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Insecticide Resistance of Vector Mosquitoes of Japanese Encephalitis Virus in Korea*

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=國文抄錄=

韓國에 있어서 日本腦炎 媒介모기의 殺蟲劑 抵抗性

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韓國에 있어서 日本腦炎 媒介모기의 殺蟲劑 抵抗性을 結定하기 위해서 1987年 6月부터 10月까지 光州, 慶山, 金海, 大田, 永川, 全州, 忠州市의 近郊에서 日本腦炎 媒介모기인 작은 빨간집모기를 採集하여 WHO 殺蟲劑 專門委員會의 17次 報告書에 依據하여 調査하였다.

작은 빨간집모기 幼蟲은 malathion, diazinon, fenthion과 fenitrothion에 對하여 높은 抵抗性을 나타 내었으며, Lc_{50} 値는 3.3~34.1ppm 이었다.

7개 地域中 3地域에서 採集한 작은 빨간집모기의 temephos에 對한 Lc_{50} 値는 96.2~121.0ppm, DDVP에 對해서는 87.5~97.5ppm으로 높은 抵抗性을 나타내었다.

작은 빨간집모기 幼蟲은 合成 pyrethroid劑인 allethrin과 phenothrin에 對하여 感受性이 높다는 것이 證明 되었고, 이중 phenothrin이 더욱 높은 感受性을 나타내었다.

幼蟲에서와 마찬가지로 成蟲에서도 全州와 光州에서 採集한 colonies가 malathion과 fenthion에 對하여 높은 抵抗性을 나타내었으며, 慶山에서 採集한 colonies에서는 幼蟲의 成績과는 다르게 fenthion에 대하여는 아주 높은 抵抗性을 나타내었다.

이런 調査에서 有機燐劑에 대한 작은 빨간집모기의 抵抗性은 아주 높음을 알았다.

Introduction

The establishment of *Culex tritaeniorhynchus* Giles as an important vector in the transmission of Japanese encephalitis in Korea and neighbouring countries re-emphasized the need for control measures against this mosquito.

The organophosphorus insecticides have been used successfully for 25 years in routine control of the vector mosquitoes and other arthropods of medical importance.

The development of insecticide resistance among mosquitoes in Korea was first reported by Whang et al.(1965) in reference to the adults of *C. tritaeniorhynchus* against dieldrin in the vicinity of Pusan city and Namweon county of Cheonpook Province. Subsequently, Lee(1969) found that *C. tritaeniorhynchus* collected from Gobu Myun, Cheonpook Province

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was highly resistant to both DDT and dieldrin, but *Anopheles sinensis* Wiedemann caught in the same area was susceptible to DDT and resistant to dieldrin.

In a recent survey on control effects of pesticide applications against the vector mosquito larvae in rice fields in Korea (Ree et al., 1981) organophosphate resistance has been discovered in *C. tritaeniorhynchus*.

In spite of the fact that this species is the major Japanese encephalitis vector in Korea, very little information is available on the resistance level of insecticide on this species.

The present paper is an attempt to survey various populations of *C. tritaeniorhynchus* in Korea for resistance to organophosphorus insecticides and to obtain information concerning the resistance levels of the insecticides against the larvae and adults of *C. tritaeniorhynchus*.

Materials and Methods

1. Test insects and the methods of rearing: All the wild mosquitoes which had taken full blood meals were collected from human and animal shelters with an insect net or with a sucking tube, and transferred into the cages.

The mosquitoes, collected in cages, were brought to the Parasitology insectarium and reared by standard methods at 27±1°C and 70—80 per cent relative humidity with 16 hour of illumination per day.

Egg rafts were deposited by females about 4 days later in the container of water.

The approximately 300 first instar hatched were reared in enamel pans measuring 50×40 cm filled to 1 cm depth of water and fed on crushed powders of laboratory mouse pellets and ebiose, and the adults were fed on 5 per cent sugar solution.

The origin and the dates of collection of the laboratory colonies used in this study are listed in Table 1 and illustrated in Figure 1.

Third or early fourth instar larvae and two to five day old female mosquitoes were used in the tests.

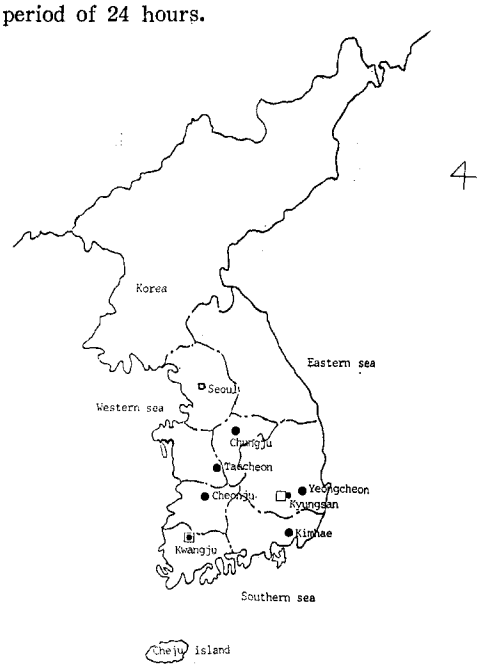
2. Insecticides used: Ethanol solutions of insecticides were prepared as the sample for testing the resistance levels of larvae, and acetone solutions were prepared for adult mosquitoes.

3. Test methods for resistance levels: Following standard WHO procedures, batches of 25 early fourth instar larvae of *C. tritaeniorhynchus* were exposed to several concentrations of insecticides.

The air temperature during testing was 25± 1°C. The mortality rates of the larvae were scored as the proportion dead or moribond after exposure for 24 hours. If the mortality of untreated mosquitoes or the pupation rate during a test exceeded 4/25, the results were discounted.

In order to measure the resistance level of adults, adult test papers were prepared by impregnating rectangles(12 by 15 cm) of Whatman No. 1 filter paper with 0.7ml of known percentage solution of insecticides in acetone.

Groups of up to 25 adults were exposed to the impregnated papers continuously for a period of 24 hours.



● Surveyed area

Fig. 1. Surveyed areas in Korea

Mortality was recorded at different intervals. Controls exposed to acetone or ethanol impregnated papers showed no mortality.

From these data the regression of the probit mortality on log of dosage was computed and the Lc_{50} and Ld_{50} were obtained.

Table 1. Details of the *C. tritaeniorhynchus* colonies used in the experiment(1987)

Location of collection		Collection date
Province	County	
Cheonnam	Kwangju	87. 8.16—17
Cheonpook	Cheonju	87. 8.30—31
Chungnam	Taecheon	87. 8.21—22
Chungpook	Chungju	87. 8.24—25
Kyungnam	Kimhae	87. 8.12—13
Kyungpook	Kyungsan	87. 7.28—30
	Yeongcheon	87. 8.05—06

Results

The organophosphate resistance levels of larval *C. tritaeniorhynchus* reared for one generation in the laboratory from collections in seven localities are compared with that of susceptible laboratory strain and listed in Table 2 and in Table 3.

In general, high resistance to organophosphate larvicides in seven colonies of *C. tritaeniorhynchus* was observed.

The larval *C. tritaeniorhynchus* was found to have high resistance against diazinon and malathion with Lc_{50} values of 17.0—29.8ppm and 10.8—34.1ppm, respectively.

The Lc_{50} values of the Kwangju and Yeongcheon colonies to diazinon were about 2,000 times as high as that of susceptible strain.

Tests with malathion showed that the resistance ratio of the Cheonju colonies, relative to susceptible strain, were the highest, being present in nearly 8,000 times, followed by Taecheon colonies with the ratios of 7,000 times and Kwangju colonies with 5,000 times.

Kimhae and Yeongcheon colonies were the least resistant.

The more increased resistance of larval *C. tritaeniorhynchus* to fenitrothion, fenthion and temephos were discovered in three localities, Cheonju, Kwangju, and Taecheon, the Lc_{50} values being 11.3—18.7ppm for fenitrothion, 16.0—27.3ppm for fenthion, and 96.2—121.0ppm for temephos.

Table 2. Resistance levels and resistance ratio of larval *C. tritaeniorhynchus* exposed to Diazinon and Malathion for 24 hours(1987)

Locality	Diazinon		Malathion	
	Lc_{50}	R.R*	Lc_{50}	R.R*
Cheonju	22.0	1,467	34.1	8,119
Chungju	21.0	1,400	19.2	4,571
Kimhae	17.5	1,167	11.6	2,762
Kwangju	29.8	1,987	22.0	5,239
Kyungsan	17.0	1,133	20.8	4,952
Taecheon	26.0	1,733	29.0	6,905
Yeongcheon	29.8	1,987	10.8	2,571
Susceptible strain**	0.015	—	0.0042	—

* R.R: resistance ratio
** Data reported by Department of Medical Entomology, NIH, Japan

These larvae also became about 2-3 times more resistant to malathion than other colonies collected from the Kimhae and Yeongcheon areas.

Table 4. shows the median lethal concentrations of larval *C. tritaeniorhynchus* exposed to DDVP and synthetic pyrethroides.

Among them, phenothrin was highly active against the larvae collected from seven localities. The Lc_{50} for larvae ranged from 0.0032(most susceptible colonies) to 0.0117ppm(most tolerant colonies), a 4-fold range of concentration.

In the allethrin, the Lc_{50} concentration ranged from 0.0280 to 0.1050ppm for various colonies. In these instances, allethrin was somewhat less active than phenothrin.

The data shown in Table 5 present the mortality rates of adult *C. tritaeniorhynchus* of 5 colonies after 1, 6 and 24 hour exposure to

Table 3. Lc_{50} values in ppm and resistance ratio of larval *C. tritaeniorhynchus* against Fenitrothion, Fenthion and Temephos (1987)

Locality	Fenitrothion		Fenthion		Temephos	
	Lc_{50}	R.R*	Lc_{50}	R.R*	Lc_{50}	R.R*
Cheonju	13.3	17,273	21.8	15,352	121.0	155,128
Chungju	7.5	9,740	7.8	5,493	47.5	60,897
Kimhae	7.8	10,130	10.3	7,254	50.0	64,103
Kwangju	18.7	24,286	27.3	19,225	96.2	123,333
Kyungsan	9.5	12,338	3.3	2,324	61.8	79,231
Taecheon	11.3	14,675	16.0	11,268	100.0	128,205
Yeongcheon	6.8	8,831	3.5	2,465	22.5	28,846
Susceptible strain**	0.00077	—	0.00142	—	0.00078	—

* R.R: resistance ratio
** Data reported by Department of Medical Entomology, NIH, Japan

Table 4. Median lethal cocentrations of larval *C. tritaeniorhynchus* exposed to DDVP and synthetic pyrethroides (1987)

Locality	Lc_{50} values in ppm of larvae		
	DDVP	Allethrin	Phenothrin
Cheonju	80.0	0.0575	0.0053
Chungju	47.5	0.0625	0.0036
Kimhae	95.0	0.0463	0.0040
Kwangju	45.8	0.0280	0.0117
Kyungsan	97.5	0.0633	0.0065
Taecheon	87.5	0.1050	0.0088
Yeongcheon	97.5	0.0280	0.0032

malathion-, fenitrothion-, and fenthion- impregnated papers.

Also in adult mosquitoes as in larvae, Cheonju and Kwangju colonies were highly resistant to malathion.

To fenthion, the Cheonju and Kwangju colonies were also highly resistant and the Kyungsan colonies, not so resistant in the larval stage, was extraordinarily resistant.

Discussion

The most important finding of this study was

Table 5. Motality rates of adult *C. tritaeniorhynchus* after 1, 6 and 24 hour exposure to Malathion- Fenitrothion- and Fenthion- impregnated papers (1987)

Concentration	Cheonju			Chungju			Kimhae			Kwangju			Kyungsan		
	1	6	24*	1	6	24	1	6	24	1	6	24	1	6	24
% Malathion															
0.05	5.0**	10.0	25.0	5.0	25.0	50.0	5.0	40.0	60.0	0	2.00	45.0	15.0	30.0	45.0
0.1	5.0	30.0	50.0	5.0	30.0	75.0	5.0	40.0	80.0	0	20.0	55.0	15.0	35.0	65.0
0.2	5.0	25.0	90.0	5.0	25.0	95.0	5.0	45.0	100.0	0	40.0	90.0	20.0	50.0	90.0
% Fenitrothion															
0.05	5.0	25.0	70.0	0	20.0	90.0	0	20.0	80.0	0	10.0	40.0	5.0	45.0	80.0
0.1	5.0	35.0	90.0	0	40.0	100.0	0	40.0	100.0	0	25.0	85.0	5.0	55.0	95.0
0.2	5.0	40.0	100.0	0	45.0	100.0	5.0	40.0	100.0	0	35.01	00.0	10.0	55.0	100.0
% Fenthion															
0.05	5.0	10.0	25.0	0	20.0	45.0	0	15.0	40.0	0	5.0	20.0	5.0	25.0	45.0
0.1	5.0	15.0	75.0	5.0	35.0	85.0	0	30.0	80.0	0	25.0	75.0	10.0	50.0	95.0
0.2	5.0	25.0	90.0	5.0	45.0	100.0	0	45.0	100.0	0	35.0	85.0	10.0	50.0	100.0

* hour
** mortality rate (percent)

the marked variation in resistance levels to a particular insecticide between colonies of *C. tritaeniorhynchus* larvae and adults collected from seven different locations in Korea, and generally provided certain evidence that the resistance ratios of larval *C. tritaeniorhynchus* against organophosphorus compounds were extraordinarily high.

After the end of World War Ⅱ, chlorinated hydrocarbon and organophosphorus compounds were introduced and widely used for the control of insects and other arthropods of medical and agricultural importance in Korea.

Since Hurlbut et al. (1952) made a survey of DDT resistance in Korean body lice and reported for the first time that the body lice was DDT-resistant, many investigations on the development of resistance to insecticides have been published.

From these results, the Korean government stopped the import of DDT in 1959 because of the fact that Korean people complained that insects and other arthropods of medical importance were not killed after DDT spraying operations.

Thereafter, malathion was introduced into Korea as a substitute for DDT, and other organophosphorus compounds have also been imported for agricultural pest control.

In the studies on insecticide resistance of vector mosquitoes in Korea, Hwang et al. (1965) carried out a survey on insecticide susceptibility of *C. tritaeniorhynchus* caught from cows outdoors in the vicinity of Pusan in August, 1964, and reported for the first time that this species was DDT-susceptible and dieldrin resistant.

Lee(1969) reported that *Anopheles sinensis* as a malaria vector species in Korea was found to be susceptible to DDT and resistant to dieldrin, but *C. tritaeniorhynchus* collected from Gobu-myun, Jeongup county, Cheonpook Province as one of the endemic areas of Japanese encephalitis was highly resistant to both DDT and dieldrin.

Subsequently, Ree et al. (1979) conducted a survey on the control effects of agricultural pesticides against mosquito populations of rice paddy breeding species in Korea, and reported that the mortality rates of *C. tritaeniorhynchus* on the first day after treatment were 33.2 per cent to BPMC, 65.6 per cent to diazinon, 32.4 per ent to fenitrothion, 99.5 per cent to padan, and 6.2 per cent to malathion.

They also indicated that most of organophosphorus and carbamate insecticides tested were available to control mosquito larvae in the rice field, while they seriously affected the population of other aquatic organisms.

In recent years, from their studies on control effects of pesticide applications against the vector mosquito larvae in rice fields in Korea, Ree et al. (1981) reported that *C. tritaeniorhynchus* and *A. sinensis* had developed high resistance to most of the insecticides as compared with the results reported by Self et al. (1974). Similar results in vector mosquitoes have been obtained by Shim et al. (1979), Ree et al. (1980), Shim and Kim(1980), and Shim and Kim (1981).

In the present study, the larval *C. tritaeniorhynchus* showed high resistance to malathion and fenitrothion with Lc_{50} values of 10.8—34.1 ppm and 6.8—18.7ppm, respectively.

The more increased resistance to temephos and fenthion was discovered in *C. tritaeniorhynchus* collected from three locations, Cheonju, Kwangju, and Taecheon, being 96.2—121.0 ppm for temephos, and 16.0—27.3 ppm for fenthion.

The results presented in Table 2 and Table 3 indicate that the resistance ratio to organophosphorus compounds in seven colonies of *C. tritaeniorhynchus*, relative to susceptible colonies, were nearly 2,000—150,000 times.

C. tritaeniorhynchus first appeared in mid-June and trapped in large numbers during the period from mid-August to early September, showed a simple sharply pointed one-peak curve. There was a gradual decrease from mid-Septe-

mber, with a very small number of them collected until early October. The main breeding sites contributing to populations of *C. tritaeniorhynchus* in Korea were evidently rice fields. The Korean have been striving for self-sufficiency in rice by increasing yields on existing paddy acreage and they have relied on chemical fertilizers and insecticides.

According to the Bureau of Agricultural production, approximately 12.0 kg of fungicides and insecticides were applied to one hectare of rice-field by farmers, 7—9 times during the period from June to September in 1987, for the control of agricultural pests.

The intensive use of fungicides and insecticides for the control of agricutural pests and medical insects, which transmit communicable diseases during the summer season, by goverment officials and by farmers have greatly influenced the populations of the mosquito larvae and their predators.

As shown in Table 4, the relatively high Lc_{50} values were obtained with all colonies tested with phenothrin. Especially high resistance levels were observed in collections from Kwangju(0.0117ppm) and Taecheon(0.0088ppm), respectively. Because the tests were conducted on larvae, against which this product is less potent, these results may not fully reflect that existing in adults.

Resistance being higher among colonies of *C. tritaeniorhynchus* in the present survey suggests that this is probably related to some difference in the opportunity of contacting insecticides and fungicides.

Summary

In order to determine the resistance levels on *Culex tritaeniorhynchus*, vector mosquitoes of Japanese encephalitis virus, collected from the suburb of seven cities, a survey based on seventeeth report of WHO Expert Committee on insecticides carried out during the period from June to October in 1987.

The larval *Culex tritaeniorhynchus* showed high resistance to malathion, diazinon, fenthion and fenitrothion with the Lc_{50} values of 3.3—34.1ppm.

Increased resistance to temephos and DDVP was discovered in three colonies of *Culex tritaeniorhynchus*, the Lc_{50} values being 96.2—121.0ppm for temephos and 87.5—97.5ppm for DDVP.

Against larval *Culex tritaeniorhynchus*, both allethrin and phenothrin proved susceptible, the latter being somewhat more active.

Also in adult mosquitoes as in larvae, Cheonju and Kwangju colonies were highly resistant to malathion.

To fenthion, the Cheonju and Kwangju colonies were also highly resistant and the Kyungsan colonies, not so resistant in the larval stage, was extraordinarily resistant.

Summarizing the results, this study indicated that the resistance levels of *Culex tritaeniorhynchus* against organophosphorus compounds are extraordinarily high.

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