International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# Effects of Rutin and Taraxerol Biologically Active Compounds In Their Binary Combinations Against Filarial Vector Culex Quinquefasciatus Larvae

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

[1]Research Scholar, Department Of Zoology, D.D.U. Gorakhpur University Gorakhpur, U.P. 273009, India
[2] Assistant Professor, Department of Zoology, Nandini Nagar Mahavidyalaya, Nawabganj, Gonda, UP-271303, India.
[3]Professor, Department of Zoology, D.D.U. Gorakhpur University Gorakhpur, U.P. 273009, India.

**ABSTRACT**
Botanical formulations could be used as eco-friendly products with proven potential as insecticide. Larvicidal activity of biologically active compounds i.e. Rutin and Taraxerol extracted from leaves of *Codiaeum variegatum* against larvae of *Culex quinquefasciatus* was studied in binary combinations. WHO protocols was adopted for the larvicidal bioassay. The active compound i.e. Rutin and Taraxerol extracted through ethyl alcohol and petrol as solvents from the leaves of *Codiaeum variegatum* used as volatile which was administered for 24h to 96h to the larvae of *Culex quinquefasciatus*. Exposure of larvae over different time durations. Sub-lethal doses (40% and 80% of LC50) (Table 1) of Rutin and Taraxerol in the ratio (1:1, 1:2, 1:5) forms significantly ($P<0.05$) altered the level of total protein, total free amino acid, glycogen and activity of enzyme acetylcholinesterase, acid and alkaline phosphatases activity in whole body tissue of *Culex quinquefasciatus* larvae. The alteration in all these biochemical parameters were significantly($P<0.05$) time and dose dependent.

**Keywords:** *Codiaeum variegatum*, Rutin, Taraxerol and *Culex quinquefasciatus*

## 1. Introduction

Vectors (Mosquitoes) is one of the greatest problem for public health which causes lymphatic filariasis, malaria,dengue, yellow fever etc. which affects lives of millions of people around the globe W.H.O. (1984),W.H.O. (1995) and Vatandoost, H.V.M. (2001).There are 3492 species of mosquitoes reported worldwide and out which more than hundred species can transmit various diseases in human and other vertebrates Rueda, L.M. (2008). Lymphatic filariasis (LF) is endemic in 82 countries of the world and recognized as potentially eradicable diseases. An estimated 300-500 million people are infected with malaria annually, resulting in 1.5-3 million deaths W.H.O. (2000). Elephantiasis is considered as the fastest spreading insect-borne disease of human in the tropic, about 30% (394 million) of the global at risk population is estimated to be in the endemic countries like African region W.H.O. (2006). Filariasis is a serious public health and economic problem in tropical and subtropical regions of the world which include India Satti et. al., (1974), El Setouhy et. al, (2003), Aiah, et. al., (2005). Malaria is a major health problem in world, about 20-40% of outpatient clinic visits and approximately 30% of total hospital admissions are due to malaria W.H.O. and UNICEF (2005). Natural products are best option because they are less harmful to environment and non-target organisms. Varieties of extracts and compounds from different plants families have been evaluated for new and promising larvicides, Ester Innocent Cosam et. al., (2008). Researchers have proved the effectiveness of plant derived secondary compounds like saponin Wiseman et. al., (2005), steroids, Chowdhury et. al., (2008), isoflavonoid, Joseph et. al., (2004), essential oil, Cavalcanti et. al., (2004), alkaloids and tannins, Khana et. al., (2007). as mosquito larvicides. Phytochemicals and their essential oils also serves as mosquito repellents, Yang et. al., (2004). *Codiaeum variegatum* is a medicinal plant used in traditional medication system and belongs to the family Euphorbiacae.

## 2. Materials And Methods

**Collection and maintenance of experimental *Culex quinquefasciatus* larvae**

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:**

Plant *Codiaeum variegatum* **(Figure 4)** (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 Rutin (**Figure 1)** 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 $35^0$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 72h at $30\text{-}40^0$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 un-dissolved part of the precipitate was dissolve 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 $194\text{-}196^0$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.

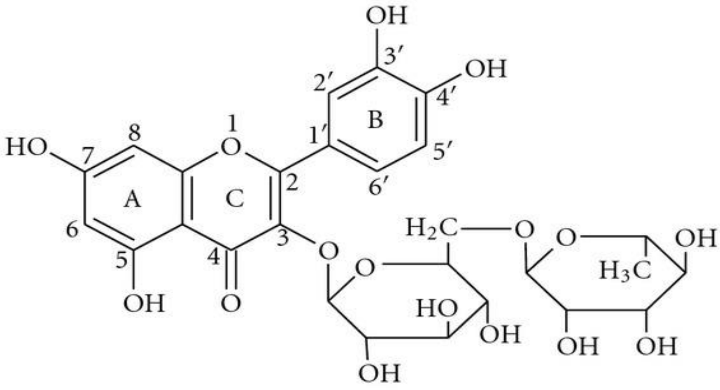



**Figure 1 :** Chemical Structure of Rutin

Pure Taraxerol (**Figure 2)** was isolated from the leaves of *Codiaeum variegatum* by the method of Chatterjee et. al., (1977**)**. The leaf of *Codiaeum variegatum* was dried in an incubator at about $35^0$C and powdered with the help of a mechanical device. From 50 g of dried powdered leaf, a concentrated solution of *Codiaeum variegatum* was extracted with petrol, for about 70 hours, in soxhlet apparatus **(Figure 3)**. After evaporation of the solvent by vacuum pump, the isolated compound in dried form was obtained. Taraxerol is soluble in organic solvents such as $CHCl_3$ and $CHCl_3$–MeOH. Confirmation of the compound was also made through IR and Rf values when compared to the authentic sample obtained from Sigma Chemical Company, USA.

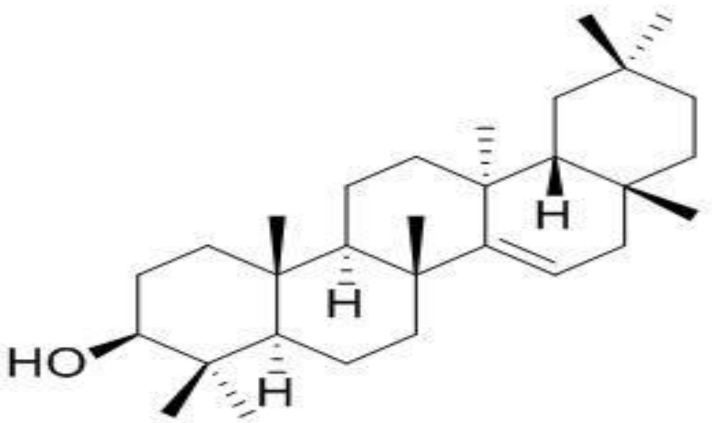



**Figure 2:** Chemical Structure of Taraxerol

**Figure 3:** Soxhlet Apparatus

**Figure** 4: *Codiaeum variegatum plant*

**Biochemical experiment:**

The late third instar larvae were treated with 40% and 80% of 24h $LC_{50}$ of Rutin and Taraxerol in binary combinations obtained from *the* leaves of *Codiaeum variegatum* 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 in (Table 1) , $LC_{50}$ 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 Sokal, R.R et. al., (1973), Prasad, S. (2003).

**Table 1:**Concentration of binary combinations of extracts used for biochemical experiments against *Culex quinquefasciatus* larvae.

| Active compounds combinations | Ratio | Sub-lethal doses (mg/L) 24h | |
|---|---|---|---|
| | | **40% of $LC_{50}$** | **80% of $LC_{50}$** |
| R + T | 1:1 | 13.59 | 27.18 |
| R + T | 1:2 | 15.48 | 30.95 |
| R + T | 1:5 | 19.34 | 38.69 |

- R – Rutin; T - Taraxerol

**Total protein**: Total protein level was estimated by the method of Lowry et. al., (1951). 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 Spies, J.R. (1957). Homogenates (10mg/mL) were prepared in 95% ethanol. Glycine was used as standard

.

**Glycogen:** Glycogen level was estimated by the method of Van der Vies (1954). Homogenate (10 mg/mL) was prepared in 5% TCA. Glucose was used as standard.

**Acetylcholinesterase activity**: Acetylcholinesterase activity was measured by the method of Ellman et. al., (1961). 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**: Acid and alkaline phosphatase activity was determined by the method of Andersch, M.A., Szcypinski, A.J. (1947), 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 Sokal, R.R et. al., (1973),

## 3. Results

In the present study exposure of Rutin and Taraxerol in their binary combinations, extracted from the leaves of *Codiaeum variegatum* plant induced significant behavioural changes in the larvae of mosquito *Culex quinquefasciatus*. The changes observed in the behaviour appeared after 4-5h of exposure. Larvae failed to emerge on the surface depicted restlessness, loss of equilibrium, lethargic and finally death. No such behavioural symptoms and mortality occurred in the control groups. This indicates that the plant moieties were responsible for altered behavior and larval mortality. The ratio results are given below-

**(A) Combination of Rutin and Taraxerol (1:1 ratio) extracts against *Culex quinquefasciatus* larvae:**

The mortality produced by Rutin and Taraxerol (1:1 ratio) for the periods ranging from 24 to 96h. The toxicity of Rutin and Taraxerol extract was time and dose dependent for *Culex quinquefasciatus* larvae. The $LC_{50}$ values of are shown in Table 16. There was a significant negative correlation between LC values and exposure periods. i.e. $LC_{50}$ values of combination extracts of *Codiaeum variegatum* leaf decreased from 41.96 mg/L (24h)>39.94mg/L (48h)>37.39mg/L (72h)>35.21mg/L (96h) in case of *Culex quinquefasciatus* larvae (Table 2;Figure 5).

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

The mortality produced by Rutin and Taraxerol (1:2 ratio) for the periods ranging from 24 to 96h (Table 3; Figure 6). The toxicity of Rutin and Taraxerol extract was time and dose dependent for *Culex quinquefasciatus* larvae. The $LC_{50}$ values of are shown in Table 17. There was a significant negative correlation between LC values and exposure periods. i.e. $LC_{50}$ values of combination extracts of *Codiaeum variegatum* leaf decreased from 49.27mg/L(24h)> 46.57mg/L (48h)> 43.17mg/L (72h)>40.30mg/L (96h) in case of *Culex quinquefasciatus* larvae (Table 3; Figure 6).

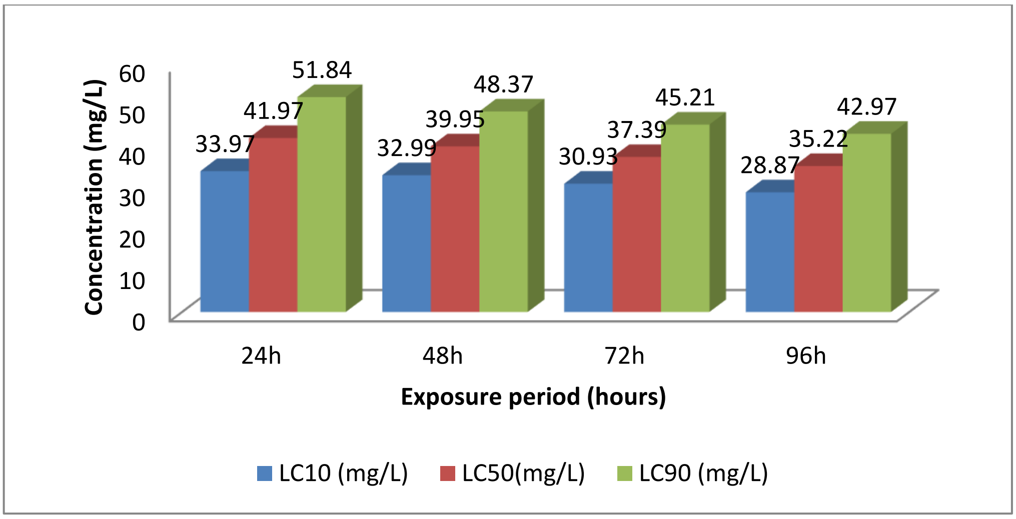
**(C) Combination of Rutin and Taraxerol (1:5 ratio) extracts against *Culex quinquefasciatus* larvae:**

The mortality produced by Rutin and Taraxerol (1:5 ratio) for the periods ranging from 24 to 96h (Table 4; Figure 7). 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 4. There was a significant negative correlation between LC values and exposure periods. i.e. $LC_{50}$ values of combination extracts of *Codiaeum variegatum* leaf decreased from 61.59mg/L (24h)>58.21mg/L (48h)>53.97mg/L (72h)>50.37mg/L (96h) in case of *Culexquinquefasciatus* larvae (Table 4; Figure 7).

**Table 2 :** Toxicity (LC values) of (**Rutin** and **Taraxerol, 1:1** ratio**)** of different concentrations extracted from ethyl alcohol of *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 | $LC_{10}$=33.97 | 23.50 | 36.76 | 13.97±7.86 | 2.84 | 0.02 |
| | $LC_{50}$=41.96 | 39.67 | 48.72 | | | |
| | $LC_{90}$=51.83 | 46.16 | 93.64 | | | |
| 48 | $LC_{10}$=32.99 | 24.53 | 35.72 | 15.43±7.70 | 3.19 | 0.08 |
| | $LC_{50}$=39.94 | 37.70 | 42.88 | | | |
| | $LC_{90}$=48.36 | 44.34 | 67.25 | | | |
| 72 | $LC_{10}$=30.93 | 21.08 | 34.07 | 15.55±7.82 | 3.16 | 0.05 |
| | $LC_{50}$=37.39 | 33.80 | 39.35 | | | |
| | $LC_{90}$=45.21 | 42.13 | 58.47 | | | |
| 96 | $LC_{10}$=28.86 | 14.89 | 32.73 | 14.83±8.33 | 2.82 | 0.16 |
| | $LC_{50}$=35.21 | 28.12 | 37.38 | | | |
| | $LC_{90}$=42.96 | 40.28 | 56.30 | | | |

- 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.



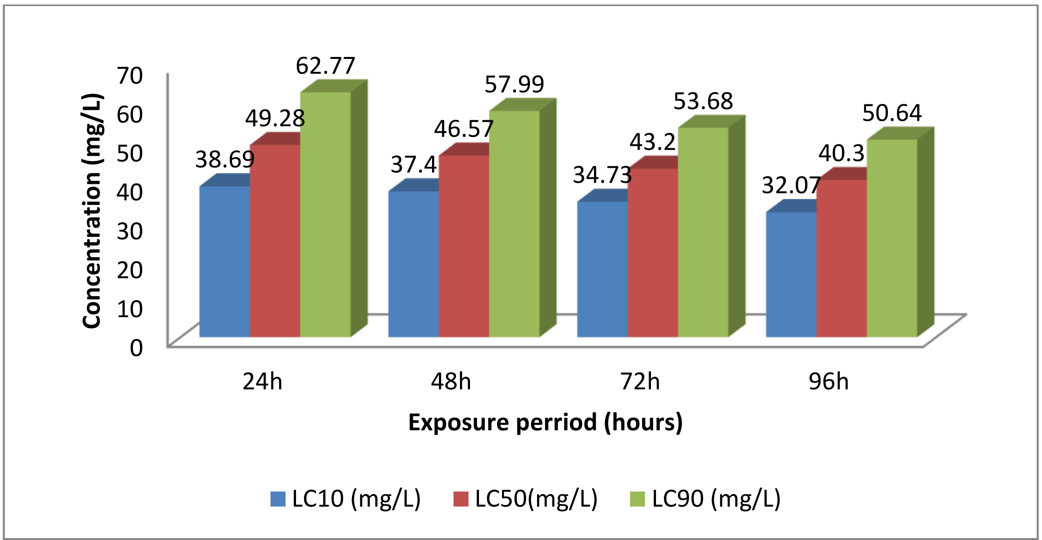

- **Figure 5:** -Bar diagram showing Rutin and Taraxerol (1:1 ratio) toxicity on *Culex quinquefasciatus* larvae extracted from the leaves of *Codiaeum variegatum* at different concentrations and at different time intervals.

**Table 3:** Toxicity (LC values) of (**Rutin** and **Taraxerol, 1:2** ratio**)** of different concentrations extracted from ethyl alcohol of *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 | $LC_{10}$=38.68 | 25.35 | 42.35 | 12.19±7.15 | 2.84 | 0.02 |
| | $LC_{50}$=49.27 | 46.20 | 58.46 | | | |
| | $LC_{90}$=62.76 | 54.97 | 123.61 | | | |
| 48 | $LC_{10}$=37.40 | 26.61 | 40.97 | 13.46±7.00 | 3.19 | 0.08 |
| | $LC_{50}$=46.57 | 43.58 | 50.58 | | | |
| | $LC_{90}$=57.98 | 52.50 | 84.63 | | | |
| 72 | $LC_{10}$=34.72 | 22.37 | 38.80 | 13.55±7.09 | 3.16 | 0.06 |
| | $LC_{50}$=43.17 | 38.44 | 45.78 | | | |
| | $LC_{90}$=53.67 | 49.50 | 72.09 | | | |
| 96 | $LC_{10}$=32.07 | 15.03 | 37.05 | 12.92±7.55 | 2.82 | 0.16 |
| | $LC_{50}$=40.30 | 31.14 | 43.15 | | | |
| | $LC_{90}$=50.63 | 47.02 | 69.00 | | | |

- 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 6 :-** Bar diagram showing Rutin and Taraxerol (1:2 ratio) toxicity on *Culex quinquefasciatus* larvae extracted from the leaf of *Codiaeum variegatum* at different concentrations and at different time intervals.

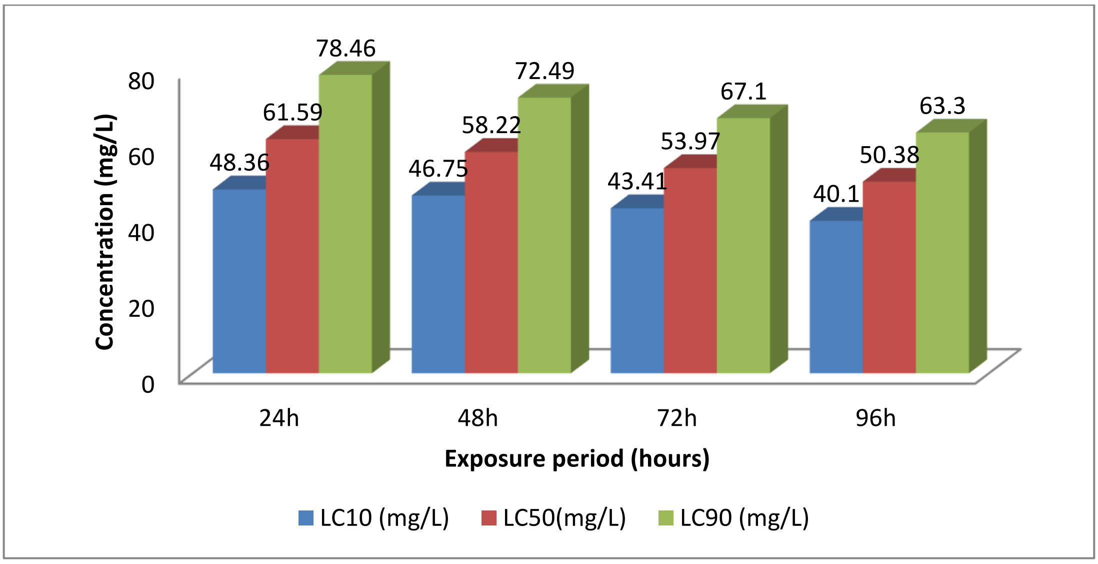
- * Values are mentioned in percentage.
- * Doses are 40% and 80% of $LC_{50}$ for period for which animals were exposed.
- * Significant ($P<0.05$) when two way analysis of variance was applied to see biochemical profile was time and dose dependent.
- * Significant ($P<0.05$) when Student 't' test was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

**Table 4:**Toxicity (LC values) of (**Rutin** and **Taraxerol 1:5** ratio**)** of different concentrations extracted from ethyl alcohol of *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 | $LC_{10}$=48.35 | 31.69 | 52.94 | 12.19±7.57 | 2.84 | 0.02 |
| | $LC_{50}$=61.59 | 57.75 | 73.08 | | | |
| | $LC_{90}$=78.45 | 68.71 | 154.52 | | | |
| 48 | $LC_{10}$=46.75 | 33.27 | 51.22 | 13.46±7.40 | 3.19 | 0.08 |
| | $LC_{50}$=58.21 | 54.47 | 63.14 | | | |
| | $LC_{90}$=72.48 | 65.62 | 105.79 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 72 | $LC_{10}$=43.41 | 27.96 | 48.50 | 13.55±7.50 | 3.16 | 0.06 |
| | $LC_{50}$=53.97 | 48.05 | 57.23 | | | |
| | $LC_{90}$=67.09 | 61.88 | 90.11 | | | |
| 96 | $LC_{10}$=40.09 | 18.78 | 46.31 | 12.92±7.99 | 2.82 | 0.16 |
| | $LC_{50}$=50.37 | 38.93 | 53.94 | | | |
| | $LC_{90}$=63.29 | 58.77 | 86.25 | | | |

- 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 7: -** Bar diagram showing Rutin and Taraxerol (1:5 ratio) toxicity on *Culex quinquefasciatus* larvae extracted from the leaf of *Codiaeum variegatum* at different concentrations and at different 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.

Bio-pesticides become advantageous due to the following reasons:

- Plant products having higher pesticidal activity.
- Bio-pesticides are non-toxic to higher animals.
- Plants are available in abundance in natural as well as in endemic areas.

- Application of bio-pesticides is simple and safe for operators.
- Bio-pesticides are cheaper than the synthetic ones.

  In the present investigation larvicidal properties of Rutin and Taraxerol in their binary combination extracted from the leaves of *Codiaeum variegatum* have been studied.

**4. Discussion**

We investegated that Rutin and Taraxerol in their binary combinations extracted from the leaves *of Codiaeum variegatum*, showed larvicidal activities against *Culex quinquefasciatus* larvae. Few plant products have showed effectiveness against mosquito population management, Sun, R et. al., (2006), Modupe Elizabeth Ojewum et. al., (2017) and 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 et. al., (2005) and Tamilselvan, P., et. al., (2015). 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 Benelli, G. P., 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 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. Borah, R et al., (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).

In the present study the Rutin and Taraxerol in their binary combinations showed larvicidal activity of *Culex quinquefasciatus* mosquitoes. Exposure to sub-lethal doses of Rutin and Taraxerol in their binary combination against 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 as ecdysial failure, abnormalities during intermediate stages, prolongation of the life span of treated instars, emergence of adultoids after treatment with Rutin and Taraxerol in their binary combination extracted with ethyl alcohol and petrol extract from the leaves of *Codiaeum variegatum*. This may be due to the effect of active moiety present in the plant extract combinations. 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 showed 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, Singh N. et. al., (1996) The decreased protein content resulted in destruction or necrosis of cells and consequent impairment in protein synthesis , Hamen C. et. al., (1986).

The total free amino acids content showed a significant increase in whole body tissue of mosquito larvae exposed to sub-lethal doses of Rutin and Taraxerol in their binary combinations. The rise in total free amino acids level in the whole body tissue depicted 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 Moorthy KS, et. al., 1984; Another reason for 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 GM. et. al., (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 Rutin and Taraxerol 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. et. al., (1986); which reduced glycogen reserves Nakano T, et. al., (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 TCA cycle Nelson DL. . et. al., (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 . et. al., (1959) has reported that this enzyme helps in the transport of metabolites across the membrane. The enzyme has been shown to be intimately associated with protein synthesis and is thus involved in the synthesis of certain enzymes Sumner et. al., (1959) ; Acid phosphatase is the lysosomal enzyme and plays a vital role in catabolism, pathological necrosis, autolysis and phagocytosis Abou-Donia MB. et. al., (1978).

## 5. Conclusion

The larvicidal activity of the Rutin and Taraxerol extracted through ethyl alcohol and petrol from the leaves of *Codiaeum variegatum* is highly toxic to larvae of *Culex quinquefasciatus* mosquito. The Binary combinations extract significantly decreased the population of the mosquito larvae by morphological, functional and physiological actions on larvae. Sub-lethal doses of biologically active compounds significantly altered the level of protein, amino acids, glycogen, enzyme activity like acetylecholinesterase, acid and alkaline phosphatase activity of *Culex quinquefasciatus* larvae. Therefore the plant extracts may be of great value for the management of vector in aquatic stages.

**6. 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. Chatterjee and A. Banerjee, F. Bohlmann (1977) Crotocaudin; a rearranged labdane type norditerpene from *Croton caudatus* geisel
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 OluwafunshoAdedokun., 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 Ocimumgratissimum 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 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): 1149–66.
38. Singh NN, Das VK and Singh S Effect of aldrin on carbohydrate, protein and ionic metabolism of a freshwater catfish Heteropneustesfossilis. 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.