



Effects of Neem Aqueous Extract (*Azadirachta indica*) against Aphids and Aphid-borne Virus in Cowpea (*Vigna unguiculata* L. Walp)

Kehinde T. Kareem^{1*}, Rachael B. Olayinka¹, Juliana A. Ugwu² and Olubusola F. Oduwaye¹

¹Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029, Moor Plantation, Ibadan, Nigeria

²Forestry Research Institute of Nigeria, P.M.B.5054, Jericho Hills, Ibadan, Oyo State, Nigeria
E-mails: kt_kareem@yahoo.com*; bolanleomoyeni@yahoo.com; dr.amaka2013@gmail.com; bussyfa@yahoo.com

*Corresponding author

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Abstract

Pests and diseases are among the major factors limiting plant growth and yields. Pests are known vectors of pathogens including viruses. Proper management of plant pests is an indirect means of controlling viral diseases in plants. This study aimed at comparing the potential of neem extract and a synthetic insecticide (lambda cyhalothrin) for the management of aphids and Cowpea aphid-borne mosaic virus (CABMV) in five different cowpea (*Vigna unguiculata*) genotypes and to determine the residual effects of the insecticide in the cowpea grains. The experimental field was laid out in randomized complete block design with three replicates. Treatments included; spraying of cowpea plots with aqueous neem extract and lambda cyhalothrin (LC) twice at foliage stage, once at flowering and podding stages. Control plots were left unsprayed. Data were obtained on growth and yields of cowpea genotypes as well as the pest and virus disease traits. Results of the Analysis of Variance (ANOVA) revealed that variations attributable to genotypes were significant ($p \leq 0.01$) for cowpea yields and virus disease traits. Treatments applied influenced the occurrence of pests and virus diseases. Neem extract reduced aphid infestations and virus diseases as much as the insecticide. Ife BPC accumulated a very high level of LC (1.14 ppm) in its grain when compared with the maximum residue level (MRL) for lambda cyalothrin in cowpea seeds. The study concludes that the use of eco-friendly bio-pesticide such as neem aqueous extract is effective for the management of aphids and aphid-borne virus in cowpea fields.

Keywords: Aphids; aqueous extract; cowpea; neem; pesticide; virus.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an annual legume belonging to the family Fabaceae, subfamily Papilionoideae and genus *Vigna* (OECD 2016). It is a staple crop grown worldwide for its high quality protein and other uses (Animasaun et al. 2015). Many countries in Africa use the tender leaves, immature pods and grains as protein sources for human beings and

livestock (Alemu et al. 2016). Globally, cowpea production was estimated to cover up to 14.5 million hectares of land with about 6.5 million metric tons annual production (Boukar et al. 2018). In 2017, cowpea was grown on about 95.6% of agricultural land area in sub-Sahara Africa and Nigeria was the largest producer followed by Niger, Burkina Faso, Mali and Senegal (FAOSTAT 2019).

The production of cowpea in Nigeria is often affected by pests and diseases which attack the crop both on the field and in storage. This has led to reduction in the yields and market values of cowpea grains. The need to curb this menace has necessitated the use of pesticides. One of the side effects of pesticides is their accumulation in the grains which has resulted in the rejection of Nigerian grown cowpea in international markets. Field pests and diseases account for about 80% to 100% losses in cowpea yields (Kareem et al. 2016, Kamara et al. 2018). Cowpea is susceptible to field insect pests which attack other legumes and they include aphids (*Aphis craccivora* Koch), bruchids, beetles, maruca and leafhoppers (Dugje et al. 2009). These pests are found on cowpea during vegetative and reproductive stages, and they transmit viruses during feeding. Infestation by aphids is the most devastating of all pest attacks in cowpea and this could result in complete (100%) yield loss (Horn et al. 2015). Symptoms induced on cowpea due to aphids feeding include chlorosis, stunting, flower abortion and plant death (Keating et al. 2015). Cowpea aphid infestations could result in the spread of about 14 viruses, of which *Cowpea aphid-borne mosaic virus* (CABMV) is the most destructive (Yang et al. 2015). *Cowpea aphid-borne mosaic virus* belongs to the genus *Potyvirus*. It is an important viral disease of cowpea transmitted in a non-persistent manner by aphids (Kareem et al. 2016). Symptoms associated with CABMV infections in cowpea include severe mosaic, leaf blistering, vein banding, leaf distortion and stunting (Elbeshehy et al. 2010). *Cowpea aphid-borne mosaic virus* infection was reported to account for about 7 to 60% losses in the yield of cowpea (Neya et al. 2015).

Management of aphids is achieved by spraying the cowpea with pesticides. However, control of viruses is difficult to achieve because they are found in the host chromosomes. Since CABMV is a vector-transmitted virus, it would be good to experiment if the control of the vector would lead to the control of the virus. It is also important to know the level of accumulation

of toxic compounds in the grains of cowpea as a result of the use of chemicals for controlling pests on the field. This is because chemicals render the grains unfit for both local and international markets. The need to substitute eco-friendly biopesticides for chemical pesticides to obtain wholesome grains free of toxic residues is paramount. Hence, this study focused on (i) the effects of spraying neem (*Azadirachta indica*) extract and lambda cyhalothrin (LC) on the growth and yields of cowpea, (ii) comparing the effectiveness of neem extract and LC for the management of aphids and CABMV in cowpea and (iii) determining the residual effects of LC in cowpea grains.

Materials and Methods

Sources of cowpea seeds

Five cowpea genotypes, namely Ife brown, Ife BPC, ART98-12, Modupe and Drum were used for the study. Four of the genotypes; Ife brown, Ife BPC, ART98-12 and Modupe were developed at the Institute of Agricultural Research and Training, Ibadan. Drum; a local genotype served as a control and was sourced from Apata market in Ibadan.

Study area and experimental design

The experiment was conducted between April and July in 2017 and 2018 at the experimental field of the Institute of Agricultural Research and Training (I.A.R. & T), Ibadan, Nigeria. The experimental field is located at latitude 7° 38'N; longitude 3° 84'E and 174.3 m above sea level in a rainforest-savannah transition zone. The mean rainfall values during the experimental period were 157 mm and 146.5 mm for 2017 and 2018, respectively, while the mean temperature values were 26.63 °C and 27 °C in 2017 and 2018, respectively.

A spacing of 60 cm × 30 cm was used for sowing the seed in a randomized complete block design with three replicates. Three seeds of cowpea were sown in each hill; seedlings were later thinned to one per hill. Based on the treatments applied, plots were grouped into three subplots, and each comprised of all the cowpea genotypes. The

first group was sprayed with lambda cyhalothrin (1 ml per liter of water). The second group was sprayed with neem extracts (50 g of neem leaves soaked in 5 L of water overnight), and the third group was not sprayed and it was regarded as the control (unsprayed). Plots were sprayed twice at foliage stage, once at flowering stage and once at podding stage. Plots were weeded twice at three weeks intervals. Data were obtained on quantitative and qualitative traits of cowpea.

Cowpea quantitative traits

Data were collected from four tagged plants to represent each subplot. Plant height and leaf number were taken at five weeks after planting (WAP). Plant heights (centimeters) were obtained by taking the length of cowpea plants from the base of the plant to the apex with a meter rule. Number of leaves was obtained by counting the number of fully expanded leaves of the tagged plants. At maturity, pod length was measured with a meter rule in centimeters, and the number of seeds per pod was also determined.

Cowpea qualitative traits

Plants were examined at 5 WAP for aphid infestations and scored for aphid abundance using a scale of 0–5 (where 0 = no aphids; 1 = number of aphids between the range of 1 and 10; 2 = aphids number ranging from 11 to 20; 3 = aphids number ranging between 21 and 50; 4 = number of aphids within the range of 50–100; 5 = > than 100 aphids) (Gobiye et al. 2016).

Plants were also observed at 5 WAP for viral disease incidence and the number of plants showing virus disease symptoms was expressed as a percentage of the total number of plants per plot. Viral disease severity was determined by scoring symptomatic plants on a scale of 1–5, where 1 = no virus symptom on plants, 2 = mild symptoms (such as chlorosis, mosaic, necrosis) ranging between 1 and 25% observed on plants; 3 = moderate symptoms (leaf deformation, leaf wrinkling, leaf reduction) ranging from 26–50% observed, 4 = severe symptoms (apical

necrosis, stunting) ranging from 51–75% observed, 5 = very severe symptoms > 75% observed on plants (Kareem et al. 2016).

Virus titer was determined by using antigen coated plate enzyme-linked immunosorbent assay according to the manufacturer's instructions (Agdia-Bioford Inc, Elkhart, Indiana, USA) with slight modifications. One gram of leaf samples collected from CABMV infected cowpea plants at 5 WAP were ground in 10 ml of Agdia's sample extraction buffer. A volume of 100 µl each of the extracted plant sap was dispensed in the microtiter wells of the ELISA plate. Positive and negative controls were included and the plate incubated. Microtiter plate was washed and 100 µl of enzyme conjugate was added and incubated. The enzyme substrate was added after 1 h and the absorbance of the optical density (OD) measured at 405 nm using an ELISA plate reader (ELx800). Absorbance reading was regarded to be positive when the OD value was twice the value of the negative control (healthy plant).

Determination of lambda cyhalothrin residues in cowpea seeds

Harvested seeds from the five cowpea genotypes sprayed with LC were taken to the Central Research Laboratory of the University of Lagos for the analysis of lambda cyhalothrin residues in the seeds. The residues were determined using GC-MS: Diluent Technologies 7890A MS Detector 5975C with 1 µL injector volume, 30 m column length, 0.25 µm film thickness, 0.32 mm internal diameter and helium gas used as the mobile phase.

Statistical analyses

The Statistical Package for Social Scientists (version 20) was used to carry out Analysis of Variance (ANOVA) using General Linear Model (GLM), and Duncan multiple range test was used for mean separation at $p \leq 0.05$.

Results

Cowpea quantitative traits

The results of the analysis of variance (ANOVA) revealed that variations

attributable to genotype were significant for plant height at 5WAP ($p \leq 0.05$), pod length and number of seeds per pod ($p \leq 0.01$). Treatment variations were significant for all the quantitative traits except number of seeds per pod. Genotypes by treatment interactions

were significant except for number of leaves at 5 WAP and pod length. The interactions between year, genotypes and treatments were not significant for any of the growth and yield parameters (Table 1).

Table 1: Analysis of variance for cowpea quantitative traits

Source	Df	F Values			
		Plant height at 5 WAP	No. of leaves at 5 WAP	Pod length (cm)	Number of seed/pod
Rep	2	2.82 ^{ns}	0.71 ^{ns}	0.61 ^{ns}	0.44 ^{ns}
Year (Y)	1	1.02 ^{ns}	0.17 ^{ns}	11.71 ^{**}	3.54 ^{ns}
Genotype (G)	4	2.74 [*]	0.79 ^{ns}	348.36 ^{**}	119.21 ^{**}
Treatment (T)	2	148.32 ^{**}	6.48 ^{**}	4.26 ^{**}	1.33 ^{ns}
G*T	8	2.79 ^{**}	1.65 ^{ns}	1.61 ^{ns}	2.14 [*]
Y*T	2	0.98 ^{ns}	0.07 ^{ns}	6.82 ^{**}	1.33 ^{ns}
G*Y	4	0.63 ^{ns}	0.45 ^{ns}	1.00 ^{ns}	0.60 ^{ns}
G*T*Y	8	0.23 ^{ns}	0.73 ^{ns}	0.70 ^{ns}	0.44 ^{ns}

Df = degree of freedom; *, ** = significant at $p \leq 0.05$ and 0.01 , respectively.

The growth performance of the genotypes indicated that there was no significant difference in the number of leaves at 5 WAP. Drum had the highest plant height (21.14 cm) followed by Ife brown and Ife BPC. The yield results revealed that ART98-12

produced the longest pod (18.07 cm), whereas Drum did not produce any pods. There was no significant difference in the number of seeds per pod produced by ART98-12, Ife BPC and Modupe (Table 2).

Table 2: Overall growth and yield performance of cowpea genotypes

Cowpea genotype	Plant height (cm) at 5WAP	No. of leaf@5WAP	Pod length (cm)	No. of seed/pod
ART98-12	18.70 ^b	8.06 ^a	18.07 ^a	12.17 ^a
Ife brown	20.07 ^{ab}	7.92 ^a	12.78 ^b	9.97 ^b
Ife BPC	19.66 ^{ab}	8.53 ^a	13.42 ^b	12.26 ^a
Modupe	19.04 ^b	8.09 ^a	13.86 ^b	12.22 ^a
Drum	21.14 ^a	8.72 ^a	0.0 ^c	0.0 ^c

Means followed by the same letter along the column are not significantly different according to Duncan multiple range test at $p \leq 0.05$.

Cowpea qualitative traits

The ANOVA for disease traits in cowpea indicated that genotypic variations were significant for all the disease traits at $p \leq 0.01$. Main effect of treatments were also significant for aphid score ($p \leq 0.01$), virus severity ($p \leq 0.05$) and virus titer ($p \leq 0.01$).

Genotypes by treatment interactions were significant for all disease traits except virus severity. The interactions between G*T*Y influenced the titer of virus (virus concentration) in cowpea plants (Table 3).

Table 3: Analysis of variance for cowpea qualitative traits

Source	Df	F Values			
		Aphid score	Virus incidence	Virus severity	Virus titer
Rep	2	0.37 ^{ns}	1.52 ^{ns}	1.31 ^{ns}	345.30 ^{**}
Year (Y)	1	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}
Genotype (G)	4	8.76 ^{**}	11.33 ^{**}	8.93 ^{**}	3.15 × 10 ^{3**}
Treatment (T)	2	25.05 ^{**}	2.79 ^{ns}	3.36 [*]	4.23 × 10 ^{3**}
G*T	8	3.37 ^{**}	2.49 [*]	1.09 ^{ns}	3.58 × 10 ^{3**}
Y*T	2	5.78 ^{**}	0.95 ^{ns}	0.99 ^{ns}	2.01 × 10 ^{3**}
G*Y	4	3.9 ^{**}	0.17 ^{ns}	0.44 ^{ns}	3.82 × 10 ^{3**}
G*T*Y	8	0.85 ^{ns}	0.73 ^{ns}	0.67 ^{ns}	1.19 × 10 ^{3**}

Df = degree of freedom, *, **: significant at $p \leq 0.05$ and 0.01 , respectively.

The genotypes' overall performance in relations to aphid attacks revealed that the responses of all the four I.A.R. & T genotypes were not significantly different from each other, and their scores ranged between 1.17 and 1.56, while the response of Drum (the control) was higher with a score of 2.17 (Figure 1). Virus incidence

was low in all the genotypes and the mean incidence was less than 10% except in Drum which had a mean incidence of 11.41% (Figure 2). The severity of the virus among the genotypes ranged from 1.33 to 2.44 with Drum having the highest severity (Figure 2).

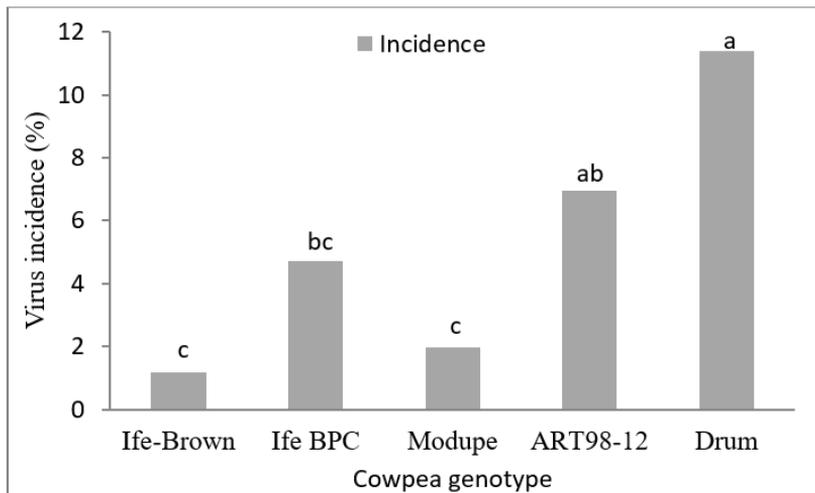


Figure 1: Overall response of cowpea genotypes to virus incidence. Means followed by the same letter are not significantly different according to Duncan multiple range test at $p \leq 0.05$.

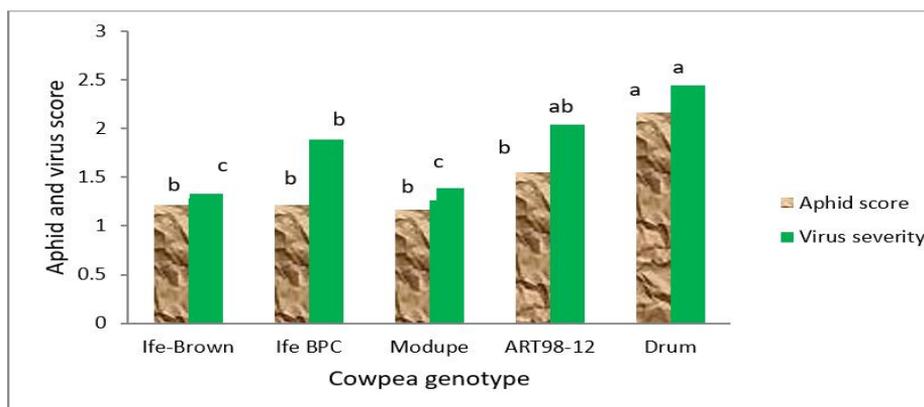


Figure 2: Overall response of cowpea genotypes to aphid attack and virus severity. Means followed by the same letter are not significantly different according to Duncan multiple range test at $p \leq 0.05$.

The genotype ‘Drum’ that was neither sprayed with neem nor with insecticide had the highest significant susceptibility to aphids and virus disease attack compared to other genotypes in 2017 and 2018. There was no aphid infestation in all the I.A.R. & T cowpea genotypes in 2017. Similar results were observed in 2018 except in ART98-12 treated with insecticide and the aphid score was 2.33.

Values of virus incidence in unsprayed Drum plots in 2017 and 2018 were 20.3% and 16.6%, respectively, while the virus severity scores were 3.33 and 3.0 in 2017 and 2018, respectively. Reduced values of virus incidence were recorded for the I.A.R. & T cowpea genotypes compared with Drum (Table 4).

Table 4: Effects of treatments on cowpea disease traits in 2017 and 2018

Treatment	Cowpea genotype	Aphid score		Virus incidence (%)		Virus severity	
		2017	2018	2017	2018	2017	2018
Neem	ART98-12	1.00 ^b	1.00 ^b	0.00 ^c	13.06 ^{ab}	1.00 ^c	2.33 ^{ab}
	Ife brown	1.00 ^b	1.00 ^b	0.83 ^c	0.00 ^d	1.33 ^{ab}	1.00 ^c
	Ife BPC	1.00 ^b	1.00 ^b	1.91 ^c	5.53 ^c	1.33 ^{ab}	2.00 ^{ab}
	Modupe	1.00 ^b	1.00 ^b	1.11 ^c	1.85 ^d	1.33 ^{ab}	1.33 ^{ab}
	Drum	1.00 ^b	2.00 ^b	2.56 ^c	3.58 ^{cd}	1.33 ^{ab}	1.67 ^{ab}
Insecticide	ART98-12	1.00 ^b	2.33 ^{ab}	0.95 ^c	2.34 ^d	1.33 ^{ab}	1.67 ^{ab}
	Ife brown	1.00 ^b	1.00 ^b	2.75 ^c	4.74 ^c	1.67 ^{ab}	1.67 ^{ab}
	Ife BPC	1.00 ^b	1.00 ^b	0.00 ^c	6.51 ^c	1.00 ^c	2.33 ^{ab}
	Modupe	1.00 ^b	1.00 ^b	1.08 ^c	1.23 ^d	1.33 ^{ab}	1.33 ^{ab}
	Drum	1.00 ^b	1.67 ^b	7.40 ^{bc}	11.96 ^b	2.33 ^{ab}	2.33 ^{ab}
No spray	ART98-12	1.67 ^b	2.33 ^{ab}	8.72 ^{bc}	5.96 ^c	2.00 ^{ab}	2.33 ^{ab}
	Ife brown	2.33 ^{ab}	1.00 ^b	13.86 ^{ab}	0.00 ^d	2.67 ^{ab}	1.00 ^c
	Ife BPC	2.33 ^{ab}	1.00 ^b	10.68 ^{abc}	3.80 ^{cd}	2.67 ^{ab}	1.67 ^{ab}
	Modupe	2.00 ^b	1.00 ^b	15.10 ^{ab}	4.05 ^c	3.00 ^{ab}	1.67 ^{ab}
	Drum	3.67 ^a	3.67 ^a	20.30 ^a	16.6 ^a	3.33 ^a	3.00 ^a

Means followed by the same letter along the column are not significantly different according to Duncan multiple range test at $p \leq 0.05$. + = CABMV positive, - = CABMV negative.

Cowpea aphid-borne mosaic was controlled in the neem and insecticide treated plots in 2017 except Drum treated with insecticide. All the plants in the untreated plots were positive to CABMV. However, in 2018, not

all the genotypes that received the neem and insecticide treatments were free of the virus. In 2018, three varieties; Ife-brown, Ife BPC and Modupe that were not treated showed negative reaction to CABMV (Table 5).

Table 5: Reactions of cowpea genotypes to *Cowpea aphid-borne mosaic virus* in 2017 and 2018

Treatment	Genotype	2017	2018
Neem	ART98-12	-	+
	Ife brown	-	-
	Ife BPC	-	+
	Modupe	-	-
	Drum	-	-
Insecticide	ART98-12	-	-
	Ife brown	-	-
	Ife BPC	-	+
	Modupe	-	-
	Drum	+	+
No spray	ART98-12	+	+
	Ife brown	+	-
	Ife BPC	+	-
	Modupe	++	-
	Drum	+++	++

+ = present, - = absent.

Concentrations of lambda cyhalothrin in cowpea seeds

The concentrations of lambda cyhalothrin in cowpea seeds treated with insecticide were compared with the Codex maximum residue

limit (MRL). The results revealed that the concentrations were below the MRL except for Ife BPC which had a very high value (1.14 ppm) far above the MRL (Figure 3).

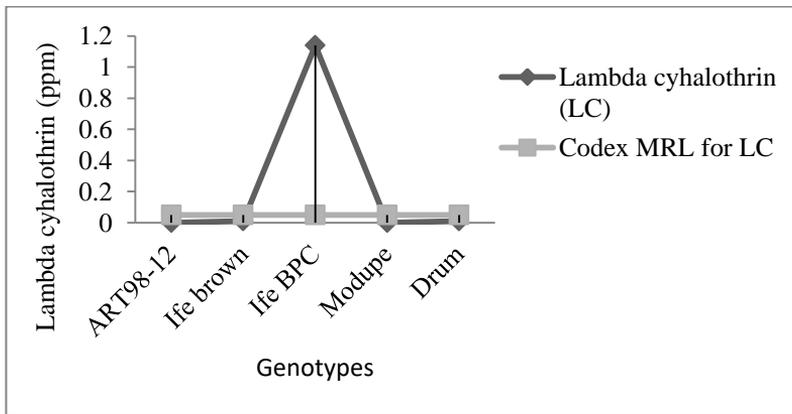


Figure 3: Concentrations of lambda cyhalothrin in cowpea seeds.

Discussion

According to the findings of this study, genotype was shown to influence plant growth at 5WAP, and also affected the yields of cowpea. This could be attributed to the genetic makeup of the cowpea genotypes. The study of Barros et al. (2013) reported genetic composition as one of the factors affecting the agronomic parameters of cowpea genotypes. Genotypic variations were

reported to affect the agronomic characters of corn (Yilmaz et al. 2007). The work of Yeasmin et al. (2016) also reported the influence of genotype on the yields of mungbean.

Treatment imposed on cowpea genotypes greatly influenced the growth and yield performance of the plants. This could be due to the fact that the applied treatments had broad spectrum activities against other pests

and pathogens such as bacteria and fungi which could hinder the normal functioning of the crop. Marei et al. (2012) and Rasoul et al. (2012) reported in their work that regular spraying of pesticidal plant extracts could facilitate direct protection against other forms of plant pathogens. In addition, there are several researches reporting the improvement of plant growth and yields after treatment with plant extracts and insecticides (Tembo et al. 2018, Dhungana et al. 2020). The results obtained on the yields of neem-sprayed cowpea compared favourably with those of the insecticide-sprayed plants. Shah et al. (2017) reported similar results in which the yields of wheat sprayed with seed extracts of neem compared favourably with the yields of imidacloprid-sprayed wheat.

The low scores recorded on aphid infestations on the I.A.R. & T cowpea genotypes implied that host-plant resistance is an effective means of minimizing aphid infestations in cowpea. Genotypic variation was significant for virus incidence and titer, which means that genetic composition will determine the tolerance of crops to diseases. The study of Kareem et al. (2016) reported that breeding for resistant genotypes against virus infections was the most effective method of preventing virus diseases in cowpea. Virus incidence was high in Drum compared to other cowpea genotypes. Report of Godfree et al. (2007) has shown that the susceptibility of plant to a particular pathogen vary considerably among host genotypes. Therefore, the use of quality genetic materials will save the stress of pumping resources into the use of chemicals. However, breeding for resistance is time-consuming and there is possibility of break in resistance. The use of biopesticides that are readily available in the environment is considered to be cheap and eco-friendly.

Treatment had a very high influence on the response of cowpea to disease traits. Neem extract was able to compete favourably with lambda cyhalothrin in reducing aphid infestations in the cowpea fields. The implication of this result is that neem could serve as a good substitute for LC and other insecticides. This agrees with the study of

Okolo and Iledun (2019) in which the insecticidal effects of neem extracts against some pests of cowpea including aphids were reported. Aphid infestations in cowpea plants were better controlled in neem-sprayed plots than the unsprayed plots. This conforms to the report of Mondedji and Nyamador (2019) in which neem leaf extracts reduced the population of Lepidopteran pests in *Solanum macrocarpon* than the control field which was not sprayed.

Neem extract and insecticide reduced the incidence and severity of CABMV in cowpea. This implies that neem is a good biopesticide for the control of viruses. Several authors have reported the use of neem oil and other plant extracts in reducing tobamovirus infections (Deepthi et al. 2007, Madhusudhan et al. 2011). Elsharkawy and El-Sawy (2015) also reported that neem and other plant extracts exhibited antiviral properties against *bean common mosaic virus* (BCMV) in bean plant. According to the ELISA results, CABMV was absent in neem and insecticide sprayed plants. The reduction in concentrations of tobamovirus by the latex of *Euphorbia tirucalli* was reported by Ramesh et al. (2009). The high concentration of LC in the grains of Ife BPC confirmed that insecticides accumulate to high concentrations in plants (Ukalska-Jaruga et al. 2020, Alengebawy et al. 2021). Therefore, the study recommends the use of neem and other plant extracts as alternatives to the use of synthetic pesticides.

Conclusion

Spraying with neem extract reduced aphids and virus occurrence in cowpea field. Since CABMV is transmitted by aphids, controlling aphids is an indirect way of protecting plants from the virus. However, CABMV is transmitted by aphids in a non-persistent manner which implies that the virus might have been transmitted before spraying due to brief probing by aphids. Therefore, farmers should not wait until viral symptoms appear before protecting their crops from viral infections. Spraying should be done at early stages of plant growth, and this should be followed by periodic spraying with plant

extracts in order to protect the fields from insect infestations which might result in viral infections.

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