IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

BIOCHEMICAL AND LARVICIDAL IMPACT OF APIGENIN AND RUTIN IN THEIR BINARY COMBINATION (1:2) AGAINST ELEPHANTIASIS VECTOR *CULEX QUINQUEFASCIATUS*

Abhay Deep Johnson [1], Vikas Chandra Verma [2] and Ajay Singh [3]

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur, U.P. 273009, India

ABSTRACT

Phytochemicals are safe for non-target organisms like man and vector borne diseases due to insecticidal nature. 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 (1:2). Standard WHO protocols was adopted for the larvicidal bioassay. The active compounds i.e. Apigenin and Rutin extracted through ethyl alcohol from the leaves of Euphorbious plants was administered for 24h, 48h, 72h and 96h to the larvae of *C. quinquefasciatus*. Exposure of larvae over 24h to 96h sub-lethal doses (40% and 80% of LC50) of Apigenin and Rutin in the ratio 1:2, 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($P<0.05$) time and dose dependent. It may act as an ecofriendly measure to manage the population of filarial vector *Culex quinquefasciatus.*

KEYWORDS- *Jatropha gossypifolia*, *Codiaeum variegatum*, Apigenin, Rutin and *Culex quinquefasciatus*

Introduction:

Mosquitoes are regarded as one of the greatest threat to public health as they act as vectors of pathogens causing lymphatic elephantiasis, malaria, dengue, yellow fever etc. which affects millions of people around the globe [1,2,3]. About 3492 species of mosquitoes are reported worldwide and more than hundred of species can transmit various diseases in human and other vertebrates [4].

Lymphatic filariasis is regarded as endemic in 82 countries of the world. Lymphatic filariasis and malaria ranks amongst the world most prevalent tropical infectious diseases. Approximately 300-500 million people are infected with malaria leading to 1.5-3 million deaths annually [5]. LF is fast spreading insect-borne disease of human in the tropics. About 30% (394 million) of the global population is at risk and is considered as endemic in countries like Africa [6]. Elephantiasis not only cause serious public health issues but also create economic issues in many tropical and subtropical regions of the world, including India [7,8,9]. One of the effective way 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 hospitalised admissions are due to malaria [10].

Phytoproducts are less harmful to environment and non-target organisms. Many phytoextracts and biologically active compounds from different plant families have been investigated for new and promising larvicides [11]. Recently, the top priority in finding new insecticides (Apigenin and Rutin) is given which must be of plant origin and must be safe for ecosystem. Researchers reported the effectiveness of plant derived secondary compounds including saponin [12], steroids [13], isoflavonoid [14], essential oil [15], alkaloids and tannins [16] as mosquito larvicides. Plant compounds and their essential oils provide alternative source of mosquito repellents [17].

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 30x30x30 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 7.6-8.1mg/L, free CO_2 4.1-5.1mg/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 and identified by Prof. S.K. Singh, Ex. plant taxonomist, Department of Botany, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, Uttar Pradesh, India, where a voucher specimen was deposited.

Extraction of active compounds:

The Apigenin was isolated from the leaves of *Jatropha gossypifolia* respectively by the method of [19]. 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 35^0C 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 30-40^0C.Confirmation of the compound was also made through IR and Rf values data of [20], when compared to the authentic sample obtained from Sigma Chemical Company, USA.

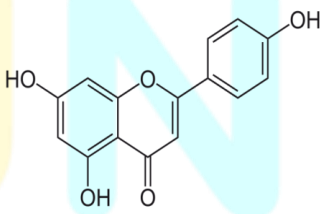

Figure 1 : Chemical Structure of Apigenin

The Rutin was isolated from the leaves of *Codiaeum variegatum* respectively by the method of [19]. 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 35^0C 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 72h at 30-40^0C. 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 un-dissolved 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 194-196^0C. Confirmation of the compound was also made through IR and Rf values when compared to the authentic sample obtained from Sigma Chemical Company, USA.

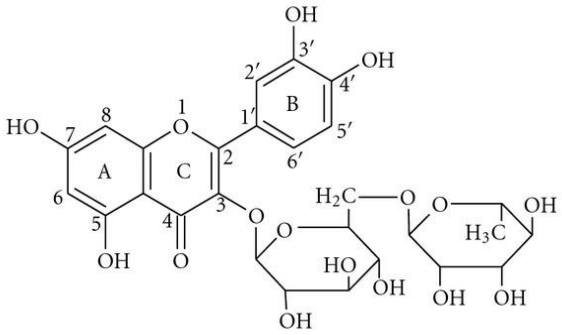

Figure 2: Chemical Structure of Rutin

Biochemical experiment:
The late third instar larvae were treated with 40% and 80% of 24h LC_{50} of Apigenin and Rutin in binary combination (1:2) obtained from *the* leaves of *Jatropha gossypifolia and Codiaeum variegatum*, respectively for 24h. Six beakers were set up for each dose and each beaker contained 50 larvae in 1L de-chlorinated tap water. 40% and 80% of 24h, 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 (24h), 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 [21,22,23].

Total protein: Total protein level was estimated by the method of [24] 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 [25] Homogenates (10mg/mL) were prepared in 95% ethanol. Glycine was used as standard

.

Glycogen: Glycogen level was estimated by the method of [26] Homogenate (10 mg/mL) was prepared in 5% TCA. Glucose was used as standard.

Enzyme activities: Acetylcholinesterase activity was measured by the method of [27] 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 412nm, caused by the enzymatic reaction, was monitor for 3 min at 25°C.

Acid and alkaline phosphatase activity was determined by the method of [28] 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 [22].

RESULT AND DISCUSSION

Combination of Apigenin and Rutin (1:2 ratio) extracts against *Culex quinquefasciatus* larvae:

This section deals with the toxic effect of binary combination of extracted compounds in the ratio 1:2, , extracted through ethyl alcohol from leaf of *Codiaeum variegatum* and *Jatropha gossypifolia* (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. Mortality was recorded after 24h, 48h, 72h or 96h. The data obtained were used for calculation the LC values (LC_{10}, LC_{50} and LC_{90}), upper and lower confidence limits, slope values, t-ration and heterogeneity.

Mortality produced by apigenin and rutin (1:2 ratio) for the periods ranging from 24 to 96h (Table 1). 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 50.66mg/L (24h)>49.33mg/L (48h)>47.62mg/L (72h)>46.12mg/L (96h) in case of *Culex quinquefasciatus* larvae (Table 1; Figure 3).

Table 1: Toxicity (LC values) of (**Apigenin** and **Rutin, 1:2** ratio**)** of different concentrations extracted from ethyl alcohol of *Jatropha gossypifolia* and *Codiaeum variegatum* leaf against *Culex quinquefasciatus* larvae at 24h to 96h exposure period.

Exposure Period (hours)	Effective dose (mg/L)	Limits (mg/L)		Slope value	t ratio	Heterogenity
		LCL	UCL			
24	LC_{10}=45.22	37.12	47.18	26.01±15.47	2.84	0.03
	LC_{50}=50.66	49.15	54.89			
	LC_{90}=56.75	53.32	77.96			
48	LC_{10}=44.53	38.00	46.47	28.79±15.21	3.20	0.07
	LC_{50}=49.33	47.83	51.25			
	LC_{90}=54.66	52.18	65.22			
72	LC_{10}=43.03	35.05	45.31	29.09±15.53	3.16	0.02
	LC_{50}=47.62	45.12	48.94			
	LC_{90}=52.71	50.75	60.50			
96	LC_{10}=41.48	29.05	44.35	27.79±16.63	2.81	0.16
	LC_{50}=46.12	40.88	47.61			
	LC_{90}=51.29	49.55	59.33			

- 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 24h.
- 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.

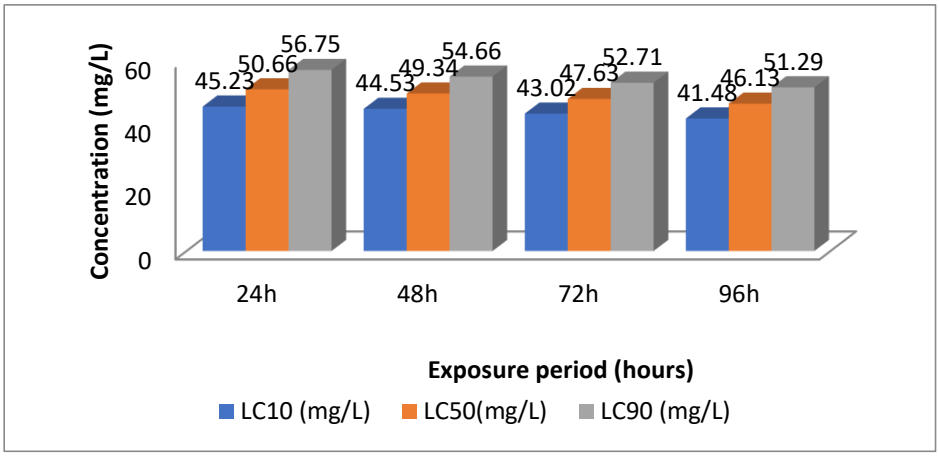

Figure 3-Bar diagram showing apigenin and rutin (1:2 ratio) toxicity on *Culex quinquefasciatus* larvae extracted from the leaf of *Jatropha gossypifolia* and *Codiaeum variegatum* at different concentrations and at different time intervals.

Biochemical experiment of binary combination (1:2) extracts :

This section deals with the biochemical effect of binary combination of Apigenin and Rutin (1:2 ratio), extracted through ethyl alcohol from the leaves of *Codiaeum variegatum* and *Jatropha gossypifolia* (family: Euphorbiaceae) against 3rd instar mosquito larvae of *Culex quinquefasciatus*.

Combination of apigenin and rutin (1:2 ratio) extracts against *Culex quinquefasciatus* larvae:

Total protein levels were reduced to 78% and 64% of control respectively after treatment with 40% and 80% of LC_{50} (24h). The levels of glycogen reduced to 70% and 56% of control in the body tissue respectively after treatment with 40% and 80% of LC_{50} (24h). Exposure of 40% and 80% of LC_{50} (24h) of binary combination of apigenin and rutin (1:2 ratio) extract significantly increased the free amino acid level to 120% and 136% of control respectively (Table 2; Figure 4).

Exposure of larvae to 40% and 80% of LC_{50} of binary combination of apigenin and rutin (1:2 ratio) extract for 24h or 96h caused significant dose dependent reduction in the AChE, acid phosphate and alkaline phosphatase activity in the body tissue of *Culex quinquefasciatus* larvae. Acetylcholinesterase (AChE) activity was reduced to 85% and 74% of control respectively after treatment with 40% and 80% of LC_{50} (24h) while in case of (96h) of 40% and 80% of LC_{50} AChE activity was also reduced to 76% and 62% of control respectively. The acid phosphatase activity was reduced to 78% and 67% of control respectively after treatment with 40% and 80% of LC_{50} (24h) while in case of (96h) of 40% and 80% of LC_{50} acid phosphatase activity was also reduced to 71% and 58% of control respectively. Alkaline phosphatase activity was reduced to 80% and 68% of control respectively after treatment with 40% and 80% of LC_{50} (24h) while in case of (96h) of 40% and 80% of LC_{50} alkaline phosphatase activity was also reduced to 76% and 61% of control respectively (Table 3; Figure 5,6,7).

Table 2: Changes in total protein, glycogen and total free amino acid in whole body tissue of *Culex quinquefasciatus* larvae after 24h exposure to sub-lethal doses (40% and 80% of LC_{50} of 24h) of (Apigenin and Rutin, 1:2 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	1.85±0.003 (100)	1.45±0.003 (78)	1.19±0.001 (64)
Glycogen	1.60±0.003 (100)	1.12±0.003 (70)	0.90±0.003 (56)
Amino acid	0.66±0.002 (100)	0.79±0.003 (120)	0.90±0.003 (136)

Table 3: Changes in acetylcholinesterase, acid and alkaline phosphatase activity in whole body tissue of *Culex quinquefasciatus* larvae after 24h or 96h exposure to sub-lethal doses (40% and 80% of LC_{50} of 24h) of binary combination of (Apigenin and Rutin, 1:2 ratio) extracted through ethyl alcohol from leaf of *Jatropha gossypifolia* and *Codiaeum variegatum* plant.

AChE	**AChE activity (µm SH hydrolyzed/min/mg protein)**		
	24h		
	0.078±0.0004 (100)	0.066±0.0004 (85)	0.058±0.0003 (74)
	96h		
	0.082±0.0004 (100)	0.062±0.0004 (76)	0.051±0.0004 (62)
Acid phosphatase	**µm *p*-nitrophenol formed/30 min/mg protein**		
	24h		
	0.180±0.005 (100)	0.141±0.0006 (78)	0.120±0.0003 (67)
	96h		
	0.200±0.003 (100)	0.141±0.0003 (71)	0.116±0.0004 (58)
	µm *p*-nitrophenol formed/30 min/mg protein		
	24h		
	0.440±0.005	0.350±0.003	0.300±0.005

Alkaline phosphatase	(100)	(80)	(68)
	96h		
	0.380±0.004 (100)	0.290±0.003 (76)	0.230±0.004 (61)

- 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.

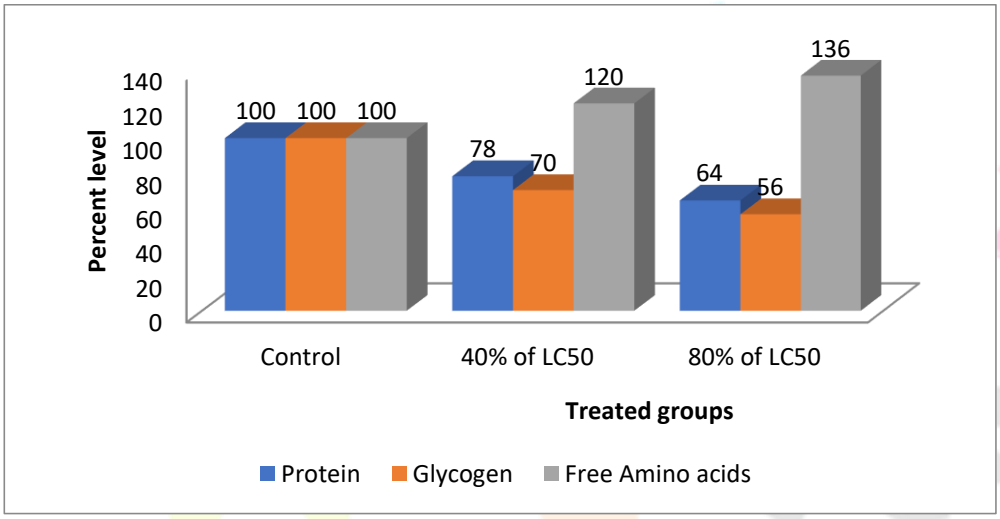

Figure 4: Bar diagram showing effect of Apigenin and Rutin (1:2 ratio) on the biochemical parameters of mosquito larvae at different concentrations.

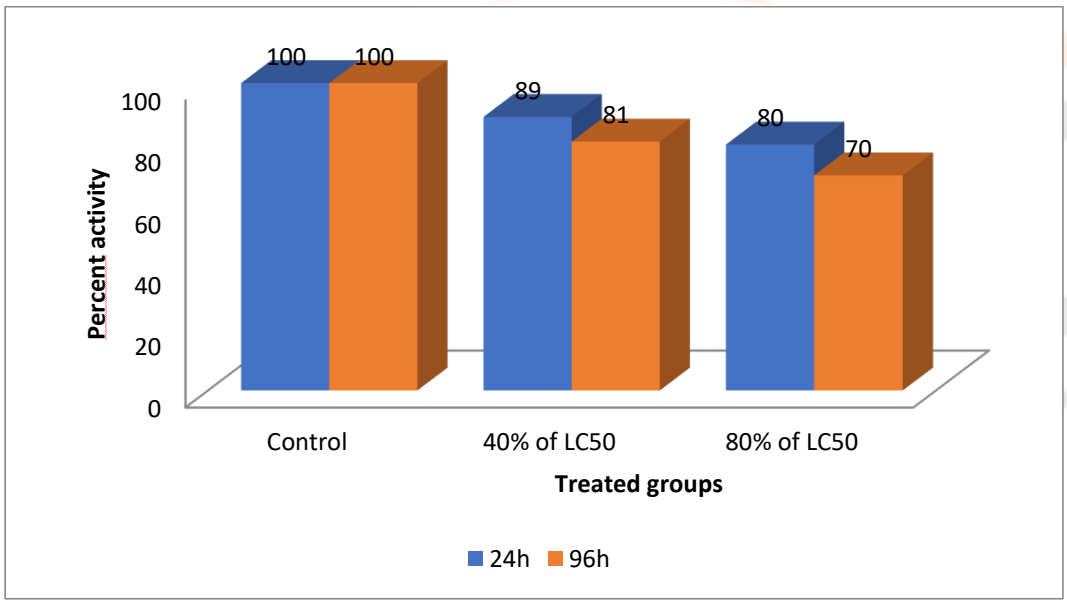

Figure 5: Bar diagram showing effect of Apigenin and Rutin (1:2 ratio) on the % activity of AchE of mosquito larvae at different concentrations.

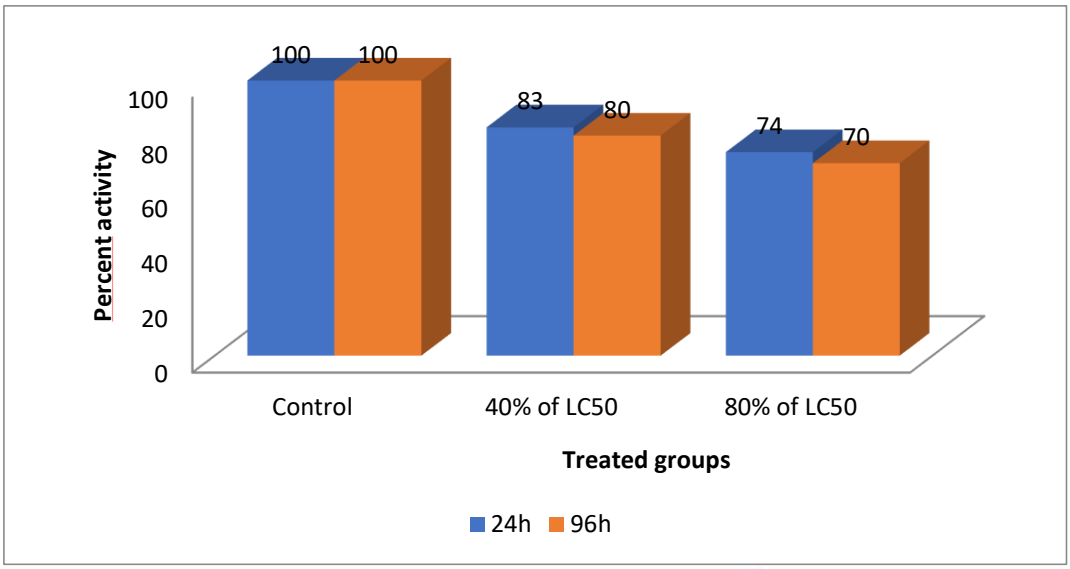

Figure 6: Bar diagram showing effect of Apigenin and Rutin (1:2 ratio) on the % activity of Acid phosphatase of mosquito larvae at different concentrations.

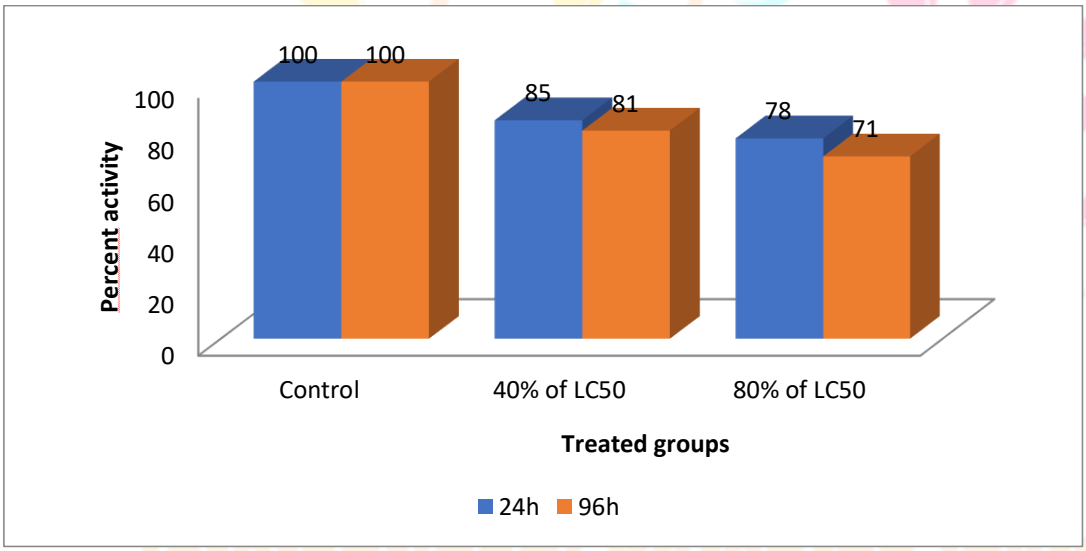

Figure 7: Bar diagram showing effect of Apigenin and Rutin (1:2 ratio) on the % activity of Alkaline phosphatase of mosquito larvae at different concentrations.

* 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.

We investigated that Apigenin and Rutin in their binary combination (1:2 ratio) extracted from the leaves *of Jatropha gossypifolia and Codiaeum variegatum* depicted larvicidal activities against *Culex quinquefasciatus* larvae. Few plant showed effectiveness against mosquito population management, [29,30]. The biologically active compounds present in the plant are classified into two categories i.e. primary metabolites which includes amino acids and chlorophyll whereas the other one is secondary metabolites which comprises of alkaloids, flavonoids, tannins and saponins [31].

Phytocompounds in their binary combination (1:2 ratio) showed insecticidal, antimicrobial, anticonstipative, antispasmodial and antioxidant activities [32,33]. The LC50 values recorded in different studies like - *Sonchus arvensis* stem extracts has LC50 value of 68.0 ppm, *Matricaria maritima* flowers extracts has LC50 value of 72.0 ppm have shown effectiveness [34]. A study has tested the effects of some plants extracts against the larvae of *Culex quinquefasciatus* included *Tagetes erectes* leaf extract has LC50 value of 100.0 ppm, *Achilea millefolium* stem extract has LC50 value of 120.0 ppm, *Tanacetum vulgare* flower extract has LC50 value of 178.0 ppm and *Otanthus maritimus* stem extract has LC50 195.0 ppm [35]. The plant extracts effectiveness on mosquito larvae is due to entrance through alimentary canal and bounding with lipids or cell metabolites resulting in moulting or cuticle hardness through Tyrosinase enzyme effectiveness or respiratory bores closing [36].

Exposure to sub-lethal doses of Apigenin and Rutin in their binary combination against larvae *of Culex* significantly significantly altered the level of total protein, total free amino acid, glycogen and enzyme activity of acetylcholinesterase, acid and alkaline phosphatase activity. There changes in *Culex quinquefasciatus* larvae showed ecdysial failure, abnormalities during intermediate stages, prolongation of the life span of treated instars, emergence of adultoids after treatment with binary combination extracted with ethyl alcohol and petrol extract from the leaves of Euphorbious plants which may be due to the effectiveness of active moiety present in the plant extract in their binary combination. The male and female in the treated groups failed to feed on sugar solution and blood of mammal as a result of which they finally died. Laboratory investigations revealed that their mouth parts were undeveloped, legs were paralyzed and the females were sterile after treatment.

The protein act as an alternative source of energy to meet the increase energy demand. Protein depletion in treated mosquito larvae may be due to their degradation and the possible utilization for metabolic activities or due to impaired incorporation of amino acids into polypeptide chains [37,38]. The decreased protein content resulted in destruction or necrosis of cells and consequent impairment in protein synthesis.

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 binary combination (1:2 ratio). The rise in total free amino acids level in the whole body tissue showed high proteolytic activity. The accumulation of free amino acids can be attributed to lesser use of amino acids and their involvement in the equilibrium of an acid base balance [39,40,41]. 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 [42]. During stress, carbohydrate level reduced to meet energy demand. The low glycogen content in body tissues of *Culex* larvae depicted fast utilization for energy generation. Glycogenolysis might be the result of increased secretion of catecholamine by the larvae in excess amount due to stress of phytoextract treatment [43] resulting in reduced glycogen reserves [44]. During anaerobic segment, breakdown of glucose or glycogen through glycolysis occurs while the next one consists of oxidation of pyruvate to acetyl Co-A to be utilized through TCA cycle [45].

Effect of binary combination of Apigenin and Rutin on enzymatic activity is one of the most important biochemical parameters, which affects physiology of body. Organs which are diseased because of toxicant resulted in increased or inhibited activities of enzyme activity. This is 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. Enzyme alkaline phosphatase plays a vital role in animal metabolism [46] by transporting metabolites across the membrane. The enzyme show association with protein synthesis and is also involved in the synthesis of certain enzymes [47]; Acid phosphatase is the lysosomal enzyme and plays a vital role in catabolism, pathological necrosis, autolysis and phagocytosis [48].

CONCLUSION

The larvicidal effect of the Apigenin and Rutin extracted through ethyl alcohol from the leaves of *Jatropha gossypifolia and Codiaeum variegatum* in their binary combination (1:2 ratio) is highly toxic to larvae of *Culex quinquefasciatus* and significantly decreased the population of the larvae. Sub-lethal doses significantly altered the biochemical parameters which includes level of protein, amino acids, glycogen and enzyme activities (acetylecholinesterase, acid and alkaline phosphatase) of *Culex quinquefasciatus* larvae. Thus the plant extract in their binary combinations are helpful in the management of filarial vector.

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 *Azadirachta indica* 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. http://www.who.int/wer.

[7] Satti, M.H. and Abdel Nur, O. (1974): Bancroftian filariasis in the Sudan. Bull.of the world Heal. Organ.,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 http://rbm.who.int/wmr2005.

[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, MM.., 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, 468 pp.

[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. 24.

[29] Modupe Elizabeth Ojewumi., Samuel Oluwafunsho Adedokun., Oladele Julius Omodara., Esther AdenikeOyeniyi., Olugbenga Samson Taiwo. and Emmanuel Omotayo Ojewumi. (2017). Phytochemical and Antimicrobial Activities of the Leaf Oil Extract of Mentha www.wjpr.net Vol 9, Issue 10, 2020. Ajay et al. World Journal of Pharmaceutical Research 1319 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 *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector. International Journal of Mosquito Research, 2(2): 01-08.

[34] Benelli, G. P., Roman M., Filippo, and et al P. (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 quinquefasciatus*. 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): 1149–66.

[38] Singh NN, Das VK and Singh S Effect of aldrin on carbohydrate, protein and ionic metabolism of a freshwater catfish *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol., 1996 ; 57. 204-210.

[39] Bradbury SP, Symonic DM, Coats JR and Atchison GJ. Toxicology of fenevalerate and its constituent's isomers to the fathead minnows (Pimephalespromelos) and blue gill (Lepomis macrochirus). Bull. Envir. Contam. Toxicol., 1987; 38: 727-735.

[40] Seshagiri Rao, K Srinivas Moorthy, Kashi Reddy B, Swamy KS, Chethy CS. Effect of benthio carb on protein metabolism of teleost, Sarotherodonmossambica. Indian J. Environ. Health, 1987; 29: 440-450.

[41] Moorthy KS, Kashi Reddy B, Swamy KS and Chethy CS. Changes in respiration and ionic content in the tissues of freshwater mussel exposed to methyl-parathion toxicity. Toxicol. Lett., 1984; 21: 287-291.

[42] Natarajan GM. Inhibition of branchial enzymes in snakehead fish (Channa striatus) by oxy demeton- methyl. Pest. Bioch. and Physiol.,1985; 23: 41-46.

[43] Hamen C. Aminotransferase activities and the amino acid excretion of bivalve mollusc and brachiopods. Comp. Biochem. Physiol., 1986; 26: 697-705.

[44] Nakano T, Tomilinson N. Catecholamine and carbohydrate concentration in rainbow trout (Salmo gairdneri) in relation to physical disturbance. J. Fish. Res. Bd. Can., 1967; 24: 1701-1715.

[45] Nelson DL and Cox MM. Lehninger Principles of Biochemistry. Macmillan worth Publishers, New York: 2002.

[46] Vorbrodt A. The role of phosphatase in intracellular metabolism. Postepy. Hig. Mws. Soaq., 1959; 13: 200-206.

[47] Sumner. The cytology and histo-chemistry of the digestive gland cells of Helix. Quart. J. Microscopic Sci., 1959; 106: 173-192.

[48] Abou-Donia MB. Increased acid phosphatase acivity in hens following an oral dose of Leptophose. Toxicology Letter, 1978; 2: 199-203.