https://dx.doi.org/10.4314/dujopas.v7i3b.11
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 7 No. 3b September 2021*

Influence of *Allium Sativum* (Garlic), *Zingiber officinale* (Ginger) and *Syzygium aromaticum* (Clove) Extract against Larvae of *Aedes* mosquitoes (*Culicidae*: Diptera)

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Abstract
Mosquito control has become more difficult due to the unsystematic use of synthetic chemical insecticides which have inauspicious effect on the environment. Botanical phyto-chemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvacidal activities. The present

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study assessed the influence of A. sativum (garlic), Z. officinale (ginger) and S. aromatic (clove) extracts against Aedes mosquitoes. Larvacidal activities of three indigenous plant extracts were studied in the range 5.0 to 30.0mg/l in the laboratory bioassay against fourth instar larvae of Aedes sp. The mortality were subjected to probit analysis to determine the lethal concentration (LC_{50}) to kill 50 percent of treated larvae of Aedes sp. Data obtained were analyzed by Comparing experimental groups and control groups with significance level established at $p<0.05$. All plants tasted showed effects after 24hrs of exposure at different concentration (mg/l). However the highest mortality was found in A. Sativum (garlic) and Z. officinale (ginger) against larvae of Aedes mosquitoes with LC_{50} = 42.50% (2.685) and LC_{50} = 30.01% (4.461) mg/l, while S. aromatic(clove) was found to have lowest mortality at LC_{50} = 24.50% (5.52) respectively. It is therefore recommended that A. sativum(Gallic), S. aromatic (Clove) has showed larvacidal effects against Aedes mosquitoes and could be served as alternative form of botanical control against mosquitoes .

Keywords: *A. sativum (garlic), Z. officinale (ginger) and S. aromatic (clove), Extracts, Aedes* mosquitoes

INTRODUCTION

Mosquito control methods mainly rely on the chemical insecticides, but has led to environmental pollution (Barnard, 1999). Mosquito control has become more difficult due to the unsystematic use of synthetic chemical insecticides which have inauspicious effect on the environment (Das *et al*., 1997), The larval stages of mosquitoes can easily be eradicated for control operations because they are less movable in larval forms than the adults (Benelli, 2015). To control or eliminate mosquito population highly efficacious pesticides have been employed (Downe, 1975). These pesticides are threatened due to the developing resistance of mosquitoes against them (Edriss *et al.,* 2012).Therefore, alternative biological mosquitocides are urgently needed. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents (Arsenaul *et al*., 2008). Phytochemical with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal and Adulticidal properties (De Souza *et al*., 2011).

Therefore, alternative biological mosquitocides are urgently needed. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents (Arsenaul *et al*., 2008). The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent (Ferdouse, 2005).

Some of the active compounds of interest are *ocimenone* from *Tagetes minuta* (Maradufu *et al.,* 1998), *rotenone* from *Derris elliptica* (Ameen *et al.,* 1983), *azadirachtin* from *Azadirachta indica* (Schmutterer, 1981), *capillin* from *Artemisia nilagiric* (Banerji *et al.,* 1990), *quassin* from *Quassia amara* (Evans, 1991), *neolignans* from *Piper decurrens* (Chauret *et al.,* 1996).

There have been many attempts to assay the activity of plant extracts against vectors of human disease, in particular through the utilization of plants for which such knowledge exists (Hafeez *et al*., 2011). Plant extracts contain botanical insecticides or phytochemicals that could be used to limit reproduction and survival of various pest species including mosquitoes (Haque *et al.,* 2009). Mosquitoes are of much concern to public health and well-beings of the global human population (James, 2016). Since these mosquitoes transmit a number of dreadful diseases like filarial, malaria, elephantiasis and dengue, control measures using non-conventional insecticides like botanicals and phytochemical derivatives are gaining much attention in recent days due to a number of favorable reasons (Gericke, 2002). the impacts of phytochemicals on mosquito control strategies reveals some encouraging information on insecticides of plant origin have been tested to be specific in action against target insects and are non-toxic on the ecosystem and man as compared to the chemical compounds (Jeyabalan *et al*., 2003).

One can speculate that people controlled and killed mosquitoes and other domestic insect pests by physically removing them or by using plant parts and plant derivative before the advent of synthetic chemicals. Larger number of human Population in this world of about 80% depends largely in plants for its health care control of infectious and non-infectious diseases (Werka *et al.,* 2007) Therefore, plants extracts are gaining acceptance in providing alternative majors in vector control of diseases and arboviruses due to their non-toxicity, specificity, and are safe to the environment and ecosystem and have lesser effects to the flora and fauna (Ormancey *et al* 2008). Therefore in the present study, I have screened three endemic plants *A. Sativum (garlic), Z. officinale (ginger) and S. aromatic (clove)* extracts leaves extract on the larvicidal activities against *Aedes* mosquitoes. The possible result of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive compounds from indigenous endemic medicinal plant source.

MATERIALS AND METHODS

Collection and Rearing of Mosquitoes

Reared mosquito larvae of *Aedes* mosquito were obtained from tidal pole or stagnant water at the Department of Biological sciences, Bayero University Kano, Nigeria.
Larvae of Aedes *sp were* maintained under standard and constant laboratory rearing conditions. Around 100 larvae were reared under relaxed conditions of low larval density (1larva/ 5 ml) in each standard rearing tray with 1L of de-chlorinated water with 50ml of bovine liver or slurry or yeast diet given daily (Lutomiah *et al.,* 2007).

Collection and Identification of Plant Materials

Plants materials such as *A. Sativum (garlic), Z. officinale (ginger) and S. aromatic (clove)* leaves were obtained from Goron-maje village, Dambatta LGA, Kano State. The plants were authenticated by an expert in plant taxonomy at the Department of Plant Biology, Bayero University Kano.

Preparation of Plant Extracts

A. sativum, Z. officinale and S. aromatic were washed in tap water, cut into small pieces and air dried. After the plants were completely dry, they were ground into powder and then macerated in an aqueous solvent at room temperature for 3 days and then filtered. The combined filter was concentrated to dryness by rotary evaporation and kept in a freezer. The prepared test concentration of each plant extract was volumetrically diluted with water.

BIOASSAY

Larvacidal Assay

A stock solution of plant extract was prepared by dissolving 100 mg of extract in 1 ml of distilled water. initial concentration of plant extract (100 mg/ml) were diluted using double fold serial dilution by transferring 1 ml of sterile plant extract (stock solution) to obtain 50 mg/ml concentration (Jidong *et al.,* 2009). The above process will be repeated several times to obtained other dilution 5.0 mg/ml, 12.0 mg/ml, 20.0 mg/ml, 25.0 mg/ml and 30.0mg/ml.

The mortality rate was subjected to probit analysis to determine the lethal concentration (LC50 and LC90) to kill 50 to 90% of the larva and pupae respectively. Determination of LC50 and LC90 values at 90% confident limit was estimated or calculated Using: LC50 = $ABY^{2,}$ (Edillo *et al.*, 2004)
Where, A= highest toxicant concentration in which none of the test organism died, and B = lowest concentration. Determination of immature stages would be determined according to the techniques of (Edillo *et al.*, 2001).

Data Analysis
Data were base on Excel (Microsoft Co.). Comparison between treatment and control groups, were performed using analysis of variance (ANOVA). Probit analysis program was used to determine LC_{50} between concentration and percentage mortality at various concentrations of plant extracts. Abbott's formula was used to analyze the data of mortality obtained through bioassay test and statistical tools (Abbott, 1999).

RESULTS

Effects of plant extract on 4thlarvae of *Aedes sp.*
The activity of plant extract is often attributed to the complex mixture of active compounds. In the preliminary screening, potential larvacidal activity of different plant extracts *A. sativum, Z. officinale and S. aromatic* were noted (table 1& fig.1) with seven (7) replicates against *Aedes* sp and percentage mortality was observed in *A. sativum* 134 (19.14) *Z. offinale* 147 (21.00) and *S. aromatic* 196 (32.42) respectively (Table 1). Data obtained were analyzed by comparing experimental groups and control groups with significance level established at $p<0.05$.

Table 1: Preliminary screenings of different Plant extracts against 4th Instars larvae of *Aedes sp*

Plant material/Extracts	Larvae 4th instars	Total Mortality/Replication	Means ± SD
A. sativum	*Aedes sp*	134(5)	19.14±2.685
Z. officinale	*Aedes sp*	147(5)	21.00±4.461
S. aromatic	*Aedes sp*	196(5)	32.42±5.52

SD= Standard deviation,

LC_{50} (Lethal Concentration at 50%) on *Aedes larvae*
All plants extracts show toxic effects on *Aedes sp* after 24h of exposed at different concentration (mg/l). However the highest mortality was found in *A. sativum and Z. officinale* against larvae of Aedes mosquitoes with LC_{50} = 42.50% (2.685) and LC_{50} = 30.01% (4.461) mg/l respectively (Table.2) while *S. aromantic* was found to have lowest mortality at LC_{50} = 24.50% (5.52).

Table 2: LC_{50} Statistical analysis of different plant with aqueous solvent (Extracts) against larvae of *Aedes sp* (mortality)

Plants Species	Solvent	LC_{50}±SE (mg/L)
A. sativum (Gallic)	*Aqueous*	30.01±7.10
Z. officinale (Ginger)	*Aqueous*	24.50±11.80
S. aromatic (Clove)	*Aqueous*	42.50±14.61

LC_{50} = Lethal concentration that kills 50% of exposed larvae; Control= Nil mortality; SE= Standard error; Comparing experimental and control group, with a significant level established at $P<0.05$.

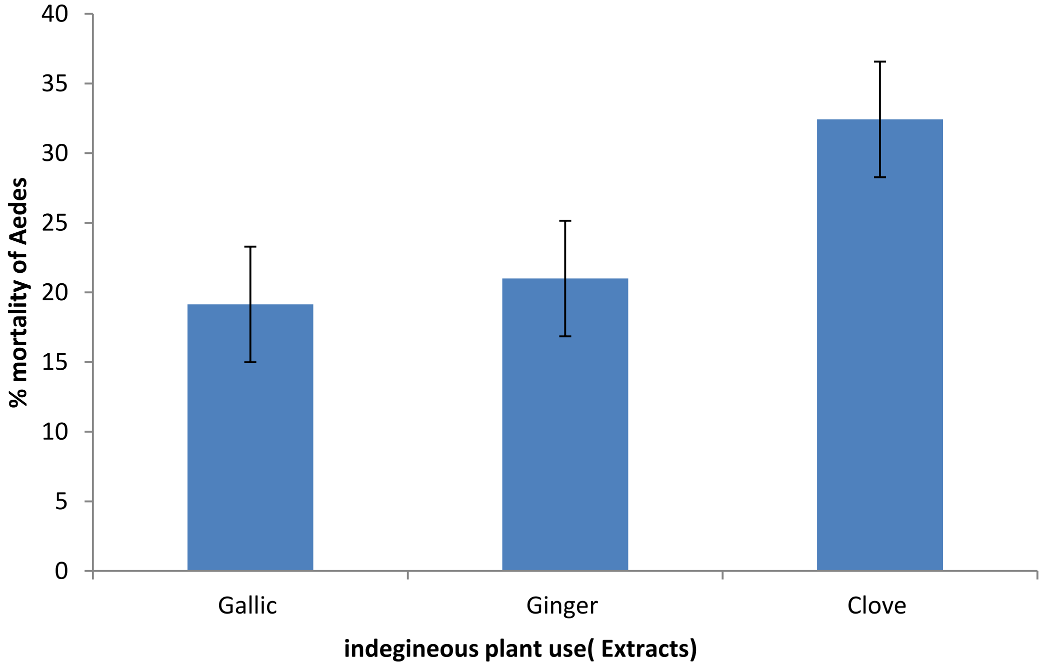

Fig. 1. Effects of plants extract on perncetage mortality of *Aedes sp.*

Table 3: Effect of plant extracts on different growth stages of the mosquito *Aedes sp.*

Concentration (ppm)	Larval Mortality (%)	Survival to pupae (%)	Adult hatched (%)	Inhibition of Adult Emergency (%)
A. sativum (Gallic)				
5.0ml	18	41	38	45
10.0ml	27	33	18	21
15.0ml	21	18	14	16
20.0ml	22	5	4	4
25.0ml	17	3	1	0.0
30.0ml	24	5	3	0.0
Control	5	91	6	-
Z. officinale (Ginger)				
5.0ml	14	32	26	20
10.0ml	21	12	9	7
15.0ml	14	8	6	4
20.0ml	26	23	18	14
25.0ml	42	19	12	9
30.0ml	25	13	8	6
Control	5	97	45	0.0
S. aromatic (clove)				
5.0ml	34	29	22	28
10.0ml	41	22	14	18
15.0ml	35	11	14	18
20.0ml	42	9	5	6
25.0ml	39	8	3	3
30.0ml	36	0	0	00
Control	0	43	28	00

*Use d the equation Abbot (Abbott, 1993) to the percentage inhibition of treatments, according to those in control (Untreated). With six (6) replicates (larvae/replicate)

Effect of plant extracts on different growth stages of *Aedes mosquitoes*.
Results of laboratory testing for three plants extracts of *A. sativum, Z. officinale and S. aromatic* were screened for larvarcidal activities such as LC_{50} (larvae mortality) and adult survival (%). From the result (Table. 3) compare with control and treatment group *A. sativum* showed highest survival from pupa to adult (41%, 38% and 45%) recorded, followed by S. aromatic with (29%, 22% and 28%) and *Z. officinale* recorded the least activity among all the concentration (32%, 26% and 20%) respectively. With increasing concentration of 5.0m, 12.0m, 20.0m, 25.0m and 30.0m when compare to control group (Table. 3)

DISCUSSION
Plant could be an alternative source for mosquitocidal because they constitute a potential source of bioactive compounds and are general free from harmful effects. In the present study experimental results of solvent extracts of three indigenous plants *A. sativum, Z. officinale and S. aromatic* were accessed and revealed to be toxic to immature stages of *Aedes*

mosquitoes. From the experiment the earlier instars larvae are more susceptible than the late instars (Murugal*et al*. 2012).

Results of laboratory testing for three plants extracts of *A. sativum, Z. officinale and S. aromatic* were screened for larvarcidal activities such as LC_{50} (larvae mortality) and adult survival (%). From the result (Table. 1) compare with control and treatment group *A. sativum* showed highest survival from pupa to adult (41%, 38% and 45%) recorded, followed by S. aromatic with (29%, 22% and 28%) and *Z .officinale* recorded the least activity among all the concentration (32%, 26% and 20%) respectively. With increasing concentration of 5.0m, 12.0m, 20.0m, 25.0m and 30.0m compare to control group (Table. 1). These results are also comparable to the earlier report of Murugal *et al*. (2012) with *Culex and Anopheles sp.*

All plants extracts shows toxic effects on *Aedes sp* after 24h of exposed at different concentration (mg/l). However the highest mortality was found in *A. sativum and Z. officinale* against larvae of Aedes mosquitoes with LC_{50} = 42.50% and LC_{50} = 30.01% respectively (Table.2) while *S. aromantic* was found to have lowest mortality at LC_{50} = 24.50%. Similar report from prathibha *et al*. (2011) with LC_{50} value in 4^{th} instars larvae. LC_{50} = 5.388, LC_{50}= 6.288, and 10.73 respectively.

Inhibition of growth was detected with the extract since all larvae grew to become pupae and subsequently emerged to adult (Table 3). Earlier report (Bangavan *et al.,* 2008) reveals that extracts of *A. aromatic,* showed larvacidal activity against the earlier and fourth instars larvae of *Aedes mosquitos'species*, while Present study showed *A. aromatic* extract has effects on both first third and fourth instar larvae of *Aedes* mosquitoes. Furthermore, results of the present study may contribute to reduction of application of synthetic insecticides, which in turn increase the opportunity for natural control of medically important pest by botanical pesticides or insecticides (Govindarajan, 2008).

CONCLUSION

The results of laboratory testing for three indigenous plants *A. sativum, Z. officinale and S. aromatic* were screened for larvae to adult survival (%) rates with effects of extracts. From the results *A. sativum* showed highest survival from pupa to adult recorded, followed by S. *aromatic* the least activity among all the concentration (5.0m, 12.0m, 20.0m, 25.0m and 30.0m) respectively. In the preliminary screening, potential larvacidal activity of different plant extracts *A. sativum, Z. officinale and S. aromatic* were noted with seven (7) replicates against *Aedes* sp and percentage mortality was observed and recorded among. However the highest mortality was found in *A. sativum and Z. officinale* against larvae of Aedes mosquitoes with LC_{50} = 42.50 and LC_{50} = 30.01 mg/l respectively. While *S. aromantic* was found to have lowest mortality at LC_{50} = 24.50.Data obtained were analyzed by comparing experimental groups and control groups with significance level established at $p<0.05$.

These researches recommended that *A. sativum* (Gallic), *S. aromatic* (Clove) has the shows more larvacidal activity against mosquito's species (*Aedes*, *Culex* and *Anopheles*) and could be served as alternative form of botanical control to insects and pest (insecticides and pesticides). Further studies on the tested plant should include mode of actions under field conditions.

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