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LARVICIDAL EFFICACY OF THE SYNERGISTIC COMBINATION OF *Allium sativum* AND *Cymbopogon citratus* AGAINST *Aedes* SPECIES LARVAE

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ABSTRACT

The *Aedes* mosquitoes are important vectors of Zika, chikungunya, yellow fever, and Ross River arboviral diseases. The challenges caused by the over-reliance on chemical insecticides in managing the vectors and as well the diseases they transmit have led to increased emphasis on developing more effective and environmentally friendly alternatives. This study investigated the phytochemicals present in *Allium sativum* and *Cymbopogon citratus* mixed together in a ratio of 1:1 and the larvicidal efficacy of their synergistic combination against *Aedes* mosquito larvae. The phytochemistry of the extracts were determined qualitatively. The test was conducted using different concentrations of 500ppm, 750ppm and 1000ppm, of the larvicidal mixture and mortalities recorded at 24, 48 and 72 hour exposure time. Data were analysed using Analysis of variance and probit analysis. Carbohydrates, tannin, flavonoid, alkaloid, and steroid phytochemicals were present in the mixture. Larval mortality was greater than 80% in all test concentrations at the 24, 48 and 72 hour exposure time. Larval mortality at the different concentrations, did not vary significantly. There was no significant variation ($p>0.05$) in the mortality of the larvae at different concentrations of the extracts; 500ppm, 750ppm, and 1000ppm as well as the different exposure time ($p>0.05$). However, a highly significant variation ($p<0.01$) was observed in the mortality of the larvae exposed at different concentrations throughout the 72 hour period. The calculated LC¬¬50 and LC90 at 72 hours were 123.79ppm and 459.09ppm respectively. The combined extracts of both plant materials showed very high larval potency against the *Aedes* mosquitoes. This mixture should be considered as possible supplementary or substitute for the control of *Aedes* sp. larvae.

Keywords: *Aedes, Allium sativum, Cymbopogon citritus, Phytochemicals, Synergistic combination*

INTRODUCTION

Aedes mosquitoes have a worldwide distribution, acting as a major pest of humans and livestock (Service, 2012). They transmit viruses such as dengue, Zika, chikungunya, yellow fever, and Ross River (Charlier *et al*., 2017). Generally, outbreaks of these diseases are rife. An estimate of over 2.5 billion persons are in danger of getting infected with Chikungunya, Dengue and Zika arthropod-borne viral diseases transmitted by *Aedes aegypti* in urban dwellings and environs annually (Guzman *et al*., 2010; Bhatt *et al*., 2013). Sub-Saharan Africa accounts for more than 90% of cases of yellow fever virus (Tomori, 2004: Service, 2012), with as estimated 51,000 to 380,000 severe cases and 19,000 to 180,000 fatalities per year (Garske *et al*., 2014; Kraemer *et al*., 2015). Although there is presently the availability of established vaccines for use in the prevention and control of the yellow fever virus (Service, 2012; Barrett, 2017), there are none for extensive use against dengue, zika, chikungunya, (Laughlin *et al*., 2012; Rather *et al*., 2017) and the Ross River viruses (Wressnigg *et al*., 2015).

Insecticide-based vector control methods are used nearly entirely in interventions meant to manage arboviral infections (Thomas, 2018). The classes of insecticides commonly used are pyrethroids, organochlorides, organophosphates, and carbamates, which all impair the normal functioning of the nerves of insects (Braga and Valle, 2007; Oliveira *et al*. 2017). However, overdependence on them and their unguarded use led to the development of insecticide resistance in mosquitoes (Araujo *et al*., 2015) and confer a negative impact on both humans and their environment (Essumang, 2015; Oliveira *et al*., 2017; Hogarh *et al*., 2018). In the event of this, exploring the floral biodiversity and the field of employing safer insecticides of botanical origin as a straightforward and sustainable means of mosquito control is one of the most effective alternative approaches in vector management (Ghosh *et al*., 2012). Plants are a rich source of bioactive organic compounds, which have several advantages over synthetic pesticides in terms of toxicity, susceptibility to resistance development, and ease of biodegradation (Nath *et al*., 2006).

Allium sativum (garlic) and *Cymbopogon citratus* (lemon grass) are plants commonly known and used for their medicinal values in many countries of the world including Nigeria (Odugbemi and Akinsulire, 2006). Previous studies on their phytochemical extracts at different concentrations have shown some insecticidal activities against larvae of mosquito vector species (Ebe *et al*., 2015; Muhammad *et al*., 2019; Sitorus *et al.,* 2020; Aminu *et al*., 2021; Castillo-Morales *et al*., 2021;). Synergistic combinations of two or more compounds can reduce the adverse effects brought on by high dosages of a single agent, access context-specific multitarget mechanisms, and reduce the chance of resistance development (Lehar *et al*., 2009; Youssefi *et al*., 2019). Even when studies on synergistic combinations of plants against vector species exist (Indhumathi *et al*., 2019; Mahanta and Khanikor, 2021), there is hardly any report on the combination of *A. sativum* and *C. citratus* against *Aedes* mosquitoes. Therefore, the aim of this study was to ascertain the efficacy of the synergistic combination of *A. sativum* and *C. citratus* extracts as possible larvicide against the larvae of *Aedes* mosquito species.

MATERIALS AND METHOD

Collection, Identification and Preparation of Plant Extracts

Fresh garlic (*A. sativum*) and lemon grass (*C. citratus*) were purchased at the New Benin market, Benin City, and identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. The plants were rinsed with water, air-dried for about 21 days, and then pulverized to powder and weighed individually, with lemon grass and garlic weighing 200 g and 300 g respectively. They were all macerated with ethanolic solvent

of 500 ml for lemon grass and 700 ml for garlic, stirred continuously for 72 hours. The residue was then separated from the filtrate using filter paper, funnel and conical flask. The filtrate was concentrated using a water bath to produce crude extract of the plant material. Both plant extracts were preserved in a refrigerator until they were needed for bioassay.

Qualitative Screening of Phytochemicals

The phytochemical constituents of the combined extracts of *A. sativum* and *C. citratus* were analysed according to Keay *et al.* (1964) and Ejikeme *et al*. (2014). Constituents determined were carbohydrates, tannins, saponins, alkaloids, flavonoids and steroids.

Collection of Mosquito Larvae

A colony of the mosquito species was reared in the Department of Animal and Environmental Biology, University of Benin according to WHO (1975). Larvae of *Aedes* species were collected in various breeding sites around Ugbowo Campus of the University of Benin. Mosquito larvae collected from the field were kept in plastic bowls of water wherein they were fed with yeast. Pupae were then reared to adults within the plastic bowls kept inside 0.4m x 0.4m x 0.4m (L x B x H) mosquito-rearing cages. Emerging adults were first fed with sugar solutions and then later with blood meals to aid egg production. The bioassay was conducted using successive generations of larvae.

Preparation of Stock solution of the Extracts

The stock solution was prepared according to standard WHO (2005) procedure with minor modifications. Both plant extracts of *A. sativum* and *C. citratus* weighing 2.5 g each were mixed in a ratio 1:1. The resulting 5 g of the combined plant extracts were dissolved in 100 ml of water to prepare a 5% stock solution.

Larvicidal Bioassay

From the prepared stock solution, 500, 750, 1000 ppm of the test concentrations were made up by adding 5, 7.5 and 10 ml of stock solution to each round plastic container and diluted to 100 ml by adding 95, 92.5 and 90 ml of water respectively. The control (0 ppm) was made up of only water (100 ml) without any added extract. Each test solution containing each test concentration was made into triplicates. Ten (10) individuals of *Aedes* sp in their second and third larval instar developmental stage were introduced into each test bowl with the defined concentrations and the control for 72 hours. Mortality in the test bowls was observed and recorded at 24, 48 and 72 hour post-exposure intervals. Larvae were recorded as dead if they stayed still at the bottom when triggered by external disturbance. No food materials were added to the treatment groups during the exposure periods.

Statistical Analysis

One-way factorial Analysis of Variance (ANOVA) was used to analyze difference in the mortality data and; ensuing significant differences were further evaluated using Duncan's Multiple Range test (DMR). Significance in comparison was set at $p<0.05$. Mortality data obtained were also subjected to probit analysis to obtain the lethal concentrations (LC50 and LC90) values of the combined extracts against the *Aedes* sp. larvae at 95% confidence limits. Analysis of data was done using Microsoft Excel 2010 and Statistical Package for Social Scientists (SPSS 16.0) accordingly.

RESULTS

Phyto-constituents of combined ethanolic Extracts of *A. sativum* and *C. citratus*

Result of phytochemical screening of combined ethanolic extracts of *A. sativum* and *C. citratus* revealed the obvious presence of carbohydrate, tannin, flavonoid, alkaloid and steroid and the absence of saponin (Table 1).

Effect of Time and Concentration on Larval Mortality

There was no significant difference ($p>0.05$) in mortality of the *Aedes* larvae exposed to each of the different concentrations; 500 ppm, 750 ppm and 1000 ppm of the extracts. Variation in the mortality of the mosquitoes exposed at the different times of exposures being 24, 48 and 72 hours across the different concentrations was also not significant ($p>0.05$).

The result of the analysis of the entire mortality data revealed a highly significant variation in the mortality of the exposed mosquitoes at 500 ppm, 750 ppm and 1000 ppm throughout the 72-hour period ($p<0.01$). The highest mortalities were recorded by the *Aedes* mosquitoes exposed to the 750 ppm and 1000 ppm test concentrations and the lowest by those exposed to the 500 ppm concentration of the extracts. However, mortalities in the test organisms didn't vary significantly to time of exposure ($p>0.05$) (Table 2).

The concentration of the combined *A. sativum* and *C. citratus* extracts that would cause 50% and 90% mortality of *Aedes* larvae were calculated respectively as 236.30 ppm and 602.59 ppm at 24 hours, 210.55 ppm and 528.24 ppm at 48 hours and 123.79 ppm and 459.09 ppm at 72 hours (Table 3).

Table 1: Qualitative phytochemical constituents of the combined ethanol extract of *Allium sativum* and *Cymbopogon citratus*

Phytochemicals					
Carbohydrate	**Saponin**	**Tannin**	**Flavonoid**	**Alkaloid**	**Steroid**
+++	-	+++	+++	+++	+++

Key: +++ extremely present, ++ slightly present, - absent

Table 2: Effect of concentration of combined *Allium sativum* and *Cymbopogon citratus* against *Aedes* sp.

Plant type	Conc. (ppm)	n	Mean ± SD (Percentage Mortality %)			F-value	P-value
			24 hours	48 hours	72 hours		
Combined *A. sativum* and *C. citratus*	0	3	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	-	-
	500	3	8.00±1.00 (80.00)	8.33±1.15 (83.33)	8.67±1.53 (86.67)	0.21	0.81
	750	3	9.67±0.58 (96.67)	9.67±0.58 (96.67)	10.00±0.00 (100.00)	0.50	0.63
	1000	3	9.67±0.58 (96.67)	10.00±0.00 (100.00)	10.00±0.00 (100.00)	1.00	0.42
	F-value		5.00	4.20	2.29		
	P-value		0.05	0.07	0.18		

Table 3: Lethal concentrations of combined *Allium sativum* and *Cymbopogon citratus* extract against *Aedes* Larvae

		Lethal Concentration (ppm)
24 Hours	LC_{50}	236.30
	LC_{90}	602.59
48 Hours	LC_{50}	210.55
	LC_{90}	528.24
72 Hours	LC_{50}	123.79
	LC_{90}	459.09

DISCUSSION

The phytochemicals identified in the combined extracts of *A. sativum* and *C. citratus* in this study were all identified in significant amounts except saponin which was absent. Gupta *et al*. (2019) confirmed the presence of phytochemicals detected in this study in the ethyl acetate extracts of *C. citratus*. In another report (Efiong *et al*., 2020), only steroids, cardiac glycosides, alkaloids, and saponins were present in oil extracts of *A. sativum* while flavonoids, phenols, tannins, and triterpenoids were absent. Phytochemicals in plants function originally as defense mechanisms in the face of attack by herbivores (War *et al*., 2012).

The phytoconstituents recorded in this study including tannin, flavonoid, alkaloid, and steroid all can cause harm to herbivores including insects. Plant alkaloids have been reported to cause significant loss in fecundity in adult species of mosquitoes (Saxena *et al*., 1993). Tannins have a substantial negative impact on phytophagous insects, causing midgut lesions, reducing the efficiency of nutrition absorption, and affecting insect growth and development through binding to proteins (Sharma and Agarwal, 1983; Sharma *et al*., 2009; Barbehenn and Peter Constabel, 2011). By affecting the behaviour, growth, and development of insects, flavonoids and their subclass group isoflavonoids both protect plants from insect pests (Simmonds, 2003). Feeding on plants that contain phytoecdysteroid compounds by phytophagous insects causes them to molt immediately and experience metabolic breakdown which leads to their eventual death (Das *et al*., 2021).

Lethal effect of the combined extracts of this study (*A. sativum* and *C. citratus*) was observed on the *Aedes* larvae after the 24-hour period of exposure in all the test concentrations. Larval mortality was high, ranging from 80.00% to 100.00%. Indhumathi *et al*. (2019) reported a remarkable larvicidal activity of combined formulations of *A. sativum, Andropraphis paniculata* and *Ruta gravelons* against *A. aegypti* compared to individual exposures. They reported 100% mortality for the extracts combined in the ratios 1:1:1, 1:4:10 and 3:2:1 and the lowest were in the combined ratio of 1:10:4 (33.4%). Of the 155 combinations of different volume ratios of essential oils to determine their mosquitocidal activity against *Culex quinquefasciatus* by Mahanta and Khanikor (2021), 1:1 ratio of mixed *A. sativum* (bulbs) L. and *Citrus paradisi* (peels) Macid was found to be the most potent against the adults and combination of *A. sativum* (bulbs) and *C. paradisi* (leaves) was found to have the most activity against the larvae with respect to their dose and synergistic interaction.

CONCLUSION

The findings of this study have shown a potent synergistic combination potential of ethanolic extracts of *A. sativum* and *C. citratus* against *Aedes* mosquito larvae. Although larval mortalities showed no clear dose dependence at the

different exposure times, the high larval mortality rates recorded at 24 hours in all the test concentrations till the end of the study are noteworthy. Due consideration should be given to this mixture as a possible future supplementary or substitute larval control tool for *Aedes* mosquito vectors.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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