Effects of Temperature on the Susceptibility of *Aedes aegypti* (L.) (Diptera: Culicidae) Larvae to a Mosquito Pathogen *Coelomomyces stegomyiae* in Uganda

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*Coelomomyces stegomyiae* is reported from Uganda for the first time. Laboratory studies were conducted to determine the susceptibility of various larval instars of *Aedes aegypti* to zygotes of *Coelomomyces stegomyiae* at various temperatures. The fungus was most effective between 23 to 28°C with mortality rate of 99.8% for first and second instar larvae, respectively. Below 20°C the infection progressed very slowly and the infection was not significant. At temperature above 28°C the infection declined and at 32°C there was only 20% mortality in the first and second instar larvae, respectively, but 0% infection for the third and fourth instar larave, exposed to 32°C. The larvae of first and second instars were more susceptible than those of the third and fourth instars. At 32°C the zygotes attached to the larval cuticle but many of them did not penetrate the cuticle to infect the larvae.

INTRODUCTION

*Coelomomyces* is a complex genus of fungi (Chytridiomycetes: Blastocladiiales) which primarily infects mosquitoes. It consists of over 40 known species of aquatic fungi, some of which cause epizootics periodically, resulting in high larval mortalities (Chapman, 1974; Roberts, 1974). Whister et al. (1974, 1975) established a culture of *C. psorophora* in *Culiseta inornata* and discovered that the copepod *Cyclops vernalis* was an intermediate host for that species.

Federici (1975) and Federici and Roberts (1976) observed *C. vernalis* as an intermediate host of *C. punctatus* and *C. dodegi* (Federici, 1980). Laird (1967) carried out a field experiment with *C. stegomyiae* against *Aedes polonesiensis* larvae. This fungus became established in the region. Eleven larvae of *A. aegypti* were found infected with *C. stegomyiae* in a tree hole in Zika forest and pond adjacent to Zika in Uganda.

The purpose of this experiment was to determine the effect of temperature on the susceptibility of *A. aegypti* larvae in the laboratory to zoospores of *C. stegomyiae*.

MATERIALS AND METHODS

In vivo production of *Coelomomyces* using copepods was done in the laboratory following the method developed by Federici (1975, 1977, 1980). Crustacean copepods were

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collected from the pond and brought to the laboratory and synchronized with the infected larvae. One hundred mosquito larvae of each instar were exposed to 40 patently infected copepods and kept under observation at various temperatures such as 15, 20, 23, 25, 28, 30, 32 and 35°C in an incubator. A 100°C calibrated-thermometer was used to check the temperature of the water inside the incubator. Larvae were exposed to infection in enamel plates. Both the copepods and larvae were examined for the presence of mycelium and sporangia using a compound microscope and a stereo-microscope.

The *A. aegypti* colony was reared on dog biscuit, beemax, honey and glucolin at 27°C and 100% relative humidity. Eggs for the experiments were surface sterilized with 5% sodium hypochlorite for 2 min and rinsed in sterile distilled water. Infection and/or mortality records were kept on a daily basis.

**RESULTS**

The results are presented in Figs. 1–4. The data has been subjected to Abbott’s formula (Abbott, 1925) to correct mortality due to factors other than the pathogen.

\[
\frac{\text{% test mortality} - \text{% control mortality}}{100 - \text{% control mortality}}
\]

**First instar**

The maximum cumulative mortality of 92.44±12% (SD) was observed on the 14th day after exposure at 28°C, while 99.79±14.25% was observed when larvae were exposed to infection at 23°C. About 34% of the infected larva had neither died nor pupated by the end of the 14th day. In all, 7% of the total larvae exposed to infection pupated at 28°C against less than 1% pupation observed at 23°C. Most of the pupae died and were found infected with sporangia when dissected under a stereomicroscope. The maximum daily mortality of 23.24±10.96% was observed on the 5th day after exposure compared to 35.60±4.96% observed on the 3rd day after exposure at 23°C. The daily mortality of 6.47% was recorded at 28°C compared to 15.71% at 23°C. The cumulative mortality of 99.7% was observed at 23°C. However, at 32°C only 20% infection occurred and only 20% mortality was recorded. There was no infection at 35°C.

![Graph](image)

**Fig. 1.** The effect of temperature on the susceptibility of *Aedes aegypti* in the first instar to *Coelomomyces aegyptiae.*
Effect of Temperature on Mosquito Larvae Exposed to *C. stegomyiae*

Fig. 2. The effect of temperature on the susceptibility of *Aedes aegypti* in the second instar to *Coelomyces stegomyiae*.

Fig. 3. The effect of temperature on the susceptibility of *Aedes aegypti* in the third instar to *Coelomyces stegomyiae*.

Fig. 4. The effect of temperature on the susceptibility of *Aedes aegypti* in the fourth instar to *Coelomyces stegomyiae*. 
Second instar

The second instar larva was slightly more susceptible than the first larva to C. steigomyiae zygotes at 28°C with mortality of 96.75±15% (SD) and this was almost the same as the maximum cumulative mortality observed at 23°C (97.35%) on the 6th day. Three percent of the larvae pupated at 28°C but the pupae died before emergence and were found infected. The daily mortality of 18.6% was observed at 28°C compared to 14.89% at 23°C. The maximum daily mortality of 35.7% was observed on the second day after exposure at 28°C to compared to 25.0% at 23°C on the second day after exposure. At 32°C the second instar larva was as susceptible as the first instar larva, but the mortality was only 20%. There was no infection at 35°C.

Third instar

The maximum cumulative mortality of 72.39±6.28% (SD) was observed on the 14th day after exposure at 28°C compared to 57.65±19% observed on the 7th day after exposure at 23°C. About 39% of the infected larvae did not die before the 7th day after exposure at 28°C, but about 7% of those larvae pupated and died before the 14th day. The maximum daily mortality of 15.66±7.76% was observed on the 5th day after exposure compared to 15.75±6.48% on the 5th day at 23°C. The daily mortality of 6.08% was observed at 28°C compared to 7.42% at 23°C. The average lethal time was twice as long at 28°C as at 23°C. At 32°C there was no significant infection within the exposed larvae compared to the control. In a few specimens, the zygotes attached to the cuticle at 32°C but did not infect. Four replicates were made with the same results.

Fourth instar

The maximum cumulative mortality of 34.73±6.06% (SD) was recorded on the 14th day after exposure at 28°C compared to 40.40±7.37% on the 7th day at 23°C. About 35% of the larvae exposed to infection pupated and died before the 14th day after exposure at 28°C, but the remaining 6% pupae emerged and the adults had mycelium in the ovaries when dissected. The maximum daily mortality of 8.37±3.94% was recorded on the fifth day after exposure at 28°C compared to 11.20±4.4% on the fifth day at 23°C. The daily mortality of 2.97% was observed at 28°C compared to 5.76% at 23°C, this was almost twice as much as at 28°C. The average lethal time was almost twice as long at 28°C as at 23°C. At 32°C there was no significant infection within the exposed larvae. The examination of larvae on the 7th day after exposure revealed that some had zygotes attached to the larval cuticle but were not infected. The controls were pathogen-free and no significant mortality was recorded.

DISCUSSION

The mortality is affected by temperature, being much lower at lower temperatures (Roberts and Yendol, 1973). The total mortality may or may not be affected depending on whether the optima of the host and parasite coincide or not. The results obtained in this study indicate that the daily mortality was lower at higher temperatures than at lower temperatures. At 28°C it was almost half (6.47%) of that at 23°C (15.71%) for first instar larvae and still almost half for the fourth instar larvae at 28°C (2.97%) of that at 23°C (5.76%). The point is clear that the growth rate of the host was higher at 28°C
than at 23°C. This implies a rapid host reaction to the infection at 28°C. It was observed that melanization and encapsulation occurred among the infected larvae. It is probable that this host reaction was higher at 28°C than at 23°C, resulting in the higher mortality at 28°C. But only 7% and 3% of the first and second instar larvae respectively exposed to the pathogen pupated and died later. A few adults that emerged from the infected population were found infected (Hall and Papierok, 1982). Ferron (1978) referred to this delay as a phenomenon of postponed mortality in the instar affected. This phenomenon coupled with the variation in moulting behavior in Aedes species larvae could account for the slower rate of mortality observed at 28°C than at 23°C. The host reaction was higher in the fourth instar than in the first instar (Stephens, 1963). Thus the susceptibility was higher among young instars than among later instars. The second instar larva was slightly more susceptible than the first instar larva at 28°C, probably because there is a breakdown in maternal immunity when the larva moults from the first to the second instar coupled with the vulnerability of the second instar just after moult.

Sweeney (1978) observed that larvae of Anopheles amictus hilli and C. fatigans when exposed to Culicinomyces sp. conidia, were killed between 15 to 27.5°C but not at 30°C. It was observed that conidia adhering to the normal infection sites of the foregut and the cuticle penetrated but hyphae did not invade the haemocoel and the infection was discarded at the next moult. Similar observations were made in this experiment at 32°C. The optimum temperature for growth for Coelomomyces was found to lie between 18°C and 28°C, hence the higher mortality rates reported at 23°C than at 28°C. Mohamed (1977) observed that the fungus Nomuraea rileyi was most effective at 20°C (80%) and lower at 30°C (43%). Getzin (see Mohamed, 1977) suggested that the reduced infective power of the fungus at 30°C might be caused by the insect ability to resist infection at higher temperatures (Hall and Papierok, 1982). The data reported here agrees with Getzin’s work.

However, at 32°C only 20% infection was observed for both the first and second instar larvae, respectively, and no sensible mortality was observed for the third and fourth instar larvae at this temperature. This may be in part due to the failure of zygote formation at 32°C or the inability of the zygote formed at 32°C to attach and penetrate and grow inside the haemocoel of the exposed larvae. A few specimens showed that the zygotes attached to the cuticle did not penetrate at 32°C. Similar observations have been reported for Leptolegnia sp. which killed 100% in first and second instars of A. aegypti and achieved only 12% mortality in the third and fourth instars, respectively. Its infectivity was high between 15–28°C and reduced at 30°C but nil at 35°C (McInnis, 1982). Thus, high temperature either enhanced resistance to infection (host reaction, humoral immunity) and/or inactivated the pathogen leading to loss of pathogenicity or infectivity.

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