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Research Article

Preservation of Cowpea (*Vigna unguiculata* (L.) Walp) *Grains* against Cowpea Bruchids (*Callosobruchus maculatus*) Using Neem and Moringa Seed Oils

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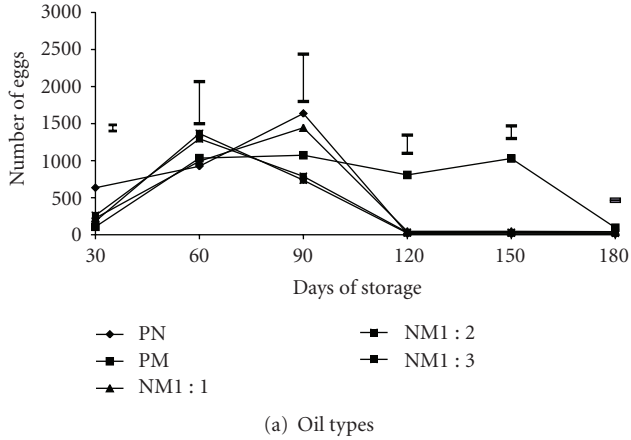
This study was conducted to determine the effects of neem (*Azadirachta indica* A. Juss) and moringa (*Moringa oleifera*) seed oils on the storability of cowpea grain. Cowpea samples were treated with various concentrations (0.5, 1.0, and 1.5 mL/200 g cowpea) of pure neem and moringa oils and their mixtures in ratios of 1:1, 1:2, and 1:3. The treated cowpea samples were stored for 180 days. Data were collected every 30 days on number of eggs laid, total weevil population, and percentage of uninfested grains and analysed statistically. Significantly different means were compared using LSD at $P < .05$. Increasing oil concentration resulted in better cowpea protection, for example, in oviposition where the control had 6513 eggs, only 8 eggs were recorded in pure neem oil-treated sample at 0.5 mL/200 g. Generally, better results were obtained with higher oil concentrations either in their pure forms or mixtures. The control had a total weevil population of 4988, while most treated samples had none. The control samples had 0% uninfested grains, while 73–94% of uninfested grains were observed in treated samples after 6 months of storage. Therefore, mixture of the oils at 1.5 mL/200 g can be effectively used to store cowpea.

1. Introduction

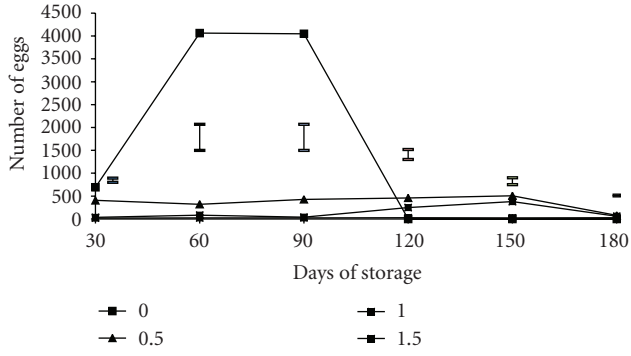
Cowpea *(Vigna unguiculata* (L.) Walp), one of the grains that suffer postharvest losses most, is a warm season, annual, herbaceous legume. It suffers heavily from insects, both in the field and when grains are stored after harvest. Yield reductions caused by insects can reach as high as 95%, depending on location, year, and cultivar [1].

The main pests during the growing season are the aphids while the main storage pests are the bruchids. The primary insect causing losses to stored cowpeas in West Africa is the cowpea weevil, *Callosobruchus maculatus*. Infestation begins in the field at low level. After the crop is placed in storage, the insect population continues to grow until the cowpea is completely damaged [2]. Another bruchid pest of cowpea is *Bruchidius atrolineatus*. This insect causes losses primarily around harvest times and does not reproduce in storage [3]. A single female weevil can reproduce herself 20-fold every 3-4 weeks. Harvested cowpea grains with a very light infestation will have a heavy infestation within 2-3 months [1].

Although insecticides are widely available, they require expensive equipment and training for their use. They are expensive, polluting, and potentially dangerous to users. Consequently, many cowpea growers in Africa do not use insecticides because they cannot obtain them; they cannot afford them; they do not have the necessary equipment or they are not taught how to apply them properly. That is why conventional insecticides are not the answer to the insect problems [1]. Insecticides, especially the dust and gaseous forms are recommended for short-term storage. The product Actellic (2%) or Actellic super and Phostoxin gas are very helpful to the farmer, but they are expensive and may not be available everywhere. Phostoxin is a fumigant that can kill humans and animals [3]. In the light of the above, indigenous preservatives are urgently required which will

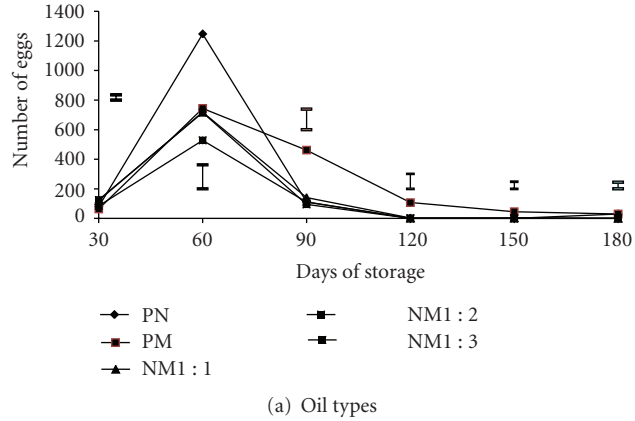


(a) Oil types

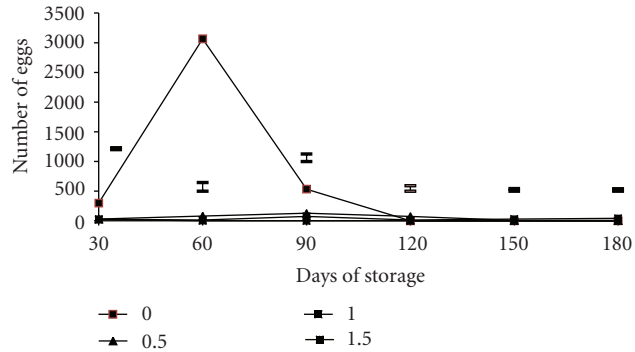


(b) Oil concentrations

Figure 1: Effect of oil types and concentrations on number of eggs laid by *C. maculates* in stored cowpea. Vertical lines are LSD bars at $P = .05$.



(a) Oil types



(b) Oil concentrations

Figure 2: Effect of oil types and concentrations on total weevil population in cowpea storage. Vertical lines are LSD bars at $P = .05$.

be indigenous to poor resource farmers, consumers, and distributors. Green plants act as a reservoir for inexhaustible source of innocuous pesticides, which are mammalian, non-toxic, and easily biodegradable than synthetic chemicals.

This study was carried out to evaluate the effects of neem and moringa seed oils on the storability of cowpea.

2. Methodology

The study was conducted in the Food Science and Technology Laboratory of the Federal University of Technology, Yola. The neem and moringa seeds used were either handpicked or plucked on the university campus, while part of the moringa seeds was obtained from settlements near the university. The cowpea grains used for the study were harvested from a farm on the same campus.

Oils extracted from neem and moringa seeds were used to treat the cowpea grains so as to test the efficacy of the oils against *Callosobrucus maculatus* on the cowpea grains in storage. The test insects used for the experiment were adults of the cowpea weevils that were obtained from highly infested grain bought from Jimeta market in Adamawa State, Nigeria and reared on uninfested cowpea grains in a ventilated chamber. The containers used in rearing the insects were plastic containers measuring 17 cm by 17 cm diameter and depth, respectively. Each container was covered with 10 mm mesh sieve to allow free air circulation and also to prevent insects from escaping. This was carried out at ambient temperature of 28°C (±2) and relative humidity of between 70–75% [4]. This was left for 10 days, at the end of which the weevils in the cowpea were sieved out and then left for another 24 hours after which they were harvested and used.

The treatments which comprised of pure neem oil, pure moringa oil, neem-moringa 1 : 1, neem-moringa 1 : 2, and neem-moringa 1 : 3 all at concentrations of 0.5, 1.0, and 1.5 mL/200 g cowpea grains were arranged in Randomized Complete Block Design (RCBD) and replicated 3 times. For each replicate, 200 grams of healthy, fresh, clean, and unbroken cowpea grains were placed in clean, dry plastic bowls measuring 5.5 cm deep by 8 cm wide and treated with 0.5, 1.0, and 1.5 mL of pure moringa oil, pure neem oil, and mixtures of neem and moringa oils in ratios 1 : 1, 1 : 2, and 1 : 3 of neem and moringa seed oils, respectively. This ration was used in order to reduce the bitter taste of neem oil on the cowpea while still maintaining its insecticidal properties, moringa oil which is tasteless and has benhenic acid (antifungal) was used to dilute the neem oil in ratios 1 : 1, 1 : 2, and 1 : 3 The oil was pipetted into a conical flask containing the 200 g cowpea grains and was shaken vigorously to ensure uniform exposure of grains to the oil.

Table 1: Interaction of oil types and various concentrations of the oils on number of eggs laid by *C. maculatus* on cowpea samples after different storage periods.

Oil type	Oil concentration (mL)						
	0.0	0.5	1.0	1.5	Mean	LSD at 0.05	Prob. of F
		30 Days					
PN	686.00	10.00	23.00	10.00			
PM	283.00	105.00	25.00	15.00			
NM1 : 1	891.00	37.00	66.00	25.00			
NM1 : 2	955.00	34.00	34.00	22.00			
NM1 : 3	649.00	34.00	27.00	26.00	190.90	185.80	<0.001
		60 Days					
PN	3667.00	10.00	20.00	8.00			
PM	2401.00	1474.00	247.00	15.00			
NM1 : 1	3787.00	55.00	80.00	47.00			
NM1 : 2	5073.00	38.00	36.00	33.00			
NM1 : 3	5387.00	32.00	34.00	15.00	1123.00	1135.70	0.001
		90 Days					
PN	6513.00	8.00	19.00	8.00			
PM	2263.00	1986.00	29.00	12.00			
NM1 : 1	5607.00	64.00	63.00	45.00			
NM1 : 2	3004.00	51.00	57.00	40.00			
NM1 : 3	2862.00	36.00	30.00	21.00	1135.90	1275.50	<0.001
		120 Days					
PN	0.00	11.00	32.00	16.00			
PM	0.00	2137.00	1078.00	16.00			
NM1 : 1	0.00	77.00	67.00	42.00			
NM1 : 2	0.00	48.00	39.00	46.00			
NM1 : 3	0.00	34.00	33.00	25.00	185.05	491.20	<0.001
		150 Days					
PN	0.00	14.00	20.00	8.00			
PM	0.00	2364.00	1737.00	18.00			
NM1 : 1	0.00	88.00	62.00	38.00			
NM1 : 2	0.00	38.00	50.00	39.00			
NM1 : 3	0.00	36.00	31.00	22.00	228.25	338.00	<0.001
		180 Days					
PN	0.00	9.70	16.00	12.30			
PM	0.00	248.00	123.30	7.70			
NM1 : 1	0.00	55.30	70.70	45.70			
NM1 : 2	0.00	42.70	53.70	45.30			
NM1 : 3	0.00	29.00	29.30	22.00	40.54	61.94	<0.001

PN = pure neem.
PM = pure moringa.
NM 1 : 1 = neem-moringa oil in ratio 1 : 1.
NM 1 : 2 = neem-moringa oil in ratio 1 : 2.
NM 1 : 3 = neem-moringa oil in ratio 1 : 3.

This was then transferred into the bowl according to the method of Singh et al. [5]. Ten male and ten female *C. maculatus* (0–24 hours old) starved for 24 hours were added to each set of treated grains. Each container was covered with 10 mm-mesh muslin cloth to allow free air circulation and also to prevent insect from escaping. The control group was left untreated. All these were kept at ambient temperature (28 + 2.5°C) for six months.

2.1. Data Collection and Analysis. Data were collected on weevil population, percentage of uninfested grains and number of eggs laid by *C. maculatus* on monthly intervals for six months. The total insect population was determined by counting the live and dead insects. Uninfested grains were counted to determine percentage of uninfested grain at each treatment. Uninfested grains were considered to be those with no visible surface damage. Data in each observational

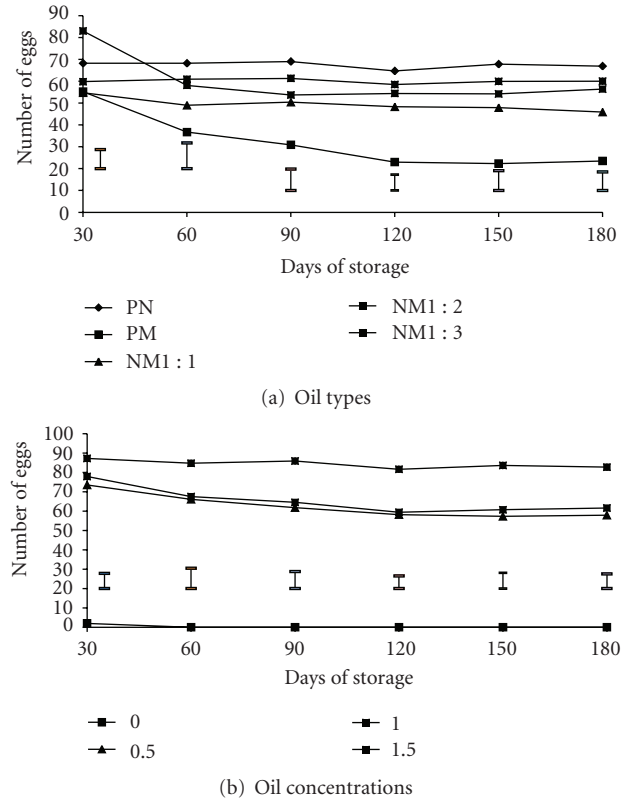


Figure 3: Effect of oil types and concentrations on the percentage of uninfested cowpea stored for 6 months. Vertical lines are LSD bars at $P = .05$.

date were statistically analyzed using analysis of variance to ascertain the significant differences between means. Least significant difference [5] was used to separate treatment means that were significantly different.

3. Result

3.1. Effects of Oil Types and Mixture on Oviposition of C. maculatus. Table 1 shows the mean numbers of eggs laid by *C. maculatus* on cowpea grains protected using various oil types for a period of 180 days. The result shows no significant difference ($P < .05$) in the activities of the oils in the first two months, but indicated significant difference ($P < .05$) in the third month between the oil types with the least number of eggs obtained from neen-moringa oil blend in the ratio of 1 : 3 and neem moringa oil in ratio 1 : 2 (737 and 788 eggs, resp.). Pure neem oil had the highest number of eggs (1637) at the third month. There was a sharp reduction in oviposition by *C. maculatus* within the remaining three months for all the oil types except for pure moringa oil-treated samples that had the highest ($P < .05$) mean number (>800) of oviposition. Pure neem oil however appeared to be the best in reducing oviposition especially within the last three months of storage with less than 16 eggs laid, while pure moringa oil was the least with up to 1030 eggs in the fifth months of storage. After 6 months of storage, pure neem oil-treated samples had the least number of eggs (9.5). This was not significantly different from neen-moringa oil in ratio 1 : 3 and neen-moringa oil in ratio 1 : 2. The least effective oil type appeared to be pure moringa with mean number of 94.7 eggs.

Table 2 shows the mean number of eggs laid by *C. maculatus* on cowpea grains treated with various concentrations of oils for a period of six months. All rates of application significantly ($P < .05$) reduced oviposition by *C. maculatus* when compared with the control in the first 90 days. The oils applied at a concentration of 1.5 mL/200 g cowpea had the least ($P < .05$) oviposition (<27 eggs laid) compared to the other concentrations, while oils applied at 0.5 mL/200 g were least effective with the highest number of eggs laid in each month similar to the control. Generally, the result shows that increased oil concentration resulted in less number of eggs laid.

The mean number of eggs laid by *C. maculatus* on cowpea grains protected using various oil types and at various concentrations at 30-day interval for a period of 180 days is presented on Table 3. There were no significant interactions ($P < .05$) between oil types and the different concentrations of these oils on the number of eggs laid in the 30 days of application. From 60 days, highly significant ($P < .05$) interactions were observed between the oil types and their concentrations on number of insect eggs laid. There were significantly ($P < .05$) more number of eggs laid in the control compared to the grains treated with all other oil types except for the pure neem oil type where there were no significant ($P < .05$) differences between the number of eggs in the control sample and 0.5 mL of pure moringa oil-treated samples.

3.2. Effects of Treatment Oils on Weevil Population in Cowpea Storage. Results of data on the effects of oil types on total weevil population (Figure 2(a)) indicated a significant difference ($P < .05$) in only mixtures of neem-moringa oils in ratios 1 : 1 and 1 : 2 in the first 30 days of storage, while there was significant difference ($P < .05$) between pure neem and all other oils at 60 days. From 90 to 180 days, the oils significantly suppressed the insect population except in pure moringa oil-treated samples which still showed significantly higher ($P < .05$) insect population. Neen-moringa oil type in ratio 1 : 3 appeared to be the most effective in reducing weevil population as it had the least weevil population altogether.

Figure 2(b) shows mean number of weevil in the stored cowpea grains after the application of various concentrations of oils. The results showed that all the oils at various concentrations significantly suppressed the insect population throughout the study compared with the control. Oils at 1.5 mL/200 g concentration however were the most potent and persistent retardant and arrested weevil population growth throughout the study as no single weevil was found in the cowpea after 60 days of storage. There was a significant difference ($P < .05$) between the weevil population of those samples treated with 0.5 mL concentration with that of other sample treated with other concentration at 90 days, while there was no significant difference ($P < .05$)

Table 2: Interaction effects of oil types and various concentrations of the oils on total weevil population of cowpea after different storage periods.

Oil type	Concentration (mL)						
	0.0	0.5	1.0	1.5	Mean	LSD at 0.05	Prob. of F
			30 Days				
PN	250.70	23.30	22.00	22.70			
PM	141.70	59.30	31.00	22.70			
NM1 : 1	406.70	23.70	28.30	25.70			
NM1 : 2	431.70	23.70	22.00	23.00			
NM1 : 3	264.70	23.70	23.70	25.70	94.80	71.41	<0.001
			60 Days				
PN	4988.00	0.00	0.00	0.00			
PM	2497.00	407.00	72.00	2.00			
NM1 : 1	2870.00	1.00	1.00	2.00			
NM1 : 2	2863.00	1.00	1.00	0.00			
NM1 : 3	2108.00	2.00	0.00	0.00	790.80	324.30	<0.001
			90 Days				
PN	442.00	0.00	0.00	0.00			
PM	852.00	629.00	368.00	0.00			
NM1 : 1	557.00	4.00	0.00	2.00			
NM1 : 2	378.00	1.00	0.00	0.00			
NM1 : 3	439.00	0.00	0.00	0.00	183.60	278.90	0.156
			120 Days				
PN	0.00	0.00	0.00	0.00			
PM	0.00	361.00	65.00	0.00			
NM1 : 1	0.00	13.00	0.00	0.00			
NM1 : 2	0.00	0.00	0.00	0.00			
NM1 : 3	0.00	0.00	0.00	0.00	22.00	202.20	0.337
			150 Days				
PN	0.00	0.00	0.00	0.00			
PM	0.00	28.70	152.70	0.00			
NM1 : 1	0.00	13.30	0.00	0.00			
NM1 : 2	0.00	0.00	0.00	0.00			
NM1 : 3	0.00	0.00	0.00	0.00	9.70	95.83	0.501
			180 Days				
PN	0.00	15.30	104.70	0.00			
PM	0.00	15.30	104.00	0.00			
NM1 : 1	0.00	3.30	0.00	0.00			
NM1 : 2	0.00	0.00	0.00	0.00			
NM1 : 3	0.00	0.00	0.00	0.00	12.10	90.24	0.683

PN = pure neem.
PM = pure moringa.
NM 1 : 1 = neem-moringa oil in ratio 1 : 1.
NM 1 : 2 = neem-moringa oil in ratio 1 : 2.
NM 1 : 3 = neem-moringa oil in ratio 1 : 3.

at all other periods between samples treated with various concentration.

Table 2 shows that all the oil types and concentrations significantly interacted to suppress the weevil population throughout the study with 1.5 mL/200 g rates emerging as the best in retarding weevil population throughout the study period. Mixtures of the oils appear to have performed better in totally suppressing weevil population than the nonmixed oils as they proved more potent and persistent reproduction retardant and arrested weevil population growth throughout the study. The result from this table also revealed that from 30 to 120 days pure moringa oil-treated samples maintained a trend of the higher the oil concentration the lower the weevil population, however at 150 and 180 days pure moringa oil-treated samples at 1.0 mL/200 g cowpea had more weevil population than 0.5 mL treated samples. The mixtures (1 : 1,

Table 3: Interaction effects of oil types and various concentrations of the oils on the percentage of uninfested cowpea after different storage periods.

Oil type	Concentration (mL)						
	0.0	0.5	1.0	1.5	Mean	LSD at 0.05	Prob. of F
			30 Days				
PN	0.30	92.50	84.70	95.10			
PM	9.40	42.00	80.60	88.80			
NM1 : 1	0.00	77.80	59.20	81.70			
NM1 : 2	0.00	80.00	81.70	90.30			
NM1 : 3	0.40	75.00	83.30	80.30	60.20	17.42	<0.001
			60 Days				
PN	0.00	92.20	87.00	93.70			
PM	0.00	17.70	38.80	90.30			
NM1 : 1	0.00	67.30	57.30	71.60			
NM1 : 2	0.00	74.90	76.20	81.30			
NM1 : 3	0.00	78.50	78.10	86.80	54.60	23.46	0.002
			90 Days				
PN	0.00	92.90	88.80	94.40			
PM	0.00	5.20	26.70	91.50			
NM1 : 1	0.00	63.40	61.40	76.70			
NM1 : 2	0.00	73.10	64.90	77.00			
NM1 : 3	0.00	74.20	80.90	90.00	53.50	19.64	<0.001
			120 Days				
PN	0.00	91.50	78.10	89.20			
PM	0.00	0.00	3.20	88.90			
NM1 : 1	0.00	58.40	61.40	73.70			
NM1 : 2	0.00	65.50	76.80	75.30			
NM1 : 3	0.00	75.70	77.40	80.70	49.80	14.58	<0.001
			150 Days				
PN	0.00	90.60	87.30	93.20			
PM	0.00	0.00	0.00	89.10			
NM1 : 1	0.00	47.80	65.20	78.50			
NM1 : 2	0.00	73.80	69.70	73.20			
NM1 : 3	0.00	74.40	81.30	83.90	50.40	18.14	<0.001
			180 Days				
PN	0.00	90.30	88.70	88.50			
PM	0.00	0.00	0.00	93.90			
NM1 : 1	0.00	44.60	64.70	74.30			
NM1 : 2	0.00	77.70	72.80	75.30			
NM1 : 3	0.00	76.90	81.80	81.40	55.50	16.93	<0.001

PN = pure neem.
PM = pure moringa.
NM 1:1 = neem-moringa oil in ratio 1 : 1.
NM 1:2 = neem-moringa oil in ratio 1 : 2.
NM 1:3 = neem-moringa oil in ratio 1 : 3.

1 : 2, and 1 : 3 NM) followed the same trend of the higher the oil concentration the lower the weevil population. Pure moringa oil at 0.5–1.0 mL/200 g cowpea appeared to be the worst in arresting weevil population having as much as 25.3 and 104.7 insects, respectively, at 180 days of storage.

3.3. Effects of Treatment Oils on Percentage of Uninfested Cowpea Grains. The effect of oil types on the percentage of uninfested cowpea stored for six months (Table 7) indicated that the percentage of infestation caused by *C. maculatus* was highest (as much as 76.5% at the end of the six months) in pure moringa oil-treated samples. In those samples, infestation continued to increase with the time the insects were allowed to feed on the cowpea (up to six months). However, less infestation was observed in other oil treated samples. Averagely pure neem oil-treated sample appeared to

have the highest percentage (69%) of uninfested grains. Pure moringa oil-treated samples appeared to have the least (22.3) percentage of uninfested samples.

The effects of concentration of oils on mean percentage of uninfested cowpea stored for 180 days are presented in Table 8. Although there was no significant difference at ($P < .05$) between samples treated with 0.5 mL and 1.0 mL/200 g cowpea throughout the storage period, there were, however, significant differences between those samples treated with 1.5 mL/200 g and all other samples treated with 0.5 mL and 1.0 mL/200 g cowpea. Oils at 1.5 mL/200 g level had the highest (82%) percentage of uninfested cowpea grains while the least (57.9%) percentage of uninfested cowpea grains was recorded in those samples treated with 0.5 mL/200 g samples at the end of 180 days of storage. Control samples however had 0% uninfested cowpea.

The interaction between oil types and concentrations is shown in Table 3. All the oil types at various rates significantly protect the cowpea samples against weevil attack compared to the control samples throughout the study except for pure moringa at lower rates (0.5 mL and 1.0 mL) that failed to protect the samples from 120 to 180 days. Pure neem and neem-moringa oil mixture in ratio 1 : 3 had the same effect on the percentage of uninfested grains. The higher the concentration of these two oils, the higher the percentage of uninfested grains. Pure neem oil at 0.5 and 1.5 mL/200 g cowpea were observed to have performed better in protecting the grains from infestation than those treated with pure neem oil at a concentration of 1.0 mL/200 g cowpea. All the oils, however, exhibited great protection ability at 1.5 mL/200 g. Pure neem oil at 1.5 mL/200 g cowpea exhibited the highest protective ability against *C. maculatus* infestation throughout the study, having had above 88% uninfested grains, while pure moringa oil at 0.5 mL and 1.0 mL/200 g cowpea had the least percentage of uninfested cowpea with 0 percent uninfested grains when compared with other oil types apart from 4 the control at the end of the storage period.

4. Discussion

The study has shown that pure neem and moringa oils and the mixtures in ratios 1 : 1, 1 : 2, 1 : 3 adequately protected uninfested cowpea grains from *C. maculatus* attack. This result is consistent with previous works of [4] who used groundnut oil at the rate of 2–8 mL/kg of cowpea to treat cowpea for storage; Schoohoven [6] and Pereire [7] used industrially refined palm oil and groundnut oil for cowpea storage while Gesellschatt fur Technische Zesammenarbeit (GTZ) [8] reported the storage of cowpea with pure neem oil. Efficacy of 10 vegetable oils against *C. maculatus* has been attributed to ovicidal effect of the oils [9], reduced oviposition [6], and reduced adult longevity.

It is clear from the study that pure neem and the mixtures of neem-moringa seed oils at 1.5 mL/200 g was more potent in protecting stored grains of cowpea against infestation and damage by *C. maculatus* as the oils drastically reduced oviposition, weevil population, and cowpea damage. It was also observed that pure moringa oil at higher rate of 1.5 mL/200 g cowpea had the same effects on the stored cowpea. The above results affirmed the report of Lale [10] and RMRDC [11] that neem oil has more than 12–15 complex constituents that have repellent, antifeedant, insect growth regulatory, and pesticidal properties. Perhaps this gives the reason why the preservation effect of these oils was so glaring in this study. Rao [12] affirmed that neem oil with main constituent of Azadirachtin is used as insect repellent, feeding inhibitors, growth retardants, and sterilant. It has both contact and systemic action on the egg and insect thereby destroying them. Lale [10] stated that some essential oils are highly lipophilic and therefore have the ability to penetrate the curticle of the insect or mite and that fatty vegetable oils kills insects by flooding their spiracles thus causing asphyxiation. Fatty vegetable oils are mainly active against eggs and so a uniform spread of the oil is necessary to maximise effectiveness. This result was also consistent with that of other workers [13–17] who used different essential oils to protect cowpea. They reported that oil extracted from *P. guineense* seed were potent in protecting stored cowpea seeds against infestation and damage by *C. maculatus* and also that adult weevils are rapidly killed and oviposition and subsequent adult emergence may be completely prevented.

Mixture of neem-moringa seed oil at 1 : 3 was found to be the best in protecting the cowpea samples perhaps the mixture of these two oils form a compound that has synergistic properties. The mixtures of the oils and invariably, the mixtures of their constituents had more effects than the individual oils in most of the cases observed. Neem and moringa are reputable for their effective medicinal activities in Africa [18, 19]. Neem and moringa are all around us; they are trees that thrive in marginal tropical areas with little care and inputs [10]. The seeds are cheap to get and their oils easy to extract traditionally or with solvent.

5. Conclusion

It was concluded from the study that pure neem oil, mixture of neem and moringa oils (neem-moringa 1 : 2 and 1 : 3 at 1 : 5 mL/200 g cowpea) could be used for the preservation of cowpea. However, if only moringa oil is to be used, the concentration should not be less than 1.5 mL/200 g cowpea.

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