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Biological Activity, Residue Analysis and Dietary Risk Assessment of Five Non-Conventional Insecticides in Cowpea

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ABSTRACT

In recent years, the problem of pesticide residues in cowpeas remains a challenge, prompting comprehensive management efforts nationwide. Therefore, the analysis of pesticides has become an important research field, especially for new pesticides developed recently. The present study was undertaken to investigate the residual activity of five non-conventional insecticides (abamectin, emamectin benzoate, imidacloprid, indoxacarb and spinosad), on the development of cowpea weevil, *Callosobruchus maculatus* at different periods of storage on treated cowpea seeds and the potential dietary risk of tested insecticides through cowpeas to consumers. Residual activity of tested insecticides on *C. maculatus* adults exposed to cowpea seeds treated with high concentration (equal 24h-LC_{90}) at different storage intervals was assessed. The adult mortality of *C. maculatus* decreased from 95, 98.5, 90, 89 and 90 % (at Zero-day of storage) to 40, 43, 50, 45 and 43% (at 90-day of storage) for abamectin, emamectin benzoate, imidacloprid, indoxacarb and spinosad, respectively. Among the five tested insecticides, abamectin, emamectin benzoate and Spinosad seemed to be more effective. The residual content of tested insecticides gradually decreased over time. In addition, data indicated that spinosad and emamectin benzoate were relatively more stable on cowpeas up to the 120 days storage interval. Data showed that all the Target hazard quotient (THQ) values for each insecticide from the consumption of treated cowpea was less than one (<1.0) suggesting that the associated health hazard with exposure to any of these insecticides, applied at selected concentrations, is insignificant for both human adults and children.

INTRODUCTION

Dry seeds of cowpea (*Vigna unguiculata* L. Walp) are an important food and cash crop for cowpea farmers in tropical and subtropical regions and exporting countries. However, its production is subject to serious pest infestations, especially the cowpea weevil, *Callosobruchus maculatus* Fab. Cowpea is an important seed pest of legumes (Fabaceae) both in the field and in storage. Crop contamination begins in the field, while

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most damage occurs during storage. *C. maculatusis* considered the most destructive of cowpeas, causing more than 90% yield reduction (Caswell 1981).

Many nations have reported that major stored-grain pests have developed resistance to grain-protectant pesticides. (Lorini and Galley, 1999). The history of resistance to pyrethroids, organophosphates, and juvenile hormone analogues makes the case for looking for an eco-friendly product with new mechanisms of action that provide effective alternatives to conventional insecticides.

Imidacloprid and indoxacarb are two examples of newly licensed agricultural insecticides from the neonicotinoid and oxadiazine insecticide groups. (Wing *et al.*, 2000). Synthesized natural insecticides, particularly spinosad and abamectin, have favourable toxicological and ecotoxicological characteristics that provide them a substantial advantage and good chances for practical application. (Andric' *et al.*, 2013). However, a little published information focusing on using such non-conventional insecticides as protectants of stored-product insects with emphasis on cowpea beetles.

Pesticide residues in cowpeas have been a persistent issue in recent years, leading to extensive control initiatives across the country (Wang *et al.*, 2023). As a result, pesticide analysis and detection methods have steadily grown in importance as a topic of study, particularly for newly produced pesticides whose long-term exposure and application risks are still unknown (Ren *et al.*, 2023). Assessments of consumption risk are frequently conducted to ascertain risk levels and make suitable risk management decisions to guarantee food safety for the general public. Amount of food ingested and the level of pesticide residues in the food are combined to determine the exposure to pesticide residues as part of the consumption risk assessment process. In order to assess food safety, the obtained exposure values are compared with the acute reference dose (ARfD) for acute risk assessment and with the acceptable daily intake (ADI) for chronic risk assessment. (Mekonen *et al.* 2015).

Therefore, this study was undertaken to investigate: 1) degradation and persistence of residual activity of five non-conventional insecticides, 2) biological activity of insecticide residue on the development of *C. maculatus* at different periods of storage on treated cowpea seeds; 3) The potential dietary risk of tested insecticides through cowpeas to consumers.

MATERIALS AND METHODS

1. Insect Rearing:

The cowpea weevil (beetle), *Callosobruchus maculatus* Fab. (Coleoptera: Chrysomelidae) was obtained from a colony maintained in the Plant Protection Research Institute, Stored-Product Insects Department, ARC, Giza, Egypt. The adults were fed on the clean dried cowpea (*Vigna unguiculata* (L.) Walp.) seeds in plastic jars and covered with a black muslin cloth. Seeds of *V. unguiculata* were commercially obtained from local markets.

2. Insecticides Used:

Commercial formulations of five insecticides were purchased from local markets to use in this study: Spinosad (TRACER® 48% SC), Abamectin (VERTIMEC® 1.8% EC), Emamectin Benzoate (PROCLAIM® 5.7% SG), Imidacloprid (COMMANDO® 35% SC) and Indoxacarb (AVAUNT® 30% WG)

3. Residual Activity of Tested Insecticides against *C. maculatus:*

To determine persistent effects of tested insecticides against *C. maculatus*, 250 g of healthy cowpea seeds were vigorously-shaken for 20-30s thorough coating with 0.35 mL of the 24 h-LC_{90} value of each tested insecticide, determined in our previous study

(Ammar *et. al.*, 2024) until coverage on the surface of the seeds was relatively consistent. The LC_{90} values used for coating cowpea seeds were: 427.3, 186.7, 1019.8, 889.4 and 408.7 ppm (equal to: 0.614, 0.301, 3.325and 1.459 and 0.992 mg AI/kg cowpea seeds) for abamectin, emamectin benzoate, imidacloprid, indoxacarb, and spinosad, respectively. Treated seeds of each treatment were stored separately in poly-ethylene bags in dark conditions and each bag was sealed in another additional plastic bags for hermetic storage.

Samples of treated seeds (25 g) were withdrawn after 10 days, 1, 2, 3, and 4 months of treatment and then 20 pairs adults (1-2- day old) are released in the 0.4 L-glass jar covered with muslin secured with elastic bands. The same number was also used for the control treated only with tap water. Each treatment was replicated three times. The insects were exposed to treated seeds were continued for 48-h and then mortality was recorded. After 10 days of treatment, both alive and dead insects were discarded, and by using a binocular microscope, the number of eggs laid for both treated and control seeds was counted. For two more weeks, the egg hatch was observed at least twice a week. At the end of the F1-generation, approximately three weeks after the initial appearance, the emergence of the F1-adult was determined and recorded. Three duplicates of each experiment were conducted.

4. Residue Analysis of Tested Insecticides in Cowpea Seeds After Different Periods of Storage:

4.1. Sample Preparation:

Samples of treated seeds (250 g) were taken after 10 days, 1, 2, 3, and 4 months of treatment from poly-ethylene bags for every tested insecticide, then transferred to the laboratory for residue analysis test. Preparation of insecticide-treated cowpea seeds for insecticide residue analysis was carried out in the Central Pesticide Residue and Heavy Metals Laboratory (QCAB), Dokki, Giza.

4.2. Sample Extraction by QuEChERS Method:

The extraction procedure is based on liquid–liquid partitioning with acetonitrile followed by a cleanup step with dispersive-SPE QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). Briefly, a 5 g sample of either dry or boiled cowpea seeds was homogenized for extraction and put into a 50 mL polypropylene (PP) centrifuge tube with 10 ml of acetonitrile, and the mixture was homogenized. Subsequently, 4 g of anhydrous MgSO4 and 1 g of NaCl salts and citrate buffer (pH 5 to 5.5) were added and vortexed with a multi-tube vortex mixer for 1 min. After a 5-minute centrifugation of the tubes at 4000 rpm, a 1.0 mL supernatant was moved to a 2-mL clear HPLC vial. A direct injection of approximately 1 µL of each sample was executed into the LC-MS/MS system. (Li *et al.,* 2020).

4.2. LC-MS/MS System:

` Agilent 1260 series liquid chromatography system equipped with Applied Bio-systems (API 6500 Q trape) tandem mass spectrometers with electrospray ionization (ESI) interface. Separation was performed on a C18 column ZORBAX Eclipse XDBC18 4.6 mm x 150 mm, 5 µm particle size (Agilent, USA). The injection volume was 25 µl. The following was the mobile phase: 10 mM ammonium format solution in methanol–water (1:9) at pH 4 ± 0.1 is solvent A; methanol is solvent B. Starting at 100% A, the linear gradient program ran for 0–13 minutes from 100% to 5% A, 13–21 minutes from 5% to 100% A, 21–28 minutes from 5% to 100% A, and 28–32 minutes from 100% A at a flow rate of 0.3 ml/min. While the nitrogen nebulizer, curtain, and other gas characteristics were optimized in accordance with the manufacturer's recommendations, the source was set in the positive mode. All chemicals shared an ion spray potential of 5500 V and a source temperature of 400°C. By injecting individual pesticide solutions into the MS detector, the collision energy and decluster potential were adjusted. Quantification and confirmation were done using the multiple reactions monitoring mode. (Li *et al.*, 2020).

4.3. Method Validation:

To ensure the validity of the analytical method for the tested pesticides, validation parameters were assessed by analyzing the limit of detection (LOD), limit of quantitation (LOQ), and matrix affect (ME). To create matrix-matched standard solutions, the standard solutions were dissolved in control matrix (cowpeas) extract solutions after being dissolved in acetonitrile as solvent standard solutions. Both the solvent standard solution and the matrix-matched standard solutions were injected three times at concentrations ranging from 0.0005 to 0.5 mg/L. Serial doses of standards (5 ng/ml, 20 ng/ml, 50 ng/ml, 100 ng/ml, and 200 ng/ml) were used to evaluate the matrix effect. Where ME was calculated as follow:

$$ME\ (\%) = (k_{matrix}\text{-}k_{solvent})/k_{solvent} \times 100$$

K_{matrix} is the slope of the matrix-matched calibration curve and $K_{solvent}$ is the solvent-only calibration curve.

Three spiking concentration levels (0.005 mg/kg, 0.05 mg/kg, and 0.5 mg/kg) were analyzed in a recovery experiment with three replications to assess the method's accuracy and precision. Before extraction, the homogenized sample was combined with the required volume of an insecticide standard solution to create the spiked sample. Relative standard deviations (RSDs) were measured in order to assess the established method's precision. Under these conditions the retention time for tested insecticides were: 14.9, 14, 8.8, 14.2 and 14.2 min for abamectin, emamectin benzoate, imidacloprid, indoxacarb, and spinosad, respectively.

4.4. Chronic Dietary Risk Assessment in the Egyptian Diet:

The risk assessment is computed by comparing the appropriate estimated daily intake (EDI), which is dependent on the concentration of pesticide residues and food consumption, with the established acceptable daily intake (ADI), (or ARfD, acute reference dose). Only pesticide residues exceeding the maximum residual limit (MRL) were considered in the risk assessment.

The estimated daily intake (EDI) was assessed by using the following formula (1), based on USEPA, (2012) and Adebiyi et. al. (2020):

$$EDI = (C \times IR \times EF \times ED) / (BW \times AT) \text{ .. (1)}$$

Where: C is the pesticide concentration in cowpea (mg/kg), IR, is average daily consumption of cowpea (0.0237 kg/ person/day) (WHO, 2012); EF, is exposure frequency (365 days/year); ED, is exposure duration (number of exposure years, assuming 70 years, equivalent to the average lifetime); BW, is the average body weight (kg) (70 kg for adults, 20 kg for children) and AT, is the average exposure time for non-carcinogens (365 days/year × ED = 25,550).

Target hazard quotient (THQ) was computed in accordance with the USEPA standard technique of assessing the risk of non-carcinogenic impacts. The usual assumption from an integrated U.S. EPA risk analysis was used to calculate the doses. Adults were considered to weigh an average of 70 kg. The THQ was determined based on the standard assumption from the integrated USEPA risk analysis equation (USEPA, 2012; and Adebiyi et. al., 2020).

$$THQ = EDI / RfD \text{ .. (2)}$$

The THQ was performed by dividing the estimated daily intake by the corresponding reference dose (RfD). The RfD is the reference dose which are considered to be safe levels of exposure over the lifetime. The established reference dose (RfD) values for selected insecticides were from EU Pesticides Database. The RfDs for Abamectin, Emamectin benzoate, Imidacloprid, Indoxacarb and Spinosad are 0.003, 0.03, 0.057, 0.02 and 0.0249 mg/kg/day, respectively.

When the THQ >1; this indicates that food is considered a risk to the consumers. When the index <1, this indicates that food is considered acceptable.

5. Statistical Analysis:

Data obtained in these tests were analyzed statistically by analysis of variance (ANOVA) and the means were compared using Lease Significant Difference (LSD) test (CoStat Statics Software).

RESULTS AND DISCUSSION

1. Residual Activity of Tested Insecticides against *C. maculatus* Exposed to Treated Seeds After Different Storage Periods:

1.1. Abamectin:

Results of the residual activity of abamectin on *C. maculatus* adults exposed to cowpea seeds treated with high concentration (equal 24h-LC_{90}) at different storage intervals are presented in Table 1. The adult mortality of *C. maculatus* decreased from ~ 95 % (at zero-day of storage) to 40% (at 90-day of storage). Persistence of abamectin on *C. maculatus* oviposition rate, egg hatch and progeny production are summarized in Table 1. Mean number of eggs deposited were severely decreased when adults were exposed to cowpea seeds treated with abamectin, at the initial period (24h- after treatment) reporting 0.5 eggs/female, then increased gradually with increasing storage periods of treated seeds reporting 56.0 eggs/ female (at 90-day), compared to 71.0 eggs/ female in the control. Consequently, the oviposition inhibition (OI %) was decreased with increasing in storage period, which ranged from 99.29 % (at 0-day) to 21.12 % (at 90-day of storage) .

Similar trend was shown for egg hatch percent, where from zero % (at 0-day) to 55.5% (at 90-day of storage), compared to 81.25% in the control. In addition, the total number of the emerged adults (F1-progeny) increased with storage time in cowpea, i.e. 0.5 (at 10-day) to 108.8 (at 90 days of storage) in comparison with 292.0 in the control.

Table 1 : Residual activity of abamectin on *Callosobruchus maculatus* adults exposed to an insecticide-treated cowpea seeds for different storage periods.

Storage period (Days after treatment)	% [a]Mortality	[b]Total No. deposited eggs ± SE	[c]Mean No. eggs/♀ ± SE	[d]Oviposition inhibition (OI) %	% Hatch ± SE	Total No. Adult emerged ± SE	[e]% Adult emergence
2-h	95	1.5 ± 1.2e	0.5 ± 0.4d	99.29	0.0	0.0f	0.0
10 days	85	9.0 ± 2.82d	6.25 ± 1.06c	91.19	4.45 ± 4.63	0.5 ± 0.35e	5.5
30 days	75	128.5 ± 43.13c	51.0 ± 2.82b	28.16	14.4± 2.68	18.0 ± 2.82d	14.0
60 days	55.5	157.5 ± 6.36c	52.5 ± 2.12b	26.76	38.35± 2.47	60.5 ± 6.36c	38.4
90 days	40	196.5 ± 21.2b	56.0 ± 1.41b	21.12	55.5 ± 2.82	108.8± 19.09b	54.96
Control	-	355.5 ± 21.2a	71.0 ± 4.24a	--	81.25 ±1.02	292.0± 12.72a	82.25

Mean values followed by the same letter(s) are not significant based on mean separation method of LSD (Least Significant Difference) at $p < 0.05$.

[a]Corrected mortality percentage based on Abbott's formula (Abbott, 1925).

[b]Total number of deposited eggs. Three replicates for each treatment (5 females + 5 males).

[c]Mean number of eggs for alive females.

[d]% OI was calculated by using the formula [OI = [(NC - NT)/NC] ×100], NT = No. eggs in untreated and NT = No. eggs laid in treated.

[e]% Adult emergence was calculated by dividing total number of emerged adults by total number of deposited eggs.

1.2. Emamectin Benzoate:

Results on the residual activity of emamectin benzoate on *C. maculatus* adults exposed to cowpea seeds treated with high concentration (equal 24 h-LC90) at different storage intervals are presented in Table 2. The adult mortality of *C. maculatus* decreased from ~ 89.5 % (at Zero-day of storage) to 43% (at 90-day of storage). The mean number of eggs deposited were significantly decreased, reporting 24.0 eggs/female (2h-after treatment). Then, increased gradually with increasing in storage periods of treated seeds, i.e. 25.0 eggs/female (at 10-day) to 66.0 eggs/ female (at 90-day), compared to 71.7 eggs/ female in the control. Consequently, the oviposition inhibition (OI %) was decreased with increasing in storage period, which ranged from 66.19 % (at 0-day) to 7.04 % (at 90-day).

Similar trend was shown for egg hatch percent, where ranged from 0.8 % (at 0-day) to 53.45% (at 90-day of storage), compared to 81.25% in the control. In addition, the total number of the emerged adults (F1-progeny) increased with storage time in cowpea, i.e. 0.5 (at 0-day) to 141.0 (at 90 days of storage) in comparison with 292.2 in the control.

Table 2: Residual activity of emamectin benzoate on *Callosobruchus maculatus* adults exposed to an insecticide-treated cowpea seeds for different storage periods

Storage period (Days after treatment)	% [a]Mortality	[b]Total No. deposited eggs ±SE	[c]Mean No. eggs/♀ ± SE	[d]Oviposition inhibition (OI) %	% Hatch ± SE	Total No. Adult emerged ± SE	[e]% Adult emergence ± SE
2-h	89.5	58.0 ± 2.82d	24.0 ± 4.65c	66.19	0.8 ± 0.68	0.5 ± 0.35f	0.86
10 days	85	39.3 ± 15.5d	25.0 ± 0.35c	64.78	7.1 ± 3.25	2.5 ± 2.12e	7.8
30 days	75	142.5 ± 31.8c	57.5 ± 3.53b	19.01	38.69 ± 0.7	55.0 ± 11.31d	38.5
60 days	50	214.5 ± 36.0b	61.5 ± 2.12b	13.38	46.15 ± 0.49	99.0 ± 15.55c	46.15
90 days	43	264.0 ± 5.65b	66.0 ± 1.41b	7.04	53.45 ± 4.3	141.0 ± 8.48b	53.4
Control	-	355.0 ± 21.2a	71.7 ± 4.24a	--	81.25 ± 10.2	292.2 ± 12.72a	82.25

Mean values followed by the same letter(s) are not significant based on mean separation method of LSD (Least Significant Difference) at $p < 0.05$.

[a]Corrected mortality percentage based on Abbott's formula (Abbott, 1925).

[b]Total number of deposited eggs. Three replicates for each treatment (5 females + 5 males).

[c]Mean number of eggs for alive females.

[d]% OI was calculated by using the formula [OI = [(NC - NT)/NC] ×100], NT = No. eggs in untreated and NT = No. eggs laid in treated.

[e]% Adult emergence was calculated by dividing total number of emerged adults by total number of deposited eggs.

1.3. Imidacloprid:

Results on the residual activity of imidacloprid on *C. maculatus* adults exposed to cowpea seeds treated with high concentration (equal 24 h-LC90) at different storage intervals are presented in Table 3. The adult mortality of *C. maculatus* decreased from ~ 90 % (at Zero-day of storage) to 50% (at 90-day of storage). The mean number of eggs deposited were significantly decreased when adults were exposed to cowpea seeds treated with imidacloprid, at initial time (2h- after treatment) reporting 14.0 eggs/female, compared to 71.0 eggs/ female in the control. Consequently, the oviposition inhibition (OI %) was decreased with increasing in storage period, which ranged from 80.28 % (at 0-day) to 14.08 % (at 90-day).

Similar trend was shown for egg hatch percent, where ranged from 3.30 % (at 0-day) to 55.42% (at 90-day of storage), compared to 81.25% in the control. In addition, the total number of the emerged adults (F1-progeny) increased with storage time in cowpea, ranged from 0.5 (at 0-day) to 135.0 (at 90 days) in comparison with 292.9 in the control.

Table 3: Residual activity of imidacloprid on *Callosobruchus maculatus* adults exposed to an insecticide-treated cowpea seeds for different storage periods

Storage period (Days after treatment)	% [a]Mortality	[b]Total No. deposited eggs ± SE	[c]Mean No. eggs/♀ ± SE	[d]Oviposition inhibition (OI) %	% Hatch ± SE	Total No. Adult emerged ± SE	[e]% Adult emergence ± SE
2-h	90	14.0 ± 1.41e	14.0 ± 1.41d	80.28	3.30 ± 1.46	0.5 ± 0.37f	3.5
10 days	80	66.5 ± 4.94d	33.25 ± 2.47c	53.16	8.95 ± 1.48	6.5 ± 0.70e	9.7
30 days	65	190.5 ± 53.03c	54.0 ± 4.24b	23.94	20.6 ± 3.11	38.5 ± 4.94d	20.2
60 days	55	169.5 ± 2.12c	55.5 ± 0.70b	21.83	41.1 ± 1.83	68.5 ± 2.12c	41.14
90 days	50	244.0 ± 11.31b	61.0 ± 28.2b	14.08	55.42 ± 4.3	135.0 ± 4.24b	55.32
Control	-	355.0 ± 21.2a	71.0 ± 4.24a	--	81.25 ± 10.2	292.9 ± 12.72a	82.25

Mean values followed by the same letter(s) are not significant based on mean separation method of LSD (Least Significant Difference) at $p < 0.05$.
[a]Corrected mortality percentage based on Abbott's formula (Abbott, 1925).
[b]Total number of deposited eggs. Three replicates for each treatment (5 females + 5 males).
[c]Mean number of eggs for alive females.
[d]% OI was calculated by using the formula [OI = [(NC - NT)/NC] ×100], NT = No. eggs in untreated and NT = No. eggs laid in treated.
[e]% Adult emergence was calculated by dividing total number of emerged adults by total number of deposited eggs.

1.4. Indoxacarb:

Results on the residual activity of indoxacarb on *C. maculatus* adults exposed to cowpea seeds treated with high concentration (equal 24 h-LC90) at different storage intervals are summarized in Table 4. The adult mortality of *C. maculatus* decreased from ~ 89 % (at Zero-day of storage) to 45% (at 90-day of storage). The data also presented persistence of indoxacarb on *C. maculatus* oviposition rate, egg hatch and progeny production. The mean number of eggs deposited were severely decreased reporting 8.0 eggs/female (2h- after treatment). Then, the oviposition rate increased gradually with increasing in storage periods of treated seeds, which ranged from 28.8 eggs/female (at 10-day) to 63.5 eggs/ female (at 90-day of storage), compared to 71.7 in the control. Consequently, the oviposition inhibition (OI %) was decreased, with increasing in storage period, which ranged from 88.73 % (at 0-day) to 10.56 % (at 90-day).

Similar trend was shown for egg hatch percent, where ranged from 2.25 % (at 0-day) to 55.42% (at 90-day of storage), compared to 81.25% in the control. In addition, the total number of the emerged adults (F1-progeny) increased with storage time in cowpea, ranged from 0.55 (at 0-day) to 157.0 (at 90 days) in comparison with 292.9 in the control.

Table 4: Residual activity of indoxacarb on *Callosobruchus maculatus* adults exposed to an insecticide-treated cowpea seeds for different storage periods.

Storage period (Days after treatment)	% [a]Mortality	[b]Total No. deposited eggs ± SE	[c]Mean No. eggs/♀ ± SE	[d]Oviposition inhibition (OI) %	% Hatch ± SE	Total No. Adult emerged ± SE	[e]% Adult emergence ± SE
2-h	89	13.5 ± 12.02	8.0 ± 4.24	88.73	2.25 ± 1.33	0.55 ± 0.27	3.70
10 days	80	56.5 ± 37.4	28.8 ± 1.69	59.43	8.7 ± 2.96	5.5 ± 4.44	9.73
30 days	65	209.5 ± 50.2	59.6 ± 2.26	16.05	23.1 ± 1.82	49.0 ± 15.55	23.38
60 days	55	213.0 ± 38.18	61.0 ± 1.14	14.08	44.08 ± 0.62	91.5 ± 13.43	42.90
90 days	45	285.0 ± 35.3	63.5 ± 2.12	10.56	55.04 ± 0.62	157.0 ± 21.12	55.08
Control	-	355.0 ± 21.2	71.7 ± 4.24	--	81.25 ± 10.2	292.9 ± 12.72	82.25

Mean values followed by the same letter(s) are not significant based on mean separation method of LSD (Least Significant Difference) at $p < 0.05$.
[a]Corrected mortality percentage based on Abbott's formula (Abbott, 1925).
[b]Total number of deposited eggs. Three replicates for each treatment (5 females + 5 males).
[c]Mean number of eggs for alive females.
[d]% OI was calculated by using the formula [OI = [(NC - NT)/NC] ×100], NT = No. eggs in untreated and NT = No. eggs laid in treated.
[e]% Adult emergence was calculated by dividing total number of emerged adults by total number of deposited eggs.

1.5. Spinosad:

Results on the residual activity of spinosad on *C. maculatus* adults exposed to cowpea seeds treated with high concentration (equal 24h-LC90) at different storage intervals are summarized in Table 5. The adult mortality of *C. maculatus* decreased from ~ 90 % (at Zero-day of storage) to 43% (at 90-day of storage). The data also presented persistence of spinosad on *C. maculatus* oviposition rate, egg hatch and progeny production. It is obvious that mean number of eggs deposited were highly decreased when adults were exposed to cowpea seeds treated with spinosad, at initial period (2h-after treatment) reporting 7.8 eggs/female. Then, the oviposition rate increased gradually with increasing in storage periods of treated seeds, which ranged from 23.65 eggs/female (at 10-day) to 66.6 eggs/ female (at 90-day of storage), compared to 71.7 in the control. Consequently, the oviposition inhibition (OI %) was decreased, with increasing in storage period, which ranged from 89.01 % (at 0-day) to 7.04 % (at 90-day).

Similar trend was shown for egg hatch percent, where 8.05 % (at 0-day) to 69.02% (at 90-day of storage), compared to 81.25% in the control. In addition, the total number of the emerged adults (F1-progeny) increased with storage time in cowpea, ranged from 1.0 (at 0-day) to 195.0 (at 90 days) in comparison with 292.9 in the control.

Table 5: Residual activity of spinosad on *Callosobruchus maculatus* adults exposed to an insecticide-treated cowpea seeds for different storage periods.

Storage period (Days after treatment)	% [a]Mortality	[b]Total No. deposited eggs ± SE	[c]Mean No. eggs/♀ ± SE	[d]Oviposition inhibition (OI) %	% Hatch ± SE	Total No. Adult emerged ± SE	[e]% Adult emergence ± SE
2-h	90	14.5 ± 7.78f	7.8 ± 1.69d	89.01	8.05 ± 4.31	1.0 ± 0.0f	6.8
10 days	80	56.0 ± 5.65e	23.65 ± 8.98c	66.69	15.15 ± 0.22	7.5 ± 0.70e	13.3
30 days	70	165.0 ± 60.8d	56.5 ± 6.36b	20.42	43.25 ± 3.33	72.5 ± 31.81d	43.9
60 days	56	214.0 ± 48.08c	61.0 ± 1.41b	14.08	54.25 ± 4.80	115.0 ± 15.55c	53.7
90 days	43	296.5 ± 40.3b	66.6 ± 1.41b	7.04	69.02 ± 4.88	195.0 ± 14.11b	65.87
Control	--	355.5 ± 21.2a	71.0 ± 4.24a	--	81.25 ± 1.02	292.2 ± 12.72a	82.25

Mean values followed by the same letter(s) are not significant based on mean separation method of LSD (Least Significant Difference) at $p < 0.05$.

[a]Corrected mortality percentage based on Abbott's formula (Abbott, 1925).

[b]Total number of deposited eggs. Three replicates for each treatment (5 females + 5 males).

[c]Mean number of eggs for alive females.

[d]% OI was calculated by using the formula [OI = [(NC - NT)/NC] ×100], NT = No. eggs in untreated and NT = No. eggs laid in treated.

[e]% Adult emergence was calculated by dividing total number of emerged adults by total number of deposited eggs.

Andrić *et al.* (2013) conducted similar research to assess the insecticidal effect of spinosad and abamectin against various populations of the red flour beetle (*Tribolium castaneum* Herbst) in treated wheat grain. They found that while spinosad at 5 mg/kg was most effective after 21 days (97% mortality), abamectin doses of 2.5 and 5 mg/kg caused high adult mortality of 94-100%. They also found that the laboratory population experienced the highest mortality (75%) 14 days after treatment with the highest dose (5 mg/kg) of spinosad, while treatment with the same dose of abamectin achieved the highest mortality (58%).

For the two avermectins tested in our present study, abamectin and emamectin, the results demonstrated high persistence of both insecticides against *C. macultus* after 3 months of seed storage showing significant reduction in F1-progeny compared to the control. Likewise, Kabir (2019) evaluated the insecticidal efficacy of abamectin against *Sitophilus zeamais* and *Tribolium castaneum* on treated maize and wheat. Abamectin was very effective against *S. zeamais* and *T. castaneum*. However, its efficacy was influenced by dose rate and exposure interval. After 14-d of exposure to maize and wheat treated at 1-1.5 mg/kg, complete adult mortality was achieved for both pest insect species, indicating that this exposure interval is sufficient to control these insect species (Kabir 2019).

Abamectin appears to be more potent than other classes of grain protectants, with particular reference to the organophosphates, whose recommended rates ranged from 4 to 12 mg/kg of grain (FAO, 1994).

The other new agricultural insecticides evaluated in our present study, as protectants against cowpea weevil, are belonging to the neonicotinoid (imidacloprid); and oxadiazine (indoxacarb) groups. Our present data showed that exposure of *C. maculatus* adults to seeds treated with either imidacloprid (at 1020 ppm) or indoxacarb (890 ppm), after 90 days of storage, remained effective against the insects indicating significant reduction in F1-progeny. Imidacloprid and indoxacarb were found to be effective against resistant strains of five stored grain beetles in a previous study by Daglish and Nayak (2012). Based on these findings, the authors discussed the possibility of employing neonicotinoid or oxadiazine insecticides as grain protectants. After adults were exposed to treated wheat for two weeks, mortality and reproduction varied depending on the species, dose, and insecticide. They discovered that *S. oryzae* was unaffected by imidacloprid at any dose, but at 10 mg/kg, none of the other species produced any viable offspring. *T. castaneum* was unaffected by indoxacarb at any dose, but at 5 mg/kg, none of the other species produced any viable offspring (Daglish and Nayak 2012).

Degradation of Tested Insecticides on Treated Cowpea Seeds and The Potential Health Risk of Pesticide Residues:

1.6. Persistence of Tested Insecticide Residues on Treated Cowpea Seeds at Different Storage Periods:

The residual content of abamectin gradually decreased over time. High degradation in abamectin residual content was observed from 0.4441 mg/kg, on 10 days of storage after treatment to 0.0983 mg/kg after 120 days of storage showing 83.9% reduction (Table 6). Moderate degradation rates were observed for imidacloprid, where the residual amounts were reduced from 2.89 mg/kg, at 10-days of storage, (12.9% reduction) to 1.388 mg/kg, at 120-days of storage (58.25% reduction). Similar trend was shown for the degradation of indoxacarb, where the residual content of the compound was gradually decreased by 15.66% and 44.91 % on 10 and 120- days after treatment, respectively.

Table 6: Mean levels of residual concentrations of tested insecticides in cowpea beans (mg a.i. /kg) at different storage periods

Insecticide	Initial Concentration mg a.i./ kg	Standard matrix (%)	Recovery (%)	Residue amount (mg a.i./ kg cowpea beans) at Storage periods (Days after treatment) (% Degradation)				
				10	30	60	90	120
Abamectin (1.8% EC)	0.6140	102.2	110.9	0.4441 (27.67 %)	0.3625 (40.96 %)	0.3200 (47.88 %)	0.2657 (56.72 %)	0.0983 (83.9 %)
Emamectin benzoate (5.7% SG)	0.3012	74.0	99.6	0.2682 (10.96 %)	0.2502 (16.93 %)	0.2311 (23.27 %)	0.2060 (31.60 %)	0.1838 (38.98 %)
Imidacloprid (35% SC)	3.3251	103.0	86.3	2.8956 (12.92 %)	2.6892 (19.12 %)	2.0340 (38.82 %)	1.6505 (50.36 %)	1.3883 (58.25 %)
Indoxacarb (30% WG)	1.4593	123.2	86.6	1.2307 (15.66 %)	1.0124 (30.62 %)	0.9903 (32.14 %)	0.8442 (42.16 %)	0.8039 (44.91 %)
Spinosad (48% SC)	0.9924	77.2	105.6	0.9636 (2.90 %)	0.9144 (8.19 %)	0.8511 (14.24 %)	0.7473 (24.69 %)	0.6997 (24.70 %)

The residual contents of emamectin benzoate were also decreased gradually from 0.268 mg/kg, after 10-days of storage (10.96% reduction) to 0.183 mg/kg, after 120-days of storage (38.98 % reduction). The data indicated that spinosad was relatively the most stable on cowpea seeds up to the 120 days storage interval with a content of 0.9636 mg/

kg (10-days of storage) and 0.6997 mg/ kg (120- days of storage) indicating 2.90% and 24.70 %, respectively.

Data indicated that Spinosad and emamectin benzoate were relatively more stable on cowpeas up to the 120 days storage interval, where caused mortality percentages by 35 and 30%, respectively, to *C. maculatus* adults exposed to treated cowpeas after 120 days of storage, compared to the other insecticides (Fig.1). Our current investigation is supported by Sanon et al. (2010), who found that cowpeas treated with a dry spinosad formulation at 0.94 ppm could be continuously protected for up to six months when the seeds were packaged in plastic and stored inside a typical warehouse condition in Burkina Faso.

Additionally, the residual contents of tested insecticides in cowpea seeds treated with selected concentrations were determined by using GC/MS analysis after boiling the cowpea seeds (Table 7). High degradation in residual contents of emamectin benzoate (90.27%), imidacloprid (88.74%), and Spinosad (79.66%) were reported after cowpea boiling. Also, the boiling process resulted in degradation in residual contents of indoxacarb (67.36%) and abamectin (62.41%).

The maximum residue levels (MRL) of the studied insecticides in/on beans are stated by EU Pesticides Database, as mentioned in the following Table:

Pesticide	MRL in beans (mg/kg)*
Abamectin	0.01
Emamectin benzoate	0.015
Imidacloprid	2.00
Indoxacarb	0.1
Spinosad	1.00

*Based on CODEX and EU Pesticides Residues Database

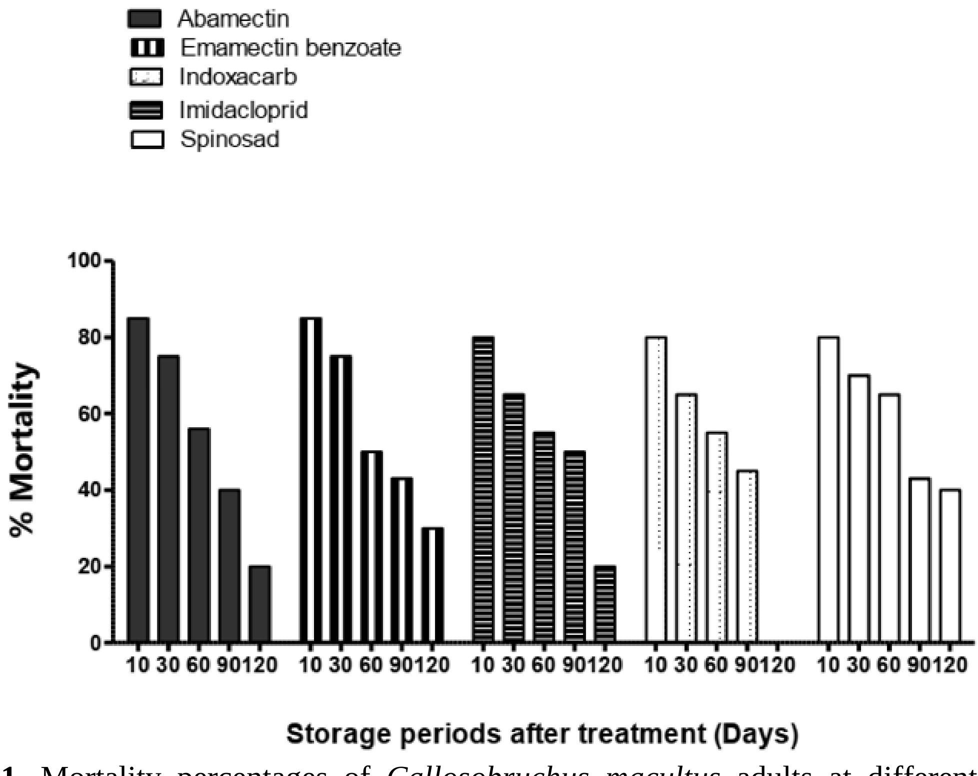

Fig. 1. Mortality percentages of *Callosobruchus macultus* adults at different storage periods after exposure to cowpea seed treated with LC_{90} of tested insecticides.

Our present study showed that the residual contents of boiled cowpea beans (**Table 7**) treated previously with emamectin benzoate, imidacloprid, indoxacarb and spinosad were 0.029, 0.374, 0.476 and 0.201 mg/kg, respectively, and not exceeded their respective MRL values reported by CODEX and EU Databases Pesticides with 0.20, 2.0, 2.0 and 1.00 mg/kg, respectively. However, the residual concentrations of abamectin (0.230 mg/kg) in boiled cowpeas exceeded its corresponding CODEX MRL value, 0.005 mg/kg, respectively.

Based on CODEX and EU Pesticides Database, the MRL values of abamectin (0.005 mg/ kg), emamectin benzoate (0.200 mg/kg), imidacloprid (2.00 mg/kg), indoxacarb (2.0 mg/kg) and spinosad (1.0 mg/kg) on beans are higher than their corresponding Acceptable Daily Intake (ADI): 0.002, 0.003, 0.060, 0.020 and 0.025 mg/kg, respectively. The MRL's primary objective is to set a legally enforceable threshold, and adherence to it can be used as an indicator of GAP compliance. Since no one would ever eat a commodity with residue levels at the MRL on a daily basis throughout their lifetime, it is illogical to assume that the product of the MRL times the prospective intake would ever surpass the ADI (Renwick, 2002).

Table 7: Mean levels of residual concentrations of tested insecticides in cowpea bean samples after boiling

Insecticide	Initial concentration mg a.i./ kg	Boiled standard matrix (%)	Recovery (%)	Residue amount (mg a.i./ kg cowpea beans) after boiling (% Degradation)	
				mg a.i./ Kg	Degradation (%)
Abamectin (1.8% EC)	0.6140	57.2	129.2	0.2308	62.41
Emamectin benzoate (5.7% SG)	0.3012	79.2	100.8	0.0293	90.27
Imidacloprid (35% SC)	3.3251	152.8	113.2	0.3745	88.74
Indoxacarb (30% WG)	1.4593	80.2	106.6	0.4763	67.36
Spinosad (48% SC)	0.9924	90.8	91.6	0.2018	79.66

1.7. Health Risk Assessment of Insecticide Residues Via Dietary Intake Of Treated Cowpea:

For the safe application of pesticides, the health risk quotient was used to evaluate dietary risk assessment based on the terminal residues data in cowpeas. In general, Data in Table 8. demonstrated that all the THQ values for each insecticide from the consumption of treated cowpea was less than one (<1.0) suggesting that the associated health hazard with exposure to any of these insecticides, applied at selected concentrations, is insignificant for both adults and children; whereas “TRQ” value > 1.0 indicates that there is an adverse effect on human health and unacceptable (Lin et al., 2020).

Likewise, data **in** Table 9. demonstrated that the “THQ” values computed for each insecticide in boiled cowpea seeds was less than one (<1.0). These results suggesting that the associated health hazard with exposure to any of such insecticides, applied at selected concentrations, is insignificant for both adults and children after subjecting to boiling process.

Analysis of pesticide residues in food is a key tool for monitoring the levels of human exposure to pesticide residues. The present study demonstrated that even the residual contents of studied insecticides reported through 120 days in/on cowpeas were higher than their respective MRL values, however, the Target Hazard Quotient was less than < 1, suggesting that the associated health hazard with exposure to pesticide is insignificant and acceptable for adults or/and children. However, the boiling of treated cowpeas resulted in high degradation in residual concentrations in/on cowpeas of all insecticides, except abamectin, reporting low values which did not exceed their MRL values. Also, though the MRL values of test insecticides exceeded their corresponding ADI, but this is an unrealistic exposure scenario because no individual would consume the commodity with residue levels at the MRL every day throughout life (Renwick, 2002). A very little research work has been published to investigate the health risk assessment of residues of new classes of insecticides via dietary intake of cowpeas. Recently, Li *et al.* (2022) showed that neonicotinoid, thiacloprid and spirotetramat degrades relatively quickly after application on cowpeas under field condition. The chronic and acute dietary exposure assessment risk quotient (RQ) values of thiacloprid in cowpeas for different consumers were lower than 100%, indicating that both insecticides have a low dietary intake risk in cowpeas. Numerous pesticide residues, primarily carbendazim, carbofuran, fenthion, acephate, chlorpyrifos, cyromazine, methamidophos, clothianidin, difenoconazole, acetamiprid, imidacloprid, and methomyl, were found in cowpea samples from the Chinese province of Hainan and exceeded the MRLs (Wei, 2024). The significant level of risk associated with pesticide residues in cowpea samples was indicated by the fact that all of the risk coefficients (R), which represent the cumulative and simultaneous impacts of several pesticides, were above 2.5.

Table 8: Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) to residues of tested insecticides due to cowpea beans consumption by adults and children.

Pesticide	Storage Periods (Days)	RfD (mg/Kg/day)	EDI (Adult)	THQ (Adult)	EDI (children)	THQ (children)
Abamectin (1.8% EC)	0	0.003	0.0002	0.0693	0.0002	0.2425
	10		0.0001	0.0501	0.00015	0.1754
	30		0.0001	0.0409	0.00012	0.1438
	60		0.0001	0.0361	0.0001	0.1264
	90		9E-05	0.0299	9E-05	0.1049
	120		3.3E-05	0.0110	3.3E-05	0.0388
Emamectin benzoate (5.7% SG)	0	0.030	0.0001	0.0034	0.0001	0.0118
	10		9.1E-05	0.0030	9.08E-05	0.0105
	30		8.5E-05	0.0028	8.47E-05	0.0098
	60		7.8E-05	0.0026	7.82E-05	0.0091
	90		6.9E-05	0.0023	6.97E-05	0.0081
	120		6.2E-05	0.0021	6.22E-05	0.0072
Imidacloprid (35% SC)	0	0.057	0.0011	0.0198	0.00112	0.0691
	10		0.0009	0.0172	0.00098	0.0601
	30		0.0009	0.0159	0.00091	0.0559
	60		0.0007	0.0121	0.00068	0.0422
	90		0.0006	0.00980	0.00055	0.0343
	120		0.0005	0.0082	0.00047	0.0288
Indoxacarb (30% WG)	0	0.0200	0.0005	0.0247	0.000494	0.0864
	10		0.0004	0.0208	0.000417	0.0729
	30		0.0003	0.0171	0.000343	0.0599
	60		0.0003	0.0167	0.000335	0.0586
	90		0.0003	0.0142	0.000286	0.0500
	120		0.0003	0.0136	0.000272	0.0476
Spinosad (48% SC)	0	0.0249	0.0003	0.0134	0.000336	0.0472
	10		0.0003	0.0131	0.000326	0.0458
	30		0.0003	0.0124	0.00031	0.0435
	60		0.0003	0.0115	0.000288	0.0405
	90		0.0003	0.0101	0.000253	0.0355
	120		0.00023	0.0095	0.000237	0.0332

RfD, USEPA' reference dose (USEPA, 2012).

Table 9: Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) for Analyzed Pesticides from Boiled Cowpea Samples Consumed by Adults and Children.

Pesticide	RfD*	EDI (Adult)	THQ (Adult)	EDI (children)	THQ (children)
Abamectin	0.003	7.81319E-05	0.02604	0.000273	0.091154
Emamectin benzoate	0.030	9.90321E-06	0.00033	3.47E-05	0.001155
Imidacloprid	0.057	0.000126797	0.00223	0.000444	0.007786
Indoxacarb	0.020	0.000161265	0.00806	0.000564	0.028221
Spinosad	0.0249	6.83162E-05	0.00274	0.000239	0.009603

*RfD, USEPA' reference dose (USEPA, 2012).

Conclusion

To conclude, the results of the present study show that novel non-conventional insecticides can be effective against the cowpea weevil, *C. maculatus*. Among the five tested insecticides, abamectin, emamectin benzoate and indoxacarb seemed to be more effective, but all insecticides provided a satisfactory level of control at the exposure intervals examined here. The insecticides of natural origin, i.e. abamectin, emamectin benzoate and Spinosad can be attractive as an alternative to the synthetic pesticides because, although they have long-term action in storage, it is harmless to mammals and lack other environmental side effects. Therefore, further investigation of the potential of these insecticides would be needed to include experiments on their performance during long-term storage.

Although the residual contents of these insecticides in/on cowpeas via 120-day storage, they have a low dietary intake risk in cowpeas, according to the Target Hazard Quotient. The residual concentrations of tested insecticides found in/on cowpeas were highly degraded by boiling.

Declarations

Ethical Approval: Not applicable.

Competing Interests: The authors declare that there were no conflicts of interest.

Authors' Contributions: All authors contributed to the study plan, sample collection and preparation of field experiment and carrying out it, interpretation of results, and writing, reviewing, and editing the manuscript.

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