Eco-friendly management of the flea beetle, *Podagrica* species (Coleoptera: Chrysomelidae) on okra (*Abelmoschus esculentus* (L.) Moench) with *Artemisia annua* L. seed extract

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ABSTRACT

Okra, grown and consumed in every ecological zone, is amongst the most sort after fruit vegetable crops in Nigeria. The crop is often infested by diverse field insect pests, especially the flea beetle *Podagrica* species, which impinges on its growth and productivity. The application of synthetic pesticides has been the generic insect pest control measure due to its touted effectiveness. These synthetics, however, has safety concerns which include; hazard to human health, amplification of toxins in the food chain, pest resurgence, domestic animals and human poisoning, environmental pollution, insect resistance, natural enemies destruction; springs, wells, rivers, and underground water contamination. Stakeholders are advocating an alternative management approach that is sustainable with less negative social and environmental impact. This study, therefore, compared the insecticidal efficacy of *Artemisia annua* (source of the antimalarial artemisinin) seed extract in comparison with Cypermethrin 10 EC, a synthetic insecticide, in controlling *Podagrica* species, the major field insect pests of okra. The trial was set out in a 7 x 4 factorial arrangement integrated into a randomized complete block design and replicated thrice. Cypermethrin 10 E.C was tested at 0.25 ml, 0.50 ml, and 0.70 ml/100 ml of water, while *Artemisia annua* extract was applied at 1.00 ml, 2.00 ml, and 3.00 ml/100 ml of water respectively. The control for both treatments was designated 0.0 ml. Data on leaf damage and flea beetle abundance were carried out from 4-10 weeks after planting (WAP) whilst the pods' weight was measured at maturity. The result shows that i) the concentrations of Artemisia extract and Cypermethrin sprayed provided comparable protection to okra against the flea beetle; ii) the yield of okra (pod weight) does not vary between *Artemisia annua* extract and Cypermethrin treated plots; and iii) flea beetle infestations vary with okra developmental stages.

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1. INTRODUCTION

Okra plant (*Abelmoschus esculentus* (L.) Moench), commonly called "lady's finger", "bhindi" or "gumbo" was initially classified as Hibiscus. It was later assigned to the genus *Abelmoschus* [1]. Okra, a very important fruit vegetable crop grown and consumed in every part of Nigeria is also cultivated in most warm temperate, sub-tropical, and tropical parts of the world [2]–[4]. The volume of Okra fresh fruits, marketed daily in departmental stores and markets and the land mass cultivated for the crop, confirms its importance to the dietary needs and economic significance [5]. The fresh fruits are also exported by some Caribbean and African countries to America and Europe where they are consumed mostly by immigrant ethnic nationalities from Pakistan, India, West, South Africa, and other subtropical and tropical countries [6]. Okra enhances the economic well-being of rural and urban dwellers [7] as it provides an avenue to prosperity for producers (large and small-scale alike) who are engaged in the okra value chain, comprising women traders and producers.

The pods contain reasonable levels of Ca, Zn, Na, K, Fe, Mg, and Mn as primary elements [8]–[11]. The seeds contain high levels of oil, with concentrations ranging from 20 to 40%. The oil is majorly Linoleic acid (47.4%), a polyunsaturated fatty acid essential for human nutrition [12], [13]. Gemede [14] has referred to okra as "a perfect villager's vegetable" because it has a balance of seed protein with amino acid tryptophan and lysine which can be compared to soybean protein but with a better efficiency ratio. The seed also has dietary fiber which makes it a good supplement to cereal, other flour diets, and legumes [15], [16]. Okra is infested by a myriad of insect pests that affect plant growth, survival, reproduction, and crop yield [17]. Crop yield in Nigeria has been described as very low, usually below 7 t/ha [18]. Insect pest infestation has been reported as the major limiting factor to the profitable production of okra [19]. The main insect pests of *Abelmoschus esculentus*, identified in Owerri, Imo state, Nigeria, humid tropical environment are; the flea beetle, *Podagrica* species; cotton strainer, Dysdercus species; aphids, Aphis gossypii whitefly, Bemisia tabaci; Nigerian grasshopper, Oedaleus nigeriensis and cotton leaf roller, Notarcha derogata [20].

Podagrica species (P. uniformis and P. sjostedtiis) are the major and most damaging insect pest of okra which causes folial damage, defoliation, and tremendous yield losses due to the insect feeding habit which defoliates and reduces the plant photosynthetic capability by making round holes in leaves resulting in the death of young plants [21]. Also, the severe infestation may lead to retarded and stunted development which reduces plant vigor and an ultimate reduction in yield [22]. *Podagrica* species also act as vectors that transmit mosaic virus which may lead to a 20-50% reduction in yield [23].

The use of synthetic pesticides such as pyrethroids by large and small-scale farmers in the management of insects is relatively high compared to the use of bio-pesticides due mainly to the quick action of the constituent active ingredients and restricted knowledge regarding bio-pesticides use. Cypermethrin is the most commonly applied amongst pyrethroids. It is a fast-acting, composite, neurotoxic insecticide with effective stomach and contact [24] which has become one of the most important insecticides in wide-scale use [25], [26]. Cypermethrin, like all other synthetic pyrethroids, kills insects by disorganizing the proper performance of the insect's nervous system [27], making it very effective and fast. In recent times, wrong uses of synthetic insecticides and administration of under or overdoses have led to detrimental consequences which include: poisoning of humans and domestic animals, deleterious effects on natural enemies, loss of honey bees, pollution of rivers, underground water, and phytotoxicity. [28]–[31].

Artemisia annua L. (Asterales: Asteraceae), otherwise called annual wormwood or sweet wormwood, has been used in China for over 2,000 years in native Chinese medicine for treating malaria and several ailments [32]. The plant is an annual crop and cultivated largely in, Turkey, Afghanistan, Australia, Vietnam, and China [33], [34] for artemisinin; in Romania, it is cultivated for its essential oils and in the United States, it is grown in small quantities for aromatic wreaths. However, in China and Africa (Tanzania and Kenya), *Artemisia annua* is cropped on large scale for the production of antimalaria artemisinin, a combination therapy (ACT) recommended by the World Health Organization to treat several-drug resistant Plasmodium falciparum and P. vivax strains [35], [36]. Researchers [37]–[40] have reported the larvicidal, growth inhibition, antifeedant and insecticidal effects of *Artemisia annua* extracts on insects.

With *Artemisia annua* now cropped in Zaria in Kaduna State; Jos, in Plateau State, and some southern states like Bayelsa, Nigeria, this study is necessary to source alternative use of the plant by comparing its insecticidal action with Cypermethrin, a standard synthetic insecticide, to determine their comparative efficiency in the management of field insect pests of okra in a humid tropical environment. The finding should form an important basis for developing an alternative, safe and eco-friendly approach to the management of *Podagrica* species in okra production.

2. MATERIALS AND METHOD

2.1. Site location

The experiment was performed at the School of Agriculture and Agricultural Technology's Teaching and Research Farm in the Federal University of Technology Owerri, Imo State, Nigeria. The site is situated in the tropical rainforest zone of southeast Nigeria, with latitude 5 °27N and longitude 7 °2E with an average temperature of 27 °C, 75% relative humidity, and rainfall that ranges from 2500 mm-4000 mm.

2.2. Land preparation, experimental design, and soil treatment

An area measuring 20.5 m x 8.0 m (164 m^2) was cleared manually pulverized, plowed, leveled, and hand hoed. The leveled field was delineated into plots and blocks and set out in a randomized complete block design (RCBD), consisting of 7 treatments and 3 replications. Twenty-one (21) beds of 2.5 m x 2.0 m (5.0 m^2) experimental plots were divided into 3 blocks with a spacing of 1 m between the blocks. A spacing of 0.5 m between the beds with a height of 0.25 m was made. Furadan 3G (carbofuran) was applied to the soil at 1.0 kg active ingredient per hectare (30 kg of product per ha) or 15 g of Furadan 3G per 5.0 m^2 bed, to control nematode infestation.

2.3. Procurement of seeds, Cypermethrin 10 EC, and *Artemisia annua* seed extraction

Okra, Lady's finger variety, was procured from the Agricultural Development Project Office in Owerri, Imo State, Nigeria, whilst *Artemisia annua* seeds were obtained from a farm in Mawingo, Central Kenya. The *Artemisia annua* seeds were taken to the laboratory for extraction with ethanol. One hundred and fifty g (150 g) of the crushed seeds were immersed in 500 ml of ethanol for four days. A fine muslin cloth was used to filter the solution to obtain the active principle.

The ethanol was removed by heating using a hot plate. The extract was measured out at 1.0 ml, 2.0 ml, and 3.0 ml. The samples that were measured out were mixed with 100 ml of water, respectively, and sprayed with a 500 ml hand sprayer. Cypermethrin 10 EC was procured from Syngenta Nigeria Limited, located at 387 Agege Motor Road, Mushin, Lagos, Nigeria. The insecticide was measured out with a syringe at 0.25 ml, 0.50 ml, 0.75 ml, and mixed with 100 ml of water, respectively. The measurement and spraying procedure were repeated every designated spraying day.

2.4. Planting of okra seeds

Okra seeds were soaked in water, 6 hours before planting. They were sowed at a spacing of 50 cm x 40 cm, 2-3 seeds per hole and thinned down to one plant per stand, 14 days after planting. This gave a plant population of 25 per bed or 50,000 plants per hectare.

2.5. Treatment layout

- Control 0.0-C
- Artemisia *annua* at 1.0 ml/100 ml of water (Ata-C_1)
- Artemisia *annua* at 2.0 ml/100 ml of water (Ata-C_2)
- Artemisia *annua* at 3.0 ml/100 ml of water (Ata-C_3)
- Cypermethrin at 0.25 ml/100 ml of water (Cyp-C_1)
- Cypermethrin at 0.50 ml/100 ml of water (Cyp-C_2)
- Cypermethrin at 0.75 ml/100 ml of water (Cyp-C_3)

These were replicated three times.

2.6. Data collection

2.6.1. Flea beetle abundance

Insect pests were collected every week from each plot using clinical hand cloves, plastic forceps, sweep nets, and sample bottles. This was effected from 6:30 am-7:30 am every Saturday, starting from two weeks after planting and at 4 to 10 WAP, respectively. The collected insects were kept in containers that had chloroform-soaked cotton wool and later recorded and identified in the laboratory.

2.6.2. Leaf damage assessment

The severity of leaf damage was determined by recording the number of damaged leaves relative to the total number of leaves. Recording the damaged leaves was done by physical counting of the cuts and holes from three randomly selected plants at 4, 6, 8, and 10 weeks after sowing. Similarly, the number of leaves was calculated by enumerating the number of leaves from three randomly selected plans at 4, 6, 8, and 10 weeks after planting.

2.6.3. Pod weight

The mature pods were harvested manually and conveyed to the laboratory and weighed with an electronic scale.

2.7. Data analysis

Data collated from the trials were analyzed using the analysis of variance (ANOVA) and significant treatment means separated using the Tukey HSD test. One-way ANOVA (treatments) was used to analyze data on pod weight, whereas two-way ANOVA (treatments and sampling interval) was performed on data from flea beetle abundance and leaf damage. A sample T-test was independently used to compare (means) the efficacy of the Artemisia extract against cypermethrin. All analysis was done using R statistical software.

3. RESULTS

3.1. Flea beetle abundance

Insects identified during the experiment were aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae); cotton strainer, and *Dysdercus* species. (Hemiptera: Pyrrhocoridae); *w*hitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae); Nigerian grasshopper, *Oedaleus nigeriensis* Uvarov (Orthoptera: Acrididae); *Podagrica* species (Coleoptera: Chrysomelidae) and cotton leaf roller, *Notarcha derogata* Fabricius (Lepidoptera: Pyralidae: Crambidae).

The most abundant (more than 70%) insect pest identified was the flea beetle, *Podagrica specie*. The other insect pests encountered were deemed insignificant and occasional to report. Flea beetle abundance differed on okra stands sprayed with Cypermethrin and Artemisia extract at the various concentrations ($F_{6,56}$ = 96.42, P<0.001; see Table 1) and on spraying intervals ($F_{3,56}$ = 205.46, P<0.001; see Table 1).

The treatment effect reveals that okra stands treated with the highest concentration of Cypermethrin (Cyp-C_3) recorded the lowest number of flea beetles although does not differ statistically with other treatments, the control and the highest concentration of Artemisia extract (Ata-C_3), see Table 2. As expected, the control plots were the most infested, and the peak of infestation was observed on the 8^{th} and 4^{th} weeks after planting. The lowest count of flea beetles sampled took place on the 6^{th} and 10^{th} weeks of WAP, see Table 3.

A two-way interaction between the treatments and spraying interval shows that plots sprayed with the highest concentration of Artemisia extract (Ata-C_3) at 8 weeks recorded the highest infestation level followed by the mid concentration of Cypermethrin (Cyp-C_2) at 4 weeks. Okra stands treated with the mid concentration of Artemisia extract (Ata-C_2) and Cypermethrin (Cyp-C_2) at 6 and 10 weeks after planting, respectively, did not record flea beetle infestation. A similar outcome was recorded on plots sprayed with the highest concentration of Artemisia extract at 6 weeks ($F_{18,56}$ = 28.76, P<0.001). Figure 1 shows statistics on T-test results. Although the mean number of flea beetle infestation was slightly higher on plots sprayed with Artemisia extract compared to Cypermethrin-treated plots, they do not differ statistically (t(72) = 0.6352, p-value = 0.2636, see Figure 1(a)).

3.2. Leaf damage assessment

The results show that the severity of leaf damage (damaged leaves relative to the total count of leaves) on okra stands differed with the treatments applied ($F_{6,56}$ = 706.34, P<0.001; see Table 1) and sampling interval ($F_{3,56}$ = 286.20, P<0.001; see Table 1). Leaf damage was lower on plots sprayed with the lowest concentration of Artemisia extract (Ata-C_1) followed by the highest concentration of Cypermethrin (Cyp-C_3), and the control plots were more damaged than other treatments Table 4. A two-way interaction reveals that the severity of damaged leaves on treated plots was lowest on plots sprayed with the lowest concentration of Artemisia extract (Ata-C_1) at 8 weeks together with the mid concentrations of Cypermethrin (Cyp-C_2) at 10 weeks after planting ($F_{18,56}$ = 63.95, P<0.001, see Table 1). This was followed by plots sprayed with the highest concentration of Artemisia extract (Ata-C_3) at 10 WAP. While the severity of leaf damage was higher on cypermethrin-treated plots, a T-test analysis revealed that it is not statistically different from plots sprayed with Artemisia extract t(72) = -1.0018, p-value = 0.3198, see Figure 1(b)).

3.3. Pod weight

The pod weight result indicates that the treatments significantly affected the okra yield ($F_{6,14}$ = 597.4, P <0.001; see Table 1). A posthoc test revealed that pod weight was significantly higher on plots treated with the mid concentration of Cypermethrin (Cyp-C_2), followed by the mid concentration of Artemisia extract (Ata-C_2) ($F_{6,14}$ = 597.4, P <0.001; see Table 1 and Table 5). As predicted, pod weight was significantly lower on the control plots. However, a T-test result showed that the yield recorded on plots sprayed with cypermethrin does not differ statistically from those sprayed with Artemisia extract (t(18) = 0.0323, p-value = 0.4812, see Figure 1(c)).

Table 1. Summary of ANOVA on the effect of insecticides, concentration and sampling time on flea beetle populations in okra field

	Df	Beetle abundance	Leaf damage	Pod weight
Sources of variance			F- value	
Treatment	6	96.42***	706.34***	597.4***
Sampling time	3	205.46***	286.20***	
Insecticides x Sampling time	18	28.72***	63.95***	

*** = highly significant at 0.05 probability level Ns = not significant

Table 2. Post-hoc test results on the flea beetle abundance on okra plots treated with Artemisia extract and cypermethrin at different concentrations

Treatments	Beetle abundance
Artemisia extract C1	0.861_{b}
Artemisia extract C2	1.110_{b}
Artemisia extract C3	2.083_{ab}
Cypermethrin C1	1.166_{b}
Cypermethrin C2	1.583_{b}
Cypermethrin C3	0.665_{b}
Control	3.500_{a}

Means with the same letters are not statistically different (p>0.05)

Table 3. Post-hoc test results on the flea beetle abundance on okra plots at different sampling times

Sampling interval (WAP)	Beetle abundance
4	2.015_{a}
2	0.492_{b}
8	2.857_{a}
10	0.904_{b}

Means with the same letters are not statistically different (P>0.05)

Table 4. Post-hoc test results on the severity of leaf damage on okra plots treated with artemisia extract cypermethrin at different concentrations

Treatments	Leaf damage
Artemisia extract C1	0.303_{d}
Artemisia extract C2	0.598_{bc}
Artemisia extract C3	0.637_{bc}
Cypermethrin C1	0.532_{bc}
Cypermethrin C2	0.643_{b}
Cypermethrin C3	0.487_{c}
Control	0.910_{a}

Means with the same letters are not statistically different (P>0.05)

Table 5. Post-hoc test results on the pod weight of okra plot treated with artemisia extract and cypermethrin at different concentrations

Treatments	Pod weight (g)
Artemisia extract C1	0.072_{bc}
Artemisia extract C2	0.075_{b}
Artemisia extract C3	0.047_{d}
Cypermethrin C1	0.031_{e}
Cypermethrin C2	0.095_{a}
Cypermethrin C3	0.067_{c}
Control	0.021_{f}

Means with the same letters are not statistically different (P>0.05)

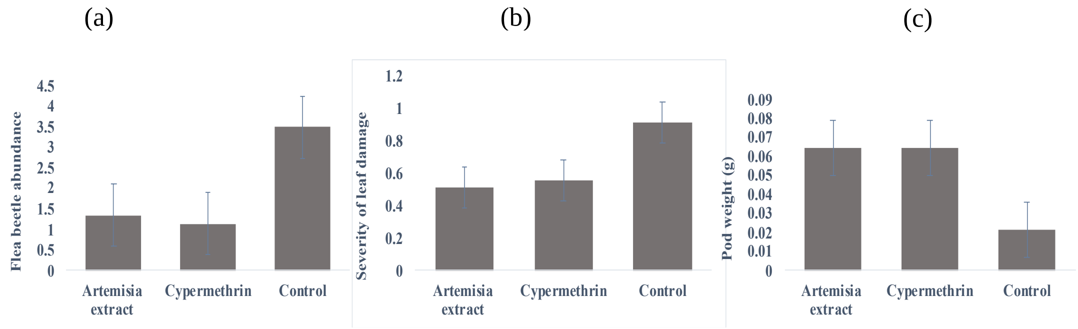

Figure 1. Statistics T-test results from (a) Flea beetle abundance, (b) the severity of leaf damage, and (c) the pod weight from Okra stands sprayed with Artemisia extracts and Cypermethrin

4. DISCUSSION

This study to examine the insecticidal action of Cypermethrin and *Artemisia annua* in controlling insect pests of Okra indicated that though Cypermethrin performed comparatively quicker than *Artemisia annua* in the reduction of the insect pests, the yield (pod weight) from both treatments did not differ. Furthermore, though the plots treated with the extracts of *Artemisia annua* had *a* higher mean value of flea beetle infestation, it did not result in lower yields nor significant damage to the leaves. This suggests that *Artemisia annua* could be harboring compounds that deterred the beetle from feeding on the leaves.

The potential antifeedant and repellent property of Artemisia species extracts has been documented: [38] had observed the antifeedant action of methanolic extract of *Artemisia annua* against elm leaf beetle (Xanthogaleruca luteola Mull.). In another study, the methanolic extract of *Artemisia annua* was reported to have powerful deterrence which also affected the biochemical metabolism of the lesser mulberry pyralid (Glyphodes Pylolais Walker) (Lepidoptera: Pyralidae) [41]. Also, a constituent of *Artemisia annua*, scopoletin, has been reported to have insect antifeedant and growth inhibitory activities against Spodoptera obliqua Walker (Lepidoptera: Arctiidae) [42]. *Artemisia annua* derivative, 1,8-Cineole, and artemisinin have been used to prevent fruit infestation by Cydia pomonella (L.) (Tortricidae), a major insect pest of apples. Apple fruits treated with extracts from *Artemisia annua* had less than 10% of the C. pomonella neonates' attack and the insect deterrence action was very high [43]. Capillin, methyl eugenol, bornyl acetate, capillary and arcurcumene isolated from Artemisia capillaries growing buds have been reported to have antifeeding activity against the cabbage butterfly, Pieris rapae craccivora larva [44], [45]. Also, the ethanolic extract of the dry seeds and leaves of *Artemisia annua* has been reported to have repellent and lethal effects on Anopheles gambiae [46]. Deterrence has been reported to be triggered by the presence of naturally occurring organic compounds like alkaloids, sterols, terpenes, flavonoids, tannins, glycosides, phenols, resins, capillin, capillarin, methyl eugenol, arcurcumene, bornyl acetate, scopoletin and Artemisinin, a secondary metabolite [42], [44]–[49]. Results indicated that flea beetle infestation was highest at the 4th and 8th weeks after planting compared to other sampling intervals. This may suggest that okra plants are attracted to the pest at both developmental stages during which period the plants could be emitting chemical cues that lure the pests. This finding presents great potential in the management of the pest using a semiochemical-based approach [50] making further studies in this line highly imperative [51].

The peak of flea beetles' infestation at the vegetative stage of okra in this experiment agrees with the reports of [52] which documented a huge population of flea beetle at 3 to 5 WAP. From this, it can be deduced that flea beetle control at the early growth stage will reduce the pests' deleterious effect on the yield and growth of the okra plant [23]. The pod weight/yield results indicated that treatment with *Artemisia annua* extract was as good as using the synthetic, cypermethrin in the control of *Podagrica* species, a major pest of okra. It has been reported that the application of leaf extracts of *Artemisia annua* 1% concentration on Solanum scabrum significantly reduced the aphid population density and had a significantly higher mean weight of dry leaves and seeds per plot [53].

5. CONCLUSION

This study shows that the seed extract of *Artemisia annua* formulation could successfully be used as a bio-pesticide in developing countries like Nigeria, which could assist in stemming the rampant dumping of large quantities of deleterious pesticides on farms. Since Artemisia is used in ethnobotany for the treatment of numerous ailments, it is easily biodegradable, environmentally friendly, safe and cultivatable.

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