DETERMINATION OF FIELD-BASED BIOLOGICAL CONSEQUENCES OF INSECTICIDE SPRAYS ON INSECT POPULATION, COWPEA GRAIN, AND YIELD IN ADOPTERS OF PBR AND NON-PBR COWPEA IN 4 AGROECOLOGICAL ZONES OF NIGERIA

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BEING A TECHNICAL REPORT SUBMMITED TO THE AFRICAN AGRICULTURAL TECHNOLOGY FOUNDATION (AATF), UNDER THE SRA PROJECT

MAY, 2023

EXECUTIVE SUMMARY

Insect pest has been a major challenge of cowpea production in sub-Saharan Africa. The legume pod borer *Maruca vitrata* is a major pest attacking cowpea which can caused a yield loss of about 20 to 80%. Chemical insecticides are widely used to manage insect pests on cowpea; however, there is always concerns on the effect of chemical residues on humans and environment as a result of indiscriminate use on cowpea production. The Pod Borer Resistant (PBR) transgenic *Maruca vitrata* cowpea; SAMPEA-20T has proven very effective in the control of the *M. vitrata* with promising grain yield. The PBR cowpea is now being cultivated by Nigerian farmers. However, no information on the influence of this new control practice on the non-target organisms in farmer's fields. Therefore, a survey was conducted to evaluate the impact of insecticide sprays on the insect population, cowpea yield, and insecticide residue in the fields of adopters and nonadopters of PBR cowpea farmers in selected areas of four agroecological zones (Sahel, Sudan, Northern Guinea, and Southern Guinea) of Nigeria. The result of the survey has found the presence of 21 insect species of which 12 were pest, 7 predators and 2 pollinators. No Maruca larvae were found in the PBR cowpea fields sprayed twice, however, Maruca larvae were found in the non-BPR cowpea sprayed 2,4, or 5 times. Other non-target organisms (NTO) were more in the PBR and non-PBR cowpea sprayed 2 times than the non-PBR cowpea sprayed multiple times (4 or 5). The PBR cowpea field sprayed twice with insecticide had the highest cowpea grain yield compared to the non-PBR cowpea field sprayed up to 4 times. The result of the chemical residue analysis using GC-MS has detected 6 insecticide compounds (Chlorpyrifos, Cypermethrin, Methyl parathion, Lamdacyhalothrin, Dimethoate, Fenpropathrin) in the samples. Only Lamdacyhalothrin and Fenpropathrin were detected in the PBR cowpea 2 sprays, with a residue concentration below the EU's MRL. The residue level of Cypermethrin sprayed 5 times (0.06mg/kg), Methyl parathion sprayed 4 and 5 times (0.05mg/kg), Dimethoate sprayed 4 (0.09mg/kg) and 5 (0.06mg/kg) times and Fenpropidin sprayed 4 (0.02mg/kg) and 5(0.03mg/kg) times in the non-PBR cowpea were higher than the EU's MRL The concentration of some of the compounds was affected by the storage period (0 or 4 weeks) of the samples. Famers need to be more aware of the significant of adopting the BPR cowpea technology.

1.0 Introduction

Cowpea is one of the most important leguminous crops being cultivated as food and fodder as well as utilized as manure in West Africa, particularly Nigeria. Nigeria is the largest producer and consumer of cowpea in the world, accounting for 61% of production in Africa and 58% worldwide (IITA, 2015). Cowpea forms an important staple leguminous diet of more than 200 million households in Sub-Saharan Africa (USAID 2012; OECD 2019). Nutritionally, humans and animals benefit from the protein-rich seeds and haulm this plant produces (Muranaka *et al*., 2016; Horn *et al.*, 2022). Unlike many other legumes, cowpea green leaves and pods can be eaten before crop maturity, helping to bridge the hunger gap between harvests (Vissoh *et al*., 2007; Horn *et al.*, 2022). This plant is resilient and able to withstands low rainfall and poor soil conditions through its unique ability to fix soil nitrogen (Timko and Singh 2008).

Cowpea is attacked and damaged by many insect pests, this includes the legume pod borer *Maruca vitrata*, flower thrips (*Megalurothrips sjostedti*), aphids (*Aphis craccivora*), Complex of pod sucking bugs (*Clavigralla tomentosicollis and Riptortus denkipes*) and the storage pest *Callosobruchus maculate* (Fatokun, *et al*., 2002). *Maruca vitrata* is one of the most damaging post flowering insect pests of cowpea. Attack by this pest occurs at all reproductive stages of cowpea growth which consequently reduce grain yield by over 80% (Ogunwolu, 1990).

In cowpea production, chemical insecticides are widely used to control insect pests; however, insecticides are not an easy viable solution for smallholder farmers in Africa due to their indiscriminate use in cowpea production. This attitude raises a number of concerns such as potential toxicity to humans' health and environment in addition to increase in the cost of production. Therefore, a safe, efficient, and environmentally friendly pest control strategy is required to reduce the use of insecticides in the pest of cowpea. In Nigeria the Pod Borer Resistant (PBR) cowpea; SAMPEA-20T, has proven very effective in the control of *M. vitrata* pest with high grain yield increase (Umar *et al*., 2022). Since its release for commercial cultivation in 2019, the PBR cowpea has being in cultivation by the Nigerian farmers. Since then, there has been high demand of the seed probably because of the reduced in frequency of insecticide spray; 2 sprays, and higher yielding advantage of the variety. In Nigeria, farmers spray their cowpea field between 5-8 times during the season due to the incidence of *M. vitrata* and other pests. This may result in the fact that most cowpea harvested has high pesticide residues which may have an effect on humans' health and the environment. However, the BPR cowpea has a protective trait against *M. vitrata* thus the reduce 2 sprays to protect the crop against other pests during its reproductive stage. There was no information on the influence of this new control practice on the non-target organisms as well as the chemical residue in the cowpea grain produced by the Nigerian farmers. Therefore, the objective of this work was to determine the impact of insecticide sprays on the insect population, cowpea grain yield, and insecticide residue in the fields of adopters and nonadopters of PBR cowpea farmers in Nigeria.

2.0 Materials and Methods

2.1 Survey location

The survey was carried out in the fields of cowpea farmers of PBR and non-PBR in four Agro-ecological zones including the Sahel savannah (Katsina state), the, Sudan savannah (Kano state) Northern Guinea savannah (Kano & Kaduna states) and the Southern Guinea savannah (Abuja, Kuje) of Nigeria (Table 1) to sample insect pests and non-pests, as well as to collect cowpea grain samples after harvest to determine insecticide residue in the grain.

Table 1 Name and GPS of the sampling area in the four agroecological zones of Nigeria

Zone	Local government	Sampling area	LONGTITUDE	LATITUDE
Sahel savannah	Sandamu	Karkarku	13˚ 00.242N	08˚ 21.159E
Sahel savannah	Matazu	Mazoji	12˚ 59.805N	08˚ 11.63E
Sahel savannah	Daura	Maiaduwa	13˚ 03.956N	08˚ 14.455E
Sahel savannah	Sandamu	Sandamu	12˚ 57.982N	08˚ 23.314E
Sahel savannah	Daura	Nafuta	13˚ 03.676N	08˚ 17.497E
Sahel savannah	Sandamu	Karkarku	13˚ 00.365N	08˚ 20.925E
Sahel savannah	Sandamu	Karkarku	13˚ 00.355N	08˚ 20.925E
Sahel savannah	Sandamu	Karkarku	13˚ 00.333N	08˚ 20.891E
Sahel savannah	Daura	Maaduwa	13°00.174N	08°20.5472E
Sahel savannah	Sandamu	Kagare	12°57.591N	08° 23.194E
Sudan savannah	Dawakintofa	Bagadawa	12˚ 06.525N	08˚ 24.078E
Sudan savannah	Dawakintofa	Bagadawa	12˚ 06.362N	08˚ 24.040E
Sudan savannah	Makoda	Tukwi	12°21'45.0"N	8°30'49.2"E
Sudan savannah	Dawakintofa	Bambarawa	12˚ 06.370N	08˚21.800E
Sudan savannah	Dawakintofa	Gajimi	12°5'37.2"N	8°21'8.0"E
Sudan savannah	Makoda	Babaruga	12°19'38.6"N	8°31'12.1"E
Sudan savannah	Makoda	Babaruga	12˚ 19.360N	08˚ 31.900E
Sudan savannah	Dawakintofa	Hayin hago	12°05'37.2"N	8°21'07.9"E
Sudan savannah	Dawakintofa	Dawakiwest	12°05'37.4N	8°20'3.01"E
Sudan savannah	Dawakintofa	Zangon dawanau	12°05'26.8N	8°24'2.3"E
Northern Guinea	Tudun wada	Yaryasa	10°42'49.4"N	7°31'44.4"E
Northern Guinea	Tudun wada	Labi	11°19'51.6"N	8°18'00.0"E
Northern Guinea	Tudun wada	Anguwan tsamiya	12˚ 18.170N	08˚ 16.410E
Northern Guinea	Kiru	Kiru	11°20'11.6"N	8°17'47.2"E
Northern Guinea	Igabi	Gidan Kanawa birnin yaro	10°42'52.2"N	7°29'19.4"E
Northern Guinea	Igabi	Birnin yaro	10°45'58.6"N	7°30'41.9"E
Northern Guinea	Igabi	Sabon yelwa	10°52'17.4"N	7°36'48.6"E
Northern Guinea	Igabi	Zagina	10°52'30.1"N	7°37'38.9"E

Northern Guinea	Sabongari	Anguwan naibi	11°10'32.7"N	7°38'19.8"E
Northern Guinea	Sabongari	Anguwan gwaiba	11°10'26.7"N	7°38'35.7"E
Northern Guinea	Igabi	Sabon yelwa	10°52'17.2"N	7°36'47.4"E
Northern Guinea	Igabi	Birnin yaro	10°45'59.01"N	7°30'42.9"E
Northern Guinea	Tudun wada	Yaryasa west	11°20'26.11"N	8°18'20.4"E
Northern Guinea	Tudun wada	Yaryasa west	11°20'12.2"N	8°18'11.4"E
Northern Guinea	Sabongari	Samauru	11°09'33.7"N	7°38'07.0"E
Northern Guinea	Tudun wada	Yaryasa west	11°20'07.3"N	8°17'54.7"E
Southern Guinea	Kuje	Kuje	8˚53.480"N	07˚13.360E
Southern Guinea	Kuje	Chibiri	8˚53.350"N	07˚ 10.470E
Southern Guinea	Kuje	Kuje	8°52'58.2"N	7°13'05.8"E
Southern Guinea	Abuja	Gui	8°53'49.1"N	7°13'36.8"E
Southern Guinea	Abuja	Gui	8°54'25.6"N	7°12'56.9"E
Southern Guinea	Abuja	Gui	8°54'18.6"N	7°12'55.7"E
Southern Guinea	Kuje	Chibiri	8°53'49.2"N	7°10'50.4"E
Southern Guinea	Kuje	Chibiri	8°53'59.2"N	7°10'50.1"E
Southern Guinea	Kuje	Kuje	8°54'23.2"N	7°12'56.7"E
Southern Guinea	Kuje	Kuje	8°53'49.0"N	7°13'34.1"E
Southern Guinea	Abuja	Gui	8°53'50.12"N	7°13'36.1"E
Southern Guinea	Kuje	Kuje	8°54'24"N	7°12'57.8"E
Southern Guinea	Kuje	Chibiri	8°53'39.2"N	7°10'47.7"E
Southern Guinea	Kuje	Chibiri	8°53'36.0"N	7°10'47.0"E
Southern Guinea	Kuje	Chibiri	8°53'29.5"N	7°12'41.5"E
Southern Guinea	Kuje	Kuje	8°54'18.6"N	7°12'55.7"E

2.2 Selection of PBR and Non-PBR farmers

In each of the locality mentioned in section 2.1 above, five PBR cowpea farmers were randomly selected from a list of PBR cowpea famers provided by seed companies, which was authenticated by a pre-survey conducted by Agric extension expert from the Institute for Agricultural Research, ABU Zaria. Similarly, a corresponding five farmers growing non-PBR cowpea in each location were also selected for the study.

2.3 Insect sampling in PBR and Non-PBR cowpea farmers' fields

In each of the PBR and non-PBR farmers field a 5m x 5 m area was demarcated from the center of each farm. Each field was sampled for Target (Maruca) and Non-Target organism (NTO) such as Thrips, Pod sucking (PSB), Aphids, Bugs, ladybird beetles, ants, syrphids, spiders and other insects available during the survey. Two methods of insect sampling, visual observation & sticky trap were used.

Visual observation: Forty (40) flowers were randomly sampled within the 5 m x 5m demarcated farmers' field. Each flower was opened and the presence of thrips and Maruca. Pod sucking bugs and Aphids occurring in each field were visually observed, counted and recorded. Other NTO and other insects were visually observed and counted.

Sampling with sticky trap: At the time of visual observation, 2 sticky traps were placed from the center of each field at a distance of 3 m apart and allowed to stay for two weeks after which each trap was retrieved for identification and recording of the insect caught. In each case of the sampling, a sample of each of the insect found was place in insect sampling bottles containing 75% ethanol solution and taken to the Department of Crop Protection, Institute for Agricultural Research, Ahmadu Bello University Zaria insect museum. All the insects observed were identified counted and recorded according to the locations where they were found.

2.4 Determination of yield in the PBR and Non-PBR cowpea fields

At maturity, dried pods within each of the 5m x 5m demarcated area were harvested, threshed and grain weight was taken. The grain weight for each of the fields was expressed as yield in kilograms per hectare.

2.5 PBR and Non-PBR cowpea grain sample collection for insecticide residue Analysis

In each farmer's field 100g were randomly sampled and the samples were bulked based on whether it was PBR cowpea and number of sprays (2,4, or 5) for non-PBR cowpea after which 600g of grain samples were collected for each sample and put in a plastic container then transported to the Department of Crop Protection, ABU Zaria. The 600 g for each sample were divided into two portions of 300 g sample each. One portion was considered as sample at harvest called 0 storage and the other was subjected to a 4 weeks storage condition, such that there was a 0 and 4-weeks storage samples.

2.5. Storage experiment

One hundred grams (100g) were weighted from each sample and placed in a clean cheese cloth bag (15cm x 14cm) and tightened. Each sample was repeated 3 times and arranged in a completely randomized design and kept on a laboratory bench for 4 weeks after which 100g of each sample was taken for extraction.

2.6 Extraction of insecticide residues from the grain samples

One hundred grams (100g) each of the PBR 2 sprays, non-PBR 2, 4 and 5 sprays for the 0- and 4-weeks storage were taken and milled to powder form at particle size of 0.1 mm, using an MFC-90D micro-hammer mill (Culatti, Zürich, Switzerland). The pesticides were extracted from the samples using a modified QuEChERS method combined with a dispersive liquid-liquid microextraction (DLLME) procedure, involving the following steps:

(i) 2.5g of thoroughly homogenized sample (flour) were weighted into a 50ml polypropylene centrifugation tubes;

(ii) 10ml of deionized water was added and mixed with glass rod.

(iii) 10 ml of acetonitrile containing 1% acetic acid was added for the extraction process.
(iv) The tubes were sealed and placed on a shaker at 130 rpm for 30 min.
(v) 4 g of anhydrous MgSO4 and 1 g of NaCl were added to induce phase separation.
(vi) The tubes were sealed and shaken gently by hand for 1 min.
(vii) The tubes were centrifuged at 5000 rpm for 4min.
(viii) Then a DLLME procedure was performed by transferring 1ml of the MeCN extract to 4mL vial tubes.
(viii) 200µl of carbon tetrachloride was added
(ix) The mixtures were rapidly transferred to a 25ml screw cap plastic tubes with conical bottom containing 4ml of deionized water.
(x) The tubes were sealed and gently shaken by hand for 30s.
(xi) The tubes were centrifuged at 5000 rpm for 1min.
(xii) 100µl of the settled volume was transferred into vials and 1µl of each of the sample extracts was injected into the GC-MS system.

2.7 Preparation of the pesticide standard solution

Certified reference mixed analytical insecticide standard containing Lambda-cyhalothrin, Cypermethrin, Dichlorvos, Dimethoate, Chlorpyrifos were obtained from the Accustandard analytical company, USA. The standards were prepared separately in acetonitrile (MeCN) at a concentration of 1000 mg / L and stored at -20 ◦C until use. A mixed standard solution of 50 mg/L in MeCN containing all the aforementioned pesticides was prepared by adding the appropriate volume of each stock solution to a 50 mL volumetric flask, and the volume was divided by adding acetone. An intermediate mixed standard solution of 10 mg / L was prepared in MeCN from the mixed standard solution of 50 mg/L. Then, working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 5.0 mg/L in MeCN were prepared by transferring the appropriate amount from a 10 mg/L intermediate mixed standard solution into 10 separate 10-ml volumetric flasks. All the standard solutions were kept in a freezer at -20 ◦C until use. The matrix-matched standard for the preparation of the calibration curve was made by adding multiple standards working solutions in the blank extracts of both matrices separately to reach the desired concentrations (0.01, 0.02, 0.05, 0.1, 0.2, 0.3, and 0.5 mg/kg) and stored at -20

2.8 Gas Chromatography/ Mass spectrometer (GC/MS) Analysis

Gas Chromograph linked to mass spectrometry (GC-MS) was used to determine the insecticide residues in the PBR and Non-PBR cowpea samples collected from farmers' fields. The sample extracts were analyzed using the Agilent Technologies network GC-MS system coupled with a universal detector. The model number of the column used was Agilent19091- 433UI capillary column with specification: 30 m x 0.25 mm id with 0.25µm film thickness (5% diphenyl, 95% dimethyl polysitoxane). The carrier gas was helium at a flow rate of 1ml/min. The oven temperature was initially programmed at 50^{o}C for 2min and then increased by 8c/min to 300 ^{o}C. A 25µl Glass Hamilton syringe was used to inject 1 µl of each sample into the GC machine. Ion

count was used to evaluate for compound identification and quantification. The spectrum of the separated compound was compared with the database of the spectrum of known compound save in the NIST reference spectra library. The recorder within several minutes recorded several peaks and print out the peaks, the retention times and quantification of the compounds. All the extraction and GC-MS analysis were performed at the Multi-User Research Laboratory, Department of Chemistry, Ahmadu Bello University Zaria.

3.0 Results

3.1 Insect sampling

Table 2 shows the average number of different insect species found per $5m^2$ PBR cowpea fields sprayed 2 times and non-PBR cowpea sprayed 2, 4 or 5 times in the northern guinea savannah of Nigeria. A total of 17 different insect species belonging to 11 different insect orders were found, of which only 1 was Lepidopteran species (*M. vitrata)*, Thysanoptera (1), Hemiptera (3), Hymenoptera (3), Coleoptera (3), Diptera (1), Homoptera (1), Araneae (1), Hemiptera (1), Orthoptera (1) and Dermaptera (1). Ten of the insect species were pest and 7 species were either predators or pollinators. There was no Maruca larvae found in the $5m^2$ PBR cowpea field sprayed 2 times with insecticides, however between 3-5 Maruca larvae were found in the non-PBR- cowpea fields sprayed 2, 4 or 5 times with insecticides, with 4 and 5 sprays had the least. All insect species were present in the PBR cowpea field sprayed 2 times. However, in the non-PBR cowpea field, some species were absent or occurred in a small number, particularly 4 and 5 sprays. This could be the result of frequent spraying, which can affect the abundance of biodiversity and the food chain in an ecosystem.

Table 2 Taxonomic order, status and number of insect species found in PBR and non-PBR cowpea famers fields in the northern Guinea savannah of Nigeria

				Average number of insect species/ $5m^2$			
Common Name	**Order**	**Species name**	**Status**	**BPR- 2 sprays**	**Non-PBR 2 sprays**	**Non-PBR 4 sprays**	**Non-PBR 5 sprays**
Cowpea pod borer	Lepidoptera	*Maruca vitrata*	Pest	0	5	3	3
Cowpea Flower Thrips	Thysanoptera	*Megalurothrips sjostedti*	Pest	2	6	4	3
Cowpea Aphids	Hemiptera	*Aphis craccivora*	Pest	10	37	33	1
Brown pod-sucking bug	Hemiptera	*Clavigralla tomentosicollis*	Pest	4	5	4	4
Pod-sucking bug	Hemiptera	*Riptortus denti pes*	Pest	1	2	0	0
Black ant	Hymenoptera	*Componotus perris*	Predator	9	4	0	0

Surgar ant	Hymenoptera	*Componotus terebrans*	Predator	4	6	3	0
Honey bee	Hymenoptera	*Apis melifera*	Pollinator	1	1	0	0
Housefly	Diptera	*Musca domestica*	Pollinator	1	1	1	0
Blister beetle	Coleoptera	*Mylabris fimbriatus*	Pest	2	1	2	2
Blister beetle	Coleoptera	*Mylabris phalerata*	Pest	1	1	1	0
Spittle bug	Homoptera	*Locris rubens*	Pest	3	1	0	0
Ladybirds beetle	Coleoptera	*Cheilomenes sulphurea*	Predator	1	0	0	0
Huntsman spider	Araneae	*Palystes Castaneus*	Predator	1	0	0	0
Leafhopper	Hemiptera	*Empoasca dolichi*	Pest	1	2	2	12
Earwig	Dermaptera	*Forficula senegalensis*	Predator	2	1	1	0
Bushcricket	Orthoptera	*Phaneroptera nana*	Pest	1	0	0	0

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Table 3 shows the average number of different insect species found per $5m^2$ PBR cowpea fields sprayed 2 times and non-PBR cowpea sprayed 2, 4 or 5 times in the southern guinea savannah of Nigeria. A total of 21 different insect species belonging to 11 different insect orders were found, of which only 1 was Lepidopteran species (*M. vitrata)*, Thysanoptera (1), Hemiptera (3), Hymenoptera (3), Coleoptera (3), Diptera (2), Homoptera (1), Araneae (1), Hemiptera (1), Orthoptera (2) and Dermaptera (1). Twelve of the insect species were pests and nine species were predators or pollinators. There was no Maruca larvae found in the $5m^2$ PBR cowpea field sprayed 2 times with insecticides, however between 2-4 Maruca larvae were found in the non-PBR- cowpea fields sprayed 2, 4 or 5 times with insecticides, with 5 sprays had the least. All insect species were found to be present in the PBR cowpea field sprayed 2 times except the Blister beetle and the Spittle bug. However, in the non-PBR cowpea field, some species were either absent or occurred in a small number, particularly the 5 sprays.

Table 3 Taxonomic order, status and number of insect species found in fields of PBR and non-PBR cowpea famers fields in the southern guinea savannah of Nigeria

				Average number of insect species/ $5m^2$			
Common Name	**Order**	**Species name**	**Status**	**BPR- 2 sprays**	**Non-PBR 2 sprays**	**Non-PBR 4 sprays**	**Non-PBR 5 sprays**
Cowpea pod borer	Lepidoptera	*Maruca vitrata*	Pest	0	4	3	2
Cowpea Flower Thrips	Thysanoptera	*Megalurothrips sjostedti*	Pest	2	2	1	3
Cowpea Aphids	Hemiptera	*Aphis craccivora*	Pest	210	150	17	0
Brown pod-sucking bug	Hemiptera	*Clavigralla tomentosicollis*	Pest	3	5	2	1
Pod-sucking bug	Hemiptera	*Riptortus denti pes*	Pest	2	0	0	0
Black ant	Hymenoptera	*Componotus perris*	Predator	0	0	2	0
Surgar ant	Hymenoptera	*Componotus terebrans*	Predator	0	2	1	0
Honey bee	Hymenoptera	*Apis melifera*	Pollinator	1	1	0	0
Housefly	Diptera	*Musca domestica*	Pollinator	1	0	1	0
Blister beetle	Coleoptera	*Mylabris fimbriatus*	Pest	1	1	1	0
Blister beetle	Coleoptera	*Mylabris phalerata*	Pest	1	2	0	1
Blister beetle	Coleoptera	*Coryna argentata*	Pest	0	0	2	0

Spittle bug	Homoptera	*Locris rubens*	Pest	0	0	1	0
Ladybirds beetle	Coleoptera	*Cheilomenes sulphurea*	Predator	2	1	1	0
Ladybird beetle	Coleoptera	*Cheilomenes vacina*	Predator	2	2	1	0
Hoverfly	Diptera	*Allograpta obliqua*	Predator/Pollinator	1	0	0	0
Huntsman spider	Araneae	*Palystes Castaneus*	Predator	1	1	1	0
Leafhopper	Hemiptera	*Empoasca dolichi*	Pest	2	3	4	0
Variegated grasshopper	Orthoptera	*Zonocerus variegatus*	Pest	1	0	0	0
Earwig	Dermaptera	*Forficula senegalensis*	Predator	1	1	0	1
Bushcricket	Orthoptera	*Phaneroptera nana*	Pest	1	0	2	0

Table 4 shows the average number of different insect species found in $5m^2$ PBR cowpea fields sprayed twice and non-PBR cowpea sprayed four times in the Sudan savannah of Nigeria. A total of 17 different insect species belonging to 9 different insect orders were found, of which only 1 was Lepidopteran species (*M. vitrata),* Thysanoptera (1), Hemiptera (3), Hymenoptera (2), Coleoptera (4), Diptera (2), Hemiptera (1), Orthoptera (2) and Dermaptera (1). Eleven of the insect species were pests and six species were predators or pollinators. There was no Maruca larvae found in the $5m^2$ PBR cowpea field sprayed 2 times with insecticides, however an average of 2 Maruca larvae were found in the non-PBR- cowpea fields sprayed 4 times with insecticides. All insect species were found to be present in the PBR cowpea field sprayed 2 times except the Blister beetle. However, in the field of non-PBR cowpea 4 sprays, some species (Black ant, Sugar ant, Blister beetle, Hoverfly, leafhopper, Earwig) were absent or occurred in small number.

Table 4 Taxonomic order, status, and number of insect species found in the PBR and non-PBR cowpea famers fields in the Sahel savannah of Nigeria

				Average number of insect species/ $5m^2$	
Common Name	Order	Species name	Status	PBR- 2 sprays	Non-PBR 4 sprays
Cowpea pod borer	Lepidoptera	*Maruca vitrata*	Pest	0	2
Cowpea Flower Thrips	Thysanoptera	*Megalurothrips sjostedti*	Pest	3	3
Cowpea Aphids	Hemiptera	*Aphis craccivora*	Pest	33	15
Brown pod-sucking bug	Hemiptera	*Clavigralla tomentosicollis*	Pest	6	2

Pod-sucking bug	Hemiptera	*Riptortus denti pes*	Pest	3	1
Black ant	Hymenoptera	*Componotus perris*	Predator	9	0
Surgar ant	Hymenoptera	*Componotus terebrans*	Predator	33	0
Housefly	Diptera	*Musca domestica*	Pollinator	1	1
Blister beetle	Coleoptera	*Mylabris fimbriatus*	Pest	1	0
Blister beetle	Coleoptera	*Mylabris phalerata*	Pest	0	1
Blister beetle	Coleoptera	*Coryna argentata*	Pest	1	1
Ladybirds beetle	Coleoptera	*Cheilomenes sulphurea*	Predator	5	1
Hoverfly	Diptera	*Allograpta obliqua*	Predator/Pollin ator	1	0
Leafhopper	Hemiptera	*Empoasca dolichi*	Pest	5	0
Variegated grasshopper	Orthoptera	*Zonocerus variegatus*	Pest	2	1
Earwig	Dermaptera	*Forficula senegalensis*	Predator	1	0
Bushcricket	Orthoptera	*Phaneroptera nana*	Pest	2	2

Table 3 shows the average number of different insect species found per $5m^2$ PBR cowpea fields sprayed 2 times and non-PBR cowpea sprayed 4 times in the Sudan savannah of Nigeria. A total of 17 different insect species belonging to 11 different insect orders were found, of which only 1 was Lepidopteran species (*M. vitrata)*, Thysanoptera (1), Hemiptera (3), Hymenoptera (2), Coleoptera (3), Diptera (2), Homoptera (1), Araneae (1), Orthoptera (2) and Dermaptera (1). Nine of the insect species were pest and 8 species were either predators or pollinators. There was no Maruca larvae found in the $5m^2$ PBR cowpea field sprayed 2 times with insecticides, however an average of 2 Maruca larvae were found in the non-PBR- cowpea fields sprayed 4 times with insecticides. All insect species were found to be present in the PBR cowpea field sprayed 2 times except Aphids and variegated grasshopper. However, in the field of non-PBR cowpea 4 sprays some species were either absence or occurred in a small number.

Table 5 Taxonomic order, status and number of insect species found in the PBR and Non-PBR cowpea famers fields in the Sudan savannah of Nigeria

				Average number of insect species/ 5m^2	
Common Name	**Order**	**Species name**	**Status**	**BPR- 2 sprays**	**Non-PBR 4 sprays**
Cowpea pod borer	Lepidoptera	*Maruca vitrata*	Pest	0	2
Cowpea Flower Thrips	Thysanoptera	*Megalurothrips sjostedti*	pest	3	3
Cowpea Aphids	Hemiptera	*Aphis craccivora*	pest	0	15
Brown pod-sucking bug	Hemiptera	*Clavigralla tomentosicollis*	pest	4	2
Pod-sucking bug	Hemiptera	*Riptortus denti pes*	pest	1	1
Surgar ant	Hymenoptera	*Componotus terebrans*	Predator	5	0
Honey bee	Hymenoptera	*Apis melifera*	Pollinator	1	0
Housefly	Diptera	*Musca domestica*	pollinator	1	1
Blister beetle	Coleoptera	*Coryna argentata*	Pest	1	1
Spittle bug	Homoptera	*Locris rubens*	Pest	1	
Ladybirds beetle	Coleoptera	*Cheilomenes sulphurea*	Predator	5	0
Ladybird beetle	Coleoptera	*Cheilomenes vacina*	Predator	1	1
Hoverfly	Diptera	*Allograpta obliqua*	Predator/Pollinator	2	0
Huntsman spider	Araneae	*Palystes Castaneus*	Predator	1	0
Variegated grasshopper	Orthoptera	*Zonocerus variegatus*	pest	0	1
Earwig	Dermaptera	*Forficula senegalensis*	Predator	1	0
Bushcricket	Orthoptera	*Phaneroptera nana*	Pest	1	2

3.2 Grain yields of PBR and non-PBR cowpea famers fields in four agroecological zones of Nigeria

Table 6 shows the cowpea grain yield of PBR cowpea 2 sprays and non-PBR cowpea 2, 4 or 5 sprays fields in the four Agro-ecological zones of Nigeria. The two sprays of PBR cowpea fields had the highest gran yield compared to the 2, 4 or 5 sprays of non-PBR cowpea fields in all the Agro-ecological zones.

Table 6 PBR and Non-PBR cowpea grain yield after harvest at four Agro-ecological zones of Nigeria

	Cowpea grain yield Kg/ha			
Location	**BPR- 2 sprays**	**Non-PBR-2 sprays**	**Non-PBR 4 sprays**	**Non-PBR 5 sprays**
Northern Guinea	1476	796	1248	1533
Southern Guinea	1720	696	1252	1557
Sahel Savannah	1226	*	919	*
Sudan Savannah	1521	*	1291	*

*****Data not available: The survey commenced at the late season when most farmers harvested their cowpea; farmers under this category in the Sahel & Sudan savannah were missed out

3.3 Determination of insecticide residue in PBR and Non-PBR cowpea grain samples in four Agro-ecological zones of Nigeria

Table 7 shows the common insecticides used by farmers to manage insect infestation during the 2022 cowpea production season in the four agroecological zones.

Table 7 Common insecticides and their active ingredients used by farmers in the 4 agroecological zones

Trade name	**Active ingredients**	**Percentage of pesticide usage by Agro-ecology**			
		Sahel	Sudan	N/Guinea	S/Guinea
Imiforce	Imidacloprid	29	0	6	13
Best action	Cypermethrin + Dimethoate	24	0	0	0
Cyperforce	Cypermethrin	40	20	25	30
Predator	Chlorpyrifos	7	20	25	0
Karate/Laraforce	Lambda cyhalothrin	0	40	13	19
Scorpion	Lambdacyhalothrin + Dimethoate	0	20	25	13

No identity (Famers cannot say the type of chemical used)	0	0	6	25

Table 8 shows the concentration of different insecticides (Chlorpyrifos, Cypermethrin, Methyl parathion, Lamdacyhalothrin, Dimethoate, Fenpropathrin) residues obtained from the PBR and Non-PBR cowpea grains samples across three different spray regimes (2, 4, 5). The concentrations were compared with the maximum residual limits of the European Union (EU MRLs). Insecticide residue analysis indicated that all the samples contained one or more insecticide compounds. Based on the overall mean concentration, Methyl parathion (0.03mg/kg), Lamdacyhalothrin (0.02 mg / kg), dimethoate (0.06 mg / kg) and Fenpropathrin (0.02mg/kg) had residues above the EU's MRLs. However, the overall mean concentration for Chlorpyrifos (0.02mg/kg) and Cypermethrin (0.04mg/kg) were below the EU's Maximum Residue Limits (MRLs). Only Lamdacyhalothrin and Fenpropathrin were detected in the PBR cowpea 2 sprays with a residue concentration below the EU's MRL The residue level of Cypermethrin sprayed 5 times (0.06mg/kg), Methyl parathion sprayed 4 and 5 times (0.05mg/kg), Dimethoate sprayed 4 (0.09mg/kg) and 5 (0.06mg/kg) times and Fenpropidin sprayed 4 (0.02mg/kg) and 5(0.03mg/kg) times were higher than the EU's MRL However, when the sample were stored for 4 weeks the residue levels of some compounds were reduced.. Other compounds were not detected in the PBR cowpea 2 sprays. The presence of a high concentration of some insecticide compounds in the 4 and 5 sprays may be due to the frequency of application during the production period. Other levels of insecticides not detected in the samples could be the result of a minimal spray frequency and gradual degradation of pesticides; as a result, the concentration in the sample could be below the detection level as in the case of PBR cowpea 2 sprays.

Table 8. Concentration (mg/kg) of the insecticide residues in the PBR and Non-PBR cowpea grain sample in four Agro-ecological zones of Nigeria

Cowpea Sample	Chlorpyrifos	Cypermethrin	Methyl parathion	Lamdacyhalothrin	Dimethoate	Fenpropathrin
PBR 2 sprays 0 storage	ND	ND	ND	0.01	ND	0.001
PBR 2 sprays 4 weeks after storage	ND	ND	ND	ND	ND	ND
Non PBR 2 sprays 0 storage	0.02	0.03	0.01	ND	0.03	0.01
Non-PBR 2 sprays 4 weeks after storage	0.01	0.02	0.01	ND	0.03	0.01
Non PBR 4 sprays 0 storage	0.01	0.04	0.05	0.02	0.09	0.02

Non-PBR 4 sprays 4 weeks after storage	0.01	0.03	0.03	0.02	0.09	0.02
Non PBR 5 sprays 0 storage	0.02	0.06	0.05	0.01	0.06	0.03
Non PBR 5 sprays 4 weeks after storage	0.02	0.06	0.04	0.01	0.05	0.03
Mean	0.02	0.04	0.03	0.02	0.06	0.02
EU's MRL*	0.05	0.05	0.01	0.01	0.02	0.01

MRL: Maximum Residue Limits; ND: not detected

4.0 Conclusion

This study has found that insecticide spray regime had a profound effect on insect population, cowpea grain yield and insecticide residue in the fields. No Maruca infestation was found in the PBR cowpea sprayed two times. The NTO were higher in the PBR cowpea sprayed 2 times than in the non-PBR cowpea sprayed multiple times (4 or 5). The grain yield was higher in the PBR cowpea sprayed 2 times than in the non-PBR cowpea sprayed 2,4 or 5 times. Residues of six compounds namely Chlorpyrifos, Cypermethrin, Methyl parathion, Lamdacyhalothrin, Dimethoate, and Fenpropathrin were detected from the PBR and non-PBR grain samples. The concentration of all the insecticide residue in the non-PBR cowpea sprayed multiple times (2,4 or 5) was higher than in the PBR cowpea sprayed only 2 times. The residue level of Cypermethrin sprayed 5 times, Methyl parathion sprayed 4 and5 times, Dimethoate sprayed 4 and5 times and Fenpropidin 4 and 5 times were higher than the EU's MRL. Therefore, for human and environmental safety, it is recommended that famers should be more aware of the significant of adopting the BPR cowpea technology.

5.0 Challenge

1) Untimely release of fund.
2) Untimely commencement of the survey in some locations which resulted in not obtaining famers that practice other spray regimes i.e., in the Sahel and Sudan Savannah some famers had already harvested at the time of the survey.
3) The standard chemical not accompanied with comprehensive method for running the standard chemical in the GC/MS as a result the laboratory conducting the analysis had to worked out the appropriate methods for running the standard; This had caused unnecessary delay for the GC analysis of the samples.

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