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A STUDY ON *REESIMERMIS NIELSENI* FOR CONTROL OF *CULEX PIPIENS FATIGANS* IN TAIWAN[1]

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ABSTRACT

Chen Pao-shu (1976). *A Study on* Reesimermis nielseni *for Control of* Culex pipiens fatigans *in Taiwan.* Bull. Inst. Zool., Academia Sinica, **15**(1): 21-28. A method for mass production of *Reesimermis nielseni* has been developed by using the Nankang strain of *Culex pipiens fatigans.* The percentage of parasitism and the production of postoparasites were both the highest when the host-parasite ratio was at 1:7.5. A lesser host consumption with a higher nematode production was economically obtained when the host density was at 4.2/cm^2.

Laboratory tests indicated that the nematode could emerge from the adult stage if the late fourth-instar *C. p. fatigans* were exposed. Its emergence always caused the death of adult mosquitoes and made female survivors castrated and incapable of developing their ovaries.

The tests also indicated that the pH value of water is an important factor in limiting the habitat range of *R. nielseni* which, when used as a biological control agent, will probably have to be restricted to the mosquito breeding habitats with a pH range of between 6.7 and 7.7.

Field trials showed that this kind of nematode could recycle in the natural breeding place of *C. p. fatigans* by exposing preparasites.

Synthetic organic insecticides are still important as a powerful weapon for fighting against mosquitoes. However, recent concern about serious environmental problems and mosquito resistance to chemical pesticides has renewed interest in alternate means of control. To the best of our knowledge, one of the most promising methods is to use the mermithid nematode (*Reesimermis nielseni* Tsai and Grundman) as an agent for control of mosquitoes. This is because such a kind of nematode has become so adapted to the life cycle of several mosquito species that it may produce high levels of parasitism and kill the hosts[1].

In July 1971, a culture of the mermithid nematode was established in the laboratory of Taiwan Provincial Institute of Infectious Diseases (formerly Taiwan Provincial Malaria Research Institute), and exploratory trials were initiated to ascertain whether or not the nematode can play the role in reducing mosquito populations in Taiwan. The preliminary results indicated a high level of nematode parasitism in *Culex*

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pipiens fatigans Wiedemann-one of the most important vectors of filariasis in Taiwan. Field trials with preparasites or mature worm eggs sometimes resulted in infection of natural populations; however, infection rates generally were low, and in no case was evidence obtained which would indicate that the parasite had become established in nature[2].

The objectives of this study were to (1) develop a method for mass production of *R. nielseni* with *C. p. fatigans*, (2) test the possibility of the parasites passing through the pupal into the adult stage of the host, (3) continue the test under field conditions in search of whether or not *R. nielseni* could complete its life cycle and produce an infectious progeny in the breeding place of *C. p. fatigans*, and (4) determine the pH factor of water that might affect the introduction of the parasite into new habitats.

MATERIALS AND METHODS

The preparasitic juveniles of *R. nielseni* used in this study were obtained from the laboratory cultures that were flooded with chlorine-free water 18 hours before exposure or application. The number of hatched nematodes was estimated on the volumetric dilution; five 0.2 ml volumes were pipetted from well-mixed cultures and the nematodes in each culture were counted under a stereoscopic dissecting microscope. The average was taken as the approximate number of nematodes per 0.2 ml of water.

For a mass production test, the Nankang colony of *C. p. fatigans* was used as hosts and a plastic pan (56 cm × 50 cm × 5 cm deep) as a rearing apparatus. Initially the pan was half filled with chlorinefree water and mosquito eggs were added. As soon as the eggs hatched, the pan was aerated and a desired number of preparasities were added. Liver powder mixed with yeast (at a ratio of 1:3) that had been finely ground and passed through a 30-mesh sieve was fed to the mosquito larvae at a rate of 0.30, 0.45 and 0.60 g per 1,000 hosts on the 1st, 2nd and 3rd days, respectively and 0.90 g each day thereafter, The pupae, after appearing, were drained into an aquarium (51 cm × 27 cm × 30 cm deep) with a screen layer inserted in the middle to let the postparasitics slip through and withhold debris, dead carcasses, etc. (see plate 3).

In an attempt to prove whether or not *R. nielseni* could pass through the pupa into the adult of the host, tests were made by exposing the late fourth-instar *C. p. fatigans* to preparasitic nematodes. Adult mosquitoes, once they emerged, were confined in a cage (33 cm^3) and reared with dextrose. A bowl with water was put in the cage for nematode emergence. All adults were dissected and examined for parasitism on the 12th day after the exposure. At the same time, the spermathecae of females were examined for the presence of sperms.

For field release trials, two *C. p. fatigans* breeding places (hereafter referred to as sites "A" and "B") were selected in Pali Township, Taipei County. Preparasitics were disseminated with a gravity type garden sprinker and postparasitics released directly into the sites. An assessment was carried out weekly at site "B" and biweekly at site "A" after treatments. Mosquitoes were collected and brought to the laboratory for further rearing until the larvae reached fourth-instar, and then examined for parasitism. All the infected larvae were re-introduced into the sites. Maximum and minimum water temperatures and pH values at both sites were taken for each assessment.

The pH limits that the nematode could tolerate were determined in the laboratory. First-instar *C. p. fatigans* were exposed to preparasitic nematodes in beakers which contained water with various pH values. A 0.1 M disodium hydrogen phosphate and a 0.1 M potassium dihydrogen phosphate solutions were used for adjusting the pH in the range of 5.7 to 7.7; mixtures of boric acid and sodium hydroxide were used to buffer the solutions in the range of pH 8.2 to 8.7.

RESULTS

Mass production:

High parasitism could usually be produced

by increasing the number of preparasitics on a constant number of hosts (Table 1). But the ideal ratio between hosts and parasites for postparasitics production seemed to be at 1:7.5. The yield of postparasitics when the ratio was 1:5 was lower than that when the ratios were 1:7.5 and 1:10. When the ratio was 1:10, the number of nematodes harvested was quite the same as at 1:7.5, but more hosts were consumed. When the number of parasites was increased to a ratio of 1:15, however, the number of nematodes harvested was lower; a possible explanation for this finding is that some of the hosts were killed by the overdose of preparasitics.

TABLE 1

Mass production test of *R. nielseni* by using the early instar larvae of Nankang strain of *C. p. fatigans* in laboratory

Culture No.	No. larvae exposed	Mosquito-Approximate		% Parasitism	Host density*
		nematode ratio	number of post-parasitic harvested		
L	600	1 : 2	254	19	0.2
2	600	1 : 2	312	38	0.2
3	600	1 : 5	1796	74	0.2
4	600	1 : 10	2897	94	0.2
5	3000	1 : 5	7000	44	1.1
6	3000	1 : 7.5	10000	81	1.1
7	3000	1 : 10	12000	85	1.1
8	6000	1 : 5	8000	50	2.1
9	6000	1 : 7.5	18000	82	2.1
10	6000	1 : 10	18000	84	2.1
11	12000	1 : 5	19000	43	4.2
12	12000	1 : 7.5	30000	80	4.2
13	12000	1 : 10	30000	84	4.2
14	20000	1 : 5	30000	41	9.1
15	20000	1 : 7.5	40000	82	9.1
16	20000	1 : 7.5	48000	83	9.1
17	20000	1 : 10	45000	83	9.1
18	20000	1 : 7.5	45000	84	9.1
19	20000	1 : 7.5	40000	84	9.1
20	20000	1 : 15	22500	100	9.1
21	20000	1 : 15	23700	100	9.1

* No. of larvae per sq. cm^2 on slide.

As for the percentage of parasitism, there was little difference among the host densities of 0.2, 1.1, 2.1, 4.2, and 9.1. However, host crowding usually caused a high mortality at the time when larvae molted to fourth-instar.

Infection of late fourth-instar *C. p. fatigans*:

The results in two trials indicated that the preparasitic nematode could penetrate and parasitize in late fourth-instar *C. p. fatigans* and could pass through pupae into adults. In the

TABLE 2

Rates of parasitism in mosquito adults by exposing late fourth-instar larvae of *C. p. fatigans* to preparasitic *R. nielseni*

No. of larvae exposed	Mosquito-nematode ratio	No. adult emerged	No. adult infected		% parasitism of the emerged adult
			Male	Female	
100	1 : 10	62	20	42	100
147	1 : 5	103	20	34	52

first trial, a 100% parasitism was noted when the mosquito-nematode ratio was at 1:10; in the second trial, a 52% parasitism was obtained at a ratio of 1:5 (Table 2). Most of the infected females engorged as in normals; some of them had sperm stored in the spermathecae. However, few if any of their ovaries had developed.

Field trial with preparasitics:

Site "A" was a 5-m^2 ground-pool, a little shaded, with a highly organic-polluted bottom, and the water was clear if undisturbed. All mosquitoes sampled in the pool were identified as *C. p. fatigans* on the treatment date of March 1, 1974. The population estimated was as high as 52 per dip at that day. A total of 1×10^4 preparasitics (2000/m^2) were introduced. Parasitism was satisfactory, up to 68% at 48 hours after treatment even though the mean of the minimum temperature of water was as low as 16.2°C (Table 3). Follow-up samplings were continued biweekly. The first recycling of *R. nielseni* occurred on the tenth week after treatment, by then the parasitism dropped to a low level of 7.2%. However, it increased a little on the 126th and 140th days of samplings (up to 23.7 and 19.7% respectively). Unfortunately, the pool was occasionally flooded by rain-falls since the 154th day (early August) after-treatment, and the population of *C. p. fatigans* suddenly decreased. On the 196th day, only few mosquito larvae or pupae could be servive in the study site.

In anticipation of the possible disappearance of the hosts, some 250 laboratory-reared first-instar *C. p. fatigans* were introduced into the site 2 days prior to the 210th and 224th-day. Surprisingly, the recyclings of *R. nielseni* re-appeared, and a few of infected larvae of *C. p. fatigans* were found repeatedly on the 238th day and in the following samplings, although, the parasitism was quite low, ranging from 0.1 to 0.9%.

Field trial with postparasitics:

Another ground-pool, with approximately 4.5 m^2 of water surface, was selected as site "B". One thousand postparasitic juvenile nematodes were released into the site. On the day of treatment, 26 April 1974, the pool yielded about 8 larvae of *C. p. fatigans* per dip; no other species of mosquito was encountered on the day.

After treatment, the mosquito population of the host in the pool gradually decreased; on the 42nd day, it dropped to only 2 larvae per dip. Thus some 250 laboratory-reared first-instar larvae of *C. p. fatigans* were transferred weekly into the pool; 48 hours later, they were collected and returned to the laboratory rearing so to determine the infection rate. This treatment continued until the end of June, and no infected larve could be found (Table 3). The pH values of the pool water during the period of the trial were pH 7.2–7.8; it had been assumed that such conditions would be suitable for the maturation of juvenile postparasitics and for the infection of *C. p. fatigans* larvae.

Hydrogen ion tolerance:

Though the pH value of water in the natural breeding place of *C. p. fatigans* had been found to be not in excess of the 6.0–8.6 range, the pH limits of *R. nielseni* could tolerate remained unknown. A test was, therefore, conducted in the laboratory, The result (Table 4) indicated that the optimal pH value for parasitism was between 6.7 and 7.2, that no parasitism was observed at pH values limits lower than 5.7 or

TABLE 3

Parasitism in larvae of *Culex pipiens fatigans* treated with *Reesimermis nielseni*

Treated site	Day after treatment	pH of water	Mean temperature of water (°C)		% larvae parasitized (no. larvae collected)	
			Minimum	Maximum	*C. p. fatigans*	other*
A (preparasitics released on 1st of March 1974)	2	7.2	16.2	19.1	68 (1200)	
	28	7.2	17.4	20.0	0 (887)	
	42	7.4	16.8	22.7	0 (1152)	
	56	7.2	18.4	21.8	0 (774)	
	70	7.2	22.2	25.7	7.2(207)	
	84	7.2	23.5	26.2	0 (112)	
	98	7.0	23.5	27.5	3.4(178)	0(30)
	112	7.2	25.1	28.9	8.6(140)	
	126	7.2	26.4	28.4	23.7(76)	
	140	7.2	27.6	29.3	19.7(122)	
	154	7.0	27.7	29.1	0.3(78)	
	168	6.8	26.5	28.1	0 (22)	
	182	7.0	26.5	28.6	0 (34)	
	196	7.0	26.0	28.7	0 (0)	
	210	7.0	25.6	29.0	0 (228)**	0(12)
	224	7.2	25.5	28.2	0 (214)**	0(35)
	238	7.2	22.3	27.0	0.1(716)	0(77)
	252	7.2	20.7	24.5	0.2(288)	0(21)
	266	7.2	18.5	22.3	0.9(1344)	0(1)
	280	7.2	17.8	22.6	0.1(1412)	0
	294	7.2	17.2	23.3	0.1(947)	
B postparasitics released on 26th of April 1974)	7	7.4	18.4	21.8	0 (254)	0(2)
	14	7.6	18.2	22.4	0 (120)	0(7)
	21	7.4	22.2	25.7	0 (88)	0(3)
	28	7.6	22.5	24.8	0 (83)	
	35	7.8	23.5	26.2	0 (15)	
	42	7.4	23.5	26.1	0 (2)	
	49	7.6	23.5	27.5	0 (176)**	
	56	7.2	24.2	28.3	0 (193)**	
	63	7.4	15.1	28.9	0 (216)**	
	70	7.4	25.5	28.6	0 (184)**	

* Other species included 135 *Culex tritaeniorhynchus summorous*, 21 *Culex fuscanus* and 32 *Anopheles sinensis*.

** A batch of 250 laboratory reared first-instar *C. p. fatigans* was introduced into the site 2 days befores collection.

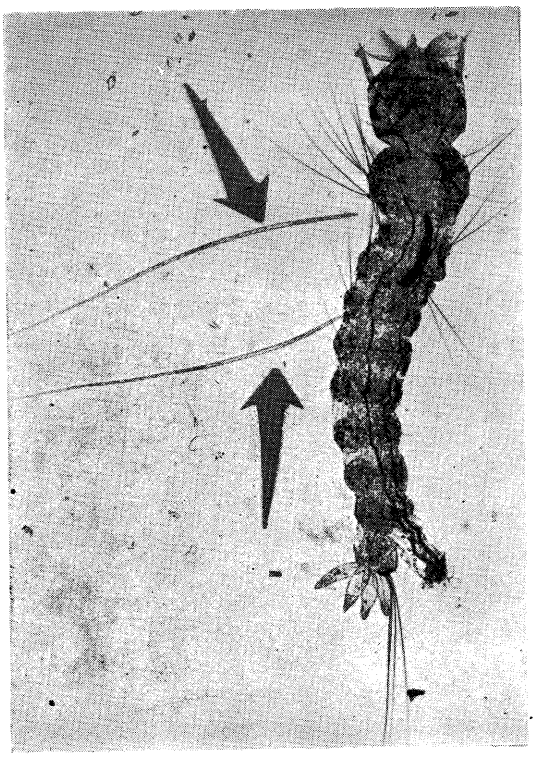

Fig. 1. Preparasitic juveniles of *Reesimermis nielseni* in the process of penetrating the cuticle of first-instar larva of *Culex pipiens fatigans.*

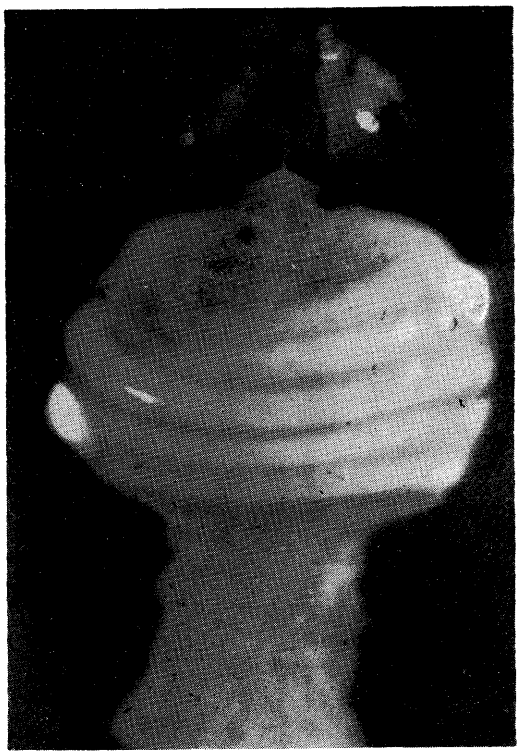

Fig. 2. *Reesimerims nielseni* coiled around thorax of fourth-instar larva of *Culex pipiens fatigans.*

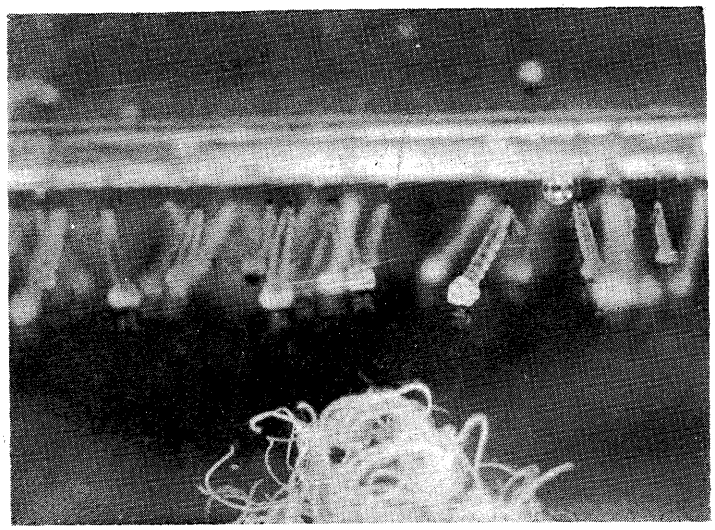

Fig. 3. Postparasitic juveniles of *Reesimermis nielseni* heaping on the bottom of container after emergence from hosts.

higher than 8.7, and that a sharp drop occurred when the pH was lower than 6.2 or higher than 7.7. The activity of preparasitic nematodes was found to be the highest at pH 7.2.

DISCUSSION

A method for mass production of *R. nielseni* has been developed by using the Nanking strain of *C. p. fatigans*. The data thus obtained clearly show that at the host-parasite ratio of 1:7.5, the percentage of parasitism and the production of postparasitic nematodes were the highest. There was little difference in the tested host densities. However, when the density was at 4.2/cm^2, lesser host consumption and higher nematode production were obtained.

The life cycle of *R. nielseni* on mosquitoes is similar to that of other aquatic mermithids. Generally, its eggs hatched, the preparasitic nematode invaded the early instar mosquito (Fig. 1) and grew rapidly to full size just before the mosquito larvae would normally pupate (Fig. 2). Then it emerged from the host (Fig. 3), killing the host in the process. Petersen *et al.*[3] indicated that the nematode could be released from the third and fourth instar mosquito larvae but not from the pupae or adults. In this study, however, when the late fourth-instar *C. p. fatigans* were exposed, the preparasitic nematodes could also penetrate into the haemocoel of mosquito larvae and pass through pupae into adults of the host. Their emergence resulted in the death of the host, and the surviving female mosquito became castrated and incapable of developing its ovaries.

Chapman *et al.*[4] conducted a field test by using the same spcies of nematode to control *C. p. fatigans* in polluted ditches and drains in Bangkok, Thailand, at doses exceeding 200,000/m^2; the resulting parasitism was low and never exceeded 27%; no recycling of the parasite was noted. Mitchell *et al.*[2] also made a field trial in Taiwan by the inundation of an area with preparasitics, resulted in infection of natural host populations in same cases; however, infection rates were generally low, and no evidence indicated that the parasite had been established in nature. In this study, field trials at site "A" showed that *R. nielseni* could recycle in the natural breeding place of *C. p. fatigans*. However, more information is needed on the factors affecting the mosquito breeding places, on the timing of parasite applications, on the cost of nematode production and, more important, on the effect of ecological factors on both host and parasite, such as the precipitation, and the changes in host density.

Table 4 indicates that the pH value of water is an important factor in limiting the habitat range of *R. nielseni* which, if used as a biological control agent, will probably have to be restricted to the mosquito breeding habitats with a pH range between 6.7 and 7.7.

TABLE 4
Effect of pH on the extent of parasitism (groups of 200 first-instar *C. p. fatigans* exposed to 2000 *R. nielseni*)

pH value	No. of larvae survived	% of survivors infected
5.7	0	
6.2	99	6
6.7	124	84
7.2	148	100
7.7	128	24
8.2	71	14
8.7	0	

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以尼爾遜寄生性線蟲防治臺灣產熱帶家蚊之研究

陳　寶　樹*

試驗結果顯示，尼爾遜線蟲可在室內以南港品系之熱帶家蚊幼蟲大量繁殖；其最經濟之蚊線蟲比率爲 1：7.5，蚊蟲密度以每平方公分水面 4.1 隻爲宜。

以第四齡期熱帶家蚊幼蟲接觸該前期線蟲，則線蟲可經由蚊蛹期至成蟲期。通常被寄生之蚊蟲於羽化後死亡，生存者則卵巢被退化而不能產卵。

室內試驗顯示以尼爾遜線蟲用爲蚊蟲生物防治時，蚊蟲滋生地之酸鹼度爲主要因子之一，其範圍需在 6.7 與 7.7 之間。

野外試驗結果，以尼爾遜前期線蟲引入熱帶家蚊滋生地，該線蟲可以繁殖衍生。

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