Biochemical Effects and Larvicidal Activities of Apigenin and Rutin in their Binary Combination (1:5) against Elephantiasis Vector Culex Quinquefasciatus

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Abstract

Larvicidal activity of biologically active compounds i.e. apigenin and rutin extracted from the leaves of *Jatropha gossypifolia* and *Codiaeum variegatum* against larvae of *Culex quinquefasciatus* was studied in their binary combination (1:5). The active compounds i.e. apigenin and rutin extracted through ethyl alcohol from the leaves of euphorbious plants was administered for 24 h, 48 h, 72 h and 96 h to the larvae of *C. quinquefasciatus*. Exposure of larvae at different interval of time for sub-lethal doses (40% and 80% of LC_{50}) of apigenin and rutin in the ratio 1:5, significantly ($p < 0.05$) altered the biochemical levels like total protein, total free amino acid, glycogen and activities of enzymes acetylcholinesterase, acid and alkaline phosphatases in whole body tissue of the larvae. The alteration in all these biochemical parameters were significantly time and dose dependent. It can be used for the management of elephantiasis vector worldwide.

Keywords: Jatropha Gossypifolia, Codiaeum Variegatum, Apigenin, Rutin and Culex Quinquefasciatus

Introduction

Mosquitoes act as vectors of pathogens causing lymphatic elephantiasis, malaria, dengue, yellow fever etc. which affects millions of people around the globe W.H.O. (1984, 1995) and Vatandoost, H.V.M. (2001). About 3492 species of mosquitoes are reported worldwide and more than a hundred of species can transmit various diseases in human and other vertebrates Rueda, L.M. (2008). It is an endemic in 82 countries of the world and recognized as one of six potentially eradicable diseases. Elephantiasis and malaria ranks amongst the world most prevalent tropical infectious diseases. Approx 300-500 million people are infected with malaria annually which finally leads to 1.5-3 million deaths W.H.O. (2000). Lymphatic filariasis is one of the fastest spreading insect-borne disease of human in the tropic, about 30% (394 million) of the global population is at risk and is considered to be endemic in countries like Africa W.H.O. (2006). Elephantiasis cause serious public health issues and economic problem in many tropical and subtropical regions of the world, including India Satti, M.H. and Abdel Nur, O. (1974), El Setouhy, M. and Ramzy, R.M.R. (2003) and Aiah, A.G. et al. (2005). One of the methods to control these diseases is to control the vectors for the interruption of disease transmission. About 20-40% of the outpatients visits clinic and approximately 30% of total hospitalized admissions are due to malaria W.H.O. and UNICEF (2005).

Plant products are eco-friendly and less harmful to the environment and non-target organisms. Biologically active compounds from different plant families have been investigated for new and promising larvicides Ester Innocent Cosam et al. (2008). The top priority in finding new insecticides i.e. Apigenin and Rutin is given, which must be of plant origin and safe for ecosystem. Scientists have found the effectiveness of plant derived secondary compounds which includes saponin Wiseman, Z. and Chapagain, B.P. (2005), steroids Chowdhury, N. et al. (2008), isoflavonoid Joseph, C.C. et al. (2004), essential oil Cavalcanti, E.S.B (2004), alkaloids and tannins Khana, V.G. and Kannabiran, K. (2007) as mosquito larvicides. Plant compounds and their essential oils provide alternative source of mosquito repellents agents Yang, Y.C. et al. (2004).

Materials and Methods

Collection and Maintenance of Experimental Insect

Fully fed adult females of Culicines were collected from the different residential areas Collections were made from human dwellings with the help of an aspirator supplied by W.H.O. and kept in 30 × 30 × 30 cm cages with cotton pads soaked in 10% glucose solution and water containing enamel bowl for egg laying.

Experimental conditions of water determined by the method of APHA/AWWA/WEF (1998) were atmospheric temperature 30.2°±1.6° C, water temperature 27.6°±1.1° C, pH 7.3-7.5, dissolved oxygen 7.6-8.1 mg/L, free CO_2 4.1-5.1 mg/L, bicarbonate alkalinity 103.5-105.0 mg/L.

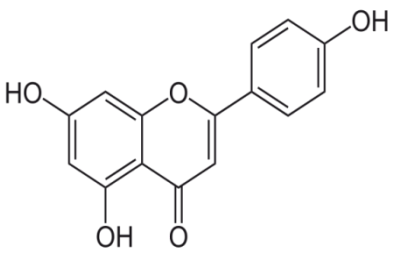
Collection of Plant Material

Plants *Codiaeum variegatum* and *Jatropha gossypifolia* (family: Euphorbiaceae) were collected locally from botanical garden of Deen Dayal Upadhyay Gorakhpur University, Gorakhpur (U.P.), India.

Extraction of Active Compounds

The Apigenin (Figure 1) was isolated from the leaves of *Jatropha gossypifolia* respectively by the method of Subramanian, S.S. et al. (1971). The leaves of these plants were washed properly in tap water and the leaves were cut by scissors then dried in shady place and finally dried in an incubator at about 35° C temperature; dried leaves were powdered by electric Grinder. About 50 g powder of leaves was subjected in Soxhlet extraction unit with about 250-300 mL ethyl alcohol for about 72h at 30-40° C. Confirmation of the compound was also made through IR and Rf values data of *Dordevice* and Mcakic (2000), when compared to the authentic sample obtained from Sigma Chemical Company, USA.

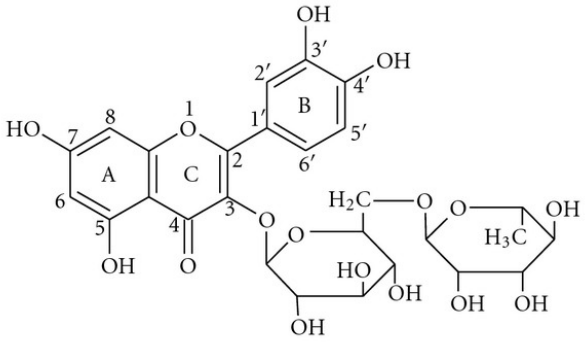
Figure 1: Chemical Structure of Apigenin



The Rutin (Figure 2) was isolated from the leaves of *Codiaeum variegatum* respectively by the method of Subramanian et al. (1971). The leaves of these plants were washing properly in tap water and cut the leaves by scissors then dried in shady place and finally dried in an incubator at about 35° C temperature;

dried leaves were powder by electric Grinder. About 50 g powder of leaves was subjected in Soxhlet extraction unit with about 250-300 mL ethyl alcohol for about 72 h at 30-40° C. In case of compound Rutin after extraction, the aqueous layer was collected and left to stand in a cold place for 72 hours; a yellow precipitate separated out from the solution. The precipitate was filtered and washed with a mixture of chloroform: ethyl acetate: ethanol (2:1:1). The undissolved part of the precipitate was dissolved in hot methanol and filtered, the filtrate was evaporating to dryness to give 280 mg yellow powder (Rutin), and its melting point was measured as 194-196° C. Confirmation of the compound was also made through IR and Rf values when compared to the authentic sample obtained from Sigma Chemical Company, USA.

Figure 2: Chemical Structure of Rutin



Biochemical Experiment

The late third instar larvae were treated with 40% and 80% of 24 h LC_{50} of Apigenin and Rutin in binary combination obtained from the leaves of *Jatropha gossypifolia* and *Codiaeum variegatum*, respectively for 24 h. Six beakers were set up for each dose and each beaker contained 50 larvae in 1 L de-chlorinated tap water. The LC_{50} value of Apigenin : Rutin in the ratios 1:1, 1:2, 1:5 was 53.66 mg/L, 50.66 mg/L and 40.65 mg/L respectively for 24 h against *Culex quinquefasciatus* larvae. 40% and 80% of 24 h, LC_{50} of ethyl alcohol extract was selected as sub-lethal dose to analyze its time and dose dependent effects in the present study and at that dose there was no mortality were observed in the treated larvae. After the stipulated time (24 h), the dead larvae were removed from the beaker and washed with water and the whole body tissue stored in deep freezer, for biochemical analysis. Control larvae were held in the same condition without any treatment. Each experiment was replicated six times and the values are expressed as mean ±SE of six replicates. Student's 't' test was applied to locate significant changes with controls Sokal, R.R. and Rohlf, F.J. (1973), Prasad, S. (2003), Lowry et al. (1951).

Total Protein: Total protein level was estimated by the method of Spies, J.R. (1957). Homogenates (10 mg/mL) was prepared in 10% tri-chloroacetic acid (TCA). Bovine serum albumin was used as standard.

Total Free Amino Acids: Total free amino acids level was estimated by the method of Van der Vies, J. (1954). Homogenates (10 mg/mL) were prepared in 95% ethanol. Glycine was used as standard.

Glycogen: Glycogen level was estimated by the method of Ellman G.L et al. (1961). Homogenate (10 mg/mL) was prepared in 5% TCA. Glucose was used as standard.

Acetylcholinesterase Activity: Acetylcholinesterase activity was measured by the method of Andersch, M.A. and Szcypinski, A.J. (1947). Homogenate (50 mg/ml, w/v) was prepared in 0.1 M-phosphate buffer, PH 8.0 for 5 min in an ice bath. The change in optical density at 412 nm, caused by the enzymatic reaction, was monitor for 3 min at 25° C.

Acid and Alkaline Phosphatase Activity: Acid and alkaline phosphatase activity was determined by the method Sun, R., et al. (2006). Homogenates (2% w/v) were C for 15 min. prepared in ice-cold 0.9% NaCl solution and centrifuged at 5000 xg at 0 Statistical analysis: Each experiment was replicated at least six times and data has expressed as mean ±SE. Student's t-test as applied for locating significant differences Prasad, S. (2003).

Results

This section deals with the toxic effect of binary combination of extracted compounds in 1:1 ratio, extracted through different organic solvents from leaves of *Jatropha gossypifolia* and *Codiaeum variegatum* plants respectively (family: Euphorbiaceae) against 3rd instar mosquito larvae of *Culex quinquefasciatus*. Mosquito larvae were exposed to four different concentrations of each extracts of both the plants.

Combination of Apigenin and Rutin (1:5 Ratio) Extracts against *Culex Quinquefasciatus* Larvae

Table 1 clearly indicate that the mortality produced by apigenin and rutin (1:5 ratio) for the periods ranging from 24 to 96 h. The toxicity of apigenin and rutin extract was time and dose dependent for *Culex quinquefasciatus* larvae. The LC_{50} values of are shown in Table 1. There was a significant negative correlation between LC values and exposure periods. i.e. LC_{50} values of combination extracts of *Codiaeum variegatum* and *Jatropha gossypifolia* leaf decreased from 40.65 mg/L (24 h) > 39.32 mg/L (48 h) > 37.61 mg/L (72 h) > 36.13 mg/L (96 h) in case of *Culex quinquefasciatus* larvae (Table 1 and Figure 3).

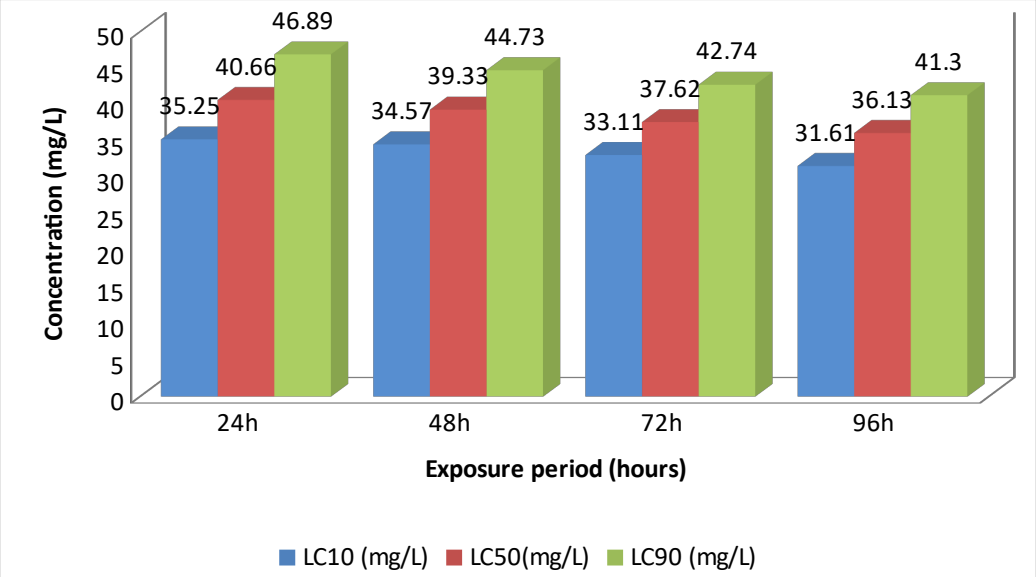
Table 1: Toxicity (LC Values) of (Apigenin and Rutin, 1:5 Ratio) of Different Concentrations Extracted from Ethyl Alcohol of *Jatropha Gossypifolia* and *Codiaeum Variegatum* Leaf against *Culex Quinquefasciatus* Larvae at 24 h to 96 h Exposure Period

Exposure Period (Hours)	Effective Dose (mg/L)	Limits (mg/L)		Slope Value	t Ratio	Heterogenity
		LCL	UCL			
24	LC_{10} = 35.25	27.53	37.18	20.698±11.60	2.84	0.02
	LC_{50} = 40.65	39.14	44.97			
	LC_{90} = 46.88	43.36	69.87			
48	LC_{10} = 34.57	28.32	36.47	22.90±11.39	3.20	0.07
	LC_{50} = 39.32	37.82	41.25			
	LC_{90} = 44.73	42.19	55.85			
72	LC_{10} = 33.11	25.58	35.33	23.12±11.60	3.16	0.02
	LC_{50} = 37.61	35.14	38.93			
	LC_{90} = 42.73	40.75	50.82			
96	LC_{10} = 31.61	20.21	34.39	22.08±12.41	2.81	0.16

	LC_{50} = 36.13	31.04	37.60			
	LC_{90} = 41.29	39.54	49.58			

- Batches of twenty mosquito larvae were exposed to four different concentrations of the extract.
- Concentrations given are the final concentration (w/v) in the glass beaker containing de-chlorinated tap water. Each set of experiment was replicated six times.
- Mortality was recorded after every 24 h.
- Regression coefficient showed that there was significant ($P < 0.05$) negative correlation between exposure time and different LC values.
- LCL: Lower confidence limit; UCL: Upper confidence limit.
- There was no mortality recorded in the control group.

Figure 3: Bar Diagram Showing Apigenin and Rutin (1:5 Ratio) Toxicity on *Culex Quinquefasciatus* Larvae at Different Concentrations and Time Intervals



- Values are mentioned in percentage.
- Doses are 40% and 80% of LC_{50} for period for which animals were exposed.
- Significant ($P < 0.05$) when two way analysis of variance was applied to see biochemical profile was time and dose dependent.
- Significant ($P < 0.05$) when Student 't' test was applied between control and treated groups.

Biochemical Experiment of Binary Combination Extracts

This section deals with the biochemical effect of binary combination of Apigenin and Rutin (1:5 ratio), extracted through ethyl alcohol (solvent) from the leaves of *Jatropha gossypifolia* and *Codiaeum variegatum* (family: Euphorbiaceae) against 3rd instar mosquito larvae of *Culex quinquefasciatus*. Total protein levels were reduced to 70% and 50% of control respectively after treatment with 40% and 80% of LC_{50} (24 h).The levels of glycogen reduced to 60% and 42% of control in the body tissue respectively after treatment with 40% and 80% of LC_{50} (24 h). Exposure of 40% and 80% of LC_{50} (24 h) of binary combination of apigenin and rutin (1:5 ratio) extract significantly increased the free amino acid level to 129% and 147% of control respectively (Table 2 and Figure 4).

Acetylcholinesterase (AChE) activity was reduced to 71% and 56% of control respectively after treatment with 40% and 80% of LC_{50} (24 h) (Table 3 and Figure 5) while in case of (96 h) of 40% and 80% of LC_{50} AChE activity was also reduced to 64% and 47% of control respectively (Table 3 and Figure 5). The acid phosphatase activity was reduced to 73% and 60% of control respectively after treatment with 40% and 80% of LC_{50} (24 h) (Table 3 and Figure 5) while in case of (96 h) of 40% and 80% of LC_{50} acid phosphatase activity was also reduced to 66% and 50% of control respectively (Table 3 and Figure 6). Alkaline phosphatase activity was reduced to 74% and 60% of control respectively after treatment with 40% and 80% of LC_{50} (24 h) while in case of (96 h) of 40% and 80% of LC_{50} alkaline phosphatase activity was also reduced to 65% and 46% of control respectively (Table 3 and Figure 7).

Table 2: Changes in Total Protein, Glycogen and Total Free Amino Acid in Whole Body Tissue of *Culex Quinquefasciatus* Larvae after 24 h Exposure to Sub-lethal Doses (40% and 80% of LC_{50} of 24 h) of (Apigenin and Rutin, 1:5 Ratio) Combination Extracted through Ethyl Alcohol from Leaf of *Jatropha Gossypifolia* and *Codiaeum Variegatum* Plant

Parameters	**Control**	**40% of LC_{50} (+, ±£)**	**80% of LC_{50} (+, ±£)**
Protein	2.00±0.003 (100)	1.40±0.003 (70)	1.00±0.003 (50)
Glycogen	1.58±0.003 (100)	0.95 ±0.003 (60)	0.67±0.003 (42)
Amino Acid	0.70±0.003 (100)	0.90±0.003 (129)	1.03±0.003 (147)

- Values are mean ±SE of six replicates.
- Values in brackets indicate percent biochemical activity with control taken as 100%.
- Doses are 40% and 80% of LC_{50} for period for which animals were exposed.
- +, significant ($P < 0.05$) when two way analysis of variance was applied to see whether enzyme inhibition was time and dose.
- £, significant ($P < 0.05$) when Student 't' test was applied between control and treated groups.

Table 3: Changes in Acetylcholinesterase, Acid and Alkaline Phosphatase Activity in Whole Body Tissue of *Culex Quinquefasciatus* Larvae after 24 h or 96 h Exposure to Sub-lethal Doses (40% and 80% of LC_{50} of 24 h) of Binary Combination of (Apigenin and Rutin, 1:5 Ratio**)** Extracted through Ethyl Alcohol from Leaf of *Jatropha Gossypifolia* and *Codiaeum Variegatum*plant

	AChE Activity (µm SH hydrolyzed/min/mg Protein)		
	24h		
AChE	0.080±0.0003 (100)	0.057±0.0003 (71)	0.045±0.0003 (56)
	96h		
	0.085±0.0004 (100)	0.054±0.0003 (64)	0.040±0.0003 (47)
Acid Phosphatase	**µm *p*-nitrophenol Formed/30 min/mg Protein**		
	24h		

	0.190±0.003 (100)	0.138±0.0005 (73)	0.114±0.0003 (60)
	96h		
	0.180±0.003 (100)	0.118±0.0003 (66)	0.090±0.0005 (50)
Alkaline Phosphatase	**µm *p*-nitrophenol Formed/30 min/mg Protein**		
	24h		
	0.420±0.005 (100)	0.310±0.003 (74)	0.250±0.003 (60)
	96h		
	0.460±0.005 (100)	0.300±0.005 (65)	0.210±0.003 (46)

- Values are mean ±SE of six replicates.
- Values in brackets indicate percent biochemical activity with control taken as 100%.
- Doses are 40% and 80% of LC_{50} for period for which animals were exposed.
- +, significant ($P < 0.05$) when two way analysis of variance was applied to see whether enzyme inhibition was time and dose.
- £, significant ($P < 0.05$) when Student 't' test was applied between control and treated groups.

Figure 4: A Chart Showing the Effect of Apigenin and Rutin (1:5 Ratio) on the Biochemical Parameters of Mosquito Larvae at Different Concentrations

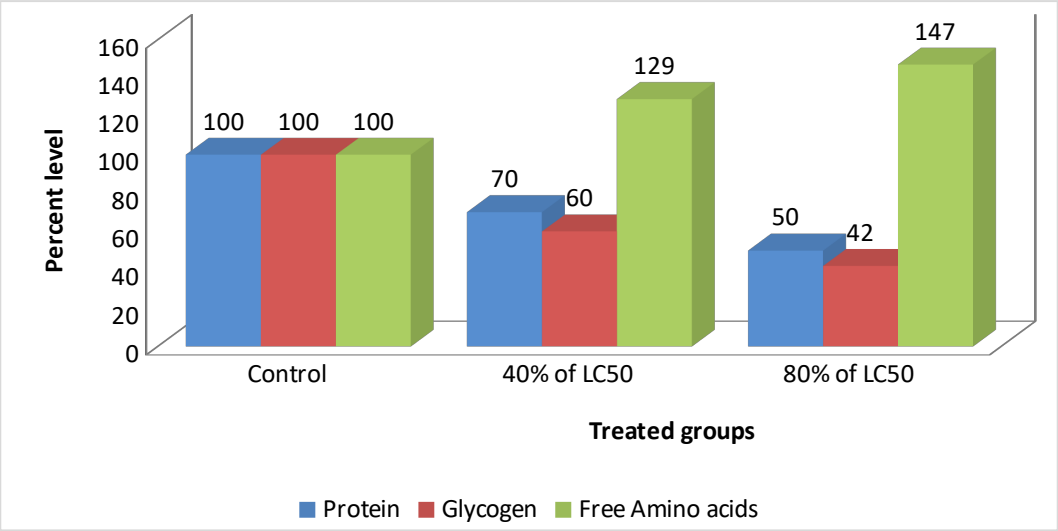
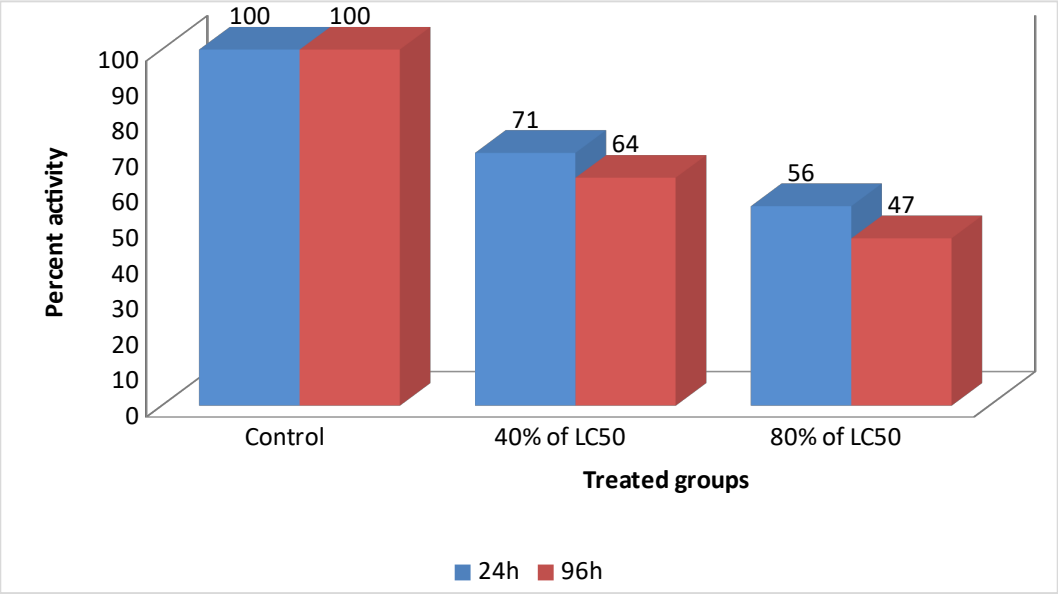


Figure 5: A Chart Showing the Effect of Apigenin and Rutin (1:5 Ratio) on the % Activity of AchE of Mosquito Larvae at Different Concentrations



- Values are mentioned in percentage.
- Doses are 40% and 80% of LC_{50} for period for which animals were exposed.
- Significant ($P < 0.05$) when two way analysis of variance was applied to see biochemical profile was time and dose dependent.
- Significant ($P < 0.05$) when Student 't' test was applied between control and treated groups.

Figure 6: A Chart Showing the Effect of Apigenin and Rutin (1:5 Ratio) on the % Activity of Acid Phosphatase of Mosquito Larvae at Different Concentrations

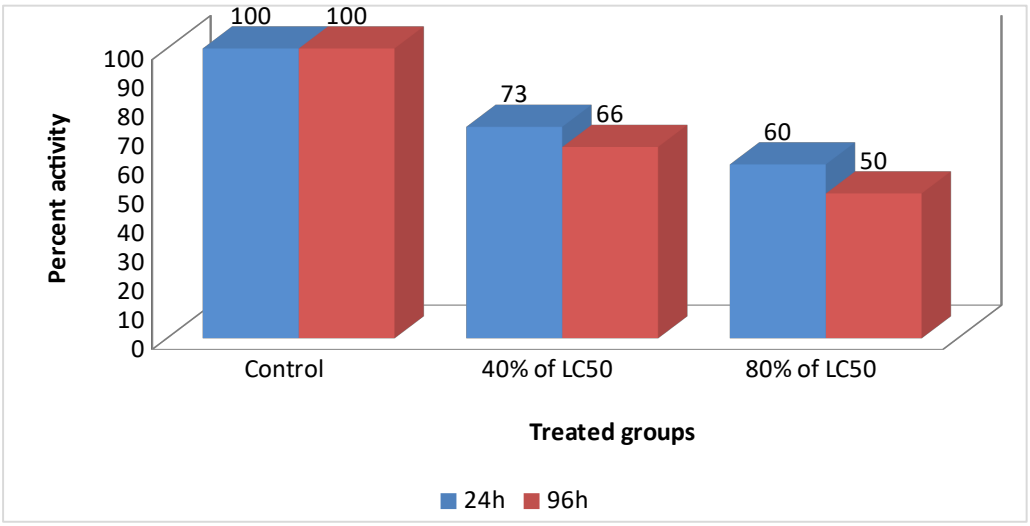
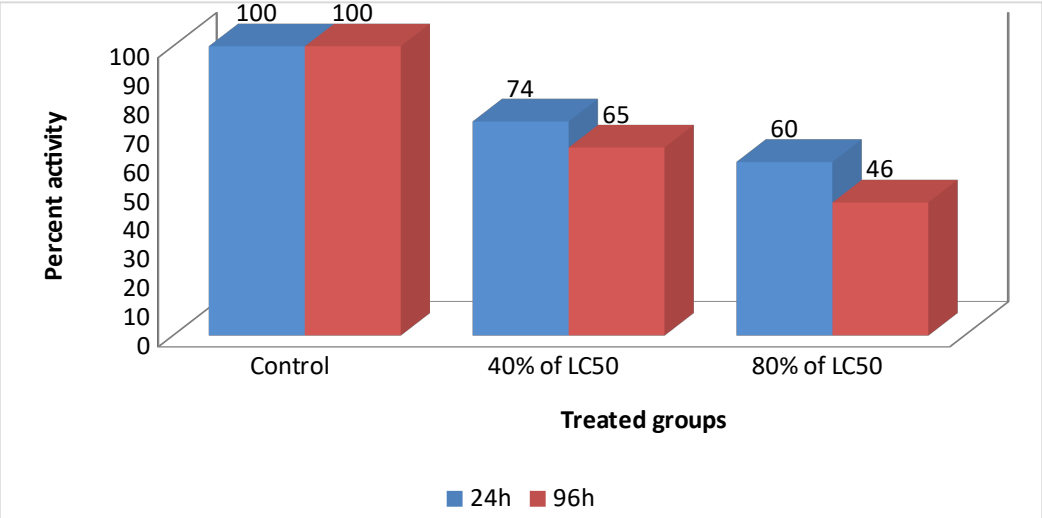


Figure 7: A Chart Showing the Effect of Apigenin and Rutin (1:5 Ratio) on the % Activity of Alkaline Phosphataseof Mosquito Larvae at Different Concentrations



- Values are mentioned in percentage.
- Doses are 40% and 80% of LC_{50} for period for which animals were exposed.
- Significant ($P < 0.05$) when two way analysis of variance was applied to see biochemical profile was time and dose dependent.
- Significant ($P < 0.05$) when Student 't' test was applied between control and treated groups.

Discussion

The Apigenin and Rutin in their binary combination (1:5 ratio) extracted from the leaves of *Jatropha gossypifolia* and *Codiaeum variegatum*, showed larvicidal activities against *Culex quinquefasciatus* larvae. Couple of plant products shows significant effect against mosquito population management Sun, R., Sacalis, et al. (2006), Modupe Elizabeth Ojewumi et al. (2017). Viswan, A. and Pushapalatha, E. (2020). The biologically active compounds present in the plant were grouped into two categories i.e. primary metabolites which includes amino acids and chlorophyll whereas the other one is secondary metabolites which includes alkaloids, flavonoids, tannins and saponins Samidurai, K., et al. (2009). Biologically active compounds in their binary combinations present in the plant extracts depicted insecticidal, antimicrobial, anticonstipative, antispasmodial and antioxidant activities Edeogo, H.O., and Mbaebie, B.O. (2005) Tamilselvan, P. et al. (2015). The LC_{50} values recorded in different studies like - *Sonchus arvensis* stem extracts has LC_{50} value of 68.0 ppm, *Matricaria maritima* flowers extracts has LC_{50} value of 72.0 ppm have shown effectiveness Benelli, G.P., Roman M., Filippo, et al. (2017). A study has tested the effects of some plants extracts against the larvae of *Culex quinquefasciatus* included *Tagetes erectes* leaf extract has LC_{50} value of 100.0 ppm, *Achilea millefolium* stem extract has LC_{50} value of 120.0 ppm, *Tanacetum vulgare* flower extract has LC_{50} value of 178.0 ppm and *Otanthus maritimus* stem extract has LC_{50} 195.0 ppm Borah, R. Kalita M.C, and Kar A. (2010). The phyto extracts effect on mosquito larvae is due to entrance of phytochemicals through alimentary canal and bounding with lipids or cell metabolites which resulted in moulting or cuticle hardness through Tyrosinase enzyme effectiveness or respiratory bores closing, Mahdi, N.S. (2001). Exposure to sub-lethal doses on larvae *of Culex* significantly altered the level of total protein, total free amino acid, glycogen and enzyme activity of acetylcholinesterase, acid and alkaline phosphatase activity. There was significant changes in *Culex quinquefasciatus* larvae like ecdysial failure, abnormalities during intermediate stages, prolongation of the life span of treated instars, emergence of adultoids after treatment with Apigenin and Rutin in their

binary combination extracted with ethyl alcohol extract from the leaves of *Codiaeum variegatum*. This may be due to the effect of active moiety present in the plant extract. The male and female in the treated groups were not able to feed on sugar solution as well as on mammal blood and finally died. Laboratory investigations depicted that their mouth parts were undeveloped, legs were paralyzed and the females were sterile after treatment. The protein is alternative source of energy to meet the increase energy demand. Protein depletion in treated mosquito larvae of *Culex quinquefasciatus* may be due to their degradation and the possible utilization for metabolic activities. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains Shaalan, E.A.C., Deon Y., Mohamed W.F.A. (2005). Singh, N.N., Das, V.K. et al. (1996). The decreased protein content resulted in destruction or necrosis of cells and consequent impairment in protein synthesis Hamen C. (1986).

The total free amino acids content showed a significant increase in whole body tissue of mosquito larvae exposed to sub-lethal doses of Apigenin and Rutin in their combination. The rise in total free amino acids level in the whole body tissue represented high proteolytic activity. The deposition of free amino acids can be attributed to lesser use of amino acids and their involvement in the equilibrium of an acid base balance Seshagiri Rao, et al. (1987) Moorthy, K.S. et al. (1984).

It can be also due to the rise of free amino acid level might be due to transamination and amination to keto acids. Stress conditions induce elevation in the transamination pathway Natarajan, G.M. (1985). During stress, carbohydrate level reduced to meet energy demand. The low glycogen content in body tissues of *Culex* larvae showed its fast utilization for energy generation caused by Apigenin and Rutin for treatment which was extracted from the leaves of *Codiaeum variegatum.* Glycogenolysis seems to be the result of increased secretion of catecholamine by the larvae in excess amount due to stress of plant extracts treatment Hamen, C. (1986) which reduced glycogen reserves Nakano, T., (1967). Anaerobic and aerobic segments are two important components of carbohydrate metabolism. During anaerobic segment, breakdown of glucose or glycogen through glycolysis occurs while the next one consists oxidation of pyruvate to acetyl Co-A to be utilized through Kreb's cycle Nelson DL (2002). Effect of toxicants on enzymatic activity is one of the most important biochemical parameters, which affect physiology of body. When an organ is diseased due to toxicant, enzyme activity is increased or inhibited due to the denaturation of active site of enzymes. Acetylcholinesterase, or acetyl-hydrolase, is a serine protease that hydrolyses the neurotransmitter acetylcholine. AChE found mainly at NMJ and brain synapse, where its activity serves to terminate synaptic transmission. It belongs to carboxyl esterase family of enzymes. Enzyme alkaline phosphatase plays an important role in animal metabolism Vorbrodt, A. (1959) has reported that this enzyme helps in the transport of metabolites across the membrane. The enzyme has been shown to be associated with protein synthesis and involved in the synthesis of certain enzymes Sumner (1959). Acid phosphatase is the lysosomal enzyme and plays a vital role in catabolism, pathological necrosis, autolysis and phagocytosis Abou-Donia, M.B. (1978).

Conclusion

The binary combination of the Apigenin and Rutin the ratio 1:5 extracted through ethyl alcohol from the leaves of *Jatropha gossypifolia and Codiaeum variegatum* is highly toxic to the larvae of *Culex quinquefasciatus* mosquitoes and significantly decreased the population of the larvae by morphological, functional and physiological actions. Sub-lethal doses significantly altered the level of biochemical parameters on the vector larvae. Therefore the biologically active compounds in their binary combinations (1:5 ratio) are helpful in the management of elephantiasis vector population worldwide.

References

[1] W.H.O. (1984): Chemical methods for the control of arthropod vectors and pests of public health importance. World Health Organization, Geneva.

[2] W.H.O. (1995): Vector control for malaria and other mosquito-borne diseases. Report of a W.H.O. study group. WHO. Tech. Rep. Ser., 857: 1-91.

[3] Vatandoost, H.V.M. (2001): Larvicidal activity of neem extract against mosquito larvae in Iran. Pestology., 25: 69-72.

[4] Rueda, L.M. (2008): Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in fresh water. Dev. Hydrobiol., 595: 477-487.

[5] W.H.O. (2000): World Health Organization, Expert Committee on Malaria. Technical Report Series, 892. Geneva.

[6] W.H.O. (2006): Global programme to eliminate lymphatic filariasis. World Health Organization, Geneva. Weekly Epidemiological Record, 81(22): 221-232.

[7] Satti, M.H. and Abdel Nur, O. (1974): Bancroftian filariasis in the Sudan. Bulletin of the World Health Organization, 51: 314-315.

[8] El Setouhy, M. and Ramzy, R.M.R. (2003): Lymphatic filariasis in the Eastern Mediterranean Region: Current status and prospects for elimination. Eastern Mediter. Heal. J., 9(4): 534-541.

[9] Aiah, A.G., Maxwell, A.A., Samuel, D., Collins, K., Samuel, O.S., Aba Baffoe-Wilmot Johnny, G. and Alan, L.S. (2005): Lymphatic filariasis in Ghana: establishing the potential for an urban cycle of transmission. Tropi. Med. and International Heal., 10(4): 387-392.

[10] W.H.O. and UNICEF (2005): World Malaria Report 2005, HO/HTM/MAL/2005.1102. Roll back malaria, World Health Organization and UNICEF.

[11] Ester Innocent Cosam, C.J., Nicholus, K.G., Manien, J.M., Mayunga, H.H., Nkunya Ahmed, H. (2008): Mosquito-larvicidal constituents from Lantana Viburnoides varkisi (A. rich) verde (Verbenacea). J. of Vec. Bor. Dis., 45: 240-244.

[12] Wiseman, Z. and Chapagain, B.P. (2005): Larvicidal effects of aqueous extracts of Balanties aegyptiaca (desert date) against the larvae of Culex pipens mosquitoes. African J. of Biotec., 4(11): 1351-1354.

[13] Chowdhury, N., Laskar, S. and Chandra, G. (2008): Mosquito larvicidal and antimicrobial activity of protein of Solanum villosum leaves. BMC Comple. Alter. Med., 8: 62.

[14] Joseph, C.C., Ndoile, M.M., Malima, R.C. and Nkunya, M.H. (2004): Larvicidal and mosquitocidal extracts, a coumarin isoflavonoids and pterocarpans from Neorautanenia mitis. Transac.of the Royal Soci. of Tro. Med. and Hygi., 98(8): 451-455.

[15] Cavalcanti, E.S.B., Morais, S.M., Lima, M.A.A., Santana, E.W.P. (2004): Larvicidal activity of essential oils from Brazilian plants against Aedes aegypti L. Memórias do Instituto Oswaldo Cruz., 99: 541-544.

[16] Khana, V.G. and Kannabiran, K. (2007): Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre and Eclipta prostrate against Culex quinquefasciatus mosquito larvae. African J. of Biotec., 3: 307-311.

[17] Yang, Y.C., Lee, E.H., Lee, H.S., Lee, D.K. and Ahn, Y.J. (2004): Repellency of aromatic medicinal plant extracts to Aedes aegypti. J. Am. Mosq. Control Assoc., 20(2): 146-149.

[18] APHA/AWWA/WEF (1998): Standard methods for the examination of water and waste water. 20th edition, American Public Health Association, New York, USA.

[19] Subramanian, S.S., Nagarjuna, S. and Sulochana, N. (1971): Flavonoids of Jatropha gossypifolia. Phytochem., 10: 1690.

[20] Dordevice and Mcakic (2000). Apigenin extracted from leaves of J. gossypifolia were confirmed by UV spectra data.
[21] Sokal, R.R. and Rohlf, F.J. (1973): In “Introduction of Biostatistics”. W.H. Freeman and Company, San Francisco, pp. 36.
[22] Prasad, S. (2003): Elements of Biostatistics. Rastogi Publications, Meerut, India, pp. 119-135.
[23] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randell, R.J. (1951): Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.
[24] Spies, J.R. (1957): Colorimetric procedures for amino acids. In: Calowick, S.P., Kaplon, N.O. (Eds.). Methods of Enzymology. Academic Press, New York, pp. 468.
[25] Van der Vies, J. (1954): Two methods for determination of glycogen in liver. Biochem. J., 57: 410-416.
[26] Ellman G.L., Courtney, K.D., Andress, V. Jr. and Stone, F.R.M. (1961): A new and rapid colorimetric determination of AChE activity. Biolog. Pharmacol., 7: 88-98.
[27] Andersch, M.A., Szcypinski, A.J. (1947): A calorimetric determination of phosphatase in biological materials. Am. J. Clin. Path., 17: 571-574.
[28] Sun, R., Sacalis, J.N., Chin, C.K., Still, C.C. (2006). Bioactive aromatic compounds from leaves and stems of Vanilla fragrans. Journal of Agricultural and Food Chemistry, 49: 51-61.
[29] Modupe Elizabeth Ojewumi., Samuel Oluwafunsho Adedokun., Oladele Julius Omodara., Esther Adenike Oyeniyi., Olugbenga Samson Taiwo, and Emmanuel Omotayo Ojewumi. (2017). Phytochemical and Antimicrobial Activities of the Leaf Oil Extract of Mentha Spicata and its Efficacy in Repelling Mosquito. International Journal of Pharmaceutical Research and Allied Science, 6(4): 17-27.
[30] Viswan, A. and Pushapalatha, E. (2020). Larvicidal effect of selected plant extracts on Aedes aegypti L. and Culex quinquefasciatus Say. International Educational Applied Scientific Research Journal, 5(2): 1-2.
[31] Samidurai, K., Jebanesan, A., Saravanakumar, A., Govindarajan, M. and Pushpanathan, T. (2009). Larvicidal, ovicidal and repellent activities of Pemphisacidula Forest. (Lythraceae) against filarial and dengue vector mosquitoes. Academic Journal of Entomology, 2(2): 62-66.
[32] Edeogo, H.O., Okwn, D.F. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7): 685-688.
[33] Tamilselvan, P., Chinnasamy, R. and Devarajan, N. (2015). Larvicidal, pupicidal and adulticidal potential of Ocimumgratissimum plant leaf extracts against filariasis inducing vector. International Journal of Mosquito Research, 2(2): 1-08.
[34] Benelli, G., Pavela, R., Maggi, F., Petrelli, R. and Nicoletti, M. (2017). Commentary: Making Green Pesticides Greener? The Potential of Plant Products for Nanosynthesis and Pest Control. Journal of Cluster Science. Springer, 28(1): 3–10.
[35] Borah, R., Kalita M.C. and Kar A. (2010). Larvicidal Efficacy of Toddalia asiatica (Linn.) Lam against Two Mosquito Vectors Aedes aegypti and Culex aquinquefasciatus. African Journal of Biotechnology, 9(17): 25–30.
[36] Mahdi, N.S. (2001). Effect of Melia azedarach L. and Azadirachta indica (A. Jass) Fruit Extracts on Biology of Anopheles Pulcherrimus Mosquito. Baghdad.
[37] Shaalan, E.A.C., Deon Y., Mohamed W.F.A. (2005). A Review of Botanical Phytochemicals with Mosquitocidal Potential. Environment International, 31(8): 1149–1166.

[38] Singh, N.N., Das, V.K. and Singh S. (1996). Effect of aldrin on carbohydrate, protein and ionic metabolism of a freshwater catfish Heteropneustesfossilis. Bull. Environ. Contam. Toxicol., 57: 204-210.
[39] Bradbury, S.P., Symonic, D.M., Coats, J.R. and Atchison, G.J. (1987). Toxicology of fenevalerate and its constituent's isomers to the fathead minnows (Pimephalespromelos) and blue gill (Lepomis macrochirus). Bull. Envir. Contam. Toxicol., 38: 727-735.
[40] Seshagiri Rao, K. Srinivas Moorthy, Kashi Reddy B., Swamy, K.S., Chethy, C.S. (1987). Effect of benthio carb on protein metabolism of teleost, Sarotherodonmossambica. Indian J. Environ. Health, 29: 440-450.
[41] Moorthy, K.S., Kashi Reddy, B., Swamy, K.S. and Chethy, C.S. (1984). Changes in respiration and ionic content in the tissues of freshwater mussel exposed to methyl-parathion toxicity. Toxicol. Lett., 21: 287-291.
[42] Natarajan, G.M. (1985). Inhibition of branchial enzymes in snakehead fish (Channa striatus) by oxy demeton- methyl. Pest. Bioch. and Physiol., 23: 41-46.
[43] Hamen C. (1986). Aminotransferase activities and the amino acid excretion of bivalve mollusc and brachiopods. Comp. Biochem. Physiol., 26: 697-705.
[44] Nakano, T. and Tomilinson, N. (1967). Catecholamine and carbohydrate concentration in rainbow trout (Salmo gairdneri) in relation to physical disturbance. J. Fish. Res. Bd. Can., 24: 1701-1715.
[45] Nelson, D.L. and Cox, M.M. (2002). Lehninger Principles of Biochemistry. Macmillan Worth Publishers, New York.
[46] Vorbrodt A. (1959). The role of phosphatase in intracellular metabolism. Postepy. Hig. Mws. Soaq., 13: 200-206.
[47] Sumner. (1959). The cytology and histo-chemistry of the digestive gland cells of Helix. Quart. J. Microscopic Sci., 106: 173-192.
[48] Abou-Donia, M.B. (1978). Increased acid phosphatase acivity in hens following an oral dose of Leptophose. Toxicology Letter, 2: 199-203.