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Studies on the Efficacy of *Azadirachta indica* A. Juss, *Piper guineense* (Schum & Thonn) seed extracts and Chlorpyrifos on Insect Pests of *Abelmoschus esculentus* (L) Moench in Ibadan South West Nigeria.

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Abstract

Field experiments were conducted during the early cropping season of 2012 at Teaching and Research Farm of Federal College of Forestry Ibadan to compare the efficacy of two selected plant extracts and synthetic insecticides (chlorpyrifos) against insect pests of okra. The experiment was laid out in a Randomized Complete Block Design (RCBD) in four replicates. The seed extracts were applied in sole and in combination, the sole treatment was at the rate of 10 ml/ liter of water, mixture of *A. indica* and *P. guineense* at 5 ml of each per liter of water and chlorpyrifos at 5 ml/liter of water using hand sprayer at two weeks intervals starting four weeks after planting until the end of harvest. The treatments significantly ($P < 0.05$) reduced leaf damage, suppressed insect population and enhanced okra pod yield. Percentage reduction in leaf damage was highest (45.41%) in plots treated with mixture of *Azadirachta indica* (neem) and *Piper guineense* (black pepper) extracts and followed closely by plots treated with chlorpyrifos (44.58%). The extract mixture of *A. indica* and *P. guineense* also ranked 1st in reducing the insect population and in enhancing the okra pod yield. Therefore, mixture of *A. indica* and *P. guineense* extracts was more effective in protecting the okra plants from insect infestation than chlorpyrifos. This technology is safe, environmentally friendly, affordable and easy to apply, thus should be adopted by farmers for the management of insect pests of okra in Nigeria.

Key words: Bio-Insecticides, Damage, Efficacy, Insect, Okra, Synthetic Insecticides

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Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) Malvaceae is a very essential vegetable crop in the tropics including Nigeria (Schippers, 2002). It is believed to be originated from East Africa and today is widely distributed in the tropics, subtropics and warmer parts of the temperate region (ECHO, 2003). It is a warm, rainy season crop that requires high day and night air temperatures, however, growers start cultivation in January when average temperatures are below 37°C as an early crop for better yield (Simmone *et al.*, 2004). The fruits, leaves and tender shoots are consumed, either in fresh or dried forms (Arapitsas, 2008). It is a good source of vitamins (A, B and C), protein, carbohydrates, fats, minerals, iron and iodine (Diaz and Ortegón, 1997). Mature fruits are harvested at 3 - 5 days intervals during the fruiting period. Consumption of 100 g of fresh okra fruit provides 20, 15 and 50% of the daily requirement of calcium, iron and ascorbic acid respectively (Schippers, 2002). Okra consumption among other fruit vegetables were found beneficial in moderating blood pressure, fibrinogen concentration and plasma viscosity in hypertensive person (Adebawoo, *et al.*, 2007). The dried seed is a nutritious material that can be used to prepare curds, or roasted and ground to be used as coffee additive or substitute (Farinde *et al.*, 2007). In Nigeria, okra is usually boiled in water resulting in slimy soups and sauces, which are highly relished. The fruits also serve as soup thickeners (Schippers, 2000) and unspecified parts of the plant reportedly possess diuretic properties (Felter, *et al.*, 2007).

Okra plants are subject to attack by several insect pests like flea beetles (*Podagrica puncticolis*, *P. paltida*), White flies (*Bemisia tabaci*), jassids (*Empoasca lubica*) and aphid (*Aphis gossypii*) attack okra. Pods and flowers are primary targets of spiny bollworm (*Earias insulana*), while the caterpillar of the American bollworm (*Heliothis armigera*) prefers the reproductive parts of the plant, including buds, flowers and fruits (Ahmed, 2000). In Nigeria, control of insect pests of vegetables is based largely on the use of synthetic insecticides, especially lambdacyhalothrin in most parts of the south-west (Farinde, *et al.*, 2007). However, due to health risks and environmental pollution emanating from the synthetic insecticides, they are being discouraged. The use of botanicals has attracted particular attention because of their specificity to insect pests, their biodegradable nature and their potential for commercial application (Bishop and Thronton, 1997; Shukla *et al.*, 2000). Neem (*Azadirachta indica*) products has been identified as “the most promising of all plants” because of its legendary insect repellent and medicinal properties, and at the present moment it is the source of most promising pesticides. More than 100 protolimonoids, limonoids or tetranortriterpenoids and some nonterpenoid constituents have been isolated from various parts of neem (Koul, *et al.*, 1990). *Piper guineense* have also been found effective against a number of insect pests of cultivated crops. (Olaifa *et al.* 1987; Oparaeke *et al.* 2004)

This work evaluated the comparative efficacy of *Azadirachta indica*, *Piper guineense* seed extracts and Chlorpyrifos (synthetic insecticides) on the major insect pests of okra in south west Nigeria.

Materials and Methods

Ripe neem fruits were picked from the ground around the mother plant; they were soaked in water for 24hour and were later macerated with hand to extract the seeds. The extracted seeds were air-dried for two weeks. Ripe fresh fruits of *Piper guineense* were purchased from a local market in Ibadan and were air dried for two weeks. The dried seeds were separately ground in a machine into fine powder. Two hundred gram (200g) of each powder was weighed into 1 litre round bottom flask and 250 ml ethanol (b.p. 56°C) was measured into it. The mixtures were thoroughly shaken with mechanical shaker for 24 hours and allowed to settle overnight and later decanted. The decanted solutions were then filtered using Whatman No.1 filter paper. The experimental plot of 12 x 10m was manually cleared with cutlass and beds were made with hoe two weeks later. Poultry manure was applied at rate of 2.5t/ha as a basal treatment. Plots of 3 x 3 m² were laid out in a Randomized Complete Block Design (RCBD) with three replications. The okra seeds were sown at the rate of 2-3 seeds per hole of 2-3 cm depth at spacing of 30cm between rows and 25 cm within rows and were later thinned to 1 plant per stand. Each sub plot consisted of 15 okra stands (15plant per replicate). Plots were weeded manually three times at three weeks interval starting from four weeks after planting. No irrigation was applied as the plots received ample rainfall.

There were five treatments including control; *Azadirachta indica*, *Piper guineense*, combination of *A. indica* and *P. guineense*, Chlorpyrifos and control. The extracts were applied as a foliar sprayer at the rate of 10 ml per liter of water with aid of hand sprayer while chlorpyrifos was applied at the rate of 5 ml per liter of water. All treatments were applied at two weeks interval starting from four weeks after planting (4WAP). Leaf damage by insect pests was assessed by counting the number of perforation on the okra leaves. Populations of major insect pests were assessed weekly during the morning hours on five randomly selected plants per sub plots (replicate) starting from four weeks after planting. The effect of various treatments on okra pod yield was estimated by harvesting mature okra pods on selected plants and taking the fresh weight using a sensitive weighing balance starting from the first week of onset of fruiting for seven weeks. All the assessment was done at weekly intervals. Data collected were subjected to Analysis of Variance (ANOVA) and significant means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

Results and Discussion

All the treatments significantly reduced the leaf damage (number of perforation) by insect pests compared to untreated control (Table 1). The extract mixture of *A.indica* and *P. guineense* was the most effective in reducing damage by insect pests on okra leaves, followed by Chlorpyrifos. Significant differences ($p < 0.01$) were observed among the treatments from 4 weeks after planting to 9 weeks after planting (4WAP-9WAP). There was a significant difference ($p < 0.05$) in number of perforation on okra leaves treated with sole neem extracts over sole treatment of *P. guineense*. The percentage reduction of leaf damage by the various treatments ranges from 45.42% - 26.71% when compared to control (Table 2). The plots treated with mixture of *A. indica* and *P. guineense* ranked first (45.42%) in reducing insect damage on okra leaves, followed by plots treated with chlorpyrifos (44.58%). The plots treated with lone *P. guineense* was least (26.71%) in reducing insect damage on okra leaves. Four major insect pests were observed at the experimental plots at the study area causing damage on okra leaves; *Sylepta derogata*, *Empoasca spp*, *Spodoptera exiqua* and *Zonocerus variegatus* (Table 3). The different treatments significantly ($p < 0.01$) suppressed the population of *Sylepta derogata* a major pests of okra compared to the untreated control. There was no significant difference on the effect of the different treatments on the population of *Empoasca spp*, *Spodoptera exiqua* and *Zonoceros variagatus* compared to control. However, plots treated with mixture of *A. indica* and *P. guineense* extracts recorded least insect count of the three insect species, followed by chlorpyrifos. The control plot recorded highest densities of the four insect species encountered.

The effect of the treatments on okra yield is shown in table 4. The okra plants started fruiting at 6 weeks after planting and harvesting commenced at 7 weeks after planting. The various treatments significantly ($p < 0.05$) enhanced okra yield compared to untreated control. Plots treated with mixture of *A. indica* and *P. guineense* recorded highest yield of okra pods, followed by chlorpyrifos while the control plots recorded least yield of okra pods. There was no significant difference ($p > 0.05$) between the effect of sole treatment of individual extracts and chlorpyrifos on the yield of okra pods from 10 weeks after planting to 13 weeks after planting (10WAP – 13WAP). The extract mixture of *A. indica* and *P. guineense* were more effective among other treatments in all the factors considered in this experiment.

The results demonstrated the potential of *A. indica* and *P. guineense* extracts to control major insect pests of okra. The mode of action of these two extracts in controlling the observed insect pests is not apparent. However, it could be contact activity for the extract of *P. guineense*, and perhaps antifeedant action for the *A. indica*. Moreover, Bernays and Chapman (1977) indicated *A.indica* (neem) as the most potent antifeedant against insects like *Locusta migratoria migratorioides* and *Schistocerca gregaria*. Rembold *et al.*, 1980 also reported that neem exhibits strong antifeedant activity against locusts as well as growth inhibiting properties. Similarly, Padi *et al.*, (2000) reported that the effectiveness of neem-based compounds is known to rely more on their ability to repel and inhibit feeding, growth and reproduction, rather than on contact activity to kill insects. Also, Saxana *et al.*, (1981b) reported that neem oil was found to be a good antifeedant for the control of rice brown hopper. Likewise, *P. guineense* have been reported by several authors for their insecticidal properties. Oparaeke *et al.*, (2004) reported that visual observations after direct spraying of *P. guineense* extracts against *Clavigralla tomentosicollis* and *Maruca* larvae on cowpea plants indicate that *P. guineense* extracts first caused illusion on them and later killed them within 10–15 minutes of contact with the extracts. Moreover, Golob *et al.*, (1999), reported that *P. guineense* powder, oil, and hexane and acetone extracts have been effective in causing mortality and reducing oviposition of various insects when applied to grains and crops such as maize or cowpea. The effectiveness of the sole treatment of *A. indica* extracts on insect damage on okra leaves was significantly ($p < 0.050$) higher than sole treatment of *P. guineense*. This could be attributed to the repellent action of the *A. indica* and the high rate of degradation *P. guineense*. Photodegradation of the active compound of *P. guineense* (piperamides) has been previously observed in studies of *Piper* species. Scott, (2004) noted that after six hours in sunlight, the active ingredient in *P. guineense* had virtually completely degraded.

The performance of the combined extracts was better than the individual extracts even more effective than the synthetic chemicals. This could be attributed to the different mode of actions of the two plant extracts used specifically the contact activity of *P. guineense* and the repellent and antifeedant activities of the neem extracts which gave double effects on the insects to achieve the best result. Therefore, the efficacy of mixtures of *A. indica* extracts and *P. guineense* extracts could not be attributed to the potency of the former but due to the complementary or synergistic activities of the latter. Similarly, Dadang *et al.*, 2009 reported that two botanical insecticide formulations containing extract mixtures of *Piper retrofractum* and *Annona squamosa* and *Aglaia odorata* and *Annona squamosa* have been tested on cabbage insect pests and was found to be more effective than synthetic insecticides.

Conclusion and Recommendations

The results presented in this study have shown that extracts of *P. guineense* and *A. indica* have great potentials as biopesticides. The mixtures of plant extracts exhibited higher insecticidal activity against the major insect pests of okra than synthetic insecticides thus indicating their potentials for development as botanicals for the management of insect pests of okra. The extracts did not show any phytotoxicity effect on treated plants and they are readily available for local farmers' use in Nigeria. Since they are biodegradable, its use will reduce potential environmental hazards associated with the use of synthetic insecticides and could provide suitable alternatives for pest control on field crops of small scale and low-input agriculture as commonly practiced in tropical countries.

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Tables

Table 1: Effect of the treatments on the number of perforation on okra leaves Weeks After Planting (WAP)

Treatments	3	4	5	6	7	8	9
<i>A.indica</i> extracts	30.25a	39.00b	44.00c	51.50b	59.00b	67.50b	66.25bc
<i>P.guineense</i> extracts	35.00a	48.50a	51.75b	54.00b	63.25b	68.75b	78.25b
Chlorpyrifos	31.25a	36.75b	40.25c	39.25b	43.50c	52.25c	59.25c
<i>A.Indica</i> + <i>P.guineense</i> extracts	31.35a	34.00b	39.75c	44.25b	40.25c	52.25c	55.25c
Control	30.50a	51.75a	62.25a	106.50a	91.75a	96.75a	109.50a
G.A	31.85	42.00	47.60	59.10	59.45	67.50	73.70
Sig. level	Ns	**	**	**	**	**	**
CV%	12.80		19.52	38.41	8.66	8.09	12.91

Means followed by the same letter within the column are not significantly different according to DMRT ($p < 0.05$)

** Significant at 1% of probability; Ns = not significant at 5% level of probability

Table 2: Effect of the treatments on the mean damage by insect pests on okra leaves

Treatments	Mean Number of perforation	Percentage (%) damage	Percentage (%) damage compared with control	Percentage (%) reduction of damage compared with control
<i>A.indica</i> extracts	337.25bc	17.90c	62.77c	38.23ab
<i>P.guineense</i> extracts	400.25b	21.24b	73.39b	24.71b
Chlorpyrifos	302.50c	16.06c	55.42c	44.58a
<i>A.Indica</i> + <i>P.guineense</i> extracts	298.00c	15.82c	54.59c	45.42a
Control	546.00a	28.98a	100.00a	0.00c

Values followed by the same letter within the column are not significantly different according to DMRT ($p < 0.05$)

Table 3: Effect of the treatments on the population of insect species insect species

Treatments	<i>Sylepta derogata</i>	<i>Empoasca spp</i>	<i>Spodoptera exiqua</i>	<i>Zonoceros variegatus</i>
<i>A.indica</i> extracts	2.16b	1.33	1.16	0.33
<i>P.guineense</i> extracts	3.16b	1.66	0.33	0.83
Chlorpyrifos	2.00b	1.33	1.00	0.50
<i>A.Indica</i> + <i>P.guineense</i> extracts	2.00b	0.66	0.33	0.33
Control	4.83a	1.33	0.60	1.16
G.A	2.83	1.20	0.70	0.63
Sig.level	**	Ns	Ns	Ns
CV%	45.97	69.72	121.78	145.00

Means followed by the same letter within the column are not significantly different according to DMRT ($p < 0.05$)

** Significant at 1% of probability; Ns = not significant at 5% level of probability

Table 4: Effect of the treatments on the fresh weight (g) of okra pods Weeks After Planting (WAP)

Treatments	7	8	9	10	11	12	13
<i>A.indica</i> extracts	32.00b	37.50b	37.00c	41.50b	43.25a	45.00b	49.00b
<i>P.guineense</i> extracts	27.25b	33.50b	35.00c	37.50b	42.00b	44.50b	46.50b
Chlorpyrifos	29.00b	39.50b	45.25b	44.75b	45.75b	50.00b	52.50b
<i>A.Indica</i> + <i>P. guineense</i> extracts	53.75a	57.50a	65.00a	68.00a	70.00a	74.50a	80.25a
Control	14.25c	15.50	12.50d	11.50c	10.25c	8.75c	7.75c
G.A	31.25	36.70	38.95	40.65	42.25	44.55	44.80
Sig.level	**	**	**	**	**	*	**
CV%	17.27	11.05	12.31	15.12	12.12	12.28	15.50

Means followed by the same letter within the column are not significantly different according to DMRT ($p < 0.05$)

** Significant at 1% of probability; Ns = not significant at 5% level of probability