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Research Article

The Insecticidal Activity of Neem and Palm Kernel Oils on Bean Weevil (*Callosobruchus maculatus* F.) (Coleoptera: Chrysomelidae: Bruchinae) Infestations of Stored Cowpea (*Vigna unguiculata* L. Walp)

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Even though it has been established that neem oil has insecticidal properties, its desirability as a plant-based insecticide is unsuitable owing to the sulfurous odour it deposits on the grain(s) that makes it bitter and impinges on its acceptability. For this reason, another oil of plant origin, palm kernel oil (PKO), was admixed with it to reduce the bitter taste and test for their bioinsecticidal potential on the insect pest of stored cowpea seeds. The general objective was to determine the combined effect of neem and palm kernel oils in controlling bean weevil infestations of stored cowpea. The results showed that the treated seeds recorded a significantly higher ($p < 0.05$) mortality of adult *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae), a reduction in oviposition, and a decrease in the number of emerged insects with the interval of treatment application, and the seeds that were treated had a significantly lower adult emergence hole ($p < 0.05$) and a reduction in % grain damage ($p < 0.05$), and the weevil perforation index (WPI) was <100%. The findings of this study suggest that admixing neem oil and palm kernel oil has the potential insecticidal efficacy of controlling the bean weevil of stored cowpea, and based on the quantity of palm kernel oil mixed with neem oil, the seeds treated tend to have varying levels of bitterness and sulfurous odour deposit.

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an annual seed legume within the pea family Fabaceae. Another name for cowpea is “black-eyed pea” or “Southern pea.” In tropical and subtropical regions, it is considered the most important food crop grown and widely consumed [1]. It is a major, cheap, and quality protein source for urban and rural African dwellers [2, 3]. In many regions, its production is restricted by several biotic and abiotic factors in the field and the harvested seeds in storage. Insect pests are among the constraining biotic factors in storage [4]. As Dubey et al. [5] reported, in temperate zones, insect infestation accounts for 5-10% of losses of stored seeds and 20-30% in tropical zones.

In West Africa, up to 100% losses of cowpea seeds can be incurred in storage in a few months when infestation surges [6], especially by the bean weevil—*Callosobruchus maculatus*. Its presence limits the storage lifespan of cowpea due to the eventual damage it causes. Researchers posit that the larval stage of this weevil makes tunnels and develops within the cowpea seeds [7, 8]. This weevil brings about decreased yield and increased quantitative and qualitative losses after harvest, which manifest as seed perforation, reduction in market value, viability loss of seeds, and weight reduction [9].

Locally, crop pest management is achieved by the use of plant extracts and plant-dried powders such as wood ash, lemongrass, clove, eucalyptus (Koul et al., 2008)[10], turmeric, ginger, neem extracts (cake, oil, bark, leaves, kernel, root, and seed), scale leaves of onions, chili peppers, and garlic [11] due to their insecticidal potentials. This system is beneficial because it poses little or no threat to the environment and the health of humans.

These plant materials used as pesticides to control the insect pest cowpea have relative advantages over synthetic chemicals; they are cheap and easily obtainable. Additionally, no particular skill is required in their usage, unlike the synthetic chemicals that require specialized skills, especially concerning calibration, which may pose a serious challenge to local farmers who do not have the basic skills [12]. Researchers [13, 14] add that some of these plant materials, particularly monoterpenes, are target-specific and biodegradable, and they can be practically used as a tool in programs related to integrated pest management.

Even though it has been established that neem oil has insecticidal properties, it is expensive, not readily sourced, and it is not widely accepted as a protectant against the cowpea weevil owing to the sulfurous odour that it deposits on the grain(s) that makes it bitter and impacts on the acceptability and marketability of treated produce [15]. For this reason, this study seeks to address this problem by admixing neem oil with another readily available oil of plant origin, palm kernel oil (PKO), to reduce the bitter taste that neem oil impinges on seeds and also to determine the combined toxicity of neem and palm kernel oils (at different concentrations) on bean weevil infestations of stored cowpea.

1.1. The Objective of the Study. The general objective of this study was to determine the combined effect of neem and palm kernel oils in controlling bean weevil infestations of stored cowpea.

2. Literature Review

2.1. Origin and Distribution of Cowpea. Cowpea (*Vigna unguiculata* L. Walp) is an annual grain legume within the pea family *Fabaceae*, genus *Vigna*, and species *unguiculata*. It originated in Africa and is widely spread across the tropics [16]. As Singh et al. [17] corroborate, in West Africa, cowpea production accounts for about 9 out of 14 million hectares of the world's total area planted with cowpea.

Cowpea is a very important grain (legume) that is chiefly grown and widely consumed in the tropics and subtropical regions of the world [18]. Singh et al. [17] and Gomez [19] emphasize that cowpea's economic importance is numerous; it is cheap and a major source of protein, vitamins, and minerals that farmers can harvest as nutritious fodder for their livestock. They further add that farmers can harvest it as seeds for consumption based on climatic and economic constraints.

2.2. The Cowpea Weevil: Callosobruchus maculatus. West Africa is known to be the place of origin of the cowpea weevil, and it is believed to have spread across the globe via the trading of legumes alongside other crops. Also, apart from its global spread through trading, this weevil has prevailed as an agricultural pest due to its tolerance for a high degree of inbreeding [20]. The cowpea weevil is sexually dimorphic; the females are easily distinguished from males. The sexes of the weevils can be visually determined by examining the anatomical (elytra, shape of the abdomen) pattern. The female of this species is dark-coloured with four elytral spots, while the male is pale brown and less distinctly spotted. Also, the plate that covers the end of the abdomen in females is large and dark-coloured along the sides but smaller without the dark areas in males [21].

2.3. Botany and Economic Importance. Taxonomically, the bean beetle is placed in the family Chrysomelidae: Bruchinae, genus *Callosobruchus*, and species *C. maculatus*. The bean weevil is a major and common field-to-storage agricultural pest of economically essential legumes, especially cowpea, in Tropical Africa [22, 23], and it is widely known as an essential pest of pulse crops in Africa and Asia under storage conditions [24, 25].

2.4. Control of Cowpea Weevil Using Plant Materials. Plant extracts and plant-dried powders that have insecticidal potentials and pose little or no threat to the ecosystem and the health of humans have been locally employed with varying levels of effectiveness in the management of crop pests such as neem oil, wood ash, lemon grass, ginger, and garlic [11]. Their insecticidal activities have been variously studied by different scholars, but over the years, the bean weevil has been chemically controlled by using synthetic insecticides [1]. Pirimiphos methyl and permethrin are effective synthetic insecticides when used against *C. maculatus*, but the hazards associated with their usage reduce their desirability [4]. Also, Ilesanmi and Gungula [26] aver that Phostoxin tablet, a fumigant, effectively protects cowpea seeds in storage against insect pests. Still, it is not readily sourced and is harmful to man and livestock.

More so, the use of various synthetic insecticides in the protection of cowpea seeds against insect attacks has not been successful because it has caused a lot of environmental setbacks, including pest resurgences, development of resistance to pesticides, lethal effects to nontarget organisms in the ecosystem, and toxic residues in food and water bodies. It may also cause direct toxicity to users [27]. All these shortfalls can be prevented by using plant-based insecticides, which are ecologically safe, cost-effective, and user-friendly [28].

There are numerous hazards associated with the use of synthetic chemicals, and these hazards are expressed based on toxicity. These hazards that are associated with the use of synthetic chemicals are a result of exposure via inhalation of droplets of chemicals and dermal contact with contaminated hands or food [29], which could have hazardous effects on users such as neurotoxic effects [30], mutagenic effects (changes in the gene or the chromosome), endocrine defects, cataract formation, oncogenic effects (production of tumours), and carcinogenic effects (production of cancer) [31].

In light of the above, it is imperative to adopt a safer insect control method as an alternative replacement for synthetic insecticides. As such, the adoption of an integrated pest management system based on the use of botanicals (insecticides derived from plants) has been employed in this study. Botanicals have advantages over synthetic chemicals in many ways. Weinzierl [32] and Scott et al. [33] posit that botanicals are of relatively lower risk to nontarget organisms; they are nontoxic to mammals; and environmentally, they do not persist because they rapidly break down and are metabolized easily by animals receiving sublethal doses [34]. Scholars [35] posit that they are more economical and more effective. The study of Dunkel et al. shows that neem oil and palm kernel oil in combination are effective as storage protectants against the cowpea weevil, beetles, and grain borers.

2.5. Analysis of the Chemical Composition of Neem Oil. Using neem oil as an insect protectant has proven effective due to neem's primary active insecticidal ingredient, azadirachtin. It is a chemical compound that belongs to the limonoid group and is a secondary metabolite found in neem seeds. It has a chemical formula of $C_{35}H_{44}O_{16}$ and a molar mass of 720.721 $g{\cdot}mol^{-1}$. It has a broad spectrum of activity and works as a repellant by disrupting the hormones involved with insect moulting, thereby preventing insect larvae from developing properly into adults. It also prevents oviposition and is a feeding inhibitor [36]. It does this by disrupting the bruchids' sense of smell so that they can avoid ingesting azadirachtin-coated cowpea seeds that can inhibit the functions of their digestive enzymes [37].

The neem oil is obtained by cold-pressing the seed kernels of the neem. The oil extracted from the seed of the tree can be used directly as an insect and mite repellant, an insecticide, a fungicide, and an antifertility agent [38] and is the source of many commercial pesticide products such as dusts, granules, and concentrates because it contains azadirachtin which has a broad spectrum of activity [36]. Neem oil also contains disulphide, which contributes to its bioactivity. Interestingly, azadirachtin has been proven to have a low acute mammalian toxicity [39, 40]. Environmentally, Boursier et al. [41] assert that it does not persist due to its fast biodegradation in sunlight.

Palm kernel oil is the edible plant oil that is derived from the kernel of the oil palm tree (*Elaeis guineensis* Jacq.), and it has a wide amplitude of usage; it is used in industries (cosmetics) and in pharmaceuticals, as a biofuel and, more recently, as a biopesticide against storage pests as evidenced in the previous studies [42, 43]. Oils such as soybean, groundnut, olive, and palm have also been recently exploited for their protectant ability against storage pests [44, 45]. All these findings align with other research works conducted to test the insecticidal potential of plant extracts of different plants against storage insect pests. Based on the literature above, the researchers tested the following hypotheses:

H1. Phostoxin causes high mortality of adult *C. maculatus* in storage

H2. Neem and palm kernel oils admixed have ovicidal properties

H3. P_t and N_0P_0 encourage the emergence of adult *C. maculatus*

3. Materials and Methods

This study was conducted at the Department of Crop Science Teaching Laboratory, Faculty of Agriculture, University of Nigeria, Nsukka. This experiment was laid on a laboratory bench in a completely randomized design (CRD) with three replications. This study was conducted at an average temperature of 27.9°C (82.2°F) and a relative humidity of 77%.

The bean weevil used for this experiment was naturally sourced from an already infested stored cowpea which was kept in a transparent white container with a white lid for an efficient light interception to breed sufficient bruchid that was needed to carry out this experiment. The breeding container had twenty (20) perforations around it, and a circular cut was made in the middle of the lid (using a red-hot knife). It was covered with muslin cloth to facilitate aeration, mating, and reproduction.

The cowpea (California black-eye cowpea—subspecies of cowpea) used to carry out this research work was locally sourced from Ogige, Nsukka main market, Enugu State, Nigeria. After purchase, the seeds were thoroughly handpicked to eliminate contaminants, dry pods, and damaged seeds.

To disinfect, the clean seeds that have been selected were sun-dried for 72 hours (3 days) at 75°F and stirred several times a day to ensure even drying and eliminate all forms of incipient infestation that may be inherent in the cowpea seeds as well as to ensure zero infestation of the cowpea seeds before experimenting.

After disinfestation, 20 g of wholesome, uninfested cowpea seeds were accurately weighed out using a professional miniscale (digital pocket scale) into each of the uniform, blue-coloured cylindrical plastic experimental containers with a white lid that were used to carry out this experiment. Each plastic container had nine (9) uniform perforations that were made with a red-hot 1-inch nail and were covered with a well-fitted muslin cloth to provide adequate aeration and prevent the escape of adult bruchid upon introduction. The experimental containers were well labelled using masking tape and coloured markers (blue and red). The introduced adult bruchid and uninfested cowpea seeds were then allowed to stay until the emergence of the first filial generation (F_1) that was used to carry out this experiment. The sexes of the emerged bean weevils were determined visually by examining the anatomical (elytra and shape of the abdomen) pattern.

Cold-pressed neem oil that was used to carry out this experiment was sourced from Royal Neem Company, Gudu District, Abuja, Nigeria while the hot-pressed palm kernel oil (PKO) that was used to carry out this experiment was sourced from Ugo Tech. Industries Limited, Emene, Enugu, Nigeria.

Using a 5 ml calibrated syringe, neem oil (N) was measured at 0 ml, 1 ml, and 2 ml. Palm kernel oil (PKO) was also measured out using a 5 ml calibrated syringe at 0 ml, 1 ml, and 2 ml, while neem oil (N) and PKO were admixed in different ratios (in three different plastic mixing containers) as follows, 1N : 1P, 1N : 2P, and 1N : 3P (using 10 ml and 20 ml syringes), and measured out at different rates of 0 ml, 1 ml, and 2 ml. Phostoxin tablet was applied at 0.2 g/kg cowpea seed.

With the aid of the digital pocket scale, 20 g each of the cowpea seeds that had been disinfested was weighed out into the experimental plastic containers that had nine (9) perforations and were covered with the well-fitted muslin cloth. The different rates of neem oil and palm kernel oil treatments were carefully measured using clean and appropriately calibrated syringes of 5 ml, 10 ml, and 20 ml. Mixing containers were employed to properly mix the oils before applying them to the different rates of treatments using clean syringes of 1 ml and 2 ml, while a Phostoxin tablet was applied at the rate of 0.2 g/kg cowpea seeds to the breeding containers labelled Phostoxin, and nothing was applied to the containers labelled N_0P_0.

After adding the oil treatments, the containers were thoroughly shaken and randomly placed on a laboratory bench for observations in a completely randomized manner. Five adult male and five adult female bruchids were anatomically selected from the transparent insect culture container, where the bruchid was bred and introduced into the perforated experimental containers.

The treatment combinations were represented as follows: N_0P_0 = no neem, no PKO; N_1 = neem at 1.0 ml; N_2 = neem at 2.0 ml; $1N_1:1P_1$ = 10 ml neem+10 ml PKO at 1.0 ml; $1N_2:1P_2$ = 10 ml neem+10 ml PKO at 2.0 ml; $1N_1:2P_1$ = 10 ml neem+20 ml PKO at 1.0 ml; $1N_2:2P_2$ = 10 ml neem+20 ml PKO at 2.0 ml; $1N_1:3P_1$ = 10 ml neem +30 ml PKO at 1.0 ml; $1N_2:3P_2$ = 10 ml neem+30 ml PKO at 2.0 ml; P_1 = PKO at 1.0 ml; P_2 = PKO at 2.0 ml; and P_t = Phostoxin tablet at 0.2 g/kg cowpea grain.

3.1. Data Collection. Data on the effect of bean weevil on the cowpea were collected as follows:

(1) Effect of treatments on the mortality of adult bruchids

This was done by counting, recording, and discarding the dead bruchids in each container at 1, 2, and 7 days after the commencement of the experiment. The insects were probed to confirm that they were dead using an office pin. The percent mortality was calculated using the following formula: percent mortality = (number of dead insects/total number of insects) × (100/1).

(2) Effect of treatments on the oviposition of adult *C. maculatus*

This was obtained by counting the number of eggs laid on ten (10) randomly selected seeds per container at 7, 14, 21, 28, 35, 42, and 49 days after infestation (DAI).

(3) Effect of treatments on the emergence of adult *C. maculatus*

This was obtained by carefully observing and counting the number of emerged adults (dead and living) on the 1st, 2nd, 3rd, and seventh days after infestation (DAI).

(4) Effect of treatments on emergence hole count, % damaged seeds, and weevil perforation index at 10 weeks after infestation

(i) The total number of emergence hole count: the total number of emergence holes per seed from ten (10) randomly selected seeds at 10 weeks after infestation (WAI) was counted.

(ii) %damaged seeds = (total number of sampled grains perforated/total number of sampled grains) × (100/1)

(iii) Weevil perforation index (WPI): the weevil perforation index was calculated as WPI = (total number of treated grains perforated/total number of infected grains perforated) × (100/1)

From the formula above, a weevil perforation index that is greater than 50% indicates the enhancement of infestation by the weevil or a negative ability of the plant material or insecticide(s) tested while a weevil perforation index that is less than 50% implies a positive protection of the seeds by the protectant (plant material). All the data collected were subjected to analysis of variance (ANOVA) according to the procedure outlined for completely randomized design (CRD). The detection of differences among treatment means for significance was done with Fisher's least significant difference (F-LSD) at a 5% probability level.

4. Results

Table 1 shows the effect of the different treatments at different dosage rates on the mortality rate of adult *C. maculatus* at 1, 2, and 7 days after infestation. The treatments had a significant effect ($p < 0.05$) on the mortality assessed for the study. From the result obtained, the botanical treatments have mortality potentials. However, Phostoxin (P_t) had a significantly high mortality effect, while the treatment N_0P_0 had the least significant effect on mortality, and this increased with time. This result supports this study's first hypothesis, which states that Phostoxin causes high mortality of adult bruchids.

Table 2 shows the effect of the different treatments at different dosage rates on the oviposition of adult *C. maculatus* at 7, 14, 21, 28, 35, 42, and 47 days after infestation. The treatment N_0P_0 successively recorded the highest oviposition (egg laying) of adult bruchid, while the botanical treatments reduced the oviposition of adult *C. maculatus*. This result agrees with this study's second hypothesis that neem and palm kernel oils admixed have ovicidal properties.

Table 3 shows the effect of the different treatments at different dosage rates on the emergence of adult *C. maculatus* at 1, 2, 3, 4, 7, 70, 77, and 84 days. All the treatments tested had no significant effect on the emergence of adult *C. maculatus* at 1, 2, 3, 4, and 7 DAI except for the treatment N_0P_0, which recorded an increasingly high adult emergence at 70, 77, and 84 DAI, and the treatment P_t also recorded a high adult emergence at 70, 77, and 84 DAI. This implies that both P_t and N_0P_0 encouraged the emergence of adult *C. maculatus* in stored cowpea seeds which can bring about qualitative and quantitative losses of stored seeds with time. This result aligns with the third hypothesis, which says that P_t and N_0P_0 encourage the emergence of adult *C. maculatus*.

Table 1: Effect of treatments on adult bruchid mortality count (%).

Treatments	N_0P_0	$1N_1:1P_1$	$1N_1:2P_1$	$1N_1:3P_1$	$1N_2:1P_2$	$1N_2:2P_2$	$1N_2:3P_2$	N_1	N_2	P_1	P_2	P_t	Mean	F-LSD (0.05)
1 DAI	2.83	6.86	7.56	6.08	4.86	6.33	7.31	6.05	7.11	5.75	7.33	9.15	6.435	1.390
2 DAI	4.43	7.30	8.78	6.33	6.33	7.08	7.97	6.55	7.78	6.33	7.56	9.33	7.148	1.206
7 DAI	4.86	8.59	9.15	8.78	7.95	8.78	8.78	8.57	9.13	8.59	8.57	9.85	8.466	0.916

DAI = days after infestation. Data represent square root transformed values.

Table 2: Effect of treatments on the oviposition of adult *C. maculatus* (DAI).

Treatments	N_0P_0	$1N_1:1P_1$	$1N_1:2P_1$	$1N_1:3P_1$	$1N_2:1P_2$	$1N_2:2P_2$	$1N_2:3P_2$	N_1	N_2	P_1	P_2	P_t	Mean	F-LSD (0.05)
7	2.39	0.71	0.71	0.88	0.71	0.71	0.71	0.71	0.88	0.88	0.88	1.29	0.96	0.653
14	2.39	0.88	0.71	0.88	0.71	0.71	0.88	0.71	0.71	0.88	0.88	0.88	0.94	0.435
21	3.45	1.17	1.30	0.88	0.71	0.88	1.00	1.17	0.71	1.17	1.30	1.56	1.27	0.902
28	5.17	1.29	0.88	1.74	1.71	0.88	1.65	1.44	1.00	1.34	1.17	1.87	1..63	1.583
35	5.56	1.05	0.88	1.05	1.05	0.88	1.00	1.17	0.88	1.34	1.05	1.52	1.45	2.043
42	6.09	0.88	1.35	1.34	1.39	0.71	1.00	1.00	0.71	0.71	1.17	1.34	1.47	2.408
49	8.99	0.88	1.05	1.34	0.88	1.05	1.34	0.71	0.88	0.71	0.88	1.17	1.66	3.732

DAI = days after infestation. Data represents square root transformed values.

Table 3: Effect of treatments on the emergence of adult *C. maculatus* (DAI).

Treatments	N_0P_0	$1N_1:1P_1$	$1N_1:2P_1$	$1N_1:3P_1$	$1N_2:1P_2$	$1N_2:2P_2$	$1N_2:3P_2$	N_1	N_2	P_1	P_2	P_t	Mean	F-LSD (0.05)
1	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	NS
2	0.71	0.88	0.71	0.71	0.88	0.71	0.88	0.71	0.71	0.71	0.71	0.71	0.76	NS
3	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	NS
4	1.05	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.74	NS
7	0.85	0.71	0.71	0.71	0.71	0.88	0.71	0.71	0.88	0.71	0.88	0.71	0.77	NS
70	8.22	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	1.17	1.37	NS
77	8.48	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	1.05	1.39	NS
84	8.56	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	1.05	1.39	NS

DAI = days after infestation; AE = adult emergence. Data represents square root transformed values.

Table 4 shows the effect of the different treatments on emergence hole count (EHC), percentage damaged seeds (% DG), and weevil perforation index (WPI). From Table 4, at 10 WAI, the control group (N_0P_0) statistically recorded the highest EHC followed by P_t, and the treatment means are different from each other ($p < 0.05$). All the treatments tested had a significant effect on the EHC of adult *C. maculatus*. The % DG was higher in the control group with a value of 59.5%, and all the treatments tested had a significant effect on the %DG. With regard to WPI, the highest statistical value was recorded in N_0P_0 (90.0) which shows a negative ability of the protectant tested. The treatments N_1 and P_1 recorded the least statistical value of 28.6%, implying that the treatments were able to protect the cowpea seeds against *C. maculatus* infestation in storage at 10 weeks after infestation.

Table 5 shows the sulfurous odour/bitterness level of the seeds treated with different dosage rates of the oils tested in this study. From Table 5, the seeds treated with $1N_2:3P_2$ and $1N_1:3P_1$ (10 ml neem+30 ml PKO at 2.0 ml and 10 ml neem +30 ml PKO at 1.0 ml) recorded low bitterness and reduced sulfurous odour. This reduced sulfurous odour/low bitterness level can be attributed to the increased quantity of palm kernel oil that was admixed with neem oil. The seeds treated with $1N_2:2P_2$ and $1N_1:2P_1$ (10 ml neem+20 ml PKO at 2.0 ml and 10 ml neem+20 ml PKO at 1.0 ml) were moderately bitter as the quantity of palm kernel oil admixed with neem oil was reduced by 10 ml. With an equal quantity of palm kernel oil admixed with neem oil, the seeds treated with $1N_2:1P_2$ and $1N_1:1P_1$ (10 ml neem+10 ml PKO at 2.0 ml and 10 ml neem+10 ml PKO at 1.0 ml) had a high sulfurous odour/bitterness level while the seeds that were treated with N_2 and N_1 (neem at 2.0 ml and neem at 1.0 ml) had a very high level of sulfurous odour/bitterness level. This is because the oil was not admixed with another oil that has the tendency to neutralize its bitterness like palm kernel oil.

5. Discussion

Although certain plant powders and plant extracts have been found to have insecticidal potentials over the years, Pavela

Table 4: Effect of treatments on emergence hole count, % damaged seeds, and weevil perforation index at 10 weeks after infestation.

Treatments	N_0P_0	$1N_1:1P_1$	$1N_1:2P_1$	$1N_1:3P_1$	$1N_2:1P_2$	$1N_2:2P_2$	$1N_2:3P_2$	N_1	N_2	P_1	P_2	P_t	Mean	F-LSD (0.05)
EHC	3.88	0.88	0.88	0.71	0.88	0.88	0.88	0.71	0.88	0.71	0.71	1.05	1.09	1.355
%DG	59.5	9.5	11.0	11.5	9.5	9.5	10.0	7.6	9.6	7.8	10.9	12.4	14.1	20.16
WPI	90.0	30.5	31.9	32.6	30.7	30.7	30.7	28.6	42.0	28.6	31.7	33.7	36.8	16.13

EHC = emergence hole count; %DG = percentage damaged seeds; WPI = weevil perforation index. Data represents square root transformed values.

Table 5: The sulfurous odour/bitterness level of the seeds treated with different dosage rates of the oils tested.

Sulfurous odour/bitterness level	Treatments	The dosage rates of tested oils
Low	$1N_2:3P_2$ and $1N_1:3P_1$	10 ml neem+30 ml PKO at 2.0 ml and 10 ml neem+30 ml PKO at 1.0 ml
Moderate	$1N_2:2P_2$ and $1N_1:2P_1$	10 ml neem+20 ml PKO at 2.0 ml; 10 ml neem+20 ml PKO at 1.0 ml
High	$1N_2:1P_2$ and $1N_1:1P_1$	10 ml neem+10 ml PKO at 2.0 ml and 10 ml neem+10 ml PKO at 1.0 ml
Very high	N_2 and N_1	Neem at 2.0 ml and neem at 1.0 ml

[46] believes that their level of effectiveness has not been well assessed. From the results obtained in this study, Phostoxin caused the highest mortality of adult bruchids. However, it is not a readily available cowpea protectant against the cowpea weevil, and it is toxic to man and eco-unfriendly. Also, the different rates of the botanical oils tested were able to cause the death (mortality) of adult bruchids. It was observed that the mortality of adult bruchids increased with time (7 DAI>2 DAI>1 DAI) following the application of the treatments, and this corresponds with the mortality result obtained by Boulahbel et al. [36], whereas the control group N_0P_0 did not cause significant death of adult bruchids at the various intervals tested. It gave the least insecticidal protection of the cowpea seeds stored. Overall, the mortality rate of adult *C. maculatus* was significantly higher in treated cowpea seeds than in untreated cowpea seeds.

As shown in the result obtained for the effect of the treatments on the oviposition of adult bruchids, both neem oil and palm kernel oils at 1 ml (N_1 and P_1) significantly inhibited oviposition of adult bruchids while the control group (N_0P_0) encouraged oviposition at all the intervals tested. This implies that the botanical oils effectively prevent adult bruchids from laying eggs even at low concentrations (1 ml). This is in concordance with the result obtained by Akami et al. [47] when they tested the synergistic effects of wood ash and essential oils on the fecundity, pupal occlusion, and adult mortality of the cowpea seed weevil. The result they obtained revealed that wood ashes from the French digbe plant (*Hymenocardia acida*) and the dwarf red ironwood plant (*Lophira lanceolata*) and the essential oils of the lippia plant *(Lippia adoensis)* could be used to reduce *C. maculatus* infestation of stored cowpea. They equally assert that the essential oil had the inherent potential of preventing oviposition and progeny production of *C. maculatus* even at low concentrations.

Based on adult emergence, the botanical oils tested had adulticidal potential, and the different ratios of the oils tested considerably inhibited the emergence of adult bruchids unlike the treatments N_0P_0 and P_t that encouraged adult emergence of adult bruchids. This corresponds with the result obtained by Oni and Ogungbite [48] and Uddin II and Sanusi [49]. Uddin II and Sanusi tested the efficacy of three plant origin oils and palm kernel oil in the control of *Callosobruchus maculatus* (F.) in stored cowpea (*Vigna unguiculata* L. Walp) and found out that all the oils tested, especially palm kernel oil, were more effective against the F_1 progeny emergence. This is because the oils tend to flood the spiracles of the bruchids, thereby causing asphyxiation, which prevents adults from emerging. This is consistent with the result obtained by Durojaye et al. [50]. Based on the results of this study, it has been shown that both neem and palm kernel oils have insecticidal potentials to be used as biopesticides of stored insect pests of cowpea seeds. Even though the fumigant, Phostoxin tablet, was effective in protecting cowpea seeds in storage against insect pests, it is not recommended for storing cowpea seeds owing to its negative residual impacts and unavailability and the difficulty in sourcing it, as well as the harm it causes to man and livestock.

Concerning the percentage of seed damage of stored cowpea seeds, grain damage was higher in untreated seeds (59.5%) than in treated seeds (<12.4%) at 10 weeks after infestation. On the evaluation of the weevil perforation index (WPI), the control group (N_0P_0) recorded the highest perforation (least protection) of the stored cowpea seeds (90%) while the botanical oils, especially N_1 and P_1, caused the least seed perforation (28.6%) at 10 weeks after infestation. This portends a positive protectant ability of the botanical oils tested against the stored insect pest.

Moreso, the seeds treated with the oils tested at different dosage rates recorded varying levels of bitterness/sulfurous odour deposit. From Table 5, it can be seen that the higher the quantity of palm kernel oil admixed with neem oil, the lesser the bitterness level/sulfurous odour that is deposited on the seeds and vice versa. Researchers [15] emphasize that the major reason why neem oil is not widely accepted as a storage pest bioinsecticide is due to its bitter taste and sulfurous odour. This then implies that even though neem oil has been confirmed to have insecticidal properties, it is still recommended that higher quantities of plant origin oil should be admixed with it to overcome the sulfurous odour that neem oil deposits on the seeds that make it bitter and impact the acceptability and marketability of the treated seeds.

Although this study has revealed the synergistic interactions between the different proportions of the tested oils resulting in toxicity and a reduction in the number of eggs deposited, it has limitations, and one of such limitation is that other experiments like sublethal experiments, repellence, antifeedant, and fertility tests need to be added. In the second place, the GC-Ms of the oils used were not shown. It is recommended that future studies in this direction should include the GC-Ms of the oils used or the company's chemical composition of the oils where it is procured. In the third place, a Phostoxin tablet was used in this study for disinfecting the cowpea seeds and also served as one of the treatments in this study. It is recommended that other disinfecting methods such as using sodium hypochlorite (NaOCl) or calcium hypochlorite solution can be employed.

Abbreviations

PKO: Palm kernel oil
N: Neem oil
DAI: Days after infestation
AE: Adult emergence
EHC: Emergence hole count
%DG: Percentage damaged grains/seeds
WPI: Weevil perforation index.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no known financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Prof. B. C. Echezona of the Department of Crop Science, University of Nigeria, Nsukka, contributed to the design and acquisition of materials for this study. He also worked on the analysis and data interpretation while Mrs. Gever Esther Rita of the Department of Crop Science, University of Nigeria, Nsukka, drafted and revised this work critically for important intellectual content.

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