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The Synergistic Larvicidal Activities of Three Local Plants on *Aedes aegypti*

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Abstract- The synergistic activity of extracts of *Lantana camara*, *Stachytarpheta indica* and *Allamanda blanchetii* against *Aedes aegypti* mosquito larvae were investigated. Ethanolic leaf extracts of these plants were tested separately and combined on *Ae. aegypti* larvae with concentration ranging from 5, 10, 20, 30 and 40mg/ml. In 72 hours bioassay experiment, mortalities were observed at the different time intervals but were highest at 40mg/ml concentration for the three plants independently, with the *L. camara* extract showing better larvicidal activity over the other plant extracts at 48 hours and 72 hours exposure. The LC_{50} of *L. camara* (6.08mg/ml), *S. indica* (8.15mg/ml) and *A. blanchetii* (6.44g/ml) indicates their ability to cause 50% larval mortality at such low concentrations. For the synergistic effects, all the concentrations exhibited high mortality at 48 hours and 72 hours exposure. The 40mg/ml showed the highest larvicidal activity (100% mortality) after 48 hours exposure with the L2:A1:S1 combination. Synergistic factor (S.F.) of 1.00, 1.27 and 0.94 were obtained for *L. camara*, *S. indica* and *A. blanchetii*. Synergism was recorded for *L. camara* and *S. indica*, while antagonism was recorded for *A. blanchetii*.

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Keywords: extracts, ethanol, synergism, mosquito, control, aedes.

I. Introduction

Mosquitoes are well known as annoying biting pests and vectors of disease-causing agents to humans and other animals. Mosquito alone transmit diseases to more than 700 million people annually (Someshwar *et al*, 2011). Though mosquito – borne diseases currently represent a greater health problem in tropical and subtropical regions, no part of the world is immune to this risk (Fradin and Day, 2002; Taubes, 1997). *Aedes* mosquitoes are widely distributed in Africa and can serve as vectors of yellow fever and dengue virus. When their distribution is combined with rapid population growth, unplanned urbanization, and increased international travel, extensive transmission of these diseases is more. Many African cities now have an increasing number of overcrowded, informal settlements, or 'shanty towns', characterized by low-grade housing, poor roads, inadequate water supplies, sanitation and waste management services and most people who live here have no access to running water and store drinking water in containers which often serve as breeding sites for *Aedes aegypti*, the primary vector of urban yellow fever. In addition, the lack of public sanitation services in many large cities prevent the removal of other artificial breeding sites such as metal cans, tires or derelict vehicles. According to the World Health Organization, there are currently 200,000 worldwide cases and 30,000 deaths from yellow fever per year, 90% of them in Africa, and as many as 50 million cases of dengue (WHO, 2009). The worldwide threat of arthropod transmitted diseases, with their associated morbidity and mortality underscores the need for effective control measures. Over the past decade, phytochemicals have been given progressively more attention as insecticidal alternatives. However, most studies on the synergistic and additive toxic effects of mixtures involving phytochemicals have been conducted on agricultural pests rather than vectors of diseases (Essam *et al*, 2005). Combined effect or synergistic effect of various control agents have proved very advantageous in the control of various pests (Caraballo, 2000; Pathak and Shukla, 1998). In this regard therefore, there is a need to also study vector control of various human diseases using a combination of botanicals, this will checkmate the menace posed by pests of public health importance. This work however tries to investigate the individual and synergistic larvicidal effects of *Lantana camara, Stachytarpheta indica and Allamanda blanchetii* on *Aedes aegypti* mosquito and to determine the active compounds that confer mosquitocidal properties on these plants and also to establish the use of these plants as alternative in the control of *Aedes aegyti* mosquito.

II. Materials and Methods

a) Plant collection

The plant materials used in this study are the fully developed leaves of *Lantana camara (Verbenaceae)*, *Stachytarpheta indica (Verbenaceae)* and *Allamanda blachetii (Apocynaceae)*. The mature leaves of *Lantana camara* and *Stachytarpheta indica* were locally collected in and around Ifakala Community in Mbaitoli Local Government Area of Imo State, South-

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East Nigeria, while the mature leaves of *Allamanda blanchetii* were collected from Amata Community in Ikeduru Local Government Area of Imo State, South-East Nigeria. The plants were identified by Dr. C. M. Duru, a botanist in the department of Biology, Federal University of Technology Owerri, Imo State, Nigeria and was later authenticated by a technologist in the department of Forestry and Wildlife Technology, Federal University of Technology Owerri, Imo State, Nigeria.

b) Sample preparation and extraction

The leaves of the plant specimens were washed with tap water and shade-dried at room temperature between 25-30°C for two weeks. The dried leaves were ground into fine powder from which the extracts were prepared. Ethanol extracts of the plants were obtained by taking 100g of the powdered-dried leaves in a container and 500ml of ethanol was added and kept for twenty-four hours with periodic shaking, then filtered and the filtrates were collected. The ethanol extracts were concentrated by rotary vacuum evaporator at 40°C and evaporated to dryness and stored at 4°C in air – tight bottle until use (Dong *et al*, 2005).

c) Test insect

The test insect used in this study is mosquito of the species *Aedes aegypti*. The eggs of *Ae. aegypti* were collected from the National Arbovirus and Vector Research Centre Enugu (NAVRC), Enugu State, South-East Nigeria. *Ae. aegypti* was obtained as egg colony on a white piece of cloth and reared in white basins containing tap water and maintained at 27±2°C. When the eggs hatched into first (1^{st}) instar larvae after two days, they were fed with yeast powder and biscuit powder in the ratio of 1:3. The larvae were reared until the required fourth (4^{th}) instar larvae emerged on the sixth (6^{th}) day.

d) Larvicidal bioassay

The bioassay were performed at a room temperature of 27±2°C, Relative humidity 70 - 85%, Photoperiod 12:12 (light : dark) and pH 7.0 of distilled water. Bioassays were set up according to a slightly modified version of the standard WHO larval susceptibility test methods (WHO, 1981) under similar conditions used for rearing. The test concentrations used for larvicidal bioassay were 5mg/ml, 10mg/ml, 20mg/ml, 30mg/ml and 40mg/ml. Each of the individual plant extracts were weighed according to required concentration and dissolved in 2ml of ethanol. 95ml of distilled water was measured with a 100ml measuring cylinder and poured into each of the containers to be used. The test concentrations dissolved with ethanol were introduced into the containers containing 95ml of distilled water. Then ten of 4^{th} instar larvae of the test insect were selected and counted using micropipette and placed into the small bottles and made up to 3ml mark which was introduced into the containers. For the combination of plant extracts, the same procedure was followed with the individual plant extracts weighed according to required amounts and mixed according to the required ratios for all the test concentrations. The larvae 4^{th} instar of *Ae. Aegypti* were subjected to various specified concentrations of the plant extracts *L. camara* (L100%), *A. blanchetii* (A100%) and *S. indica* (S100%) independently and in combinations in the ratios: L1:A1:S1; L2:A1:S1; L1:A2:S1 and L1:A1:S2 respectively. For each of the concentrations, three (3) replicates were maintained. A control was also maintained by adding 2ml of ethanol to 95ml of distilled water and ten (10) fourth (4^{th}) instar larvae in 3ml of distilled water introduced. The larvae were fed with yeast powder and biscuit powder at the ratio of 1:3 on daily basis (sprinkled on the surface of the water surface). The larval mortality were counted and recorded in percentages (%) at 24, 48 and 72 hours intervals. Dead larvae were removed to avoid decomposition. The median lethal concentration LC_{50} was determined using Levenberg-Marquardt algorithm(1964).The synergistic factor (SF) was calculated using the formula: SF = LC_{50} value of individual plant extract / LC_{50} value of plant with assumed synergist (Susan and Vincent, 2005)

e) Phytochemical analysis

i. *Test for Tannins*

About 0.5g of the plant extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 1% ferric chloride was added and observed for brownish-green or blue-black coloration. The presence of Tannins, Flavonoids, Saponins, Alkaloids, Cardiac Glycosides was noted and recorded.

ii. *Test for Flavonoids*

Dilute ammonia (5ml) was added to a portion of an ethanol filtrate of the extract. Concentrated sulphuric acid (1ml) was added and observed for yellow coloration that disappears on standing. The presence or absence of flavonoids was noted and recorded.

iii. *Test for Saponins*

5ml of distilled water was added to 0.5g of the extract in a test tube. The solution was vigorously shaken and observed for a stable persistent froth. The presence or absence of saponins was noted and recorded.

iv. *Test for Alkaloids*

1ml of plant extract was shaken with 5ml of 2% HCL and heated on a steam bath and filtered. 1ml of the filtrate was treated with o.5ml of Wagners reagent and observed for a reddish-brown precipitate. The presence or absence of alkaloids was noted and recorded.

v. *Test for Cardiac Glycosides*

1ml of lead sub-acetate was added to 2ml of plant extract, shaken and filtered. The filtrate was extracted in an equal volume of chloroform. The

chloroform layer was evaporated to dryness in a dish over water bath. The residue was dissolved in 3ml of 3.5% ferric chloride in glacial acetic acid and left to stand for 1 minute. 1ml of concentrated H_2SO_4 was run down the side of test tube and observed for a blue colour at the interface which is a positive test for deoxy-sugars (Evans, 2002). The presence or absence of cardiac glycosides was noted and recorded.

f) Statistical analysis

Data were analyzed using appropriate software (Microsoft Excel was used for calculating means and standard deviations and performing of analysis of variance (ANOVA). Table Curve 2D Systat, USA was used for mathematical modeling of data). The parameters were estimated by iterative minimization of least squares using Levenberg-Marquardt algorithm (Marquardt, 1964).

III. Results

The present study showed that the extract of the leaf of *Lantana camara Stachytarpheta indica* and *Allamanda blanchetii* expressed larvicidal activity against the 4th instar larvae of *Aedes aegypti*. The mosquito larvae exposed to the plant extracts showed significant changes in behavior. The most pronounced change in behavior observed in the *Ae. aegypti* larvae was the inability to stay at the surface of the water and also showed restlessness which finally resulted to death. These changes may be attributed to the presence of toxic compounds present in the plants. There was no change in behavior observed in the control.

Table 1 : Mean larval mortality of ethanolic leaf extracts of *Lantana camara, Allamanda blanchetii and Stachytarpheta indica* on the 4thstage larvae of *Ae. Aegypti*

Plant	Concentration (mg/ml)	Mean larval mortality (%) Time Interval (hours)		
		24	48	72
Control(with no extract)	0	0	0(0)	1(10)
Lantana camara	5	0(0)	1(10)	2(20)
	10	7(70)	8(80)	9(90)
	20	9(90)	10(100)	10(100)
	30	9(90)	10(100)	10(100)
	40	10(100)	10(100)	10(100)
Starchytarpheta indica	5	0(0)	1(10)	2(20)
	10	1(10)	3(30)	6(60)
	20	4(40)	7(70)	7(70)
	30	5(50)	9(90)	9(90)
	40	7(70)	10(100)	10(100)
Allamanda blanchetii	5	1(10)	3(30)	4(40)
	10	2(20)	7(70)	8(80)
	20	5(50)	8(80)	9(90)
	30	8(80)	9(90)	10(100)
	40	9(90)	10(100)	10(100)

Table 1 represented the dose dependent larvicidal effects of ethanolic leaf extracts of *L. camara*, *S. indica* and *A. blanchetii* on the larvae of *Ae aegypti* at 72 hours exposure. The table showed that the leaf extracts of *L. camara* recorded the highest percentage mortality at 48 hours and 72 hours of exposure followed by *A. blanchetii* and then *S. indica*. The result also showed that the 40mg/ml concentration recorded the highest percentage mortality on *Aedes aegypti* larvae at the various time intervals.

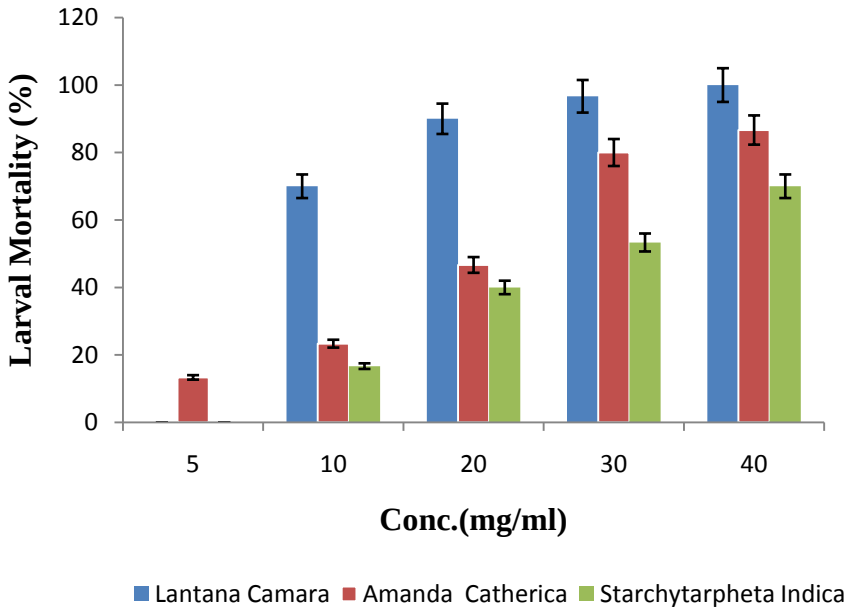

Figure 1 : Comparison of Mean larval mortality(%) of the ethanolic extract of the three plants: *Lanata camara*; *Allamanda blanchetii* and *Starchytarpheta indica* at different concentrations (mg/ml) against *Ae. Aegypti* 4th instar larvae

Table 2 : Mean Larval Mortality of combinations of ethanolic leaf extracts of *Lantana camara, Allamanda blanchetii and Stachytarpheta indica*_on 4th stage Larvae of *Ae. Aegypti*

Concentratiomg/ml	Mean larval mortality (%)		
	Time interval(hour)		
	24	48	72
Control(with no extract)	0(0)	0(0)	1(10)
5			
L1 : A1 : S1	3(30)	3(30)	5(50)
L2 : A1 : S1	4(40)	9(90)	10(100)
L1 : A2 : S1	4(40)	7(70)	8(80)
L1 : A1 : S2	3(30)	6(60)	7(70)
10			
L1 : A1 : S1	6(60)	8(80)	9(90)
L2 : A1 : S1	6(60)	9(90)	10(100)
L1 : A2 : S1	6(60)	8(80)	9(90)
L1 : A1 : S2	5(50)	7(70)	9(90)
20			
L1 : A1 : S1	7(70)	9(90)	10(100)
L2 : A1 : S1	8(80)	9(90)	10(100)
L1 : A2 : S1	7(70)	8(80)	9(90)
L1 : A1 : S2	6(60)	8(80)	9(90)
30			
L1 : A1 : S1	9(90)	9(90)	10(100)
L2 : A1 : S1	9(90)	10(100)	10(100)
L1 : A2 : S1	8(80)	9(90)	9(90)
L1 : A1 : S2	7(70)	8(80)	9(90)
40			
L1 : A1 : S1	9(90)	10(100)	10(100)
L2 : A1 : S1	9(90)	10(100)	10(100)
L1 : A2 : S1	8(80)	10(100)	10(100)
L1 : A1 : S2	7(70)	10(100)	10(100)

L = Lantana camara; A = Allamanda blanchetii; S = Stachytarpheta indica

Table 2 represented the dose dependent larvicidal effects of combination of the ethanolic leaf extract of *L. camara*, *A. blachetii* and *S. indica* on the larvae of *Ae. aegypti* at 72 hours exposure. The combination of the plant extracts at different test concentrations showed high percentage mortality even at low concentration at 48 and 72 hours exposure. The table also showed that the 40mg/ml concentration recorded the highest percentage mortality at 72 hours exposure while the L2:A1:S1 combination recorded the highest percentage mortality over the other combinations at 48 hours exposure. The high larvicidal activity showed by the ratio L2:A1:S1 gives rise to the *L. camara* plant extract being considered as the synergist in this present study.

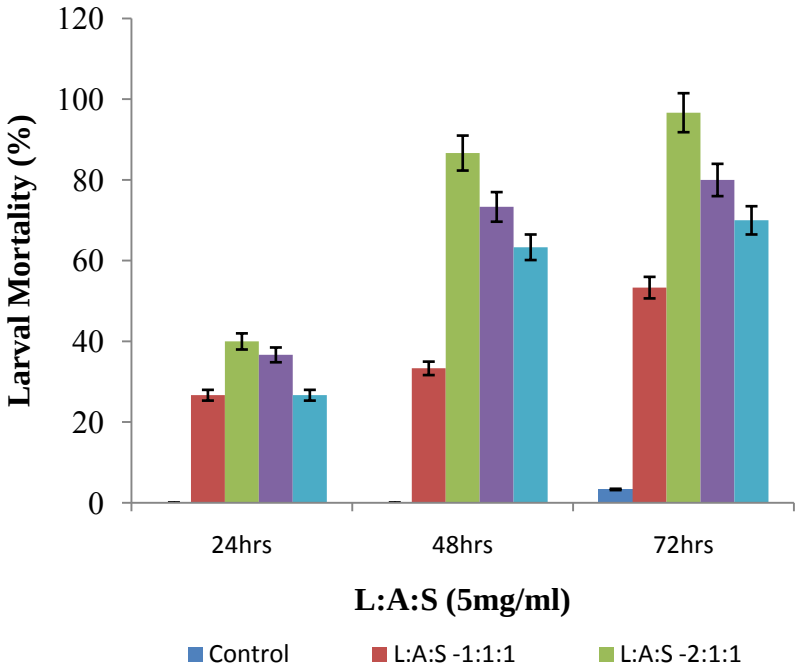

Figure 2 : Mean mortality (%) of the 5mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* 4th instar larvae

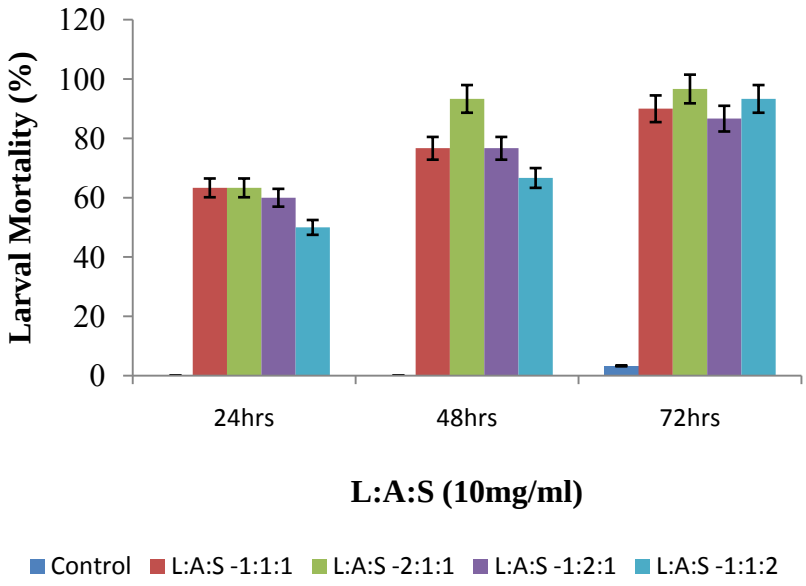

Figure 3 : Mean mortality (%) of the 10mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* '4th instar larvae

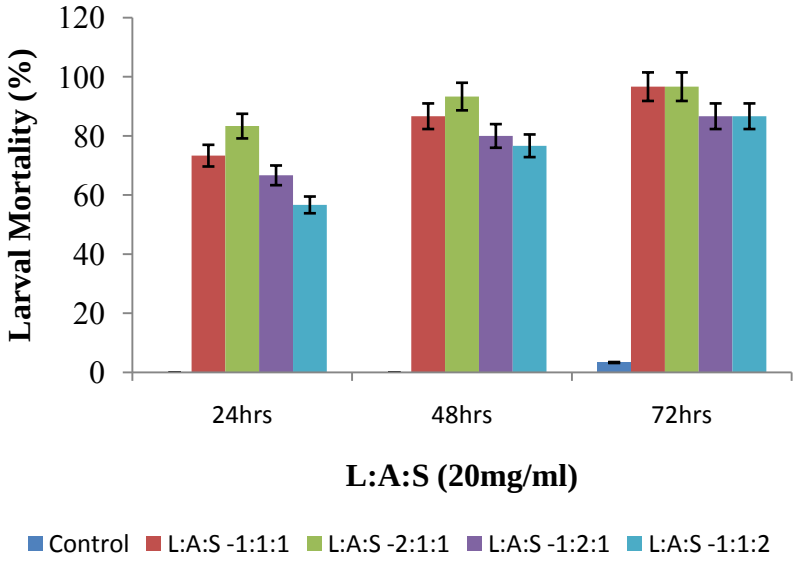

Figure 4 : Mean mortality (%) of the 20mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* '4th instar larvae

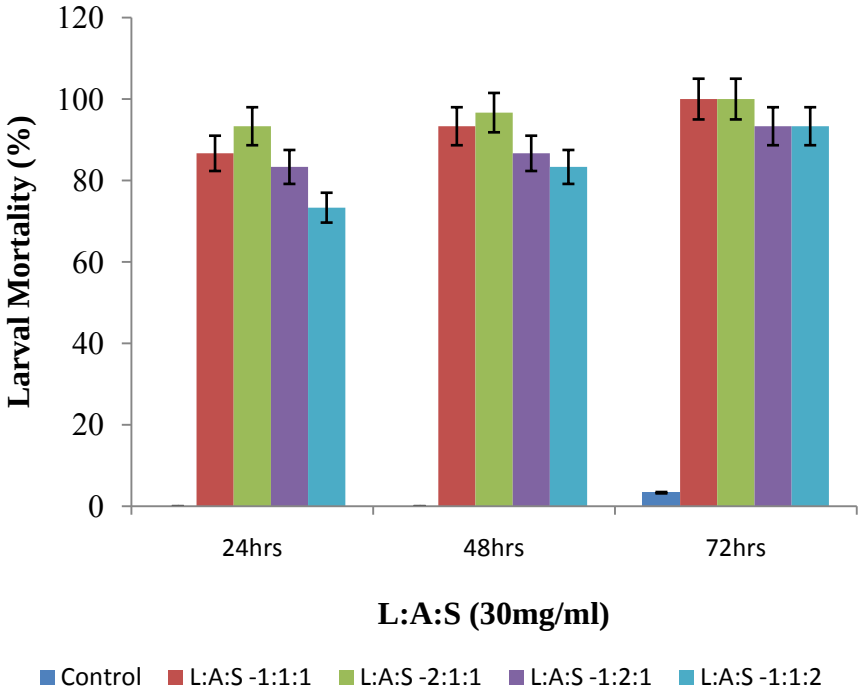

Figure 5 : Mean mortality (%) of the 30mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* 4th instar larvae

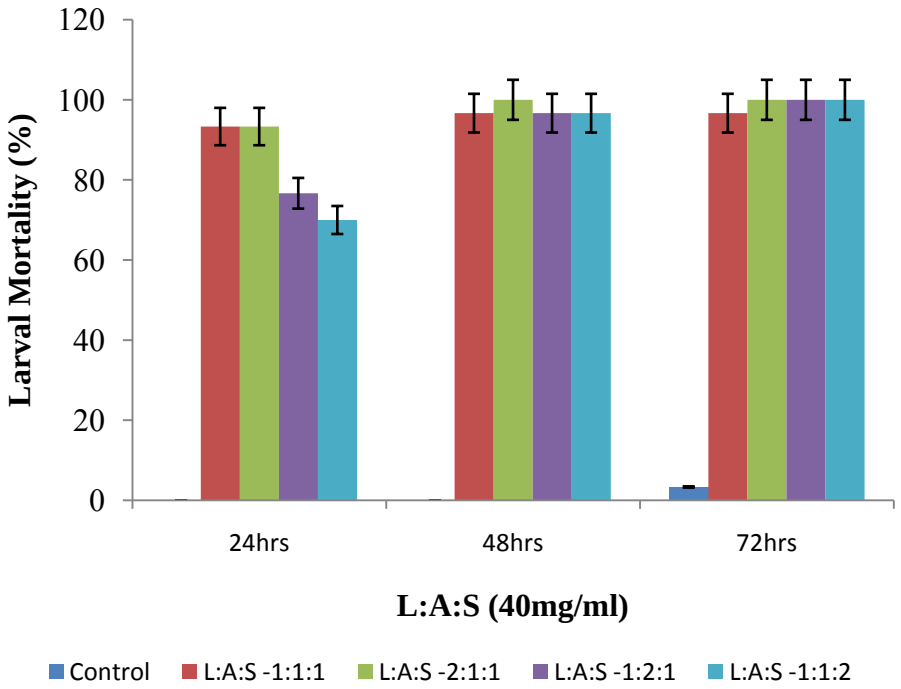

Figure 6 : Mean mortality (%) of the 40mg/ml concentration combinations of the ethanolic leaf extract *of Lanata camara*, *Allamanda blanchetii* and *Starchytarpheta indica* at different time interval s against *Ae. aegypti* '4th instar larvae

Table 3 : Synergistic factor of ethanolic leaf extracts of *L. camara*, *S. indica* and *A. blanchetii* against the 4th larvae of *Ae. Aegypti*

Plant	Synergistic factor	Synergism	Antagonism
Lantana camara	1.00	*	
Stachytarpheta indica	1.27	*	
Allamanda blanchetii	0.94		**

** = synergism; ** = antagonism; ≥ 1 = synergism; ≤ 1= antagonism*

Synergism was observed between *L. camara* and *S. indica* while antagonism was noted between *L. camara* and *A. blanchetii* (Table 3).

Table 4 : Phytochemical analysis of leaf extracts of *L. camara*, *S. indica* and *A. blanchetii*

Phytochemicals	Plant		
	L. camara	*S. indica*	*A. blanchetii*
Flavonoid	+	+	-
Tannin	+	+	+
Alkaloid	+	+	-
Saponin	+	+	+
Cardiac glycosides	+	+	+

+ = present, - = absent

The phytochemicals present in the extracts of *L. camara*, A. *blachetii* and *S. indica* are represented in Table 4. The result showed that tannin, saponin and cardiac glycosides are present in all the three plants while flavonoid and alkaloid are present only in *L. camara* and *S. indica* and absent in *A. blanchetii*.

IV. Discussions

The ethanolic leaf extracts of *Lantana camara*, *Starchytarpheta indica* and *Allamanda blanchetii* were found to exhibit larvicidal activities individually and in combination indicating the mosquitocidal potentials of the leaves of these plants. The result of this study shows that mortalities increased with concentration ($P \leq 0.05$), this confirms the report of (Pelah *et al*, 2005) and (Mehra and Hiradhar, 2000) that there is a positive correlation between concentration and the percentage of the larval mortality.

However, on the individual basis, L. *camara* exhibited a higher larvicidal effect than *S. indica* and *A. blachetii* as was observed with their LC_{50}. The LC_{50} values of the three plants show that they can cause 50% larval mortality at 6.08mg/ml, 6.44mg/ml and 8.15mg/ml (which are low concentrations) which makes them preferable to synthetic insecticides. But combined effects or synergistic effects of various control agents have proved very advantageous in the control of various pests (Seyoum *et al*, 2002). Shaalan *et al* (2002) reviewed different mosquito larvicidal plant species with growth retarding, reproduction inhibiting, ovicides,

synergistic, additive and antagonistic action of botanical mixtures. Susan and Vincent (2005) reported the result of the mixture of *Pongamia glabra* and *Annona squamosa* extracts which exhibited synergistic effect against larvae of mosquitoes. A high larval mortality was recorded for the combination of *L. camara*, *S. indica* and *A. blachetii* at all test concentrations indicating synergism between the plant extracts. Antagonism was recorded for *A. blanchetii*. The 40mg/ml recorded the highest larval mortality after 48 hours exposure and the L2: A1: S1 combination was found to be the best combination over other combinations proposing *L. camara* as a good synergist in combination with other plants in the control of *Ae. aegypti* larvae. This combination exhibited relatively higher larval mortality at various concentrations as compared to others. The effects of these plant extracts on larval mortalities could be attributed to the various chemical components observed in these plants. Chiasson *et al* (2001) and Silva *et al* (2008) report that the bioactivity of the essential oil results from interaction among structural components, particularly the major constituents, the other compounds, even trace elements, which can also have a vital function due to coupled effects, additive action between chemical classes and synergy or antagonism. Several of these secondary metabolites are produced by some plants for their own defense from their enemies, and have been found to have good larvicidal activity such as steroids, essential oils, triterpenes, etc. (Chowdhury *et al*, 2008). Wiesman and Chapagain (2006) reported that saponin extracted from the fruit of *Balanites aegyptica* showed 100% larvicidal activity against *Ae. aegypti* mosquito larvae. A commercial saponin mixture extracted from *Q. saponaria* bark showed increasing toxicity (100% larval mortality) in *Ae. aegypti* and *Cx. pipiens* when both saponin concentration and duration of the experiment were increased (Pelah *et al*, 2005) while the apiperidine alkaloid from *Piper logum* fruits was found to be active against *C. pipiens* mosquito larvae (Lee, 2000) and cardiac glycoside exhibited acaricidal effect against larval and adult stages of the camel tick (Al-Rajhy *et al*, 2003). The results of this present study showed that tannin, saponin and cardiac glycosides are present in all the three plants (*L. camara, S. indica* and *A. blanchetii*), while flavonoid and alkaloid were found to be present in *L. camara* and *S. indica* but absent in *A. blanchetii*. The presence of these secondary metabolites may be responsible for the larvicidal activity against *Ae. aegypti* mosquito larvae, thereby proposing their use as control agents against *Ae. aegypti* mosquito. Someshwar *et al* (2011) observed that some secondary metabolites in combination may be responsible for better effect of larvicidal activity. This present study noted such observation and supports the use of the combination of the leaf extracts of *L. camara, S. indica* and *A. blanchetii* as a better control agent against *Ae. aegypti* mosquito. Susan and Vincent (2005) established that *Pongamia* extract acted as a powerful synergist with *A. squamosa* against mosquito larvae. The performance of combined application of *Neem* and *Karanja* oil cake was reported to be better against the mosquito larvae than their individual application (Shadia *et al*, 2007). According to Lokesh *et al* (2010) in their study, the results obtained showed the combination effects of *T. foenum* and *N. oleander* leaf extracts were much effective on mosquito larvae than the individual extracts. Someshwar *et al* (2011), established that 100% mortality of mosquito larvae was recorded when 0.2% crude extracts of *C. caudatus* fruits and *T. acuminate* flower at 1:1 combinations was applied and it was found to be the best. Though, the individual applications of plant extracts are good against mosquito, the synergistic effects is well established (Shadia *et al*, 2007). It has been observed that individual botanical insecticides are slow acting, time consuming, and active only at high concentration which makes them impractical and uneconomical for field application (Mohan *et al*, 2007; Narasimhan *et al*, 1998). However, the importance of proper selection of plant extracts as synergists in mixed formulations with different botanicals is being increasingly recognized in mosquito management (WHO, 1981). So use of these combinations in mosquito control can be of greater use (Lokesh *et al*, 2010).

V. Acknowledgement

Our in-depth gratitude goes to the staff and management of National Arbovirus and Vector Research Institute (NARVC), Enugu, for making their laboratory facility available for this work, especially Mr Emma Nwosu and Mr Goddy Uwakwe for their immense contributions to the success of this work.

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