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Evaluation of the Insecticidal Potential of Leaf Oil Extracts of *Acalypha wilkesiana* (Muel.) Arg. against the Cowpea Weevil, *Callosobbruchus maculatus* Fabricius (Coleoptera: Chrysomelidae, Bruchidae)

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Abstract **Original Research Article**

The extraction of *Acalypha wilkesiana* oil was carried out in the laboratory using a Soxhlet`s apparatus with six solvents, namely, ethanol, methanol, acetone, pet-ether, n-hexane and water. The biological activities of the different solvent extracts was investigated on *Callosobruchus maculatus* in the laboratory. Adult mortality, oviposition, adult emergence, long time storage ability and germinability were used as indices. The results obtained showed that all the different extracts were potent on *C. maculatus*. Ethanol extract was the most toxic because it recorded 100 % mortality within 96 hours of exposure. Aqueous extract was the least toxic among the extracts because it was only able to achieve 72.75 % mortality within 96 h. The extracts caused reduction in oviposition and adult emergence. Weevils in cowpea treated with ethanol and pet-ether extracts recorded the lowest number of eggs (12.25 and 12.45 respectively) which are not significantly ($p < 0.05$) different from the oviposition observed in methanol, acetone and pet-ether extracts. Adult emergence in ethanol and pet-ether extracts was totally suppressed while the aqueous extract recorded the highest adult emergence among the extracts used. Ethanol and pet-ether extracts totally prevented infestation and damage of cowpea seeds within the 90 days of storage. The percentage germination of all the treated seeds was generally high, ranging from 78.50 % to 95.25 %. The highest germination of 92.25 % was recorded in ethanol extract which is not significantly different from that of methanol extract. The results from this findings showed that extracts of *A. wilkesiana* were effective in protecting cowpea seeds against *C. maculatus.* This extract can be used as sustainable substitute to chemical insecticide in the control of *C. maculatus*.

Keywords: *Acalypha wilkesiana*, *Callosobruchus maculatus,* solvents, mortality, oviposition, adult emergence.

INTRODUCTION

One major cause of food shortage in the world is the problem of storage. The harvested produce is the net result of all the prior efforts of crop husbandry. These products have to be stored for some period of time before consumption [1]. Adequate grains supply all year round in most developing nations has been seriously hampered by contamination, attack by insects, rodents, deterioration in storage and adequate storage techniques [2]. In Nigeria, pulses occupy a prominent place in the nutrition of the populace. The edible grains are cheap source of protein [3]. Cowpea (*Vigna unguiculata* (L.) Walp is an important staple food and cash crop of significant economic importance in Nigeria and around the world [4]. It is used to control malnutrition in the rural area [5] because it is cheaper than other sources of protein, such as fish, meat and egg [6]. Cowpea seeds and leaves are consumed in fresh form as green vegetables in some African countries [7].

The production and storage of cowpea have been faced with many problems throughout West Africa. These are problems associated with diseases and the limited use of fertilizers and irrigation input [8] but the major constraints associated its storage is infestation by cowpea weevils, *Callosobruchus maculatus* [9-11]. The cowpea bruchid, *Callosobruchus maculatus* (Fabricius) is the major pest of stored cowpea in Nigeria [12]. *C. maculatus* is a field-to-store pest of cowpea. It is prone to infestation in the field and during the period of storage. The attack of cowpea initially begins in the field, and rapidly builds up during storage. Up to 100 % infestation of cowpea seeds can occur after three to six months storage [13]. This activity of the weevils can result into about 60 % weigh loss [14]. The damage caused by *C. maculatus* has been described by many researchers [15]. The infestation often results in weight loss, reduction in nutritive value, reduced commercial value, physical damage such as presence of exit holes and reduction in seed viability [16-18, 10].

In an attempt to control the storage insets of cowpea, synthetic insecticides are widely used [19]. Many problems have been faced as a result of the use of synthetic insecticides. Such problems as environmental pollution, ozone layer depletion potential, resistance of pest and affordability by poor-resource farmers. In Nigeria, inexperience and illiteracy have led to the abuse and misuse of these convectional insecticides and these have resulted to several repercussions, such as acute and chronic poisoning, sudden death, blindness, skin irritation and pest resurgence of man [20, 21]. The negative effects posed by the chemical insecticides have led to the need for cheap, effective, biodegradable organic pesticides [22]. In this scenario, the use of insecticidal botanicals stand out as a promising alternative for the control. This plants are usually affordable, biodegradable and ecologically friendly [23-25].

Acalypha wilkesiana is a medicinal plant, an evergreen shrub with antimicrobial properties [26] use for treatment of gastrointestinal disorders and fungal skin infection. The leaves are used as vegetables in management of hypertension [27]. The present research investigated the insecticidal potential of different leaf extracts of *Acalypha wilkesiana* on *C. maculatus* in stored cowpea.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh leaves of Acalypha wilkesiana were collected from the campus of Ekiti State State University, Ado-Ekiti, Nigeria. The leaves were air-dried for 15 days under the shade of a Flamboyant, *Delonix regia* tree at ambient temperature. Thereafter, the leaves were pulverized into fine powders using an electric Binatone blender (Model BLG400). The pulverized leaves were sieved with a 1.0 mm^2 mesh and stored in an air-tight plastic container for subsequent use.

Preparation of leaf extraction

The oil extraction of the pulverized leaves was done in a Soxhlet`s exractor with six differents solvents which include ethanol, methanol, acetone, pet-ether, n-hexane and distilled water at 60°C. The aqueous extract was obtained by soaking the powder in warm water at the temperature of 60°C to 70°C for 12 h after which the residue was filtered off using a muslin cloth. In order to concentrate the filtrate extracted, excess solvent was recovered from the different extracts by using a rotary evaporator. The resulting oil extracts were poured into labeled specimen bottles separately and kept in the refrigerator until needed.

Insect Culture

The cowpea bruchids, *Callosobruchus maculatus* used to establish the insect culture were obtained from naturally infested cowpea seeds from the food store of Ekiti Anglican Diocesan High School, Ado-Ekiti, Nigeria. The insects were raised under laboratory conditions (28°C ± 2°C and 70% relative humidity) on Ife brown cowpea seeds inside a transparent plastic container, covered with muslin cloth for six weeks. Freshly emerged *C. maculatus* adults (0 - 24 h old) were used for the experiments.

Insect mortality Bioassays

Fifty grams (50 g) of pristine Ife brown cowpea seeds variety was measured with weigh balance into each of the seven Kilner jars and 0.4 mL of each oil extract was measured and mixed with the cowpea seeds in the six jars while the seventh jar was not treated with extract (control). The grains and the extracts were thoroughly mixed together with a glass rod to enhance uniform coating of the seed. Each of the treated and untreated grains was replicated four times. Each treatment was air-dried for two h after which 20 (Twenty) newly emerged (0-24 hours) *C. maculatus* adults were introduced into each of the jars and covered with a muslin cloth, held in place with rubber bands for proper ventilation and to prevent the exit of the weevils. All treatments were arranged in a completely randomized design and the experiments were monitored for 96 h. The number of dead insects from each jar was counted and recorded at an interval of 24 h for 96. The insects were confirmed dead when they failed to respond after probing their abdomen with sharp pin.

Oviposition and adult emergence Bioassays

Twenty grams (20 g) of clean and pristine Ife brown cowpea seeds variety were measured into seven Petri dishes (5 cm diameter). Thereafter, 0.2 mL of each oil extract was mixed with the grains in six of the Petri dishes while the seventh Petri dish (control) was not treated with oil extracts. The grains and the extracts were thoroughly mixed together with a glass rod to enhance uniform coating of the grain. Each of the treated and untreated grains was replicated four times. Each treatment was air-dried for 2 h. Copulating pairs of newly emerged adult *Callosobruchus maculatus* were introduced into the Petri dishes. Each treatment and control was replicated four times and laid in a completely randomized design (CRD). The experiment was kept in the laboratory till after the death of the insects (seven days).

After seven days, the total number of eggs laid were counted and recorded. Thereafter, the experiment was kept for thirty days to allow for the emergence of adults weevils. The number of adults that emerged from each Petri dish was counted and recorded.

Assessment of damage

One hundred grams (100 g) of disinfested cowpea seeds were measured into transparent plastic containers and admixed with 0.4 mL of oil extracts (i.e ethanol, methanol, n-hexane, pet-ether, acetone, and water). They were air-dried for 2 h. Each of the treated and untreated cowpea seeds were replicated four times. After that, 20 copulating pairs of adult *C. maculatus* were introduced into each treatment. The untreated (control) cowpea seeds were similarly infested. The jars were covered with muslin cloth held in place with rubber bands. The experiment was left in the laboratory for 90 days. The extent of damage was assessed using seed emergent holes and weight loss as indices. This was done by randomly picking 200 samples of cowpea seeds from each replicates, after which number of grains with emergent holes were counted and recorded. Weight loss was calculated by subtracting the final weight of the seeds from the initial weight (50 g). Seed damage was also assessed using Weevil Perforation Index (WPI) as described by [28]. Weevil Perforation Index value exceeding 50 was regarded as enhancement of infestation by the weevils.

Effects of *Acalypha wilkesiana* leaf oil extracts on seed viability

Twenty grams (20 g) of pristine cowpea seeds were measured into Petri dish and 0.2 mL of each oil extract was added to each Petri dish and mixed with glass rod. They were left for 1 h to air dry and then covered with Petri plate. Each treatment was replicated four times. The experiment was left in the laboratory for ninety days. After 90 days 50 (fifty) seeds were selected at random and placed on moisten filter paper in Petri dishes (5 cm diameter). The seeds were treated with Apron plus to prevent moldiness. Fifty seeds were randomly selected from each treatment and the control after storage for 90 days. The seeds were treated with Apron plus to prevent moldiness and fungi growth. The seeds were germinated on moistened filter papers in Petri dishes. The germination process was monitored for seven day after which the number of germinated seeds in each petri dish were counted and recorded.

Data Analysis

The data obtained was subjected to one-way analysis of variance (ANOVA) at 0.05 significant levels, treatment means were separated by new Duncan`s Multiple Range Test.

RESULTS

Effects of *Acalypha wilkesiana* leaf oil extracts on mortality of *Callosobruchus maculatus*

Effects of leaf oil extracts of *A. wilkesiana* on mortality of adult *C. maculatus* is presented in Table 1. The results show that the effects of the treatment were solvent and period of exposure dependent. There was significant ($p < 0.05$) differences between the treatments at all period of application. Ethanol oil extract achieved 100 % mortality of *C. maculatus* within 96 hours of exposure and was significantly ($p < 0.005$) different from the mortality obtained in other extract used except for methanol extract which is not significantly different. Aqueous extract was the least toxic among the extracts because it achieved 72.75 % mortality by the 96th h of exposure while the untreated cowpea seeds did not record beetle mortality throughout the duration of the experiment.

Table 1: Cumulative mortality of adult *Callosobruchus maculatus* treated with different solvent extracts of *Acalypha wilkesiana* leaf.

Leaf extract	Percentage	Mortality (in H)	Post-treatment	
	24	48	72	96
Control	0.0 ± 0.00^c	0.0 ± 0.00^d	0.0 ± 0.00^d	0.0 ± 0.00^e
Ethanol	17.25 ± 1.24^a	38.75 ± 2.11^a	$78. \pm 3.44^a$	$100.\pm 0.00^a$
Methanol	18.50 ± 1.04^a	35.25 ± 1.22^b	76.45 ± 3.44^a	98.50 ± 3.58^a
n-hexane	$14.50\pm .1.25^b$	32.15 ± 1.28^b	68.25 ± 2.33^b	92.75 ± 4.33^b
Acetone	17.20 ± 1.11^a	28.75 ± 1.44^c	65.35 ± 3.12^b	88.25 ± 3.82^c
Pet-ether	15.25 ± 1.33^{ab}	34.15 ± 2.12^b	78.50 ± 3.44^a	90.25 ± 2.15^b
Aqueous	12.25 ± 0.77^b	28.25 ± 1.35^c	52.25 ± 1.33^c	72.75 ± 1.12^d

Each value is mean ± standard error of four replicates. Values followed by the same letter in the same column are not significantly ($p > 0.05$) different using New Duncan`s Multiple Range Test.

Effects of *Acalypha wilkesiana* leaf extracts on oviposition and adult emergence of *Callobruchus maculatus*

The effect of *A. wilkesiana* leaf extract on oviposition and adult emergence of *C. maculatus* is presented in Table 2. Under ethanol and pet-ether extracts treatment *C. maculatus* recorded the lowest number of eggs (12.25 and 12.45 respectively) which are not significantly ($p < 0.05$) different from oviposition in methanol, acetone and pet-ether extracts. There was no adult emergence recorded in ethanol and pet-ether extracts. Adult emergence in methanol and n-hexane extracts are not significantly ($p < 0.05$). The highest number of emergence was observed in the seeds treated with aqueous extract.

Table 2: Effects of *Acalypha wilkesiana* leaf oil extracts on oviposition and adult emergence of *Callobruchus maculatus*

Leaf extract	No of eggs laid (within 7 days	Percentage adult emergence (within 30 days)
Control	32.50 ± 2.40^{a}	78.20 ± 3.44^{a}
Ethanol	12.25 ± 1.50^{d}	0.0 ± 0.00^{d}
Methanol	13.75 ± 2.33^{d}	4.25 ± 0.43^{c}
n-hexane	16.50 ± 1.32^{c}	5.25 ± 0.58^{c}
Acetone	13.25 ± 1.45^{d}	6.20 ± 1.33^{c}
Pet-ether	12.45 ± 2.13^{d}	0.0 ± 0.00^{e}
Aqueous	19.50 ± 1.22^{b}	10.25 ± 1.55^{b}

Each value is mean ± standard error of four replicates. Values followed by the same letter in the same column are not significantly ($p > 0.05$) different using New Duncan`s Multiple Range Test.

Effects of *Acalypha wilkesiana* leaf extracts on seed damage

The effects of *A. wilkesiana* leaf extract on seed damage was presented in Table 3. All the plant oil extracts used caused reduction in infestation. Ethanol and pet-ether extracts completely prevented infestation and damage of the treated cowpea seeds. There was no seed damage, no weight and weevil perforation index was zero. Weevil perforation index in methanol (14.39), n-hexane (9.25), acetone (12.20) and aqueous (14.82) oil extracts were regarded as positive protectant ability of the extract. All the extract used showed positive protectant ability. There was 69.13% seed damage in the control experiment (untreated seeds) as a result of the feeding activity of the larvae on the cowpea seeds.

Table 3: Effects of *Acalypha wilkesiana* leaf extracts on seed damage after storing for ninety days

0.2 mL leaf Extract	No of seeds	No of damaged Seeds	Percentage seed damaged	Weight Loss	Weevil perforation Index (WPI)
Control	200.00	138.25 ± 5.22	69.13 ± 3.23^{a}	28.23 ±2.25^{a}	58.30 ± 3.53^{a}
Ethanol	200.00	0.00 ± 0.00	0.00 ±0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
Methanol	200.00	8.75 ± 1.38	4.33 ± 0.53^{c}	6.25 ± 1.23^{c}	14.39 ± 1.38^{b}
n-hexane	200.00	6.50 ± 0.42	3.25 ± 0.63^{c}	4. 12 ± 0.77d	9.25 ± 2.11^{d}
Acetone	200.00	10.10 ± 1.11	5.10 ± 1.12^{bc}	6.11 ± 1.14^{c}	12.20 ± 1.44^{c}
Pet-ether	200.00	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
Aqueous	200.00	18.25 ± 2.21	9.13 ± 1.44^{b}	7.14 ± 1.13^{b}	14.82 ± 2.12^{b}

Each value is mean ± standard error of four replicates. Values followed by the same letter in the same column are not significantly ($p > 0.05$) different using New Duncan`s Multiple Range Test.

Table 4: Effects of *Acalypha wilkesiana* leaf oil extracts on germination of cowpea seeds after storing for ninety days

A. Wilkesiana leaf extract	Percentage germination
Control	31.25 ± 2.42^{c}
Ethanol	95.25 ± 3.35^{a}
Methanol	88.75 ± 4.45^{b}
n-hexane	90.20 ± 3.88^{ab}
Acetone	86.33 ± 4.33^{b}
Pet-ether	92.50 ± 2.84^{a}
Aqueous	78.50 ± 1.02^{d}

Each value is mean ± standard error of four replicates. Values followed by the same letter in the same column are not significantly ($p > 0.05$)
different using New Duncan`s Multiple Range Test.

Effects of *Acalypha wilkesiana* leaf oil extracts on germination of cowpea seeds after storing for ninety days

Effect of *A. wilkesiana* leaf oil extracts on germination of cowpea seeds is shown in Table 4. The percentage germination of all the treated seeds was generally high, ranging from 78.50 % to 95.25 %. The untreated seeds recorded the lowest germination percentage of 31.25.

DISCUSSION

Food security has been hampered by the activities of stored product pest in the world as a whole. In order to eradicate the use of synthetic chemical insecticides, as a result of their adverse effects, the use of botanicals in various forms is gradually taking over [29]. The use of indigenous plant product and other available materials to protect cereal and legumes have been reported by many workers [22, 30-31, 10]. Some researchers have discovered the use of plant oil as effective grain protectants [32]. The results obtained from this research work indicate that extracts of *Acalypha wilkesiana* leaf oil extracts are useful bio-insecticides for the control of cowpea weevils, *Callosbruchus maculatus*. All the different solvent extracts tested were effective in causing beetle mortality, reducing seed damage and depressing oviposition and adult emergence of *C. maculatus.* The result on beetle mortality in this finding is in conformity with the result obtained by [33] who recorded 100 % mortality within 96 hours of application with oil extracts of *Acalypha wilkesiana.* The presence of some phytochemical compounds in plants are known to play important roles in the bioactivity of the plant. The bioactivity of the plants lies in these phytochemical compounds, such as alkaloid, tannins, saponins flavonoid, resins and glycosides which produce a definite physiological action on the body [34]. These compounds have been reported to demonstrate biochemical functions against the insects and effected insecticidal, repellent, anti-molting, nematicidal and antimicrobial activities against stored products [35, 36].

The result of *Acalypha wilkesiana* oil extracts in suppressing oviposition, adult emergence and causing reduction in infestation has been established [37]. Plant oil has suffocating effects on insect pests by preventing the easy passage of atmospheric air onto them, due to the blockage of the spiracles by the oil. The egg mortality and failure to hatch on the seeds treated with the oils has been probably attributed to the toxic component and to the physical property, leading to changes in surface tension and oxygen tension within the egg [38].

The oil of *A. wilkesiana* suppressed infestation and consequent seed damage. The result on seed damage is in conformity with the results obtained by [10] who recorded drastic reduction in seed damage with cashew kernel oil extracts. The reduction in seed damage and weight loss observed in the study may be attributed to reduced oviposition and consequent reduction in the number of eggs that hatched, thereby reducing feeding by the larvae which subsequently lower the percentage seeds damage and weight loss [39].

CONCLUSION

The results presented in this study have shown that the different solvent used to extract *Acalypha wilkesiana* have great potentials as biopesticides, caused adult mortality of *Callosobruchus maculatus*. The potency of the oil extracts caused adult mortality, suppressed oviposition and adult emergence, prevent infestation and seed damage. Ethanol extract was the most toxic while Aqueous extract was the least toxic among the extract. Thus, having potential to provide suitable alternatives for pest control on cowpea seeds. However, further studies are necessary to elucidate the effects of various concentrations and optimum spraying conditions for *Acalypha wilkesiana* extracts to discover the most effective combination of concentrations and spraying that would offer adequate protection to the crop.

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Declaration of Interest: Authors declare no competing interest.

REFERENCES

1. Adesuyi, S. A. (1997). Relative Resistance of some Varieties of maize to attack by *Sitophilus zeamais* (Mots.). *Rep. Nigeria Stored Products Institutes*, 1976/1977 Technical Report, 79-82.
2. Odeyemi, O. O., & Daramola, A. M. (2000). Storage practice in the tropics: Food storage and pest problem. 1st edition, Nigeria: Dave Collins, 2: 235pp.
3. Ofuya, T. I. (2003). Bean Insects and man, an inaugural lecture series 35, The Federal University of Technology, Akure.
4. Musa, A., & Olaniran, J. O. (2015). Studies on the efficacy of *Jatropha curcas* L. seed oil on adult mortality and emergence of seed beetle, *Callosobruchus maculatus* (F.) (Coleopters: Chrysomelidae) in cowpea. *International Journal of Phytofuels and Allied Sciences*, 4(1), 1-12.
5. Ileke, D. K., & Olotuah, O. F. (2012). Bioactivity of *Anacardium occidentale* (F.) and Allium sativum (L.) powders and oil extracts against cowpea bruchid, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae). *International Journal of Biology*, 4, 96-103.
6. Philips, R. D., Mcwatters, K. H., Chnnan, M. S., Hung, Y. C., Beuchat, L. R., Sefa-Dedeh, S., Sakyi-Dawson, E., Enwere, J. (2003). Utilization of cowpeas for human food. *Field Crops Research*, 82, 193-213.
7. Ghaly, A. E., & Alkoaikk, F. N. (2010). Extraction of Protein from Commom Plant Leaves for Use as Human Food. *American Journal of Applied Science*, 7(3), 323-334.
8. Brisbe, E. A., Adugbo, S. E., Ekanem, U., Brisibe, F., & Figueira, G. M. (2011). Controlling Bruchid Pest of Stored Cowpea Seeds with Dried Leaves of *Artemisia annua* and Two Other Common Botanicals. *African Journal of Biotechnology*, 10(47): 9586-9592.
9. Musa, A. K., Oyerinde, A. A., & Owolabi, F. O. (2009). Evaluation of efficacy of mixed leaf powders of *Vernonia amygdalina* L. and *Ocimum gratissimum* Del. against *Callosobruchus maculatus* (F.) (Coleoptera; Bruchidae). *Academic Journal of Entomology*, 2(2), 85-87.
10. Adedire, C. O., Akinkurolere. R. O., & Obembe, O. M. (2011). Efficacy of cashew kernel extracts in the control of maize weevil, *Sitophilus zeamais*

(Coleptera: Curculionidae). *Archive of Phytopathology and plant Protection,* DOl: 10. 1080/03235408.2011.598259

11. Ojo, D. O., & Ogunleye, R. F. (2013). Comparative effectiveness of the powders of some underutilized botanicals for the control of Callosobruchus maculatus (Coleoptera: Bruchidae). *Journal of plant diseases and protection*, *120*, 227-232.
12. Ojo, D. O., & Ogunleye, R. F. (2013). Comparative effectiveness of the powders of some underutilized botanicals for the control of Callosobruchus maculatus (Coleoptera: Bruchidae). *Journal of plant diseases and protection*, *120*, 227-232.
13. Maina, Y. T. (2011). Effects of different level of infestation and storage duration on the development of *Callosobruchus maculatus* (Fab.) in stored cowpea *Vigna Unguiculata* (L.) Walpers. *Production Agriculture and Technology*, 7(1), 49-54.
14. Umeozo, O. C. (2005). Effect of the infection of *Callosobruchus maculatus* (Fab.) on the weight loss of stored cowpea (*Vigna unguiculata* (L.) Walps.). *Journal of Applied Science and Environmental Management*, 9(1), 169-172.
15. CABI. (2004). *Callosobruchus maculatus* (Cowpea weevil). Available at www.cabi.org/isc/datasheet/10987.
16. Ofuya, T. I. (2001). Biology, ecology and control of insect pest of stored legumes in Nigeria In: Pests of Stored cereals and pulses In Nigeria, biology, ecology and control. Nigeria Dove Collins Publications, 2001.
17. Adedire, C. O., & Akinneye, J. O. (2004). Biological activity of tree marigold, *Tithonia diversifolia* on cowpea bruchid, *Callosobruchus maculatus* (Coleoptera: Bruchdae), *Annal of Applied Biology*, 144, 185-189
18. Ugwu, J. A., Kareem, K. T., & Aluko J. O. (2021). Insecticidal Activities of Aqueous Extracts of Five Nigerian Aromatic Spices against Garden Eggplant Defoliators and Fruit Borer Leucinodes orbonalis Guenee: Lepidoptera: Crambidae; *Tanzania Journal of Science* 47(3), 1322-1329.
19. Lowenberg-DeBoer, J., & Ibro, G. (2018). A Study of the Cowpea value Chain in Kano State, Nigeria. From A Pro-Poor and Gender Perspective. A paper commissioned by the GATE Project 56pp.
20. Akunne, C. E., & Okonkwo, N. J. (2006). Pesticides: Their abuse and misuse in our Environments. *Book of proceedings of the 3rd Annual National Conference of the Society for Occupational Safety and Environmental Heaith (SOSEH), Awka*, 130-132 pp.
21. Omoleye, A. A. (2008). Fundamental of Insect Management. Corporate Publishers, Lagos, 223pp.
22. Rise, L. O. (2000). Efficiency of Certain Plant Extracts Against Plant Pest. *Journal of Plant Science*, 168, 30-45.
23. Magloire, N. S. (2005). The Genetics, Morphological and Physiological Evaluation of African Cowpea Genotype. Thesis presented in accordance with the requirements for the degree *Magister Scientiae Agriculturae* in the Faculty of Natural and Agricultural Sciences, Department of Plant Science (Plant Breeding) at the University of Free State, South Africa, 131p.
24. Ekeh, F. N., Oleru, K. I., Ivoke, N., Nwani, C. D., & Eyo, J. E. (2013). Effect of *Citrus sinensis* Peel Oil on the Oviposition and Development of Cowpea Beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in Some Legume Grains. *Pakistan Journal of Zoology*, 45(4), 967-974.
25. Campus, A. C. T., Radunz, L. L., Radunz, A. L., ossil, A. J., Dionello,, R. G., & Ekker, S. L. (2014). Insecticidal and repellent activity of the essential oil from carqueja doce on bean weevil. Revista Brasileira de Engenharia Agricola e Ambiental, 18(8): 861-865.
26. Ogundaini, A. O. (2005). From green into Medicine: Taking a lead from nature. An Inaugural Lecture at Oduduwa Hall, Obafemi Awolowo University, Ile-Ife, Nigeria, 12-15pp.
27. Ikewuchi, J. C., Anyadiegwu, A., Ugono, E. Y., & Okungbowa, S. O. (2008). Effects of *Acalypha wilkesiana* Muell. Arg. On plasma sodium and potassium concentration of normal rabbit. *Pakistan Journal of Nutrition*, 7(1), 130-132.
28. Fatope, M. O., Nuhu, A. M., Mann, A., & Takeda, Y. (1995). Cowpea weevil bioassay: a simple prescreen for plants with grain protectant effects, *Int J Pest Management*, 41, 44-86.
29. Adesina, J. M. (2013). Insecticidal potential of *Mormordica charantia* (L.) leaves powder against maize weevil, *Sitophilus zeamais* (Mots) (Coleoptera: Curculionidae) infestation. *International Journal of Biosciences*, 3, 28-38.
30. Katembwe, M. (2003). Natural Control of pest and diseases. Kumbaba Publishers, Cameroun 432pp
31. Ewete, T. I. (2007). Use of plant ash for reducing *Callosobruchus maculatus* damage on cowpea seedlings. *Journal of Agricultural Science*, 107, 467-470.
32. Oparaeke, A. M., & Bunmi, O. J. (2006). Insecticidal potential of cashew, *Anacardium occidentale* L. for the control of beetle, *Callosobruchus subinnotatus* (PiC.) (Coleoptera: Bruchidae) on babarra groundnut, Voandzeia subtrranea (L.) Verde. *Archive Phytopathology and plant Protection*, 39, 247-251.
33. Omage, K., & Marchal A. A. (2014): Medicinal Potential of Acalypha wilkesiana Leaves *Advances in Research*, 2(11), 655-665.
34. Mobolanle, D. A., Yeye E. O. E., & Ewete, F. K. (2015). Qualitative Phytochemical screening of *Acalypha fimbriata*, and its methanol extract as protectant against *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored maize. *Journal of Natural Science Research*, 5(6), 136-141.
35. Al-Rajhy, D. H., Alahmed, D. M., Hussein, H. I., & Kheir, S. M. (2003). Acaricidal effects of cardiac

glycosides, azadirachtin, and neem oil against the camel tick, *Hyalomma dromedaril* (Acari: Ixodidae). *Pest Management Science*, 59(11), 12250-1254.

36. Udebuani, A. C., Abara, P. C., Obasi, K. O., & Okuh, S. U. (2015). Studies on the insecticidal properties of *Chromolaena odorata* (Asteraceae) against adult stage of *Periplaneta americana. Journal of Entomology and Zoology Studies*, 3(1), 318-321.
37. Dauda, Z., Maina, Y. T., & Richard, B. I. (2012a). Insecticidal activities of garlic (*Alium sativum* (L.) oil on *Callosobruchus maculatus* (F.) In post-harvest cowpea (*Vigna unguiculata* (L.) Walp.). *Journal of Biology*, *Agriculture and healthcare*, 2, 28-35.
38. Singh, S. R., & Tailor, J. A. (1978). Pest of grain legumes and their control in Nigeria. In: Singh, S. R., Van Emden, I. I. F., Tailor, T. A. eds. *Pest of grains legumes, ecology and control*, London, U. K., Academic press, 329-336 pp.
39. Alabi, O. Y., & Adewole, M. M. (2017). Essential oil extract from Moringa olifera roots as cowpea seed protectant against cowpea beetle. *Africa Journal of Crop Science*, 25(1), 71-81.