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Specialty Section: This article was submitted to Sciences section of NAPAS.

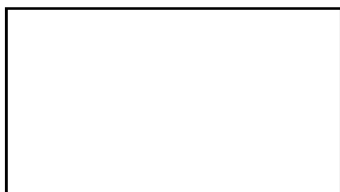
Submitted date: 7th Feb.,2023
Accepted date: 16th April,2023
Published date:

Citation: Mazza Benjamin, Abdullahi Gambo * , Medugu Marcel Alexander, and Aliyu Ahmed Santuraki (2023). **Bio-Pesticidal Potentials of *Elaeis Guineensis* l. Kernel Oil Against Cowpea Bruchid *Callosobruchus Maculatus* (fab.) on Stored Cowpea *Vigna Unguiculata* l. (walp) Seeds** - Nigerian Annals of Pure & Applied Sciences. 6(1):91 - 101. DOI:10.5281/zenodo.7338397

Publisher: cPrint, Nig. Ltd

Email: cprintpublisher@gmail.com

Access Code



<http://napas.org.ng>

Bio-Pesticidal Potentials of *Elaeis Guineensis* l. Kernel Oil Against Cowpea Bruchid *Callosobruchus Maculatus* (fab.) on Stored Cowpea *Vigna Unguiculata* l. (walp) Seeds

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Abstract

The cowpea bruchids *Callosobruchus maculatus* L is the most devastating postharvest insect pest, which attacks cowpea both in the field and store leading to significant economic losses. Laboratory experiments were conducted to determine the insecticidal effects of *Elaeis guineensis* kernel oil at five different application rates against *Callosobruchus maculatus* on cowpea (*Vigna unguiculata*) seeds. *Callosobruchus maculatus* adult mortality, F1 progeny emergence, as well as the percentage (%) insect damage seeds, percentage weight loss, beetles perforation index and seed viability of cowpea seeds were assessed. The result revealed that sample treated with 0.8 ml /100g recorded the highest mortality rate of 66.67%, the least (8.33%) was recorded on the control. The oviposition was highest in the control with the mean of 2905.67 eggs while the least was on sample treated with 0.8 ml /100g with the mean of 122.33 eggs. The highest weight loss (24.27%) was recorded on the control while the least (1.00%) was recorded on sample treated with 0.8 ml /100g. The highest insect damaged seeds (64.25%) were recorded on the control while the least (3.03%) was recorded on sample treated with 0.8 ml of oil /100g. F1 progeny emergence was highest in the control (305.00) and the least (11.00) was on sample treated with 0.8 ml/100 g. Germination was highest (100%) in the control while the least (20%) was on sample treated with 0.8 ml /100g. It was concluded that *E. guineensis* kernel oil is effective in reducing the attack and other negative effects of *C. maculatus* on stored cowpea seed and can be used as a safer alternative to toxic insecticides in the protection of cowpea seeds against this beetle.

Key words: Perforation, mortality, seed damage, weight loss, oviposition, progeny

Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. (Fabaceae), is grown in all countries in sub-Saharan Africa and in Asia, South America, Central America, the Caribbean, the United States of America (USA) and around the Mediterranean Sea (Hall, 2004). In Africa, cowpea is the second most important grain legume (NRC, 2006), and is commonly consumed in the form of dry grains or young pods (Adenakan *et al.*, 2013). Its grain provides high amounts of carbohydrates, vitamins and minerals (Khalid *et al.*, 2012), proteins (Frota *et al.*, 2017); and is an income source for the rural and suburban inhabitants (Boukar *et al.*, 2016). It, therefore, serves as a cheap source of plant protein to low income peasant farmers who cannot afford animal proteins (Yusuf *et al.*, 2011; Ekeh *et al.*, 2013). Dried grains are prepared into "moi-moi", "Akara" (Kosai) or eaten in combination with other crops such as rice, yam etc and can be used as forage also (Muhammad *et al.*, 2017). But, the production of cowpea in all cowpea belts is constrained by many factors. The nature and magnitude of these constraints differ among production areas. However, the major constraints for cowpea grain production in most cowpea producing nations are insect pest's infestation in both the field and in storage after harvest (Ronner and Giller, 2013).

The cowpea bruchids *Callosobruchus maculatus* L is the most devastating postharvest insect pest, which attacks cowpea both in the fields and stores leading to economic losses (Adams *et al.*, 2018). It is regarded as the most important and most common storage pest of cowpea in Africa (Fakayode *et al.*, 2014) and Asia (Deshpande *et al.*, 2011) causing up to 80.2% grain damage, in a month after infestation (Adams *et al.*, 2018). And without control measure all the stored cowpea can be consumed by bruchids in the first 10 to 12 months of storage (Gómez, 2004). The impact of this storage pest induces reduction in seeds wholesomeness or conversion of nutrients which leads to reduced germination rate and nutrient level of seeds (Swella and Mushobozy, 2007). It also causes loss in quality as a result of contamination with

filthy materials composed of insect fragments, exuviae, excreta and molds (Musa and Adeboye, 2017). All these damages reduce the degree of usefulness, making the seeds unfit either for planting or human consumption (Ali *et al.*, 2004). The huge post-harvest losses as a result of deterioration in quantity and quality of Cowpea caused by this insect are major obstacles to achieving food security in developing countries.

The use of insecticides as sprays or fumigants to protect cowpea seeds from bruchids can be effective and is at the moment the most widely used control method. But these chemicals pose health hazard to farmers and consumers, causes environmental pollution. The insecticides are also expensive. Furthermore, insects develop resistance to insecticides, necessitating the application of larger amounts (Boyer *et al.*, 2012). For these reasons, non-chemical approaches for control of bruchids which is cheap, easy to apply, and safe to the environment and consumers is being aggressively advocated (Wudil *et al.*, 2020). Naturally occurring plant products have been used to protect agricultural products against pests for many years globally (Ugwu *et al.*, 2021; Wudil *et al.*, 2020; Beizhou *et al.*, 2012).

Oils extracted from some plant materials are used in *Callosobruchus maculatus* control. For instance, Aliyu and Ahmed (2006) and Raja *et al.* (2001) reported the effect of *Arachis hypogaea* oil and *Mentha arvensis*, *M. spicata*, *M. piperata* and *Cymbopogon nardus* on *Callosobruchus maculatus*. Palm kernel oil is an important and versatile raw material for both food and non-food industries accounting for more than 28 million tons of the world's annual consumption, 95 million tons of vegetable oil consumption (RSPO, 2008). Palm kernel oil is used in various food products such as cooking and frying oils, margarine, frying fats, shortenings, non-dairy creamer, ice cream, cookies, crackers, and biscuits (Akinniran *et al.*, 2013). Non-food uses of palm oil and palm kernel oil are either directly or through the oleo chemical routes. Direct applications include the use of crude palm oil (CPO) as a diesel fuel substitute, drilling mud, soaps and epoxies

palm oil products; polyols, polyurethanes and polyacrylates (Ahmad, 2003). In view of numerous safe uses of oil palm products as raw materials for industrial uses and cooking human diets, there is the need to explore its potential as a cowpea seeds protectant against infestation by *C. maculatus*. Therefore, this study evaluated the potentials of *E. guineensis* kernel oil in suppressing cowpea bruchid *C. maculatus* damage on stored cowpea *V. unguiculata* seeds.

Materials and Methods

Source of cowpea seeds and *E. guineensis* kernel oil used in the study

Seeds of improved iron beans Black-eye variety used for the study was obtained from Girei market. The seeds were sorted out to remove the damaged and infested seeds. The sorted out and cleaned seeds were sterilized in an oven at 60 °C for 3 hours to reduce moisture content and to disinfect it from hidden insect infestation. Finally, the disinfected seeds were packaged in polyethylene bags and kept until needed (Ileke et al., 2020). The *E. guineensis* kernel oil of good quality used for the study was locally obtained from Wukari in Taraba State of Nigeria, from a reputable refiner.

Insect culture

The initial insects used to establish a laboratory colony of *C. maculatus* was obtained from a batch of infested *farin hantsi* (Susceptible variety) local cowpea cultivar collected from Girei market. The insects were cultured on the cowpea seeds (black-eyed variety) which were obtained from Girei market. Prior to using the cowpea seeds as a substrate for *C. maculatus* culture, the seeds were disinfected in an oven at 60 °C for 30 minutes. Ten (10) pairs of the insect were introduced into the rearing bottle of 1L capacity containing 250 g cowpea seeds. The bottles were covered with muslin cloth and fastened with rubber band and kept in the laboratory under prevailing temperature and relative humidity for one (1) months until the emergence of the F₁ progeny. The F₁ generation was then used for the experiment at 2 days old (Ileke et al., 2020).

Experimental design and treatment procedure

The experiment was carried out in a completely randomized design (CRD) with four different rates of the oil (0, 0.2, 0.4, 0.6, and 0.8 ml)/100 g of cowpea seeds in 1000 ml glass jar and replicated three times. The oil doses were measured by the use of micropipette. For treatment application, batches 100g of cowpea seeds were weighed into 1000 ml containers. The respective oil rates (0.0, 0.2, 0.4, 0.6, and 0.8) was measured out and added to the grains and mixed thoroughly by shaking the jar vigorously for one minute. Ten pairs of the reared *C. maculatus* aged two days were introduced into each glass jar using an aspirator containing the treated grains which are then covered with muslin cloth and fastened with rubber band to avoid escape by insect and to allow for aeration. The containers were then left undisturbed in the laboratory under ambient temperature and relative humidity for observation and data collection. The study was conducted in the Entomology laboratory (9 °N and 10 °N and longitude 12 ° E and 35 °E) of the Department of Crop Protection, Modibbo Adama University Yola.

Data collection

Data was taken on mortality rate, oviposition, grain weight loss, grain damage, progeny production and seed viability. Beetle perforation index was also computed for each application rate. The *C. maculatus* adult mortality rate was taken at 7, 14, 21, and 28 days after treatment (DAT) for each set of treatment and used to compute the mortality percentage as;

$$\% \text{mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Adults Oviposition rate: the number of eggs laid by introduced adult insect was taken for each treatment (oil application rate).

Weight loss: the reduction in weight recorded for each sample in a set of treatment. The weight of grains before and after each period was taken and readings on weight loss were calculated at post

experimental period (Yakubu, 2015). And used to compute percentage weight loss as follows;

$$\% \text{ weight loss} = \frac{[UaN - (U + D)]}{UaN} \times 100$$

Where U = weight of undamaged fraction in sample

N = total number of grains in sample

Ua = average weight of one undamaged kernel

D = weight of damaged fraction in sample (Adams and Schulten, 1978; Boxall, 1986 as adopted by Wudil *et al.*, 2020).

Grain damage is the visible infraction on the wholesomeness of the grains as a result of insect feeding damage. Separation of the damaged and undamaged grains was done using grain tunneling and holes as the criteria (Tefera *et al.*, 2011); these were counted and the percentage of damaged grain was calculated according to the method described by Odeyemi and Daramola (2000).

$$\% \text{ grain damage} = \frac{\text{number of grains damaged}}{\text{total number of grains}} \times 100$$

Beetle Perforation Index (BPI) was also evaluated after the analysis of damage as described by Fatope *et al.* (1995)

$$\text{BPI} = \frac{\% \text{ damaged grain in treated}}{\% \text{ damaged grains in the control}} \times 100$$

F_1 Progeny emergence is the number of offsprings of the introduced adults that emerges after the experimental period. After the mortality data assessment, all the dead and live insects were removed from each treatment and the seeds were kept in their set ups under the same experimental conditions to further assess F_1 progeny emergence. All emerged adults were removed on daily bases until 28 days.

Progeny inhibition rate (IR) was also calculated using the method described by Tapondjoun, *et al.* (2002) thus:

$$\% \text{ IR} = \frac{C_n - T_n}{C_n} \times 100$$

where

C_n = number of emerged insects in the control; and

T_n = number of emerged insects in the treated.

Seed viability test was carried out after the experimental period to see if the oil affects viability by placing randomly selected seeds on a wetted filter paper in a petri dish. The number of seeds that germinated normally was counted after 7-10 days. The percentage germination (viability index) was calculated as:

$$\text{GP}(\%) = \frac{\text{NSC}}{\text{TNS}} \times 100$$

Where GP = Germination percentage,

NSC = Number of seeds germinated after 7-10 days

TNS = Number of seeds tested in each petri dish. (Ogendo *et al.*, 2004; Acheampong *et al.*, 2019).

Statistical Data Analysis The data obtained above was subjected to analyses of variance (ANOVA) appropriate to Completely Randomized Design using statistical analysis software (SAS) version 9.4. Means were separated using Least Significant Difference (LSD) at probability of 5% where significant differences were recorded.

Results

Effect of the different rate of *Elaeis guineensis* kernel oil on the mortality of adult *C. maculatus*

The analysis of variance (ANOVA) for the efficacy of the different rates of the oil on the mortality of the *C. maculatus* shows significant difference ($P \leq 0.05$) between the treatments. The treatment percentages mortality indicated that the highest mortality (9.53, 28.10, 52.86, and 73.24%) was recorded on sample treated with 0.8 ml/100 g at 24, 48, 72, and 96 Hours After Treatment respectively, and the least mortality of 0.00, 0.00, 0.03, and 1.2% was recorded in the control at 24, 48, 72, and 96 HAT respectively (Table 1).

Table 1: Effect of the different rate of *Elaeis guineensis* kernel oil on the mortality of adult *C.maculatus*

Effect of the different rate of *Elaeis guineensis* kernel oil on the adults oviposition, progeny emergence and progeny inhibition rate

Result for effect of different rate of *Elaeis guineensis* kernel oil on the adults oviposition, progeny emergence and progeny inhibition rate is presented in Table 2. The ANOVA for the adults oviposition indicates a significant difference ($P \hat{A} 0.05$) among the treatments with respect to adults oviposition deterrence. The result indicates that the control samples had the highest number of oviposition with the mean of 2905.67 of eggs $100g^{-1}$ seeds, while the sample treated with 0.8 ml/100 g recorded the least with mean of 122.33 eggs $100g^{-1}$ seeds (Table 2). Also, the least mean number of F1 progeny (11.0) emerged on samples treated with 0.8 ml/100g while the highest (305.00) was recorded on control samples. Similarly, the highest % progeny inhibition rate (96.39) was recorded on samples treated with 0.8 ml/100 g and the least (0.00%) was on the control samples (Table 2).

Table 2: Effect of the different rates of *Elaeis guineensis* kernel oil on mean oviposition, F1 progeny emergence and progeny inhibition rate of the adult *C. maculatus*.

Effect of the different rate of *Elaeis guineensis* kernel oil on the insect damage grain, % weight loss, beetle perforation index (BPI), and seed viability of the

treated cowpea seeds.

The result of Effect of the different rate of *Elaeis guineensis* kernel oil on the insect damage grain, % weight loss, beetle perforation index (BPI), and seed viability of the treated cowpea seeds is in Table 3. The ANOVA for the efficiency of the different rates of the oil on the insect damaged seed showed a significant difference ($P \hat{A} 0.05$). The result for mean comparison for mean percentages damaged seeds (Table 3), indicated that the highest damage (64.25%) was recorded in control, followed by 0.2 ml, 0.4 ml, 0.6 ml, and the least damage (3.03%) was recorded in treatment with concentration of 0.8 ml/100 g of cowpea seeds.

The ANOVA for weight loss indicates a significant difference among the treatments ($P \hat{A} 0.05$). The percentage mean comparison (Table 3) shows that, the control had the highest weight loss (24.27%) and the lowest (1.00%) was recorded in the sample treated with 0.8 ml/100 g of seeds. The result for beetle perforation index (BPI) showed that all samples except the controls very low BPI. The least perforation index of 4.72 was recorded on samples treated with 0.8 ml / 100g while the highest >50 was recorded on control samples (Table 3).

The effects of the different rates of the oil on the seed viability of the cowpea seed showed a significant difference ($P \hat{A} 0.05$). The percentage comparison (Table 3) indicates that the highest (100%) was recorded in the control, and the least (20%) was recorded in the sample treated with 0.8 ml /100g.

Table 1: Effect of the different rate of *Elaeis guineensis* kernel oil on the mortality of adult *C. maculatus*

Rate ml $100g^{-1}$	24 HAT	48 HAT	72 HAT	96 HAT
0.0	0.00	0.00	0.03	0.12
0.2	5.11	11.91	40.72	56.40
0.4	7.38	22.87	42.15	59.14
0.6	8.57	25.71	48.86	67.14
0.8	9.53	28.10	52.86	73.24
LSD	0.36	0.68	0.91	1.37
P values	0.0001	0.0193	0.0001	0.0003

HAT-hours after treatment

Table 2: Effect of the different rates of *Elaeis guineensis* kernel oil on mean oviposition, F1 progeny emergence and progeny inhibition rate of the adult *C. maculatus*.

Rate ml 100g ⁻¹	Mean oviposition	F ₁ Progeny Emergence	Progeny Inhibition Rate
0.0	2905.67	305.00	0.00
0.2	587.00	26.67	91.26
0.4	290.00	21.67	92.90
0.6	183.33	12.33	95.96
0.8	122.33	11.00	96.39
LSD	90.47	9.12	1.25
<i>P</i> values	0.00001	0.00000003	0.0001

Table 3: Effect of the different rate of *Elaeis guineensis* kernel oil on, insect damaged seeds, % weight loss, beetle perforation index, and viability of the cowpea seeds.

Rate (ml 100g ⁻¹)	% IDS	%WT loss	BPI	Seed Viability (%)
0.0	64.25	24.77	>50	100
0.2	6.63	6.83	10.32	90
0.4	5.21	4.87	8.11	80
0.6	3.38	1.43	5.26	30
0.8	3.03	1.00	4.72	20
LSD	1.19	1.05	2.10	39.67
<i>P</i> values	.0000003	0.0000001	0.001	0.0000001

IDS= Insect damaged seeds, WT=Weight, BPI=Beetles perforation index

Discussion

Dose-dependent mortality in insects to insecticides was reported by Ekeh et al. (2013). Similarly, 73% mortality was recorded on the samples treated with 0.8 ml /100g while (1.2%) was recorded on the control 96 hour after treatment. This indicates the effectiveness of the different application rate of *E. guineensis* kernel oil tested in our study in causing mortality in *C. maculatus*; hence, points to its promising potentials as a safe and eco-friendly management option for this pest on cowpea seeds. This will go a long way in reducing the undesirable aspects of the use of synthetic

insecticides in the control of *C. maculatus* on cowpea especially in resource - poor families that cannot afford synthetic insecticides that are often expensive.

Significant reduction in the number of eggs laid was seen from this study. From the results obtained, the control has the highest number of eggs (2905.67) and the least was obtained from the sample treated with 0.8 ml /100g (122.33). This shows a 95.8% enhancement of reduction in the oviposition of the *C. maculatus* between the control and the highest dose. This implies that the capacity to lay eggs by adult *C. maculatus*

was drastically reduced in the treatments which indicated the effectiveness of *E. guineensis* kernel oil with respect to oviposition deterrence. Oviposition deterrence of plants products in *C. maculatus* and other insects was earlier elucidated in literature. For instance, Yusuf *et al.*, (2011) investigated the insecticidal activities of seven plant materials: citrus peel powder (CPP), Acacia leaf powder (ALP), Occimum leaf powder (OLP), mahogany bark powder (MBP), hot pepper powder (HPP), ginger powder (GP) and mahogany wood ash (MWA) and a synthetic insecticide, pirimiphos-methyl dust (PMD) (0.1-0.5g/100g cowpea seeds) as standard. Their results demonstrated that the seven botanicals tested have shown their potential of discouraging oviposition, emergence of F₁ generation and substantially reduce damage on cowpea seed by *C. maculatus*. Oils extracted from some plant materials have also been demonstrated to be effective in *C. maculatus* control. Aliyu and Ahmed (2006) and Raja *et al.*, (2001) separately reported the effect of groundnut (*Arachis hypogaea* oil and *Mentha arvensis*, *M. spicata*, *M. piperata* and *Cymbopogon nardus* respectively on *C. maculatus*. Ravinder (2011) also compared insecticidal action of seven plantmaterials namely: citrus leaf powder (CLP), Acacia leaf powder (ALP), Occimum leaf powder(OLP), mahogany bark powder (MBP), hot pepper powder (HPP), ginger powder (GP) and mahogany wood ash (MWA) with pirimiphos methyl and found that citrus leaf powder was as effective as pirimiphos in exhibiting insecticidal actions against *C. maculatus*.

F₁ progeny emergence has been drastically impaired in samples treated with all rate of the oil used in this study. From the result obtained, it showed that the F₁ progeny emergence was highest (305.00) indicated the effectiveness of *E. guineensis* kernel oil in suppressing the F₁ progeny emergence of *C. maculatus* on stored cowpea seeds.

Progeny inhibition rate observed in this study is significant (up to 96%). Though the mode of action of vegetable oils on insect pests of stored products is not yet fully

understood (Nana *et al.*, 2014), the reduced oviposition and inhibited progeny development clearly indicates that *P. guineensis* kernel oil demonstrated oviposition deterrence as well as toxicity to eggs (ovicidal). This could be due the modification storage micro-environment with respect to grain texture (Obeng-Ofori, 1995; Haghtalab *et al.*, 2009), anoxia (Don-Pedro, 1989) or the oil acting as anti-feedants, thus, depressing insect penetration in the grain for feeding (Bekele and Hassanali, 2001; Haghtalab *et al.*, 2009).

The seed damage caused by the *C. maculatus* was significantly highest in the control (64.25%) while the least was recorded in samples treated with 0.8 ml/100 g (3.03%). The parameter used in determining seed damage was based on the number of perforation or holes created by the bruchids on the grains (Ileke *et al.*, 2020). From our study, it was obvious that the control samples might be damaged up to 100% before any of the treated samples could exhibit any meaningful damage. This can be attributed to protectant effect of the *E. guineensis* kernel oil applied. Severe *C. maculatus* infestations is reported to be capable of causing 100% damage of the stored cowpea and grain loss within few months of infestation (kang *et al.*, 2013; Tarver *et al.*, 2007)

Weight loss observed showed that *E. guineensis* kernel oil is effective in reducing weight loss of stored cowpea attacked by *C. maculatus*. Highest weight loss (24.27%) and the lowest weight loss were recorded in sample treated with 0.00 and 0.8 ml/100g (1.00%), *E. guineensis* kernel oil. This shows a significant reduction in the damage rating of *C. maculatus* on cowpea seed. Brooker (1967) and Fatope *et al.*, (1995) earlier reported that a single *C. maculatus* is able to cause 3.5% weight loss. More recent studies revealed that *C. maculatus* infestation on cowpea seeds can cause up to 60% weight loss (Tanzubil, 1996; Umeozor, 2005).

Beetle perforation index (BPI) is an index of protectant potential of products (Ileke *et al.*, 2020). BPI values for all *E. guineensis* kernel oil rates tested in this study were dismally low (range 4.72-10.32). Beetle

perforation index *BPI* value that exceeded 50 will be regarded as negative protectant or enhancement of damaged by cowpea bruchid (Fatope *et al.*, 1995). Therefore, our findings implies that the *E. guineensis* kernel oil tested is a good cowpea seed oil protectant against *C. maculatus*.

The result of the study also indicate that the application of *Elias guineensis* kernel oil at the rate of 0.1- 0.4 ml/100g did not affect the germination rate of the seeds. However, increase in the rate of the application 0.6ml, and 0.8 ml/100g reduced the germination percentage of the grains. Impairment of seed germination after treatment with vegetable oils has been reported in literature. Aliyu and Ahmed (2006) and Raja *et al.* (2001) independently reported the effect of groundnut oil and *Mentha arvensis*, *M. spicata*, *M. piperata* and *Cymbopogon nardus* respectively on the effect of groundnut oil on the germination process of cowpea seed stored for 12 weeks. The germination process of the stored cowpea was not affected by the application of groundnut oil at the rate of 4 ml /Kg. However, when the rate of application was increased to 6 ml /Kg, the rate of germination decreased and the reduction peaked at 8 ml /Kg. Therefore, storage of cowpea seeds intended for planting should not exceed 0.4 ml /100g of *E. guineensis* oil. However, grains meant for consumption can be treated with rate up to 0.8 ml/100g.

Conclusions

The study has revealed that *E. guineensis* kernel oil is effective in reducing the attack of *C. maculatus* on cowpea seeds and could be utilized for its eco-friendly management. However, the use of *E. guineensis* kernel oil to control *C. maculatus* should not exceed 0.4 ml/100g if the seeds are intended for planting but can be increased to 0.8 ml / 100g where the seeds are intended for consumptions.

Acknowledgments

We gratefully acknowledge all the facilities provided by the Department of Crop Protection, Modibbo Adama University, Yola

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