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Bio-pesticide Actions of Aqueous Extract of *Ocimum gratissimum, Piper nigrum, Xylopia aethiopica, Azadirachta indica* and *Zingiber officinale* Formulation on Cowpea Leaves

[*1] IKECHI – NWOGU, GC; [2]OMEKE, CC

Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State – Nigeria
**Corresponding Author Email: chinyerum.nwogu@yahoo.com; Tel.: +2348032325098*

ABSTRACT: The emergence of widespread insecticide resistance and the potential environmental problems associated with synthetic pesticides have indicated more interest for bio-pesticide usage in pest control. This study investigates the efficacy of a bio-pesticide formulation based on the aqueous extract of five plants namely *Ocimum gratissimum, Piper nigrum, Xylopia aethiopica, Azadirachta indica,* and *Zingiber officinale* in reducing cowpea leaf damage in event of bean beetle attach. Cowpea seeds were sown in four (4) different pots containing loamy soil and kept separately in the field at a distance of 10meters. Seven 7 days after foliage production, pots 1 and 2 were treated with 250ml of the formulations daily for 7 days, while pot 3 and 4 were not treated. From observations, the treated Cowpea plants were not attacked by bean beetle, while the untreated Cowpea plants were attacked by bean beetle, causing great damage on the foliage and this can lead to wilting, chlorosis and other fungal and bacterial diseases. Synthetic pesticides used for pests control, have adverse effects on crops and environment, but bio-pesticides formulated in this research is environmentally friendly, target specific and biodegradable. The extracts showed significant effect at $p<0.05$.

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Cowpea, *Vigna unguiculata* (L.) Walp also known as black-eyed pea, (Heuzé *et al.,* 2015), is an annual herbaceous plant that belongs to the Fabaceae family. The revenue source of millions of people in West and Central, Africa depends on cowpeas (Kamara *et al.,* 2018). According to Gómez, (2004) families in the rural regions derive food, animal feed and cash income from cowpea production. This buttresses the statement by Langyntuo, *et al.* (2003) that Cowpea is one of the most economically important indigenous African legume crop. Cowpea is cultivated mainly for its edible seeds and pods, which are rich in protein. The seeds are consumed after cooking and the leaves are used as vegetable. Also fresh leaves are also used as pot herb especially in East Africa. According to Anele (2012), cowpea can be used as forage or for hay or silage similarly Samireddypalle *et al.* (2017), reported that the dried leaves, stems and pod walls of cowpea known as haulm, could be a worthy source of income for farmers that keep livestock.

Insects are major factor affecting the yields of African cowpea crops and they affect each tissue component and developmental stage. Insect pressure is responsible for over 90% loss in yield (Jackai and Daoust, 1986). Gómez (2004), reported that cowpea suffers severely from insects attack in the field and during storage. Although insecticides are available to handle this problem but majority of farmers, cannot afford them, do not have the necessary equipment to apply, or do not how to apply them properly. Besides, these pesticides pollute the environment and pose as potential danger to humans.

In recent years primarily due to increased pressure to reduce the use of synthetic chemicals which are toxic to the environment, biological control of agricultural pests, has gained importance (Carson, 1962). Prabhu *et al.* (2011), stated that plant products have been used traditionally in many parts of the world against these pest and these products are generally known as Bio-pesticides. Suresh *et al.,* (2014) defined bio-pesticides as formulations made from naturally occurring substances that control pests by non-toxic mechanisms and in an eco-friendly manner. According to Vasantharaj, (2008), bio-pesticides are less toxic than chemical pesticides and are safer to the environment, maintains the health of the soil and sustain its life by increasing soil organic matter, effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding pollutions and it is difficult for insects to develop resistance to bio-pesticides. The objective of this work was to screen the protective effect of a bio-pesticide

formulated from aqueous extract of five plants (*Ocimum gratissimum, Piper nigrum, Xylopia aethiopica, Azadirachta indica,* and *Zingiber officinale)* on cowpea leaf damage when attached by bean beetle.

MATERIALS AND METHODS

Study Environment: The study was conducted in November, 2018 under the normal ambient conditions of the Mycology/Pathology Laboratory of the Department of Plant Science and Biotechnology University of Port Harcourt Choba, Nigeria. During this study, the temperature and relative humidity were 28°C and 68% separately.

Collection of Materials: Plant species: *Ocimum gratissimum* and *Azadirachta indica*, were collected from a garden in the University of Port Harcourt Choba while *Xylopia aethiopica, Zingiber officinale* and *Piper nigrum* were bought from Choba Market close to the University of Port Harcourt Choba and taken to the laboratory for this study.

Experimental site: Plants were grown in six (6) different polyethene bags. The experimental field was divided into two parts separated 10m apart. The experimental plots representing the treatments were labelled Pot 1, 2 and 3 for the treated plants and Pot 4, 5 and 6 for the untreated plants.

Treatments: Treatments were sprayed early in the morning between 7 am and 8 am, 2 time daily for 2 weeks as recommended by Anjarwalla *et al.* (2016). The experimental design applied to each variety was fully randomized, consisting of 8 treatments, each of which was repeated 2 times.

The different treatments were: T1, negative control representing plots that received no insecticidal treatment; T2, plots treated with leaves extract of *Ocimum gratissimum* + *Xylopia aethiopica;* T3, plots treated with leaves extract of *Ocimum gratissimum* + *Xylopia aethiopica* + *Azadirachta indica;* T4, plots treated with leaves extract of *Ocimum gratissimum* + *Xylopia aethiopica* + *Azadirachta indica* + *Zingiber officinale;* T4, plots treated with leaves extract of *Ocimum gratissimum* + *Xylopia aethiopica* + *Azadirachta indica* + *Zingiber officinale* + *Piper nigrum*

Preparation of Extracts: This study covered five plant species; *Ocimum gratissimum, Azadirachta Indica, Xylopia Aethiopica*, *Zingiber officinale* and *Piper nigrum*. The leaves of *Ocimum gratissimum, Azadirachta Indica,* tubers of *Zingiber officinale,* seeds and pods of *Piper nigrum* and *Xylopia Aethiopica*, were collected and pounded in a mortar to produce a fine crushed plant material in accordance to Dabire *et al*. (2008) and according to the modified method of the prescribed guidelines used by Anjarwalla *et al.* (2016), water was used as a solvent to extract the required material from the five plants for use as pesticides.

Formulation of Bio-pecticides: The aqueous extracts of the 5 plants were obtained and applied, using different formulations:

Formulation A: 220g of *Ocimum gratissimum* leaves dissolved in 1000ml of 70% ethanol + 460g of *Xylopia aethiopica* pods dissolved in 1000ml of 70% ethanol + 260g of the leaf of *Azadirachta indica* dissolved in 1000ml of 70% ethanol + 920g of *Zingiber officinale* tubers dissolved in 1000ml of 70% ethanol + 70g of *Piper nigrum* pods dissolved in 1000ml of 70% ethanol.

Formulation B: 110g of *Ocimum gratissimum* leaves dissolved in 1000ml of 70% ethanol + 230g of *Xylopia aethiopica* pods dissolved in 1000ml of 70% ethanol + 130g of the leaf of *Azadirachta indica* dissolved in 1000ml of 70% ethanol + 460g of *Zingiber officinale* tubers dissolved in 1000ml of 70% ethanol + *35g of Piper nigrum pods dissolved in 1000ml of 70% ethanol.*

Formulation C: 350g of *Ocimum gratissimum* leaves dissolved in 1000ml of 70% ethanol + 700g of *Xylopia aethiopica* pods dissolved in 1000ml of 70% ethanol + 400g of the leaf of *Azadirachta indica* dissolved in 1000ml of 70% ethanol + 1400g of *Zingiber officinale* tubers dissolved in 1000ml of 70% ethanol + 100g of *Piper nigrum* pods dissolved in 1000ml of 70% ethanol

Bioassay: After one week of planting, the cowpea began to produce leaves then application test was carried out on the cowpea leaves using different aqueous extracts (Formulation A, B and C) to evaluate the contact action and toxicity ratings of the plant extracts and the control was without any treatment. Among the three Formulations A, B and C, formulation C, performed actively.

Observation: From the observation made, the cowpea pest started eating up the leaves from day 4 after

germination and cause severe damage to the foliage (Table 1).

Statistical Analysis: The data obtained from all the treatment were subjected to analysis using One-Way ANOVA.

Table 1: Showing Field Observation

Treatments	Observations	Inference
Untreated Cowpea Pots 1-3	Days 1-3 (slight patches appear in young leaf)	Mild damages occurred
	Day 4-7 (leaves of pot 1-3 were eaten by pests)	Pest cause severe damage on the foliage
Treated Cowpea Pots 4-6	Days 1-3 (young leaf grew well no patch was noticed)	Pesticide was very effective
	Days 4-7 (The leaves grew bigger and broader, with no appearance of leaf patch)	It also increased the growth rate of the plant

RESULTS AND DISCUSSIONS

The experiments conducted for evaluating pesticidal activities of roadside and wasteland weed (*Ocimum gratissimum*) and some locally sourced plants for cowpea protection, revealed that the formulation c extracts (PLATE 1) of *Xylopia aethiopica*, *Azadirachta indica*, *Zingiber officinal*, *Ocimum gratissimum* and *Piper nigrum* had effective pesticidal effects on pest of cowpea.

This means *Piper nigrum* powder can as well be used for cowpea grain storage (Shazia *et al.,* 2006). This corresponds with the work of Simon, (2012) that *Azadirachta indica, Xylopia aethiopica, Zingiber officinal* and *Ocimum gratissimum* are effective in the control of pests. This could be as a result of the alkaloid and terpenoid present in the extracts.

Plate 1: Showing Formulated Biopesticide in Spray Bottle

Phytochemical Analysis: From the Phytochemical carried out, it was discovered that alkaloid and terpenoid are present in the extracts (Table 2).

Table 2: Phytochemical Screening of *Xylopia aethiopica*, *Azadirachta indica*, *Zingiber officinal*, *Ocimium gratissimum* and *Piper nigrum* extracts.

Plants	Terpenoids	Alkaloids
Xylopia aethiopica	+	+
Piper nigrum	+	+
Zingiber officinale	+	+
Azadirachta indica	+	+
Ocimium gratissimum	+	+

Note: + shows presence of terpenoids and alkaloids

The presence of alkaloids discourage insects from ingesting plants. They protect plants against attack of predators (insects and herbivores) by acting as defense compounds (Waller and Nowacki, 1978 & Matsuura and Fett-Neto, 2015). Also Dambolena *et al.* (2016) in their research, confirmed that terpene have potentials for insect management. After the plants germinated and foliage formed, pests attacked the untreated pots causing damages on the foliage (Plate 2a). While the treated pots were not invaded by pests, instead the leaves of the cowpea plants blossomed (Plate 2b). The untreated cowpea that was devastated in its foliage resulted to malnutrition and stunted growth. From the results, the formulated bio-pesticide, helped repel field pests such grasshoppers and aphids from causing damages on the treated cowpea plant. As far as the formulation C, is concerned, pest damage was under control (Fig. 1). Results of the bio-pesticide spray on foliage in treated and untreated Cowpea plant are presented in Fig 1 and the data obtained revealed a significant effect at ($p<0.05$).

Among the three formulations: A, B and C, formulation C, showed pesticidal activities. The result showed that formulation C containing 350g of *Ocimum gratissimum* leaves + 700g of *Xylopia*

aethiopica pods + 400g of the leaf of *Azadirachta indica* + 1400g of *Zingiber officinale* tubers + 100g of *Piper nigrum* pods all dissolved in 1000ml of 70% ethanol had pronounced pesticidal activity. In agreement with this research work Burkill, (1985), observed that *Xylopia aethiopica* can be used as insecticides. Onolemhemhen *et al*. (2011), also worked on the efficacy of *xylopia aethiopica* and *piper guineense* seeds powder on *sitophilus oryzae* mortality and observed the efficacy of these plant in the control of insect pests especially *Sitophilus oryzae.* Extracts of *Piper guineense* mixed with extracts of *Allium sativum* L., *Azadirachta indica* A. Juss, and *Xylopia aethiopica* (Dunal) A. Rich likewise provided effective pest control, leading to significant decline in the population of the test pests as compared with untreated controls (Oparaeke *et al.,* 2007). Addo, (2017), furthermore confirmed the effect of aqueous ginger (*Zingiber officinale*) extracts on the management of major pests of cabbage (*Brassicae oleracea* var.capitata). similarly, Ukoroije *et al.* (2019), studied the efficacy of *Ocimum gratissimum* leaf powder and ethanol extract on adult *Periplanata americana* and detected that there was significant difference in mortality in both increase in concentration levels and exposure time at 5% level of significance in both leaf powder and ethanol extract.

Plate 2a: Showing untreated Cowpea Plant

Plate 2b: Showing treated cowpea plant

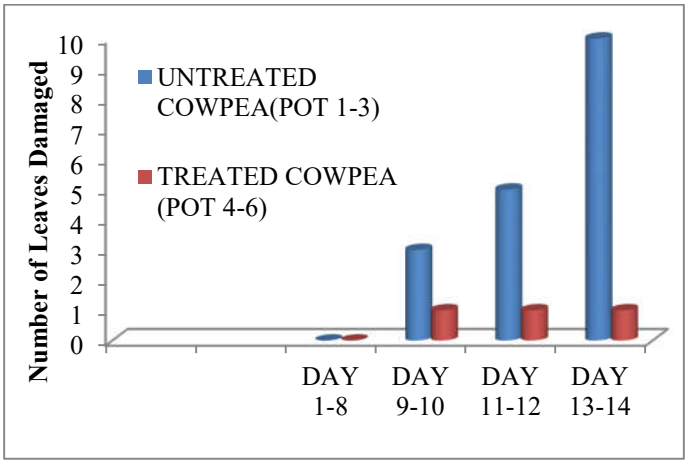

Fig 1: Chart Showing Pest Damage and Effect of the Pesticide

The insecticidal properties of *Ocimum gratissimum* and *Vernonia amygdalina* Leaf Powders were reviewed by Enobakhare and Law-Ogbomo (2007) their experiment also revealed that *Ocimum gratissimum* was more effective than *Vernonia amygdalina* as stored grain protectants at all dosage level of application. This study is consistent with the previous work of Ogendo *et al.* (2008) using *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. From the study, *Ocimum gratissimum* oil and its constituents are

potential alternatives to synthetic fumigants in the treatment of durable agricultural products. Man has been searching for means to substitute synthetic pesticides. This study has led to the use of biological pesticides. Several biological sources of pesticides have been explored, and one of these great sources comes from plants.

Conclusion: The current study showed that *Xylopia aethiopica*, *Azadirachta indica*, *Zingiber officinal*, *Ocimium gratissimum* and *Piper nigrum* have strong pesticidal activity. Biological pesticides have proven beyond reasonable doubt, to be the most environmentally friendly form of pesticide for crops so the uses of this formulation, would serve as help in preventing pest attack on crops and improve the fertility of the soil. It is recommend that farmers who grow legumes in general and other vegetables, utilize bio-pesticides as it will gun down pests and still maintain the natural state of the environment.

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REFERENCES

Addo, A (2017). The Effect of Aqueous Ginger (*Zingiber officinale*) extracts on the Management of Major Pests of Cabbage (*Brassicae oleracea* var.capitata). An MS.c Thesis submitted to the Department of Theoretical and Applied Biology, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi. pp. 41-58.

Anele, UY; Sudekum, KH; Arigbede, OM; Luttgenau, H; Oni, AO; Bolaji, OJ; Galyean, ML (2012). Chemical Composition, Rumen Degradability and Crude Protein Fractionation of Some Commercial and Improved Cowpea (*Vigna unguiculata* L. Walp) Haulm Varieties. *Grass Forage Science,* 67 (2): 210-218.

Anjarwalla, P; Belmain, S; Ofori, DA; Sola, P; Jamnadass, R; Stevenson, PC (2016). *Handbook on Pesticidal Plants*. World Agroforestry Centre (ICRAF), Nairobi, Kenya. pp. 27- 30

Burkill, HM (1985). The Useful Plants of West Tropical Africa, Volume 1. Published by Jstor Global Plants.

Dabire, CLB; Ba, MN; Sanon, A (2008). Effects of Crushed Fresh *Cleome viscosa* L. (Capparaceae) Plants on the Cowpea Storage Pest, *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae). *International Journal of Pest Management,* 54: 319-326

Dambolena, JS; Zunino, MP; Herrera, JM; Pizzolitto, RP; Areco, VA; Zygadlo, JA (2016). "Terpenes: Natural Products for Controlling Insects of Importance to Human Health—A Structure-Activity Relationship Study," Psyche, vol. 2016, Article ID 4595823, 17 pages, 2016. https://doi.org/10.1155/2016/4595823.

Gómez, C (2004). COWPEA: Post-Harvest Operations. Edited by Mejía, Danilo in Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

Heuzé, V; Tran, G; Nozière, P; Bastianelli, D; Lebas, F (2015). *Cowpea (Vigna unguiculata) Forage*. Feedipedia, a Programme by INRA, CIRAD, AFZ and FAO. Available at: https://feedipedia.org/node/233. Retrieved on 07/11/2019.

Jackai, LEN; Daoust, RA (1986). "Insect Pests of Cowpeas". *Annual Review of Entomology*. 31: 95–119

Kamara, AY; Omoigui, LO; Kamai, N; Ewansiha, SU; Ajeigbe, HA (2018) *Improving Cultivation of Cowpea in West Africa.* In: Achieving Sustainable Cultivation of Grain Legumes Volume 2: Improving cultivation of particular grain legumes. Burleigh Dodds Series in Agricultural Science. Burleigh Dodds Science Publishing, pp. 1-18.

Langyintuoa, AS; Lowenberg-DeBoerb, B; Fayec, J; Lambertb, MD; Ibrod, G; Moussad, B; Kergnae, A; Ushwahaf, S; Musaf, S; Ntoukamg, G (2003). Cowpea Supply and Demand in West and Central Africa. *Field Crops Research*, 82: 215–231.

Matsuura, H; Fett-Neto, A (2015). Plant Alkaloids: Main Features, Toxicity, and Mechanisms of Action. *Plant Toxins, pp.1-15.*

Onolemhemhen, PO; Ulebor, JU; Umeri, C (2011). The Efficacy of Xylopia Aethiopica and *Piper guineense* Seeds Powder on *Sitophilus Oryzae* Mortality. *Journal of Agriculture and Social Research* (JASR) Volume 11(2):161.

Oparaeke, AM; Dike, MC; Amatobi, CI (2007). Control of Cultivated Cowpea Pests with Mixtures of Extracts from *Piper guineense* and Other Plants. *Journal Herbs, Spices & Medicinal Plants,* 13(1): 93-102.

Prabhu, K; Murugan, K; Nareshkumar, A; Ramasubramanian, N; Bragadeeswaran, S (2011). Larvicidal and Repellent Potential of *Moringa oleifera* against Malarial Vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, 1(2): 124–129.

Shazia, OWM; Reuben, MM; Makundi, R; Robert, N; Misangu, R; Bukheti, K; Maulid, M; Herman, FL; Christine, G; Ishengoma, DGM; Loth, SM (2006). Control of Cowpea Weevil (*Callosobruchus maculatus* L.) in Stored Cowpea (*Vigna unguiculatus* L.) Grains using Botanicals. *Asian Journal of Plant Sciences,* 5: 91-97.

Simon, K O (2012). Plants as Potential Sources of Pesticidal Agents: A Review, Pesticides - Advances in Chemical and Botanical Pesticides, R.P. Soundararajan, IntechOpen, DOI: 10.5772/46225. Available at: https://www.intechopen.com/books/pesticides-advances-in-chemical-and-botanical-pesticides/plants-as-potential-sources-of-pesticidal-agents-a-review.

Ukoroije, B; Abowei, J; Otayoor, R (2018). The Efficacy of *Ocimum gratissimum* Leaf Powder and Ethanol Extract on Adult *Periplanata americana* under Laboratory Condition. Open Access Library Journal 5(4):1-5.

Waller, GR; Nowacki, EK (1978). The Role of Alkaloids in Plants. In: Alkaloid Biology and Metabolism in Plants. Springer, Boston, MA. pp. 143-181.