https://dx.doi.org/10.4314/dujopas.v9i2b.11
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 9 No. 2b June 2023*

Evaluation of Seed Extracts of *Azadirachta indica*. Juss (Meliaceae) as Pesticide for the Treatment of Stored Bean Seeds

Musah, M., Mohammed, M., Mathew, J. T. and Umar, M. T.

Department of Chemistry,
Ibrahim Badamasi Babangida University,
Lapai, Nigeria
Email: mkwagana@gmail.com

Abstract

This study sought to ascertain the potentials of extracted oil and various partition portions of the seed extract of *Azadirachta indica* in the control of insect (*Callosobrochus maculatus*) infestation of stored bean seeds. The extracted oil significantly enhanced feeding deterrence in the insect, grain damage was greatly reduced as well as weight loss and oviposition of the insects. The extracted oil at different doses significantly reduces oviposition and adult emergence of *C. maculatus* in the treated bean seeds. Methyl allyl disulphide, n-propyl benzoate, n-hexadecanoic acid, 9,12-octadecadienenoic acid, Methyl nonanoate, Methyl hexadecanoate, Linoleic acid, Oleic acid and Campesterol were the major components in the extracted oil through GC-MS analysis. Regression analysis of the available data on individual in the treated bean seeds confirm that significant reduction of the oviposition and adult emergence of *Callosobrochus maculatus* decreases with increasing doses of the extract. It was also observed that feeding deterrent, oviposition and adult emergence of the *Callosobrochus maculatus* of the bean seeds were found to reduce as the polarity of the partition portion of the extracts increases significantly. The preliminary phytochemical screening of the n-hexane and methanol extract revealed the presence of alkaloids, saponins, carbohydrates, steroids, triterpenes, glycosides, tannins and flavonoids. The findings have demonstrated the bio-efficacy for the use of the extracted oil and various partition portion of the seed extract against insect infestation of the stored beans, hence strengthening the possibility of using it as an alternative to synthetic chemicals for the preservation of grains.

Keywords: *Azadirachta indica,* Beans, Bio-efficacy, *Callosobrochus maculatus*, Extract, Pest

INTRODUCTION

Agriculture in the developing countries today is adversely affected by numerous pests like bacteria, fungi, weeds, virus and insects, leading to reduction in yields and poor productivity. Green Revolution technology of crop production has increase food production in developing countries through the intensive use of inputs like agrochemicals, fertilizers and pesticides etc. The agrochemicals have helped a lot in increasing agricultural productivity, but they have caused adverse effects on the soil health, water quality, develops problems like insect's resistance, generic variation in plants, toxic residues in food and feeds. Dependent on agrochemicals and indiscriminate use of synthetic pesticide causes a lot of detrimental effects in our environment. Recognizing the ill effects of the agrochemicals such as pesticides resistance, pest resurgence, and outbreak of secondary pest, pest residue in the produce, soil,

**Author for Correspondence*

air and water, it's therefore pertinent to develop an alternative to those synthetic agro – inputs (Tudi *et al*., 2021; Qaim, 2020).

Post-harvest deterioration causes economic losses due to obvious decay and adverse changes in the odour, taste, appearance and nutrition value. *Callosobruchus maculatus* is a cosmopolitan pest that infests a wide range of stored cereals especially beans. This insect is one of the most damaging pests of the kidney bean in the tropical and subtropical environment. It causes losses of up to 30% of stored beans, its oviposition and growth are continuous, and the larvae feed on the seeds. *Callosobruchus maculatus* is one of the most damaging pests of the kidney bean in the tropical and subtropical environment. It causes losses of up to 30 % of stored beans. Its oviposition and growth are continuous, and the larvae feed on the seeds. After emergence from the seeds, the adults reproduce either in the field or in the stored seeds in a continuous cycle (Kalpna *et al*., 2022). Larvae reduce product quality by their presence and by the production of frass and webbing, and they also cause direct damage by feeding. The insect pests cause great damage to crops both in the field and in stores. In this research, we examined the bio-efficacy of the various seed extract of *Azadirachta indica* against *Callosobruchus maculatus*. This is with a view to curtail the diminishing natural resources and also to protect the post-harvest loses without adversely affecting the environment.

MATERIALS AND METHODS

Collection of samples

Seed portion of *Azadirachta indica* (Melaceae) were collected from a farm land in Lapai town, Niger State of Nigeria in the month of January, 2022. Botanical identification of the plant was performed at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria. A voucher specimen No. (DC1226) was deposited.
The bean seeds with the insect (*Callosobruchus maculatus*) were collected from Lapai market, Niger state. The unaffected beans seed was obtained from Institute of Agricultural Research (IAR), Zaria.

Callosobruchus maculatus was identified in the laboratory based on the sex. Females are darker overall, while males are brown. The plate covering the end of the abdomen is large and dark in colour along the sides in females, and smaller without the dark areas in males.

Plate 1: Male *Callosobruchus maculates*

Plate 2: Female *Callosobruchus maculates*

Preparation of plant sample

The dried seed sample was decorticated, sliced into small pieces and air dried at room temperature for two weeks. This was grinded into powdered form using pestle and mortar (1.500g).

The infested bean seeds and those damaged by insects were separated from the unaffected seeds. The seeds were disinfected using methanol and kept in a deep freezer for 72 hours to eliminate any contamination (Chrinius *et al.,* 2015). The seeds were removed from the deep

freezer, kept at room temperature and relative temperature to equilibrate and to maintain the moisture content of the seeds (Milind and Dev, 2012).

Extraction of the oil and partition of various portions of the seed.
The powdered material was further subjected to exhaustive de fattening using cold maceration techniques with solvent of low polarity i.e n-hexane (100%). The marc was further dried at room temperature and was further resubmitted for another cold maceration techniques using methanol (100%). The n-hexane and methanol portion of the seed extract where further concentrated using rotary evaporator to obtain n-hexane and MeOH extract. The portion of the n-hexane and methanol extract were subjected to phytochemical screening using standard protocol (Zhang *et al*., 2018; Abubakar and Haque, 2020). The concentrated methanol extract portion was further resubmitted to partition techniques using various solvent of increasing polarity i.e Chloroform (3×500 ml),Ethyl acetate (4×600 ml)and n-butanol (5×500 ml). The various partition portion of the extract were concentrated using rotary evaporator to afford Chloroform (5.62 g), Ethyl acetate (4.32 g), n-butanol (6.31 g) and Aqueous residue (8.16g). The various extract partition portions including the n-hexane extract (16.62ml) were dried and kept in a desiccator while the oil was stored in a clean sealed glass container for subsequent use.

The n-hexane portion of the extract was subjected to GC-MS analysis. Analysis was done using a Varian 3800 gas chromatography equipped with a Agilent MS capillary column (30 m × 0.25 mm i.d.) connected to a Varian 4000 mass spectrometer operating in the EI mode (70 eV; m/z 1 – 1000; source temperature 230 °C and a quadruple temperature 150 °C). The column temperature was initially maintained at 200 °C for 2 min, increased to 300 °C at 4 °C/min, and maintained for 20 min at 300 °C. The carrier gas was Nitrogen at a flow rate of 1.0 mL/min. The inlet temperature was maintained at 300 °C with a split ratio of 50:1. A sample volume of 1 µL in chloroform was injected using a split mode, with the split ratio of 50:1. The mass spectrometer was set to scan in the range of m/z 1-1000 with electron impact (EI) mode of ionization, runtime were 40 minutes. Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC – MS compounds present in the samples were identified. The compounds were identified by comparing their retention times, mass spectra with the authentic standards and the fragmentation patterns of the MS with NIST and wiley GC-MS computer library (Corrias *et al*., 2020; Shah *et al*., 2021).

Culturing and Maintenance of the Insects
The n-hexane extract and various partition portion of the seed extract of *A. indica* were tested for their in vivo effects on insect (*C. muculatus*) mortality, oviposition and adult emergence. A stock solution of the n-hexane and various extract of the seed was prepared by dissolving 100 µl of the extracts in 1.9 ml of acetone. Twenty five seeds of beans were filled in a glass vials (6.3×2 cm diameter) and treated separately with different close i.e 100 µl, 10 µl, 1.0µl and 0.1µl of the various extracts (Hematpoor *et al*., 2017). The seeds were then dressed by continuous shaking for five minutes for proper mixing of the extracts onto the seeds and for the control, the seeds were dressed in requisite amount of acetone in place of the extracts. The treated and infested bean seeds were kept in humidity control cabinet (27×2 c and RH 80±5 %). The treated and infested bean seeds were incubated for 24, 48, 72 and 96 hours and requisite control set were kept for each treatment (Ileke *et al*., 2020). After 24 hrs, the mortality of *C. maculatus* was observed in each vial and all the insects (live and dead) were removed. The numbers of eggs laid on the seeds of treated and controlled seeds were counted after three days of starting the

experiment. The numbers of adult *C. muculatus* that emerged on the beans in each treated and control set was counted for 10, 20, 40 and 80 days of storage.

RESULTS AND DISCUSSION

In this study, the preliminary phytochemical studies of the n-hexane and methanol seed extracts of the *A. indica* presented in Table 1 showed the presence of flavonoids, alkaloids, saponins, glycosides, carbohydrates, triterpenes, tannins and steroids. Many reports suggested that flavonoids of plants belonging to various families exhibit antimicrobial activities against bacterial and fungal pathogens (Shaba *et al*., 2013; Abubakar *et al*., 2015a). Flavonoids, present at high levels in most plants have many biological effects including anti-allergic, anti-inflammatory, antihepatotoxic, anti-ulcer, anti-viral and anti-spasmolytic and are of interest in the investigation of disease processes and as potential new drugs (Tsado *et al*., 2018; Musah *et al*., 2022).

Tables 1 - 7 present the preliminary phytochemical screening of the seed extracts of *Azadirachta indica*, effects of the various extracts on the adult mortality, oviposition and adult emergence of *C. maculatus* on treated beans, while the GC-MS Spectra is presented in figure 1.

Table 1: Preliminary Phytochemical screening of the seed extracts of *Azadirachta indica*

CONSTITUENTS	TEST	OBSERVATION	PORTIONS OF EXTRACT	
Carbohydrate			H_E	M_E
General Test	Molisch	Red colouring	-	+
Sugar Test	Aniline	Red colour	-	-
Sugar (Monosaccharide)	Barfoed's	Red ppt	-	+
Red. Sugar	Fehling's	Red ppt	-	+
Tannins	Lead Ethanoate	White ppt	-	+
	Iron (III) Chloride	Blue – Black	-	+
	Ethanoic acid	White ppt	-	+
	Methanol's	Red ppt	-	-
Saponins	Frothing	Persist frothing	-	+
Sterols	Liberman B.	Blue or green	+	+
Saponin Glycoside	Fehlings Solution	Red ppt	-	+
	Tetraoxosulphate(iv) acid	Brick red	-	+
Phlobatannins	Hydrochloric Acid	Red ppt	-	-
Carotenoids	Carr price's	Blue to red colour	+	+
Emodol	Borntrager's	Red colour	+	-
Flavones aglycones	Shibata's	Red to Orange	-	-
Terpenoids	Liebermann B.	Pink to Red colour	+	+
Alkaloids	Mayer's	Buff ppt	-	-
	Wagner's	Dark brown ppt	-	+
	Dragendorff's	Redish brown	-	+
Flavonoids	Shinoda	Deep red	-	+
	Tetraoxosulphate (vi) acid	Deep Yellow	-	+
Cardiac glycoside	Legal's	Deep red colour	-	+
	Kedd's	Violet colour	-	+
	Keller – kilanis	Reddish brown	-	+
	Baljet	Orange to Deep red	-	+
	Lieberman	Bluish green	-	+

Key: - = Absent, **+ =** Present.
H_E = n-Hexane; M_E = Methanol; CL = Chloroform; Eta=Ethylacetate; n-But = n-Butanol; Aq = Aqueous

The presence of alkaloids in *A. indica* is also of great importance to humans because of their medicinal values as significant quantities are used as anti-malarial, analgesic and as stimulants. Alkaloids rich fraction from *Prosopis juliflora* (Fabaceae) was reported to be most sensitive against gram negative *K. Pneumonia* with a comparable zone of inhibition with that of standard drug (amphicillin) (Uzor, 2020). This therefore, gives credence to some of the

ethnomedical uses of the plant (Sofowora *et al*., 2013; Aziz *et al*., 2018). Many studies have established the usefulness of medicinal plants as a great source for the isolation of active principles for drug formulation (Tanko *et al*., 2020). The presence of some phytochemical compounds reported from *A. indica* could be linked to the biological activities such as antimicrobial, anti-inflammatory, immune-modulatory and anti-cancer activities (Pant *et al*., 2017). Research findings indicated that the antimicrobial properties of plants are conferred on them by the presence of secondary metabolites (Pant *et al*., 2017). Plants rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids etc., have been found in-vitro to have antimicrobial properties (Michal *et al*., 2012). Phenolic compounds are essentially representing varieties of natural antioxidants, which are used as nutraceuticals and also in control of human pathogens (Abubakar *et al*., 2015b). Triterpenoidal saponin extracted from aqueous and ethanol extracts of *Allophylus cobbe* and *Allophylus serratus* was found to have potential antibacterial activities against *B. subtilis*. Saponin was reported to be active against six strains of *E. coli* compared to standard drugs streptomycin (Najjar-Tabrizi *et al*., 2020).

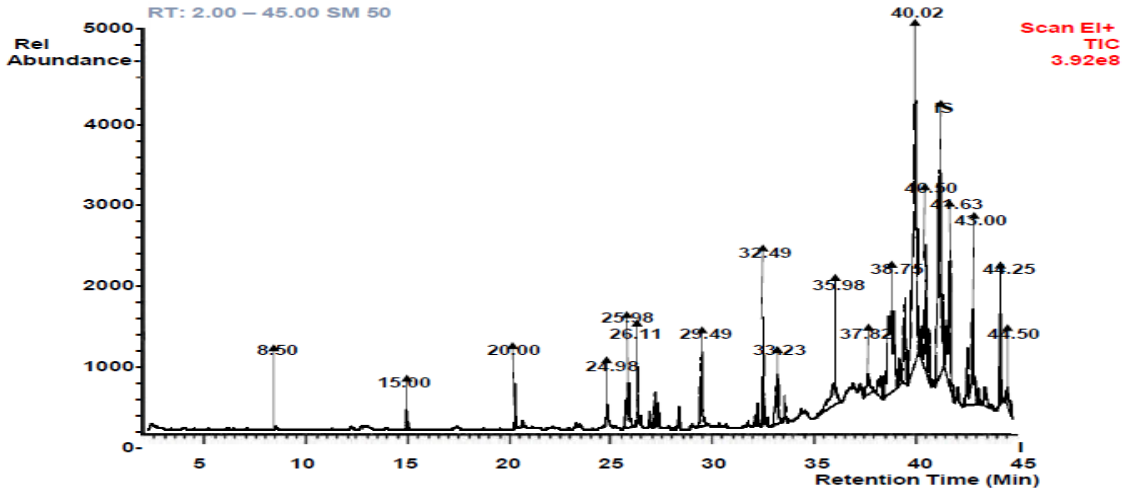

Figure 1: GC-MS Spectra of *A. indica* Seed.

Table 2: GC-MS Analysis of the n-hexane seed extract of *Azadirachta indica*

	Compound	MF	(%) Composition
1	Decane	$C_{10}H_{22}$	2.13
2	Propyl cyclohexane	C_9H_{18}	4.33
3	Phytol	$C_{20}H_{40}O$	2.74
4	Methyl allyl disulphide	$C_4H_8S_2$	0.65
5	Hexadecane	$C_{16}H_{34}$	3.06
6	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	21.44
7	n-propyl benzoate	$C_{10}H_{12}O_2$	0.81
8	Methyl, 14-methyl pentadecanoate	$C_{17}H_{34}O_2$	1.75
9	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	25.78
10	Methyl nonanoate	$C_{10}H_{18}O_2$	4.87
11	Methyl hexadecanoate	$C_{17}H_{34}O_2$	10.13
12	Linoleic acid	$C_{18}H_{32}O_2$	3.30
13	Oleic acid	$C_{18}H_{34}O_2$	5.28
14	Methyl 9,12,15-octadecatrienoate	$C_{19}H_{32}O_2$	3.42
15	Octadecanoic acid	$C_{18}H_{36}O_2$	4.52
16	Campesterol	$C_{28}H_{48}O$	3.97
17	Squalene	$C_{30}H_{50}$	1.83
	Total		**100**

The GC-MS spectra of the seed extract of *A. indica* is presented in figure 1. Results revealed the presence of seventeen compounds (Table 2). Some of the major compounds included: Methyl allyl disulphide, n-propyl benzoate, n-hexadecanoic acid, 9,12-octadecadienenoic acid, Methyl nonanoate, Methyl hexadecanoate,Linoleic acid, oleic acid and Campesterol. The neem oil showed a significant activity in the growth regulation, Oviposition and feeding deterrent of the *Callosobrochus maculatus* after 10- 80days of application on the bean seeds. The insecticidal constituents of many oils are mostly triterpenoids and monoterpenoids (Bright *et al*., 2001). Triterpenoids and monoterpenoids have been reported to inhibit reproduction of stored insects at various steps of their life cycle. This include oviposition, ovicidal effects and larvicidal effect on neonate larva before the penetration of the seeds or a larvicidal effect on the larva within the seed, therefore inhibiting the emergence of imagos (Silvério *et al*., 2020; Şengül *et al*., 2022).

Table 3: Effect of n-hexane extract on the adult mortality, oviposition and adult emergence in *C. maculatus* on treated beans.

Dose of n-hexane Extract (µl)	Percentage Adult mortality (%)	Percentage reduction in eggs (%)	Percentage reduction in adult Emergence (Days after treatment)			
			10	20	40	80
100	87	78	81	72	64.3	50.3
10	66	57	73	68	61.4	42.4
1.0	54	58	66	54	43.2	33.5
0.1	38	42	62	42	36.3	31.2

The various partition portions of the seed extract of *A. indica* exhibited botanical activities in the protection of stored beans against *C. muculatus* by enhancing feeding deterrence, oviposition as well as weight loss of the insects. These findings are in conformity with those of Viteri *et al*., (2018) who investigated the effect of essential oil of *Cymbopogon martinii, Caesulia axillaris* and *Mantha arvensis* for the protection of stored grains from *C..chinensis, S. oryzae* and *T. castaneum* for the first 12 months of storage. The various extracts from the seed of *A. indica* was known to possess repellant, ovicidal, antifeedant, growth retardant and insecticidal activities against various stored grain insects (Viteri *et al*., 2018). The adult mortality of the various extract of the seed might be attributed to the contact toxicity or the abrasive effect on the post cutile which might also interfere with the respiratory mechanism of the insect (Wafaa *et al*., 2017; Quandahor *et al*., 2022). The results of the previous and the present study indicated that the extracted oil from the seed might be useful for managing coleopterpous insects in an enclosed compartments such as glasshouse, warehouse and silos. Moreso, the oviposition, mortality and adult emergence of *C.maculata* were found to vary significantly with different doses of the various seed extract. It was observed that *C. maculatus* females laid significantly few eggs on the beans treated with different doses of *A. indica* oil and various extract than the control, ,hence indicating that the oil and various extract possess deterrent principles responsible for the oviposition in *C. muculatus* (Moura *et al*., 2019). The fewer eggs were hatched on the treated beans than the control at 10, 20,40 and 80 days after treatment. Female *C. maculata* were found to deposit their eggs on the beans seed surface while the neonate larva have to penetrate through the outer ectoderm of the bean seed into the endosperm where the biologically active principles from the oil and various extract of the *A.indica* might have a deterred effect. The reduction in the emergence of adult *C. maculatus* might be attributed to the following factors which include egg mortality or larva mortality or the reduction in the hatching of the eggs. The oviposition inhibitors have the advantage of attacking a pest at the early state of its life cycle. The insect is

deterred from laying its eggs on the food stuff, hence retarding the growth in population of the insect (Sayah *et al*., 1996).

Table 4: Effect of chloroform extract on the adult mortality, oviposition and adult emergence of *C. maculatus* on treated beans.

Dose of Chloroform Extract (µl)	Percentage Adult mortality (%)	Percentage reduction in eggs (%)	Percentage reduction in adult Emergence (Days after treatment			
			10	20	40	80
100	82%	73%	70	61	52	48
10	61%	54%	62	53	48	41
1.0	50%	53%	51	46	35	32
0.1	32%	40%	43	40	31	28

Table 5: Effect of Ethyl acetate extract on the adult Mortality, Oviposition and Adult emergence of *C. maculatus* on treated beans.

Dose of Ethyl acetate Extract (µl)	Percentage Adult mortality (%)	Percentage reduction in eggs (%)	Percentage reduction in adult Emergence (Days after treatment			
			10	20	40	80
100	80%	70%	61	53	47	36
10	58%	50	50	46	42	30
1.0	47%	51	43	41	34	26
0.1	30%	36	28	36	27	19

The Antifeedant effect observed in this specie of *C. maculatus* is highly correlated with the sensory response of chemoreceptors on the insect's mouthparts. Feeding behavior depends upon both neural input from the insects' chemical senses (taste receptors on tarsi, mouthparts and oral cavity) and central nervous integration of this 'sensory code (Chaudhary *et al*., 2017). Active constituents from the seed extract of *A. indica* stimulates specific 'deterrent' cells in chemoreceptors and also blocks the firing of 'sugar' receptor cells, which normally stimulate feeding. This results in an inhibition of feeding, culminating in starvation and death of these species by feeding deterrence alone (Kumar and Prabhakar, 2012). The effects of the *A. indica* seed extracts on growth and molting could be observed on the *C. maculatus* insects resulting to increased mortalities, abnormal molts and delayed molts in the insects. These IGR effects are thought to be caused by disruptions of the complex interactions between molting hormone (20-0H ecdysone from the prothoracic glands) and juvenile hormone (JH from the corpora allata) at the molt (Liu *et al*., 2018). The disruption can be explained by a blockage of release of morphogenetic peptides from the brain, which controls the release of the hormones from their endocrine glands. These peptide hormones, are prothoracicotropic hormone (PTTH) from the pars inter cerebralis neurosecretory cell - corpus cardiacum complex, which stimulates the synthesis and release of ecdysone from the prothoracic glands; the allatotropins from the brain - cc complex which stimulates JH release; and the allatostatins, also from the brain (lateral neurosecretory cells), which inhibit JH release (Hasebe and Shiga, 2021). It is the modification of haemolymph ecdysteroid levels by these indirect effects of the seed extracts that in large part causes the well described insect growth regulatory (IGR) effects of the extracts. Delayed or reduced JH titers in addition cause subtle changes of cuticle structure with either larval or adult characteristics being displayed (Riddiford, 2020).

Table 6: Effect of n-Butanol extract on the adult Mortality, Oviposition and Adult emergence of *C .maculatus* on treated beans.

Dose of n-Butanol Extract (µl)	Percentage Adult mortality (%)	Percentage reduction in eggs (%)	Percentage reduction in adult Emergence (Days after treatment			
			10	20	40	80
100	76	63	48	42	40.3	31
10	52	48	39	32	28	22
1.0	45	50	31	27	24	18
0.1	25	32	24	20	17	12

Test: 7: Effect of Residual Aqueous extract on the adult Mortality, Oviposition and Adult emergence of *C .maculatus* on treated beans.

Dose of Residual Aqueous Extract (µl)	Percentage Adult mortality (%)	Percentage reduction in eggs (%)	Percentage reduction in adult Emergence (Days after treatment			
			10	20	40	80
100	72	60%	30	22	18	17
10	43	40	22	16	14	10
1.0	41	46	19	12	08	06
0.1	20	28	11	05	03	01

The effect of the various *A. indica* extract was found to cause profound effects on reproductive processes of both male and female *C. maculatus* insects. The extract interferes with both the synthesis of vitellogenin by the fat body and its uptake by the eggs, resulting in reduced fecundity and sterility (Nanfack-Minkeu and Sirot, 2022), again due to disruption in JH levels and ovarian ecdysteroid production. As spermatogenesis tends to occur as part of morphogenesis and males are often able to mate soon after emergence (Parthasarathy *et al*., 2010). However, effects of the various extracts on female fecundity have been noted and testes development has been shown to be inhibited. In addition, the meiotic processes responsible for the production of mature sperm in adult males were interrupted and blocked prior to metaphase (Kumar and Singh, 2015).

CONCLUSION

It could be concluded that, the bio-efficacy of the neem seed oil and the partition portion of the neem seed extract against *C. maculatus* increases with an increase of dose concentration. However, both the neem oil and the partition portion of the seed extract can be used preferably as a post-harvest in the management of seed infested with *C. maculatus* and safely stored for consumption, since there is no any side effect of the extracts on human health.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the Tertiary Education Trust Fund (TETFund) for funding this study and the management of IBB University, Lapai for creating an enabling environment for research to thrive.

REFERENCES

Abubakar, A. R., and Haque, M. (2020). Preparation of Medicinal Plants, Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of pharmacy and Bioallied sciences*, 12(1), 1–10.

Abubakar, I., Mann, A. and Mathew, J. T. (2015a). Evaluation of Phytochemical, Anti-nutritional and Antioxidant Potentials of Flower and Seed Methanol Extracts of *Senna alata* L. Grown in Nigeria. *American Journal of Applied Chemistry*. 3(3), 93-100

Abubakar, I., Mann, A. and Mathew, J. T. (2015b). Phytochemical composition, Antioxidant and Anti-nutritional properties of root-bark and leaf methanol extracts of *Senna alata L.* Grown in Nigeria. *African Journal of Pure and Applied Chemistry*, 9(5), 91-97.

Aziz, M.A., Khan, A.H. and Adnan, M. (2018). Traditional uses of medicinal plants used by Indigenous communities for veterinary practices at Bajaur Agency, Pakistan. *J Ethnobiology Ethnomedicine* 14, 11.

Bright, A., A, Babu, A., Ignacimuthu, A. and Dorn, S. (2001). Efficacy of crude extracts of *Andrographis paniculata* nees on *Callosobruchus chinensis* L. during post-harvest of cowpea. *Indian Journal of Experimental Biology,* 39,715-718.

Chaudhary, S., Kanwar, R. K., Sehgal, A., Cahill, D. M., Barrow, C. J., Sehgal, R. and Kanwar, J. R. (2017) Progress on *Azadirachta indica* Based Biopesticides in Replacing Synthetic Toxic Pesticides. *Front. Plant Sci*. 8,610.

Chrinius, H., Musa M., Olalekan, B.A., Usman, J. M., Eprhaim, A. A. and Suleiman, B. (2015). The Efficacy of *Agave Sisalana* n-Hexane Extract in the Control of *Callosobruchus maculatus* (Fabricius) (Colloptera, Bruchidae) Pest. *J. App. Biol. Biotech.*, 3 (04), 001-003.

Corrias, F., Atzei, A., Giglioli, A., Pasquini, V., Cau, A., Addis, P., Sarais, G., and Angioni, A. (2020). Influence of the Technological Process on the Biochemical Composition of Fresh Roe and Bottarga from *Liza ramada* and *Mugil cephalus*. *Foods,* 9(10), 1408.

Hasebe, M., and Shiga, S. (2021). Oviposition-promoting pars intercerebralis neurons show period-dependent photoperiodic changes in their firing activity in the bean bug. *Proceedings of the National Academy of Sciences of the United States of America*, 118(9), e2018823118.

Hematpoor, A., Liew, S. Y., Azirun, M. S., and Awang, K. (2017). Insecticidal activity and the mechanism of action of three phenylpropanoids isolated from the roots of Piper sarmentosum Roxb. *Scientific reports*, 7(1), 12576.

Ileke, K.D., Adesina, J.M. and Nwosu, L.C. (2020). Perforation index assessment of cowpea seeds against cowpea bruchid, *Callosobruchus maculatus* (Fabricius) [Coleoptera, Chrysomelidae], infestation using *Piper guineense*. *JoBAZ* 81, 60.

Kalpna, P., Ahmad, H. and Rajesh, K. (2022). Management of stored grain pest with special reference to *Callosobruchus maculatus*, a major pest of cowpea, A review. *Heliyon,* 8, e08703.

Kumar, P., and Prabhakar, N. R. (2012). Peripheral chemoreceptors, function and plasticity of the carotid body. *Comprehensive Physiology*, 2(1), 141–219.

Kumar, N., and Singh, A. K. (2015). Trends of male factor infertility, an important cause of infertility, A review of literature. *Journal of human reproductive sciences*, 8(4), 191–196.

Liu, S., Li, K., Gao, Y., Liu, X., Chen, W., Ge, W., Feng, Q., Palli, S. R., and Li, S. (2018). Antagonistic actions of juvenile hormone and 20-hydroxyecdysone within the ring gland determine developmental transitions in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 115(1), 139–144.

Michal A., Aneta W. C., Grzegorz C., Lankiff A. and Wieslaw K. (2012), Effect of Saponin against Clinical E. coli Strains Eukaryotic Cell Lines. *Journal of Biomedicine and Biotechnology*, 12,1-6.

Milind P, and Dev C (2012) Orange, Range of Benefits. *International Research Journal on Pharmacy,* 3(7), 59– 63.

Moura, E. D. S., Faroni, L. R. A., Zanuncio, J. C., Heleno, F. F. and Prates, L. H. F. (2019). Insecticidal activity of Vanillosmopsis arborea essential oil and of its major constituent α-bisabolol against *Callosobruchus maculatus* (Coleoptera, Chrysomelidae). *Scientific Reports*, 9(1), 3723.

Musah, M., Mohammed, M., Mathew J. T. and Umar, M. T. (2022). Isolation and characterization of bioactive constituent of n-butanol seed extract of *Azadirachta indica* plant. *Bima Journal of Science and Technology*, 5(4), 91-106.

Najjar-Tabrizi, R., Javadi, A., Sharifan, A., Chew, K. W., Lay, C. H., Show, P. L., Jafarizadeh-Malmiri, H. and Berenjian, A. (2020). Hydrothermally extraction of saponin from *Acanthophyllum glaulosum* root - Physico-chemical characteristics and antibacterial activity evaluation. Biotechnology reports (Amsterdam, Netherlands), 27, e00507.

Nanfack-Minkeu, F., and Sirot, L. K. (2022). Effects of Mating on Gene Expression in Female Insects, Unifying the Field. *Insects*, 13(1), 69.

Pant, D. R., Pant, N. D., Saru, D. B., Yadav, U. N. and Khanal, D. P. (2017). Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. *Journal of Intercultural Ethnopharmacology*, 6(2), 170–176.

Parthasarathy, R., Sheng, Z., Sun, Z., and Palli, S. R. (2010). Ecdysteroid regulation of ovarian growth and oocyte maturation in the red flour beetle, Tribolium castaneum. *Insect Biochemistry and Molecular Biology*, 40(6), 429–439.

Qaim, M. (2020). Role of New Plant Breeding Technologies for Food Security and Sustainable Agricultural Development. *Applied Economic Perspectives and Policy*.13, 1-12.

Quandahor, P., Gou, Y., Lin, C. and Liu, C. (2022). Potato (Solanum tuberosum L.) Leaf Extract Concentration Affects Performance and Oxidative Stress in Green Peach Aphids (Myzus persicae (Sulzer). *Plants*, 11, 2757.

Riddiford L. M. (2020). *Rhodnius*, Golden Oil and *Met*, A History of Juvenile Hormone Research. *Frontiers in Cell and Developmental Biology*, 8, 679.

Sayah, F., Fayet, C., Idaomar, M. and Karlinsky, A. (1996), Effect of azadirachtin on vitellogenesis of *Labidura riparia* (Insecta, Dermaptera), *Tissue and Cell*, 28, 741-749.

Şengül D. M. Ş. and Canpolat, E. (2022). Plant-Based Bioinsecticides for Mosquito Control, Impact on Insecticide Resistance and Disease Transmission. *Insects*, 13(2), 162.

Shaba, E. Y., Mathew, J. T., Inobeme, A., Mustapha, S., Tsado, A.N. and Amos, J. (2013). Phytochemical and Antimicrobial Screening of the Fruit Pulp of *Canarium schweifurthii* (ATILE). *Nigerian Journal of Chemical Research,* 18(1), 6-10.

Shah, M., Murad, W., Ur Rehman, N., Mubin, S., Al-Sabahi, J. N., Ahmad, M., Zahoor, M., Ullah, O., Waqas, M., Ullah, S., Kamal, Z., Almeer, R., Bungau, S. G., and Al-Harrasi, A. (2021). GC-MS Analysis and Biomedical Therapy of Oil from n-Hexane Fraction of *Scutellaria edelbergii Rech*. f., In Vitro, In Vivo and In Silico Approach. *Molecules (Basel, Switzerland)*, 26(24), 7676.

Silvério, M. R. S., Espindola, L. S., Lopes, N. P., and Vieira, P. C. (2020). Plant Natural Products for the Control of Aedes aegypti, The Main Vector of Important Arboviruses. *Molecules*, 25(15), 3484.

Sofowora, A., Ogunbodede, E., and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary and alternative medicines*, 10(5), 210–229.

Tanko E., Dauda B.E.N., Mann A., Oyeleke S.B., Fadipe L. A. and Mathew J.T. (2020). Phytochemical and Antibacterial Studies of *Ensete gilletii* (E.A.J. De Wildman) Stem Extract and Fractions. *Nigerian Research Journal of Engineering and Environmental Sciences*, 5(1), 390-398.

Tsado A. N, Abdulkadir A., Shaba A. M., Mathew J. T., Umar A. M., Chirama D. N. (2018). Phytochemical, Antioxidant and Antimicrobial Potentials of Methanol Seed Extracts of *Carica Papaya*. *World Wide Journal of Multidisciplinary Research and Development*, 4(3), 24-28.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., and Phung, D. T. (2021). Agriculture Development, Pesticide Application and Its Impact on the Environment. *International journal of environmental research and public health*, 18(3), 1112.

Uzor P. F. (2020). Alkaloids from Plants with Antimalarial Activity: A Review of Recent Studies. *Evidence-based complementary and alternative medicine, eCAM*, 2020, 8749083.

Viteri, J. L. O., Haddi, K., Faroni, L. R. D., Heleno, F. F., Pinto, F. G., and Oliveira, E. E. (2018). Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils. *PloS one*, 13(11), e0207618.

Wafaa M. H., Rowida S. B. and Hussein A.H. S. (2017) Botanical insecticide as simple extractives for pest control, *Cogent Biology*, 3(1), 140-144.

Zhang, Q. W., Lin, L. G., and Ye, W. C. (2018). Techniques for extraction and isolation of natural products, a comprehensive review. *Chinese medicine*, 13, 20.