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BIOLOGICAL CONTROL OF MOSQUITOES IN THAILAND

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Abstract

The development of biological control of mosquitoes in Thailand started in 1944 when mosquito fish, ***Gambusia affinis*** *was introduced and released in many areas, including Bangkok, to control malaria mosquito larvae. Other examples of biological control by exotic fish which show considerable promise are* ***Poecillia reticulata*** *and* ***Oreochromis niloticus (Tilapia nilotica).*** *These fish are useful against mosquitoes breeding in temporary standing water or man-made breeding sites but are unable to survive in running water. It is, therefore, necessary to seek and import other natural enemies. Over 50 natural enemies in Thailand have been discovered, including twenty species of non-insect predators, fourteen species of insect predators, and seventeen species of pathogens.*

Mosquito pathogens such as ***Bacillus thuringiensis*** *var.* ***israelensis*** *and* ***Bacillus sphaericus,*** *predaceous aquatic insects such as* ***Diplonychus*** *spp.,* ***Enithares*** *spp.,* ***Ranatra*** *spp. and* ***Toxorhynchites*** *spp. and other predaceous aquatic invertebrate including* ***Hydra*** *and* ***Planaria*** *are able to destroy high numbers of mos quito larvae. Terrestrial vertebrates, for example, the small house geckos,* ***Hemidactylus frenatus*** *and* ***Platyrus platyrus,*** *actively destroy adult mosquito. The biology, life history, mass production, and effectiveness of these organisms are being studied to assess their value as biological control agents.*

Introduction

Mosquitoes are one of the major insect problems of the world and are the most prominent of the blood-sucking arthropods that annoy man and other warm-blooded animals. In Thailand, they are vectors of pathogens that cause malaria, dengue haemorrhagic fever, filariasis, and Japanese encephalitis. Thus, control measures are needed to reduce their annoyance and the transmission of disease to man or domestic animals.

The development of residual insecticides over the past 40 years provided a relatively simple and inexpensive tool controlling many of disease vectors. However, vector control no longer can be dependent solely on the use of chemicals because of the 1) emergence and spread of insecticide resistance in many species, 2) concern of environmental pollution from inappropriate use of insecticides and 3) high cost of new chemical insecticides. These problems can be countered by integrated pest management (IPM) measures[1,2].

Integrated pest management involves careful timing and placement of insecticides to avoid damage to existing natural limitation factors and provides for the use of biological control measures. Biological control can be defined as the action of predators, parasites of pathogens in maintaining another organism's population density at a lower average than would occur in their absence.[3] Research on the biological control of insects of medical importance in Thailand has been neglected in the past. Interest in IPM has increased considerably in recent years, and there seem to be definite possibilities for success with certain problems. A significant result of the support for IPM in Thailand has been the use of the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) as a biological control agent (microbial insecticide)[4,5].

In 1979, Hembree[6] listed 17 mosquito pathogens that occur in Thailand. There are also over 34 invertebrate and vertebrate predators of mosquitoes[7]. Although biological control agents occur under natural conditions, without the aid of man, they seldom reduce mosquito vector population levels to a point low enough to interrupt disease transmission. Even when these agents drastically reduce vector populations, they do not ordinarily do so until after the vectors have reached undesirably high levels. For this reason, biological control agents require careful manipulation for maximum effectiveness. Biological control is most effective when parasites or predators of the pest are introduced into an area to control an imported pest[3,8].

Promising Biological Control Agents in Thailand

Among the many agents under development, the Committee of the National Biological Control Research Center of Thailand noted that the following two agents have the greatest potential for mosquito control[9].

Formulations of *Bacillus thuringiensis* var. *israelensis* (Bti) recently have become operational against many species of *Aedes, Culex* and *Anopheles* in the laboratory and during small-scale field trails[4,5]. Although Bti has potential use against a wide range of mosquitoes, it does not recycle in the environment at sufficient levels to provide any significant residual activity[5,10]. However this new microbial insecticide now is marketed in Bangkok and is easy to use. Safety studies carried out on mosquito pathogens have shown non-toxicity for mammals and other nontarget organisms''.

The fresh water fish, *Gambusia affinis,* first was introduced in the year 1944 by the Division of Malaria, Thailand and released in many areas, including Bangkok, to control *Anopheles.*[12] In general, the use of indigenous rather than introduced fish should be encouraged because introduced fish may not survive their new ecosystem [11]. But in Thailand, introduced fish show considerable promise. In addition to *Gambusia,* other examples of exotic fish with potential include *Poecilia*'' *reticulata Oreochromis niloticus*[7,9] *(Tilapia nilotica)* These fish are effective against mosquitoes breeding in temporary standing water, but are of limited usefulness since they are unable to invade running water due to their specialized breeding habits.

Research Findings on Biological Control Agents in Thailand

Bacteria

Jenkins[13] listed 22 species of bacteria from mosquitoes, but Singer[14] listed only 2 species which can be used as bacterial pathogens worldwide. Hembree[6] mentioned that a gram-positive, spore-forming bacillus w as found in *Ae. aegypti, Ar. albopictus,* and *Armigeres subalbatus* in Thailand. Kalyawong[15] reported that five strains of sport-forming bacteria exhibited some larvicidal actions when tested against *Cx. quinquefasciatus* larvae. Chapman[16] commonly found a bacterial pathogen in larval populations of *Cx. quinquefasciatus* and *Ae. aegypti* in Bangkok.

Bacillus sphaericus. Many species of *Aedes, Culex, Anopheles* and *Armigeres* have shown varying degrees of susceptibility to *B. sphaericus* in the laboratory[4,17]. The toxin of this species is located in the spore coat[18,19]. It is more active than Bti against *Cx. quinquefasciatus*[17] The LC-50 (Lethal Concentration-50, in 24 h) values of *B. sphaericus* 1593 for 3rd and 4th stage larvae of *Cx. quinquefasciatus* in polluted water were 0.06 and 0.11 ppm. In our preliminary study, there was no significant difference in larval mortality in polluted water and tap water with this species, such as presented for 3rd stage larvae (Figures 1 and 2). Singer was optimistic about the use of this pathogen in Thailand (personal communication and ref20).

Bacillus thuringiensis var. *israelensis (Bti).* The microbial insecticide, Bti, can be used to control larval mosquito populations in Thailand. This variety which is particularly active against mosquitoes is now commercially available in Bangkok and has been extensively tested against non-target fauna. The effects fo *B. thuringiensis* var. *israelensis* were determined in *Ae. aegypti, Cx. quinquefasciatus, An. dirus* and *An. mininus* larvae[4,5,17,21]. Our results show that toxic activity is greatest against *Aedes* and *Culex* spp (Figures 3 and 4), and slightly less active against *Anopheles.* The LC-50 values (24 h) for Bti against *Ae. aegypti* were found 0.12 and 0.23 ppm for 3rd and 4 th stage larvae, respectively (Table 1). The LC-50 values (24 h) of Bti in *Cx. quinquefasciatus* were 0.15 and 0.22 ppm; in *An. dirus* were 0.34 and 0.64 ppm; and in *An. minimus* were 16.5 and 18 ppm for 3rd and 4th stage larvae, respectively[5]. The LC-50 values increase as larvae mature or with shorter exposure times. A concentration equal to or greater than the LC-50 can control 90 to 100% of the larval mosquito population for a period of one week.

The toxin crystal is formed alongside the spore. Larval enzymes digest the crystal, releasing the toxin within seconds of ingestion, and larvae are killed within hours of ingesting a lethal dose[21]. The bacteria do not persist longer than a few days and regular treatments are necessary during the mosquito breeding season[5]. However, since natural enemies are unharmed, treatments can be applied frequently to suppress new infestations of the target species.

According to available evidence, there is no cross-resistance with chemical insecticides, nor in there an indication of the development of resistance to Bti itself[11].

Fungi

Many different types of fungi are considered to have mosquito control potential. Jenkins[13] reported 47 species of fungi as pathogenic for mosquitoes. The classes of fungi are based on the differences in life-cycles and the 47 pathogenic species belong to the fungal classes Chytridiomycetes, Oomycetes, Zygomycetes, and Deuteromycetes (Fungi Imperfecti).

Fungi of the genus *Coelomomyces* (Class Chytridiomycetes) are obligate parasites most commonly reported from mosquito larvae. Hembree[22] reported that *Coelomomyces* sp. infected mosquitoes in the field and laboratory. He found

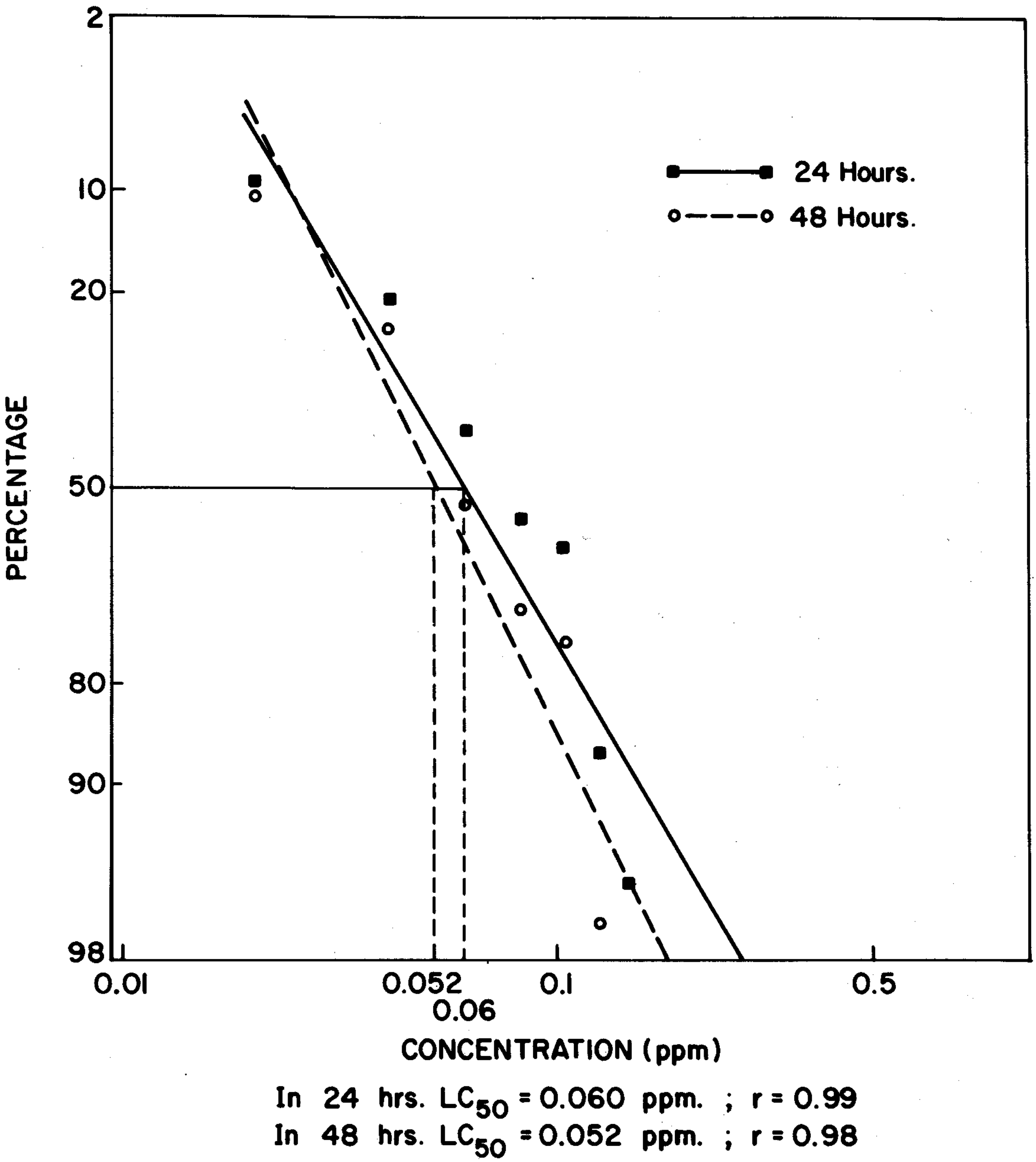


Fig. 1 The toxicity of *Bacillus sphaericus* 1593 to the larvae of *Culex quinquefasciatus* (3rd. instar) in polluted water.

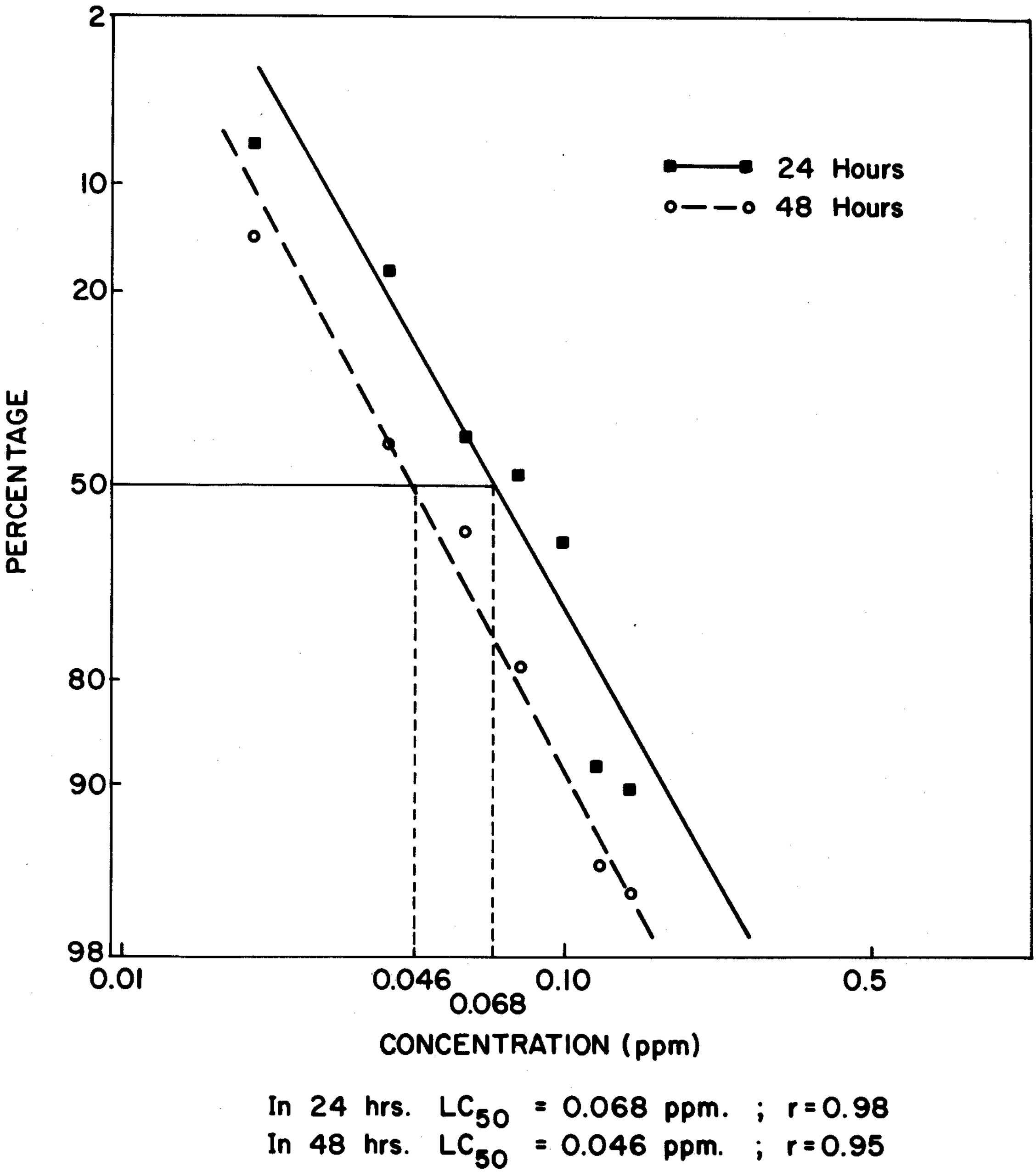


Fig. 2 The toxicity of *Bacillus sphaericus* 1593 to the larvae of *Culex quinquefasciatus* (3rd. instar) in tap water.

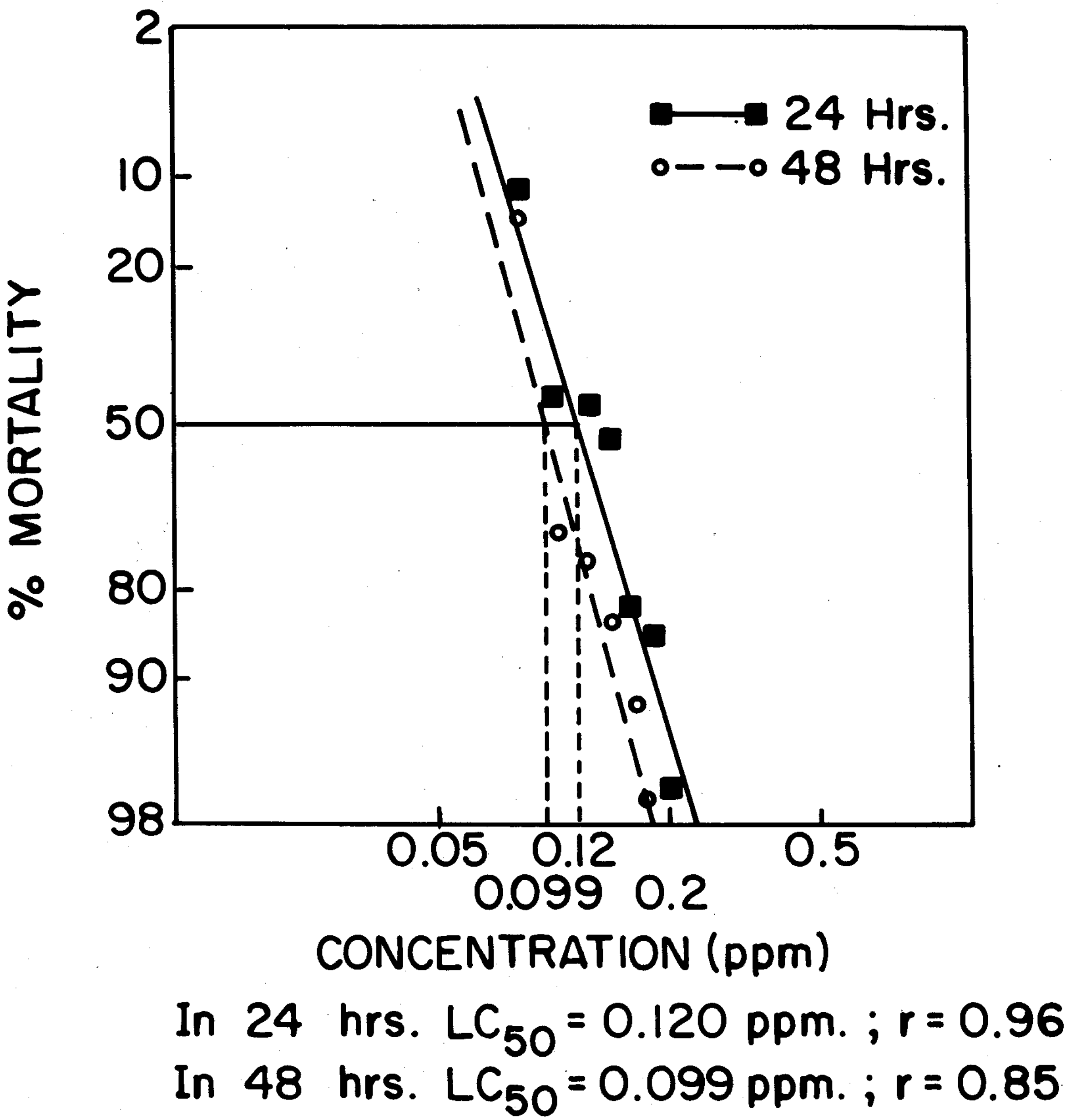
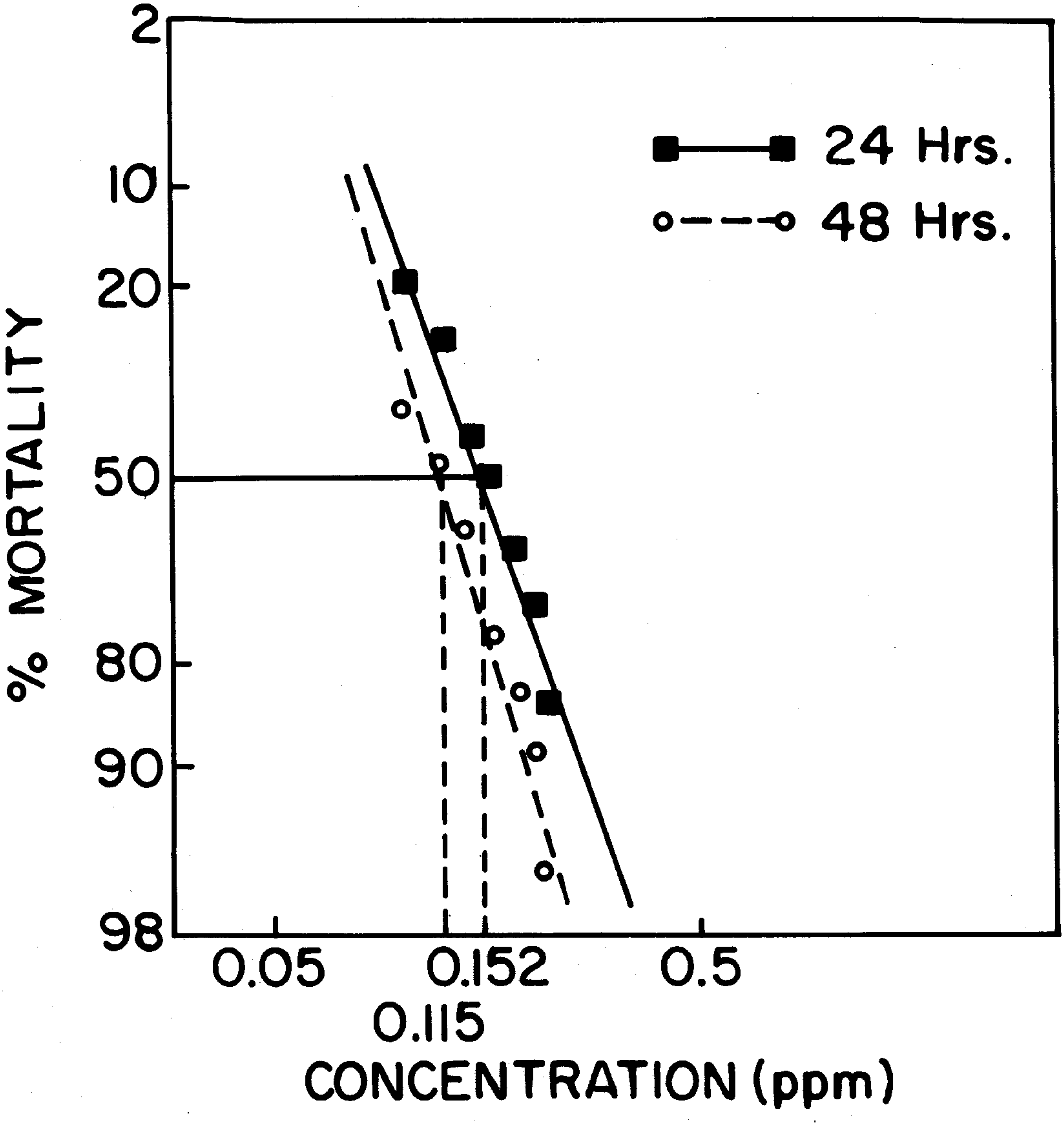


Fig. 3 The toxicity of *Bacillus thuringiensis* var. *israelensis* to the larvae of *Aedes aegypti* (3rd. instar) in tap water.



In 24 hrs. LC_{50} = 0.152 ppm. ; r = 0.97
In 48 hrs. LC_{50} = 0.115 ppm. ; r = 0.96

Fig. 4 The toxicity of *Bacillus thuringiensis* var. *israelensis* to the larvae of *Culex quinquefasciatus* (3 rd. instar) in tap water.

Coelomomyces sp. in larvae of *An. aconitus, An. bengalensis, An. nivipes, An. vagus, Ae. albopictus, Ae. mediopunctatus, Ae. pseudoalbopictus. Cx. vishui, Cx. fuscocephala,* and *Cx. tritaeniorhynchus* in several locations in Thailand. The results of recent field observations and laboratory trials indicate that some species of *Coelomomyces* have broad host ranges, with a few species being capable of attacking mosquitoes of different genera [22]

Most species of *Coelomomyces* are difficult to study and evaluate[11] since they are obligate parasites that require both a crustacean and a mosquito host for development. Nevertheless, as highly specific obligate parasites which seek out mosquito larvae, they may prove extremely valuable for vector suppression in the future.

Protozoa

Chapman[16] cited Jenkins[13] who stated that 83 protozoan species are known from mosquitoes, many of those species, especially the ciliates, flagellates, schizogregarines, and eugregarines, have been insufficiently studied because of their low pathogenicity, or because they are commensal or facultative parasites. Hembree[6] listed 3 microsporidia in *Ae. aegypti;* 1 microsporidian each in *Cx. quinquefasciatus* and *An. vagus;* 1 or possibly 2 microsporidia in *Culex* sp.; 1 helicosporidia in *Ae. aegypti* and *Cx. quinquefasciatus;* eugregarines in *Ae. aegypti, Ae. albopictus, Ae. chrysolineatus* and *Ar. subalbatus;* and a flagellate in *Toxorhynchites splendens* in several locations of Thailand

A protozoan pathogen, *Helicosporidium parasiticum,* was found in *Aedes aegypti* in Thailand in 1974[23]. Because of its transmissibility and virulence to medically important mosquitoes, it was considered as a biological control agent[11]. Studies to determine storage characteristics of *Helicosporidium* sp. from *Ae. aegypti* showed that frozen material remained highly infectious through 16 weeds of storage[23]

Nematodes

Among the various biological control agents, the nematode *Romanomermis culicivorax* holds special promise for control of *Cx. quinquefasciatus* populations.[24] Chapman reported that a field trial was conducted in Bang Khen, a suburb of Bangkok in July-August, 1972. the overall infection rates obtained at high dosages of juveniles (up to 252,000/m^2, 5/cc and 1,700/larva) were disappointingly low. Even these dosage rates were impractically high considering the amount of inoculum that would be required for large-scale field programs. Pupal surveys before and after treatments did not reflect any significant changes in the population due to the nematode treatment. No consistent changes in the population densities of *Cx. quinquefasciatus* could be detected after treatment[24].

TABLE 1 THE TOXICITY (LC_{50}) OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND *B. SPHAERICUS* 1593 AGAINST MOSQUITO LARVAE.

Species	Instar	Bacteria	Source of Water	Container	Concentration ppm. (LC_{50}) 24 h	48 h
Aedes aegypti	1	*B. thuringiensis*	1	aluminum	0.0100	0.0039
	2	var. *israelensis*		bowl	0.0380	0.0290
	3				0.1200	0.0990
	4				0.2300	0.1800
Aedes aegypti	1	*B. thuringiensis*	1	ant guard	—	—
	2	var. *israelensis*		jar	0.0170	—
	3				0.1400	—
	4				0.2000	—
Culex quinquefasciatus	1	*B. thuringiensis*	1	aluminum	0.0094	0.0027
	2	var. *israelensis*		bowl	0.0400	0.0300
	3				0.1520	0.1150
	4				0.2200	0.1500
Culex quinquefasciatus	1	*B. sphaericus*	1	aluminum	0.0023	0.0015
	2	1593		bowl	0.0190	0.0140
	3				0.0680	0.0460
	4				0.1700	0.1350

Species	Instar	Bacteria	Source of Water	Container	Concentration ppm. (LC_{50}) 24 h	48 h
Culex	1	*B. sphaericus*	2	aluminum	0.0014	0.0009
quinquefasciatus	2	1593		bowl	0.0200	0.0160
	3				0.0600	0.0520
	4				0.1100	0.1500
Anopheles dirus	3		1		0.34	N.D.
	4				0.64	N.D.
Anopheles minimus	3		1		16.5	N.D.
	4				18	N.D.
Anopheles dirus	4		3		1.4	N.D.
			4		2.6	N.D
Anopheles minimus	4		3		10.3	N.D.
			4		14	N.D.

1. tap water
2. pond water
3. rain water
4. breeding site water
5. N.D. = not determined

Chapman[16] mentioned that *Neoaplectana carpocapsae* (DD-136) was tested with some success against mosquitoes in the laboratory and in the field. Some laboratory data from Chulalongkorn University[25] showed that the first, second, third, fourth and pupal stages of *Cx. quinquefasciatus* were reduced to 74.6%, 66.6%, 58.7%, 44% and 7.4% mortality respectively by a proportion of 1 mosquito larva : 200 nematodes *(N. carpocapsae)*. Also, the percent mortality of the different stages of *Ae. aegypti* was 30%, 86.6%, 73.3%, 39.3% and 9.3% respectively by the same dosage of preparasites.

Predators of Mosquitoes

Invertebrates

The predatory mosquitoes of the genus *Toxorhychites* spp., especially *Tx. splendens* deserve special mention. The adults of this genus are non-biting and feed on nectar and pollen, thus aiding pollination. The female oviposits in small bodies of water, such as water tanks where *Aedes* mosquitoes are present. *Toxorhychites* larvae feed voraciously on other mosquito larvae. There are approximately 9 species of this beneficial mosquito in Thailand. In the laboratory at Mahidol University first, second, third and fourth stage mosquito larvae of *Aedes aegypti* were consumed by 4th stage *Tx. splendens* each day at the rate of 940, 315, 60 and 20 larvae respectively [26,27]. In a field trial in two small residential areas of suburban Bangkok, six second instar *Tx. splendens* larvae were released into each of fifteen and forty gallon water tanks in per area. We found that the *Ae. aegypti* larval densities were reduced by more than 90%[20] after release of *Tx. splendens* larvae.

There are also a number of aquatic insects in Thailand, such as *Crocothemis servillia* (Odonata: Libellulidae), *Coeneura* sp. (Odonata : Coenagrionidae), *Dyplonychus* sp. (Hemiptera: *Belostomatidae*), *Ranatra varipes* (Hemiptera: Nepidae), *Anisops bouveri* and *Enitheres* sp. (Hemiptra : Notonectidae), that are useful predators of mosquito larvae. Other invertebrates that are important in the natural regulation of mosquito populations include species of *Limnocythere* (Crustucea), *Hydra* and *Planaria*[7]

Reptiles

The Thai small house gecko, *Hemidactylus frenatus* (Reptilis: Geckkonidae) plays an important role in the natural regulation of *Culex* populations[7]. This small house gecko can be seen commonly around residential areas in Bangkok. This predator has been observed to feed on a hundred adult mosquitoes per night[7]. More study is required, however, before the operational feasibility of vector control campaigns based on small house gecko releases in villages can be established.

Fish

Fish are one of the most important natural regulatory agents of mosquito vectors of disease. Jenkins[13] showed that 189 species of fish around the world are predators of mosquito larvae. Many species are efficient predators and little expenditure is involved in their introduction into a particular biotope[11]. Larvivorous fish have been used since 1905 as biological agents for mosquito control in anti-malaria campaigns. For example, *G. affinis* was introduced successfully from Texas, where it is native, to the Hawaiian Islands, and to Thailand in 1944[12]. It was the first recorded use of biological control for mosquitoes in Thailand.

Sesa (1965) reported that *Poecilia reticulata* was introduced and released in a polluted breeding site of *Cx. quinquefasiatus* in Bangkok[28]. Because of the ecological acceptability of these fish in Thailand, they are used now in many areas. In the same year *Oreochromis niloticus* was introduced into Thailand from Japan as a gift to H.M. the King, who initiated its mass rearing for a protein source for Thai people[29,30]. The result of using *O. niloticus* as a biological control agent of mosquito larvae in the laboratory shows that they can eat *Culex* spp. at the rate of 321 ± 136 larvae per day for one fish (size 1.1 – 2.0 cm long), 349 ± 189 larvae per day for one fish (size 2.1 – 3.0 cm long), 476 ± 163 larvae per day for one fish (size 4.1 – 5.0 cm long)[7,29]. They also eat *Aedes* and *Anopheles* larvae. The main criteria for the selection of fish for larval control are a marked preference for mosquito larvae over alternative sources of food, small size (less than 6 cm. long, which makes for easier colonization and access to shallow depths), rapid maturation, high fecundity, and harmlessness to nontarget fauna in the aquatic ecosystem. *Oreochromis nilolicus,* for example, shows many of these favourable attributes and can withstand a wide range of climatic and ecological conditions.

In general, the use of indigenous rather than introduced fish should be encouraged. Examples of native fish with high efficiency include *Oryzius* spp., *Dermogenys* spp. *Panchax* spp, and *Trichopsis* spp[7,11,31]. Most of these species of fish can be released in the rice fields and in *Anopheles* ecological habitats. *Trichopsis vittatus* can live in the polluted breeding sites of Culex found commonly in suburban Bangkok. Indigenous fish are better adapted to local conditions and also are established easily and economically with community help in rearing[11,31] .

Discussion and Conclusion

The development of new biological agents should encompass strategies to circumvent the important problem of insecticide resistance. Biological control agents and juvenile hormone analogs[32] could possibly play an important role in (1) serving as alternatives to Chemicals or (2) in integrated medical pest management strategies. Research in this area is critical, since a number of mosquitoes species already show multiple insecticide resistance[1,2,3,8,11]. Improved formulations of Bti for controlling different vector species under different ecological conditions need to be developed. Formulartions with different contents of active ingredients are required for different operational purposes[11]. Research is needed on the production of formulations for specific areas of operation. For example, formulations that will persist in polluted water in Bangkok are needed and certain formulations of light weight are needed for use in villages in the forest near the border of Thailand and Kampuchea. The carrier for wettable powder formulations, such as sand, clay, or agricultural byproducts, usually can be found locally, reducing shipping costs. Another problem in developing new formulations is to define optimum storage conditions in order to maximize effectiveness. For example, Bti begins to decompose much more readily when the powder formulation contains more than 5% moisture[11].

Additional research is required on integrated pest management in Thailand. For example, the combined use of larviparous fish (eg. *O. niloticus*) and microbial insecticides (eg. Bti) to control vector anophelines (eg. *Anopheles dirus)* in a village situation should be tested.

One of the key factors limiting the isolation, identification, development and use of biological agents for the control of mosquitoes is the lack of trained personnel in endemic areas. An understanding of the ecology of vector species is even more important for biological control strategies than for the relatively simpler techniques of chemical control. For instance, some migrant laborers for the gem industries in Chantaburi Province dig holes for gem mining and leave many suitable breeding sites for *Anopheles dirus*. Local entomologists should receive training in all of these aspects of biology and ecology of the prevalent vectors.

Operational personnel must understand how a biological control agent functions. For example, the pupal stage of mosquitoes which do not feed, will not be affected by Bti and the timing of operational treatments will be crucial for successful control.

The biological control of mosquitoes may reduce the problem of environmental contamination and hazards to man, as well as other factors such as residues in natural areas and the resistance of mosquitoes to pesticides. To expedite these needed research programs, a cooperative approach will be the best method of solving existing and future problems.

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บทคัดย่อ

การควบคุมยุงโดยชีวินทรีย์ในประเทศไทยนั้น ได้เริ่มปฏิบัติกันมาตั้งแต่ปี พ.ศ. 2487 จากรายงานของข่าวสารมาลาเรียกล่าวว่า ได้มีการปล่อยยุงกินยุง *Gambusia affinis* เพื่อควบคุมลูกน้ำยุงก้นปล่องหลายแห่งในขณะนั้น หลังจากนั้นได้มีการนำปลาหางนกยูง *Poecilia reticulata* เข้ามาอีก ซึ่งปรากฏปลาทั้งสองชนิดสามารถอยู่รอดและสถาปนาตัวเองได้เป็นอย่างดี ในสภาพธรรมชาติ โดยเฉพาะในเขตกรุงเทพฯ ในปี พ.ศ. 2508 ได้มีการนำปลานิล ***Oreochromis niloticus*** เข้ามาเพื่อใช้ในการเพาะเลี้ยงเพื่อการบริโภค ต่อมาได้พบว่าปลานิลขนาดเล็ก ๆ หรือในขณะยาว์วัยสามารถเป็นปลาที่ใช้กินลูกน้ำได้ดีอีกตัวหนึ่ง ปัจจุบันได้มีการสำรวจศัตรูธรรมชาติของลูกน้ำยุงต่าง ๆ มากกว่า 50 ชนิด คือ มีตัวห้ำที่มิใช่แมลง 20 ชนิด แมลงตัวห้ำของลูกน้ำยุง 14 ชนิด และดรคต่าง ๆ ของยุงอีก 15 ชนิด

การควบคุมยุงโดยชีวินทรีย์ที่สำคัญในประเทศไทยคือ การใช้แบคทีเรีย *Bacillus thuringiensis* var. *israelensis* และ *B. sphaericus* ในรูปสารกำจัดแมลง

สำหรับในรูปศัตรูธรรมชาติพบว่า แมลงตัวห้ำที่สำคัญคือ แมลงดาสวน *Diplonychus* spp. มวนวนยักษ์ *Enithares* spp. มวนแมลงปล่อง *Ranatra* spp. และยุงยักษ์ *Toxorhychites* spp. สามารถกินลูกน้ำได้วันละมากกว่า 20 ตัว นอกจากนั้นก็มีสัตว์น้ำจืด เช่น พลานาเรีย *Dugesia tigrina* และไฮดรา *Hydra* sp. ที่สามารถกินลูกน้ำยุงบ้างตามแหล่งเพาะพันธุ์ หนองน้ำ และคลองบึงทั่วไป จากการหาศัตรูธรรมชาติใหม่ ๆ ของลูกน้ำยุงพบว่าจิ้งจกบ้าน 2 ชนิด *Hemidactylus frenatus, Platyrus platyrus* สามารถกินยุงตัวเต็มวันได้ดีที่สุด ในจำพวกสัตว์เลื้อยคลานด้วยกัน