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# Assessment of different rates of neem extracts as bio-pesticide for the control of insect vectors and associated viral diseases in okra

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**Neem extract as bio-pesticide is effective in reducing insect pest populations. However, farmers lack information on the effective application rate. This study ascertained the effective neem extract concentration for reducing insect vector numbers, incidence and severity of the viral diseases. Three rates 20, 30 and 40 ml/L of neem extract, commercial pesticide Akape® and water as control were sprayed as treatments on field grown three okra varieties *F1 Sahari, F1 Kirene*, and *Asuntem*. The populations of flea beetle and whiteflies as well as the incidence and severity of Okra mosaic virus (OkMV) and Okra yellow vein mosaic virus (OYVMV) were monitored. 20 ml/L produced significantly ($p < 0.05$) the lowest flea beetle and whiteflies count. The 20 ml/L rate also caused significantly ($p<0.05$) the lowest severity of insect pest damage and the least severity of OkMV and OYVMV diseases in all three okra varieties. Importantly, a yield of 207 kg/ha from the okra variety *F1 Kirene* obtained at the rate 20 ml/L was the highest ($p>0.05$) compared to the other neem extract concentrations and okra varieties. The cultivation of *F1 Kirene* could be combined with 20 ml/L neem extract to obtain effective disease and pests control for good yield.**

**Key words:** Chemical control, flea beetle, pests and diseases, plant protection, whitefly, yield loss.

## INTRODUCTION

Okra (*Abelmoschus esculentus*L. Moench) is an annual plant of the family Malvaceae (Daliu et al., 2020). The crop is widely grown over the world, notably in tropical and subtropical climates as well as in warmer temperate zones, for its immature fruits and fresh, sensitive leaves. Okra is an important vegetable that is utilized in food, medicine, and industry. The immature fruits of okra are consumed as a cooked vegetable or used as a thickening

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in soups and stews. Okra is abundant in beneficial nutritional ingredients, primarily insoluble and soluble fiber, gums and pectins that are important for keeping the healthy wellbeing of the consumer (Nasab et al., 2022). Additionally, the dried seeds are nutritious and contain up to 20% protein (Ofori et al., 2020). Okra fruits and leaves also contain significant amount of calcium and vitamins A and C. Along with other crucial amino acids for diets, the vegetable also offers a unique balance of lysine and tryptophan (Ofori et al., 2020).

Okra cultivation, processing and marketing serve as a decent means of rural employment and income generation for many resource-poor farmers (Harris et al., 2019). The contribution of okra to the economy of Ghana is, therefore, quite significant. For example, in 2020 alone, the total production of okra in Ghana was 67,600 tonnes up by 0.44% from the previous year. Despite its huge potential for employment and income generation, current low yield levels at an average yield of 1.5 to 4.5 $tha^{-1}$ compared to an estimated 10 to 15 t/ha that could be obtained under good management including seed-borne fungi infections management (Cobbinah and Kwoseh, 2021). In Ghana, the major production constraints limiting significant yields and fruit quality with considerable economic losses are insect pest attacks and the incidence of viral diseases (Epidi et al., 2008). The effect of these biotic factors results in significant economic losses with respect to poor fruit quality and reduced market premium. The most prevalent viral disease of okra in Ghana is the O*kra mosaic virus* (OkMV), transmitted by flea beetles (*Podagrica spp*). Another virus of economic importance in okra agriculture is the whitefly (*Bemisia tabaci*) transmitted *Okra yellow vein mosaic virus* (OYVMV) (Ugwu et al., 2021; Onunkun, 2012; Brunt et al., 1996). The low yields of the vegetable discourage good adoption of it by local farmers for commercial cultivation. The level of commercialization of the crop therefore lags the rate of increasing demand for food, medicinal, and industrial use.

To overcome the menace of these biotic factors, in general, many okra farmers rely heavily on the use of synthetic chemicals in controlling or managing these insect vectors and viral diseases. Chemical control methods are, however, proving increasingly ineffective and continually pose damaging effects on the environment, human and animal health (Alengebawy et al., 2021). Consequently, all over the world, excessive use of agrochemical is currently becoming increasingly unattractive and agricultural practices are being fashioned to cut down on chemical application (Atwood et al., 2022; Scott et al., 2005). A paradigm shift, therefore, could be the use of biopesticides in the form of plant extracts. Dayan et al. (2009) affirmed that plant extracts and exudes possess active ingredients which have the potential to repel some insect pests without any negative effects on non-targeted species and the environment. The use of plant extracts from locally available plant materials such as neem (*Azadirachta indica*), has been found to be environmentally friendly and a cheaper approach to the control of pests and viral diseases in crops (Adhikari et al., 2020). The main active component of neem is azadirachtin, which is well-known for being a key component in insecticides. Neem extracts include azadirachtins in varying quantities, which offers a range of insecticidal properties. By blocking oviposition and halting males' sperm production, it promotes sterility in insects and functions as an antifeedant, repulsant, and unpleasant agent. Azadirachtin is regarded as an active chemical, as are its structural analogues (Song et al., 2018; Chaudhary et al., 2017)

Many reports on plant extracts as bio-pesticides have confirmed neem extracts as one of the most effective for the control of a wide variety of insect pests (Baba and Malik, 2015) and is therefore, widely used in particularly vegetable farming across most developing regions including sub-Saharan Africa. A major gap in the use of bio-pesticides, however, is the lack information on the right minimal effective concentration required to control insect pests or diseases (Dimetry, 2014). Consequently, farmers apply plant extracts as bio-pesticides without taking into consideration the minimal concentration of the extract required to cause significant insecticidal effect. In this regard, the widespread use of neem extract by vegetable farmers, justifies the need to ascertain the effective minimal concentration of the extract that is required to substantially reduce the population of whiteflies and flea beetles. This will in turn minimize the severity of plant damage as well as incidence and severity of their associated viral diseases. Therefore, the main objective of this work was to ascertain the most efficacious formulation of the neem extract that can significantly reduce whitefly and flea beetle populations as well as incidence and severity of OkMV and OYVMV diseases in three field grown okra varieties.

## MATERIALS AND METHODS

### Study area, experimental design and cultural practices

The field experiment was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) between July 2020 and March 2021. The study area is located at Kwabenya, Accra on latitude 5° 40' N, longitude 0° 13' W with Ochrosol (Ferric Acrisol) soil type, derived from quartzite Schist. The site is well drained and has an elevation of 76 m above sea level within the coastal savannah agro-ecological zone. The maximum and minimum average temperatures for the period of study were 30.7 and 26.0°C respectively with average annual rainfall of 220 mm. The highest and lowest relative humidity is between 75 and 60% (Akaho et al., 2003).

The experimental field was lined and pegged and laid out into four blocks with five plots per block, each plot consisting of three sub plots. Seeds of three varieties of okra namely *F1 Sahari (FIS), F1 Kirene (FIK)*, and *Asuntem (AST)* were acquired and planted in a randomized complete block design with four replications. Four seeds were planted per stand and later thinned to one plant per stand two weeks after emergence leaving twenty plants per sub plot. There were twenty plants per sub plot and five plants out of the twenty were tagged as experimental plants on which data was taken. Routine cultural practices like watering, fertilizer application and weed control were carried out as and when necessary from germination of the seeds until maturity when the okra fruits were harvested. N.P.K (15-15-15) fertilizer was applied 21 days after seed emergence at the rate of 250 kg $ha^{-1}$.

**Methods of statistical analysis**

Data on disease incidence and severity were analyzed using Statsgraphics Centurion software (version 16.1) and Microsoft Excel Software (2010 edition). The quantitative data were subjected to Analysis of variance (ANOVA) and where significant differences were obtained (p-value of 0.05 or less), Duncan’s multiple range test was employed to separate means.

**Okra varieties used, neem extract preparation and application**

The three okra varieties used in the study were *F1 Sahari*, *F1 Kirene* and *Asuntem*. These varieties were selected based on their susceptibility to OkMV and OYVMV (Appiah et al., 2020). To prepare the neem extracts, fresh leaves were harvested by hand plugging from neem trees of varied ages found growing in the vicinity of the research community. The leaves were washed clean of any dust or chemical contaminants, blended, and prepared following the method of Biswas (2013), using 50 g neem leaf per litre of water. The resulting extract was sieved with a fine mesh. The final volume of the extract stock solution was obtained by diluting 15 ml of extract into 1 L solution made up of 900 ml of distilled water and 100 ml of 5% solution of powdered detergent (brand name *Omo*) as surfactant. The stickiness and adherence of the extract was enhanced by the addition of the detergent solution. Three different extract concentrations: 20, 30 and 40 ml/L were formulated from a stock of crude-the first product of extraction-neem extract by dilution with distilled water. The treatments comprised the three rates of 20, 30 and 40 ml/L of neem extract, the commercial pesticide Akape® and distilled water as control. These were sprayed as pesticides on field grown okra plants using a portable hand sprayer. The spraying was done at 10 ml of extract solution per plant on twenty plants per sub plot but data was taken on five tagged plants none of which was a border plant. The spraying was done across all four replicated experimental blocks. The rates of the chemical pesticide used were based on the chemical producer’s rate of application. The plants were sprayed at 15 days after emergence and continued weekly until the 9th week. Spraying was done early in the morning before sunrise to avoid any likely photo-degradation of the extracts. Spraying was done on both the adaxial and abaxial sides of the okra leaves.

**Total flavonoid analysis in neem leaves**

The amount of flavonoids in the neem extract was determined with 100 g powder prepared from leaves and analyzed at the Noguchi Memorial Institute for Medical Research laboratory for quantification of the total flavonoids in each sample. The sample powder was made by first allowing leaves to air dry for a week, then cutting them into pieces and using a mechanical blender to grind them into powder. To determine the total flavonoids, a serial dilution was done using methanol to obtain seven different concentrations of Quercetin, plant flavanol belonging to the poly-phenolic flavanoid family found in many plants. It is a typical reference standard for measuring flavanoids (Widiyana and Illian, 2022; Mathur and Vijayvergia, 2017). Two concentrations (10 and 5 mg/ml) of the extract were prepared by dissolving the extract in appropriate amount of distilled water. To 100 µl of each Quercetin test sample, 100 µl of aluminium chloride was incorporated (Widiyana and Illian, 2022; Mathur and Vijayvergia, 2017). The resulting solution was kept at room temperature for 20 min, after which a 415 nm absorbance reading was recorded. The data from Quercetin was used to plot a standard calibration curve from which the flavonoid content of the neem extract was extrapolated. From the absorbance, the total flavonoid content of the extract was calculated using the linear equation obtained from the standard calibration curve and expressed as quercetin equivalents.

**Analysis of phyto-constituents in neem leaves**

In this analysis, 500 g of neem leaves was air dried, grounded and sieved to obtain a fine powder. 100 g of the powder was analyzed using the respective appropriate protocols described by Widiyana and Illian (2022) and Mathur and Vijayvergia (2017) to determine the presence or absence of the following phyto-constituents; saponins, polyuronides, reducing sugars, alkaloids, phenolic compound, cyanogenic glycosides, flavonoids, anthracenosides, triterpenes and phytosterols.

**Flea beetle and whitefly population count**

Data on insects count were collected from five okra plants randomly selected from the middle rows-within sub plot rows excluding border rows, with five plants per row. Five topmost fully expanded okra leaves were carefully examined by observing both the abaxial and adaxial surfaces. Insects found on the surfaces of the leaves were identified, counted manually, and recorded. The insect count was carried out on weekly basis, between 6:00 to 8:00 am when the insects are known to be less active.

**Insect vector leaf damage assessment**

The extent to which insects had caused physical damage to the leaves of the okra plants in the field was assessed by using a rating scale developed by Boateng et al. (2019) as follows: 1 = very mild damage (1 to 15 holes); 2 = mild damage (16 to 30 holes); 3 = moderately severe damage (31 to 45 holes); 4 = very severe damage (46 to 60 holes); 5 = extremely severe damage (more than 60 holes). The adaxial and abaxial sides of the leaves were examined visually for holes created by insect feeding, assessed and scored for severity of damage using the damage rating scale described above. Leaf damage was determined by counting the total number of perforations created by the insects in all leaves found on the five randomly selected test plants. This was then divided by the total number of leaves on the five selected test plants to obtain the average number of perforations per leaf.

**Estimation of disease incidence (DI) and symptom severity**

In cases where the initiation of symptom development was observed, it was monitored and recorded for six consecutive weeks. Based on visual examination of symptoms, and the procedure outlined by Karri and Acharyya (2012), the disease incidence within the okra field was estimated as follows:

$$\text{Disease Incidence} = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ per\ plot} \times 100\%,$$

Severity of symptoms was scored on a five point scale (0 - 4), modified after Ali et al. (2005), where; 0 = No symptom; 1 = Very

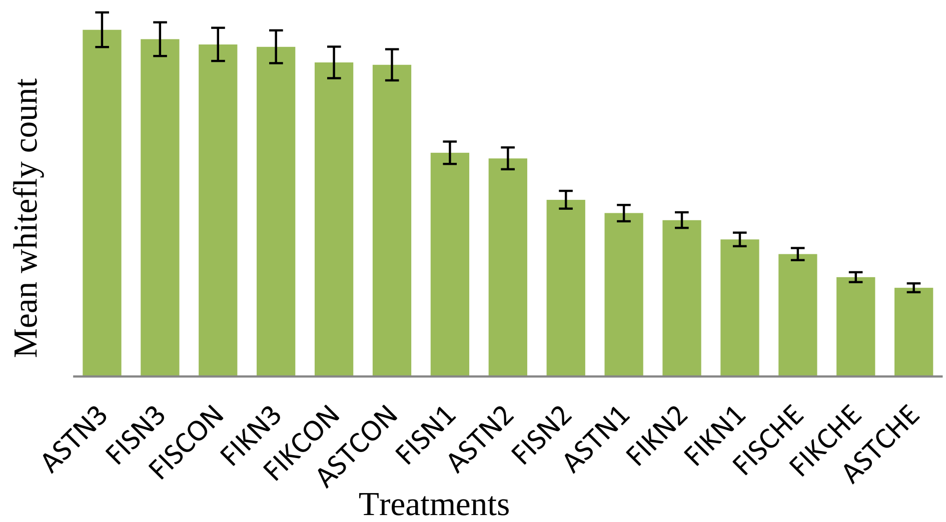


**Figure 1.** Mean whitefly count observed in pesticide treated okra varieties. Varieties: AST, Asuntem; F1S, F1 Sahari and F1K, F1 Kirene. Treatments: N1, 20 ml/L; N2, 30 ml/L, N3, 40 ml/L, CHE, Chemical pesticide and CON, control (no pesticide spray).
**Source:** Authors.

mild symptoms, initial vein clearing, initial leaf yellowing, mild curling and blistering; 2 = Leaf completely yellow and inter-veinal regions remain green or yellow and blistering; 3 = Severe curling, yellowing, stunting and blistering; 4 = Severe yellowing, curling, blistering and deformed pods (all leaves of the plants get affected).

## RESULTS

### Total flavonoids in neem leaves

Flavonoids are synthesized by plants in response to microbial infections (Baba and Malik, 2015). *In vitro* analysis of flavonoids proves its potency in suppressing the effects of a range of microorganisms (Baba and Malik, 2015). Results from the analysis show that total flavonoids in neem is very high (647.37 mg/100 gQE).

### Array of phyto-constituents in neem leaves

The phytochemicals that were found to be present were saponins, reducing sugars, polyuronides, flavonoids, phenolic compounds and phytosterols all of which play important role in the potency of the plant extracts as biopesticides. The following were however, absent or could not be detected; cyanogenic glycosides, alkaloids, anthracenosides and triterpenes.

### Whiteflies population on okra varieties treated with neem extract

The mean whitefly population observed in pesticide-treated okra varieties over a period of 9 weeks is shown in Figure 1. Generally, the mean whitefly count recorded ranged between 7.96 to 39.57 for the variety *Asuntem* treated with synthetic chemical and with 40 ml/L of neem extract respectively. Chemical treated plants showed significantly ($p<0.05$) lower mean whiteflies count than the neem extract treated okra plants in all the varieties tested (Figure 1). Among the three concentrations of neem extract applied, 20 ml/L neem extract treated okra plants, *F1 Kirene* (17.88), *Asuntem* (23.95) and *F1 Sahari* (29.01) gave significantly ($p<0.05$) reduced mean whitefly count compared to the 40 ml/L neem extract treated plants of *F1 Kirene* (37.90), *Asuntem* (39.57) and *F1 Sahari* (38.22). Compared to the control, the mean whiteflies count recorded in the 40 ml/L neem extract-treated okra varieties, was not significant ($p>0.05$) (Figure 1). Among the treatments, *F1 Kirene* treated with 20 ml/L gave the least mean whiteflies count of 17.88 followed by 22.79 in F1 *Kirene* while the highest mean whiteflies count of 39.57 was recorded in *Asuntem* treated with 40 ml/L extract.

### Effect of neem extract on flea beetle populations in treated okra varieties

The mean flea beetle population observed on treated okra varieties is indicated in Figure 2. Generally, the mean flea beetle population ranged between 5.03 (*F1 Kirene* with chemical treatment) and 84.71 (*Asuntem* with control treatment). Chemical treated okra varieties [*F1 Kirene* (5.03), *Asuntem* (6.22) and *F1 Sahari* (8.57) had significantly ($p<0.05$) lower mean population of flea beetle compared to those treated with 40 ml/L of neem extract [*Asuntem* (65.88), *F1 Kirene* (72.68) and *F1 Sahari* (80.88] and the controls *F1 Kirene* (77.08), *F1 Sahari* (84.59) and *Asuntem* (84.71). Of the three concentrations

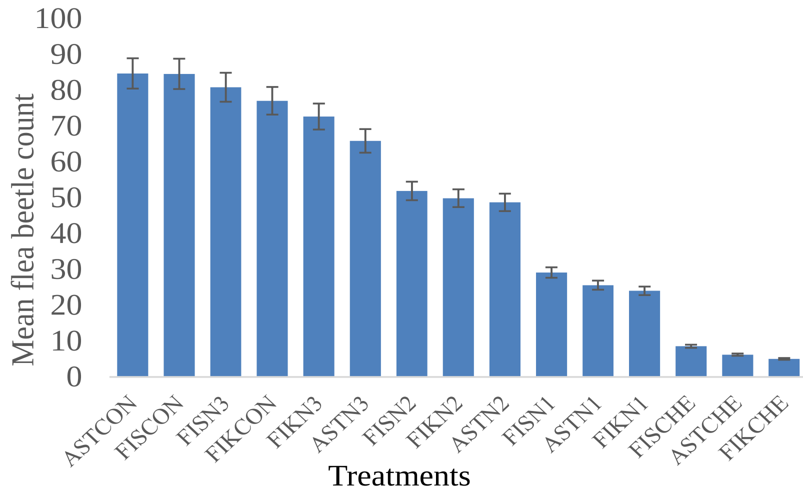


**Figure 2.** Mean flea beetle count observed in pesticide treated okra varieties. Varieties: AST, Asuntem; F1S, F1 Sahari and F1K, F1 Kirene. Treatments: N1, 20 ml/L; N2, 30 ml/L, N3, 40 ml/L, CHE, Chemical pesticide; CON, control (no pesticide spray).
Source: Authors.

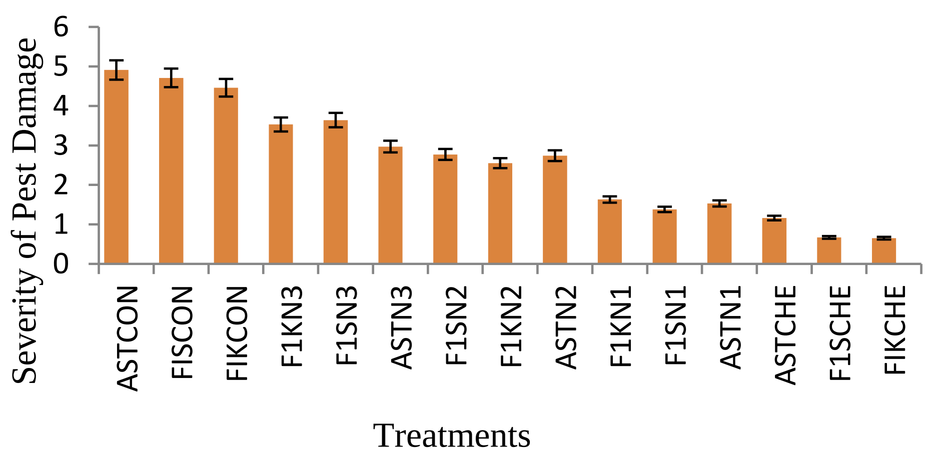


**Figure 3.** Mean severity of insect pest damage in pesticide treated okra varieties. Varieties: AST, Asuntem; F1S, F1 Sahari and F1K, F1 Kirene. Treatments: N1, 20 ml/L; N2, 30 ml/L, N3, 40 ml/L, CHE, Chemical pesticide and CON, control (no pesticide spray).
Source: Authors.

of neem extract applied, the 20 ml/L resulted in significantly ($p < 0.05$) lower flea beetle populations in *F1 Kirene* (24.04%), *Asuntem* (25.61%) and *F1 Sahari* (29.41%) compared to the 30 ml/L extract application in *F1 Kirene* (49.89%), *Asuntem* (48.73%) and *F1 Sahari* (51.92%) and the 40 ml/L treatment of *F1 Kirene* (72.68%), *Asuntem* (65.88%) and *F1 Sahari* (80.88%) (Figure 2).

**Severity of insect pest damage in neem extract treated okra varieties**

Mean severity of insect pest damages on the three okra varieties is shown in Figure 3. The severity of insect pest damage progressively increased from 0.65 (very mild damage) in *F1 Kirene* treated with chemical to 4.91 (very severe damage) in *Asuntem* without any pesticide treatment (control). Okra varieties treated with 20 ml/L neem extract had significantly ($p<0.05$) lower insect pest damage as in *F1 Sahari* (1.38), *Asuntem* (1.53) and *F1 Kirene* (1.63), compared to the 40 ml/L neem extract treated plants of *F1 Sahari* (3.64), *Asuntem* (2.97) and *F1 Kirene* (3.53) which had mild to moderately severe damages and the control treatments in *F1 Sahari* (4.72), *Asuntem* (4.91) and *F1 Kirene* (4.46) which had very severe damage. Nevertheless, all the neem treated okra varieties recorded lower pest damage than the controls.

**Table 1.** Incidence (%) of OMV and OYVMV diseases in field grown okra treated with neem extract and chemical pesticide for 9 weeks.

| Okra variety | Disease incidence (%) | | | | | |
|---|---|---|---|---|---|---|
| | Pesticide treatment | | | | | |
| | 20 ml/L | 30 ml/L | 40 ml/L | Chemical pesticide | Control | Means |
| *Asontem* | 34.6[b] | 44.3[b] | 48.90 [b] | 26.80 [c] | 53.60 [a] | **41.64** [b] |
| *F1 Kirene* | 33.5[b] | 39.20 [b] | 43.60 [b] | 17.70 [c] | 58.10 [b] | **38.42** [b] |
| *F1 Sahari* | 35.8[b] | 41.90 [b] | 59.70 [a] | 26.01 [c] | 66.62 [a] | **46.01** [b] |
| **Means** | **34.63** [b] | **41.80** [b] | **50.73** [a] | **23.50** [c] | **59.44** [a] | **42.02** |
| **STDEV** | **1.15** | **2.55** | **8.21** | **5.04** | **6.61** | |
| **CV (%)** | **1.32** | **6.51** | **67.32** | **25.41** | **43.73** | |

Lsd (P<0.05): Cultivar = 3.18; Pesticide = 2.60; Cultivar x Pesticide = 3.92.
Source: Authors.

Furthermore, damage done to plants of *Asontem* treated with synthetic chemical produced very mild damage (1.16) which was not different (p>0.05) from okra varieties protected with 20 ml/L neem extract; *F1 Sahari* (1.38), *Asuntem* (1.53) and *F1 Kirene* (1.63) which also showed very mild pest damage (Figure 3).

## Effect of neem extract on the incidence of OkMV and OYVMV in treated okra varieties

The percentage incidence of OkMV and OYVMV diseases in the three okra varieties treated with the different concentrations of neem extract (20, 30 and 40 ml/L) are shown in Table 1. Generally, the mean incidence of diseases was moderately high with a mean incidence ranging between 30.0 and 42.02%. Among the okra varieties, *F1 Kirene* had significantly (p<0.05) the lowest mean disease incidence (38.42%) compared to *F1 Sahari* (46.01%) and *Asontem* (41.64%) okra varieties. *F1 Sahari* (46.01%) and *Asontem* (41.64%) were, however, not significantly different (p>0.05) with respect to mean disease incidence. Furthermore, among the treatments, the chemical had the lowest mean disease incidence (23.50%) while 40 ml/L neem extract resulted in the highest mean disease incidence (50.73%). Even though the 20 ml/L produced lower disease incidence (34.63%) than the 30 ml/L neem extract (41.80%), the difference between them was not significantly different (p>0.05; Table 1).

## Severity of OMV and OYVMV in okra varieties treated with neem extract

The mean severity of OkMV and OYVMV diseases on the three okra varieties treated with 20, 30 and 40 ml/L concentrations of neem extract are shown in Figure 4. Generally, the mean severity of viral diseases was moderately low, ranging between 0.32 in *Asuntem* with chemical treatment and 2.35 in *Asuntem* control plants. The *F1 Sahari* okra variety exhibited the highest disease severity with chemical (1.04), 20 ml/L (1.23), 30 ml/L (2.12), 40 ml/L (1.83) and control (2.35) throughout the experiment. The variety *Asuntem* showed the lowest disease severity with chemical treatment having the least disease severity (0.32). Of the three concentrations of neem extract applied, 20 and 30 ml/L significantly (p<0.05) reduced the severity of the diseases in all the three okra varieties with *Asuntem* having the least (0.64) while *F1 Sahari* recorded the highest (1.23). Nonetheless, neem extract concentration 40 ml/L was effective in reducing viral severity in the varieties *Asuntem* and *F1 Sahari* but ineffective in *F1 Kirene.*

## Yield obtained in okra from different application rates of neem extract

Yield of three okra varieties treated with 20, 30 and 40 ml/L of neem extract and a chemical pesticide in the field are presented in Table 2. Okra varieties treated with chemical pesticide had significantly (p<0.05) higher yield (234.03 kg/ha) compared to the control (101.43 kg/ha) and the okra varieties treated with 30 and 40 ml/L neem extracts (138.87 and 125.90 kg/ha, respectively). However, difference between the yield of okra varieties treated with chemical pesticide (234.03 kg/ha) and 20 ml/L neem extract (207.62 kg/ha) was not significant (p>0.05). Comparing varieties, *F1 Kirene* produced significantly (p<0.05) the highest yield (207.62 kg) followed by *F1 Sahari* (139.18 kg/ha) and *Asuntem* (127.94 kg/ha).

# DISCUSSION

## Array of total flavonoids and phyto-constituents in neem leaves

Secondary metabolites, a bioactive substance with a variety of actions, are abundant in plants. According to

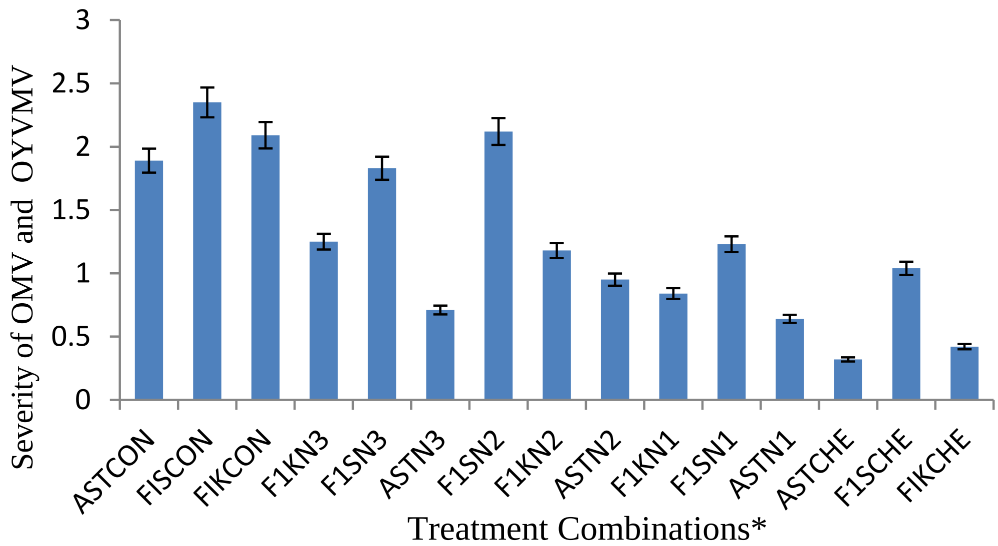


**Figure 4.** Severity of OkMV and OYVMV diseases in okra varieties treated with Neem extract and chemical. Varieties: AST, Asuntem; F1S, F1 Sahari and F1K, F1 Kirene. Treatments: N1, 20 ml/L; N2, 30 ml/L, N3, 40 ml/L, CHE, Chemical pesticide and CON, control (no pesticide spray). Source: Authors.

**Table 2.** Yield (kg/ha) of field grown okra varieties treated with neem extract and chemical pesticide.

| Treatment (ml/L) | Yield (kg/ha) | | | Mean |
|---|---|---|---|---|
| | Okra variety | | | |
| | ***Asontem*** | ***F1 Sahari*** | ***F1 Kirene*** | |
| 20 | 155.70[a] | 132.00[b] | 285.30[a] | 191.00[a] |
| 30 | 122.00[b] | 122.70[b] | 171.90[a] | 138.87[b] |
| 40 | 109.80[b] | 120.70[b] | 147.20[b] | 125.90[b] |
| Akape®* | 174.30[a] | 219.70[a] | 308.10[a] | 234.03[a] |
| Control | 77.90[c] | 100.80[c] | 125.60[c] | 101.43[c] |
| **Mean** | **127.94[b]** | **139.18[b]** | **207.62[a]** | **158.25** |
| **STDEV** | **38.04** | **46.43** | **83.29** | |
| **CV (%)** | **28.31** | **44.14** | **16.88** | |

*Akape® = chemical pesticide.
Source: Authors.

studies, flavonoids, a kind of secondary metabolite, exhibit anticancer, antioxidant, antibacterial, anti-inflammatory, and antiviral properties. The leaves of neem (*Azadirachta indica* Juss.) contain a lot of flavonoids. The results of the analysis show that the total flavonoids content in neem is very high (647.37 mg/100 gQE). This high level of flavonoids indicates that neem has the potency to prevent or reduce pathogen attack and disease incidence and severity. This is consistent with work done by Alzohairy (2016), who reported that constituents of neem extracts are effective in restricting the creation of free radicals, thus preventing disease initiation. Phytochemicals are naturally occurring in medicinal plants and offer protection from various diseases. Their properties are important attributes of many potent medicinal plants. Medicinal plants have so many phytochemicals each of which possesses important medicinal properties (Nasab et al., 2022). The phytochemicals that were detected to be present were saponins, reducing sugars, polyuronides, flavonoids, phenolic compounds and phytosterols all of which play important roles in the potency of the plant extracts as biopesticides. The analysis was carried out to detect the presence of the following phytoconstituents; saponins, polyuronides, reducing sugars, alkaloids, phenolic compound, cyanogenic glycosides, flavonoids, anthracenosides, triterpenes, and phytosterols. This analysis was only qualitative showing the presence or absence of particular phyto-constituents but could not quantity their levels in the sample. The observation is consistent with the report of Widiyana and Illian (2022) who also detected in neem leaves several including the secondary metabolites: Flavonoids, steroids/triterpenoids, tannins, and saponins, utilizing the UV-Vis spectrophotometric method.

### Whiteflies populations in okra varieties treated with neem extract

Among the three concentrations of neem extract, the 20 ml/L neem extract treatment produced the lowest mean whitefly count compared to the 30 and 40 ml/L treatment. Islam et al. (2010) similarly found that lower concentration (20 ml/L) of castor plant extracts was most effective in reducing the population of thrips and aphids insect pests in chili pepper. The lesser potency of the 40 ml/L neem extract in significantly reducing the population of whiteflies suggests that high rate of bio-pesticide application beyond an optimum concentration may result in a decreased rate of lethality of pests and disease-causing organisms (Khuhro et al., 2014). It is, therefore, suggested that farmers apply the required concentration of neem extracts on their crops to ensure high efficacy against insect pests and diseases. The synthetic chemical 'Akape®' significantly decreased the population of whitefly in the okra varieties. This observation is consistent with the findings of Jackai and Oyediran (1991) and Jackai and Adalla (1997) who reported that synthetic insecticides are the main means of insect pest control both in the field and in storage. The high level of efficacy of 'Akape®' against whiteflies and other insect pests has been reported (Tanzubil, 1991).

### Effect of neem extract on flea beetle populations in treated okra varieties

Of the three concentrations of neem extract applied, okra varieties treated with 20 ml/L significantly caused the lowest flea beetle population compared to the 30 ml/L extract and the 40 ml/L treatments. This finding is consistent with the report of Islam et al. *(*2010) who observed lower neem rates being more effective in chilli pepper but contradicts the results of Santos et al. (2004) and Mondédji and Nyamadorin (2019) who found higher concentrations of aqueous neem extract to result in more effective reduction in aphid and lepidopteran populations respectively. Okra varieties treated with chemical pesticide had significantly the lowest mean population of flea beetle compared to those treated with 40 ml/L of neem extract and the controls. Like findings of Islam et al. (2010) who reported on the toxicity of diatomaceous earth and mono-terpenoids against the storage pests *Callosobruchus maculatus*, there was a significant reduction in mean population of flea beetle in the synthetic pesticide-treated okra varieties. Despite their negative effect on non-target organisms and the environment, synthetic pesticides, when applied in recommended doses, have the potential of reducing pest infestations.

### Severity of insect pest damage in neem extract treated-okra varieties

The severity of insect pest damage progressively increased from very mild damage in chemically treated *F1 Kirene* to very severe damage in *Asuntem* without any pesticide treatment (control). Okra varieties treated with 20 ml/L neem extract had significantly reduced insect pest damage compared to 40 ml/L neem extract treated plants which exhibited moderate to very severe damage in the control treatments. Neem extract at a 40 ml/L concentration reduced viral severity in the *Asuntem* and *F1 Sahari* varieties but not in the *F1 Kirene* variety. The mild damage recorded in okra varieties treated with 20 ml/L neem extract could possibly be ascribed to the ability of the extract to significantly induce mortality of both whitefly and flea beetle vectors compared to the 30 ml/L and 40 ml/L neem extract. Nevertheless, all the neem treated okra varieties recorded lower pest damage than the controls which confirm the efficacy of neem extract to effectively reduce pest damage leading to increased yields (Jackai and Oyediran, 1991; Tanzubil, 1991). Furthermore, plants of *Asuntem* treated with synthetic chemical produced very mild damage, similar to the level of damage observed in okra varieties protected with 20 ml/L neem extract. The similarity in performance of the 20 ml/L neem extract and the synthetic chemical in reducing the severity of pest damage to the okra varieties confirm the efficacy of neem extract against insect pest damage (Tanzubil, 1991) especially, when applied in the right dose.

### Effect of neem extract on incidence of okra mosaic disease and okra yellow vein mosaic disease in treated okra varieties

Nearly all plants of the different okra varieties expressed symptoms of viral infections irrespective of treatment except the chemical treated okra varieties. Okra is generally affected by various viral diseases during its transition through growth and development but OkMV and OYVMV are the most important and prevalent (Sardana et al., 2005; Aziz et al., 2011). The incidence of viral diseases observed may be due to the increased populations of flea beetle and whiteflies in the okra fields probably brought about by seasonal variation (Asare-Bediako et al., 2017). Among the okra varieties, *F1 Kirene* had significantly the lowest mean disease incidence compared to *F1 Sahari* and *Asuntem* okra varieties. Furthermore, among the treatments, the chemical pesticide had the lowest mean disease incidence while 40 ml/L neem extract resulted in the highest mean disease incidence. The lower disease incidence of *F1 Kirene* in all treatments is inconsistent with the report by Appiah et al. (2020) which rated *F1 Kirene* as the most susceptible okra cultivar to OkMV and OYVMV, in a study where no insect control was applied.

Generally, the mean severity of viral diseases was moderately low, ranging between healthy no symptom plants in *Asuntem* with chemical treatment and very mild symptoms of initial vein clearing, blistering, mild mosaic with curling in *Asuntem* with control no chemical treatment. The *F1 Sahari* okra variety exhibited the highest disease severity with chemical whereas the variety *Asuntem* showed the lowest disease severity with treatments. *Asuntem* had previously been rated as tolerant to OkMV and OYVMV (Appiah et al., 2020), therefore, the significant reduction of the disease severity in this variety probably confirms the ability of the variety to tolerate the presence of the disease on the field (Ugwu et al., 2021). Of the three concentrations of neem extract applied, 20 ml/L reduced severity of the diseases in all the three okra varieties significantly. These results suggest that, the 20 ml/L concentration of neem extract is the appropriate and most effective application rate that is helpful in managing OkMV and OYVMV diseases in okra.

### Yield obtained in okra with different application rates of neem extract

Okra varieties treated with chemical pesticide produced significantly the highest yield compared to the control and the okra varieties treated with 30 and 40 ml/L neem extracts. However, the difference between the yield of okra varieties treated with chemical pesticide and 20 ml/L neem extract was not significant. The better yield resulting from the application of synthetic chemical supports the reports of Epidi et al. (2008) and Powers et al. (2009). The observed results of the yield levels of the okra varieties treated with synthetic chemicals and 20 ml/L neem extract is similar to that of Khuhro et al. (2014) who reported that high potency of bio-pesticides is achieved with the appropriate minimal effective rate of application. This explains the superior performance of 20 ml/L of neem extract in producing comparatively better yields in okra. Comparing varieties, *F1 Kirene* produced significantly the highest yield followed by *F1 Sahari* and *Asuntem*. Pesticide plant extracts boost crop output by reducing insect pests. Because plant extracts frequently produce lower pest densities than those seen on the controls, higher yields were obtained in neem extract treated plants than the control without treatment. The high yields recorded in this trial may be explained by the low level of disease severity recorded during the production season compared to previous findings. More often, crops give appreciable yields in the presence of diseases with lower severity levels (Aziz et al., 2011). These low severity levels may be attributed to the effectiveness of the bio-pesticides application.

## Conclusion

Among the three concentrations 20, 30 and 40 ml/L of neem extract applied, the 20 ml/L neem extract treatment outperformed the other two concentrations in all parameters measured. Neem extract at 20 ml/L gave the lowest flea beetle and whitefly populations, lowest severity of insect pest damages and significantly lowest mean disease incidence. Neem extract applied at 20 ml/L also significantly reduced disease severity due to mixed-infection of OMV and OYVMV. There was a significant interaction between the okra cultivars and various pesticides applied. For instance, *F1 Kirene* treated with neem extract recorded significantly higher yield compared to same variety without any pesticide treatment. *F1 Kirene* produced significant the highest yield followed by *F1 Sahari* and *Asuntem*. Plant extracts that are pesticides increase crop yield by decreasing insect pests. Higher yields were obtained in neem extract treated plants than the control without treatment because plant extracts often produce lower pest densities than those seen on the controls.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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