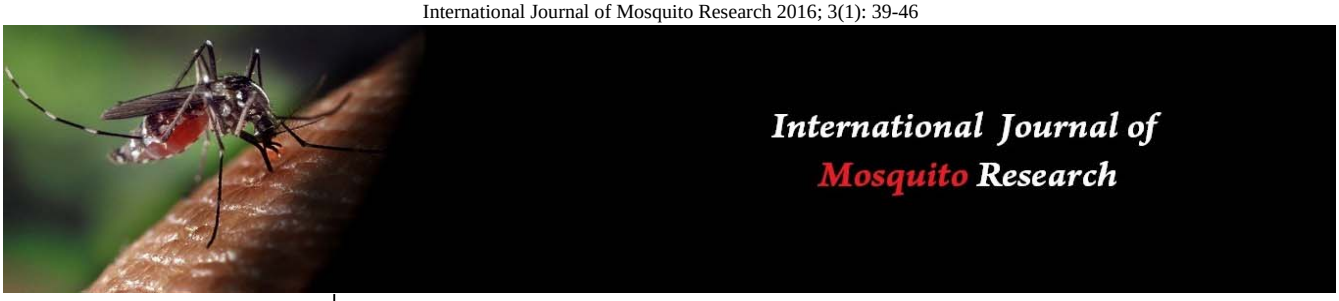

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Impact of sublethal conventional and biorational larvicidal stress on fitness status in nutritionally challenged *Aedes aegypti* larvae

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Abstract
In the present study we manipulated larval stressors (nutritional and larvicidal stress) by fabricating two larval microhabitats under laboratory conditions, aiming to determine the physiological and biochemical fitness of *Aedes aegypti* larvae. Sublethal exposures (LC_{10}, LC_{25} and LC_{50} for 24, 48 and 72 hr) with selected larvicides along with nutritional challenge adversely affected larval developmental process and fitness status of *Ae. aegypti* larvae *viz.* prolonged larval and pupal period, larval and pupal deformities, malformations. Maximum significant (P<0.05, P<0.005 and P<0.001) decrease of glycogen (14-87%), sugar (32–92%) and lipid (48–92%) content was noticed in Bti, neem oil, permethrin followed by pyriproxyfen and minimum decline with triflumuron treatment. Collectively these findings represented that all applied stressors drastically affected the nutritional status of larvae and hindered larval metamorphosis thereby suppressing the adult emergence.

Keywords: *Aedes aegypti*, fitness status, nutrition depletion index, nutritional stress, larvicidal stress

1. Introduction
Aedes aegypti, principal vector of dengue fever, chikungunya and yellow fever, is widely distributed in tropical and subtropical regions of the world. It is a peridomestic, diurnally active mosquito is being adapted to utilize all forms of manmade aquatic habitats like air coolers, septic tanks, discarded and unused artificial containers, metal cans, tire dumps, tree holes and bird baths which are especially close to human habitation. In these unpredictable habitats mosquito larvae are exposed to various types of challenging factors in their unstable and dynamic environmental as well as ecological conditions including nutritional scarcity, overcrowding, struggle for food, ever changing temperatures [1], presence of toxic metals and some physiological stressors [2, 3] that can affect larval metamorphosis and survival period. Adult mosquito fitness is also affected by environmental conditions experienced during larval growth period. Several larval stress studies were conducted and revealed that artificially/naturally induced stress during larval developmental period can cause significantly smaller females, changes in adult mosquito phenotype and immunity that can increase their susceptibility to pathogens including arboviruses [4, 5, 6] andreduced fecundity of female mosquitoes [7].
Larval nutrition and availability of food are the two important factors which influence larval survivorship and development as well as the vectorial capacity and size of emerging matures [8, 9, 10]. The factors like starvation, competition, temperature and overcrowding have also been associated with alterations in teneral energy reserves, fecundity [11] and reproductive capacity of female mosquitoes [12,13,14]. In mosquito life cycle, pupal phase is non feeding stage, hence energy reserves collected during the larval developmental period and sugar feeding as adults plays a crucial role for adult mosquito existence and reproductive success [11, 15-16]. In *Ae. aegypti* larvae lipids are mainly acquired during larval feeding phase for long-term maintenance [13, 17] whereas, sugars and glycogen reserves are primarily required for flight and these determinants also control the mating success of adult mosquitoes [18]. This implies that nutritional depletion at larval stages may affect survivorship, reproductive and vectorial capacity of adult female mosquitoes. Therefore, it is important to know what factors influence the larval nutritional intake and larval metamorphosis process and thereby altering the adults physiological conditions. Keeping these facts in view, the present study was planned to explore the comparative effectiveness of various conventional and biorational larvicides on the fitness status of nutritionally challenged *Ae. aegypti* larvae following sublethal exposure.

2. Materials and methods

Present study was conducted in the Agra city located in the extreme South-West corner of Uttar Pradesh (27°10'N78°05'E) for the period 2012-2014.

2.1 Larvicides and preparation of stocks

Permethrin, pyriproxyfen, triflumuron were procured from Sigma–Aldrich (USA). Neem oil (1300 ppm) and Bacticide (*Bacillus thuringiensis israelensis*) spores were purchased from, RYM Exports (Mumbai, India) and Biotech International Ltd. (New Delhi, India), respectively.Stock solutions (0.1%) of larvicides were prepared according to the standard method recommended by WHO [19]. Serial dilutions were made from stocks to prepare different sublethal concentrations (LC_{10}, LC_{25} and LC_{50} for 24, 48 and 72 hr) in distilled water.

2.2 Determination of sublethal concentrations

Batches of 25 larvae of early IV instar were transferred to beakers containing 200 ml of distilled water. Various sublethal concentrations (LC_{10}, LC_{25} and LC_{50}) of conventional and biorational larvicides were selected on the basis of conducted efficacy studies against IV instar *Ae. aegypti* larvae which ranged between 0.00005-0.005 ppm (permethrin), 0.0005-0.005 ppm (pyriproxyfen), 0.0008-0.005 ppm (triflumuron), 0.03-0.5 ppm (neem oil), 0.08-0.5 ppm (Bti). Suitable volumes of dilutions were added to the beakers containing 200 ml of water to attain desired sublethal concentrations, starting with the lowest concentration. Similar conditions were maintained for controls with distilled water only and experiments were conducted in triplicates.

2.3 Assessment of the fitness status of *Aedes aegypti* larvae

2.3.1 Physiological status

Early fourth instar larvae were used for this study. After determining the sublethal concentrations (LC_{10}, LC_{25} and LC_{50}) of various larvicides for 24, 48 and 72 hr we set up the experiment in triplicates consisting of beakers with 200 ml of distilled water and sprinkled with larval food (10 mg of tetramine fish food per beaker and twice a day). Batches of 25 larvae were transferred to each beaker. The experimental setup was continued until adult emergence. Similar conditions were maintained for controls but without larvicidal treatments. We observed the physiological status of treated larvae in terms of larval and pupal developmental days, larval pupal deformities, adult emergence inhibition and growth index. For each consecutive day dead and alive larvae and pupae were counted followed by adults. Growth index was calculated as follows:

$$\text{Growth index} = \frac{\text{Adult emergence (\%)}}{\text{Total larval days}}$$

2.3.2 Nutritional status

For this study we maintained two larval micro-habitats under laboratory conditions. Which are as follows: (1) Larvicidal and nutritionally challenged larvae (LNC) where larvae were reared under larvicidal and nutritional stress. For this group we also maintained controls without larvicidal stress but they are nutritionally challenged. (2) Larvicidal stress and optimal nutrition (LON) where larvae were reared under larvicidal stress but supplied with optimal food whereas, in control larvae maintained in normal conditions i.e. without larvicidal and nutritional stress. *Ae. aegypti* larvae were exposed to above said stress conditions. Each beaker consists of 20 IV instar larvae and filled with 200 ml of distilled water and sprinkled with larval food (10 mg of tetramine fish food per beaker and twice a day) according to the test groups. After 24, 48 and 72 hr of exposure the survived larvae from various treatments were taken out to measure the biochemical fitness parameters.

2.3.3 Extraction and estimation of energy reserves

From each treatment larvae were picked up and crushed in 200 µl of sodium sulfate solution and further processed for extraction of glycogen, sugar and lipid [20]. Glycogen and sugar content of control and treated mosquito larvae was estimated by using the hot anthrone assay. The absorbance was recorded at 625 nm [21]. Lipid content was measured by using vanillin–phosphoric acid reagent [22] and the absorbance was read at 525 nm. The amount of glycogen, sugars and lipid in each sample was calculated in µg by using standard graphs. The values of sugars, glycogen and lipid in µg were converted into joules [23]. The total nutrition (glycogen + sugar + lipid) depletion index (NDI) was calculated as follows:

$$NDI = [(C - T)/(C + T)] \times 100$$

Where C is the control total energy reserve and T is total energy reserves (glycogen+sugar+lipid) present in treated larvae.

2.4 Statistical analysis

Significant difference in adult emergence inhibition and growth index were determined by using student t-test while the effect of nutrition and larvicidal treatments on larval energy reserves, significant differences in control and treatments were analysed using Two-way ANOVA and further Bonferroni post hoc test. Statistical tests were analysed using Graph Pad Prism version 6.0 (Graph Pad Software, Inc., San Diego, USA) and considered significant at $P<0.05$, $P<0.01$ and $P<0.001$.

3. Results

3.1 Effect of larvicidal stress on physiological status

3.1.1 Larval and pupal duration

Ae. aegypti larvae reared under the larvicidal-stress treatment exhibit a visibly prolonged larval and pupal developmental period when compared with controls. The untreated larvae showed a larval developmental period (from early IV instar larvae to pupae) of 3.6 days whereas, larvae treated with neem oil (8 days) showed maximum prolongation in larval duration followed by pyriproxyfen (6.3 days), permethrin (6 days), triflumuron (6 days) and Bti (4 days) (Fig. 1). In contrast pupae emerged from stress induced larvae showed reduced pupal duration when compared with controls. The duration of pupal period in untreated larvae was noticed as 2.3- 3.3 days which were reduced by 0.3-1.7 (permethrin), 0.4- 1 (pyriproxyfen), 0.7-1.7 (triflumuron) and 1-2 (Bti) days. But pupae which were emerged from pyriproxyfen and neem oil treatment prolonged the pupal developmental period (Fig. 1) when compared with control.

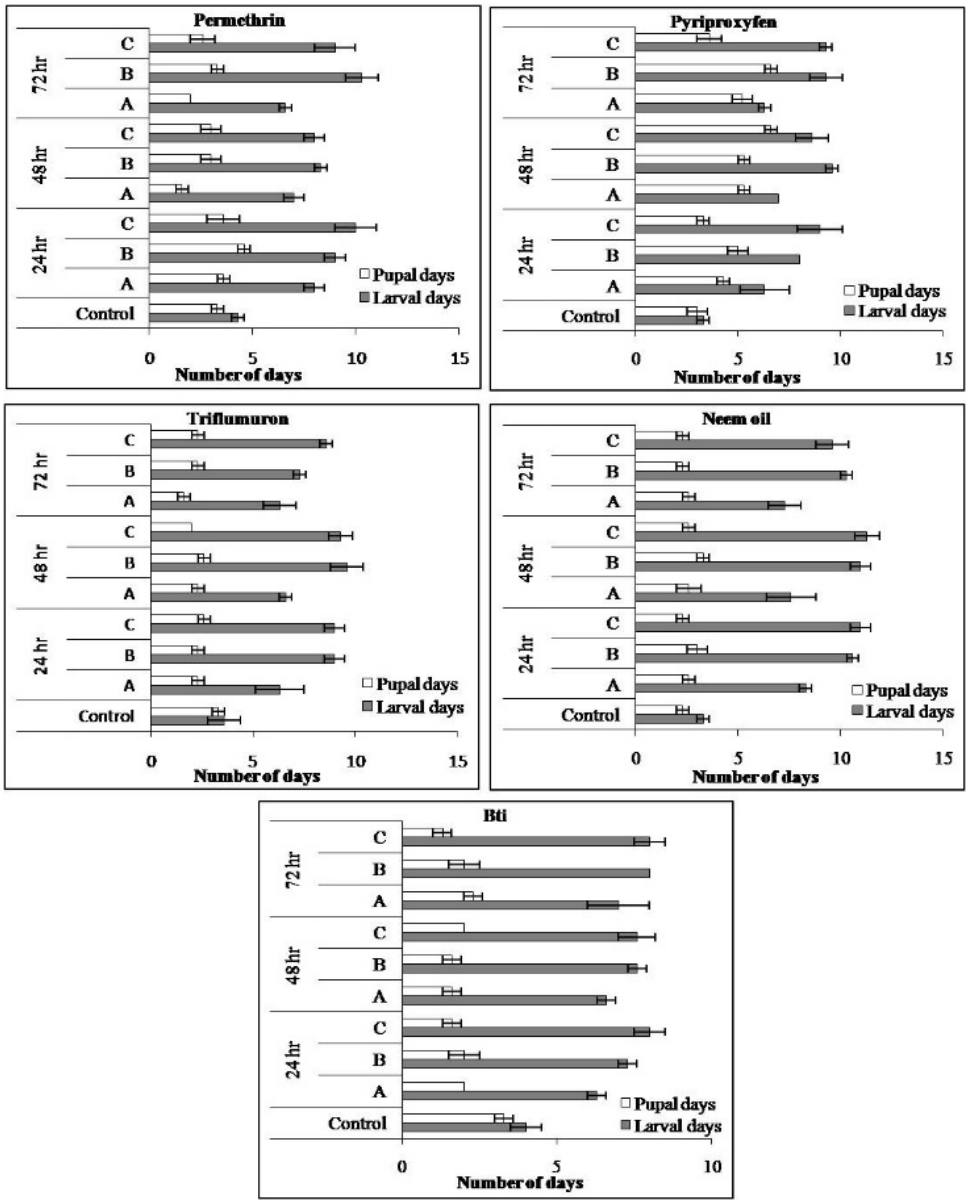

Fig 1: Mean larval and pupal developmental period of *Ae. aegypti* following treatment with various larvicides. Alphabets indicate: A - LC_{10}; B - LC_{25}; C - LC_{50}

3.1.2 Stress induced metamorphosis in *Ae. aegypti* larvae

Highest level of disturbance such as larval and pupal deformities, malformations and changes in physiological processes was noticed in larvae treated with permethrin, neem oil and Bti (Table. 1). Most notably, morphological and behavioural changes were observed in larvicidal stress induced larvae such as curling up, vigorous body movements and discoloration of larvae. Overall examination of malformed pupae showed features like incomplete larva-pupal transformation, partially emerged pupae appended with larval head capsule, underdeveloped pupae and incompletely developed dark colour pupae with outsized abdomen. The metamorphic abnormalities were also found in newly emerged adults from stress induced pupae such as incomplete pupal-adult transformation process, partially emerged dead adults from pupal exuvia and lack of ability in matures to shack their pupal skin, which remain attached to their appendages. Maximum adult abnormalities were observed with pyriproxyfen (2.6-6.6%) and neem oil (1.3-6.6%) followed by permethrin (1.3-5.3%) exposure. Whereas, treatment with Bti and triflumuron did not exhibits any apparent adult abnormalities. Maximum significant (P<0.05, P<0.005 and P<0.001) inhibition in adult emergence was noticed in pyriproxyfen (94%) and permethrin (89%) treatments followed by triflumuron (77%), neem oil (64%) and Bti (58%) exposed larvae. Significant reduction (P<0.05, P<0.005 and P<0.001) in growth index was observed in every treatment when compared with untreated individuals. We recorded highest growth index reduction in pyriproxyfen (60-98%) and permethrin (51-90%) followed by triflumuron (50-85%), neem oil (55-82%) and Bti (26-66%) exposed larvae. Growth index was found to be dose and time dependent. Maximum reduction in growth index level was seen in 24 hr (LC_{50}) larvicidal treatment followed by 48 hr (LC_{50}) and 72 hr (LC_{50}) larvicidal treatments.

Table 1: Physiological status of *Ae. aegypti* larvae treated with various conventional and biorational larvicides.

Hr	Treatment	Adult Emergence Inhibition (%)	Adult Malformation (%)	Growth Index
	Control	2.6±1.3	0±0	12.7±0.5
24	LC_{10}	50.6±3.5^{c}	4.0±0.5	4.1±0.2^{a}
	LC_{25}	69.3±7.0^{d}	5.3±2.6	2.0±0.4^{a}
	LC_{50}	84.0±3.1^{a}	1.3±0.7	1.2±0.4^{a}
48	LC_{10}	48.0±3.1^{c}	1.3±0.3	6.2±1.4^{d}
	LC_{25}	64.0±5.9^{d}	2.6±1.3	3.1±0.4^{c}
	LC_{50}	89.0±2.6^{a}	4.0±2.3	1.2±0.3^{a}
72	LC_{10}	42.6±5.8^{d}	4.0±2.3	6.6±0.9^{d}
	LC_{25}	61.3±7.0^{d}	4.0±1.3	2.8±0.6^{c}
	LC_{50}	77.3±6.6^{c}	4.0±0.5	1.7±0.4^{a}
	Control	2.6±1.3	0±0	15.4±0.8
24	LC_{10}	54.6±1.3^{a}	2.6±0.8	5.3±0.5^{c}
	LC_{25}	74.6±2.6^{a}	4.0±2.3	2.3±0.1^{a}
	LC_{50}	85.3±1.8^{a}	4.0±1.7	1.3±0.2^{a}
48	LC_{10}	56.0±2.3^{a}	2.6±1.3	4.6±0.l^{c}
	LC_{25}	89.3±2.3^{a}	5.3±1.3	2.2±0.08^{a}
	LC_{50}	89.3±2.7^{a}	2.6±0.8	0.9±0.2^{a}
72	LC_{10}	49.3±2.6^{a}	6.6±3.5	6.1±0.4^{d}
	LC_{25}	72.0±2.3^{a}	4.0±2.3	2.2±0.1^{a}
	LC_{50}	94.6±1.2^{a}	4,0±1.6	0.3±0.08^{a}
	Control	1.3±1.3	0±0	14.2±1.0
24	LC_{10}	42.6±5.3^{d}	6.6±1.3	6.6±0.4^{d}
	LC_{25}	53.3±1.3^{a}	9.3±2.6	4.1±0.1^{d}
	LC_{50}	76.0±2.3^{a}	4.0±2.3	2.1±0.2^{c}
48	LC_{10}	37.3±1.3^{a}	5.3±1.3	7.0±0.5^{d}
	LC_{25}	50.6±3.5^{c}	4.0±2.3	4.2±0.4^{d}
	LC_{50}	74.6±1.3^{a}	2.6±0.6	2.3±0.03^{d}
72	LC_{10}	37.3±1.3^{a}	1.3±0.5	7.9±0.7^{d}
	LC_{25}	46.6±1.8^{a}	4.0±2.3	5.5±0.1^{d}
	LC_{50}	77.3±2.6^{a}	2.6±1.3	2.1±0.2^{d}
	Control	5.3±1.3	0±0	16.8±1.2
24	LC_{10}	38.6±1.7^{a}	4.0±2.3	5.5±0.2^{d}
	LC_{25}	41.3±2.1^{c}	6.6±3.5	4.2±0.1^{d}
	LC_{50}	61.3±1.3^{a}	6.0±0.6	2.8±0.1^{d}
48	LC_{10}	24.0±4.0^{d}	0±0	7.3±0.4^{d}
	LC_{25}	45.3±2.6^{c}	3.0±0.8	3.7±0.2^{c}
	LC_{50}	58.6±3.5^{c}	2.6±0.6	2.9±0.2^{c}
72	LC_{10}	25.3±2.6^{d}	2.6±0.6	7.5±0.4^{d}
	LC_{25}	49.3±4.6^{d}	1.3±0.8	4.0±0.5^{d}
	LC_{50}	64.0±4.0^{c}	2.8±0.9	2.9±0.2^{c}
	Control	2.6±1.3	0±0	13.3±2.4
24	LC_{10}	22.6±1.3^{c}	2.6±1.3	9.3±0.4^{p}
	LC_{25}	34.6±2.6^{c}	0±0	7.0±0.5^{p}
	LC_{50}	57.3±3.1^{a}	4.0±1.6	4.4±0.4^{p}
48	LC_{10}	18.6±2.8^{d}	0±0	9.8±1.0^{p}
	LC_{25}	33.3±3.5^{d}	4.0±1.6	7.1±0.6^{p}
	LC_{50}	58.6±2.9^{d}	3.0±0.8	4.4±0.0^{p}
72	LC_{10}	25.3±5.3^{d}	0±0	8.4±0.8^{p}
	LC_{25}	33.3±3.5^{a}	2.0±0.2	6.6±0.08^{p}
	LC_{50}	57.3±2.6^{b}	2.6±0.3	4.5±0.3^{p}

Data represented as mean ± S.E.M (n=3). Alphabets indicate significant differences between control and treatments ($^{a}P<$ 0.0001, $^{b}P<$ 0.001 $^{c}P<$0.0005, $^{d}P<$0.005 and p - non significant)

3.2 Effect of nutritional and larvicidal stress on nutritional status

The two different fabricated larval rearing conditions produced significant effects on the nutritional status in all treatment groups when compared with controls. LNC group has recorded significantly lesser nutrient levels (presented in terms of energy Joules/larva) than LON group.

3.2.1 Glycogen

Glycogen content was significantly ($P<0.05$, $P<0.005$ and $P<0.001$) affected in both treatment groups (Fig. 2). In LNC group maximum reduction in glycogen content was observed in Bti (87%) treatment following neem oil (86%), permethrin (84%), pyriproxyfen (64%) and triflumuron (60%) treatment after 24 hr (LC_{50}) which was slightly elevated at 48 hr and 72 hr post treatments when compared with respective control group. While in LON group food provided to the larvae had slightly elevated the levels of glycogen but significantly affected by larvicidal treatments. LON group also exhibited reduced glycogen level at 24 hr larvicidal treatment which was increased slightly at 48 hr and 72 hr exposure.

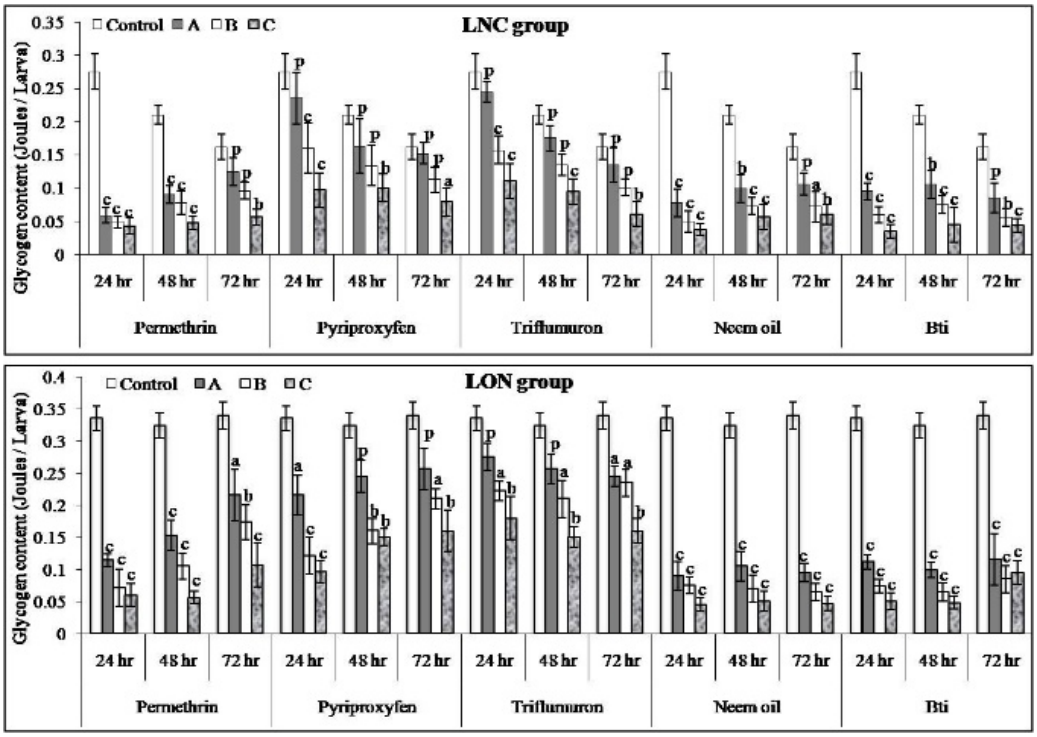

Fig 2: Effects of larvicidal treatment following with or without food on glycogen content of *Ae. aegypti* larvae. Data represented as mean ± S.E.M (n=3). Alphabets indicate significant differences per larva between control and treatments ($^{a}P< 0.05$, $^{b}P< 0.01$, $^{c}P < 0.001$ and p - non significant).

3.2.2 Sugar

Sugar profile of treated and untreated larvae in both the groups (LNC and LON) has been depicted in Fig. 3. The result revealed that the highest amount of sugar decrease was recorded at 24 hr (LC_{50}) in larvae exposed to neem oil (92%) followed by permethrin (86%), Bti (84%), pyriproxyfen (67%) and triflumuron (59%) treated larvae (LNC group). In LON group sugar content was found to be decreased significantly (P<0.05, P<0.005 and P<0.001) by 90%, 88%, 88%, 77% and 75% in 24 hrs (LC_{50}) treatments of permethrin, neem oil, Bti, pyriproxyfen and triflumuron, respectively.

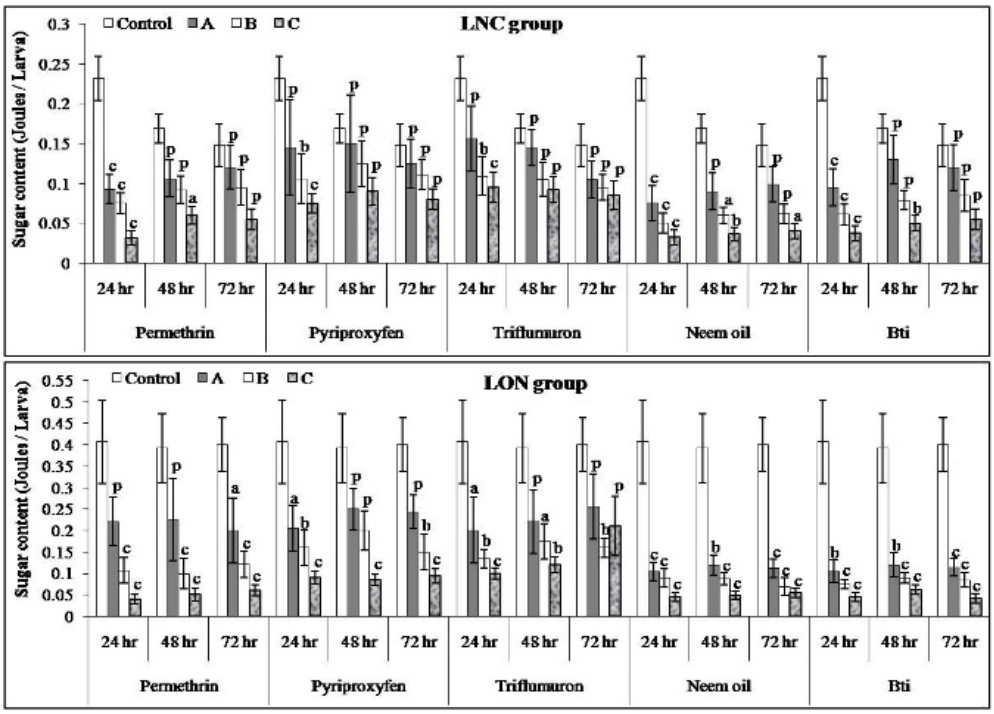

Fig 3: Effects of larvicidal treatment following with or without food on sugar content of *Ae. aegypti* larvae. Data represented as mean ± S.E.M (n=3). Alphabets indicate significant differences per larva between control and treatments ($^{a}P< 0.05$, $^{b}P< 0.01$, $^{c}P < 0.001$ and p - non significant).

3.2.3 Lipid

Lipid content was significantly decreased ($P < 0.01$ and $P < 0.001$) in both treatment groups LNC and LON (Fig. 4). In LNC group, lipid profile maximum decrease was observed in 72 hr (LC_{50}) treatment followed by 48 hr (LC_{50}) and 24 hr (LC_{50}) treatments. For LNC group, highest amount of reduction in lipid profile was recorded by permethrin (92%), neem oil (82%) and then by Bti (81%). Whereas, in LON group highest drop off in lipid content was noticed at 24 hr (76%-86%) treatment followed by 48 hr (73%-85%) and 72 hr (74%-84%) treatments.

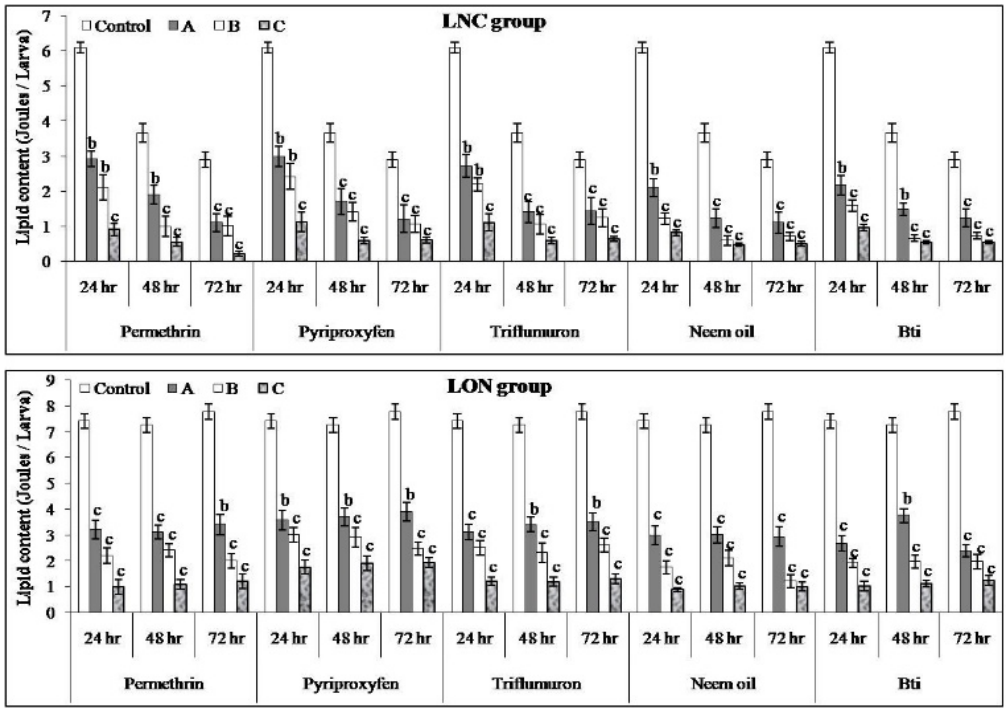

Fig 4: Effects of larvicidal treatment following with or without food on lipid content of *Ae. aegypti* larvae. Data represented as mean ± S.E.M (n=3). Alphabets indicate significant differences per larva between control and treatments ($^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ and p - non significant).

3.3 Nutrition depletion index (NDI)

NDI depicts percent depletion in total energy reserves of treated and untreated larvae. By using NDI we can compare the effectiveness of applied conventional and biorational larvicides in terms of their reduction in larval energy reserves. NDI was found to be concentration dependent where maximum depletion in total energy reserves was observed in larvae treated with LC_{50} concentration of every applied larvicide. It is considered that nutritional depletion occurs when the NDI is superior to 75%, moderate when it is between 50-75% and low when it is below 50%. In LCN group permethrin (74-81%) was found to be superior than neem oil (76-68%) and Bti (72-66%) originated as superior to moderate followed by pyriproxyfen (67-61%) and triflumuron (66-60%) were found as moderate whereas, in LON group neem oil (78-76%) was considered as superior, permethrin (76-72%) and Bti (75-71%) were considered as superior to moderate while pyriproxyfen (61-58%) and triflumuron (69-66%) were recorded as moderate to low.

4. Discussion

Dengue and chikungunya are frequently emerging diseases in India due to positive ecological and environmental conditions for the breeding of *Ae. aegypti*, a major vector responsible for the transmission of these diseases. They become very well established owing to their incredible ability to adjust to any type of environment ranging from artificial containers to tree holes near human surroundings. Larvicides/insecticides which were applied on *Ae. aegypti* larvae comprise various damaging and depressing effects on their growth and development by inducing alterations at molecular, cellular, metabolic and biochemical level. In current study, a comparative effect of different conventional and biorational larvicides has been tested on IV instar larvae of *Ae. aegypti* with respect to their physiological and nutritional status.

4.1 Physiological status

Mosquito is a holometabolous insect which passes through complete (egg, larvae, pupae and adult) and complicated metamorphosis process. In this study we observed larval and pupal developmental days, malformations, and emergence inhibition of adults. The mode of action of all these applied conventional and biorational larvicides may be clearly diverse but physiological and behavioural changes occurred in larvae are comparable with each other. Utmost level of disturbance in normal behavioural, morphological and physiological processes was noticed in larvae treated with pyriproxyfen and permethrin followed by triflumuron, neem oil and Bti. Senthilkumar*et al*. [24] also reported this type of behavioural changes in treated larvae like curling up, vigorous body movements and discoloration shows kind of neurotoxicity. In the current study we noticed extended larval duration time due to exposure of larvae to various sublethal treatments. This kind of extensions in larval stage was also reported by few researchers when larvae treated with neem extract [25, 26]. We have also recorded that pupae emerged from treated larvae

exhibited reduced pupal duration in all treatments except for neem oil treated pupae which showed delay in pupal duration. Our results were comparable with Shaalan *et al*. [27] as they also showed reduction and delay in larval and pupal period when treated with secondary metabolites of some plant species.

In this study, we also observed various anomalies and malformations such as partially emerged pupae with oversized abdomen, dark colour dwarf sized pupae, half emerged adults from pupal exuvia and dead adults appendages attached with pupal skin in newly emerged pupae and adults from stress induced larvae. This type of regulatory effects during moulting process were under the control of the juvenile hormones (JH) [28] and ventral nerve cord neurosecretory cells, which releases the tanning hormone [29]. Hence, conventional and biorational larvicides exhibited inhibiting effects on larvae and influenced JH synthesis process which hindered larval metamorphosis processes and thereby suppressing the adult emergence.

4.2 Nutritional status

This study showed that starvation, feeding and larvicidal treatments have detrimental effects on the larval energy reserves, and consequently on larval development and behaviour of *Ae. aegypti*. Our results were comparable with other researchers that had reported depletion of larval energy reserves when exposed to any type of stress (environmental, chemical and nutritional) [20, 24, 29, 30, 31].

Ae. aegypti larvae treated with conventional and biorational larvicides exhibited significantly lower energetic reserves (glycogen, sugars and lipids) than untreated larvae. Such a decrease in energetic resources can have drastic consequences for the vectorial capacity of female mosquitoes [32]. In the present study, drastic decrease in glycogen and sugar content was noticed in both treatment groups LNC and LON. Maximum decrease of glycogen and sugar content was noticed in Bti, neem oil, permethrin followed by pyriproxyfen and minimum decline with triflumuron treatment. Highest drop off in these indices was observed at 24 following 48 and 72 hr treatment. It is well recognized that throughout larval development, extensive biosynthesis of energy reserves such as protein, carbohydrate and lipids occur in larvae which act like precursors for the transformation of larvae into pupae and adults [33]. Carbohydrates, such as sugars and glycogen, are the fuels for mosquito flight and distance flown by mosquitoes and has been dependent on availability of sugars [23].

Lipid content was also found to be drastically decreased in the treated larvae at each and every concentration (both groups LNC and LON) suggesting that treatment with these conventional and biorational larvicides reduced feeding and appropriate use of supplemented nutrients in group LON. Highest drop off in lipid content was noted at 72 hr followed by 48 hr and 24 hr which are in contrast with glycogen and sugar content. While maximum reduction was observed in permethrin, neem oil, Bti and least in pyriproxyfen and triflumuron. Similar, decrease in feeding was reported in *Anopheles*, *Culex* and *Aedes* following treatment of neem [34]. Lipids are the foremost energy reserves for insect continued existence and reproductive capacity of females [23]. Lipids are also an important source of the acetyl groups needed to synthesize the enzymes constitutive amino acids [32].

It is a universally accepted fact that the stress response as a whole is characterised by depleted larval energy reserves in both treated groups LNC and LON. Another possibility is that energy resources might have been utilized by the larvae either to restore the damage or defend itself from the applied stress on the larvae in the form of starvation and larvicidal treatments. We observed that both the groups (LNC and LON) showed significantly reduced energy levels indicating that larvicidal stress was not affected much by presence or absence of food. However, it is clear that applied conventional and biorational larvicidal stress has great impact on potential fitness of larvae and subsequently on emerging adults, particularly in conditions where larvae were nutritionally challenged.

5. Conclusion

In the present study we manipulated larval stressors (nutritional and larvicidal stress) by fabricating two larval microhabitats under laboratory conditions, aiming to determine the physiological and biochemical fitness of *Ae. aegypti* larvae.The impact of externally induced stress signs were measured by various parameters in the form of alterations occurred in physiological (developmental time, malformations, emergence inhibition) and nutritional status (glycogen, sugar and lipid) of IV instar *Ae. aegypti* larvae. Sublethal exposures (LC_{10}, LC_{25} and LC_{50} for 24, 48 and 72 hr) with selected larvicides along with nutritional challenge adversely affected larval developmental process and fitness status of *Ae. Aegypti* larvae. All stressors had a consistent effect of significant reduction in energy reserves relative to the controls. Maximum level of disturbance such as prolonged larval and pupal period, larval and pupal deformities, malformations and changes in physiological processes was noticed in larvae treated with permethrin, neem oil and *Bacillus thuringiensis israelensis* (Bti). Maximum significant ($P<0.05$, $P<0.005$ and $P<0.001$) decrease of glycogen (14-87%), sugar (32–92%) and lipid (48–92%) content was noticed in Bti, neem oil, permethrin followed by pyriproxyfen and minimum decline with triflumuron treatment. Highest drop off in these indices was observed at 24 hr (LC_{50}) and least at 72 hr. Nutrition depletion index was found to be concentration dependent depicting maximum reduction at LC_{50} sublethal exposure. Collectively these findings represented that all applied stressors drastically affected the nutritional status of larvae and hindered larval metamorphosis thereby suppressing the adult emergence.

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7. References

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