CHAPTER 5

CONCLUSIONS

L. giganteum, an introduced strain (ATCC 36942) was tested for the control of mosquito larvae in the laboratory. The mosquito species used were An. dirus, Ae. aegypti and Cx. quinquefasciatus which are important vectors. L. giganteum was tested in mosquitoes both for susceptibility and infectivity in various environments.

Observations of L. giganteum growing in mosquito cell lines, after inoculation for 3 days indicated the cell culture slough off the surface of the culture flask and became smaller and degenerate. Some mosquito cells were seen clumping around the fungal mycelium. These results might explain the infection process of L. giganteum by using substrates inside the cell to produce protease enzymes (Dean and Domnas, 1983) at the fungal cell surface.

Studies of the infection of L. giganteum in mosquito larvae by histopathological methods showed that the fungal mycelium spread in mosquito organs, especially in the head and in the digestive tract. This supported the fact that the fungi were ingested by mosquito larvae and that the zoospores germinated and grew inside the larvae. Death was most likely caused by the toxicity of aglycones (McInnis and Domnas, 1974) and difficient ingestion of food.

Three genera of mosquito larvae were tested for susceptibility to infection by L. giganteum. The most susceptible genera were the culicine mosquitoes, i.e., Cx. guinquefasciatus and Ae. aegypti. Only Ae. aegypti was examined further because this genus is reported to be an important vector of viral-borne diseases in Thailand, while Culex sp. is only an annoying mosquito.

The infective units for infection were zoospores of L. giganteum which required specific media. Suitable concentrations of sterol in the medium promoted the virulence of zoospores. Sunflower seed extract was the best medium for production of zoospores for infection mosquito larvae. L. giganteum was cultured in SFE broth for 1 week and then transferred to SFE agar for 5 days. The SFE culture agar was suspended and used as the inoculum. The minimum inoculum size of L. giganteum to kill more than 50% of the mosquito larvae was 23.4 cm^2 of SFE agar. This produced approximately 6 x 10^6 zoospores/ml. Larval age was important in the infection rate. Mosquito larvae, 1 - 2 day old, were the most susceptible to infection by L. giganteum, while a low infection rate was detected in late instar larvae, pupae and adults.

Environmental conditions also limited the rate of fungal infectivity. Water with various acidities were tested. The optimum pH for infection was between 6.0 and 7.5. The optimum temperature range for the infection of L. giganteum was 20 - 28 $^{\circ}C$, while at lower and higher temperatures, the rate of infection decreased.

It also was observed in this study that when the biological oxygen demand (BOD) and chemical oxygen demand (COD) were increased, oxygen in the suspension water was reduced, and the infection rate was low. This would be very important in the eutrophic habitats of many mosquito species, where *L. giganteum* must compete with saprophytes and scavengers in the completion of its life cycle.

This organism is specific for larval mosquitoes. There were two genera of mosquito hosts infected, *Aedes aegypti* and *Culex quinquefasciatus*. *L. giganteum's* ability to produce a dormant stage in vitro has made it a very promising biological agent for application against pest and vector mosquitoes in some habitats within Thailand.

Recommendations

1. Practical use of *L. giganteum* for biological control of mosquito larvae requires a simple and rapid means of mass producing infective zoospores.

2. The recently successful development of an *in vitro* culture technique for zoospores will make possible a carefully designed, large - scale field trial to assess the efficiency of the fungus as a practical biological control agent for mosquitoes. A suitable medium for promoting zoospore formation is important for mass production of zoospores of *L. giganteum* in the control of mosquitoes in the future.

3. The oomycete fungus, *L. giganteum*, has shown excellent potential as a biocontrol agent of mosquitoes. In suitable habitats, *L. giganteum* is able to infect and kill a substantial proportion of mosquito larval populations.

4. It is clear from the results reported here that *L. giganteum* has some restrictive environmental limitations. The results indicate that a number of factors may come into play in limiting its

effectiveness for infection of mosquito larvae, i.e., low dissolved oxygen, extremes of pH, high temperatures, and the presence of organic pollutants.

5. Reports on surveys of microbial parasites of mosquito larvae in Thailand do not mention a local strain of *L. giganteum*, even though suitable environments for *L. giganteum* exist. Further field studies are needed.

6. Histopathological studies, results showed that *L. giganteum* produced zoospores and oospores inside larvae. The oospore remained dormant until stimulated to germinate, so this organism can be prolonged and could be a good mosquito control agent in nature.