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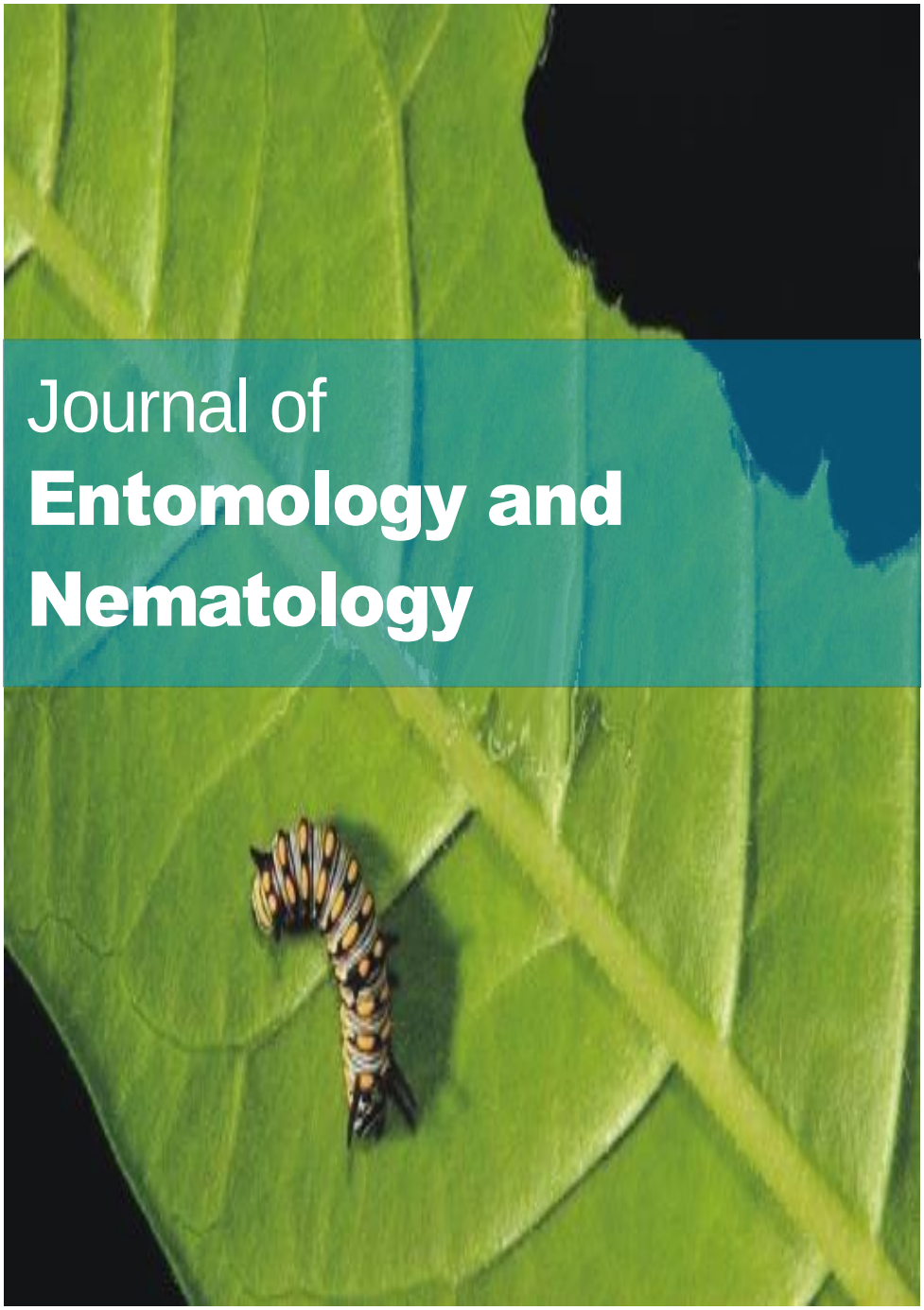

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Full Length Research Paper

Seasonal pattern of Bancroftian Filariasis transmission in Ebonyi State, Nigeria

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Bancroftian filariasis in Nigeria is endemic with 22.1% of the population thought to be infected. The main mosquito genera implicated with *Wuchereria bancrofti* transmission are *Anopheles* and *Culex*. The study was carried out to compare the infectivity rates of the vectors between the high transmission (rainy) and the low transmission (dry) seasons. Mosquitoes were sampled from houses and compared from six sentinel villages (3 each from Ohaukwu and Abakiliki Local Government Areas) of Ebonyi State, Nigeria. Day resting indoor collection (DRI) by Aspirator and Pyrethrum Spray Catch (PSC) were used to collect mosquitoes between 7:00 and 11:00am. After morphological identification, female parous mosquitoes were dissected in search for infective (L_3) larvae of *W. bancrofti*. A total of 4,840 female mosquitoes were dissected. More mosquitoes were caught in the rainy season than in the dry season. Infectivity rates of vectors in Ohaukwu villages were 3.54 and 5.41% in the rainy and dry seasons, respectively, whereas in Abakiliki villages these were 1.85 and 1.19%, respectively. There was no significant difference in the overall infectivity rates between the two seasons in both Ohaukwu and Abakiliki villages (p>0.05). Similarly, no significant difference in the total/average transmission potentials were found between the seasons (p>0.05). *Anopheles gambiae* sl was the main vector in both study sites followed by an *Anopheles funestus* and *Culex quinquefasciatus*. There was a difference in infectivity rates of *Anopheles* species between the wet and dry seasons (p<0.05), whereas no significant difference exist in infectivity rates of *Anopheles* species and *Cx. quinquefasciatus* (p>0.05). Findings were discussed in the context of on-going plans to eliminate filariasis and the transmitting vectors.

Key words: Bancroftian filariasis, rainy season, dry season, *Wuchereria bancrofti*.

INTRODUCTION

Wuchereria bancrofti which causes bancroftian filariasis is the only etiologic agent in Africa (Michael and Bundy, 1997).The disease is prevalent and widespread in Nigeria which is the third most endemic country in the world (after India and Indonesia) and estimated 22.1% of the population is thought to be infected (Eigege et al., 2003). The epidemiology of the disease regarding the abundance of the proven vectors (*Anopheles* and Culicine species) is somewhat different when compared with other ecological zones of the world.

In Nigeria, the prevalence of bancroftian filariasis or (filariasis index) between the northern parts with lowest

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rainfall and southern parts with highest rainfall parts vary considerably (Nwoke, pers. Communication, 2007). However, no explanation was offered to these findings regarding vector infectivity or infection rates. Studies elsewhere have revealed that the peak transmission of filariasis was during the long rains and after the short rains in Mambru and Jaribuni villages of Kenya (Wijers and Kinyanjui, 1977). The hot dry season transmission was interrupted in Mambru, but was rather low in Jaribuni. Wijers and Kaleli (1984) in related study concluded that transmission season coincides with the long rain during which filariasis vectors were in abundance. However, no clear reports on comparison between vector infectivity rates between the seasons were shown.

Clearly, further work is urgently needed to evaluate the exact importance of both the vector capacities and seasonal filariasis transmission in parts of Africa. In Nigeria limited works exist on vector infectivity infection rates both natural and experimental (Anosike et al., 2003; Mbah, 2010; Awolola et al., 2006; Amaechi, 2009). Infectivity and infection studies of mosquitoes on filariasis between the dry and wet seasons are lacking. The present study therefore examines the seasonal infection/infectivity rates in rural endemic areas of Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study sites

The study was conducted in six (6) sentinel villages, three (3) each from Ohaukwu (Orijiriafor, Okpochiri and Ndiagu Obu) Abakiliki (Mgbabeluzor, Okarie Echida and Okpochiri) Ebonyi State, Nigeria ($7°31^1$ -$8°\ 18^1$N- $50\ 36^1$ - $6°\ 15^1$E) Figure 1. The ecology of the area has been described in details (Amaechi et al., 2010; Richards et al., 2013). The inhabitants are Igbos who mainly live in mud walled and thatched houses. Houses with block walls and or iron sheets roofing are extremely rare. They are peasants growing mainly maize, rice, yam and cassava. Livestock kept includes cattle and goats with some animals tethered inside human dwellings. The sites were selected based on preliminary ICT-survey (Carter Center, 2009. Unpublished data) indicating the presence of bancroftian filariasis in the area.

ETHICAL CONSIDERATION

Ethical clearance and permission was approved by the Post Graduate Research Board of the Zoology Department of Imo State, University of Owerri, Nigeria and Ebonyi State Ministry of Health. Team members were trained on entomological survey methods. Informed verbal consent was obtained from residents of household on mosquito collections.

Mosquito sampling technique/laboratory processing

The prevalence of infective mosquitoes was assessed in households. Houses were visited twice monthly during the mornings and indoor resting mosquitoes were collected by pyrethrum spray catch (PSC) and mechanical aspirator (WHO, 2002). The time and period of collection were chosen to catch fully engorged vectors and reflected the seasons (rainy and dry) of the areas. As much as possible houses were of similar construction to avoid the effect of variability. Indoor resting mosquitoes were collected in 20 selected houses and at least one sleeping room in each house was used for mosquito collection (Mboera et al., 2006). Mosquitoes caught were taken to a temporary dissection center as time allowed. Visual identification (for morphology) was made using different keys and characteristics (Nwoke, 2007, unpublished). Blood fed females were dissected to determine parity by observing the degree of ovarian trachioles (Detinova, 1962). The body parts (head and mouth parts, thorax and abdomen) were macerated and examined for the presence of living filarial larvae. For more exact identification of filarial larvae stages and species, the preparations were stained with haematoxyline (Nelson, 1959) and examined with microscope. Recovery of larval stages of *W. bancrofti* was done according to Nelson and Pester (1962); larval stages were categorized by sizes rather than by appearances (Nathan, 1981).

Statistical analysis

Data were analyzed by chi square using Epi Info 6 computer software statistical analysis programme to compare the infectivity rates of the vectors between the rainy and dry seasons. Yates correction was also used to compare infectivity rates of the vectors; the sentinel villages and the mosquito vector species.

RESULTS

Cumulatively, 4,840 blood fed mosquitoes were dissected. Table 1 shows that infection and infectivity rates of the vectors were (8.82% versus 15.22%) and (3.54% versus 5.41%) in the rainy and dry seasons, respectively. Table 2 shows the infection and infectivity rates of LF vectors in Abakiliki. Infection and infectivity rates were found to be 2.30% versus 1.62% and 1.85% versus 1.19% for rainy and dry seasons. Infectivity rates differed significantly between the rainy and dry seasons in Ohaukwu ($p>0.05$) but not in Abakiliki ($p<0.05$). In both seasons and study sites, *Anopheles gambiae* was the predominantly species (Tables 1 and 2).

Considering the infectivity rates of the vectors independently, the order of vector importance of the three main vectors in Ohaukwu and Abakiliki was *A. gambiae, Anopheles funestus* and *Culex quinquefasciatus* (Tables 1 and 2). This was the same trend in both seasons. Statistical significant difference in infectivity rates were found between *Anopheles* species ($p<0.05$). However, no significant difference existed between infectivity rates of *Anopheles* spp. and *Cx. quinquefasciatus* (Table 3). The highest number of infective larvae (L_3) per mosquito in Ohaukwu was 5 with an average of 5 which occurred in the rainy season. In Abakiliki, the highest number of infective larvae per mosquito was 4 (*An. gambiae*) with an average of 2 during the rainy season. There was only one infective larva of *Cx. quinquefasciatus* which

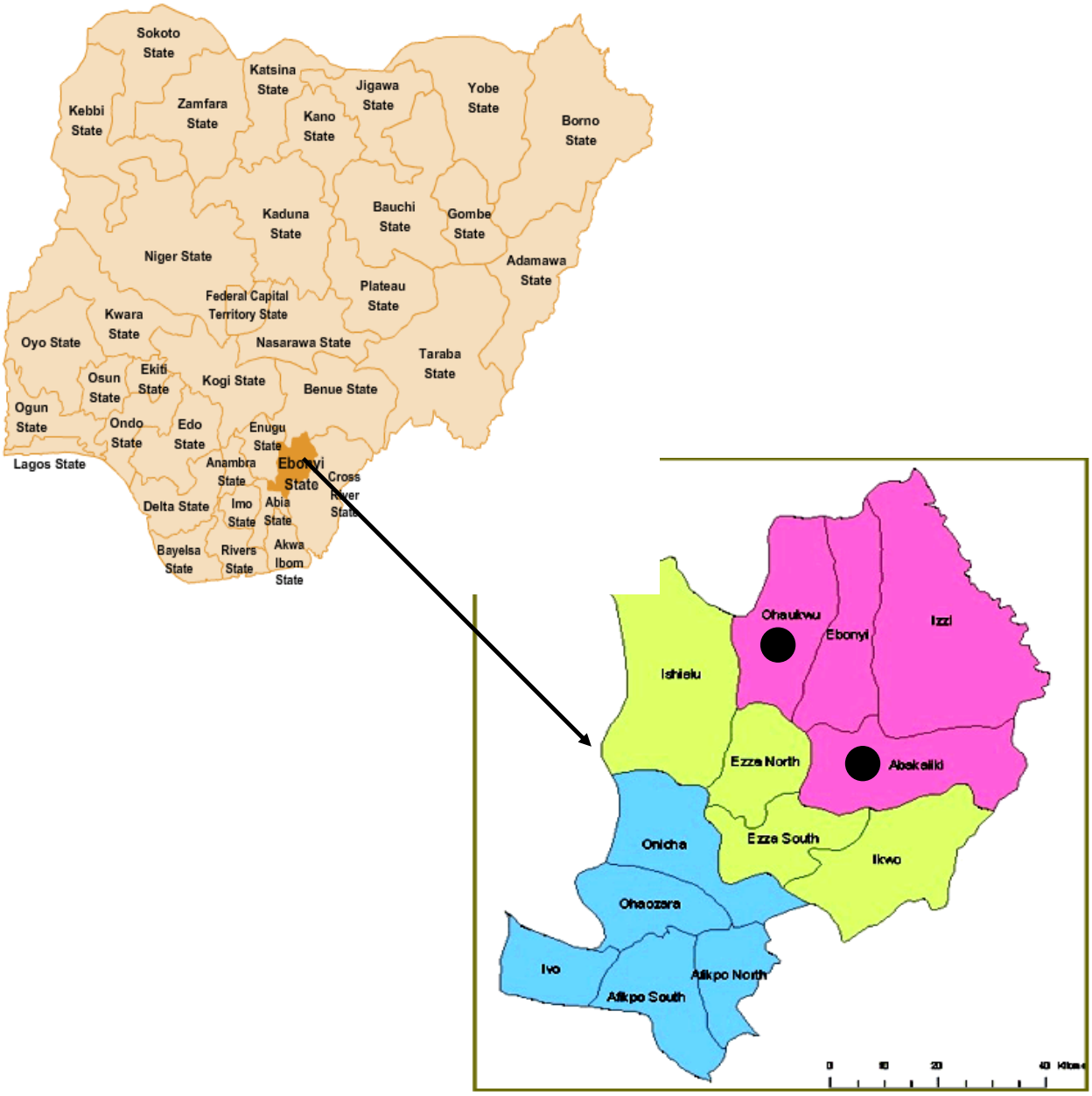

Figure 1: Map of Ebonyi State showing the study areas.

occurred during the dry season. No significant difference in the total/average transmission potential (TP^{+}) were observed between the seasons ($p<0.05$).

DISCUSSION

Although the Pyrethrum Spray Catch (PSC) and Mechanical Aspirator (MA) method records only the mosquitoes that rest indoors, it is widely used for estimating the abundance, seasonal densities, host preference and vector infection status as in this study. Previous study has shown that mosquito infectivity rates are low during the dry season and high in the rainy season. However, this was not the case for both study sites: Orijiriafor, Okpochiri, Ndiagu Obu rural villages in Ohaukwu and Mgbabeluzor, Okarie Echida and Obeagu Ibom villages towns in Abakiliki. This observation contradicted those of Kasili et al. (2009) in Kenyan Coast and in Philippines (Valeza and Grove, 1979) and could

Table 1. Overall infection and infectivity rates of LF vectors during the transmission (July - September) and non-transmission (October - December) 2013 seasons in Ohaukwu.

Mosquitoes species	Number			No. containing larvae stages			Rates %			TP^+
	Parous	Infection	Infective	Diss	Parous	Infected	L_1	L_2	L_3	
Rainy season										
An. gambiae sl	1,348	1,085	44	24	64	57	3.26	10.76	4.23	65
An. funestus sl	363	243	4	6	0	6	1.10	3.31	1.65	11
Cx. quinquefasciatus	66	31	0	0	0	0	0.00	0.00	0.00	0
Mn. africana	2	2	0	0	0	0	0.00	0.00	0.00	0
Ae. aegypti	1	0	0	0	0	0	0.00	0.00	0.00	0
Total	1,780	1,361	48	30	64	63	2.70	8.82	3.54	176/59
Dry Season										
An. gambiae sl	731	699	26	19	45	48	3.56	15.32	6.57	48
An. funestus sl	168	146	13	13	21	6	7.74	23.81	3.57	20
Cx. quinquefasciatus	99	58	0	0	0	0	0.00	0.00	0.00	0
Mn.africana	1	1	0	0	0	1	0.00	0.00	0.00	0
Ae. aegypti	0	0	0	0	0	0	0.00	0.00	0.00	0
Total	999	904	39	32	66	54	3.90	15.22	5.41	68/23

Table 2. Overall infection and infectivity rates of LF vectors during the transmission (July - September) and non-transmission (October - December) 2013 seasons in Abakiliki.

Mosquitoes species	Number			No. containing larvae stages			Rates (%)			TP^+
	Parous	Infection	Infective	Diss	Parous	Infected	L_1	L_2	L_3	
Rainy season										
An. gambiae sl	782	742	5	0	3	12	0.64	1.92	1.5	12
An. funestus sl	227	198	7	1	0	9	3.08	4.41	3.96	9
Cx. quinquefasciatus	49	34	0	0	0	0	0.00	0.00	0.00	0
Mn. africana	77	59	1	0	1	0	1.30	1.30	0.00	0
Total	1,135	1,033	13	1	4	21	1.15	2.30	1.85	21/7
Dry season										
An. gambiae sl	601	527	6	3	0	7	0.99	1.66	1.16	7
An. funestus sl	152	138	3	1	0	3	1.97	2.63	1.97	3
Cx. quinquefasciatus	92	49	1	0	0	1	1.09	1.09	1.09	1
Mn.africana	81	69	0	0	0	0	0.00	0.00	0.00	0
Total	926	783	10	4	0	11	1.08	1.62	1.19	11/4

be attributed to human behavior and difference in ecological settings. Abakiliki, though not urbanized settlement has had resulting sanitary conditions due to shift in ecology/gradual modified ecology from rural to rural/urban. The state capital is few kilometer away from these villages resulting in improved economic status through trading and rapid transportation of farm products. These could explain the parity in infectivity rates.

The only significant difference in infectivity rates of vectors was between *Anopheles* species (*An. gambiae* sl and *An. funestus* sl) in both seasons. Similar observation have been observed elsewhere by Manyi et al, (2016). These observations probably depicted *Anopheles* spp. as the most important bancrofti filariasis vectors in terms of infectivity rates. Similar findings were reported at other sentinel sites of Nigeria (Amadi and Udonsi, 2004; Mbah, 2010; Awolola et al., 2006). Thus, confirmation of evidence that their infectivity status has not changed in rural African communities in the last seven decades (Taylor, 1930; Kuhlow, 1987). The increase in number of *Anopheles* spp. in both Ohaukwu and Abakiliki accounted for infectivity rates in the seasons. Previous reports has

Table 3. Comparison of filarial infectivity rates between LF vectors.

Species	Filarial infectivities		
	-ve	+ve	P*
An. gambiae sl	3,303	159	S
An. funestus sl	870	40	-
Cx. quinquefasciatus	305	1	NS
An. gambiae sl	3,303	159	-
An. funestus sl	870	40	NS
Cx. quinquefasciatus	305	1	-

P*= Chi square with Yate's correction ; NS = not significant, P> 0.05; S = significant, P< 0.05.

shown that *An. gambiae* ss mosquito predominate in rainy season whereas *Anopheles arabiensis* in the dry season. The predominant status of *An. gambiae* in both seasons demands for identification beyond morphological criteria to ascertain the contribution of sibling species in filariasis transmission.

Cx. quinquefasciatus when compared with *An. gambiae, An. funestus* was few in number with low infectivity rate(1.09%) found only in the dry season. Wijers (1977) have found them to have reduced longevity. By implication, even if the number could be high as observed herein, very few may live to support *W. bancrofti* larvae. This could account for its low contribution to infectivity rates. Previous works have posited that it is an urban vector Manyi et al (2014) in Makurdi (North Central, Nigeria) found high infectivity rates in Anopheles and its involvement together with *Anopheles* spp. in Abakiliki further supports changed sanitary condition of the area. There has been large population movement in the South Eastern part of Nigeria as a result of human workforce (labour). Ebonyi population has been actively involved in this process. Therefore, the introduction of *W. bancrofti* into other areas is possible and because of the abundance and compatibility of the parasite and vectors the infection might well become established in the local population that is not at present a focus for bancrofti filariasis.

In both Ohaukwu and Abakiliki, *A. gambiae* was more abundant in the rainy season than in the dry season. The number of *A. funestus* in Ohaukwu and Abakiliki declined during dry season because their breeding sites were mainly clear water and vegetation near the water sources which were rare in the dry season. The observed proportions of *Culex* in both sites and seasons were due to open polluted water trenches which ramify throughout the area (foul water bodies for cassava fermentation and local sponge making from palm oil heads). In the dry months, they are filled with larvae and pupae of *Culex* mosquitoes whereas pit latrine became water-logged in the rainy seasons thus providing excellent breeding sites for *Culex* mosquitoes.

Apparently, the abundance of these vectors was due to prevailing weather conditions; rains with large numbers appearing during the long rains and very few during the drier months. In both sites, *Culex* was present though not the most important in the transmission of *W. bancrofti*. Therefore, it appears that the great risk of infection from infective mosquitoes in both Ohaukwu and Abakiliki is due to the bites of *An. gambiae* and *An. funestus*. Though, it has been noted that *Culex* is an urban vector and could serve as a potential vector for urban cities (Onwuliri and Anosike, 1989). Results of this study have shown *Culex* to be a contributing vector of filariasis transmission.

Mansonia africana (non-filarial vector in Nigeria) and *Aedes aegypti* were encountered, indicative of the level of nuisance, the inhabitants of the area got from these mosquitoes. Their dissection results probably reflected feeding habit and anatomical variation rather than absence of *W. bancrofti*.

The transmission potentials (TP^+) were comparable for both seasons and clearly indicated no changes in vector-contact rates with infected people. The difference in vector infectivity rates between the seasons is independent on vector abundance but the actual species of the mosquito vector. The results of this study therefore revealed that there is a difference between the rainy and dry seasons and the abundance of *An. gambiae* sl and *An. funestus* sl in rainy seasons could be the main reasons for this. The implication of these findings to the Nigerian Lymphatic Filariasis Elimination Programme in vector control cannot be overemphasized.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest

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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 exctract 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; Mondédji 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 (Anacarciaceae) 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

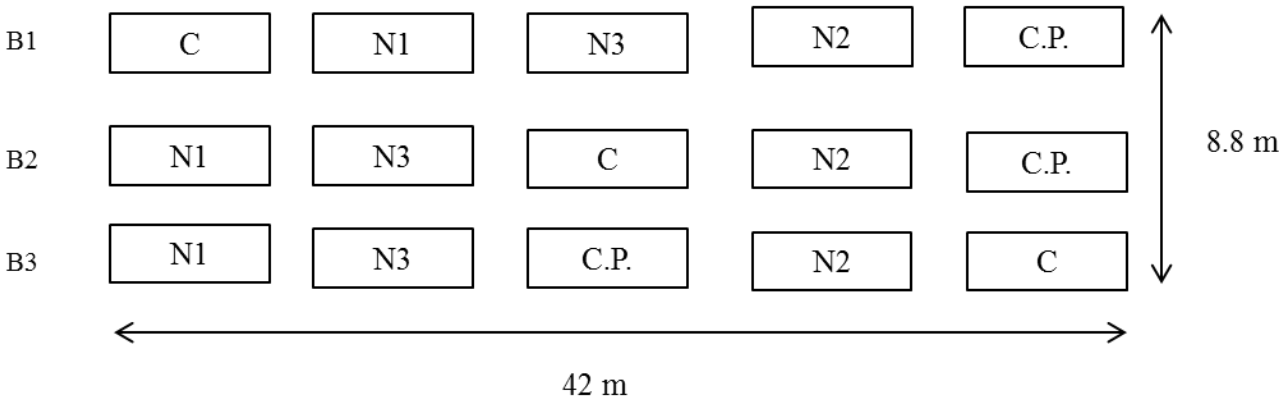

Figure 1. Experimental plots arrangement. B: Block; C: Plot untreated (Control); C.S.: plot treated with chemical insecticide (Cydim Super); N1: plot treated with the low dose of the aqueous neem leaf extract; N2: plot treated with the medium dose of the aqueous neem leaf extract; N3: plot treated with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

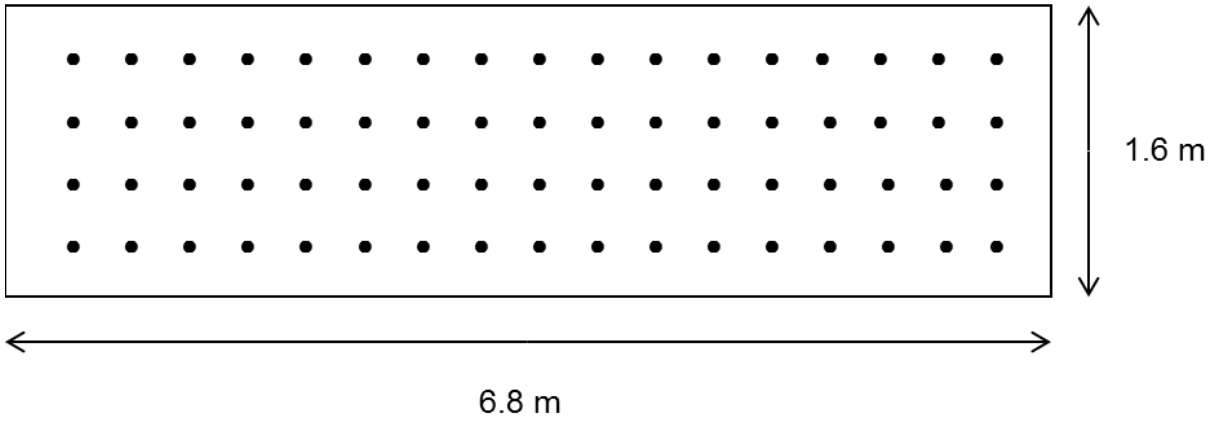

Figure 2. Arrangement of *S. macrocarpon* plants on plots (aligned points).

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.

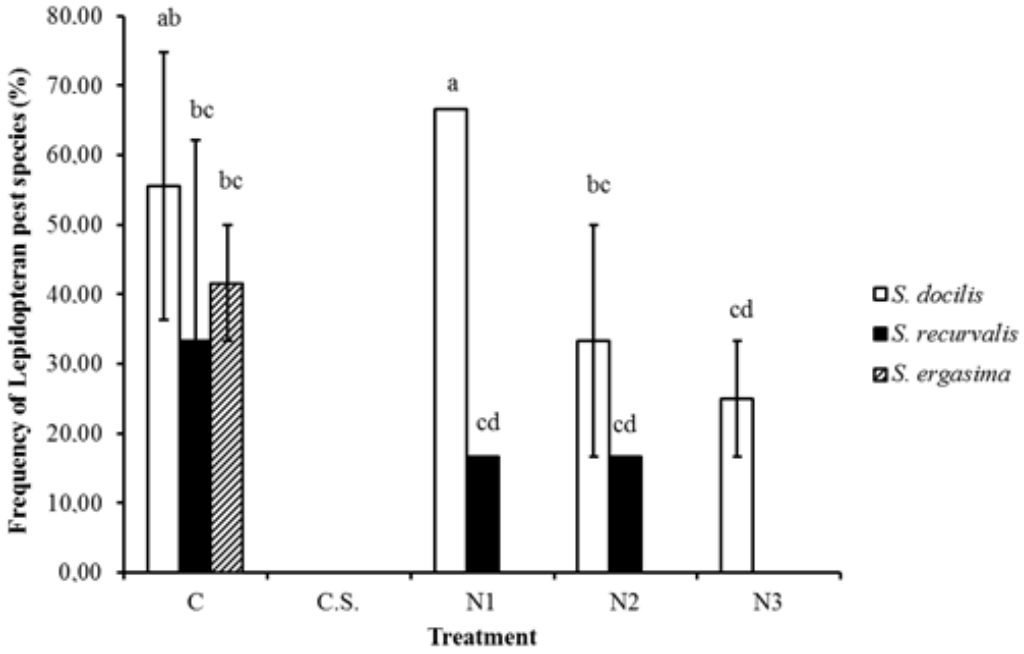

Figure 3. Mean frequency (X ± SD) of Lepidopteran larvae (*S. docilis* Butler (Noctuidae), *S. recurvalis* F. (Crambidae) and *S. ergasima* Meyrick (Gelechiidae)) following treatment. Different letters over the columns indicate statistically significant differences ($F_{(14, 44)} = 14.096$; df = 14; 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 ± 19.25%). However, the frequencies were lower (33.33 ± 16.67% and 25.0 ± 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 ± 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

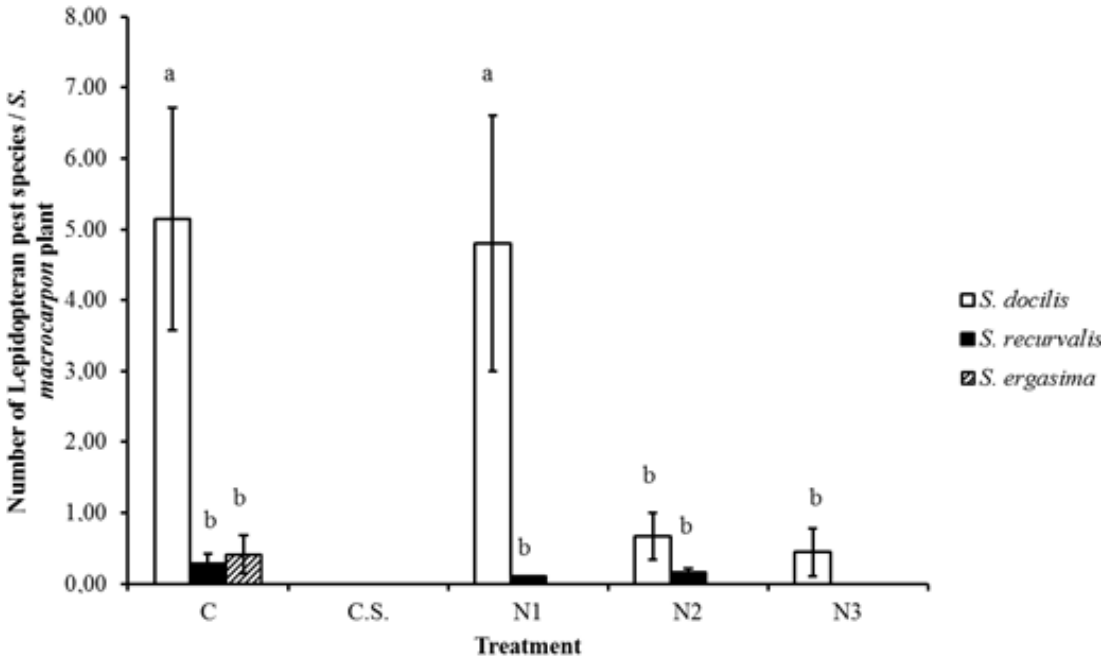

Figure 4. Mean numbers (X ± SD) of Lepidopteran larvae (*S. docilis* Butler (Noctuidae), *S. recurvalis* F. (Crambidae) and *S. ergasima* Meyrick (Gelechiidae)) per *S. macrocarpon* plant following treatment. Different letters over the columns indicate statistically significant differences ($F_{(14, 44)}$ = 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 ± 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, respectivly ($F_{(4 ; 14)}$ = 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

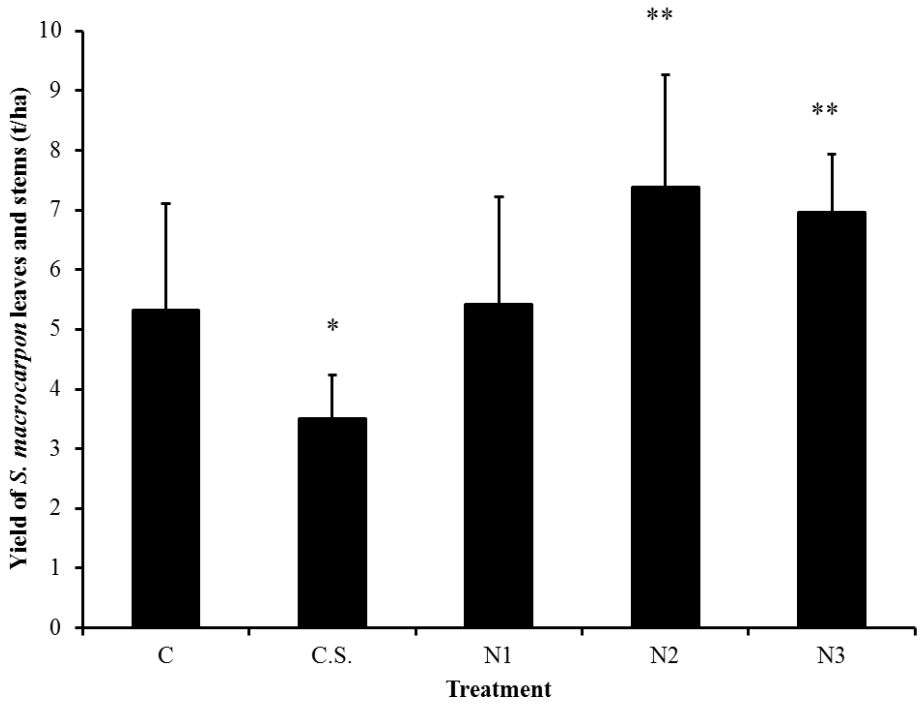

Figure 5. Mean yield (X ± SD) of *S. macrocarpon* following treatment. Columns with different numbers of asterisk over indicate statistically significant differences with LSD test ($F_{(4, 14)}$ = 3.111 ; df = 4 ; C.S.≠ N2 : P = 0.010; C.S. ≠ N3 : P = 0.019), B: Block; C: Plot untreated (Control); C.S.: 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; Mondédji 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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