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A Comparative Account on Prevalence of Liver Fluke Metacercariae in Different Larval Stages of Anopheles Stephensi and Aedes Aegypti Larvae at Varying Temperature, Light Intensities and Altering P^H in Laboratory Conditions

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***Abstract*: In present exercise mass level rearing of cercariae of liver fluke and mosquito larvae of two representative species viz. An. stephensi and Ae. aegypti was done in laboratory to set up an experiment for the purpose of estimation of prevalence of metacercariae in different stages of mosquito larvae, which may cause their death. Metacercariae prevalence was observed on different ecological parameters viz. temperature, light and pH value and it was noted that metacercariae prevalence may act as prominent biological control tool for mosquito larvae. Both representative species i.e. An.stephensi & Ae.aegypti were found affected with metacercariae up to remarkable level which may also cause death of mosquito larvae.**

***Keywords*: Anopheles stephensi, Anopheles aegypti, Biological control of mosquito, cercariae, effect of temperature on metacercariae prevalence in mosquito larvae ,metacercariae, metacercariae prevalance ,trematode.**

1. Introduction

There are a large number of entomophilic parasites and parasitoids which affect the growth and development of their respective hosts; mosquito larvae are not exception to this as these are found infected with different protozoans, bacteria, nematodes, brachanoides and sometimes with metacercariae stage of trematodes. A very little literature is available on efficiency between mosquito larvae and larval trematodes such as cercaria and metacercaria and very less laboratory trials have done on this aspect; whereas it may be proven a better natural tool to control of mosquito larvae. The basic principle in this concept is that the cercaria larvae escape from fresh water snail and can encyst in another host , usually an aquatic insect (may be a mosquito larva) ; and in case of severe infection , it lead to death of larva. In present study two very common species of mosquito's viz. Anopheles stephensi and Aedes aegypti were taken to study in comparative account as previous one is vector of Malaria and later one spreads Dengue.

2. Material and Method

For conduction of present experiment, mass rearing of cercaria and mosquito larvae of desired species was required. Hence for the mass production of cercariae adult snails(secondary hosts for trematode parasites) were collected from field , transported to laboratory and were kept in aquarium on natural conditions, food given to them was spinach leaves (Singh et.al,1981) ,water lettuce and fish food. Many of these snails were infected with trematode larvae. To examine the snails for the presence of trematode larvae, snails were kept in sunlight for 20 minutes and live snails were examined microscopically for larval trematodes by squeezing their digestive glands afterwards cercaria larvae were examined under microscope for identification.

For mass culture of mosquito larvae rectangular trays of size 45cmx30cmx4cm were used. These trays were filled with the water of same site from where larvae were collected; these trays were now covered with cages. When adults emerged after metamorphosis they were kept in separate cages for identification. For the nutrition of adult mosquitoes 5% glucose solution soaked cotton swab were kept in cages and for blood meal domestic rats were kept there. Female mosquitoes were allowed to lay their eggs in petri dished which were kept in these cages .By above method the colonies of desired species were established for experimental work.

For inoculation of cercariae in to mosquito larvae; mosquito larvae of different instars were kept in water containing petri dishes and cercariae were transferred, and before transferring number of cercariae was counted. These petri dishes were kept in desired ecological conditions viz. temperature (25oC, 32oC and 40oC), pH (6.5, 7.0 and 7.5) and light (darkness, dim and day light). Later on mosquito larvae were examined for the

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presence of metacercariae.

3. Observation and Discussion

Prevalence of liver fluke cercariae was almost nil in 1st instar larval stage at all temperature value i.e. 25oC, 32oC and 40oC in An. stephensi while at 25oC & 32oC the maximum prevalence was recorded in 4th instar larvae.3rd instar larvae exhibited maximum prevalence at 40oC.The pupae also exhibited antagonism efficiency at all the key temperature ranges but to a less extent in comparison to 2nd,3rd & 4th instar larvae. The maximum encystment of liver fluke metacercariae in An. stephensi was noted in abdominal region in comparison to head and thorax. In Ae. aegypti also, there was no response of 1st instar larvae towards the liver fluke ceracariae at all temperature values i.e. 25oC, 32oC and 40oC. However, 2nd, 3rd, 4th instar larvae and pupae exhibited prevalence of liver fluke metacercariae. The maximum efficiency was observed in 3rd instar larvae at 32oC followed by 4th instar larvae at 40oC and 25oC.The 2nd instar larvae exhibited prevalence of liver fluke metacercariae less than 3rd and 4th instar larvae but more than pupae at all three values of temperature.

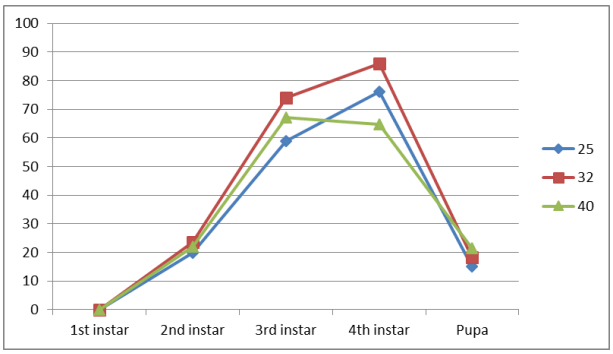

Fig. 1. Graph 1 X-Axis-Stages of larvae & Y-Axis- Percentage prevalence of metacercariae at different temperatures(in ^{o}C) in *An.stephensi* larvae at different stages.

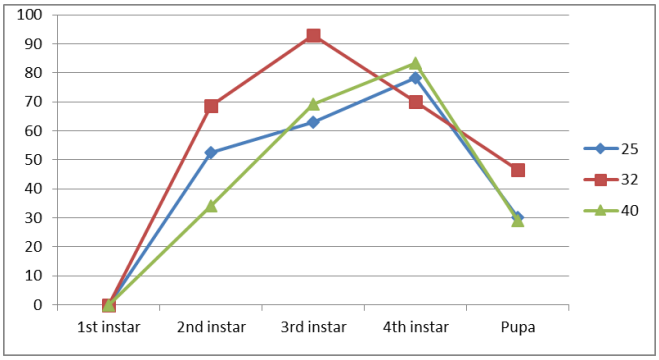

Fig. 2. Graph 2 X-Axis-Stages of larvae & Y-Axis- Percentage prevalence of metacercariae at different temperatures(in ^{o}C) in *Ae.aegypti* larvae at different stages.

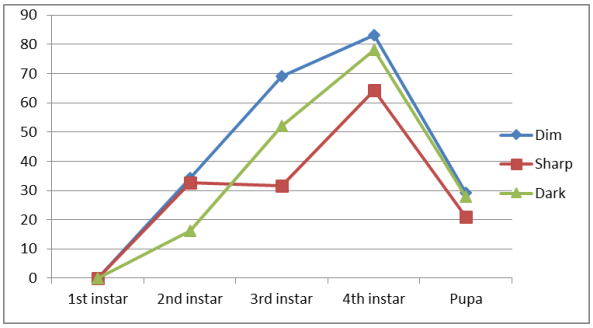

Fig. 3. Graph 3 X-Axis-Stages of larvae & Y-Axis- Percentage prevalence of metacercariae at different light conditions in An.stephensi larvae at different instars & pupa.

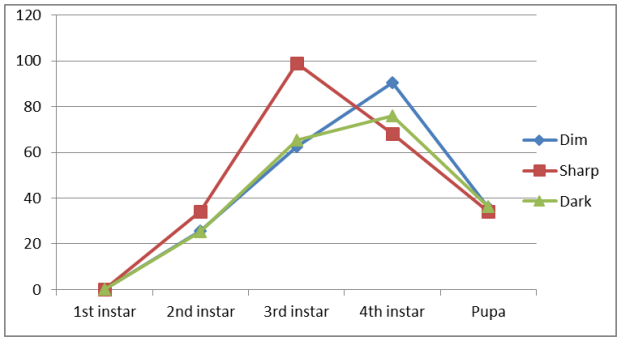

Fig. 4. Graph 4 X-Axis-Stages of larvae & Y-Axis- Percentage prevalence of metacercariae at different light conditions in Ae.aegypti larvae at different instars & pupa.

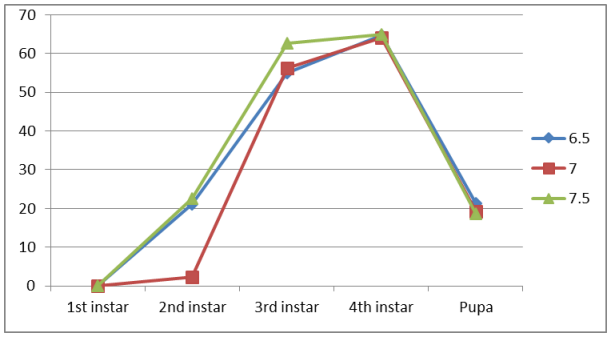

Fig. 5. Graph 5 X-Axis-Stages of larvae & Y-Axis- Percentage of prevalence of metacercaiae at different pH Values in An.stephensi larvae at different instars & pupa.

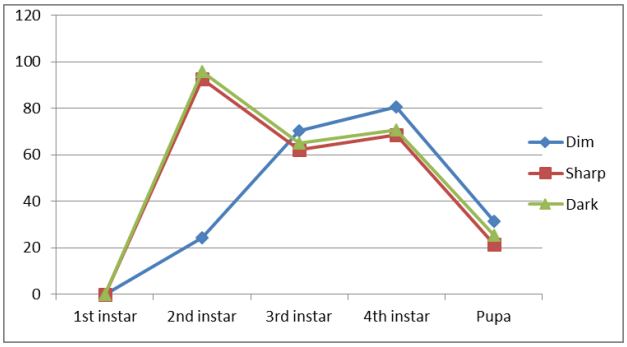

Fig. 6. Graph 6 X-Axis-Stages of larvae & Y-Axis- Percentage of prevalence of metacercaiae at different pH Values in *Ae.aegypti* larvae at different instars & pupa.

Table 1
Larval stages

Larval Stages	Temperature						Light conditions						pH Value					
	25ºC		32ºC		40ºC		Dim		Sharp		Dark		6.5		7		7.5	
	An.	Ae.	An.	Ae.	An.	Ae.	An.	Ae.	An.	Ae.	An.	Ae.	An.	Ae.	An.	Ae.	An.	Ae.
1st instar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2nd instar	20	52.6	23.6	68.6	22.1	26.7	34.16	25.6	32.7	34.2	16.2	25.2	21.2	24.16	2.2	92.8	22.6	95.82
3rd instar	58.8	62.9	74.1	92.9	67.2	70.8	69.1	62.5	31.52	99	52.1	65.2	54.9	70.2	56.2	62.1	62.61	65.1
4th instar	76.2	78.2	85.9	70.1	64.8	90.1	83.24	90.6	64.2	68.2	78.2	75.9	64.75	80.7	64	68.4	64.9	70.7
Pupa	15.1	30.1	18.2	46.6	21.4	24.5	29.1	36	20.9	34	27.9	36.2	21.4	31.4	19.2	21.33	18.7	25.3

It was observed that metacercariae encysted in Ae. aegypti larvae was more in abdomen in comparison to thorax and head region as similar to the An. stephensi & more encystment was present in 4th instar in comparison to 3rd & 2nd instar larvae whereas no metacercaria stage was found in 1st instar larvae. In An. stephensi there was no prevelance of metacercariae at all three light conditions viz. darkness, dim and day light. 4th instar larvae exhibited maximum prevalence at all levels of light conditions ,minimum prevelance was recorded in 2nd instar larvae in dark light whereas pupae exhibited less antagonism efficiency in all levels of light conditions. Similar to An. stephensi there was no response of 1st instar larvae of Ae.aegypti towards with reference to metacercariae prevalence in all light conditions. The maximum prevalence was noted in 3rd instar larvae in full light condition followed by 4th instar larvae in dim light condition. The pupae were at minimum level of prevalence in full light. In An. stephensi the prevalence of metacercariae was found maximum in 4th instar larvae at pH 7.5. The first instar larvae did not show any response towards antagonism efficiency at all pH levels, whereas in Ae.aegypti maximum prevalence was found at pH 6.5 and 7.5 it was maximum in 4th instar larvae at pH 6.5 followed by 2nd , 3rd instar larvae and pupae on the other hand at 7.5 pH the maximum prevalence was seen in second instar larvae followed by 4th & 3rd instar and pupae.

4. Conclusion

Present study suggests that metacercariae prevalence may act as prominent biological control tool for mosquito larvae. Both representative species i.e. An. stephensi & Ae.aegypti were found affected with metacercariae. Finally it was concluded that 32oC temperature condition for metacercariae prevalence in An. stephensi was proved best range and for Ae.aegypti 40oC. Dim light was best condition for metacercariae prevalence in An. stephensi whereas sharp light for Ae.aegypti. pH Value was not proved so effective for An. stephensi but for Ae.aegypti best performed for metacercariae prevalence at 7.5 pH .

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