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CHEMICAL COMPOSITION AND INSECTICIDAL ACTIVITY OF VOLATILE OILS of *Allium cepa* BULB AND *Carica papaya* LEAF AGAINST *Balanogastris kolae* INFESTATION ON STORED KOLANUTS

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ABSTRACT

This study investigated the phytochemicals present in the hexane extracts of red *Allium cepa* bulbs and *Carica papaya* leaves and the insecticidal activity of their essential oils against *Balanogastris kolae*. Saponins, flavonoids, tannins, alkaloids, phenols, and cardiac glycosides were found in the hexane extracts of *A. cepa* bulbs and *C. papaya* leaves. The GC-MS analysis of these essential oils showed that the most abundant compounds in *A. cepa* oil were the aromatic compounds, which was about 32% of the whole constituents, followed by about 26% organosulfur compounds. In the *C. papaya* leaf oil, β-Bisabolene (9.3201%), Linalool (2.0773%) and Limonene (1.6953 %) were prominent. The contact toxicity assay showed that the insecticidal activity of essential oil of *C. papaya* leaf were stronger than that of *A. cepa* bulb at lower concentrations because after 24 hours of exposure to treatments, percentage mortality of *B. kolae* was higher in *C. papaya*, ranging from 65.27 (5% concentration) to 87.50 (15% concentration) compared to that of *A. cepa* which ranged from 8.06 (10% concentration) to 73.31 (20% concentration). Both *A. cepa* and *C. papaya* oil have no fumigant property as there was no statistical difference in percentage mortality across all the treatments. Conclusion: C. *papaya* leaf and *A. cepa* bulb essential oils can be used as a bio-insecticide (contact toxicity) against *B. kolae*, as they compared favourably with synthetic insecticide, 2, 2 - Dimethyl dichloro vinyl phosphate (DDVP). This is the first report on the use of the essential oils of *A. cepa* bulbs and *C. papaya* leaf against *Balanogastris kolae*.

Keywords: *Allium* cepa, *Carica papaya, Balanogastris kolae*, Bio-insecticide, GC-MS.

INTRODUCTION

Plants are potential sources of secondary metabolites known as numerous chemical substances produced by the plant cells through metabolic pathways (Ghasemzadeh and Neda, 2011). They are multifunctional metabolites typically involved in plant defence and environmental communication. They are associated with plant colour, taste, and scent (Jan *et al.,* 2021). Few secondary metabolites protect plants against stresses, both biotic (bacteria, fungi, nematodes, insects or grazing by animals) and abiotic (higher temperature and moisture, injury or presence of heavy metals) (Jan *et al.,* 2021; Pagare *et al.,* 2015).

Agricultural produce in storage faces several challenges, including principal damages from insect pests. As a result of damage done by insects, stored products such as seeds, chips and tubers lose their value for sales, consumption or sowing. Kolanut, a traditional plant belonging to the family *Sterculiaceae* is one of such produce. It is a crop native to the tropical rainforests of Africa, Nigeria inclusive. Kolanut is a major cash crop cultivated in Nigeria where it has different levels of significance to the three major existing ethnic groups, the Hausa, Igbo and Yoruba (Azeez, 2015). It has been found to contain caffeine and theobromine, hence they are used as stimulants, in the manufacturing of beverages and pharmaceutical products. More so, kola nuts contain tannins which makes them useful in the textile industry. Similarly, they are used in the production of wines, soft drinks and in animal feed formulations (Ndagi *et al.*, 2012). However, the adult kola weevil, *Balanogastris kolae (Desbrochers)*, a member of the *Curculionidae* is the most common insect pest of kolanut. They are identified as field-to-store pests of kola because their infestation begins on the field and continues in storage, causing losses to stored nuts (Azeez, 2015). Undetected infestation of kola nuts in storage by *B. kolae* larvae (which seriously feeds on the nut and consequently reduce it to some brownish powdery mass) results in huge losses as high as about 60% of the total production in a year (Popoola *et al.*, 2020; Asogwa *et al.,* 2015).

A good number of farmers and traders resort to the use of synthetic pesticides mostly in liquid form to control this menace. This is discouraged due to the toxic effects of the residues of this conventional pesticides on food and the environment. Hence, attention has been drawn to developing environmentally friendly botanical pesticides which are naturally occurring chemicals extracted from plants (El-Wakil *et al.*, 2015).

Essential oils extracted from aromatic plants are excellent alternatives to synthetic or chemical pesticides currently used for combating insect pest infestation and damage (Wafaa *et al.,* 2017).

Several studies have been conducted on the insecticidal potency of essential oils from different plant samples. The effect of the essential oils extracted from *Rosmarinus officinalis L., Mentha pulegium L., Zataria multiflora,* and *Citrus sinensis* (L.) Osbeck var. hamlin on adults of *Tribolium castaneum, Sitophilus granarius, Callosobruchus maculatus,* and *Plodia interpunctella* was investigated and were found to have good fumigant toxicity (Mahmoudvand *et al.,* 2011).

Gharsan *et al.* (2018) reported that *Allium cepa* L. (onion) oil caused 100% insect mortality at all concentrations against *Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae)*. Also, Khater *et al.* (2009) investigated the efficacy of *A. cepa* essential oil against *Haematopinus tuberculatus (buffalo louse), Musca domestica, Stomoxys calcitrans, Haematobia irritans* and *Hippobosca equine*. The findings revealed 100% of the lice died by application of the *A. cepa* oil. Therefore, it is highly effective as a pour-on solution (contact toxicity) on water buffaloes infested by insects. (Khater *et al.*, 2009)

Furthermore, there are numerous reports on the insecticidal properties of extracts from different parts of *C. papaya* (Dwi, 2015; Chand *et al.*, 2017 and Gupta *et al.*, 2020), but the information on the use of its essential oil is scanty. Rahayu *et al.* (2020) found that ethanolic leaf extract of *C. papaya* is very active as a biological insecticide in controlling the population of the German cockroach. Also, Okore *et al.* (2017) reported that *C. papaya* essential oil could serve as a good alternative for chemical insecticides in the control of *Callosobruchus maculatus* and *Sitophilus zeamais* on stored grains in a dose-dependent manner.

Information on the use of essential oils of *A. cepa* and *C. papaya* against *B. kolae* is currently not well documented. Hence, this paper presents the chemical constituents of the volatile oils of *A. cepa* bulbs and *C. papaya* leaves and their bio-insecticidal activity against *B. kolae*.

MATERIALS AND METHODS

Collection and preparation of the plant materials: *Allium cepa* (AC) and *Carica papaya* (CP)

AC (red onion) bulbs were purchased from a local market in Ibadan, Oyo State, Nigeria, while the CP leaves were harvested from the Department of Crop Protection and Environmental Biology crop, Garden, University of Ibadan. The onions were prepared by peeling off the dried outer layers, rinsed, sliced thinly and then air-dried at room temperature for seven days. *Carica papaya* leaves were air-dried for two weeks at a temperature of 27° C and 30% relative humidity. The dried plant materials were ground to a rough powder using a silver crest SC – 1589 blender and stored in air-tight containers until they were ready for use.

Crude extraction of AC and CP

Successive extraction was carried out on the dried and pulverized AC (red onions) bulbs and CP leaves with hexane, ethyl acetate and methanol via maceration. 1 kg of each plant sample was placed in flat bottom flasks (10 L) and soaked in 4 L of hexane, the container was sealed using foil paper and kept for 72 hours with periodic random shaking to achieve homogenization. The mixture was then filtered and the filtrate mixture was concentrated using a rotary evaporator at a reduced temperature (50°C). The condensed extract was stored in an air-tight container at about 4°C till further investigation. The above-stated procedure was used for ethyl acetate and methanol solvents to obtain each of their crude extracts.

Qualitative phytochemical analysis of the Hexane extract of the plant samples

The hexane extract of *Allium cepa* (AC) and *Carica papaya* (CP) were screened for their phytochemical constituents such as alkaloids, cardiac glycosides, saponins, phenols, flavonoids and tannins using standard procedures (Sofowora 1993).

Extraction of the essential oil

Hydro-distillation of 300 g of ground AC and CP in an all-glass Clevenger apparatus for three hours was carried out. The essential oil obtained was stored at -4°C for further analysis and use.

Kolanut samples collection and insect culture

Two plastic containers, 4 L capacity each containing infested and clean kola nuts were bought from a local market in Osogbo, Osun State, Nigeria. The infested nuts were kept in the laboratory at 28°C ± 0.2 and 83-90% RH for a period of two weeks for the emergence of adult weevils, while the clean kolanuts were preserved inside a black polythene bag and then placed in a sack bag to keep them fresh. *B. kolae* culture was raised in the laboratory from adult weevils collected from the infested kola nut following the method described by Asogwa *et al.* (2009). Twenty weevils, ten male and female were brought into a black polythene bag containing 50 nuts and left on the laboratory bench for 30 days to enable oviposition and development of the first generation of adult weevil emergence. Teneral adult emergents picked from this culture were used for the bioassays.

Toxicity tests using the essential oils against kola weevil

Contact assay

Ten teneral adults of *B. kolae* male and female (5: 5) of the same age were placed inside a 10 mL transparent dish. A 0.2 mL of the different concentrations (1%, 5%, 10%, 15% and 20%) of each of the essential oils of AC bulbs and CP leaves was introduced to the dorsum of insects to have contact with the oil. Two kola nuts were placed inside each of the 1-litre containers for the treated insects to serve as food and the lid of the container was perforated to achieve aeration. Each of the treatments was replicated four times in a complete randomized design. There were two controls, one in which only hexane was used against the insect and the other untreated. Mortality counts were recorded after 24, 48, 72 and 96 hours of exposure (Azeez, 2015) and corrected for mortality using Abbott's formula (Abbott, 1925; Noudegbessi *et al.*, 2021). 2, 2 - Dimethyl dichloro vinyl phosphate (DDVP) was used as standard in which 0.5 mL of the stock pesticide was diluted in 100 mL of water and applied following the same procedure used for the oil samples.

Fumigant assay

Ten adult (*B. kolae* males and females) of the same age were placed inside a 1.5 L cylindrical container with two kola nuts. A 0.2 mL of the different concentrations (1%, 5%, 10%, 15% and 20%) of the essential oil of AC bulbs and CP leaves was introduced on a filter paper strip of 2.5 cm diameter. Filter paper strip impregnated with essential oil was placed inside the cylindrical container. Each fumigant test was replicated four times along with the control. For the control, no essential oil was placed in the container. Adult mortality was recorded after 1, 6, 12 and 24 hours of the exposure and corrected for mortality using Abbott's formula (Abbott, 1925; Noudegbessi *et al.,* 2021). 2 mL of 0.5% concentration of 2, 2-Dimethyl dichlorovinyl phosphate (DDVP) was used as standard following the same procedure.

Statistical analysis

Mortality data were corrected using Abbott's formula. Data collected on contact and fumigant bioassays were analyzed with analysis of variance (ANOVA) using R statistical software version 4.1.3. ANOVA was used to compare the differences in the corrected mortality recorded from adults treated with AC and CP essential oils at 1%, 5%, 10%, 15% and 20% concentration levels. Significant means were separated using Tukey HSD Test at $p < 0.05$.

Gas Chromatography-Mass Spectrometry Analysis

Gas Chromatography–Mass Spectrometry of the essential oils was conducted at the Biocontrol Centre, International Institute of Tropical Agriculture, Republic of Benin. Capillary gas chromatography was performed using Hewlett–Packard 8890 gas chromatograph equipped with a flame ionization detector and fused silica capillary column HP-5 MS (30 m × 0.25 mm, 0.25 µm film thickness); injector and detector temperatures were 270°C and 300°C, respectively. The components of the essential oils of AC bulbs and CP leaves were separated by the GC and identified by mass spectrometry (GC–MS) using Agilent 8890 gas chromatography coupled to Agilent 5977B mass spectrometry detector. The GC settings were as follows: the initial oven temperature was held at 60°C for 1 min and ramped at 10°C min−1 to 180°C where it was held for 1 min, and then ramped at 20°C min−1 to 280°C and held there for 15 min. The total run time was 42 minutes. The sample (1 µL, diluted 1:100 in acetone) was injected, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 mLmin^{-1}. Spectra were obtained over the scan range of 20 to 550 m/z at 2 scans s^{-1}. The constituents of the oils were identified via gas chromatography by comparing their retention times using the library (Adams 2. L, Adams 2. L and NIST 11. L). Further identification was made by comparison of their mass spectra with those stored in the libraries.

RESULTS

Qualitative phytochemical analysis

The preliminary phytochemical study showed that the hexane extract of AC bulbs and CP leaves contained flavonoids, alkaloids, saponins, and phenols. However, tannins were present only in CP extract and not found in the AC bulb, while cardiac glycosides were found only in the AC leaf

Table 1: Phytochemical constituents of hexane extract of AC bulbs and CP leaves

Phytochemicals	Occurrence of Phytochemicals	
	***Allium cepa* (AC) bulbs**	***Carica papaya* (CP) leaf**
Flavonoids	+	+
Alkaloids	+	+
Tannins	-	+
Saponins	+	+
Phenols	+	+
Cardiac glycoside	+	-

+ = present; - = absent

Laboratory bioassays of AC bulbs and CP leaves essential oils against *B. kolae*

Contact toxicity test

Table 2a shows the effect of AC and CP as a contact insecticide on *B. kolae*, using five different concentrations (1%, 5%, 10%, 15% and 20%). After 24 hours of exposure to treatments, the percentage mortality of *B. kolae* was higher in CP, ranging from 65.27 (5% concentration) to 87.50 (15% concentration). AC generally displayed a lower percentage mortality compared to CP ranging from 8.06 (10% concentration) to 73.31 (20% concentration). The differences between the percentage mortality of treatments with AC and CP were statistically significant for 1%, 5%, 10%, and 15% concentrations, but were not significant for 20% concentration. The mean corrected mortality for CP was significantly higher than that of AC ($p<0.01$) across all the concentrations, except at 20%, where the mean mortality for *C. papaya* was not significantly different from that of *A. cepa* ($p > 0.05$). This result showed that the essential oil of CP leaf has greater contact toxicity on *B. kolae* adults than AC at lower concentrations (1%, 5%, 10% and 15%). It is interesting to note that there is no significant difference at higher concentrations (20%), meaning the two essential oil samples caused mortality of the adult weevils at higher doses, comparing favourably with the synthetic pesticide, DDVP, as there is no statistical difference among them.

Table 2a: Weevils mortality (%) at 24 hours caused by contact toxicity of *Allium cepa* (AC) and *Carica papaya* (CP) essential oils and 2, 2-Dimethyl dichlorovinyl phosphate (DDVP).

	Weevils Mortality (%)				
Concentrations	**1%**	**5%**	**10%**	**15%**	**20%**
AC	48.06±10.1a	10.28±4.09a	8.06±5.28a	15.83±3.09a	73.61±3.1a
CP	74.56±21.0a	65.27±12.8b	72.86±10.3b	87.50±7.22b	71.61±121a
DDVP	100.0±0a	100.0±0c	100.0±0c	100.0±0b	100.0±0a

Mean±SE. within a column followed by the same letters are not significantly different using Tukey Test at $p < 0.05$.

Fumigant toxicity test

A pairwise comparison of percentage mortality of the essential oils of CP and AC as fumigants against adult *B. kolae* is in Table 2b below at five different concentrations (1%, 5%, 10%, 15% and 20%). Across all the concentrations, AC treatment resulted in higher percentage mortality than CP except at 15% concentration where AC had just 3.70% mortality, but observed differences were not statistically different.

Table 2b: Weevils mortality (%) after 24 hours of exposure to fumigant toxicity from *Allium cepa* (AC) and *Carica papaya* (CP) essential oils and DDVP.

	Weevils Mortality (%)				
Concentrations	**1%**	**5%**	**10%**	**15%**	**20%**
AC	14.81±9.80a	18.52±3.70a	18.52±9.80a	3.70±3.70a	11.11±0a
CP	8.33±5.32a	11.11±4.54a	5.90±3.42a	14.58±5.71a	6.25±6.26a
DDVP	100.0±0b	100.0±0b	100.0±0b	100.0±0b	100.0±0b

Mean±SE. within a column followed by the same letters are not significantly different using Tukey Test at $p < 0.05$

Chemical characterization of constituents of the essential oils of AC bulbs and CP leaves

Hydro-distillation of the AC bulbs and CP leaves gave essential oils with distinct odours with good yields of 0.95% and 0.57%, respectively. Compounds identified in AC bulbs and CP leaves are in Fig. 1 and 2. The GC-MS analysis revealed the presence of 19 and 21 main chemical components for both AC and CP oils, respectively.

The most abundant compounds in AC oil were the aromatic compounds, which were about 32% of the whole constituents, followed by about 26% organosulfur compounds, some of which are: Dipropyl disulfide (2.6779 %), Dipropyl-trisulfide (1.9285%), and 3,4-dimethyl- Thiophene (1.4308%), and a good number of hydrocarbons.

In the CP leaf oil, terpenes; β-Bisabolene (9.3201%), Linalool (2.0773%), β-pinene (1.9839%) Limonene (1.6953 %) and Farnesene (1.5184%) are prominent with a few esters, ketones, hydrocarbons such as 2-methyl- 1, 5-Hexadien-3-yne (5.5633%), and 13-Docosenamide, (Z)- (5.1693%).

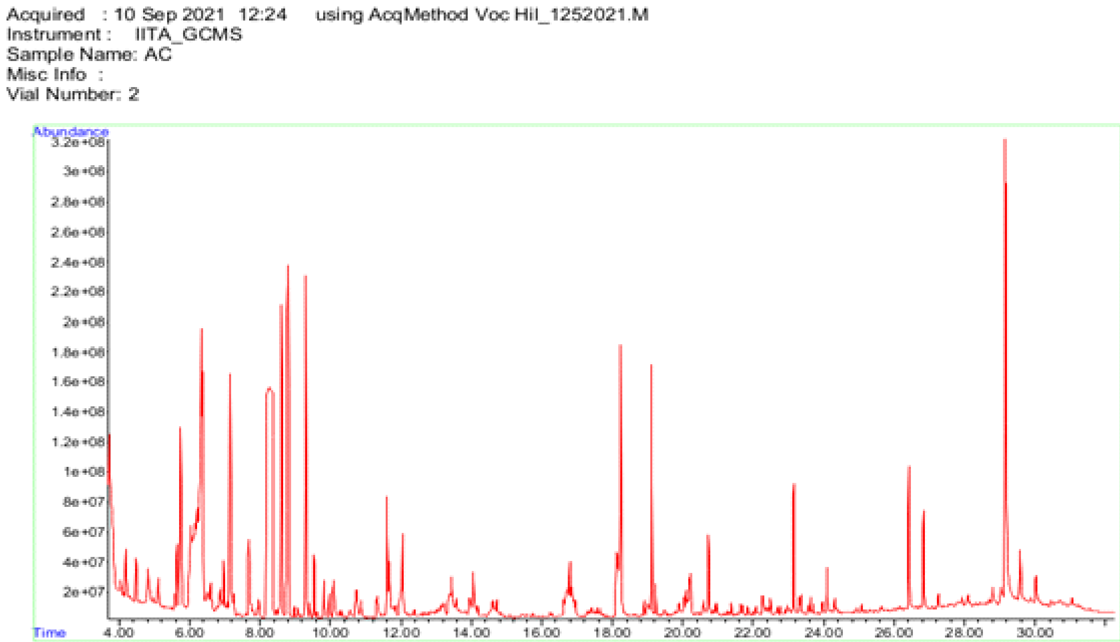

Figure 1: GC-MS chromatogram of AC essential oil

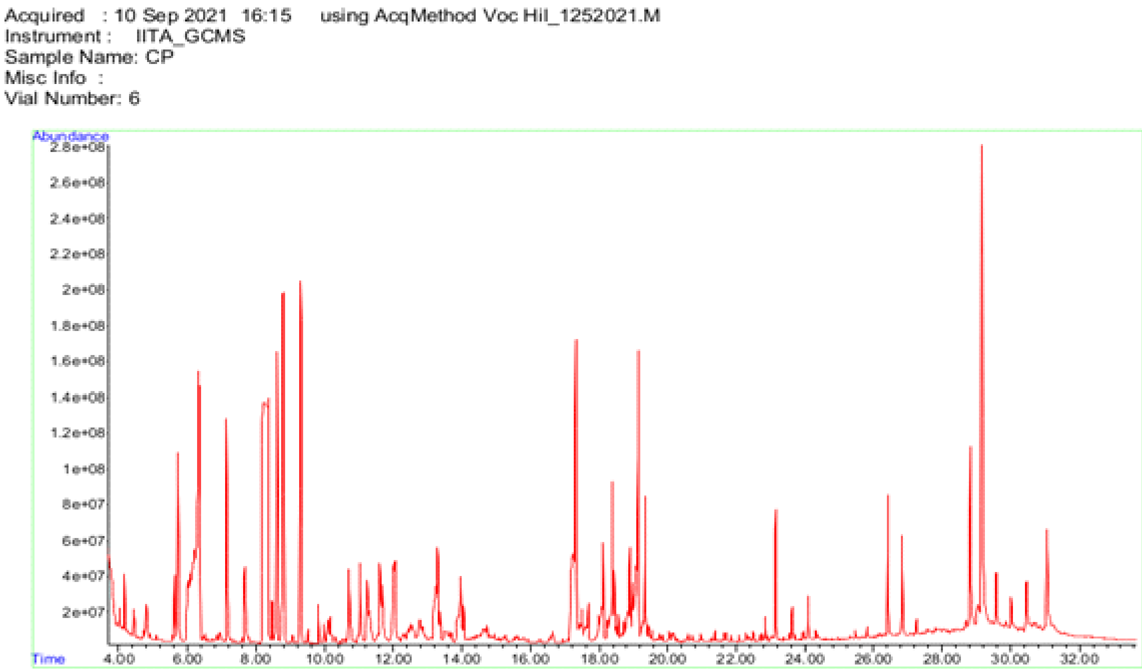

Figure 2: GC-MS chromatogram of CP essential oil

DISCUSSION

The findings of this research confirmed the insecticidal properties of the tested plant samples can be due to their phytochemical constituents. Phytochemicals such as saponins, flavonoids, tannins, alkaloids, phenols, and cardiac glycosides have been reported to possess pesticidal activities against pathogens and insects (Ghosh *et al.*, 2010) and were found in AC and CP. This finding corroborates a similar qualitative work on red onion done by Efiong *et al.* (2020), although tannins were reported to be present in the study. A similar report by Ayoola and Adeyeye, 2010 revealed the presence of saponins, cardiac glycosides and alkaloids in CP leaves whereas tannins were absent. The variations observed may be due to environmental factors and the method of extraction that was supported by previous studies (Usman *et al.*, 2017; Liu *et al.*, 2016).

Generally, saponins have a vast range of biological activities which have roles in plant defence (Hussain *et al.*, 2019). This is usually attributed to the fact that most consist of a hydrophobic triterpene and a hydrophilic carbohydrate chain (amphipathicity) (Mugford *et al.*, 2013). This enables them to permeate the plasma membrane where they form a complex with sterols, causing the formation of pores that exert their toxic effect (De Geyter, 2012 Armah *et al.* 1999). Also, saponin molecules affect the insect pest indirectly by forming a strong bond with several digestive enzymes, thus allowing them to damage the mucous lining of many cells in the digestive system of the insects leading to their mortality (Qasim *et a*l., 2020). Similarly, flavonoids are very important in plant resistance to pathogens as their compounds are transported to the site of infection and trigger a hypersensitivity reaction, which consequently causes cell damage or death (Mierziak *et al.*, 2014). Tannins have a strong destructive effect on phytophagous (plant-feeding) insects by affecting their growth and development as the tannins bind to the proteins, reducing nutrient absorption efficiency, and causing a mid-gut abscess (Munyore, and Rioba, 2020; Barbehenn and Constabel, 2011). Alkaloids are natural substances that play an important insecticidal role (Rattan, 2010). Most of these alkaloids are toxic to some degree and appear to serve primarily in defence against attack by herbivores (Pagare *et al.,* 2015). Previous reports consolidate our findings in the current study. The essential oils of *C. papaya* and *A. cepa* tested had no fumigant toxicity effect on *Balanogastris kolae*. This suggests *that A. cepa* and *C. papaya* have weak fumigant activity as there was no or little absorption of active constituents of the oil through the insect's spiracles. This result agrees with Tunaz *et al.,* (2009), which stated that *Allium cepa* did not have any fumigant activity against adult cockroaches, *Blattella germnica* (L).

The result obtained from the GC-MS analysis of the AC and CP oils showed the presence of many components with insecticidal properties, confirming the different toxicity tests conducted in this study. Results on the composition of these oils is similar to the report obtained by Cantrell *et al.* (2020); El-Wakil *et al.* (2015) and Abdel-Lateef *et al.* (2018), most especially, the sulphur compounds with some slight variations which may be due to differences in the environmental condition and the method used for the extraction (Babarinde *et al.*, 2017 and Liu *et al.*, 2016). Ndoye *et al.*, (2016) noted that the essential oil of AC was rich in organosulfur compounds, such as dipropyl trisulfide and dipropyl disulfide found to be among the constituents reported in this study, which may be responsible for its appreciable biological activities.

Furthermore, the predominance of the terpenes in the GC-MS analysis of the CP essential oil in this present study agrees with the findings of Igwe, (2015) which stated that terpenes dominate the constituents of the essential oil of CP leaf. Both AC bulb oil and CP leaf oil had contact toxicity against *B. kolae*. Gharsan *et al.*, 2018 corroborate this as it was reported that onion oil showed strong potency against *Oryzaephilus surinamensis* (L.) with insect mortality attaining 100%. Khater *et al.,* 2009 further support this as it was found that AC had strong insecticidal activity against lice and flies. More so, in support of this observation, Rahayu *et al.*, 2020 stated that ethanolic leaf extract of CP was very active as a biological insecticide in controlling the population of the German cockroach. Equally, the investigation conducted by Dwi, (2015) confirmed this report as the *C. papaya* leaf extract was found to possess remarkable larvicidal activity against *Aedes aegypti*.

The greater activity of the CP leaf oil may be a result of the high content of the terpenes which summed up to 38% of the whole constituents of the oil. For instance, limonene and β-bisabolene have been reported to have insecticidal and antimicrobial activities (Djilani, and Dicko, 2012), consolidated our findings. Liu *et al.* (2012) isolated limonene and caryophyllene from the essential oil of *Illicium pachyphyllum* fruits and found that they have strong insecticidal properties against grain storage insects, *Sitophilus zeamais* and *Tribolium castaneum*. The mode of action of limonene, which is one of the components of CP oil has been found to involve the removal of the protective wax layer from the exoskeletons of insects immediately after it makes contact with the insects, causing them to suffocate and die (Abdallah *et al.*, 2017).

More so, Pavela (2008) noted that the structural characteristics of terpenoids can enhance their insecticidal properties. The prominence of terpenes in the CP essential oil provides a vital defence strategy against insect pest infestation (Pagare *et al.*, 2015).

The efficiency of CP leaf oil at lower concentrations may be attributed to the synergistic effect exhibited by its chemical constituents since the majority of them have been reported to have biological activity (Bhalla *et al.*, 2013; Djilani, and Dicko (2012) and Sun *et al.*, (2019)). This view is supported by Silva et al., 2019 and Shareef *et al.,* 2020 in which the combined effects of several plant extracts against some pathogens were established.

CONCLUSION

This study showed that *Allium cepa* bulb volatile oil contains mainly hydrocarbons and organosulfur compounds. In contrast, *Carica papaya* leaf volatile oil contains some sesquiterpenoids and monoterpenoids with some esters, ketones and alcohols. The AC and CP oils used in this research were active against *Balanogastris kolae* as contact toxicity in a dose-dependent manner. As essential oil concentrations increased, adult mortality also increased in the contact toxicity test. However, none of the essential oils had fumigant properties against the weevils. Hence, CP leaf and AC bulb essential oils can be used as a bio-insecticide (contact toxicity) against *B. kolae,* a significant insect pest of kola nut in storage as they are both compared favourably with synthetic insecticide (DDVP). This study is the first report on the use of the essential oils of *Allium cepa* (AC) bulbs and *Carica papaya* (CP) leaf against *Balanogastris kolae*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHORS' CONTRIBUTION

Odeyemi E.F. - Conceptualization, investigation and writing
Olaoluwa O. O. and Alabi O.Y. - Supervision, validation and resources,
Tamò M. and Kpongbe H. – GC-MS analysis,
Badmos S.T. – Extraction of essential oil

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