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Full Length Research Paper

Evaluating the biocidal efficacy of aqueous solution of three botanicals against insect pest species of garden eggs (*Solanum aethiopicum*) under open field conditions

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The biocidal activities of extracts from the leaves of *Annona muricata*, the leaves of *Chromolaena odorata* and the nutshell liquid of *Anacardium occidentale* were evaluated against the insect pests of garden eggs in the field in this study. The study showed that the aqueous extract of all three botanicals had varying levels of insecticidal activities and hence has the potential of managing insect pests of garden eggs. The efficacy of the botanicals varied across all three growing seasons. *Anacardium occidentale* nutshell liquid recorded the least mean population of whiteflies (*Bemisia tabaci*) in the field during the dry and minor rainy seasons. It also recorded the least mean population of leafhoppers (*Empoasca* spp.) during the major rainy season. *Annona muricata* leaves *Chromolaena odorata* leaves recorded the least mean population of leafhoppers (*Empoasca* spp.) during the dry and minor rainy seasons respectively. All botanicals used showed no significant difference in the yield and marketability of fruits across all seasons compared with the standard Emamectin benzoate that was used as a check.

Keywords: Biocidal activities, Annona muricata, Chromolaena odorata, Anacardium occidentale, Empoasca spp, Leucinodes spp, Bemisia tabaci, Garden eggs.

INTRODUCTION

Garden eggs, *Solanum* spp., are found mainly in tropical Africa, Asia and South America. They are primarily grown for their immature fruits and in some varieties, the leaves. In Ghana, garden eggs are one of the most important vegetable crop grown and consumed (Schippers, 2000). The fruits are used as thickeners in soups and stews and are sometimes consumed raw. Different parts of the plant are believed to have medicinal properties

The crop provides a direct source of income for many farmers and other actors along the value chain such as retailers and exporters The cross-border export in garden eggs from Ghana in the West African sub-region is limited, with about 3% of the total production making it to the European and United Kingdom markets. The ban on vegetables from Ghana by the European Union in October 2015 further reduced the small volumes of garden eggs exported when large numbers of insect pests of quarantine importance like fruit flies (non-European Tephritidae), the eggplant fruit and shoot borer (*Leucinodes spp*), thrips (Thysanoptera), false codling moth (*Thaumatotibia leucotreta*) and whitefly (*Bemisia tabaci*) were found in vegetable consignments bound for the European market (Fening and Billah, 2019).

Garden egg production has been plagued with a lot of limitations including several insect pest infestations. As many as 19 different insect pests including *Leucinodes* spp. have been reported to be major insect pests of garden eggs in Ghana (PPRSD, 2020). As a result of insect pests' infestation, Ghana's current average yield of garden eggs is 8 metric tonnes/hectare/year even though it has the potential to reach 15 metric tonnes/hectare/ year (MoFA, 2010). More so, the presence and activities of these insect pests have a detrimental impact on the vegetable export industry, that is, on the economy of the exporting country, the livelihoods of the value chain actors as well as a food security threat since garden eggs are heavily consumed by many.

Farmers rely mostly on synthetic insecticides and apply several sprays of chemicals per growing season to manage insect pests of garden eggs, particularly the fruit and shoot borers found in garden eggs. The misuse of synthetic insecticides has led to an increase in the cost of production, residual toxicity, pesticide resistance, pest resurgence, potential health and environmental threats and lethality towards beneficial organisms, humans and animals (Javed *et al*., 2017). There is therefore a need for safer and more sustainable solutions for the management of these insect pests. The alternatives include the use of botanicals with biocidal activities. It is important to establish the biocidal activities of botanicals to add to the already known ones. This will provide a wide range of choices that can be explored for the management of insect pests.

Botanicals are known to act as repellents, antifeedants, and growth inhibitors on several insect species and can be used in the effective management of insect pests (Degri and Sharah, 2014). They are also known to degrade rapidly in sunlight, air and moisture, break down into less toxic or nontoxic compounds and pose less risk to non-target organisms at the same time leaving very little residue on the produce (Sarwar, 2015). Phytochemical analysis has shown that the insecticidal activities of botanicals are due to the constituent compounds which can be isolated and synthesised for use (Buxton et al, 2018). In this study, we evaluated the biocidal insecticidal activities of the aqueous leaf extracts of *Annona muricata* and *Chromolaena odorata,* and *Anacardium occidentale* nutshell liquid as potential botanicals against various insect pests of garden eggs in the field.

MATERIALS AND METHODS

Study site

The studies were conducted at the University of Ghana Experimental Farm, on the Legon campus (5.6569 ^{o}N, 0.1921^{o}W) in three seasons i.e. the minor rainy season (September-November 2020), the dry season (February-April 2021) and the major rainy season (May-August 2021).

The field was laid out in a Randomised Complete Block Design (RCBD) consisting of five treatments which were replicated four times. The treatments were as follows: *(i)* aqueous leaf extracts of *A. muricata*, *(ii)* aqueous leaf extracts of *C. odorata*, *(iii) A. occidentale* nutshell liquid, *(iv)* a standard insecticide check (Emamectin benzoate) and *(iv)* only water (control).

Nursery

A commonly cultivated garden egg cultivar in Ghana (Legon 1) was used as the test crop. The seeds were obtained from the Department of Crop Science of the University of Ghana and were nursed in seed trays. All nursery practices were carried out for six weeks and then transplanted at a planting distance of 0.8 m x 0.8 m. The total field size measured 24 m x 15.8 m and was made up of twenty (20) plots separated by 1m alleys. Each plot measures 3.2 m x 4 m and is made up of twenty (20) plants, while the six (6) middle plants were tagged for data collection. Watering was done every day while weeds were controlled manually by hoeing. Other agronomic practices such as fertilizer application and earthling-up of soil to improve aeration were employed during the growing periods.

Preparation of aqueous plant extracts and application

The leaves of *A*. *muricata* and *C. odorata* were air-dried in the laboratory for two weeks while the nuts of *A*. *occidentale* were air-dried for two months after which they were pulverised into powder.

One hundred grams (100g) of each powdered botanical was added to 1L of water (10 % w/v) in plastic bowls with a lid. The mixture was stirred for 2 minutes and left to stand in the dark for 48 hours at room temperature, then filtered using a clean muslin cloth. About 5mL of a soapy solution was added to each filtrate before it was applied.

The application of the treatments commenced two weeks after transplanting (WAT). Emamectin benzoate at a rate of 3 mL/L was used as a standard insecticide check whiles water only was used as a control. The application was done weekly using a CP- 15 knapsack

Table 1. Insects pests present during the various seasons

Insects	Dry Season	Minor Season	Major Season
Whitefly (*Bemisia tabaci*)	+	+	-
Leafhopper (*Empoasca* spp.)	+	+	+
Fruit and Shoot Borers (*Leucinodes* spp.)	+	+	+

+: Insects present. -: Insect absent

Table 2. Effect of the aqueous extract of botanicals on whitefly (*Bemisia tabaci*) population.

Treatment	Dry Season	Minor Season	Major Season
Annona muricata leaves	25.5 ± 8.3 [a]	17.8 ± 5.4 [ab]	0.0 ± 0.0[a]
Chromolaena odorata leaves	25. 6 ± 9.0 [a]	19.3 ± 4.8 [ab]	0.0 ± 0.0[a]
Anacardium occidentale nutshell liquid	25.50 ± 8.1 [a]	16.1 ± 3.6 [a]	0.0 ± 0.0[a]
Emamectin benzoate	26.1 ± 9.0 [a]	20.9 ± 5.0 [b]	0.0 ± 0.0[a]
Control	42.7 ± 12.1 [b]	34.6 ± 9.5 [c]	0.0 ± 0.0[a]

The differences between values with the same letter are not significant.

sprayer between 3 pm – 5 pm on the spraying day.

Field observation and data collection

Data collection started two days before the first application of treatments and was carried out twice a week: two days before and after treatments were applied over six weeks during each season. Data collection methods employed were direct observation for the presence and the number of insects found on the 6 randomly selected record plants on each plot.

Data collected include insect pests found on each record plant per treatment, number of fruits per plant, number of damaged fruits per plant, number of undamaged fruits per plant and number of fruit and shoot borers that emerged from infested fruits. The yield was calculated as follows:

$$\text{Yield (kg/ha)} = \frac{Weight\ (kg)\ x\ 1\ hectare}{Total\ field\ area}$$

Data analysis

Analysis of variance (ANOVA) was determined at a 95% confidence interval for all data collected with the aid of the GenStat Statistical Package (12th edition). Insect count data were log-transformed before data analysis. The mean separation was done using LSD at 5%.

RESULTS

Insect complexes found on the garden eggs

Several insects were collected; however, in terms of their numbers, whitefly (*Bemisia tabaci*), leafhoppers (*Empoasca* spp.) and fruit and shoot borers (*Leucinodes* spp.) were collected and identified as the major insects. These insects were reported in all the treatments in this study as shown in Table 1. The *Empoasca* spp and *Leucinodes* spp were found in all three seasons while *Bemisia tabaci* was recorded in only two seasons (dry and minor seasons) but absent during the major season. The fruit and shoot borers were recorded when the harvested fruits were dissected and incubated.

Effect of the botanicals on the population of whiteflies.

All the treatments showed various levels of insecticidal activities against the whiteflies as shown in Table 2. During the dry and minor seasons, there was no significant difference (p>0.05) in the number of whiteflies between the three botanicals. However, the *A. occidentale* treatment recorded significantly (p<0.05) the lowest whiteflies population (16.1 ± 3.6) differed significantly when compared to the emamectin benzoate treatment (20.9 ± 5.0). The control on the other hand significantly (p<0,05) recorded the highest number of white flies for both dry (42.7 ± 12.1) and minor seasons and (34.6 ± 9.5). Whiteflies were absent in the major season.

Effect of the botanicals on the population of leafhopper (*Empoasca* spp.)

The mean leafhopper population recorded for the botanicals did not differ significantly (p>0.05), while the control treatment significantly (p<0.05) recorded the highest mean leafhopper population in all three seasons as shown in Table 3. There was also a general increase in the leafhopper population across the seasons, with the major season recording the highest numbers. *Annona*

Table 3. Effect of the aqueous extract of botanicals on leafhopper (*Empoasca* spp.) population

Treatment	Dry Season	Minor Season	Major Season
Annona muricata leaves	3.6 ± 2.0 [a]	20.8 ± 12.4 [a]	38.8 ± 5.8 [a]
Chromolaena odorata leaves	3.9 ± 7.0 [a]	20.7 ± 11.4 [ab]	34.6 ± 6.4 [a]
Anacardium occidentale nutshell liquid	4.0 ± 1.5 [a]	21.1 ± 11.7 [ab]	33.4 ± 8.2 [a]
Emamectin benzoate	3.4 ± 4.0 [a]	20.8 ± 11.8 [ab]	32.1 ± 3.0 [a]
Control	7.4 ± 3.0 [b]	25.8 ± 13.4 [b]	71.4 ± 12.4 [b]

The differences between values with the same letter are not significant.

Table 4. Presence of fruit borer (*Leucinodes* spp.) larvae in fruits

Treatment	Dry Season	Minor Season	Major Season
Annona muricata leaves	2.3 ± 1.2 [cd]	0.9 ± 0.5 [a]	2.7 ± 1.5 [a]
Chromolaena odorata leaves	1.4 ± 1.4 [a]	1.2 ± 1.2 [a]	3.3 ± 3.3 [a]
Anacardium occidentale nut shell liquid	2.4 ± 2.4 [bc]	1.0 ± 1.0 [a]	2.3 ± 2.3 [a]
Emamectin benzoate	1.4 ± 0.7 [d]	0.6 ± 0.4 [a]	1.7 ± 1.2 [a]
Control	1.8 ± 0.7 [d]	1.5 ± 0.6 [a]	7.3 ± 2.6 [b]

The differences between values with the same letter are not significant.

muricata leaves showed the least mean (3.6 ± 2.0) followed by *C. odorata* leaves (3.9 ± 7.0) and then finally *A. occidentale* nutshell liquid (4.0 ± 1.5) in the dry season. During the minor season, *C. odorata* leaves recorded the least mean population (20.7 ± 11.4) followed by *A. muricata* leaves (20.8 ± 12.4) and finally *A. occidentale* nutshell liquid (21.1 ± 11.7). There was no significant difference in the mean population for the botanicals during the major season, but *A. occidentale* nutshell liquid recorded the least mean population (33.4 ± 8.2), followed by *C. odorata* (34.6 ± 6.4) and *A. muricata* (38.8 ± 5.8) leaves.

Effect of botanicals on eggplant fruit and shoot borers (*Leucinodes* spp.) in the field

As shown in Table 4, the three botanicals showed different levels of insecticidal activities against the eggplant fruit and shoot borers (EFSB) in all seasons. Generally, the population of the EFSB was lower in the botanicals treatment than in the control. During the dry season, a significant difference ($p<0.05$) in the number of the EFSB was recorded. However, the *C. odorata* leaves treatment recorded the least number of EFSB larvae (1.2 ± 1.4) followed by *A. muricata* leaves (1.3 ± 1.2) and then *A. occidentale* nutshell liquid (1.4 ± 2.4). During the minor season, though the mean number of EFSB recorded for the botanicals did not differ significantly ($p>0.05$) when compared to the standard insecticide (Emamectin benzoate), *Annona muricata* leaves recorded the least number of EFSB larvae (0.9 ± 0.5), followed by *A. occidentale* nutshell liquid (1.0 ± 1.0) and then *C. odorata* leaves (1.2 ± 1.2). Similarly, there was no significant difference ($p>0.05$) in the mean EFSB numbers recorded between the botanicals and Emamectin benzoate in the major season. However, *A. occidentale* nutshell liquid recorded the least number of EFSB larvae (2.3 ± 2.3), followed by *A. muricata* leaves (2.7 ± 1.5) and then *C. odorata* leaves (3.3 ± 3.3) in ascending

Assessment of damage caused by EFSB to harvested fruits

Fruits were described as damaged if there are EFSB larvae or signs of larval activities, i.e., a remnant of frass/excreta or tunnel when cut open. Otherwise, the fruits are described as undamaged as shown in Plate **1**.

Generally, the fruit damage observed in the botanicals treatment was lower compared to the control and the standard check across all the seasons (Figure **1**). The *C. odarata* treatment recorded the least fruit damage in the dry season, whereas the A muricata and A occidentale recorded the least fruit damage of 5.0 fruits each during the minor season.

It is also observed that, more fruits were damaged in the major season than in the minor and dry seasons. However, for all treatments, the minor season had fewer fruit damage compared with the other seasons.

Effect of botanicals on the yield of garden eggs.

The yield of garden eggs for all the treatments was measured in terms of the number and weight of fruits per treatment. Fruit yield varied between treatments across the seasons, but generally, the highest yields per treatment were recorded in the major season as shown in

Plate 1. Harvested garden egg fruit cut open to show damage (left) and undamaged content because of the activity of EFSB

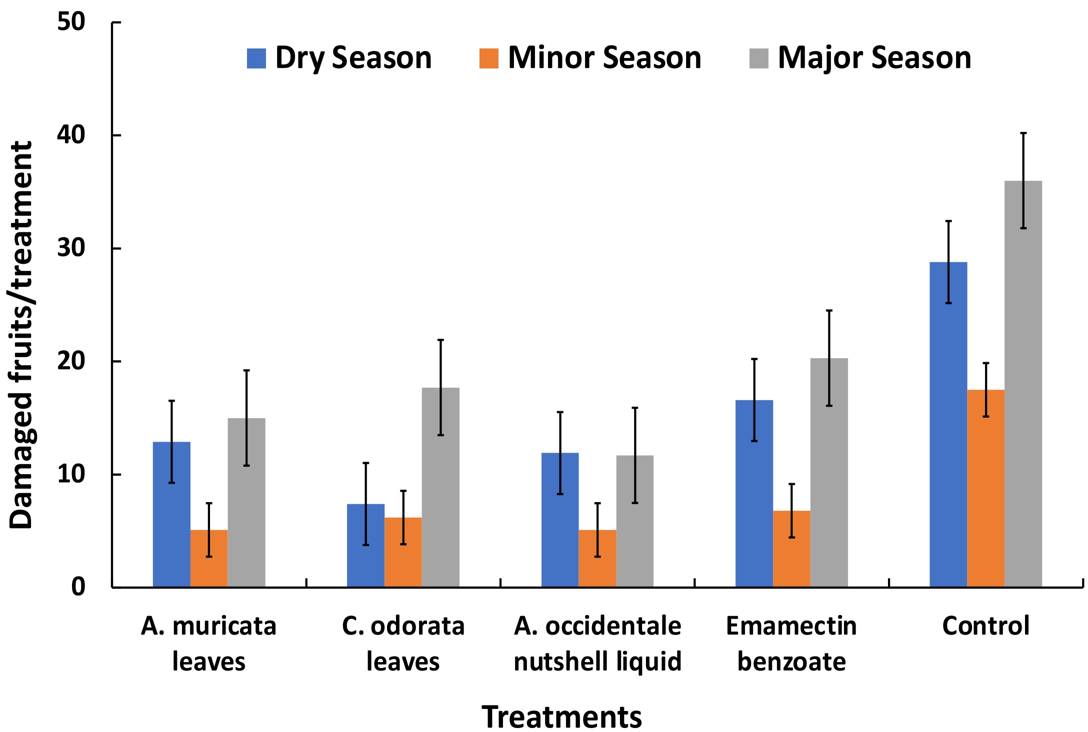

Figure 1. Garden eggs fruit damage observed in the treatments for the three seasons

Figure 2.

In the dry season, the standard check, Emamectin benzoate, recorded the highest yield (2.9kg), whereas the control (1.8kg) was the least. During the minor and major seasons, the *C. odorata* leaves significantly recorded the highest yield, 7.7 kg and 10.3 kg respectively.

Yield of marketable and unmarketable fruits

In general, damaged fruits are fruits that showed activities of the EFSB **(Plate 2)** were classified as non-marketable. Marketable fruits had no blemish i.e., had no entry or exit hole created by the EFSB, and were fit to be

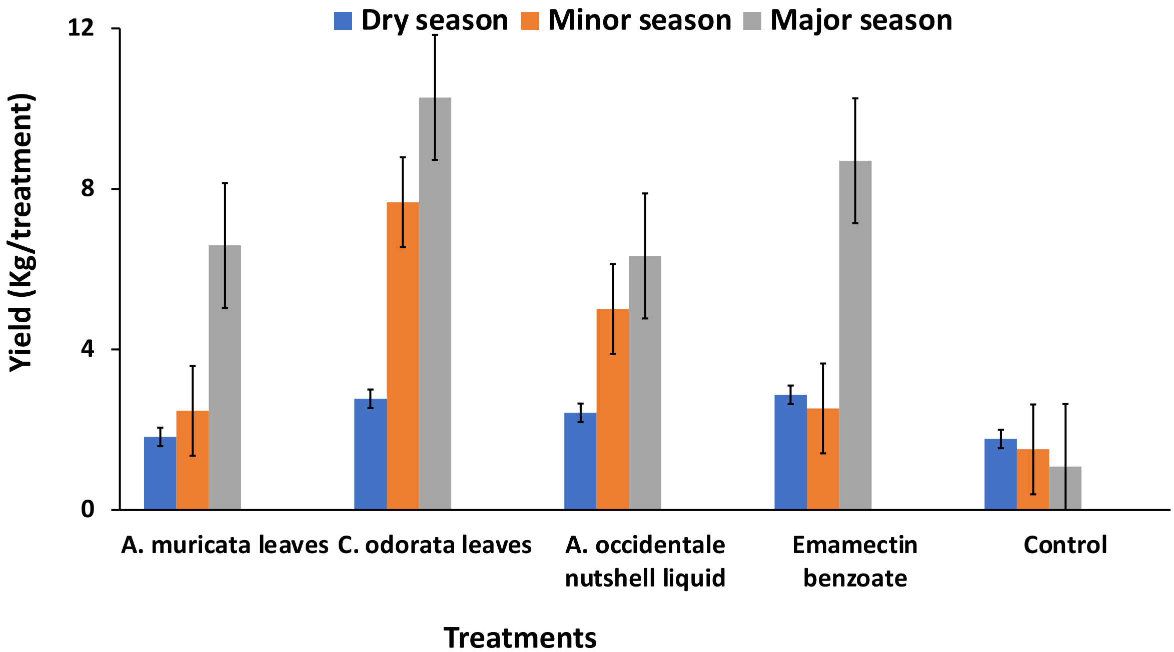

Figure 2. The yield of garden eggs for the treatments for the three seasons

Plate 2. Harvested garden egg fruits showing marketable (left) and unmarketable (right) fruits

sold on the market.

Generally, all the treatments had more marketable fruits than unmarketable fruits per season except for the control, where unmarketable fruits were more during the dry and minor seasons as shown in **Figure. 3a – c.**

With an emphasis on the botanicals, *A. occidentale* nutshell liquid recorded the highest number of marketable fruits followed by *A. muricata* and *C. odorata* during the dry season (Figure 3a), whereas *C. odorata* recorded the highest number of marketable fruits followed by *A occidentale* shell liquid and *A. muricata* in the minor (Figure 3b) and major seasons (Figure 3c).

DISCUSSION

Effect of botanicals on Whitefly (*Bemisia tabaci*), Leafhopper (*Empoasca* spp.) and fruit and shoot borer species (*Leucinodes* spp.) in the field.

The populations of the insect pests recorded varied throughout the seasons. Whiteflies (*B. tabaci*) were absent during the major rainy season which is similar to

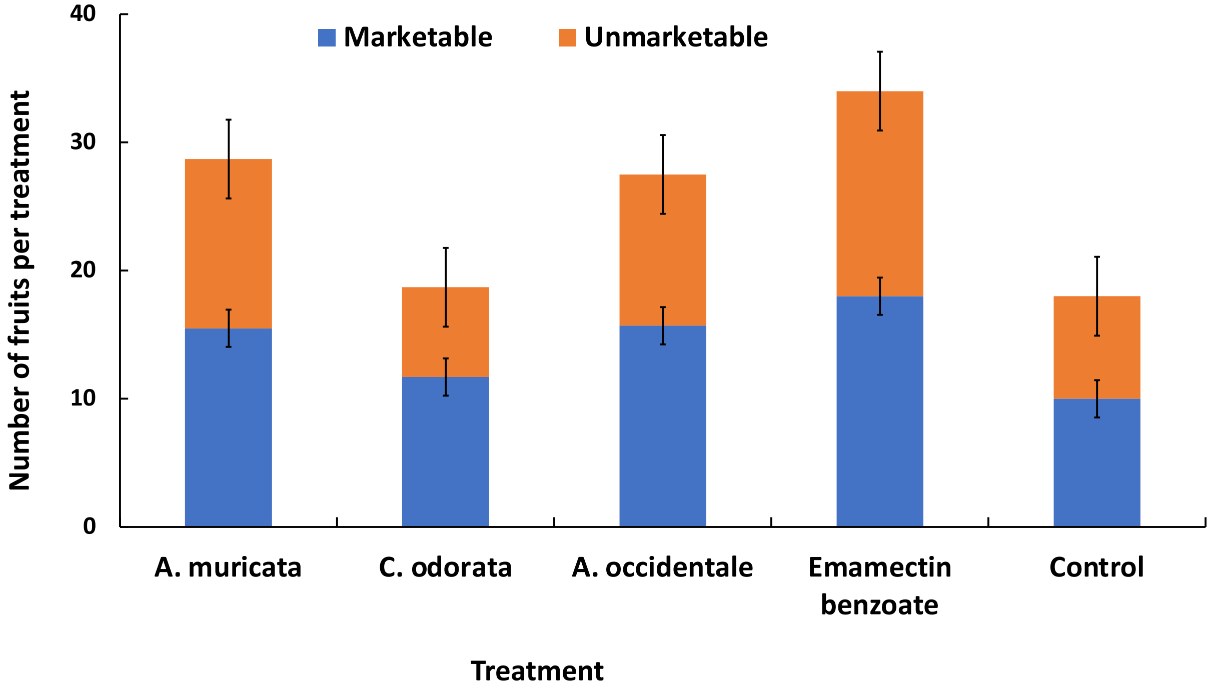

Figure 3a. Number of marketable and unmarketable fruits harvested per treatments in the dry season.

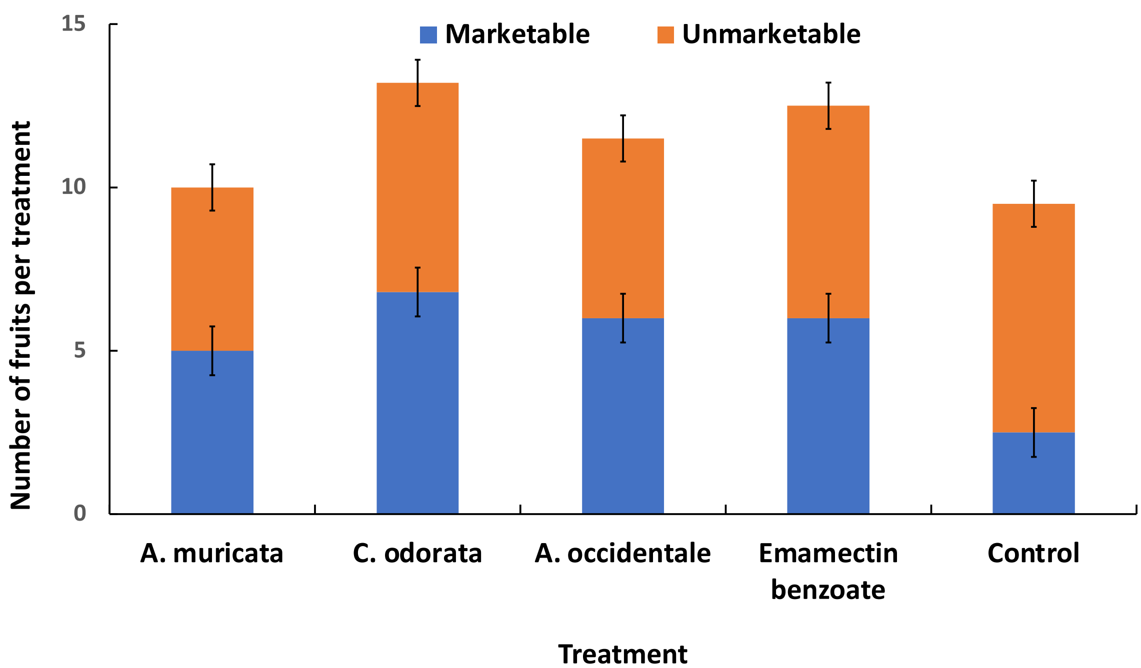

Figure 3b. Number of marketable and unmarketable fruits harvested per treatments in the minor season.

the findings of Appiah *et al*. (2012). This could be attributed to abiotic factors such as temperature and rainfall. Shivanna *et al*. (2011) confirmed the fact that abiotic factors such as precipitation have an impact on the abundance of insect pests such as whiteflies and leafhoppers.

The effects of the botanicals recorded on the insect pests in the field across the three seasons may be

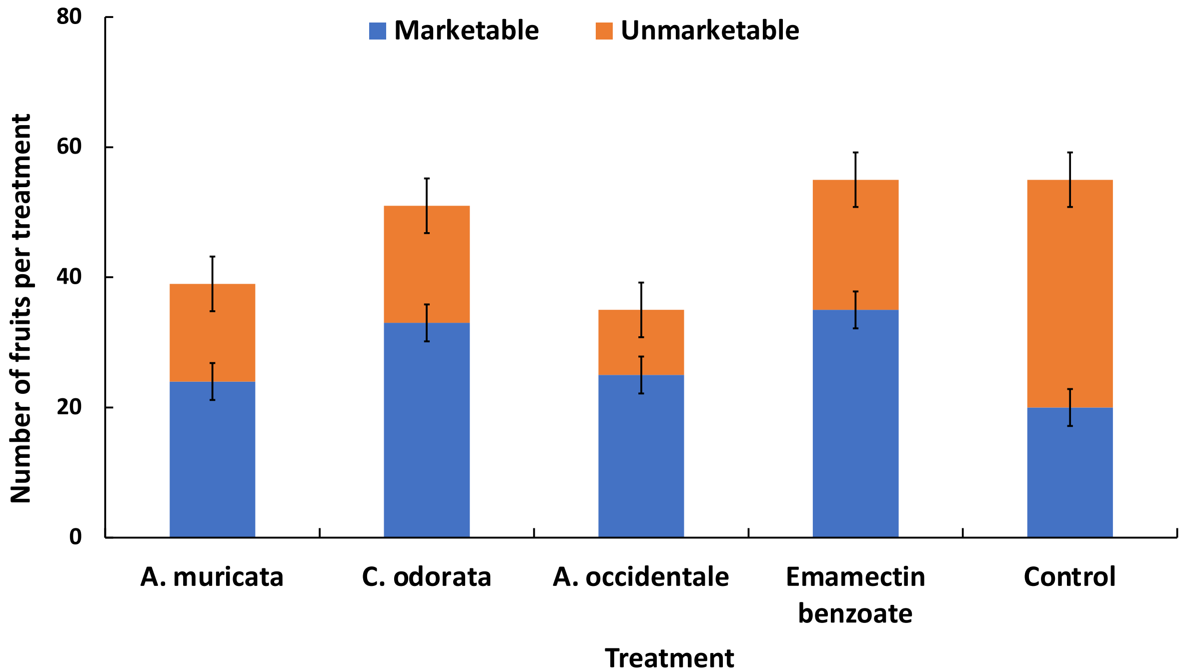

Figure 3c. Number of marketable and unmarketable fruits harvested per treatments in the major season.

attributed to the presence of active compounds that have insecticidal properties. According to Moghadamtousi *et al*. (2015), *A. muricata* is a great source of annonaceous acetogenin compounds which have insecticidal properties among other properties. As such, the extracts of *A. muricata* and other Annonaceae plants could be used in the management of whiteflies (*Bemisia tabaci*) (Soares *et al*., 2021), and the leaf extract showed toxicity against the larvae of the diamondback moth (*Plutella xylostella*) and *Callosobruchus maculatus* (Predes-Trindade *et al*., 2011). It also showed insecticidal activities against the larvae of *Aedes aegypti* (Bobadilla *et al*., 2005).

Lawal *et al*. (2015) reported that *C. odorata* leaves contain volatile compounds like phytol, caryophyllene oxide, oleic acid, hexadecanoic acid and di-n-octyl phthalate which have insecticidal properties. The leaf extracts of *Chromolaena odorata* are efficacious against *Plutella xylostella* (Ezena *et al*., 2016; Amego, 2019), *Sitophilus zeamais* (Lawal *et al.,* 2015) and have shown different levels of insecticidal activities against *Callosobruchus maculatus* (Osariyekemwen and Benedicta, 2017).

Anacardium occidentale nutshell liquid is made up of compounds like anacardic acid, cardanol and cardol which are responsible for its insecticidal activities (Oliveira *et al*., 2011). Studies have reported that it has insecticidal activity against *Helicoverpa armigera* (Mahapatro, 2011), and stored product pests like *Sitophilus oryzae* and *Tribolium castaneum* (Buxton *et al*., 2017; 2018). The findings of Andayanie *et al.* (2019) showed that *Anacardium occidentale* shell liquid is efficacious against whitefly (*Bemisia tabaci*). It also has larvicidal activities against the larvae of *Aedes aegypti* (Oliveira *et al*., 2011) and *Crocidolomia pavonana* larvae found in cabbages (Nenotek *et al*., 2022).

The fruit damage caused by the *Leucinodes* spp. larvae is an indicator of the presence of the insects and whether or not the insecticides are working against the insect pests. But the variations in the mean number recorded in each season attests to the efficacy of the botanicals used in this study. The varying efficacy of the botanicals against EFSB could be due to the different levels of photodegradation, loss through volatilization or abiotic oxidation because of the differences in the amount of rainfall, temperature and sunlight hours experienced in each season (Angioni *et al*., 2005). Although the protection against the fruit borers (*Leucinodes* spp.) offered by the botanicals varied across the three different seasons, it confirms the findings of Lawal *et al*. (2015), Ezena *et al*. (2016), Buxton *et al*. (2017; 2018) and Soares *et al*. (2021) who reported that leaves of *A. muricata* and *C. odorata,* and *A. occidentale* nutshell liquid have insecticidal properties.

Effect of botanicals on the yield of garden eggs

The yields recorded after the application of the botanicals varied. This may be attributed to the varying effects of the botanicals on the number of *Leucinodes* spp., other insect pests and the number of damaged fruits recorded in each season. Ogbuehi and Onuh (2019) recorded high yield when *A. muricata* leaf extracts were tested on flea beetles (*Podagrica* spp.) on okra, and further suggested that there was a direct relationship between the number of infestations and high fruit yield recorded. Also, higher yield was recorded in *A. muricata*, *C. odorata* and *A. occidentale* treated garden compared to the Control. This result corroborates that these botanicals have insecticidal properties (Lawal *et al*. 2015; Buxton *et al*., 2017,2018; Ogbuehi and Onuh 2019).

CONCLUSION AND RECOMMENDATIONS

This study has shown that *Annona muricata*, *Chromolaena odorata* and *Anacardium occidentale* aqueous extracts have different levels of insecticidal activities against whiteflies (*Bemisia tabaci*), leafhoppers (*Empoasca* spp.) and fruit borers (*Leucinodes* spp.) which were the main insect pests of garden eggs in the field at the time of the study.

It can be recommended that growers could incorporate these botanicals into their insect pest management strategies, and alternate between the three botanicals at different times of the year to derive the maximum insecticidal benefits from the botanicals used

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