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Microbial Control Agents—Their Effect on the Biology of Mosquitoes

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Biological control agents have gained advocacy for use in the control of mosquito vectors of human diseases in recent years. The World Health Organization have under consideration several bacterial and fungal agents which are ready to go into operational use. Several bacterial and fungal agents were isolated indigenously from sick/dead mosquito larvae at the Vector Control Research Centre and their effect on the biology of the hosts was studied. Larvae infected by bacterial pathogens lose their normal turgor and motility, become dark brown and a majority of them die before pupating. The posterior midgut region of the infected larvae appears swollen. The larvae ultimately die due to intoxication by different types of toxins produced by bacterial pathogens. Fungal pathogens gain entry into mosquito larvae through ingestion or through the spiracles/cuticle. Fungal mycelia were found along the midgut, in muscle tissues and in caeca. The sporangia were found distributed throughout the body and most of the infected larvae died. The few adults that emerged from infected pupae were found to be weak fliers. Egg maturation does not take place in infected adults. The fungal pathogens kill their host through depletion of nutrients or through suffocation, by blocking the respiratory siphon or through the production of lethal toxins.

Key Words: **Mosquito vector, Microbial Control,** ***Fungal mycelia,*** **Lethal toxins**

Introduction

Development of alternate methods of the control of mosquitoes has gained importance in recent years in view of the well known disadvantages of using chemical pesticides. The scientific community and environmentalists have also rightly expressed concern about chemical pollution. During the second world war, many sophisticated and efficient chemical insecticides were developed which have been used to successfully control mosquitoes. Among the alternatives to the chemical insecticides which are being advocated are the so-called "living insecticides". Although some bacterial and fungal

entomopathogens have been isolated from mosquitoes, only a few have been developed for mosquito control. Commercial scale fermentation technology is available, formulated products are compatible with other control methods, and there is no evidence that the microbial agents are harmful to man, animals, plants or the environment and therefore the microbial control of mosquitoes is seen as having a high potential. Certain microbial agents and their mode of action on mosquitoes are reviewed in this study.

Fungal Agents

(i) *Coelomomyces*

The members of the genus *Coelomomyces* infest 12-45% of mosquito larvae under natural conditions (Shemanchuk 1959, Muspratt 1946, Chandrahas & Rajagopalan 1979). In most cases the haemocoel is filled with mycelium which at maturity is transformed almost entirely into reproductive bodies, the oval shaped, light brown, thick-walled sporangia (Couch 1967). Attachment of mycelium to the gastric caecae, gut and fat body of the larvae has been noted (Roberts 1974). It has been observed that infected fourth instar larvae may live for several days, frequently more than 14, without pupating or dying (Umphlett 1969).

In laboratory experiments also, the average time interval between the initiation of a new infection and the appearance of patently infected larvae is higher than the normal larval development period—approximately 18 days (Federici & Roberts 1976). The fungus grows at the expense of the fat body of the infected larva and the larva fails to pupate since the developing pupa depends on the stored food in the fat body for its nourishment (Couch 1967). Large larvae with light infections sometimes pupate and the adults produced may fail to completely emerge from their pupal cases and those which emerge are weak fliers (Roberts 1974). In infected female adults, the fungus is confined to the ovaries, where each follicle is affected and the females fail to mature eggs (Anon 1977).

(ii) *Lagenidium*

Larvae of *Aedes*, *Anopheles* and *Culex* species are susceptible to the members of the genus *Lagenidium* (Mc Cray et al. 1973, Mattingly 1972). Infection is initiated by biflagellate zoospores, which encyst in the buccal cavity or the cuticle of the host and then send a thin germ tube through to the haemocoel (Roberts 1974). The mycelium ramifies throughout the haemocoel and the insect dies without tissue invasion or indication of toxins. Biochemical parameters of *Culex pipiens fatigans* larvae infected by *L. giganteum* were analysed for protein, amino acids, sugars, O.D. phenolase, GOT, alkaline phosphatase, trehalase and Chitobiase". Infected larvae exhibited significant decreases in rate of synthesis of the above, compared to control. The progress of mycosis is correlated with the decreased rate of synthesis of the above compounds, and it has been concluded that the larvae die from starvation as a consequence of utilization of endogenous reserves by the parasite.

(iii) *Metarrhizium*

This fungus invades the cuticle of the larvae through the perispiracular valves, which is followed by blocking of spiracles, leading to death before significant invasion of the haemocoel occurs (Roberts 1974). Also digestion of the ingested spores leads to the release of the encapsulated fungal toxins into the gut of the mosquito larvae and subsequent death of the larvae is correlated with the total number of spores digested (Crisan 1971). Moreover some of the ingested spores germinate in the midgut

or hindgut of the larvae and penetrate to the haemocoel (Roberts 1974). The toxins of this fungus, "destruxin A and B" induce immediate paralysis in larvae (Roberts 1966).

Bacterial Agents

(i) *Bacillus sphaericus*

The cells of the bacterium enter mosquito larvae via alimentary canal and invasion of haemocoel occurs when the host is near death with subsequent bacteremia (Kellen et al. 1965). The infected larvae lose normal turgor and motility 3 days after exposure. All the bacterial cells are restricted within the peritrophic membrane of the midgut, and the posterior midgut region appear swollen (Davidson et al. 1975). The speed of the activity of the bacterium prevents mid-to-late instar larvae from pupating and emerging into adults (Ramoska & Burgess 1978). The larvae become dark brown and die (Kellen et al. 1965). Larval populations of *Psorophora columbiae* were reduced by 90% and bite count data suggest a reduction in adult emergence due to *B. sphaericus* treatment (Ramoska & Burgess 1978).

(ii) *Bacillus thuringiensis*

The cells of the bacterium showed effective larvicidal activity when applied to early larval instars (Goldberg & Ford 1973). *Aedes* larvae were more susceptible than *Culex* larvae and small-scale field tests showed considerable mortality within 24 hr (Reeves & Garcia 1971). The development of surviving larvae is retarded. Also only 20% and 2% of the *B. thuringiensis* treated larvae of *Aedes stimulaus* and *Ae. aegypti* respectively emerged as adults, though larval mortality was not very high (Shaikh & Morrison 1966).

In the gut of the insect the toxic component of the bacterium is released initially by the action of non-enzymic alkaline components on the crystalline parasporal bodies. The toxic action after the dissolution of the parasporal crystal body in the gut of the insect results in paralysis of the gut within minutes after ingestion of the crystals (Faust 1975). The development of paralysis is accompanied by progressive increase in the alkalinity of the blood so that equillibrium occurs between the highly buffered midgut and relatively poorly buffered blood. The larvae later develop a general and fatal paralysis of the whole body. The δ-endotoxin stimulates glucose uptake in the larval midgut and a general breakdown of control of permeability occurs as levels of K^+ and other ions increase in the haemolymph. Histological effects occur with noticeable "balooning"—extrusion of the columnar cells that produces a severe disruption of the gut wall as the cells burst and release their contents.

That microbial agents kill mosquitoes have been observed in many cases but the fact that infection of a mosquito larvae extends the period of time spent by the insect in the larval stage, very likely even insects with a sublethal infection, becomes a second significant pressure exerted by the pathogens on infected mosquito populations. The retardation of larval development in vector species by the pathogens can contribute substantially to the control of the vector population. The longer the duration of the immature stadia, the greater are the chances of its destruction by diverse forces, e.g., predators, parasites, pathogens, etc.

Therefore, the use of microbial agents appears to be one of the major alternatives to chemical insecticides for situations where the local strains of mosquitoes have developed resistance and where the excessive use of insecticides causes concern of environmental pollution and where environmental methods can not be applied. For Example, in Pondicherry and in the adjoining Tamil Nadu,

paddy is cultivated almost throughout the year along the coastal belt. These paddy fields receive heavy and frequent applications of chemical insecticides for the control of insect pests. In spite of the presence of higher dose of insecticides several species of mosquito vectors have been found breeding profusely in paddy fields (Anonymous 1976) indicating resistance development in paddy field breeding mosquitoes. In such situations additional applications of insecticides will have no effect and the use of microbial control agents appear to have the potential to control mosquito breeding without endangering the normal biological activity in the ecosystem.

References

Anonymous 1977 Pathogens of medically important arthropods, eds. D W, Roberts M A Strand *WHO Bulletin* **55** 419

—— 1976 *Annual Report of the Vector Control Research Centre* Pondicherry, India

Chandrahas R K and Rajagopalan P K 1979 Mosquito breeding and the natural parasitism of larvae by a fungus, *Coelomomyces* and a mermithid nematode, *Romanomermis* in paddy fields in Pondichery; *Indian J. mea. Res.* **69** 70

Couch J N 1967 Sporangial germination of *Coelomomyces punctatus* and the conditions favouring the infection of *Anopheles quadrimaculatus* under laboratory conditions; *Proc. US-Japan Seminar Microbiol. Cont. Ins. Pests, Fukuoka* 23

Crisan E V 1971 Mechanism responsible for release of toxin by *Metarrhizium* spores in mosquito larvae; *J. Inv. Pathol.* **17** 264

Davidson E W Singer S and Briggs J D 1975 Pathogenesis of *Bacillus sphaericus* strain SSII—1 : Infections in *Culex pipiens quinquefasciatus* (=*C. pipiens fatigans*) larvae *J. Inv. pathol.* **25** 179

Domnas A, Giebel P E and Mc. Innis Jr T M 1974 Biochemistry of mosquito infection: Preliminary studies of biochemical change in *Culex pipiens quinquefasciatus* following infection with *Lagenidium giganteum*; *J. Inv. Pathol.* **24** 304

Faust R M 1975 Toxins of *Bacillus thuringiensis*: Mode of action pp. 31-48; in *Biological Regulation of vectors*—A Conference Report, Usdhew Publication No. (NIH) 77-1180

Federici B A and Roberts D W 1976 Experimental laboratory infection of mosquito larvae with fungi of the genus *Coelomomyces*. II. Experiments with *Coelomomyces punctatus* in *Anopheles quadrimaculatus*; *J. Inv. Pathol.* **27** 341

Goldberg L J and Ford I 1973 Aquatic control of *Culex tarsalis* mosquito larvae using combinations of *Bacillus thuringiensis* (HD-1) with two selected growth regulators, Altosid SR-10 (Zoecon) and Monsanto 585; *Abstr. Pap. 5th Int. Colloq. Insect Pathol. Microb. Control* (Oxford), 46

Kellen W R, Clark T B, Lindegren J E, Ho B C, Rogoff M H and Singer S 1965 *Bacillus sphaericus* Neide as a pathogen of mosquitoes; *J. Inv. Pathol.* **7** 448

Mattingly P F 1972 Mosquito eggs XX. Egg parasitism in *Anopheles* with further notes on Armigeres; *Mosq. syst.* **4** 86

Mc Cray et al. 1973 Laboratory observations and field tests with *Lagenidium* against California mosquitoes; *Proc. papers of 41st Ann. Conf. Calif. Mosq. Control Assc.* 128

Muspratt J 1946 On *Coelomomyces* fungi causing high mortality of *Anopheles gambiae* larvae in Rhodesia; *Ann. Trop. Med. Parasitol.* **40** 17

Ramoska W A and Burgess J 1978 Field application of a bacterial insecticide; *Mosq. News* **38** 60

Reeves E L and Garcia C 1971 Pathogenicity of bicrystalliferous *Bacillus* isolate for *Aedes aegypti* and other aedine mosquito larvae; *Proc. 4th Int. Colloq. Insect Pathol. College Park, Md.* 228

Roberts D W 1974 Fungal infections of mosquitoes; pp. 143–193 in *Mosquito Control*, ed. A Aubin et al. (Canada: University of Quebec Press)

—— 1966 Toxins from the entomogenous fungus *Metarrhizium anisopliae*. I. Production in submerged and surface cultures, and in inorganic nitrogen media; *J. Inv. pathol.* **8** 221

Shaikh M W and Morrison F O 1966 Susceptibility of nine insect species to infection by *Bacillus thuringiensis* var *thuringiensis*; *J. Inv. Pathol.* **8** 350

Shemanchuk J A 1959 Note on *Coelomomyces psorophorae* Couch a fungus parasitic on mosquito larvae; *Can. Entomologist* **91** 744

Umphlett C J 1969 Infection levels of *Coelomomyces punctatus* an aquatic fungus parasite in a natural population of the common malaria mosquito, *Anopheles quadrimaculatus*; *J. Inv. Pathol.* **15** 305