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

Ecological And Behavioral Factors to Aid in Modelling Environmental Mosquito Control & To Design Larval Robots

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Abstract: The anatomical location of human pathogens in mosquitoes ranging from Plasmodium, Wucheriria, flavivirus and other viruses are in salivary glands of the mosquitoes which is important for transmission. Further Dengue virus (DENV) is known to exist from egg stage to adult stage in mosquitoes. The other important fact in mosquito borne transmission is the blood meal that renders them capable of egg laying. Hence hormonal, ecological, behavioral control of mosquitoes with environmental thyroxine, larval behavior pattern, predatory dragonfly larva, air turbulence g-force was studied. Environmental contamination of shallow water bodies with animal urine could introduce thyroxine in water bodies where mosquito larva is breeding. Modeling such an outcome, the results indicated significance influence of thyroxine on mosquito larva resulting in alteration of eclosed mosquito size, weight, sex ratio, antennal hairs. The second mode of ecological control with predatory dragonfly larva in water bodies modeled with introduced coal resulted in excellent mosquito larval control. The third mode of using air turbulence g-force (with home appliances like ceiling fan) induced mosquito control was also found to be effective in reducing the no of hatched larva when the adult mosquitoes are exposed to g-force but it did increase the longevity of male mosquitoes. Further based on mosquito larval behavior pattern, it was observed that mosquito larva does have the tendency to be social and tend to meet each other in water bodies that can be used for mosquito control by design of larval robots that can deliver larvicidal compounds on one-to-one basis.

Key words: Mosquito, thyroxine, bio control, dragonfly larva, G-force, robots

1.1 Introduction

It is established that there are different cascade of events post blood meal in a mosquito culminating in egg laying. It is also known that mitochondrial remodeling takes place as a consequence of blood meal. Hence by exposing mosquitoes to g-force that would involve lots of energy might involve mitochondria. Hence g-force exposure in mosquitoes after blood meal might influence their egg laying capacity that was

studied in this research. Further it is known that the size of the mosquitoes influences its vector nature and hence by using overcrowding as tool at larval stage different sized mosquitoes were reared. Size of the vector (*Aedes*) has been negatively correlated to dengue virus infectivity capacity (1) and has also been controversially reported earlier (2). Factors like overcrowded larval densities have been associated with reduced size in *Aedes* pupa (3).

It is known that insect hormones do influence molting, although the mammalian hormone thyroxine is not known to be expressed in invertebrates including the larval stages of mosquitoes, it might have an effect similar to insect hormones as T3 can mimic insect juvenile hormone structure and function in *Locusta migratoria* and *Rhodnius prolixus* (4). If Dengue virus can aid in vector size by influencing gene expression or by interaction with hormonal proteins of *Aedes* in context to size and molting is questionable and not yet answered. But it is known that in humans one of the causes of profuse sweating is hypothyroidism and sweating does attract mosquitoes to humans. It is also reported that hypothyroidism occurs in dengue infected patients (5) but if dengue virus can alter thyroxine levels in humans is yet unknown although it is known that karyopherins are viral targets and karyopherins are involved in thyroxine import in cells.

Thyroxine is also released in urine by mammals and it animal urine contamination of shallow water bodies is quiet common. Sometimes *Bacillus thurinigiensis*, the well-known larvicidal bacteria is dispersed with bio urine. It is well known that *Bacillus thuringiensis* is very effective in killing of mosquito larva. It has been documented that B. *thuringiensis var Israelensis* is very effective in elimination of mosquito larva but its maintenance in water is unknown but could be transmitted by dead infected larvae (6). Further it has been shown that the type of water body that the mosquito larva is in could influence the effect of the BT larvicide (7). To increase the efficacy of aquatic delivery floating baits have been used like corn oil, lecithin, wheat flour (8) and polystyrene beads (9). It is also known that dragonfly nymph present in aquatic systems can be mosquito larvicidal (10). But its use in harsh environment for biocontrol is not reported as in case of mine drainages. It is further known that the acidic mine drainage of aquatic system could be lethal to fishes (11).

Further the large genome with 1% MITE pony transposon of *Aedes* as compared to its diverged partner Anopheles (12) and the presence of *Aedes* at geographical locations involving deserts (middle east) where in sand storms are common triggered the possibility that the air turbulence might influence gene expression and an easier way to monitor was to study stress transposons like MITE pony (12,13). Instead of air turbulences, g-force was used to induce stress. Further as it has been shown that dengue virus carrying *Aedes* have their stress genes repressed, it would be interesting to question if synthetically induced stress by g-forces could influence infectivity capacity of *Aedes*.

Materials and methods

i.Larval rearing and groups for thyroxine exposure

From a single cohort of *A.albopictus* larvae (L1) reared in domestic purpose water tank, different groups of larvae were segregated with the same number of larvae in each group as tabulated (Table 1). Each group

had 20 larvae per litre of water (duplicates) and were sprinkled with 2 baker's yeast granules per day for fed conditions and nothing for unfed. They were all maintained at room temperature with actual daylight and night cycles. Thyroxine treatment (l-thyroxine was used at 225ppm) in groups as mentioned in Table 1.

L1- L4 well fed Control	L1-L4 unfed Control	GII T225@ L1	GI- T225@ L4	GIII- starvation rescue @L3	GV- T225@ L4	GII- overcrowded	GII starvation rescue @ L4	GIII – starvation rescue @L4 and exposure to g-force
GI	GII	GIII	GIV	GV	GVI	GVII	GVIII	GIX

ii. G-force exposure: Mosquitoes, post 72 hours eclosion in the rearing vessel was exposed to 60rpm/6hours using a gadget (3 feet in radius with 60rpm speed) and was done at room temperature.
iii. Exposure to dragonfly larva: In the third experiment, it was noticed that as such there were no dragon fly larva in coal laden water container but there was mosquito larva. But when the dragon fly larva (Figure 3) was transferred from the water container without coal to container with coal it survived to eliminate the mosquito larva (no mosquito larva visible post 2 weeks).

iv. Larval behavioural assay: It was observed that when 2 mosquito larvae were introduced in a plastic container as shown in figure 1 and its movement observed for 15', it was noted that the two larvae tend to come in contact with each other now and then and don't tend to go away from one another.

v. In the second experiment floating clay balls with B.Ti (Figure 5) as mentioned in methodology, helped in elimination of the mosquito larva (no mosquito larva visible post 2 weeks). Hence floating objects could be used as BT introducing tools in water.

vi. Adult mosquito thyroxine exposure: Female mosquitoes (n=10) post blood-meal were allowed to feed with thyroxine (pharmaceutical grade- l-thyroxine sodium) at 2000PPM with 10% sucrose solution in the first gonotrophic cycle. The control mosquitoes (n=10) were fed only with 10% sucrose solution without exposure to thyroxine. Experimental group mosquitoes were exposed at 19-25 hours PBM in one group (n=15 mosquitoes); 42-48 hours PBM in another group (n=15 mosquitoes) and control (n=15) were unexposed to g-force. During oviposition interval (48hrs PBM) post gforce exposure cups were introduced with water having ground yeast granule (2 grain per litre) to stimulate oviposition and later (72hrs PBM) water level was increased to submerge melanised eggs on the vessel walls. As transparent vessels were used and as the oviposition of eggs was all along the circumference of the cup, it enabled to count the eggs visually after melanisation.

Results

As shown in Table 1-2, Figure 1, Thyroxine treatment of larvae at first instar showed significant delay in larva to pupa molting under starved conditions and by the introduction of feed, it was possible to initiate molting and the sizes of the thyroxine exposed group was significantly larger than the unexposed group under starved conditions and also showed dosage dependent increase in size. There was no significant

difference in well fed control and optimally crowded to that of thyroxine treated well fed groups. The larval to pupal molting and pupal eclosing was seen to be more synchronized in thyroxine treated groups and at reduced span when compared to untreated groups but not at the rate of compensating the size of the adults as seen in untreated starved controls where the size was 50% of that of thyroxine treated groups or. Double treatment groups at first instar and then at third instar where larvae where starved in-between restored size to that of well-fed control. In figure 2, the sequence similarity of DNA aptamer binding with thyroxine is shown to be present in parasites like Leishmania, crabs, insects. In figure 3, the predatory dragonfly larva is depicted. In figure 4, the behavioral pattern of 2 mosquito larva is demonstrated. In figure 5, clay balls filled with *B. thuringiensis* is demonstrated. In figure 6, the effect of g-force and thyroxine on adult mosquito is demonstrated.

Discussion

The mammalian Thyroxine is known to influence insect molting including mosquito larva (4,14). Hence thyroxine, introduced in water bodies through animal urine could have an influence on molting. Further at molecular level, it can be observed that thyroxine binding aptamer sequence (15) has orthologues in leishmania, crabs, insects.

It has also been shown in leishmanial parasite that Fe2O3 could act as an attractant (16) and nano-drugs composed of Fe2O3 bound to PEI has been shown to be effective in killing of parasite (16,17,18). Interestingly the drug levothyroxine administered for hypothyroidism has Fe2O3 bound to thyroxine and leishmania has DNA sequence showing affinity for thyroxine. Since infectious diseases can cause hypothyroidism that gets administered with drugs like levothyroxine, it's likely that the environmental urine pollution in areas of diseases like Dengue, Leishmania could have a different effect on mosquito larva breeding in contaminated areas as compared to larva breeding in areas where healthy people live in. This article shows that exposure to thyroxine (Figure 1, Table 1,2) led to alteration in size of eclosed adult, alteration in sex ratio, weight. Interestingly, thyroid transcription factor is a candidate gene for HOX genes (19) and HOX genes (Antenapedia) could influence the Drosophila deformities are known (20).

Further urine contamination with Pest control compounds like Neonicotinoids are known to inhibit molting in insects (21,22) and these pesticides have been detected in urine as a result of bio accumulation and pollution,

The next mode of mosquito larval control by introduction of predatory dragonfly larva in water bodies (Figure 3) that were devoid of dragonfly larva due to introduced coal was successful in elimination of the mosquito larva. It is to be noted that coal mines have genotoxic effect that doesn't kill mosquito larva but the predatory species of mosquito larvae (23) thus affecting its survival.

In Figure 4 it is demonstrated that 2 mosquito larvae tend to be social and tend to meet one another and hence this could be extended to build novel models of larval robots. It has been demonstrated that *C. elegans* could act as robots (24) and by loading *B. thuringiensis* it could be used as a larval robot to interact with mosquito larva exploiting its social behaviour.

g-force exposed mosquitoes had decreased hatching rate and decreased number of eggs than unexposed which might indicate diapauses (Figure 6). Although it is known that low temperature and short photoperiod causes diapause in eggs (25), g-force exposure in adult mosquitoes resulting in diapause eggs has not been reported. But as g-forces could introduce stress it might have influence on mosquitoes' egg laying ability and possibly the development of the egg. g-force exposed male mosquitoes lived for an average of 15 days when compared to 8 days in control. Oviposition interval exposed female mosquitoes

had earlier mortality (7days) than pre oviposition interval exposed mosquitoes and controls (18 days). As g-force has influence on egg laying, possibly it might have influence on certain stress related genes and might have effect on the mosquito's vector nature for various pathogens. Thyroxine exposed mosquitoes laid diapause eggs and lesser number of eggs as compared to controls.

REFERENCES

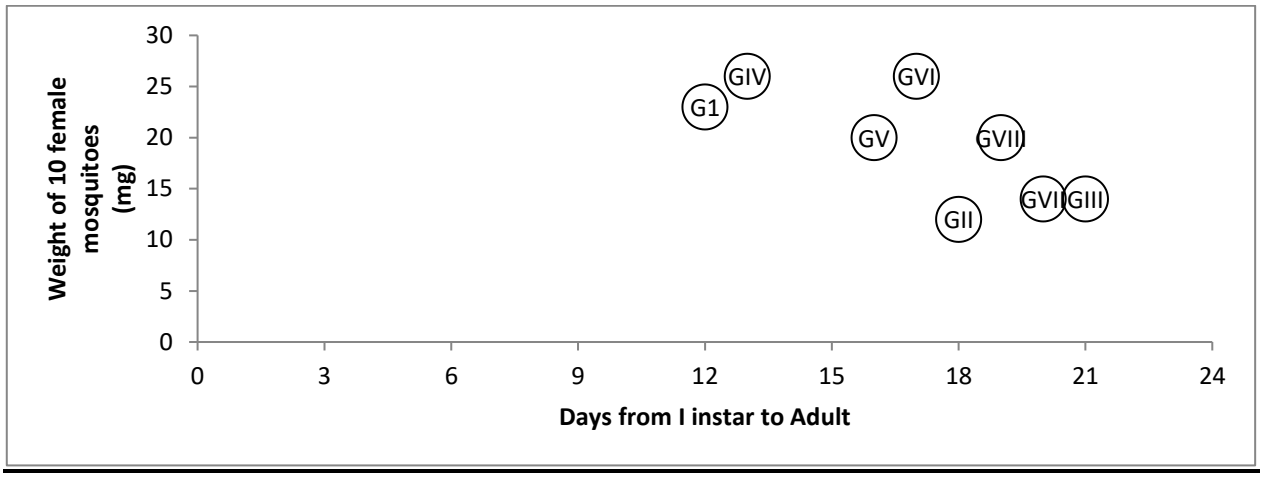
1. Alto, B. W., Lounibos, L. P., Mores, C. N., & Reiskind, M. H. (2008). Larval competition alters susceptibility of adult Aedes mosquitoes to dengue infection. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1633), 463-471.
2. Sumanochitrapon W, Strickman D, Sithiprasasna R, Kittayapong P, Innis BL. Effect of size and geographic origin of Aedes aegypti on oral infection with dengue-2 virus. Am J Trop Med Hyg. 1998 Mar;58(3):283-6. doi: 10.4269/ajtmh.1998.58.283. PMID: 9546404.
3. Hawley, W. A. (1985). The effect of larval density on adult longevity of a mosquito, Aedes sierrensis: epidemiological consequences. *The Journal of Animal Ecology*, 955-964.
4. Kim Y, Davari ED, Sevala V, Davey KG. Functional binding of a vertebrate hormone, L-3,5,3'-triiodothyronine (T3), on insect follicle cell membranes. Insect Biochem Mol Biol. 1999 Oct;29(10):943-50.
5. Parshad O, Melville GN, Prabhakar P. Thyroid function in dengue fever, meningitis, encephalitis, poliomyelitis and other febrile conditions. West Indian Med J. 1986 Jun;35(2):126-9. PMID: 3739343.
6. Zaritsky A, Khawaled K, Barak Z, Chipman DM, Rabi T. Biological control of mosquitoes by the larvicidal activity of Bacillus thuringiensis var. israelensis delta endotoxin. Acta Microbiol Pol. 1986;35(3-4):207-14. PMID: 2436447.
7. Dawson D, Salice CJ, Subbiah S. The Efficacy of the *Bacillus thuringiensis israelensis* Larvicide Against *Culex tarsalis* in Municipal Wastewater and Water from Natural Wetlands. J Am Mosq Control Assoc. 2019 Jun;35(2):97-106. doi: 10.2987/18-6771.1. PMID: 31442132.
8. Aly C, Mulla MS, Schnetter W, Xu BZ. Floating bait formulations increase effectiveness of Bacillus thuringiensis var. israelensis against Anopheles larvae. J Am Mosq Control Assoc. 1987 Dec;3(4):583-8. PMID: 3504944.
9. Curtis CF, Malecela-Lazaro M, Reuben R, Maxwell CA. Use of floating layers of polystyrene beads to control populations of the filaria vector Culex quinquefasciatus. Ann Trop Med Parasitol. 2002 Dec;96 Suppl 2:S97-104. doi: 10.1179/000349802125002446. PMID: 12625923.
10. Samanmali C, Udayanga L, Ranathunge T, Perera SJ, Hapugoda M, Weliwitiya C. Larvicidal Potential of Five Selected Dragonfly Nymphs in Sri Lanka over *Aedes aegypti* (Linnaeus) Larvae under Laboratory Settings. Biomed Res Int. 2018 Dec 3;2018:8759459. doi: 10.1155/2018/8759459. Erratum in: Biomed Res Int. 2019 Feb 5;2019:3950875. PMID: 30627580; PMCID: PMC6304608.
11. Talukdar B, Kalita HK, Basumatary S, Saikia DJ, Sarma D. Cytotoxic and genotoxic affects of acid mine drainage on fish Channa punctata (Bloch). Ecotoxicol Environ Saf. 2017 Oct;144:72-78. doi: 10.1016/j.ecoenv.2017.06.007. Epub 2017
12. Bonin A, Paris M, Després L, Tetreau G, David JP, Kilian A. A MITE-based genotyping method to reveal hundreds of DNA polymorphisms in an animal genome after a few generations of

artificial selection. BMC Genomics. 2008 Oct 6;9:459. doi: 10.1186/1471-2164-9-459. PMID: 18837997; PMCID: PMC2579443.

13. Zhijian Tu, Molecular and Evolutionary Analysis of Two Divergent Subfamilies of a Novel Miniature Inverted Repeat Transposable Element in the Yellow Fever Mosquito, *Aedes aegypti*, *Molecular Biology and Evolution*, Volume 17, Issue 9, September 2000, Pages 1313–1325, https://doi.org/10.1093/oxfordjournals.molbev.a026415
14. Kannan S (2013) Effect of Thyroxine on Mosquito Larvae during Winter Months in Dengue Prone Regions of India. Trop Med Surg 2: 159. doi:10.4172/2329- 9088.1000159
15. Lévesque D, Beaudoin JD, Roy S, Perreault JP. In vitro selection and characterization of RNA aptamers binding thyroxine hormone. Biochem J. 2007 Apr 1;403(1):129-38. doi: 10.1042/BJ20061216. PMID: 17163839; PMCID: PMC1828902.
16. Kannan S, Harel Y, Levy E, Dolitzky A, Sagiv AE, Aryal S, Suleman L, Lellouche JP, Michaeli S. NanoLeish-IL: A novel ironoxide-based nanocomposite drug platform for effective treatment of cutaneous leishmaniasis. J Control Release. 2021 Jul 10;335:203-215. doi: 10.1016/j.jconrel.2021.05.019. Epub 2021 May 19. PMID: 34019947.
17. Kannan S, Harel Y, Israel LL, Lellouche E, Varvak A, Tsubery MN, Lellouche JP, Michaeli S. Novel Nanocarrier Platform forEffective Treatment of Visceral Leishmaniasis. Bioconjug Chem. 2021 Nov 17;32(11):2327-2341. doi: 10.1021/acs.bioconjchem.1c00381. Epub 2021 Oct 19. PMID: 34664934.
18. Daniel Itshak-Levy, L.L. Israel, B. Schmerling, S. Kannan, H. Sade, S. Michaeli, Jean-Paul Lellouche, Disaggregation, stabilization,and innovative functionalization/surface engineering of detonation nanodiamonds via ultrasonication-promoted ceric ammonium nitrate treatment, Diamond and Related Materials, Volume 104, 2020, 107738, ISSN 0925-9635, https://doi.org/10.1016/j.diamond.2020.107738.
19. Guazzi S, Lonigro R, Pintonello L, Boncinelli E, Di Lauro R, Mavilio F. The thyroid transcription factor-1 gene is a candidate target for regulation by Hox proteins. EMBO J. 1994 Jul 15;13(14):3339-47. doi: 10.1002/j.1460-2075.1994.tb06636.x. PMID: 7913891; PMCID: PMC395231.
20. Toegel, J.P., Wimmer, E.A. & Prpic, NM. Loss of *spineless* function transforms the *Tribolium* antenna into a thoracic leg with pretarsal, tibiotarsal, and femoral identity. *Dev Genes Evol* **219**, 53–58 (2009). https://doi.org/10.1007/s00427-008-0265-5
21. Krishnan N, Jurenka RA, Bradbury SP. Neonicotinoids can cause arrested pupal ecdysis in Lepidoptera. Sci Rep. 2021 Aug 4;11(1):15787. doi: 10.1038/s41598-021-95284-0. PMID: 34349192; PMCID: PMC8339065.
22. Li AJ, Kannan K. Profiles of urinary neonicotinoids and dialkylphosphates in populations in nine countries. Environ Int. 2020 Dec;145:106120. doi: 10.1016/j.envint.2020.106120. Epub 2020 Sep 17. PMID: 32949879; PMCID: PMC7578109.
23. NATH, P., DEKA, A., & TALUKDAR, M. (2022). EFFECTS OF COAL MINING ON FISHES OF MEGHALAYA, INDIA: A REVIEW. *UTTAR PRADESH JOURNAL OF ZOOLOGY*, *43*(7), 1–8. Retrieved from https://www.mbimph.com/index.php/UPJOZ/article/view/2987
24. Xianke Dong et al. Toward a living soft microrobot through optogenetic locomotion control of Caenorhabditis elegans.*Sci. Robot.***6**,eabe3950(2021).DOI:10.1126/scirobotics.abe3950

25. Xiaoxia Guo and others, Survival and Replication of Dengue-2 Virus in Diapausing Eggs of *Aedes albopictus* (Diptera: Culicidae) , *Journal of Medical Entomology*, Volume 44, Issue 3, 1 May 2007, Pages 492–497, https://doi.org/10.1093/jmedent/44.3.492

26.



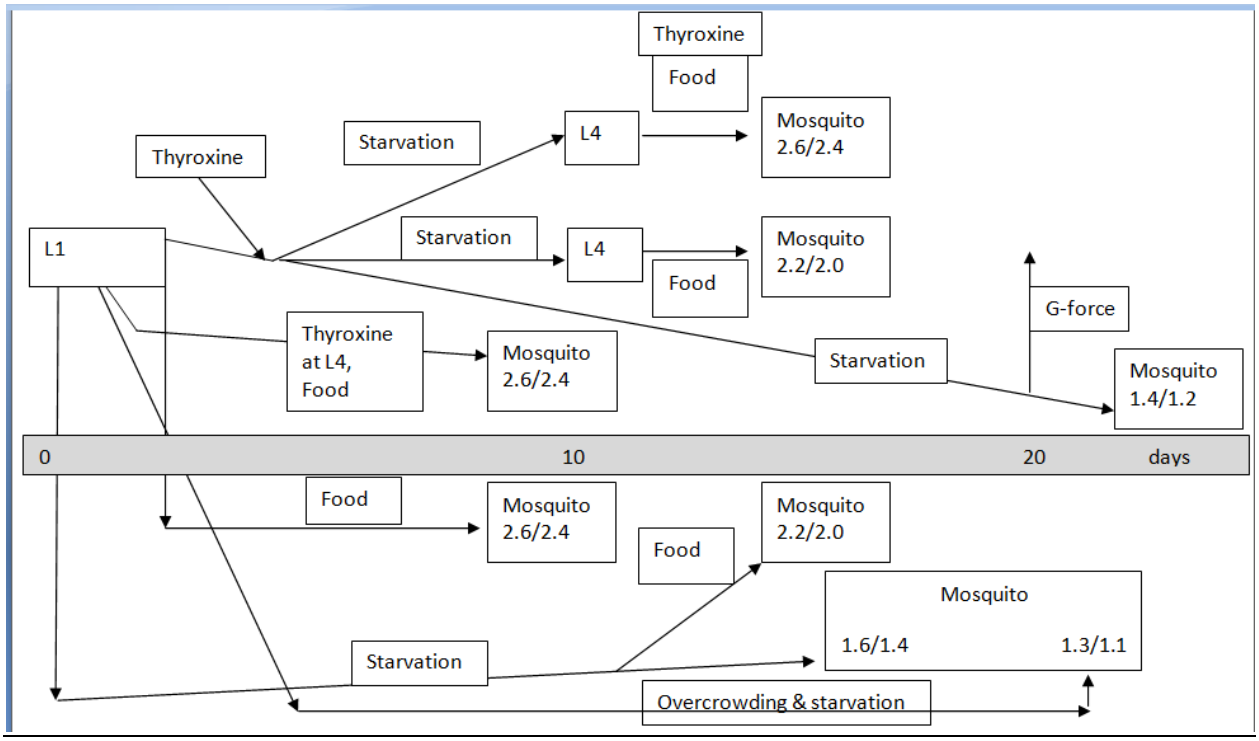


Figure 1 The figure depicts the time taken to eclose from first instar larvae (L1) and the weight of the mosquito (average weight of 20 mosquitoes - female/male in mgs). The larval stages are represented as L1,L4. Thyroxine does show increase in weight in fed conditions and extended eclosing time in starved conditions. Unexposed starved controls had sporadic eclosing. All the L1 to eclosing duration time in the figure represents 90% of the larvae becoming mosquitoes.

Leishmania donovani strain LdCL chromosome LdCL_19, complete sequence	Leishmania don…	42.1	42.1	100%	0.48	100.00%	734853	CP029518.1
Leishmania infantum strain TR01 isolate Lin_TR01 chromosome 19, complete sequence	Leishmania infa…	42.1	42.1	100%	0.48	100.00%	744660	CP027817.1
Leishmania donovani strain pasteur chromosome 19, complete sequence	Leishmania don…	42.1	42.1	100%	0.48	100.00%	722078	CP022634.1
Leishmania donovani strain MHOM/IN/1983/AG83 isolate late passage chromosome 19, partial sequence	Leishmania don…	42.1	42.1	100%	0.48	100.00%	695617	CP019526.1
Leishmania donovani strain MHOM/IN/1983/AG83 isolate early passage chromosome 19, partial seque…	Leishmania don…	42.1	42.1	100%	0.48	100.00%	695631	CP018585.1
Leishmania donovani BPK282A1 complete genome, chromosome 19	Leishmania don…	42.1	42.1	100%	0.48	100.00%	757180	FR799606.2
Leishmania chagasi strain MCER/BR/1981/M6445/Salvaterra isolate M6445 chromosome 19	Leishmania cha…	42.1	42.1	100%	0.48	100.00%	742498	CP048182.1
Leishmania chagasi strain MHOM/HD/2017/M32502/Amapala isolate M32502 chromosome 19	Leishmania cha…	42.1	42.1	100%	0.48	100.00%	742509	CP048146.1
Leishmania infantum genome assembly, chromosome: 19	Leishmania infa…	42.1	42.1	100%	0.48	100.00%	706124	LR812952.1
Leishmania donovani genome assembly, chromosome: 19	Leishmania don…	42.1	42.1	100%	0.48	100.00%	718213	LR812639.1
Leishmania infantum JPCM5 genome chromosome 19	Leishmania infa…	42.1	42.1	100%	0.48	100.00%	742501	FR796451.1
Epinotia ramella genome assembly, chromosome: 24	Epinotia ramella	40.1	40.1	95%	1.9	100.00%	16504716	OX388224.1
Chrysodeixis includens genome assembly, chromosome: 21	Chrysodeixis in…	40.1	40.1	95%	1.9	100.00%	11009237	OW796110.1
Epinotia bilunana genome assembly, chromosome: 22	Epinotia bilunana	40.1	40.1	95%	1.9	100.00%	16921967	OX346274.1
PREDICTED: Eriocheir sinensis serine/arginine repetitive matrix protein 1-like (LOC127007245), transcr…	Eriocheir sinensis	40.1	40.1	95%	1.9	100.00%	4266	XM_050877969.1
PREDICTED: Eriocheir sinensis serine/arginine repetitive matrix protein 1-like (LOC127007245), transcr…	Eriocheir sinensis	40.1	40.1	95%	1.9	100.00%	4463	XM_050877968.1
PREDICTED: Eriocheir sinensis serine/arginine repetitive matrix protein 1-like (LOC127007245), transcr…	Eriocheir sinensis	40.1	40.1	95%	1.9	100.00%	4467	XM_050877967.1
Epinotia demarniana genome assembly, chromosome: 23	Epinotia demar…	40.1	40.1	95%	1.9	100.00%	16444000	OX244282.1
Chrysodeixis includens genome assembly, chromosome: 30	Chrysodeixis in…	40.1	40.1	95%	1.9	100.00%	5842607	LR824033.1
Acomys kempi genome assembly, chromosome: 13	Acomys kempi	40.1	72.4	100%	1.9	100.00%	86063037	OU015367.1
Venustampulla echinocandica Uncharacterized protein (BP5553_05957), partial mRNA	Venustampulla …	38.2	38.2	90%	7.4	100.00%	1557	XM_032014580.1
Bradyrhizobium zhanjiangense strain CCBAU 51778 chromosome, complete genome	Bradyrhizobium…	38.2	38.2	90%	7.4	100.00%	9342022	CP022221.1

Figure 2 Blast sequence prediction for the Thyroxine binding DNA

Figure 3 Dragon fly nymph in water containers with coal to eliminate mosquito larva

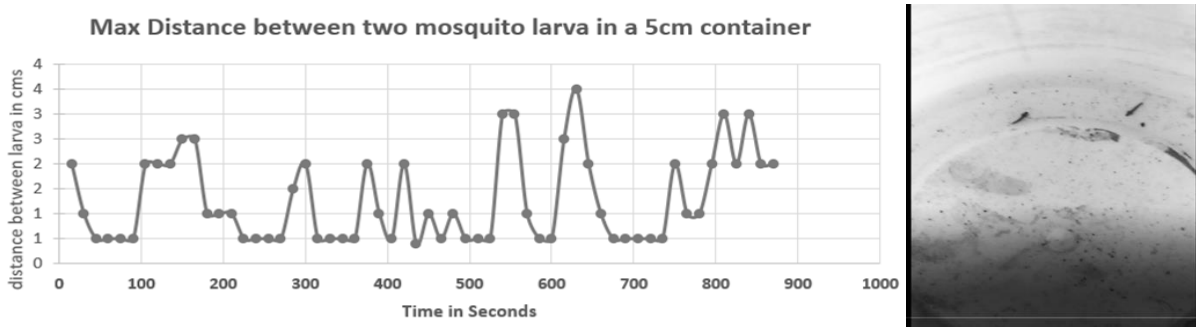


Figure 4 Time distance plot between 2 mosquito larvae in a plastic container observed for 15'- the video link is given herewith. Video

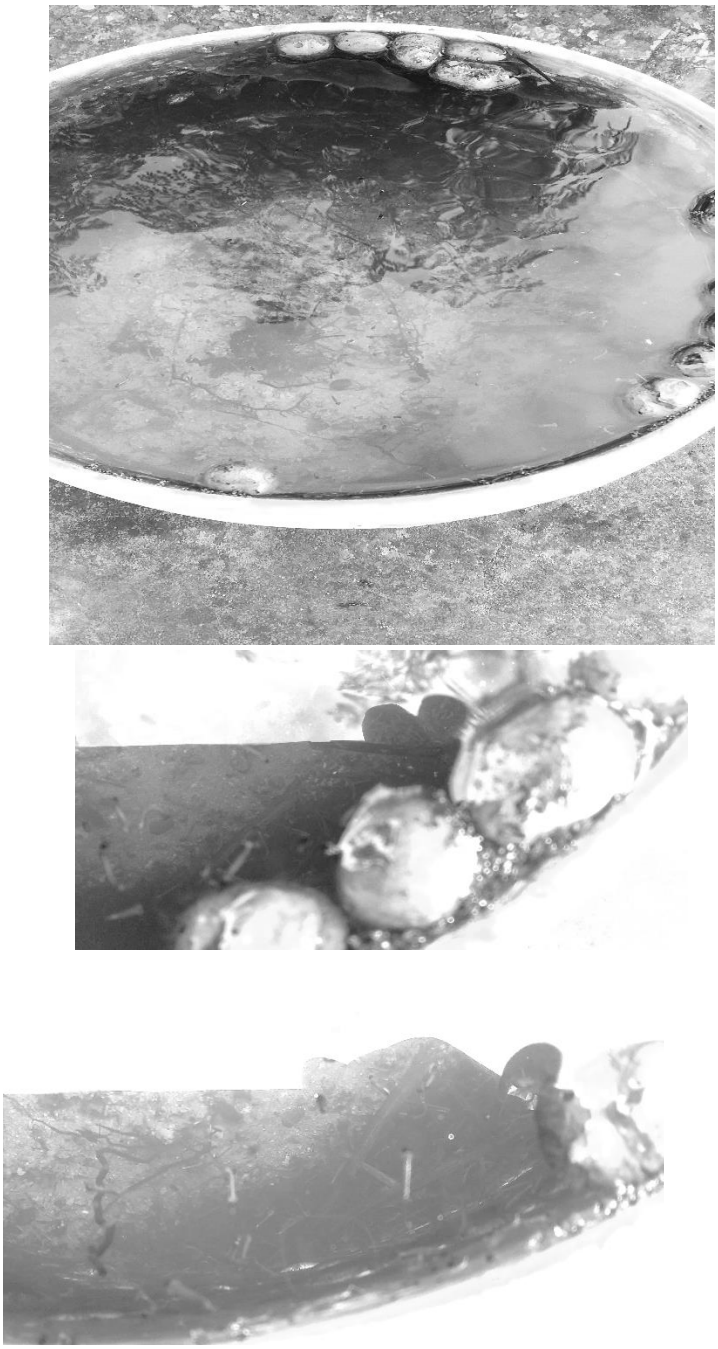


Figure 5 Floating clay balls along with dead mosquito larva killed by BT introduced in water containers

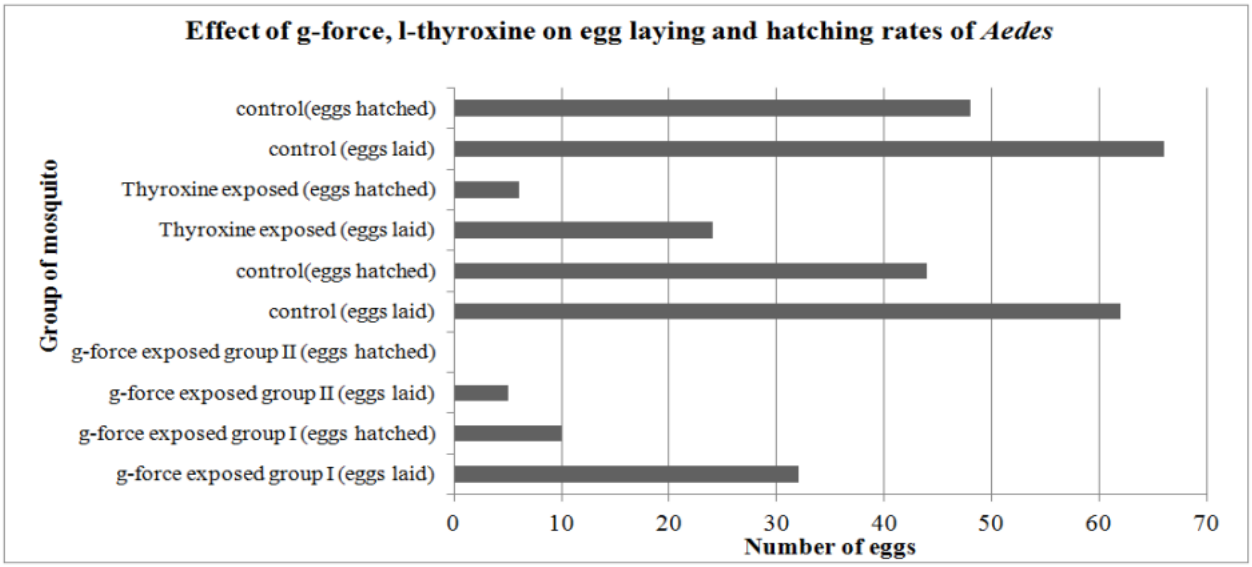


Figure 6 Effect of g-force, thyroxine on mosquito

Group	Weight (mg) (average of twenty mosquitoes) Female/male	90% IVth instar to pupal molting	90% eclosing with a given pupal population	Sex ratio	Observed mortality
GI	2.6 / 2.4	3 days	72 hours	1.5	0%
GII	1.7/ 1.5	7 days	96 hours		0%
GIII					
GIV	2.6/2.4	3 days	72 hours	0.6	0%
GV	2.3/2.1	5 days	96 hours		0 %
GVI	2.6/2.4	3 days	64 hours	1.1	0%
GVII	1.3/1.1	10 days	96 hours		Not known
GVIII					
Environmental sample	2.3/2.1				

Table 1 Effect of thyroxine exposure at mosquito larval stage on adult mosquitoes

Chi square significance of *Aedes* weight (n=20, df=1)
(Observed frequency/expected frequency) Duration from 100% IVth instar to 90% Adult

Group	Chi square	P value
G1/ES female	0.782609	P<0.5
G1/ES male	0.857143	P<0.5
G2/ES female	3.130435	P<0.1
G2/ES male	3.428571	P<0.1
G3/ES female	2.3	P<0.01

G3/ES male	2.1	P<0.01
G4/ES female	0.782609	P<0.5
G4/ES male	0.857143	P<0.5
G5/ES female	0	NOT SIGNIFICANT
G5/ES male	0	NOT SIGNIFICANT
G6/ES female	0.782609	P<0.5
G6 /ES male	0.857143	P<0.5
G7 /ES female	8.695652	P<0.01
G7/ES male	9.52381	P<0.01
G8/ES female	23	P<0.01
G8/ES male	2.1	P<0.5
G2/G5 female	4.235294	P<0.1
G2/G5 male	4.8	P<0.1
G2/G6 female	9.529412	P<0.01
G2/G6 male	10.8	P<0.01
G5/G6 female	0.782609	P<0.5
G5/G6 male	0.857143	P<0.5

Table 2 Significance comparing two groups of mosquito larva