Chemistry Research Journal, 2023, 8(6):39-46
Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Pesticidal Effect of Vernonia Amygdalina against Acanthoscelides Obtectus on Stored Common Bean (Phaseolus vulgaris L.) Grains

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Abstract

The usage of synthetic pesticides posed adverse residual effects on human and the environment. The research revealed the phytochemicals and bioactivities of leaf powder and extracts of *Vernonia amygdalina* against the bean weevil, *Acanthoscelides obtectus*. The leaves were sorted, rinsed, dried, pulverized and subjected to maceration with hexane, ethyl acetate and methanol. The leaf powder contained all phytochemicals tested, however, tannins, saponins and steroids, oxalates were absent in the hexane, ethyl acetate and methanol extracts respectively. The mortality count of the weevil was studied by exposing the weevil to 10 g of *V. amygdalina* treated bean grains at 0.1, 0.5, 0.9, 1.3 g dosage for five days under laboratory conditions. The results indicated that at minimum dosage of 0.5 g, hexane and methanol extracts provided effective mortality (100 %) of the weevils for at least 48 h. No grain weight loss and damage with the hexane and methanol extracts at dosage higher than 0.5 g. Therefore, the hexane and methanol *V. amygdalina* extracts could be used as biopesticides at a minimum dosage of 0.5 g per 10 g. This will reduce the usage of synthetic pesticides, thereby preventing their adverse residual effects on human and the environment.

Keywords: Pesticidal, *Vernonia amygdalina,* stored bean grains, mortality, *Acanthoscelides obtectus*, extract

1. Introduction

Beans being a major source of protein is grown and consumed in most developed and developing countries. However, a large amount of stored bean grains is often lost by invasion of pests after harvest. In Nigeria, the *Acanthoscelides obtectus* (common bean weevil) is one of the most common bean pest, as it grows very rapidly on untreated grains, causing damage and gross depreciation in nutritional value of infested grains [1]. Report has it that on the average, as high as 40 - 45 % losses occur in agricultural production before and after harvesting caused by attack of variety of pests including insects, nematodes, virus and bacteria, induced diseases and competition by weeds [2]. In addition, about one third of global agricultural production valued at several billion dollars is destroyed annually by over 20,000 species of insects in field and storage [3]. Weevil larvae bore into seed pods either in the field or in storage, and continuously feed inside until adults to infest more seeds. This infestation can spread rapidly and lead to significant loss of nutritional value and low yield. Depending on the storage period and storage conditions, bruchid can infest the entire quantity of stored beans within a few months, thereby reducing the weight, quality, viability of bean seed, market values, and consumption interest [1]. A simple way developing countries obtain better crop yields and increase agriculture production is the use of crop protection chemicals. Consequently,cheap chemicals such as dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) are in use [4].

It is no reservation that huge number of bean weevil can be reduced considerably by chemical pesticides. Usually, synthetic pesticides are used to reduce post-harvest losses. These pesticides prevent, destroy, repel, sterilize, or mitigate pests. Unfortunately, the use of synthetic pesticides to prevent insect infestations contaminate both food and environment, yet currently popular due to ease of use and cost-effectiveness [5,6]. The organochlorine, organophosphate, carbamate, and pyrethroid insecticides are of a particular concern because of their toxicity and persistence in the environment as they build up along the food chain, in soil and water bodies. Organochlorine residues cause breast cancer, polychlorinated biphenyls (PCBs) lead to reduced sperm count and male sterility [4,7,8]. These synthetic pesticides fat-soluble which readily accumulate in fat tissues of animals. The compounds have higher atomic weight of chlorine which is typically denser than water compared to hydrogen chlorinated organic compounds. Pesticides like DDT, aldrin, Eldrin, especially, lindane have long persistence and highly toxic, yet are the most prevalent pesticides in the environment, and are still in use in most parts of the world including Nigeria [8,9] reported grain marchants in Mubi grain market of Adamawa State, Nigeria mostly use dichlorvos, permethrin and aluminium phosphate insecticides. Many traders used crude methods like mere observation for spot traces of applied chemicals and smelling to conclude the grains to be fit for re-sale and safe for consumption within a minimum of one month, with no regard for the dosages applied and effective biodegradability. Other pesticides with brand names such as DDForce, phostoxin, jule, Rambo are used solely or in combination for grain treatment against pests [10].

The incessant indiscriminate use of these chemicals cause harmful side effects on human as some cause sterility, have impending cancer-causing effects, induce oxidative stress, affect the nervous system, leading to memory loss and may cause death by acute or protracted poisoning [2]. The chemical components of these pesticides are also responsible for development of pesticide resistance in insects, and also destroy beneficial parasites, predators and pollinators that are nature components of ecosystem and environmental pollution [3]. Human population is exposed to these chemicals primarily through the consumption of pesticide contaminated farm produce, leading to long-term health hazards.

The use of natural insecticides has become an alternative for preventing the excessive accumulation of toxic residues in human and the environment. Plant insecticides provide great potential benefits as they are harmless to both farmers and consumers, and also environmentally safe. Botanical insecticides have short persistence and little bioaccumulation in the environment due to rapid degradation. Their diversity and redundancy of phytochemicals in extracts are additional advantage. The presence of numerous analogues of one compound, is known to increase the efficacy of extract through analogue synergy. This reduces the rate of metabolism of the compounds by insects and prevents pesticide resistance. Therefore, over the past years, there has been a growing interest in plants as a source of pesticides that are safer for human health and the environment. Such products are easy to obtain at low cost. Botanically derived insecticides from plants like Pyrethrum, nicotine and rotenone are in use [8]. Therefore, when incorporated into integrated pest management programs, botanical pesticides can greatly decrease the use of conventional pesticides. Better still, they can be used in rotation or in combination with other insecticides. This will possibly decline the overall quantity of conventional pesticides used and also mitigate or delay the development of resistance by pests. Inspite of the potentials, advantages and effectiveness of biopesticides, they are still grossly underutilized in Nigeria, and in most part of the world. This has left much to be desired of and exploited in order to achieve food safety and sustainability.

A number of plants have been evaluated for pesticidal properties against various insects. This pesticidal potential is due to the phytochemical constituents such as alkaloids, terpenoids, steroids, phenols, saponins, tannins, flavonoids amongst others, which make them useful as alternatives to synthetic insecticides. Plant derived phytochemicals can act as larvicide, insect growth regulators, and repellent. It was reported that when plant's phytochemicals are mixed with stored-grains, oviposition rate become reduced, offspring production is suppressed and adult insects suffer from toxicity effect. Consequently, this result in obvious low infestation and yield loss. It has been reported that essential extracts of leaf of some plants demonstrated high larvicidal and insecticidal activity against insects [7,11,12,13,14,15]. However, several researchers had unveiled the potential of diverse botanicals against weevil on maize, bambara, cowpea, but very few studies on the effect of *Vernonia amygdalina* extracts on *Acanthoscelides obtecus* which attack common bean grain are available. Thus, this research was aimed at evaluating the pesticidal activities and of *Vernonia amygdalina* which is locally available, for the control of bean weevils (*Acanthoscelides obtecus*) on stored bean grains.

A B C

Figure 1: (A) Bean weevil (Acanthoscelides obtecus), (B) Infected bean grains, (C) Bitter leaf (Vernonia amygdalina)

2. Materials and Methods

Sample Collection

Fresh leaves of *Vernonia amygdalina* and clean, uninfected, undamaged bean seeds were purchased from the Wurukum Market, Makurdi, Benue State-Nigeria. Adult bean weevils (*Acanthoscelides obtectus*) were sourced from infected bean seeds, from stored beans bought from the Ugbokolo market, in Okpokwu Local Government area of Benue State-Nigeria.

Preparation and Extraction of Bitter Leaf

The *Vernonia amygdalina* leaves were separated from the stem, rinsed with distilled water and air-dried under for six weeks. It was then pulverized to powder with the aid of a mortar and pestle, and stored in clean dried screw-capped glass container. 1,300 g of *V. amygdalina* leaf powder was macerated with 2,350 mL of hexane, ethyl acetate and methanol at room temperature for 48 h with intermittent shaking. The filtrates were concentrated using a rotary evaporator at 45 ^oC, and kept in fume hood for the solvents to evaporate leaving the extracts (*Vernonia amygdalina* hexane extract, VHE; *Vernonia amygdalina* ethyl acetate extract, VEE; and *Vernonia amygdalina* methanol extract, VME). The yield of extracts were calculated using the equation as given [11].

$$\%\ \text{Extract yield} = \frac{\text{Mass of extract obtained}}{\text{Mass of sample used}} \times 100$$

Phytochemical Screening

Phytochemical tests were carried out on the VLP, VHE, VEE and VME for the qualitative determination of phytochemical constituent as described [15,16].

Test for Alkaloids

About 0.5 g of sample was diluted with 10 mL of acid alcohol, boiled and filtered. 2 mL of dilute ammonia was added to 5 mL of the filtrate. 5 mL of chloroform was then added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendoff's reagent) was regarded as positive for the presence of alkaloids.

Test for Cardiac glycosides

About 0.5 g of sample diluted to 5 mL in water was added 2 mL of glacial acetic acid containing one drop of ferric chloride solution. The mixture was carefully poured on the surface of 1 mL concentrated sulphuric acid. A reddish-brown ring at the interface of the layer indicate the presence of glycosides.

Test for Flavonoids

About 0.5 g of sample was dissolved in sodium hydroxide and HCl was added. An intense yellow colouration observed which turned colourless after the addition of a few drops of HCl, indicate the presence of flavonoid.

Test for Oxalate

A few drops of glacial acetic acid was added to 3 mL portion of sample. A greenish-black colouration indicate the presence of oxalates.

Test for Phenols

About 0.5 g of sample was treated with 5 % aqueous ferric chloride. A deep-blue or black colour indicate the presence of phenols.

Test for Saponins

About 0.5 g of sample was added to 5 mL of distilled water in a test tube. The solution was shaken vigorously. The formation of a stable persistent foam (froth) indicate the presence of saponins.

Test for Steroids

About 2 mL of acetic anhydride and 2 mL sulphuric acid was added to 0.5 g of sample. The colour change from violet to blue or green indicate the presence of steroids.

Test for Tannins

About 0.5 g of sample was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride was added. A brownish-green or a blue-black colouration indicate the presence of tannins.

Test for Terpenoids

About 2mL of chloroform was added to 0.5 g of sample. Concentrated H_2SO_4 (3 mL) was carefully added. A reddish-brown colouration at the interface indicate the presence of terpenoids.

Bioactivity

The bioactivity was carried out following the method described [7]. About 0.1, 0.5, 0.9 and 1.3 g each of *V. amygdalina* extract was weighed and added to 10 g of undamaged and uninfected bean grains in separate glass beakers. The contents of the beakers were gently shaken to ensure thorough mixing. Twenty (20) adult bean weevils were then introduced into the beakers, covered with calico cloth held tight with rubber band. Another twenty adult bean weevils were introduced into a beaker containing 10 g untreated, undamaged and uninfected bean grains, which served as control. These treatments were carried out in triplicate. The mortality of the weevils was assessed after 24 h interval for five days. Weevils were considered dead when there was no response after been probed with sharp object. On day six, all insects, both dead and alive were removed from each beaker. The percentage mortality of weevil, percentage grain weight loss and percentage grain damage were determined using the equations below.

$$\%\text{ mortality} = \frac{\text{Number of dead insect}}{\text{Number of initial insect}} \times 100$$

$$\%\text{ grain weight loss} = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100$$

$$\%\text{ grain damage} = \frac{\text{Number of perforated grains}}{\text{Total number of grains}} \times 100$$

3. Results and Discussion

Table 1: Yield of extracts

Extract	Yield (g)	Percent (%)
Hexane	29.5	2.3
Ethyl acetate	115.7	8.9
Methanol	125.2	9.6

The yield of the various extract increased with increasing polarity of the organic solvent used as shown in Table 1.

Table 2: Phytochemical composition *V. amygdalina* extracts

Phytochemical	VLP	VHE	VEE	VME
Alkaloids	+	+	+	+
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Oxalates	+	+	+	-
Phenols	+	+	+	+
Saponins	+	+	-	+
Steroids	+	+	-	+
Tannins	+	-	+	+
Terpenoids	+	+	+	+

Key: + = Present, - = Absent;
VLP = *V. amygdalina* leaf powder, VHE = *V. amygdalina* hexane extract,
VEE = *V. amygdalina* ethyl acetate extract, VME = *V. amygdalina* methanol extract

Table 2 indicated all phytochemicals tested were present in the VLP while tannins, saponins and steroids, oxalates were absent in the VHE, VEE and VME respectively.

Table 3: Mean mortality count at various dose of the *V. amygdalina* leaf powder and extracts

Plant sample	Mass (g/10 g)	Exposure duration (h)				
		24	48	72	96	120
	0.1	7.00±1.65	12.00±1.00	16.00±1.00	18.33±1.16	19.67±0.58
VLP	0.5	7.33±0.58	12.33±0.58	16.00±1.00	18.00±0.00	19.67±0.58
	0.9	7.67±1.08	12.33±1.53	15.33±1.53	18.33±1.16	19.67±0.58
	1.3	8.33±1.08	14.46±1.03	18.00±1.73	19.00±1.53	19.67±0.58
	0.1	14.33±1.15	18.67±0.58	20.00±0.00	20.00±0.00	20.00±0.00
	0.5	15.33±1.53	19.67±1.00	20.00±0.00	20.00±0.00	20.00±0.00
VHE	0.9	15.67±1.15	19.33±0.58	20.00±0.00	20.00±0.00	20.00±0.00
	1.3	16.33±0.58	19.67±0.58	20.00±0.00	20.00±0.00	20.00±0.00
	0.1	3.00±1.00	6.00±1.00	10.00±1.00	14.70±0.58	17.30±0.58
	0.5	5.00±1.00	9.00±1.00	14.00±1.00	18.00±1.00	20.00±0.00
VEE	0.9	5.00±1.00	10.30±1.08	15.30±0.58	20.00±0.00	20.00±0.00
	1.3	6.00±1.00	13.00±1.00	18.70±1.53	20.00±0.00	20.00±0.00
	0.1	15.33±1.15	17.33±1.15	20.00±0.00	20.00±0.00	20.00±0.00
	0.5	16.33±1.15	18.33±1.15	20.00±0.00	20.00±0.00	20.00±0.00
VME	0.9	16.67±1.53	19.33±0.58	20.00±0.00	20.00±0.00	20.00±0.00
	1.3	18.67±0.57	19.67±0.58	20.00±0.00	20.00±0.00	20.00±0.00
Control	0.0	0	0	0	0	0

Values are replicate of three ± standard deviation.
VLP = *V. amygdalina* leaf powder, VHE = *V. amygdalina* hexane extract,
VEE = *V. amygdalina* ethyl acetate extract, VME = *V. amygdalina* methanol extract

Table 4: Percentage mortality at various dose of the *V. amygdalina* leaf powder and extracts

Plant sample	Mass (g/10 g)	Exposure duration (h)				
		24	48	72	96	120
	0.1	35	60	80	92	98
VLP	0.5	37	62	77	90	98
	0.9	38	62	90	92	98
	1.3	24	73	90	95	98
	0.1	72	93	100	100	100
	0.5	77	95	100	100	100
VHE	0.9	78	95	100	100	100
	1.3	82	98	100	100	100
	0.1	15	30	50	73	87
	0.5	25	45	70	90	100
VEE	0.9	25	45	70	90	100
	1.3	30	65	93	100	100
	0.1	72	93	100	100	100
	0.5	77	93	100	100	100
VME	0.9	78	95	100	100	100
	1.3	82	98	100	100	100
Control	0.0	0	0	0	0	0

Values are replicate of three ± standard deviation.
VLP = *V. amygdalina* leaf powder, VHE = *V. amygdalina* hexane extract,
VEE = *V. amygdalina* ethyl acetate extract, VME = *V. amygdalina* methanol extract

Effect of *V. amygdalina* on Mortality Count of Bean Weevil

Generally, as shown in Tables 3 and 4, the mortality count of *V. amygdalina* against the bean weevil increased with time of exposure and treatment concentration. There was an increase in the percentage mortality of *A. obtectus* as the concentration of the VLP, VHE, VEE and VME increased along 0.1 g, 0.5 g, 0.9 g, and 1.3 g respectively. The percentage mortality ranged between 35 – 98 % for VLP, 72 – 100 % for VHE, 15 – 100 % for VEE and 72 – 100 % for VME. Deaths of the weevils beyond 50 % were recorded at all concentrations within the first 24 h for VHE and VME. These two extracts showed 100 % mortality at 0.1 g concentration and beyond for the day 3 of the bioactivity test. Although VEE showed a complete mortality at 0.9 g and 96 h, it presents a better and higher mortality efficacy than the VLP. Researchers reported a similar trend mostly at higher amount of treatment samples used [7,11,12,13,15]. The obtained results revealed the VHE and VME are comparatively effective in terms of percentage mortality against the weevil. Other studies have affirmed that *V. amygdalina* contain biologically active and toxic compounds to common bean pests. The most well-known active constituents that make it efficient as pesticide are sesqiterpene lactones: vernodalin (**1**), vernodalol (**2**), and 11,13-dihydrovernodalin (**3**). These compounds act as insecticides by deterring insect feeding (insect feeding deterrent) [14,17].

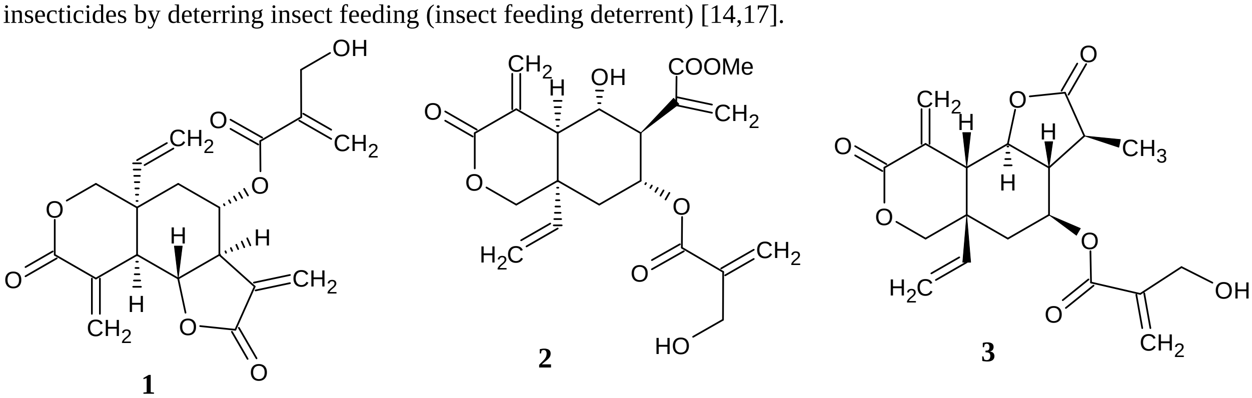

Figure 2: Structure of Vernodalin (1), Vernodalol (2), and 11,13-dihydrovernodalin

Table 5: Percentage grain weight loss and damage (g/10g)

Sample	Grain weight loss (%)				Control	Grain damage (%)				Control
	0.1g	0.5g	0.9g	1.3g		0.1g	0.5g	0.9g	1.3g	
					40					49
VLP	19.0	13.0	9.0	6.0		27.0	25.0	16.0	14.0	
VHE	0.3	0.1	0.0	0.0		0.4	0.2	0.0	0.0	
VEE	2.8	1.9	0.9	0.0		4.0	3.7	1.6	0.0	
VME	0.3	0.1	0.0	0.0		0.4	0.2	0.0	0.0	

VLP = *V. amygdalina* leaf powder, VHE = *V. amygdalina* hexane extract, VEE = *V. amygdalina* ethyl acetate extract, VME = *V. amygdalina* methanol extract

Percentage Grain weight loss and damage

The grain weight loss and damage assessment of the beans treated with *V. amygdalina* generally decreased with increase in the amount of treatment sample used (Table 5). The bean grains (10 g) treated with 0.9 and 1.3 g of VHE and VME recorded no (0 %) weight loss and damage. This implied effective preservation of the bean grains against the weevil. Meanwhile, the percentage weight losses and damage were highest with VLP at all concentrations.

4. Conclusion

This research, under laboratory conditions has derived *V. amygdalina* based pesticides against *A. obtectus.* It revealed that VHE and VME gave comparable mortality counts against the *A. obtectus* at all dosage considered and functioned at best (100 %) after two (2) days of treatment. Similarly, the VEE mortality counts were also comparable, but less effective against the weevils compared to the VHE and VME at least for the first three days. The leaf powder (VLP) showed the least mortality count, therefore, the least effective against *A. obtectus* at all treatment dosage. Therefore, hexane and methanol *V. amygdalina* based extracts could be used as pesticides at a minimum dose of 0.9 g/ 10 g bean grains to prevent *A. obtectus* invasion during storage. This will reduce the use of synthetic pesticide, eliminate the risks of toxic residues in foods and ensure the continued availability of insect-free bean grains.

Acknowledgements

We sincerely appreciate the technologists at the Chemistry Laboratory, Benue State University, Makurdi - Nigeria for providing their facility and services for this research.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1]. Kamfwa, K., Beaver, J.S., Cinchy, K.A., and Kelly, J.D. (2018). QTL mapping of resistance to Bean Weevil in common Bean. Crop science, 58(6):1-9.

[2]. Pavela, R. (2016). History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects–a review. Plant Protection Science, 52(4):229-241.

[3]. Joseph, B. and Sujatha, S. (2012). Insight of botanical based biopesticides against economically important pest. International Journal of Pharmacy & Life Sciences, 3(11), 2138-2148.

[4]. Nnamonu, L.A. and Onekutu, A. (2015). Green Pesticides in Nigeria: An Overview. Journal of Biology, Agriculture and Healthcare, 5(9):48-62.

[5]. Tinkeu, L.S.N., Madou, C., Djakissam, W., Goudoum, A. and Ndjouenkeu, R. (2016). Post-harvest storage systems and insect pests occuring on Bambara groundnuts (Vigna subterranean (L.) Verde) in the Sudano-Guinean savannah of Cameroon. Journal of Entomology Zoology Studies, 4(2), 167-173.

[6]. Hikal,W.M., Baeshen, R.S., and Said-Al Ahl, H.A.H. (2017). Botanical insecticide as simple extractives for pest control. Cogent Biology, 3 (1):1-16.

[7]. Asemave, K. and Anure, T.T. (2019). The bioactivities of neem (seed, leaves and their extracts) against callosobruchus maculatus on vigna subterranean L. Progress in Chemical and Biochemical Research, 2(3):92-98.

[8]. Maton, S.M., Dodo, J.D., Nesla, R.A., Ali, A.Y. (2016). Environmental Impact of Pesticides Usage on Farmlands in Nigeria. International Journal of Innovative Research and Development, 5(4): 311-317.

[9]. Dahiru, B., Abdullahi, G. and Bukar, N. (2014). Pesticides use among grain merchants in Mubi grain markets of Adamawa state, Nigeria. Agrosearch, 14(1):1-13.

[10]. Yusuf I.B., Azeez I.A. and Bolaji O.M. (2019). Assessment of Knowledge and Practice of Pesticide Application Among Beans and Maize Sellers in an Urban South Western Nigerian Market. African Journal of Biomeical. Research, 22(1):27- 32.

[11]. Ogunshina, O.O., Oladimeji, M.O. and Lajide, L. (2011). Insecticidal action of hexane extracts of three plants against bean weevil, Callosobruchus maculatus (F.) and maize weevil, Sitophilus zeamais motsch. Journal of Ecology and the Natural Environment, 3(1): 23-28.

[12]. Obadofin, A. A., Fatoba, T. A. and Fatunsin G. F. (2015). Insecticide Repellency of Plant Extracts against Callosobruchus maculatus. Journal of Environmental Issues and Agriculture in Developing Countries, 7(3):10-18.

[13]. Tawose, F.O. and Bagbe, A.S. (2021). Comparativeness of Insecticidal Action of Local Medicinal Plants against Stored Grains Pest (Weevils). Annals of Experimental Biology, 9 (4): 1-6.

[14]. Tlankka, N.S., Mbega, E.R., Ndakidemi, P.A. (2020). Potential of Indigenous Pesticidal Plants in the Control of Field and Post-Harvest Arthropod Pests in Bambara Groundnuts (Vigna subterranea (L.) Verdc.) in Africa: A Review. American Journal of Plant Sciences, 11, 745-772.

[15]. Adeniyi, S.A., Orjiekwe, C.L., Ehiagbonare, J.E. and Arimah, B.D. (2010). Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (Vernonia amygdalina, Sida acuta, Ocimum gratissimum and Telfaria occidentalis) against beans weevil (Acanthscelides obtectus). International Journal of the Physical Sciences, 5(6):753-762.

[16]. Agbo, E.O., Ogenyi, J.O., Adah, C.A. and Agber, C.T. (2021). Phytochemical screening and invitro antimicrobial activity of Cyperus rotundus leaf extracts. Nigerian Annals of Pure and Applied Sciences, 4(1):36-45.

[17]. Mwanauta, R.W., Mtei, K.A. and Ndakidemi, P.A. (2014). Prospective Bioactive Compounds from Vernonia amygdalina, Lippia javanica, Dysphania ambrosioides and Tithonia diversifolia in Controlling Legume Insect Pests. Agricultural Sciences, 5, 1129-1139.