LARVICIDAL EFFECTS OF ETHANOL EXTRACTS OF LEAVES AND FRUITS OF *Physalis angulata* L. ON THE LARVAE OF *Anopheles* MOSQUITOES FROM EBONYI STATE, NIGERIA

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ABSTRACT

The larvicidal activities of ethanol extracts of leaves and fruits of Physalis angulata L. on the larvae of Anopheles mosquitoes were investigated. Larval mortalities were recorded within 30 minutes of contact with the plant extracts. Leaf extracts of 5%, 10%, 15% and 20% concentrations caused 61%, 80% 91% and 92% mortalities while the same concentrations of fruit extracts caused 38%, 47%, 72% and 83% mortalities respectively. A combination of leaf and fruit extracts exerted synergistic effects and caused higher mortality rates of 67%, 84%, 91% and 95% mortalities at the same concentrations and time. The results also showed that the larvicidal effects of the leaf, fruit and synergy extracts increased with increase in concentration. Significant result was observed for the synergy with 20% extract causing 95% mortality. Analysis of variance (ANOVA) showed that there was no significant difference (P>0.05) among the leaf, fruit and synergy extracts. Preliminary phytochemical analysis revealed the presence of alkaloid (2.0%), cyanide (0.30%), flavonoid (15.5%), phytate (0.02%), saponin (2.0%) and tannin (0.05%) in the leaf and 0.01%, 0.2%, 13.2%, 0.01%, 1% 0.03% respectively in the fruit.

Keywords: Larvicidal, Effects, *Physalis angulata, Anopheles*, Synergy, Ebonyi State

INTRODUCTION

Many plants contain chemical compounds which are toxic to insects, the presence of such bioactive compounds appear to be a natural endowment (Mehrotra and Aggrawal, 2003). Some of these compounds have been known for a very long time, but few are commercially developed and processed into insecticidal products used for insect pest control (Nathanson, 1993). During the last 25 years, interest in Botanical Insecticides (BIs) has increased as a result of environmental concerns and insect population that are resistant to conventional chemical (Lesile, 2005). Nnamani *et al.* (2008) reported that synthetic insecticides which inhibit or kill insects have become popular targets of environmental conservationists. This is because they are considered to be important sources of man-made pollutants which are detrimental to the non-targeted organisms and thus threatens biodiversity. Their negative impacts on the environment are becoming of global concern; such includes environmental contamination, bioaccumulation and residues in food and feeds among others (Clark, 2000).

Nathanson (1993) reported that insect larvae exposed to cocaine-sprayed, displayed marked behavioral abnormalities, including rearing, tremors and walk-off activities. The result indicated that cocaine toxicity stems from its ability to block re-uptake of octopamine, a key insect neurotransmitter and hormone which regulate movement, behaviour and metabolism. Lesile (2005) affirmed that the advantages of BIs are their potentials ability of degrading rapidly, with less persistence in the environment and reduced risks to non-targeted organisms. He concluded that it may be applied shortly before harvest without leaving excessive residues.

The genus *Physalis* belonging to the nightshade family of Solanaceae, it includes about 120 species with herbaceous characteristics and perennial habits. They are distributed around the temperate and tropical zones of the world. Some of them are used as food as well as in popular medicine (Branch and Silva, 1983).

Phytochemical studies on *P. angulata* revealed that it contains many types of biological active compounds including flavonoids, alkaloids and many different types of plant steroids. *P. angulata* is used in the treatment of hepatitis B, bacterial infections, cancer (leukemia), mycoplasma, mycobacterium infections, dermatitis, psoriasis, skin infections, kidney stone and viral infections (Silva, 2005). Pietro (2000) reported that *P. angulata* steroids showed powerful immune-system support. Cancer studies indicated that extracts of *P. angulata* was capable of killing several types of malignant cells *in vitro* and also promote the reduction in size of cancerous tumors in mice with lymphocytic leukemia (Hwang, 2004).

The preponderance of these plants within the mosaic of the lowland rainforest vegetation zone of South Eastern Nigeria provides an adequate means of their utilization as cheap sources of biological agent for study on the production of Botanical Insecticides (BIs). The aims of this present work were to (i) determine the larvicidal effects of leaf and fruit extracts of *Physalis angulata* on the larvae of *Anopheles* mosquito, (ii) to compare the above effect with their synergy and (iii) to compare the mortality rates of these extracts with that of chemical insecticide (Doom).

MATERIALS AND METHODS

Test Organisms: *Anopheles* mosquito larvae were obtained from a colony initiated from mosquitoes collected indoors from Students' Hostel in Ebonyi State University Abakaliki on December 2007. Mosquitoes were also provided with the opportunity of having blood meal on an albino rat (shaved) that was kept in a cage covered with mosquito net. The colony of larvae was maintained at ambient temperature in plastic bucket, half filled with tap water. Some quantity of pond water was introduced to the colony every week to provide the larvae with microorganisms to feed on.

Preparation of Plant Extracts: The whole plant of *P. angulata* were collected from Ntezi Aba Community in Abakaliki, Ebonyi State and washed. The leaves and fruits were separated and oven dried at 75ºC. The dried leaves and fruits were then ground using pestle and mortar. Ten grams of the pulverized leaves and fruits were introduced into 200ml of ethanol in glass beakers respectively. They were left for 24 hours under room temperature after which it was sieved. The synergy was obtained by mixing 50 ml each of leaf and fruit extracts to make 100 ml.

Bioassays: Mosquito larvae were subjected to different concentrations of extracts (leaf, fruit and synergy) – 1ml, 2ml, 3ml and 4ml -in Petri dishes containing 19ml, 18ml, 17ml and 16ml of water converted to 5%, 10%, 15% and 20% respectively. Three replicates of the above were made. Fifty newly molted fourth instars larvae each were introduced into the concentrations with a syringe from the colony. Percentage larval mortality was recorded after 30 minutes. Phytochemical screening of the leaves and fruits were carried out.

Statistical Analysis: Analysis of variance (ANOVA) for significant difference was done using SPSS version 11.1.

RESULTS AND DISCUSSION

The results of the effects of the various concentrations of extracts on the test organisms were recorded on the table below. Table 1 shows the preliminary phytochemical screening of the leaf and fruit, while table 2 shows the mean standard deviation of the mortalities of leaf, fruits, synergy extracts of *Physalis angulata* and synthetic Doom on the larvae of *Anopheles* mosquito.

Table 1: Percentage composition of phytochemicals in leaf and fruits of *Physalis angulata*

Phytochemicals (%)	Plant Material	
	Leafy	Bark
Alkaloid	2.0	0.0
Cyanide	0.39	0.2
Flavonoid	15.5	13.2
Phytate	0.02	0.01
Saponin	2.0	1.0
Tannin	0.05	0.03

The larvicidal effects of leaf, fruit and synergy extracts of *Physalis angulata* showed that the extracts exerted insecticidal potentials on the test organisms when compared with the synthetic insecticide. Highest mortalities were recorded at 20 % concentrations for all extracts with 92.67 ± 0.30 % mortality for the leaf extracts, 83.33 ± 0.01 % mortality for fruit extract and 95.33 ± 0.02 % mortality for synergy (Table 2). The extracts also interfered with adult emergence, because those that survived could not carry out metabolic activities in order to continue growth. This was in accordance with the work of (Pushpalatha and Muthukrishnan, 1999) who showed that ethyl acetate soluble fractions of *Calophyllum inophyllum* and petroleum ether fractions of *Rhinacanthus nasutus* showed high larvicidal activities, irrespective of the species of mosquitoes tested; the extracts killed 50 % of the treated larvae.

The results also showed that a rise in the concentration of the extracts gave a corresponding rise in the rate of mortality (Table 2).

Table 2: Mean deviation of the mortalities rate of anopheles mosquito larvae exposed to different extracts

Concentration of Extract (%)	Leaf Extract	Fruit Extract	Synergy	Synthetic Doom
5	3.33 ± 0.33	38.00 ± 0.10	61.33 ± 0.04	99.20 ± 0.02
10	85.3 ± 0.09	67.33 ± 0.07	80.67 ± 0.03	100 ± 0.02
15	91.3 ± 0.06	82.67 ± 0.01	91.33 ± 0.02	100 ± .0.20
20	92.67 ± 0.30	83.33 ± 0.01	95.33 ± 0.02	100 ± 0.20

The results also revealed variation in larval mortality with time irrespective of the concentration of botanical extracts. This is because 10 % concentration of leaf extracts caused a higher mortality than 5 % concentration of the extract while 20 % had the highest impact. It showed that the results were dose and exposure duration dependent. This was in agreement with the work of Aliero (2003) which showed that exposure of *Anopheles* mosquito larvae to aqueous extracts of *Azadirachta indica* (neem) seed oil, leaf and bark for 12 hours led to 100, 98 and 48 percent mortalities respectively. In another studies, Shaalan *et al.* (2005) had confirmed that the extract of *Callitris glaucophylla* induced dose dependent mortalities of *Aedes aegypti* larvae and that the extract completely prohibited adult emergence. The result of this study is in line with earlier reports of Jeyabalan *et al.* (2000) and Nnamani *et al.* (2008) that larval mortality was dose dependent with the highest dose of 4 % *Draceana aborae* and *Vitex doniana* plant extracts invoking 98 % mortality.

The extracts affected pupicidal, adulticidal activities and significantly decreased fecundity and longevity of the larvae because those that survived were incapacitated in further developments. Significant result was observed for synergy with 20 % extract causing 95 % mortalities. High mortality was observed for synergy and leaf extracts, with synergy inducing mortality at a dose that is exceptional and worthy of consideration for field trials. Analysis of variance (ANOVA) showed that there was no significant difference (P>0.05) between the extracts of leaf, fruit, synergy and time.

The phytochemical screening showed that a combination of different secondary metabolites were present in the leaves and fruits. This agreed favourably with the report of (Isman, 1997), that natural defense of plants against herbivore consists almost of mixtures of closely related compounds, rather than a single toxicant alone. He further pointed out that Rotenone contains six or more insecticidal isoflavonoids, glycosides and tannins which caused the death of insects.

However, further studies on the stability and phytotoxicity of these bio-active compounds could enhance our knowledge and facilitate its large scale production for commercial use. More research on the mosquito populations must be done to better understand each of the species. Laboratory results are often unreliable when attempting to accurately predict what will occur in natural habitat. Therefore, on-site observational studies on the natural habitats of these organisms are required and more research on the toxicity level of *Physalis angulata* is recommended.

REFERENCES

ALIERO, B. L. (2003). Larvicidal effects of aqueous extracts of *Azadirachta indica* (neem) on the larvae of *Anopheles* mosquito. *African Journal of Biotechnology,* 2(9): 325 – 327.

BRANCH, L. C. and SILVA, M. F. D. (1983). Folk medicine of Alter Chao, Para, Brasil. *Acta Amazonica*, 13: 737.

CLARK, G. (2000). Mosquito vector control and biology in Latin America. *Journal of American Mosquito Control Association,* 16(4): 295 – 312.

HWANG, J. K. (2004). Anticariogenic activity of some tropical medicinal plants against *Streptococcus* mutants. *Fitoterapia,* 75(6): 596 – 598.

ISMAN, M. B. (1997). Neem and other botanical insecticides: Barriers to commercialization. *Phytoparasitica,* 25(4): 339 – 344.

JEYABALAN, D., ARUL, N. and THANGAMATHIN, P. (2002). Studies on effects of *Pelargonium citrosa* leaf extracts on malaria vector, *Anopheles stephensi* Liston. *Journal of Applied Entomology,* 92: 141 – 152.

LESLIE, A. R. (2005). *Handbook of Integrated Pest Management.* Lewis Press, USA.

MEHROTRA, R. S. and AGGARWAL, A. (2003). *Plant Pathology.* Tata McGraw-Hill Publishing, Company, New Delhi, India.

NNAMANI, C. V., OSELEBE, H. O. and OGBONNA, A. N. (2008). Effect of leaf extracts of

Draceana aborea l. and *Vitex doniana* sweet on the larvae of *anopheles* mosquito. Animal Research International, 5(2): 835 – 837.

NATHANSON, J. A. (1993). Cocaine and other botanical insecticides. *Proceeding of the National Academy of Science, 90:* 9645 – 9649.

PIETRO, R. C. (2000). In vitro antimycobacterial activities of *Physalis angulata* L. Phytomedicine, 7(4): 335 – 338.

PUSHPALATHA, E. and MUTHUKRISHNAN, J. (1999). Efficacy of two tropical plant extract for the control of mosquitoes. *Journal of Applied Entomology,* 123(6): 369 – 373.

SHAALAN, E. A., CANYON, D. V., YOUNES, M. W. and MANSOUR, A. (2005). Effects of sub-lethal concentrations of synthetic insecticides and *Callitris glaucophylla* on the development *Aedes aegypti. Journal of Vector Ecology,* 30 (20): 298.

SILVA, M. T. (2005). Studies on antimicrobial activity *in-vitro* of *Physalis angulata* L. (Solanaceae) fractions and physalin B bringing out the importance of assay determination. *International Journal of Pharmacology,* 100(7): 779 – 782.