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Impregnating storage materials with neem seed oil against *Callosobruchus maculatus* Fabricus (Coleoptera: Chrysomelidae: Bruchinae) in Stored Bambara Seeds (*Vigna subterranean* L.) Verdcourt

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ABSTRACT Neem (*Azadirachta indica* A. Juss.) seed oil (NSO) is acclaimed to have some form of insecticidal action against more than 400 insect species in at least 10 to 13 orders. The main delimitating factor in the wide acceptance of this well tested plant as a storage pest bioinsecticide is its foul sulfurous smell and bitter taste which impinges on the acceptability and marketability of treated produce. To ameliorate this shortfall, therefore, an assessment of the potential of impregnating different storage materials; [plastic containers (PLC), Bagco bags (BCB), Black polyethylene bags (BPB), white polyethylene bags (WPB) and calico bags (CAB)], with NSO in the management of *Callosobruchus maculatus* (F.) in stored Bambara seeds (*Vigna subterranea* L.), was conducted in the laboratory. The experiment was laid out in a 4 x 5 factorial arrangement fitted into a completely randomized design (CRD). Factor A represented four concentrations of NSO (0.00, 0.50, 1.00, 1.50 ml/ 100g seed), while factor B were the five different storage materials. The treatments were replicated four times Generally, seeds stored with impregnated storage materials recorded more bruchids mortality, reduced oviposition rate, decreased number of emerged insects (F_1 and F_2) and had less damaged seeds (and hence decreased weevil perforation index, WPI) than the control. Concentration performance was dose related though the highest (1.50 %) did not differ statistically (P=0.05) from the medium (1.00 %) concentration. Seeds stored in BPBs had the highest mortalities, but recorded the lowest in number of eggs oviposited, egg/seed ratio, F_1, F_2 and cumulative emergence, respectively. These results differed significantly (P=0.05) with the seeds stored in other materials. BPB stored seeds were also the least damaged with lowest exit holes/seed, seeds with holes and WPI, respectively. The performance of BPB was closely followed by seeds stored in WPBs in all the parameters tested. Conversely, seeds stored in BCBs recorded the lowest mortalities but the highest in all other parameters assessed. Other storage materials (PLC and CAB) had better storage quality than BCB materials. Impregnation of storage materials with NSO could be a better option to direct seed application with its attendant drawback. The use of NSO impregnated black polyethylene bags, within the scope of the storage materials screened, to control damage by *C. maculatus* in stored Bambara seeds, should be encouraged.

1. INTRODUCTION

Bambara groundnut (*vigna subterranean* (L.)Verdcourt is a leguminous crop of the order Fabales, family Fabaceae and sub family, Faboidea, with creeping stems and branching just above ground level. The seeds are variously coloured from white to cream, red, black or brown, sometimes mottled; blotched or striped [1, 2]. The legume is an indigenous African crop. Though [3] reported that Bambara groundnut originated from northeastern Nigeria and northern Cameroon where the wild forms are still found, [4] were of the plausible view that the crop originated from the Sahelian region of present day West Africa, from the Bambara tribe near Timbuktu who now live mainly in Central Mali, hence the name. It is the third most important legume in terms of consumption and socioeconomic impact in semi-arid Africa, trailing behind peanut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L. Walp.) [5]. At an estimated 100,000 metric tonnes per year, Nigeria has been noted as the highest producer of the crop in Africa [6]. In Nigeria, the nuts are known as Gurjiya or Kwaruru (Hausa), Okpa (Igbo) and Epa – Roro [2].

The unique properties and composition of Bambara nut makes it a balanced food with almost all the vital nutrients that promotes good health for people living in Africa [7]. They are rich source of minerals, energy and protein, with as much as 25.2% protein, 65% carbohydrates and 6% lipid on a dry weight basis [8] and contain more lysine and methionine than either cowpea or groundnuts [9]. Immature seeds are consumed fresh or grilled. They can also be boiled, either shelled or unshelled, and eaten as a meal or mixed with immature groundnuts or green maize [3]. The seeds are either pounded to flour or boiled to a stiff porridge, soup and various fried or steamed food such as 'akara', 'moi-moi' and 'okpa' [10]. The porridge keeps well and is traditionally used on journeys [11].

The major bruchid species that infest Bambara groundnuts are; *Callosobruchus maculatus* Fabricus, *C. subinotatus* Pic., *C. chinensis* Linnaeus *and Zabrotes ubfasciatus* Boheman [12]. Of these, *C. maculatus* is the major storage insect pest of Bambara groundnut seeds [8, 12, 13]. They are field – to - store agricultural insect pests of Africa and Asia that presently range throughout the tropical and subtropical world [14] and are the most destructive on account of their shorter life cycle [15]. The weevil can cause as much as 99% yield loss in susceptible grain legumes. The damage results in quantitative and qualitative losses. Quantitative damage includes reduction in kernel weight, caused by the burrowing larvae as they feed and qualitative losses could be as a result of chemical changes in grains; contamination with insect body products, frass, pathogenic and toxicogenic microorganisms and or losses in seed viability [16, 17, 18, 19].

To combat these debilitating storage pests, farmers and traders in West Africa use storage structures and other available materials like earthenware pots, gourds, mud silos, jute sacks, Bagco bags, metal drums plastic containers and local granaries [12, 20] for grain storage. These are, most often, integrated with synthetic pesticides.

The unabated use of toxic synthetic chemicals has given rise to problems of toxicity, pest resurgence and elevation of secondary pests, development of pesticide resistant populations, deleterious effects on populations of non-target organisms, residues in food chain, high costs of most of the chemicals, contamination of the environment, non-availability and the falsification and adulteration of pesticides [21, 22, 23, 24].

Botanical insecticides have long been reported to be safer to use than synthetics in view of what [25] dubbed the 'desirable soft modes of action' of some highly effective natural plant products with potentials for use as pest control agents. In the last four decades, therefore, considerable efforts have been directed at the integration of insecticidal products from local available plants for use in produce storage [26].

Neem (*Azadirachta indica* A. Juss.) is a foremost natural plant product of choice amongst Nigerian subsistent farmers for crop and produce protection. The main delimitating factor in the wide acceptance of this well tested plant as a storage pest bioinsecticide is its foul sulfurous smell and bitter taste [27, 28] which could readily impinge on the acceptability and marketability of treated produce. To find a way to stem this limiting factor, therefore, readily available storage materials were impregnated with different concentrations of neem seed oil for storing bambara groundnut seeds.

If found effective, farmers could easily soak these storage materials in concentrated neem seed oil for effective storage of grains without fear of compromising the sensory and organoleptic properties of the stored seeds.

2. MATERIALS AND METHODS

2.1 Location

The experiment was conducted in the Department of Crop Science and Technology Laboratory, Federal University of Technology, Owerri which lies between latitude 5^0 31'58" N and longitude 7^0 0'43" E in the Southeastern agroecological zone of Nigeria. The environmental condition is characterized by temperature usually above 27^0C, average annual rainfall of 2500 mm and relative humidity of 78 % during the rainy season [29].

2.2 Bambara Seeds: Procurement and Disinfestation

Local, untreated Bambara groundnut landrace (Okpa Nsukka) which came in a motley of colours; white, cream, black and red, were purchased from a local market in Nsukka, Enugu State, Nigeria. The grains were handpicked to remove damaged ones and contaminants. The selected seeds were put in a white polyethylene bag, firmly tied with rubber band and inserted into a transparent airtight plastic bucket and disinfested by deep-freezing for two weeks. The seeds were later air-dried in the laboratory for 24 h prior to use.

2.3 Collection of Neem seeds and Extraction of Neem Seed Oil (NSO)

Neem seeds were collected within the premises of the School of Agriculture and Agricultural Technology in the Federal University of Technology, Owerri, Nigeria. The seeds were dried under shade for one week, ground into fine particles, sealed in thick polythene sachets and kept in a dry cool place for future use.

A simple and replicable cold bulk extraction procedure was used to extract oil from the ground seeds. The ground seed was put into a big glass bottle and 1.5 litre of ethanol added, agitated for proper mixing and allowed to settle. After 2 days, the liquid oil was separated from the chaff by placing a clean calico cloth at the mouth of a funnel. The filtrate was then poured through the funnel into a 500 ml beaker. The collected neem oil was placed on hot plate at a low temperature (60^0C) for 3hours, to allow the ethanol to evaporate.

2.4 Insect Culture

Adult *C. maculatus* were sourced from infested Bambara seeds from Nsukka, Enugu State, Nigeria. 0.50 kg clean Bambara seeds was weighed out into two plastic breeding containers, respectively, and the bruchids introduced into them. The containers were then firmly covered with clean calico cloth. The introduced bruchids were discarded after 7 days. The seeds, with eggs, were allowed to stay until emergence after about 22 days. On emergence, the freshly emerged bruchids were used for the experiment.

2.5 Storage Materials

Five different storage materials were procured for the experiment: 100 ml plastic containers (PLC), 15 x 10 cm (length x width) Bagco** bags (BCB), Black polyethylene bags (BPB), white polyethylene bags (WPB) and calico bags (CAB). The BPBs and WPBs are made of high density polyethylene (HDPE, 80 microns thick).

2.6 Neem Seed Extract Rates/Preparation of Storage Materials

The NSO was measured out as follows: NSO_0 (0.00 ml, Control); NSO_1 (0.50 ml); NSO_2 (1.00 ml) and NSO_3 (1.50 ml). These rates were dissolved in 100 ml of water in 300 ml bowls with lids, respectively. The storage materials were immersed in the neem seed solution, left overnight and later spread under shade to dry. For the plastic containers, the solution was poured into the containers, covered and left overnight as others. The solution was discarded the following day and the container left to dry.

2.7 Bioactivity Tests

One hundred grammes of selected bambara groundnut seeds from the disinfested lot was weighed into the different storage materials (PLC, BCB, WPB, BPB and CAB) which had been soaked in the different concentrations of NSO solution and dried under shade. Five pairs of male and female *C. maculatus* adults aged between 24 – 48 hours were then introduced into each of the storage materials. Virgin males may not produce fully formed spermatophores until 24 hours after emergence [14]. The sexes of *C. maculatus* were determined by examining the elytral pattern. Female *C. maculatus* are usually maculate whereas the male are plain. The females are dark coloured and possess four elytral spots. In contrast, males are brown and less distinctly spotted. In most strains, the females are larger than males [30, 31]. The bags were tightly folded and firmly tied at the top whilst the plastic containers were covered with untreated calico cloth held firmly with rubber band. Untreated storage materials with the 10 bruchids served as the control. These treatments were replicated four times.

2.7.1 Effect of Treated Storage Materials on Mortality of Adult *C. maculatus*

Data were collected on the effect of the treatment on the mortality of the adults. This was done by counting, recording and discarding the number of dead insects in each storage materials, 24hrs, 48hrs and 7 days after commencement. The percent mortality was calculated thus:

$$\text{Percent Mortality} = \frac{\text{No. of dead insects}}{\text{Total No. of insects}} \times \frac{100}{1}, \tag{1}$$

**Bagco bags are synthetic, tough, sisal - like polypropylene (PP) bags intrinsically woven, with air spaces, to mimic the traditional jute bags. The bags are very popular with Nigerian farmers for produce storage. They are basically different from the Purdue Improved Crop Storage (PICS) bags [32] because they lack the two high density polyethylene inner bags.

2.7.2 Effect of Treated Storage Materials on Oviposition of Adults *C. maculatus*

The number of eggs laid on the seeds was recorded on the 7th day on a sub sample of 10 randomly selected seeds. This was achieved by counting the number of eggs per seed and the distribution, that is, the number of the seeds with eggs on the seeds in treated storage materials and the control.

2.7.3 Effect of treated Storage Materials on Emergence of Adult *C. maculatus*

The total number of adults that emerged (dead and living) was counted on the respective days and recorded. The cumulative count was gotten by summing up the number of adults that emerged for days 1, 2, 3, and 7, respectively.

2.7.2 Effect of Treated Storage Materials on Percentage Damaged Bambara Seeds

Damage was assessed by counting the total number of emergence holes per seed. The number of holes per sample of 10 randomly selected seeds and the distribution of the seeds with holes were recorded for damage assessment. The percentage damage (PD) of the perforated grains was calculated thus:

$$PD = \frac{\text{Total number of sampled grains perforated}}{\text{Total number of sampled grains}} \times \frac{100}{1}, \tag{2}$$

The Weevil Perforation Index (WPI) was then calculated [33]:

$$WPI = \frac{\text{Total number of treated bambara grains perforated}}{\text{Total number of untreated bambara grains perforated}} \times \frac{100}{1}, \tag{3}$$

A WPI value exceeding 50% is regarded as enhancement of infestation by the weevil, or a negative ability of the plant material or insecticides tested [26].

2.8 Experimemtal Design and Treatment

The experiment was laid out in 4 x 5 factorial arrangement fitted into a completely randomized design (CRD). Factor A represented four concentrations of neem seed oil (0.00, 0.50, 1.00 and 1.50 ml/100 g seed), while factor B were the five different storage materials [100 ml plastic containers (PLC), 15 x 10 cm (length x width) Bagco bags (BCB), black polyethylene bags (BPB), white polyethylene bags (WPB) and calico bags (CAB)]. The experiment was replicated four times. Total treatment combination was 80 (4 x 5 x 4).

2.9 Data Analysis

All data collection were subjected to analysis of Variance (ANOVA) procedures and means separated using least Significant Different (LSD) at P = 0.05 level of significance.

3. RESULTS

The effects of the various neem seed oil impregnated storage materials on mortality and oviposition of adult *Callosobrocus maculatus* is depicted in Table 1. Seeds stored in black polyethylene bags (BPB) recorded the highest bruchid mortalities at 24 hours (5.19) and 48 hours (3.25) of storage, respectively. BPB stored seeds also had the highest cumulative mortality (8.13 insects) at 72 h. This was closely followed by seeds stored in White Polyethylene Bags (WPB) with 5.00 (24 h) and (5.75 and 7.94) cumulative insect mortalities for 48 and 72 h, respectively. The result significantly (P=0.05) differed from the effect of Bagco Bag materials (BCB) which recorded the lowest mortality with 0.50 and 1.38 dead insects at 24 and 48 hours, respectively. The cumulative mortality of insects in BCB materials were also the lowest (5.63) at 72 h. Plastic Containers (PLC) and Calico (CAB) storage materials gave same- range statistical results which had more mortalities than those stored in BCBs.

On neem extract concentrations, the result showed that the highest (1.50 %) concentration inflicted the highest mortality on the bruchids at 24 hours (4.35) and highest cumulative mortalities (6.00 and 9.60) at 48 and 72 h, respectively. The result did not differ statistically (P=0.05) with 1.00 % concentration which recorded the second highest mortality of 4.20 bruchids at 24 h and cumulative mortalities of 5.45 and 8.75 insects for 48 and 72 h, respectively. These results recorded highly significant differences (P=0.05) when compared with the control (0.00 % concentration) which had the least number of dead insects at 24 hours (0.56), 48 hours (1.10) and 72 h (2.85), respectively.

There were significant (P=0.05) interactions of storage materials with the concentration levels of neem seed extracts as also shown in Table 1. The interaction between BPB and WPB materials with 1.00 % concentration of neem seed oil inflicted the highest (10.00, respectively) mortalities on the target insects across the trial period. This was followed by WPB bags at 1.50 % neem oil concentration interaction.

Seeds stored in BPBs recorded the least number of eggs (26.81) which differed significantly (P=0.05) with the seeds stored in BCBs which had the highest (36.44) number of eggs. Similarly, 1.50 % concentration recorded the lowest (23.50) number of eggs which was significantly different (P=0.05) from the control (0.00 %) with the highest number of eggs (49.70). There were no statistically significant differences in the number of eggs laid between the highest (1.50 %) and medium (1.00 %) concentrations.

The interaction effect result indicated that treatment materials significantly differed from each other. Interaction of WPBs with 1.50 % concentration gave the least (17.50) number of eggs oviposited. This was followed by Calico bags (CAB) which interacted with 1.00 % concentration to give 20.75 eggs. However, the results differed significantly (P=0.05) from the interaction effect between the BCBs and 0.00 % concentration which recorded the highest number of eggs (63.25).

Table 2 records the effect of the NSO impregnated storage materials on *C. maculatus* emergence. The main effect of the storage materials showed that BPBs recorded the lowest number of emerged adult bruchids at Day 1(0.00), Day 2 (0.06), Day 3 (0.19) and Day 7 (8.44) insects, respectively. Seeds stored in WPBs had the next low number of emerged insects; 0.06 (day 1), 0.19

(day 2), 0.44 (day 3) and 10.69 at day 7. These result differed statistically (P=0.05) with BCBs which had the highest emergence of the insects with 1.13, 1.50, 2.25 and 16.50 emerged insects, respectively.

On the main effect of NSO, results showed that the highest concentration (1.50 %) did not record any emergence (0.00) of insects at the various trial days. The lower concentration (1.00 %) performed almost as excellent as the highest rate. The lowest rate (0.50 %) allowed minimal bruchids emergence. These results differed significantly from the control (0.00 %) which recorded 1.80, 3.60, 5.25 and 43.45 emerged insects on Days; 1, 2, 3 and7, respectively.

The interactive effect showed that the various storage materials recorded zero (0.00) emergence of adult *C. maculatus* with 1.50 % concentration of NSO after one week trial. This result was however statistically different (P=0.05) from the storage materials which interacted with zero (0.00 %) concentration of the oil to give the highest number of emerged adult insects.

The total number of F_1, F_2 and cumulative emergence of adult *C. maculatus* is presented in Table 3. The result from the table showed that treated cowpea seeds stored in BPB recorded the lowest number of emerged F_1 insects (8.69), total number of F_2 emergence (36.90) and cumulative emergence (45.59), respectively. Following closely where seeds stored with WPB with 11.38, 41.70 and 55.26 bruchids for F_1, F_2 and cumulative emergence, respectively. Contrarily, BCB stored seeds had the most number of F_1 emerged bruchids (21.38), total number of F_2 emergence (65.55) and total cumulative emergence (86.93). These results differed significantly (P=0.05) from each other.

Analysis of NSO concentration levels showed that highest concentration (1.50 %) recorded zero emergences all through the experiment. This result did not differ significantly from medium concentration level (1.00 %) which recorded the second lowest emergence on total number of F_1 (0.15), F_2 (0.10) and cumulative emergence (0.25), respectively. Both of the stated results were significantly different from the control (0.00 % concentration) which recorded the highest number of F_1 (54.10), F_2 (195.00) and cumulative emergence (249.10), respectively.

The interaction result showed that BCB stored without neem seed oil (0.00 % concentration) recorded the highest emergence (78.00), (243.00) and (321.00) for total number of F_1, F_2 and cumulative emergence, respectively. This was followed by the interaction between Plastic Container (PLC) with the control. However, the lowest emergence was recorded by the interaction of the storage materials with 1.50 % concentration which had 0.00 values for F_1, F_2 and cumulative emergence, respectively. These results differed significantly (P=0.05) from each other.

The damage assessment of the various treatment materials is shown in Table 4. The result showed that Bambara seeds stored in BPBs were the least damaged with only 3.94 holes/seed, 3.06 seeds with holes and 6.75 WPI, respectively. Seeds stored with WPBs were next with 4.94 holes/seed, 3.69 seeds with holes and 9.69 WPI, respectively. The grains stored in the Bagco Bags (BCBs) were the most damaged amongst the storage materials with 5.88 holes/seed, 4.19 seeds with holes and 11.69 WPI. The results significantly differed (P=0.05) on number of holes/seed and number of seeds with holes but did not show any statistical differences on the WPI.

The main effect of NSO concentrations showed that seeds treated with 1.50 % and 1.00 % concentrations showed no significant difference (P=0.05) with zero (0.00) holes/seed, number of seeds with holes and WPI, respectively. However, the result showed that significant differences existed when compared with the control (0.00 % concentration) which recorded the highest number of holes/seed, seeds with holes and WPI, respectively.

The interactive effect of storage materials with neem seed extract concentrations at 1.00 % and 1.50 % also gave zero (0.00) number of holes/seed. This result did not differ statistically (P=0.05) when compared with the interaction of BCBs with the control (0.00 % concentration) which recorded the highest number (16.00) of holes/seed.

On number of seeds with holes, the interaction of the various storage materials with 1.00 % and 1.50 % concentrations of neem seed oil recorded zero, but differed statistically (P=0.05) with CABs and PLCs which recorded the highest (10.00) with the control (0.00 %), respectively.
Similarly, the Weevil Perforation Index (WPI) result showed that storage materials interaction with 1.00 % and 1.50 % concentrations of neem seed oil recorded zero WPI, respectively.

Table 1: Effect of Neem Seed Oil Impregnated Storage Materials on the Mortality and Oviposition of Adult C. *Maculatus*

	NEEM_SEED OIL EXTRACT (CONCENTRATION)				
STORAGE MATERIAL	**0.00**	**0.50**	**1.00**	**1.50**	**Mean**
Mortality at 24hrs					
BCB	0.75	0.25	0.25	0.75	**0.50**
CAB	0.25	1.25	0.00	1.50	**0.75**
BPB	0.50	0.75	10.00	9.50	**5.19**
PLC	0.75	0.75	0.75	0.75	**0.75**
WPB	0.50	0.25	10.00	9.25	**5.00**
Mean	**0.55**	**0.65**	**4.20**	**4.35**	
LSD $_{0.05}$ (Storage Material) = 0.530					
LSD $_{0.05}$(Neem Extract) = 0.474					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 1.061					
Mortality at 48 h					
BCB	1.25 (2.00)	0.75 (1.00)	1.25 (1.50)	2.25 (3.00)	**1.38 (1.88)**
CAB	2.00 (2.25)	1.75 (3.00)	3.25 (3.25)	3.00 (4.50)	**2.50 (3.25)**
BPB	0.75 (1.25)	2.25(3.00)	0.00 (10.00)	0.00 (9.50)	**3.25 (5.94)**
PLC	0.75 (1.50)	1.25 (2.00)	1.75 (2.50)	2.25 (3.00)	**1.50 (2.25)**
WPB	0.75 (1.25)	1.50 (1.75)	0.00 (10.00)	0.75 (10.00)	**0.75 (5.75)**
Mean	**1.10 (1.65)**	**1.50 (2.15)**	**1.25 (5.45)**	**1.65 (6.00)**	
LSD $_{0.05}$ (Storage Material) = 0.643					
LSD $_{0.05}$(Neem Extract) = 0.575					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 1.286					
Mortality at 72 h					
BCB	2.25 (4.25)	3.00 (4.00)	4.00 (5.50)	5.75 (8.75)	**3.75 (5.63)**
CAB	2.00 (4.25)	3.75 (6.75)	6.50 (9.75)	5.50 (10.00)	**4.44 (7.69)**
BPB	2.50 (3.75)	6.25 (9.25)	0.00 (10.00)	0.00 (9.50)	**2.19 (8.13)**
PLC	2.25 (3.75)	6.00 (8.00)	6.00 (8.50)	6.75 (9.75)	**5.25 (7.69)**
WPB	2.25 (3.50)	6.50 (8.25)	0.00 (10.00)	0.00 (10.00)	**2.19 (7.94)**
Mean	**2.85 (4.50)**	**5.10 (7.25)**	**3.30 (8.75)**	**3.60 (9.60)**	
LSD $_{0.05}$ (Storage Material) = 1.230					
LSD $_{0.05}$(Neem Extract) = 1.100					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 2.460					
Oviposition					
BCB	63.25	30.25	27.25	25.00	**36.44**
CAB	45.50	34.50	20.75	24.75	**31.38**
BPB	30.00	30.50	22.50	24.25	**26.81**
PLC	55.50	34.25	23.25	26.00	**34.75**
WPB	54.25	44.25	26.25	17.50	**35.56**
Mean	**49.70**	**34.75**	**24.00**	**23.50**	
LSD $_{0.05}$ (Storage Material) = 4.017					
LSD $_{0.05}$(Neem Extract) = 3.593					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 8.035					

KEY: BCB: Bagco Bag; CAB: Calico Bag; BPB: Black Polyethylene Bag; PLC: Plastic Container WPB: White Polyethylene Bag; ( ): Data in brackets represent the cumulative mortalities for 48 and72 hours, respectively.

Table 2: Effect of Neem Seed Oil Impregnated Storage Materials on F_1 Emergence of *C. maculatus*

	NEEM_SEED OIL EXTRACT (CONCENTRATION)				
STORAGE_MATERIAL	**0.00**	**0.50**	**1.00**	**1.50**	**Mean**
Day1.					
BCB	4.25	0.25	0.00	0.00	**1.13**
CAB	1.50	0.00	0.00	0.00	**0.38**
BPB	0.00	0.00	0.00	0.00	**0.00**
PLC	3.25	0.00	0.00	0.00	**0.81**
WPB	0.00	0.00	0.25	0.00	**0.06**
Mean	**1.80**	**0.06**	**0.06**	**0.00**	
LSD $_{0.05}$ (Storage Material) = 0.695					
LSD $_{0.05}$(Neem Extract) = 0.622					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 1.391					
Day 2.					
BCB	6.00	0.00	0.00	0.00	**1.50**
CAB	5.75	0.00	0.00	0.00	**1.44**
BPB	0.25	0.00	0.00	0.00	**0.06**
PLC	5.25	0.00	0.00	0.00	**1.31**
WPB	0.75	0.00	0.00	0.00	**0.19**
Mean	**3.60**	**0.00**	**0.00**	**0.00**	
LSD $_{0.05}$ (Storage Material) = 0.801					
LSD $_{0.05}$(Neem Extract) = 0.717					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 1.602					
Day3.					
BCB	8.50	0.00	0.50	0.00	**2.25**
CAB	8.50	0.00	0.00	0.00	**2.13**
BPB	0.75	0.00	0.00	0.00	**0.19**
PLC	6.75	0.75	0.00	0.00	**1.88**
WPB	1.75	0.00	0.00	0.00	**0.44**
Mean	**5.25**	**0.15**	**0.10**	**0.00**	
LSD $_{0.05}$ (Storage Material) = 0.646					
LSD $_{0.05}$(Neem Extract) = 0.577					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 1.291					
Day7.					
BCB	59.25	6.75	0.00	0.00	**16.50**
CAB	38.00	5.50	0.00	0.00	**10.88**
BPB	31.00	2.75	0.00	0.00	**8.44**
PLC	52.25	7.50	0.00	0.00	**14.94**
WPB	36.75	6.00	0.00	0.00	**10.69**
Mean	**43.45**	**5.70**	**0.00**	**0.00**	
LSD $_{0.05}$ (Storage Material) = 3.409					
LSD $_{0.05}$(Neem Extract) = 3.049					
LSD $_{0.05}$(Storage Material._ Neem Extract) = 6.817					

KEY: BCB: Bagco Bag; CAB: Calico Bag; BPB: Black Polyethylene Bag; PLC: Plastic Container
WPB: White Polyethylene Bag

Table 3: Effect of Neem Seed Oil Impregnated Storage Materials on Total Number of F_1, F_2 and Cumulative Emergence of Adult *C. maculatus.*

	NEEM_SEED OIL EXTRACT (CONCENTRATION)				
STORAGE_MATERIAL	**0.00**	**0.50**	**1.00**	**1.50**	**Mean**
Total Number of Emergence (F_1)					
BCB	78.00	7.00	0.50	0.00	**21.38**
CAB	53.75	5.50	0.00	0.00	**14.81**
BPB	32.00	2.75	0.00	0.00	**8.69**
PLC	67.50	8.25	0.00	0.00	**18.94**
WPB	39.25	6.00	0.25	0.00	**11.38**
Mean	**54.10**	**5.90**	**0.15**	**0.00**	
LSD $_{0.05}$ (Storage Materials) =	6.56				
LSD $_{0.05}$(Neem Extract) =	5.87				
LSD $_{0.05}$(Storage Materials _ Neem Extract) =	13.12				
Total Number of Emergence (F_2)					
BCB	243.00	19.20	0.00	0.00	**65.55**
CAB	227.20	17.80	0.00	0.00	**61.25**
BPB	135.80	11.80	0.00	0.00	**36.90**
PLC	224.20	16.20	0.50	0.00	**60.23**
WPB	144.80	22.00	0.00	0.00	**41.70**
Mean	**195.00**	**17.40**	**0.10**	**0.00**	
LSD $_{0.05}$ (Storage Materials) =	12.36				
LSD $_{0.05}$(Neem Extract) =	11.05				
LSD $_{0.05}$(Storage Materials _ NeemExtract) =	24.71				
Cumulative Emergence					
BCB	321.00	26.20	0.50	0.00	**86.93**
CAB	280.95	23.30	0.00	0.00	**76.06**
BPB	167.80	14.55	0.00	0.00	**45.59**
PLC	283.45	23.70	0.75	0.00	**76.98**
WPB	192.30	28.75	0.00	0.00	**55.26**
Mean	**249.10**	**23.30**	**0.25**	**0.00**	
LSD $_{0.05}$ (Storage Materials) =	14.11				
LSD $_{0.05}$(Neem Extract) =	12.62				
LSD $_{0.05}$(Storage Materials _ Neem Extract) =	28.23				

KEY: BCB: Bagco Bag; CAB: Calico Bag; BPB: Black Polyethylene Bag; PLC: Plastic Container
WPB: White Polyethylene Bag

Table 4: Effect of Neem Seed Oil Impregnated Storage Materials on Percentage Damage and Weevil Perforation Index (WPI)

	NEEM_SEED OIL EXTRACT (CONCENTRATION)				
STORAGE_MATERIAL	0.00	0.50	1.00	1.50	Mean
Number of Holes/Seed					
BCB	16.00	7.50	0.00	0.00	**5.88**
CAB	15.25	6.25	0.00	0.00	**5.38**
BPB	12.50	3.25	0.00	0.00	**3.94**
PLC	14.50	6.75	0.00	0.00	**5.31**
WPB	14.25	5.50	0.00	0.00	**4.94**
Mean	**14.50**	**5.85**	**0.00**	**0.00**	
LSD $_{0.05}$ (Storage Material) =	0.974				
LSD $_{0.05}$(Neem Extract) =	0.871				
LSD $_{0.05}$(Storage Material._ Neem Extract) =	NS				
Number of Seeds with Holes					
BCB	9.50	7.25	0.00	0.00	**4.19**
CAB	9.75	6.00	0.00	0.00	**3.94**
BPB	9.50	2.75	0.00	0.00	**3.06**
PLC	10.00	5.50	0.00	0.00	**3.88**
WPB	10.00	4.75	0.00	0.00	**3.69**
Mean	**9.75**	**5.25**	**0.00**	**0.00**	
LSD $_{0.05}$ (Storage Material) =	0.658				
LSD $_{0.05}$(Neem Extract) =	0.589				
LSD $_{0.05}$(Storage Material._ Neem Extract) =	1.317				
Weevil Perforation Index					
BCB	-	46.75	0.00	0.00	**11.69**
CAB	-	39.50	0.00	0.00	**9.88**
BPB	-	27.00	0.00	0.00	**6.75**
PLC	-	46.50	0.00	0.00	**11.63**
WPB	-	38.75	0.00	0.00	**9.69**
Mean	**-**	**39.70**	**0.00**	**0.00**	
LSD $_{0.05}$ (Storage Material) =	NS				
LSD $_{0.05}$(Neem Extract) =	5.310				
LSD $_{0.05}$(Storage Material._ Neem Extract) =	NS				

KEY: BCB: Bagco Bag; CAB: Calico Bag; BPB: Black Polyethylene Bag; PLC: Plastic Container
WPB: White Polyethylene Bag

4. DISCUSSION

The effects of neem seed oil (NSO) impregnation on adult mortality of *C. maculatus* increased progressively by the day (24, 48 and 72 h, respectively). As was earlier reported by [34], there was a direct relation between the concentration and degree of lethal effectiveness as the highest concentration (1.50 %) exerted the highest mortalities (at 24 h and cumulative mortalities at 48 h and 72 h), respectively. This performance did not differ statistically (P=0.05) with 1.00 % concentration indicating that the medium concentration could be apposite for the treatment. These treatments performed better than untreated storage materials which had the least number of dead insects during the period under review. Conversely, increased neem dosage decreased the number and distribution of eggs, number of F_1, F_2 and cumulative emergence and damage of stored seeds.

All parts of the neem tree, *Azadirachta indica*, are insecticidal although the seeds possess the largest concentrations of azadirachtin, a steroid-like tetranortriterpenoid, and other tetranortriterpenes; including salannin, meliatril, nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid which possess a wide range of biological activities [35, 36]. Modes of action include feeding and oviposition deterrence, developmental/growth impairment, repellency, juvenile hormone mimicry and toxic/insecticidal effects against some 400 to 500 insect species in at least 10 to 13 orders [36, 37, 38].

The use of neem extacts in storage pests' control have been well documented against *C. maculatus* on Bambara groundnut [39], *C. maculatus* on cowpea [15, 40, 41], *Trogoderma granarium* on groundnut [42] and other stored products - legumes, sorghum, maize, wheat, rice, potato tubers, etc. [43]. Dry neem leaves has been used to protect cloths from deterioration due to insect and fungal attack, preserve them, making the fabric last longer and smell good [44].

The effect of the different storage materials; plastic containers (PLC), Bagco bags (BCB), Black polyethylene bags (BPB), white polyethylene bags (WPB) and calico bags (CAB) presented different protection abilities when impregnated with NSO. Generally, however, seeds stored with impregnated storage materials recorded more bruchids mortality, reduced oviposition rate, decreased number of emerged insects (F_1 and F_2) and had less damaged seeds (and hence decreased WPI) than the control.

Indo – Pakistani farmers have been known to traditionally, for stored – grain pest control, soak empty sacks overnight in water containing 2 – 10 kg neem leaves/ 100 litres of water . The soaked sacks are then dried before filling them with grain [45]. [46] had reported that jute bags impregnated with aqueous extracts from two insecticidal plants, *Dysphania* (*Chenopodium) ambrosioides* (L.) Mosyakin and Clemants and *Lantana camara* Linnaeus, significantly reduced seed damage to stored legume seeds by *Acanthoscelides obtectus* Say and *Callosobruchus maculatus* compared with the untreated control after 6 months of storage. Later work by [47] showed that 100 % cotton fabrics impregnated with neem chitosan nanocomposites, prepared using multiple emulsion/ solvent evaporation method, had increased antimicrobial activity than the other fabric treatments.

Comparatively, Bambara seeds stored in black polyethylene bags recorded the highest mortalities, least number of eggs oviposited, lowest number of eggs/seed ratio and the least F_1, F_2, cumulative emergence, respectively which differed significantly (P=0.05) with the seeds stored in other materials. Seeds stored in BPBs were also the least damaged with the lowest number of holes/seed, seeds with holes and least WPI, respectively.

This excellent storage ability could be as a result of the colour of the BPB materials. It has been stated that dark colours absorb, comparatively, more light and reflects very little. Since light is energy; the absorbed energy would necessarily increase the black material's temperature. Hence, dark colours become better radiators of heat which would, invariably, reflect on items stored with such materials [48, 49]. Heat generation through the use of black materials has been an age-long core method of storage pest control. In Sub-Saharan Africa, the method of spreading grains on dark paper or black polyethylene sheet whilst exposed to sunlight for at least seven hours, has proved effective in reducing bruchid infestation with minimal or no effect on grain quality and germination [50, 51, 52].

In an experiment to access the effect of solar heat and colour in the storage of adzuki bean (*Vigna angularis*) (Willd.) Ohwi and Ohashi against the seed beetle, *C. maculatus*, [53] found that square storage boxes painted black from inside trapped higher solar energy and that exposure of the various developmental stages of the bruchids to heat for up to 45 minutes raised the temperature between and within the seeds well in excess of the lethal level and resulted in complete control.

The performance of BPB was closely followed by seeds stored in White Polyethylene Bags (WPB) in all the parameters tested. Though white materials are poor retainers of heat, the HDPE polyethylene materials used in this study, with approximate wall thickness of 80 microns, could have eliminated oxygen from the stored seeds which could have led to asphyxiation, desiccation and death of the adult insects, their eggs, larvae and pupa. The action of the BPBs and WPBs could be compared to what happens when grains are stored in the Purdue Improved Cowpea Storage (PICS) bags [32]. Sealed PICS bags work, as do other hermetic storage containers, by excluding oxygen and raising the temperature of stored grains [54, 55].

Bagco Bag (BCB) materials recorded the lowest mortalities, highest number of eggs laid, total cumulative emergence (F_1 and F_2), highest number of holes per seed/ number of seeds with holes and highest weevil perforation index. Though Bagco bags (without the HDPE liners) are very popular with farmers in Nigeria – most probably because of the ease of procurement as used industrial discards, they have proven to be the least effective storage material. Bagco bags are tough, sisal - like polypropylene (PP) bags intrinsically woven, with air spaces, to mimic the traditional jute bags. They therefore, lack the 'hermetic' quality of polyethylene bags as the stored seeds remain well aerated – a conducive environment for bruchids to thrive. It should be noted that the tough outer woven 'Bagco – like' encasement of the PIC technology bags are primarily meant for ease of handling [32].

Other storage materials; plastic containers (PLC) and calico bags (CAB) had better storage quality than BCB materials but had more damaged seeds and higher WPI than those stored in BPBs and WPBs.

Conclusion and Recommendation

This study suggests impregnation of storage materials with neem seed oil could be a better option to direct application with its attendant offensive odour and bitter taste of treated seeds. The use of black polyethylene bags, within the limits of the storage materials screened, to control the damage by *C. maculatus* in stored Bambara seeds, should be encouraged. White polyethylene bags could be the next best option but storage with bagco bags (without the polyethylene lining) should be avoided. This work also suggests that the medium concentration (1.00) of NSO could be good enough to reduce the negative effect of *C. maculatus* on stored bambara seed.

It is recommended that further trials be carried out with normal - sized storage materials (50 - 100 kg bags and 100 – 200 kg plastic containers) to ascertain real situation applicability of this work.

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