block; C: Plot untreated (Control); C.S.: plot treated with chemical insecticide (Cydim Super); N1: plot treated with the low dose of the aqueous neem leaf extract; N2: plot treated with the medium dose of the aqueous neem leaf extract; N3: plot treated with the high dose of the aqueous neem leaf extract.

using analysis of variance (ANOVA) followed by a Student Newman Keuls (SNK) comparison tests when ANOVA was significant at the 5% level. For yield, data were submitted to LSD comparison tests at the 5% level.

RESULTS

Selepa docilis Butler (Noctuidae), *Spoladea recurvalis* F. (Pyralidae) and *Scrobipalpa ergasima* Meyrick (Gelechiidae) were the Lepidopteran pests recorded on *S. macrocarpon*.

Effects of neem leaf extracts on the frequency of Lepidopteran pests of *S. macrocarpon* plants

Figure 3 shows that the mean frequency of different

species of Lepidopteran was from 0 to 66.67% all treatments combined. The low dose of neem extract (N1) did not reduce the frequency of *S. docilis* (66.67%) compared to that obtained on the control (C) ($55.56 \pm 19.25\%$). However, the frequencies were lower ($33.33 \pm 16.67\%$ and $25.0 \pm 8.33\%$) on plots treated with the medium (N2) and the high (N3) doses of neem extract respectively. *S. recurvalis* was less frequent than *S. docilis* in general. Its frequency was $33.34 \pm 28.87\%$ on the control (C) and 16.67% at the level of plots treated with the low (N1) and the medium (N2) doses of neem extract. *S. recurvalis* was not present on plots treated with the high dose of neem extract (N3). *S. ergasima* was present only on the control (C). Its frequency was 41.67 ± 8.33 . The three species of Lepidopteran were absent on the plots treated with the synthetic insecticide (C.S.). The

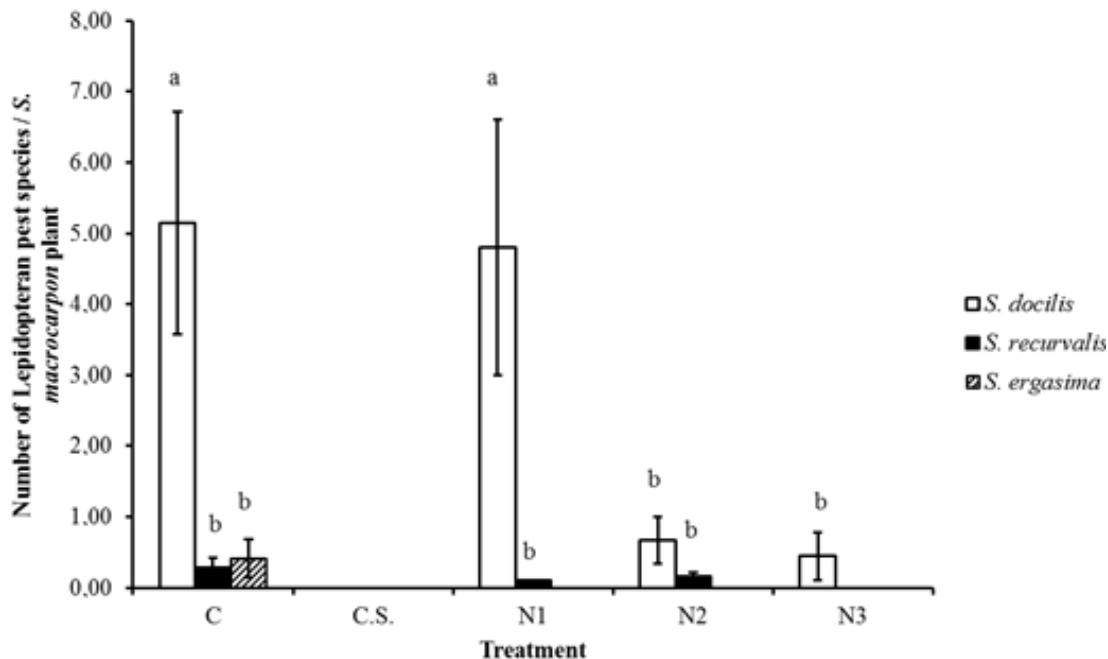


Figure 4. Mean numbers ($X \pm SD$) of Lepidopteran larvae (*S. docilis* Butler (Noctuidae), *S. recurvalis* F. (Crambidae) and *S. ergasima* Meyrick (Gelechiidae)) per *S. macrocarpon* plant following treatment. Different letters over the columns indicate statistically significant differences ($F_{(14, 44)} = 21.829$; $df = 14$; $p = 0.000$). B: Block; C: Plot untreated (Control); C.S.: plot treated with chemical insecticide (Cydim Super); N1: plot treated with the low dose of the aqueous neem leaf extract; N2: plot treated with the medium dose of the aqueous neem leaf extract; N3: plot treated with the high dose of the aqueous neem leaf extract.

neem extract and especially the high dose allowed to obtain a low frequency or outright absence of the three species of Lepidopteran ($F_{(14, 44)} = 14.096$; $P = 0$).

Effects of neem leaf extracts on the number of Lepidopteran pests of *S. macrocarpon*

Figure 4 shows that the mean number of *S. docilis* was 5.15 ± 1.57 larvae (caterpillars) / plant on Control plots (C). The numbers of *S. docilis* were 4.80 ± 1.80 ; 0.67 ± 0.33 and 0.44 ± 0.33 larvae / plant on the plots treated with low (N1), medium (N2) and high (N3) doses of neem leaf extract, respectively. Those of *S. recurvalis* were 0.29 ± 0.13 larvae / plant on Control plots (C); 0.11 and 0.16 ± 0.05 larvae / plant on the plots treated with low (N1) and medium (N2) doses of neem extract, respectively. No larva of *S. recurvalis* was found on plots treated with high dose of neem extract (N3). The number of *S. ergasima* was 0.41 ± 0.27 larvae / plant only on Control (C). No larva of *S. docilis*, *S. recurvalis* or *S. ergasima* was found on plots treated with synthetic insecticide (C.S.). Medium (N2) and high (N3) doses of neem leaf extract significantly control the number of different Lepidopteran pests species larvae on *S. macrocarpon* plant compared to Control (C) ($F_{(14, 44)} = 21.829$; $P = 0$). But synthetic insecticide Cydim Super

(C.S.) has better control the number of Lepidopteran pest species larvae on *S. macrocarpon* than the neem leaf extracts.

Effects of treatments on the yield of *S. macrocarpon*

The mean yield of *S. macrocarpon* leaves and stems varied according to treatment. The mean yield of Control plots was $(5.33 \pm 1.78$ t/ha). Those of plots treated with synthetic insecticide (C.S.), the low (N1), medium (N2) and high (N3) doses of neem leaf extract were 3.51 ± 0.72 t/ha; 5.42 ± 1.80 t/ha; 7.39 ± 1.88 t/ha and 6.97 ± 0.96 t/ha, respectively ($F_{(4, 14)} = 3.111$; $P = 0.066$) (Figure 5). A comparison of the mean yield obtained on plots treated with synthetic insecticide (C.S.) with yields obtained on the plots treated with the medium (N2) and high (N3) doses of neem extract using LSD test, showed significant differences ($P = 0.01$ and $P = 0.019$, respectively).

DISCUSSION

In this study, three species of Lepidopteran pests *S. docilis* Butler, *S. recurvalis* F. and *S. ergasima* Meyrick were recorded on *S. macrocarpon* in the field. Those

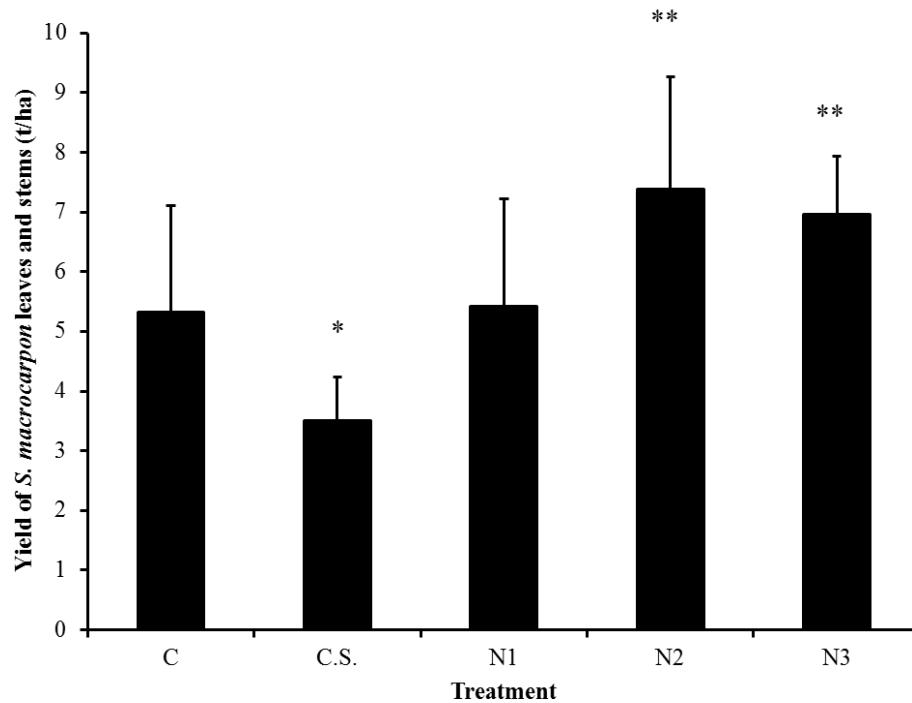


Figure 5. Mean yield ($X \pm SD$) of *S. macrocarpon* following treatment. Columns with different numbers of asterisk over indicate statistically significant differences with LSD test ($F_{(4, 14)} = 3.111$; df = 4; C.S. ≠ N2 : P = 0.010; C.S. ≠ N3 : P = 0.019), B: Block; C: Plot untreated (Control); C.S.: plot treated with chemical insecticide (Cydim Super); N1: plot treated with the low dose of the aqueous neem leaf extract; N2: plot treated with the medium dose of the aqueous neem leaf extract; N3: plot treated with the high dose of the aqueous neem leaf extract.

Lepidopteran pests have been encountered or recorded among important insect pests that caused damage to egg plants *Solanum* spp or other african indigenous vegetables like *Amaranthus* spp (Koba et al., 2007; Omburo, 2016; Oso and Borisade, 2017). Among the three species, *S. docilis* was the most representative in terms of frequency and numbers followed by *S. recurvalis* and then *S. ergasima*.

The different treatments and doses of neem leaf extract influenced the frequency and numbers of the three Lepidopteran pests on the vegetable crop. The Control plots were more attacked by these different kinds of Lepidopteran pests throughout the study period compared to the plots that were treated with the different doses of neem leaf extract and the synthetic insecticide. Research results showed the vulnerability of Control plots to insect pests (Horna and Gruère, 2006). The neem leaf extract tested was a total extract which contained the following families of compound: athraquinones, tannins, triterpenes, coumarins and flavonoids (Lagnika, unpubl.). Some of these chemical substances present in the neem leaf extract might have prevented the insects from feeding on the leaves. The neem tree (*A. indica* A. Juss) is known to be an important source of triterpenoids (Afshan, 2002; Siddiqui et al., 2004). According to

Gisbert et al. (2006), neem plants also contain salannin which makes the plant unpalatable and therefore, discourages being fed on by insects. The presence of triterpenoids and salannin in the neem leaf extract might have acted as an antifeedant and therefore repelled the insects from feeding on the leaves of *S. macrocarpon* treated with the extracts. Neem seeds oil extracts, water and ethanolic neem leaf extracts are known to inhibit the growth of various insects species (Charleston et al., 2005b; Aggarwal and Brar, 2006; Egwurube et al., 2010; Shannag et al., 2014; Mondéjji et al., 2015). Amtul (2014) reported that *A. indica* derived compounds inhibit digestive alpha-amylase in insect pests. Thus, *A. indica* extracts are potential bio-pesticides in insect pest management.

The effect of different treatments and doses of neem leaf extract also influenced the yield of the *S. macrocarpon*. The plants sprayed with the neem leaf extract grew taller than those sprayed with synthetic insecticide Cydim Super. This indicated that the yield was affected by the active ingredient in the neem leaf extract called meliantriol which prevented insect infestation of the plant and allowed *S. macrocarpon* plants to grow in height and to produce more leaves. The mean weight values of plants sprayed with the medium and the hight

doses of the extract were higher than those plants sprayed with the synthetic insecticide. There was a significant difference among the treatments ($P < 0.05$) with LSD test post hoc between yields obtained on plots treated with the synthetic insecticide and those treated with the medium and the hight doses of neem leaves extract. Agbenin et al. (2005) reported that azadirachtin and/or neem extracts enhanced plant growth, and increased the yield in different crops including garden egg. The *S. docilis* is a defoliating caterpillar that gnaws at the leaf blades, leaving only the vein. The larvae of *S. recurvalis* skeletonize the leaves before rolling them to provide shelter during pupation. Flower bud *S. ergasima* caterpillar occurs on leaves, flowers and fruit of crop plants. It damages flowers and young fruits of eggplants. Thus, despite their fairly frequency and higher numbers on control plots, these Lepidopteran did not reduce yields on the latter. However, they could reduce the market value of the vegetable because of the galleries left on *S. macrocarpon* leaves reduced to veins by *S. docilis*, and the presence of *S. recurvalis* and *S. ergasima* larvae in *S. macrocarpon* leaves folded by them.

Conclusion

Three Lepidopteran pest species (*S. docilis*, *S. recurvalis* and *S. ergasima*) were found on *S. macrocarpon*. Treatments have effects on the three Lepidopteran pests. Neem extract in general and the high dose in particular reduced the frequency and the number of the Lepidopteran larvae on *S. macrocarpon*. The effectiveness of different doses of neem leaf extract revealed that the high dose of neem leaf extract was better than other doses of the extract. Although neem extract failed to kill all the Lepidopteran pests found on *S. macrocarpon* like synthetic insecticide, the use of neem leaf extract is an eco-friendly management method. The neem extract produced better yield than synthetic insecticide.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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