VECTOR POPULATION, INCIDENCE AND SEVERITY OF THREE VIRUSES OF SOME SOYBEAN (*GLYCINE MAX* [L.] MERILL) VARIETIES IN IBADAN, DERIVED SAVANNAH AGRO-ECOLOGY

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SUMMARY

Soybean crop is the richest source of complete plant protein, essential for human nutrition. The crop is susceptible to several viruses prevailing in Nigeria. This study was undertaken to investigate the occurrence of natural infections of viruses and their vectors on five soybean varieties, and evaluate the yield and resistance status of the varieties. The experiment was conducted at the National Cereal Research Institute Experimental Field, Ibadan station in 2017 and 2018 planting season and laid-out in a Randomized Complete Block Design with three replications. Each plot size was 4 m x 1.5 m. Leaf samples showing virus-like symptoms were collected and tested for *Soybean mosaic virus* (SMV), *Cowpea mottle virus* (CMeV) and *Cowpea mild mottle virus* (CPMMV) using direct antigen coating-enzyme-linked immunosorbent assay (DAC-ELISA). Insect vector counts was carried out weekly using insect traps. It was observed in both years that the percentage insect counts was high at 4 week after planting (WAP) then peak at 6 WAP and later declined from 6 to 8 WAP for both *Bemisia tabaci* and *Aphis glycines.* The variety differed significantly in virus disease incidence and severity. All the varieties tested positive for CPMMV and negative for SMV in both years. TGX 1904 and TGX 1835-10F in 2018 tested positive for CMeV. TGX 1448-2E produced significantly ($P \leq 0.05$) higher grain yield of 3785.7 kg/ha than other varieties in 2017, it also had significantly ($P \leq 0.05$) higher grain yield of 3235.4 kg/ha than TGX 1904-6F (1007 kg/ha), TGX 1835-10F (1429.6 kg/ha) and TGX 1987-10F (1737.7 kg/ha) in 2018. Only TGX 1987-62F is resistant to the viruses. CPMMV transmitted by *B. tabaci* is the most prevalent virus associated with soybean in the study area. Some symptomatic soybean varieties tested negative to the three viruses, hence, further studies involving diagnostic test for more viruses is recommended to ascertain their resistance status.

Keywords: Soybean viruses, *Bemisia tabaci*, *Aphis glycines*, Virus incidence, Disease severity.

SOYBEAN (*Glycine max* [L.] Merill) is often described as a miracle crop. It is the leading source of oil and protein all over the world. The crop contains 40-42 % protein, 18-22 % oil comprising of 85 % unsaturated fatty acids and 15 % saturated fatty acids, 28 % carbohydrate and good amount of other nutrients like phosphorus, calcium, vitamins and iron (Antalina, 2009). It is the richest source of complete plant protein, containing all amino acids essential for human nutrition (Aaron and Michelfelder, 2009). Its nutritional values account for the various ways it is used in human diets today. It is used as a soup condiment especially for thickening purpose, today there are variety of food and drinks derived from soybean such as Soya milk, Soya "garri", Soya "eba" and Soya "Nune" or "Dawadawa" (Osho, 2003). In Nigeria the haulms and post-processed pulp (soybean meal) serve as important sources of animal feed. This haulm also improves the soil condition, and on decay supplies nutrients to subsequent crops. It also contributes to improvement in cereal based cropping systems in the Guinea savanna of Nigeria (Yusuf *et al*., 2006).

About 358.65 million metric tons of soybeans were produced worldwide in 2018/2019 farming season with Nigeria accounting for only 1.05 million metric tons of this production estimate (WAP, 2020). Nigeria is the largest producer and consumer of soybean in sub-Saharan Africa with a low yield of less than 1 ton per hectare (IITA, 2009). Several abiotic and biotic constraints threaten soybean production, resulting in reduced yield and quality. The major biotic factors are weeds, pests and diseases (Hartman *et al*., 2011). More than 300 diseases have been reported on soybean (Hartman *et al*., 1999). Losses attributed to soybean diseases alone are estimated at 11% (Hartman *et al*., 1999).

Soybean is susceptible to several viruses transmitted by aphids (*Aphis glycines*), bean leaf beetle (*Cerotoma trifurcata*) and whiteflies (*Bemisia tabaci* Gennadius. (Homoptera: Aleyrodidae) prevailing in Nigeria (Dugje *et al*, 2009). *Cowpea mild mottle virus* (CPMMV; genus *Carlavirus*, family *Flexiviridae*) transmitted by *B. tabaci* is the most prevalent virus associated with soybean mosaic disease in Nigeria (Dugje *et al*, 2009). This insect transmit at least 21 viruses in Nigeria, most of which belong to either the geminivirus or the carlavirus group. Yield losses caused by this virus range from about 15 to 100 percent (Alegbejo, 2001b). The insects also transmit different viruses belonging to the genus *Begomovirus*, family *Geminiviridae*. *Soybean mosaic* (SMV) disease occurs in all soybean-producing regions worldwide. Plants grown from SMV-infected soybean seeds provide the primary inoculum source (Wang and Ghabrial, 2002). SMV is transmitted by at least thirty two migratory aphid species belonging to 15 different genera. *A. glycines,* can acquire and transmit SMV to soybean plants in a non-persistent manner (Hill *et al.*, 2001). Soyabean mosaic virus causes several symptoms, including mosaic symptoms (light and dark green areas, chlorosis), necrosis (necrotic areas and stem tip necrosis), and seed mottling (Zheng *et al.,* 2005). These symptoms vary with soybean cultivar, virus strain, plant age at time of infection, and environment (Irwin *et al.,* 2000). Yield losses due to SMV infection range from 8 to 50% under natural field conditions (Hill, 1999), and can lead to total crop loss during severe outbreaks (Liao *et al.*, 2002). Most severe symptoms are observed in plants infected at early stages of growth (pre flowering) resulting in significant pods reduction. Furthermore, coinfection of soybean plants by SMV with other soybean viruses (e.g. *Bean pod mottle virus*) can have synergistic effects leading to yield losses greater than those caused by either virus alone (Lu *et al*., 2010). Viral Infection on soybean plants affects both the quality and quantity of soybean produced, and there is direct relationship between yield loss and genetic architecture of the host plant (Adamu *et al*., 2015).

These virus diseases have been identified on soybean several years ago. In view of dynamism in biological activities and new varieties being developed, there is need to update its status. More so, the National Cereal Research Institute (NCRI) have about twenty three varieties of soybean with no adequate information on their reaction to virus diseases under natural field conditions in Southwestern Nigeria. Hence, the objectives of this study were, to determine natural occurrence of plant viruses and their vectors in five soybean varieties, evaluate the yield and resistance level of the soybean varieties to virus diseases.

MATERIAL AND METHODS

Study location

The experiment was conducted at the Research Farm of National Cereal Research Institute (NCRI), Ibadan Out-Station in 2017 and 2018 planting seasons, Latitude 7^0 22^1N and Longitude 3^0 58^1E with mean annual rainfall of 1150-1250mm.

Source of Seeds

The five soybean varieties (TGX 1904-6F, TGX 1835-10F, TGX 1987-10F, TGX 1987-62F and TGX 1448-2E) Used in this study were sourced from NCRI Badeggi, Niger State.

Experimental design and seed planting

The experiment was laid out in a randomized complete block design with three replicates, each plot size was 4 m x 1.5 m and separated apart by 1 m border. Three seeds of each soybean varieties were sown per hill at a depth of 2 to 3 cm and at a spacing of 50 cm between the rows and 5 cm within the row, the seedlings were thinned to one plant per stand one week after seedling emergence. Farm maintenance such as weed control was carried out using standard management practices.

Disease incidence and severity

Disease incidence (DI) was determined from six to ten weeks after planting (WAP) by counting the number of plants showing virus-like diseased symptoms and expressing them as percentage of total number of plants in each plot. The degree of virus symptoms expression from different plots (Disease severity (DS)) was assessed weekly from six to ten WAP using a modified scale of 1-5 by Asadi (2005) where 1 = no visible symptoms, 2 = mild leaf mottling, 3 = chlorosis and mottling, 4 = stunted with severe mottling and chlorosis and 5 = stunted, severe mottling, leaf bunching, chlorosis with leaf defoliation.

Determination of Soybean virus resistance level

The resistance level (RL) of the soybean varieties to the virus disease was assessed based on the mean severity, using modified scale of 1 – 5 by (4): 1 = (1.0 – 1.99) highly resistant (HR), 2 = (2.0

– 2.99) moderately resistant (MR), 3 = (3.0 – 3.99) moderately susceptible (MS), 4 = (4.0 – 4.99) susceptible (S) and 5 = (5.00 and above) highly susceptible (HS).

Estimation of insect vector population

Virus vector population in the field were monitored by insect counts obtained using insect traps. This was carried out by placing a plastic bowl containing water with liquid detergent mixed with 0.5% formalin preservative solution within the plot to trap the insects (Alegbejo, 2001a). The solution was changed weekly, and the insects were Identified and counted with the aid of a light microscope and using standard entomological reference.

Sample collection and virus detection using direct antigen coating - enzyme-linked immunorbent assay (DAC-ELISA)

Leaf samples expressing virus-like symptoms such as mosaic, mottling, chlorosis, leaf deformation, etc. were collected from each plot, bagged, labelled and kept on dry ice while in the field and brought into the laboratory where they were preserved at 4^{o}C for further analysis. These samples were tested using DAC-ELISA for the presence of *Cowpea mottle virus* (CMeV), SMV, and CPMMV using homologous rabbit polyclonal antiserum. Approximately 0.1 g of the leaf sample was ground in 1 ml of carbonate (0.015 M Na_2CO_3 and 0.0349M $NaHCO_3$) coating buffer (1:10 w/v); 100 µl was dispensed into each well of the ELISA plate. The plate was incubated at 30^{o}C for 1 hour and later washed three times with phosphate buffered saline (PBS) containing 0.05% (v/v) Tween-20 (PBS-T) at three minutes interval between each wash. Polyclonal antiserum was cross-adsorbed in healthy soybean leaf sap extract (1:20 w/v) diluted in the conjugate buffer (PBS-T containing 0.02% (w/v) egg albumin and 0.2% (w/v) PVP - 40,000). All the antisera used were diluted at 1:15,000 (v/v) and 100 µl polyclonal antisera were used for virus detection. After incubating for 1 hr at 37^{o}C, the plate was washed three times with PBS-T. One hundred microliter (100 µl) of alkaline phosphatase conjugated anti-rabbit antibody diluted at 1:1500 (v/v) in conjugate buffer was used as secondary antibody and the plate was incubated at 37^{o}C for 1 hr. The plate was washed three times with PBS-T, and 100 µl of 0.001 g/ml of p-nitrophenyl phosphate in 10% (v/v) diethanolamine buffer (pH 9.8) was added per well and incubated at room temperature for 1 hr. After 1 hr the absorbance of the contents of the ELISA wells (2 per sample) was read at 405 nm in a BIO-RAD Multiscan ELISA reader (ELx 800, universal Microplate Reader). The samples were considered positive to CMeV, SMV, and CPMMV if the absorbance reading was at least twice that of the healthy soybean leaf sap control, which served as negative control.

Data collection and analyses

Data on disease incidence, disease severity, yield parameters were collected on five randomly selected plants within the plot and subjected to analysis of variance (ANOVA) using SAS (2003), and the mean values were separated at 5% significant level of probability using Least Significant Difference (LSD). Vector count were logarithm transformed before analysis.

RESULTS

The vectors population varied over the weeks. It was observed that in 2017 planting season, the percentage insect counts increased from 4 WAP (*B. tabaci* 22.5% and *A. glycines* 10.0%), then peaked at 6 WAP (*B. tabaci* 28.0% and *A. glycines* 12.5%) and later declined from 6 to 8 WAP (*B. tabaci* 17.0% and *A. glycines* 10.0%). The same trend was observed in 2018 planting season, at 4 WAP (*B. tabaci* 24.3% and *A. glycines* 9.0%) and at 6 WAP (*B. tabaci* 30.5% and *A. glycines* 10.0%) then at 6 to 8 WAP (*B. tabaci* 17.5% and *A. glycines* 8.7%) (Figure 1).

The ELISA confirmed the presence of CPMMV and CMeV on all the varieties showing symptoms of mosaic and mottling pattern in the field. As presented in Tables 1 and 2, all soybean varieties tested negative for SMV and positive for CPMMV in both years. TGX 1448-2E in 2017, TGX 1904 and TGX 1835-10F in 2018 tested positive for CMeV. From Table 1, TGX 1835-10F and TGX 1987-10F had significantly ($P \leq 0.05$) higher disease incidence of (25.48%, 26.11% and 28.40%) and (23.92%, 27.04% and 28.37) respectively, from 6-10 WAP than others varieties, while TGX 1904-6F had the least disease incidence among the varieties at 10 WAP in 2017. There were no significant (P ≥ 0.05) differences in disease severity among all the varieties in 2017, However, TGX 1904-6F showed the least disease severity of 2.83, whereas this was significantly higher in TGX 1987-10F (3.24) and TGX 1448-2E (3.33) at 10 WAP in 2018. Also, TGX 1904-6F had significantly (P≤ 0.05) least disease incidence than other varieties at 6 WAP in 2018.

In comparing the grain yield among the soybean grown under natural virus infections, TGX 1448-2E produced significantly (P ≤ 0.05) higher grain yield of (3785.7 kg/ha) than other varieties in 2017 (Table 1), while it also had significantly higher grain yield of 3235.4 kg/ha than TGX 1904-6F (1007 kg/ha), TGX 1835-10F (1429.6 kg/ha) and TGX 1987-10F (1737.7 kg/ha) in 2018 (Table 2), TGX 1448-2E was moderately susceptible, TGX 1987-62F was highly resistant while TGX 1904-6F, TGX 1835-10F and TGX 1987-10F were moderately resistant to the virus disease in both years.

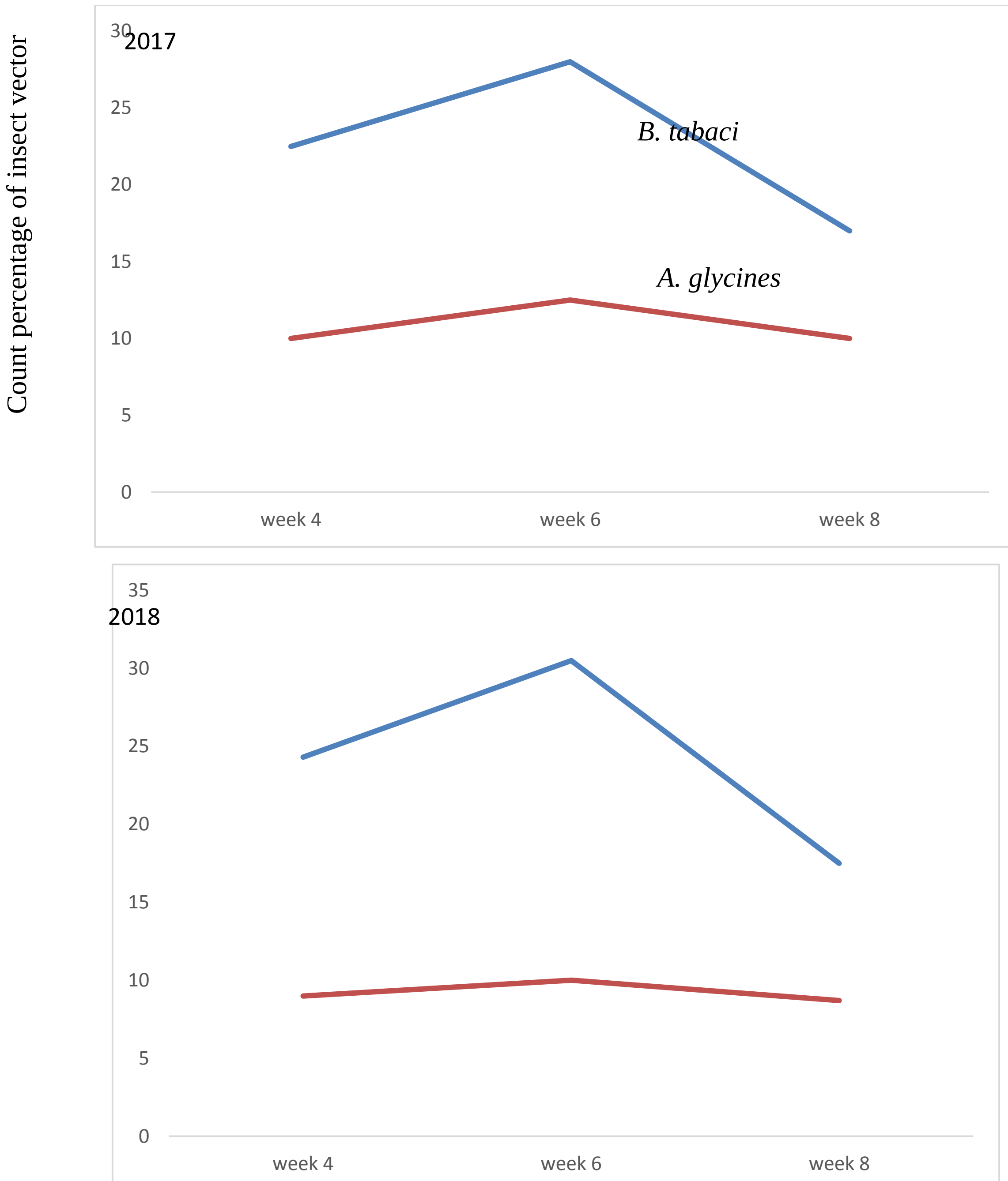

Figure 1: Populations of insect vector trapped weeks after planting on soybean plant during 2017 and 2018 seasons.

Table 1: Virus disease incidence, severity, serological indexing and disease resistance of five soybean varieties in 2017 planting season

Variety	Incidence			Severity							
	6WAP	8WAP	10WAP	6WAP	8WAP	10WAP	SMV	CPMMV	CMeV	GY(Kg/ha)	RL
TGX 1904-6F	13.40	16.46	17.34	2.12	2.60	3.12	-	+	-	1203.4	MR
TGX 1835-10F	25.48	26.11	28.40	2.13	2.20	3.07	-	+	-	1792.8	MR
TGX 1987-10F	23.92	27.04	28.37	2.54	2.77	3.10	-	+	-	2115.5	MR
TGX 1987-62F	10.62	20.28	22.94	2.30	2.64	3.19	-	+	-	2066.9	HR
TGX 1448-2E	14.71	19.05	20.65	2.52	2.77	3.38	-	+	+	3785.7	MS
LSD	4.84	4.11	3.03	0.80	0.61	0.63				944.93	

Virus assayed by Direct Antigen Coating - Enzyme-Linked Immunosorbent Assay (DAC-ELISA) +; positive, -; negative, GY = Grain yield, RL= resistance level; HR= highly resistant; MR= moderately resistant; MS= moderately susceptible. *Soybean mosaic virus* (SMV), *Cowpea mild mottle virus* (CPMMV), *Cowpea mottle virus* (CMeV); WAP= weeks after planting.

Table 2: Virus disease incidence, severity, serological indexing and disease resistance of five soybean varieties in 2018 planting season

Variety	Incidence			Severity							
	6WAP	8WAP	10WAP	6WAP	8WAP	10WAP	SMV	CPMMV	CMeV	GY(Kg/ha)	RL
TGX 1904-6F	15.71	22.99	28.17	2.07	2.44	2.83	-	+	+	1007.0	MR
TGX 1835-10F	23.03	27.64	32.03	2.27	2.33	3.07	-	+	+	1429.6	MR
TGX 1987-10F	20.43	25.56	30.27	2.34	2.67	3.24	-	+	-	1737.7	MR
TGX 1987-62F	22.30	25.87	29.58	2.00	2.74	3.00	-	+	-	2079.3	HR
TGX 1448-2E	20.37	24.63	32.07	2.33	2.93	3.33	-	+	+	3235.4	MS
LSD	4.17	3.13	3.44	0.40	0.51	0.40				1463.4	

Virus assayed by Direct Antigen Coating - Enzyme-Linked Immunosorbent Assay (DAC-ELISA) +; positive, -; negative, GY = Grain yield, RL= resistance level; HR= highly resistant; MR= moderately resistant; MS= moderately susceptible. *Soybean mosaic virus* (SMV), *Cowpea mild mottle virus* (CPMMV), *Cowpea mottle virus* (CMeV); WAP= weeks after planting.

DISCUSSION

The major insect pests observed in the field, during the study period in both years, were *A. glycines* mostly located on the under surface of leaves, either singly or in colonies and *B. tabaci* (Genn.). Symptomatically, in both years, mottling and mosaic symptoms were more conspicuous in the different soybean varieties sampled. These virus symptoms were caused as a result of aphids' damage on the crop, by directly feeding on the leaves, and indirectly by transmitting a wide array of viruses. *B. tabaci* has been reported as vector of more than 110 plant viruses as well as a serious pest of many crops worldwide, according to Jones (2003), Fontes *et al*. (2010) and Navas-Castillo *et al*. (2011). Some authors also opined that spread of plant viruses within and among soybean fields is through the activity of aphids species of 15 different genera in a non-persistent manner (Arif and Hassan, 2002). CPMMV was the commonest virus associated with all soybean variety used in this study, which agrees with the findings of Dugje et al. (2009) that CPMMV transmitted by *B. tabaci* is the most prevalent virus associated with soybean mosaic disease in Nigeria.

The decline in the vector population after it has peaked can be attributed to the age of plant and preference of *B. tabaci* for younger plant. This agrees with the finding of Gusm-ao (2000) who observed the preference of *B. tabaci* for younger leaves in tomato and cucumber (Moura *et al.*, 2003) may be explained by the nutritional value of these leaves. Also, Leite (2000) reported that *B. tabaci* lays its eggs on the younger leaves, and attributed it to the tenderness or to the higher nutritional quality of these leaves, which favours feeding and tends to decline as the plant aged. According to Ragsdale *et al*. (2004), soybeans are initially infested during the plant's vegetative growth and as the plants begin to flower and form pods soybean aphids become less within the plant. Honek and Martinkova (2004) also concluded that the sudden decline following the peak in population abundance of *Metopolophium dirhodum* (Hemiptera: Aphididae) on crops of small grain cereals is attributed to the joint effect of natural enemies and plant senescence.

Differences in the soybean variety resistance reaction to the virus can be attributed to the individual genetic makeup of the varieties, the nature and time of infection. These observations are in agreement with Gergerich and Dolja (2006) who stated that susceptibility or resistance to virus infection is determined by plant genotype. Susceptibility as a result of SMV infection are greater when soybean plants are infected prior to flowering, according to Lu *et al*. (2010) or when seed-to-seedling transmission rates and vector intensity values are high during the first 4 to 5 weeks after seedling emergence (Irwin *et al.,* 2000). The virus-free seeds used, inability of vectors to infect the soybean at flowering or time of leaf sample collection could be adduced for the absence of SMV in the samples tested.

Some varieties did not test positive for some virus despite having mosaic and mottling symptoms on the field, this may be due to individual genetic makeup of these varieties and its ability to withstand the virus attack or these symptoms were caused by other pathogens or unidentified viruses, this result corresponds with that of Njukeng *et al*. (2013) and Gug *et al*. (2005) which they report that not all the samples showing virus disease-like symptoms tested positive for viruses.

The soybean varieties TGX 1448-2E and TGX 1987-62F in which the grain yield was not significantly affected by the viruses could be described as the best among the varieties. Because production yield is one of the most valuable characters in soybean production, this agrees with the conclusion of Dilnesaw *et al.* (2014), that soybean variety with high yield are easily adopted by farmers. Agnew *et al*. (2000), also noted that tolerance is one of the mechanisms by which plants adapt to the stresses imposed by pathogens which may contribute to high yield in diseased plants, which has been exhibited by these two varieties.

CONCLUSION

This study shows that the commonest virus associated with the soybean are CPMMV and CMeV. The symptoms associated with these viruses as observed in the fields included mottling, mosaic, leaf defoliation and yellow vein banding. These symptoms can then be aided by other more reliable indexing technique such as serological testing using DAS-ELISA, Antigen coated plate-enzyme-linked immunosorbent assay (ACP-ELISA) and others virus detection methods for proper virus identification. Although only TGX 1987-62F is resistant to the viruses used in the study, TGX 1448-2E produced higher grain yield, despite been moderately susceptible. Cultivation of virus tolerant variety is a desirable option for optimal soybean production. Some symptomatic soybean varieties tested negative to the three viruses hence, further studies are recommended on diagnostic test for more viruses, as well as on the factors influencing insect vector population and the degree of disease they cause.

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