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Integrated Control of Vector Mosquitoes with Native Fishes (*Aplocheilus* and *Aphyocypris*) and *Bacillus thuringiensis*(H-14) in Natural Rice Fields of Korea*

천적포식어(*Aplocheilus* and *Aphyocypris*)와 미생물제제 *Bacillus thuringiensis* (H-14)의 병합처리에 의한 논에서 서식하는 질병매개모기의 종합적방제

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ABSTRACT A confined field assessment on the integrated control of vector mosquitoes (*Anopheles sinensis* and *Culex tritaeniorhynchus*) was conducted by timely treatment of *Bacillus thuringiensis*(H-14) formulation in the presence of larvivorous fish (*Aplocheilus latipes*) in natural rice fields of 30,000 M^2 at Yongam, South Korea Cholla Province from July through October in 1988. In presence of larvivorous fish (*Aplocheilus*) at lower density of 0.6 fish per M^2 water surface, mosquito vector control rates ranged 55.0~57.6% from July through August, and when the mosquio density started to increase with over 10 larvae on the average per sample, *B.t.*(H-14) formulation treatment at the rate of 1.0 Kg/ha made 100% vectors reduction in 24 hours, the control sustained above 98% until the test was terminated on October 11th. In the fish introduced rice paddies with *Aphyocypris chinensis* at the density of above 1.5 fish per M^2, a satisfactory degree of vector control was obtained by 88.2~96.7% in 2 week period until September 21st. In the absence of larvivorous fishes, *B.t.*(H-14) treatment made 100% control in 24 hours, however, vector population rebound appeared in day 7, required to make additional treatment to suppress population down.

KEY WORDS *Aplocheilus latipes, Aphyocypris chinensis, Bacillus thuringiensis israelensis*(H-14), *Anopheles sinensis, Culex tritaeniorhynchus*, integrated control

抄 錄 1988년 7월부터 10월까지 약 4개월간 질병매개모기 및 뇌염다발지역인 전라남도 영암군 덕진면 소재 약 30,000M^2의 자연수답을 선정, 이에 서식하는 질병매개모기인 중국얼룩날개모기(*Anopheles sinensis*)와 작은빨간집모기(*Culex tritaeniorhynchus*)를 대상으로 포식천적어송사리(*Aplocheilus latipes*) 및 왜몰개(*Aphyocypris chinensis*)방사와 미생물제제(*Bacillus thuringensis* H-14)를 병합처리하여 방제효과를 조사하였다. 포식천적어송사리가 0.6 fish/M^2가 존재하는 논에서는 7월부터 8월까지 55.0~57.6%의 자연 방제가 이루어졌으며, 한 표본당 평균 10마리이상으로 증가될 때 미생물제제(*B.t.* H-14)를 1 Kg/ha의 농도로 처리한 결과, 24시간후 100%의 방제를 보였으며, 10월 11일 본 실험이 끝날때까지 98%의 방제율을 유지하였다. 천적어가 존재하지 않는 논에서는 수면 1M^2 당 1.5마리의 비율로 포식어(*Aphyocypris*)를 방사한 결과 9월 21일(방사후 2주)까지 88.2~96.7%의 만족할 만한 방제율을 유지하였다. 천적어가 존재하지 않는 또 다른 논에서 미생물제제(*B.t.* H-14)를 1 Kg/ha의 농도로 단독 처리한 결과, 24시간 후 100%의 방제율을 보였으나, 7일후에는 개체군밀도의 회복현상을 나타내었으며 *B.t.*(H-14) 2차 처리후 모기유충의 개체군밀도를 억제할 수 있었다.

檢索語 송사리, 왜몰개, 미생물제제(*B.t.* H-14), 중국얼룩날개모기, 작은빨간집모기, 병합적방제

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Recent intrest in mosquito vector control has been directed toward an integrated methods of using indigenous fish predators with timely treatment of bacterial pathogen, *Bacillus thuringiensis israelensis* (*B.t.* H-14), in particular against Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles, in parsley field, marshes and lotus field in South Korea.

During the past years, a series of confined field trials of either predator fish or fish combined with *B.t.*(H-14) had been conducted in different vector breeding habitats, indicating that either of a single treatment rarely yield a continous successful mosquito larval suppression except that the fish population in summer increased and proliferated enough to make an efficient larval predation at the critical mosquito breeding period from late July to mid-August where *C. tritaeniorhynchus* used to reach peak density(Yu *et al.* 1985, Yu *et al.* 1986).

In natural rice field, a rather successful results of controlling encephalitis vector, *Cx. tritaeniorhynchus* in a month period at the release rate of 1.0 mature fish *Aphyocypris chinensis* over 1-M^2 rice paddies of Yongam one of the most disease endemic areas in South Korea was reported previously by Yu and associated workers in 1983.

In marsh area, Yu and associate workers reported a significant results of reducing (*Cx. tritaeniorhynchus*) at the release rate of 1.0 mature fish per 1-M^2 in marsh of Yongam-Kun. However, it required that an augmentation of treatment, preferably using *B.t.i.* (H-14) during August whereas the vector population increased in an abrupt manner over 3 folds(Yu *et al.* 1984).

In case of parsley fields, an integrated trials of native fish predator (*Aphocypris chinensis*) followed by *B.t.*(H-14) treatment in late August made a satisfactory level of control against both *Culex tritaeniorhynchus* and *Anopheles sinensis* the level of suppression had sustained until October (Yu *et al.* 1986).

Thus, this report discusses the results of an integrated effects in mosquito control by the treatment of *B.t.* (H-14) combined with a native fish available in natural rice fields in Yongam the disase endemic area particularly with heavy infestation of encephalitis vector *Culex tritaeniorhynchus*, and the mosquito control effect of each agent compared with the integrated was also elaborated.

MATERIALS AND METHODS

A confined field plot of ca. 30,000M^2 natural rice field located in Duckjin-Myun, Yongam-Kun, South Cholla province near Kwangju, one of the highest density recorded for the encephalitis vector areas was selected and aquatic sampling was begun from July through October in 1988 (Fig. 1).

Field plots of 3 different rice paddies were chosen, and random selction was made 2∼3 paddies in 3 different groups out of a total of 30 paddies; (i) the first group being "a-paddies" in the presence of fish predator at low density of 0.6 fish per M^2 water surface needed for complementary treatment with *B.t.* (H-14), (ii) the 2nd group being "b-paddies" where *Aphyocapris chinensis* fish introduced at the rate of 1.5 fish per M^2, (iii) the 3rd group being "c-paddies" in the absence of fish predators either used for *B.t.* (H-14) treatment alone and others were left as control.

Mosquito larval density before and after the fish introduction was estimated by taking random sampling of mosquito larvae by means of 500ml dipper with 6∼8 replicates per rice paddy usually consisted of two dips were pooled, and in similar manner, aquatic sam-

Table 1. Average number of mosquito larvae and % reduction before and after the treatment of *Bacillus thuringiensis* (H-14) R153.78 in the presence of lower density of larvivorous fish, *Aplocheilus latipes* in natural rice paddies at Yongam near Kwang-ju area, south Cholla Province, July-October, 1988

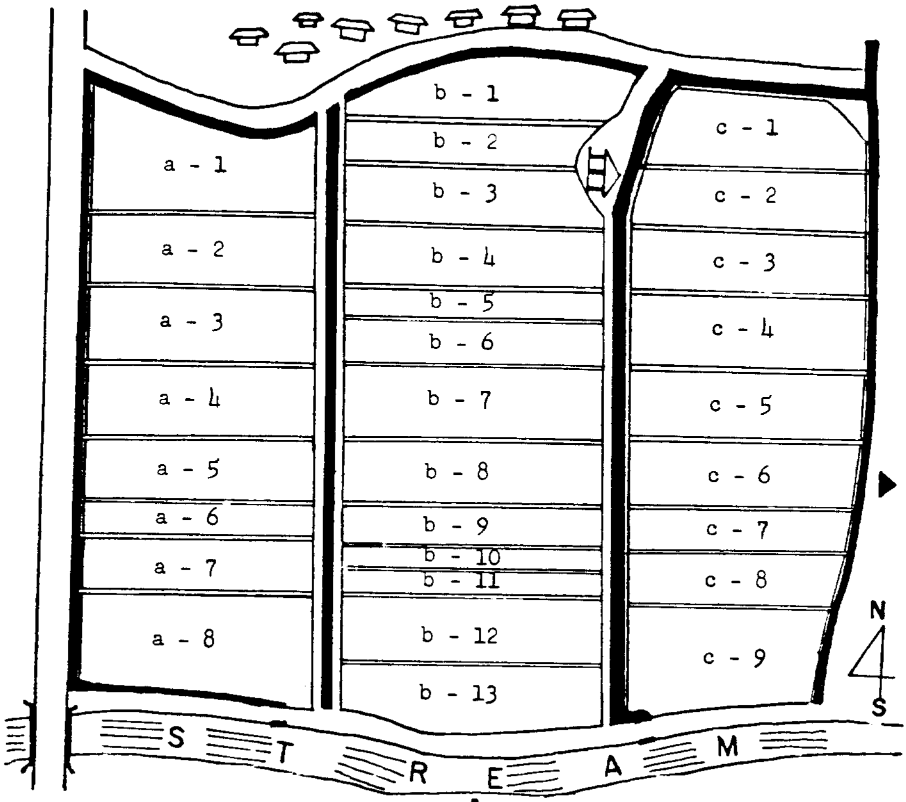
Sample	*Culex tritaeniorhynchus*		*Anopheles sinensis*		Total No. Mosquitoes	
Date	Cont.	Treat	Cont.	Treat	Cont.	Treat
Natural breeding						
Jul. 16	0.0	0.0	2.0	1.5	2.0	1.5
% Reduction[a]		—[b]		25.0%		25.0%
Jul. 28	0.25	0.1	2.75	1.25	3.0	1.35
% Reduction[a]		60.0%		54.5%		55.0%
Aug. 18	1.25	0.75	3.0	1.25	4.25	2.0
% Reduction[a]		40.0%		58.3%		53.0%
Sep. 2	4.5	2.0	10.20	4.25	14.75	6.25
% Reduction[a]		55.5%		58.5%		57.6%
Total	6.0	3.75	18.0	8.25	24.0	11.1
Average	1.5	0.94	4.5	2.06	6.0	2.78
% Reduction[a]		37.3%		54.2%		53.7%
Post-treatment (*B.t.* H-14, 1 kg/ha)						
Sep. 3	8.25	0.0	12.25	0.0	20.50	0.0
% Reduction[a]		100.0%		100.0%		100.0%
Sep. 10	11.75	0.13	9.0	0.25	20.75	0.38
% Reduction[a]		98.9%		97.2%		98.2%
Sep. 21	5.13	0.0	2.25	0.0	7.38	0.0
% Reduction[a]		100.0%		100.0%		100.0%
Oct. 11	2.0	—[c]	0.25	—[c]	2.25	—[c]
% Reduction[a]		—[b]		—[b]		—[b]
Total	27.13	0.13	23.75	0.25	50.88	0.38
% Reduction[a]		97.68%		97.63%		98.06%

[a] % Reduction=(A−B)/A×100.
Whereas, A=No. mosquito larvae survived in control.
B=No. mosquito larvae survived in treatment.
[b] Represents value of % reduction statistically not available.
[c] Field sampling was unable to be taken owing to complete dryness of the rice paddies at the time.

ples were taken by the use of aquatic net of 25 cm diameter with 120 cm wooden handle attached in such that the net sweeping of 8 ~10 times per each of the sample with 4 replicates per rice paddy water. The mosquito larval and aquatic samples were brought back to the laboratory and identified according to the characters described in each of the specific key of the corresponding taxa (Usinger 1971, Lacasse & Yamaguti 1955).

Relative density of native fishes before and after the fish introduction was estimated by setting fish trap of 12×25 cm aquarium folding type for the confirmation of either presence or absence of the released fishes in the treatment and control paddies respectively, and the fish caught were identified according to the specific key (Chyung 1977).

The control effectiveness was compared based on mean number of mosquito larvae of 2 species predominantly breeding in rice fields, *Culex tritaeniorhynchus* encephalitis vector, *Anopheles sinensis* malaria vector with emphasis placed on encephalitis vector which



A

B

Fig. 1. Natural rice paddies used for field trial with the native larvivorous fishes (*Aplocheilus latipes* and/or *Aphyocypris chinensis*) integrated with *Bacillus thuringiensis*(H-14) compared with each of the single treatment either with fish predator or *B.t.* (H-14).
A : Schematic mapping of the rice paddy in 3 different groups.
B : overall view of 3 ha natural rice paddies in Yongam County.

Table 2. Average number of mosquito larvae per two, 500 ml dips and % reduction after the fish release (*Aphyocypris chinensis*) at the rate of 1.5 fish per 1-M² water surface in natural rice paddies at Yongam near Kwang-ju area, South Cholla Province, July-October, 1988

Sample	*Culex tritaeniorhynchus*		*Anopheles sinensis*		Total No. Mosquitoes	
Date	Cont.	Treat	Cont.	Treat	Cont.	Treat
Pre-treatment						
Jul. 16	0.0	0.0	2.0	4.25	2.0	4.25
Jul. 28	0.25	0.25	2.75	3.5	3.0	3.75
Aug. 18	1.25	1.5	3.0	2.25	4.25	3.75
Sep. 2	4.5	4.25	10.25	8.75	14.75	13.00
Total	6.0	6.0	18.00	18.75	24.00	24.75
Average	1.5	1.5	4.5	4.69	6.00	6.19
Post-treatment						
Sep. 3	8.25	6.0	12.25	8.5	20.5	14.5
% Reduction[a]		27.3%		33.3%		31.3%
Sep. 10	11.75	1.25	9.0	1.25	20.75	2.5
% Reduction[a]		89.4%		86.6%		88.2%
Sep. 21	5.13	0.25	2.25	0.0	7.38	0.25
% Reduction[a]		95.1%		100.0%		96.7%
Oct. 11	2.0	—[b]	0.25	—[b]	2.25	—[b]
Total	27.13	7.5	23.755	9.75	50.88	17.25
% Reduction[a]		84.9%		60.5%		67.1%

[a] % Reduction=[(A×B′/A′−)/A×B′/A′]×100.
Whereas A =No. mosquito larvae sampled in control of post-treatment.
B =No. mosquito larvae sampled in treatment of post-treatment.
A′=No. mosquito larvae sampled in control of pre-treatment.
B′ =No. mosquito larvae sampled in treatment of pre-treatment.

[b] Field sampling was unable to be taken owing to complete dryness of the rice paddies at the time.

raised the problem responsible for transmitting the above disease in this area of endemic foci.

The statistical analysis was performed to obtain mean number of mosquito larvae reduction rate in the treatment, and the mosquito reduction rate in the treatment was calculated in comparison with the control plot with the following formula;

$$\% \text{ Reduction} = \frac{(A \times B'/A' - B)}{A \times B'/A'} \times 100$$

Whereas, A=No. of larvae sampled from control of post-treatment
B=No. of larvae sampled from treatment of post-treatment
A′=No. of larvae sampled from control of pre-treatment
B′=No. of larvae sampled from treatment of pre-treatment

RESULTS AND DISCUSSION

Natural fish breeding rice paddy (a-plot)

The pre-treatment sample data indicated that the *Culex tritaeniorhynchus* and *Anopheles sinensis* population increased, marking 4. 25 larvae per sample on the average in mid September which increased up to late September also marking 14. 7 larvae in control, while the treatment plot also indicated 2. 0 and 6. 25 larvae in the same period marking ca. 53% or above natural conttrol in the presence of native fish *Aplocheilus latipes* at rather lower density of 0. 6 fish per/M².

Table 3. Average number of mosquito larvae per two, 500 ml dips and % reduction after the treatment of *Bacillus thuringiensis* (H-14) R153.78 at higher density of mosquito larvae in rice paddies at Yongam near Kwang-ju area, South Colla Privince, July-October, 1988

Sample	*Culex tritaeniorhynchus*		*Anopheles sinensis*		Total no. Mosquitoes	
Date	Con.	Treat	Con.	Treat	Con.	Treat
Pre-treatment						
Jul. 16	0.0	0.0	2.0	2.0	2.0	2.0
Jul. 28	0.25	0.5	2.75	1.0	3.0	1.5
Aug. 18	1.25	1.25	3.0	4.25	4.25	5.5
Sep. 2	4.5	3.75	10.25	8.0	14.75	11.75
Total	6.0	5.5	18.0	15.25	24.0	20.75
Average	1.5	1.38	4.5	3.81	6.0	5.19
1st-Treatment (R153.78, 1 kg/ha)						
Sep. 3	8.25	0.0	12.25	0.0	20.5	0.0
% Reduction[a]		100.0%		100.0%		100.0%
Sep. 10	11.75	7.25	9.0	5.75	20.75	13.0
% Reduction[a]		32.93%		24.8%		27.9%
2nd-Treatment (R153.78, 1 kg/ha)						
Sep. 11	9.25	0.0	8.5	0.0	17.75	0.0
% Reduction[a]		100.0%		100.0%		100.0%
Sep. 21	5.13	0.0	2.25	0.0	7.38	0.0
% Reduction[a]		100.0%		100.0%		100.0%
Oct. 11	2.0	—[b]	0.25	—[b]	2.25	—[b]
% Reduction[a]		—[c]		—[c]		—[c]
Total	36.38	7.25	32.25	5.75	68.63	13.0
% Reduction[a]		78.34%		79.0%		78.2%

[a] % Reduction=[(A×B′/A′−B)/A×B′/A′]×100.
Whereas A =No. mosquito larvae sampled in control of post-treatment.
B =No. mosquito larvae sampled in treatment of post-treatment.
A′=No. mosquito larvae sampled in control of pre-treatment.
B′ =No. mosquito larvae sampled in teatment of pre-treatment.
[b] Field sampleling was unabel to be taken owing to complete dryness of the rice paddies at the time.
[c] Represents value of % reduction statistically not available.

The treatment of *B.t.*(H-14) at the dosage rate of 1.0 kg/ha yielded 100.0% control against *Culex tritaeniorhynchus* and *Anopheles sinensis* in 24 hours; the rates of mosquito control were subsequently sustained above 98.0% in October 11th until the experiment was terminated(Table 1, Fig. 2).

Fish release rice paddy (b-plot)

Fish introduction of *Aphocypris chinensis* at the release rate of 1.5 mature fish over 1-M^2 rice paddy surface area resulted in significant encephalitis vector *Culex tritaeniorhynchus* reduction by 89.4% in comparision with that control rice paddy, and *Anopheles sinensis* larvae was reduced at the rate of 86.6% respectively, while the control rice paddy recorded with an average number of *Culex tritaeniorhynchus* and *Anopheles sinensis* per sample of 11.75 and 9.0 larvae respectively (Table 2).

During September, mosquito larval population attained the highest level of density probably influenced by combined environmental

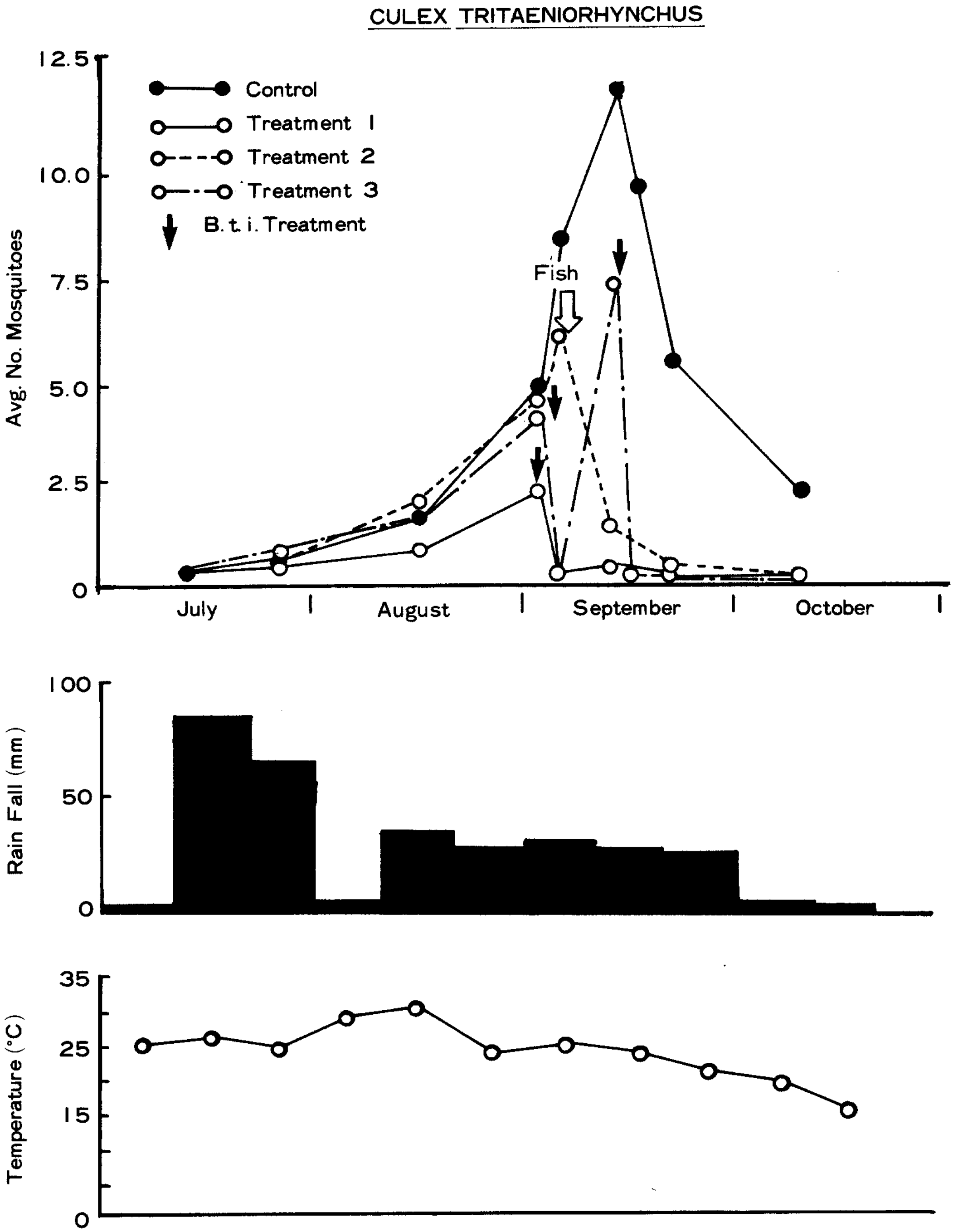


Fig. 2. Population curve of *Culex tritaeniorhynchus* Gile, the main vector of Japanese encephalitis before and after the integrated treatment of *B.t.*(H-14) R. 153. 78 in the presence of lower density of native fish, *Aplocheilus latipes* in natural rice paddies at Yongam, South Cholla Province, Korea. July-October, 1988.

factors of rainfall recorded in mid-September together with higher temperature above 25°C throughout the period (Fig. 2).

Bacillus thuringiensis (H-14) treatment rice paddy(c-plot)

In the absence of larvivorous fishes, 1st treatment of *B.t.*(H-14) at the dosage rate of 1.0 kg/ha yielded 100.0% control against *Culex tritaeniorhynchus* and *Anopheles sinensis* in 24 hours however vector mosquito rebound appeared in day 7 (Table 3).

The second treatment of *B.t.*(H-14) at the similar dosage rate as mentioned above, yielded a satisfactory level of control by 100.0 % mosquito reduction. The mosquito supression had been sustained until the experiment was terminated on October 11th (Table 3, Fig. 2).

The results of our field trial in natural rice paddies where the encephalitis vector, *Culex tritaeniorhynchus* predominantly bred, used to responsible for the outbreaks of the disease indicates that (1) in rice paddies of native fish already present at rather lower density under 1.0 per M^2 water surface area should be effectively controlled the vector population with integration of *B.t.* (H-14) treatment at the dosage rate of 1.0 kg/ha in critical vector breeding period, (2) in case of using larvivorous native fishes, the release rate should be at or more than 1.5 mature fish per M^2 preferably to introduce one month earlier from the period of vector population inbrease, and (3) in absence of fishes, *B.t.* (H-14) treation required two times or more to suppress the vector population down due parimarily to the mosquito population rebound used to appear in a week period, in particular, rice paddy condition in Korea.

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