toxicity.

It appeared that the lethal toxicity and knock-down effect of DDT emulsions were not correlated with anyone of physical properties such as boiling point, dipole moment, dielectric constant and surface tension of solvents used, and that these were also not correlated with the lethal toxicity and knockdown effect of the solvents.

From the consideration of additivity of HLB value of the solvent and the surfactant, a significant correlation between knockdown effectiveness of *p, p'*-DDT emulsion and the HLB value was observed. In other words, the peak of lethal activity was 4.4, and that of knockdown speed existed in more lipophilic site than 3.8 of HLB value; therefore, it was presumed that the more lipophilic site than the lethal action was necessary for the rapid paralysis of insect.

The knockdown speed of *p, p'*-DDT emulsion was not correlated with the physical properties of solvents used and also with biological activities of the solvent alone.

Studies on Some Biological Activities of *N*-(2-Methyl-4-Chlorophenyl)-*N'*, *N'*-Dimethylformamidine (Galecron) to the Rice Stem Borer, *Chilo suppressalis* Walker. Tadayoshi Hirano*, Hidetaka Kawasaki, Hiroshi Shinohara**, Tadaharu Kitagaki (Life Science Research Institute, Kumiai Chemical Industry Co., Ltd., Kikugawa, Shizuoka-Pref.) and Shigeki Wakamori** (Chemical Research Institute Kumiai Chemical Industry Co., Ltd., Shimizu) Received August 5, 1972, *Botyu-Kagaku*, 37, 135, 1972.

20. ガルエクロンのニカメイチュウに対する種々の生物活性について 平野忠美*, 川崎秀高, 篠原寛**, 北垣忠温 (クミアイ化学工業株式会社, 生物科学研究所) 若森薫熙 (クミアイ化学工業株式会社, 化学研究所) 47. 8. 5 受理

ガルエクロンのニカメイチュウに対する種々の生物活性について検討した. その結果を要約すると次の通りである.

1) 直接殺虫力は低かったが, 忌避作用および, 殺卵力はすぐれていた.
2) 水面施用により, 残効性の長いすぐれた食入防止効果を示した.
3) γ-BHC 抵抗性ニカメイチュウに対して感受性ニカメイチュウよりややすぐれた食入防止効果を示した.
4) 食入幼虫に対して, スミチオンと同等の殺虫効力を示した.
5) ガルエクロンの数種誘導体を合成し, その食入防止効果を検討したが, いずれもガルエクロンより著しく劣った.
6) ガルエクロンはニカメイチュウのコリンエステラーゼ, アリエステラーゼ及びメラニン生成活性のいずれをも阻害しなかった.

Introduction

Galecron was evaluated as an excellent acaricide with ovicidal and adulticidal activity[1] and it has mainly been used for control of European red mite, *Panonychus ulmi Koch*, since 1967 in Japan. However, in 1967, it was indicated that silk worm, *Bombyx mori L.* was inhibited on feeding mulberry leaves exposed to Galecron. The present paper reports some biological activities of Galecron to the rice stem borer *Chilo suppressalis* Walker, one of most important lepidopterous insect to be controled in paddy field.

Materials and Methods

Insect:

Eggs and larvae of the rice stem borer were the normal strain which was collected from the

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** Head Office, Kumiai Chemical Co., Ltd., Chiyoda, Tokyo. クミアイ化学工業株式会社本社 開発室 This work was presented at the Annual Meeting of the Japanese *Soc. Appl. Ent. Zool.*, Tokyo, April 1971.

field in Odawara, Kanagawa-pref. in 1966 and had been reared on the rice seedlings in a constant temperature room (30°C, 16 hr illumination). The γ-BHC-resistant strain was established to repeat the pressure in laboratory.[2)]

Plant:

The rice seedlings, *Oriza sativa L.* and the potted young rice plants.

Compounds:

Following 12 compounds were tested: Galecron, and its five derivatives, Sumithion, γ-BHC, EPN, Diazinon, Cartap, DDVP and phenylthiourea. These materials were applied as acetone solution or emulsifiable concentration.

Direct insecticidal activity test:

Ten of 3rd-instar larvae was placed in a vinyl chloride resin cup which contains the rice seedlings dipped into the emulsion of test compounds. They were hold in a constant temperature room (25°C) and the mortality was observed 72 hrs after treatment. Each test was replicated 4 times.

Repellent activity test:

Each 3g of the rice seedlings dipped in Galecron emulsion and the untreated rice seedlings were placed, on the opposite direction, at the same distance from the center in petri dish (135×35 mm) and the prehatching eggs were laid at the center. The light condition was constant at each point and the test was examined at three replications. After incubation for 5 days at 30°C, the number of larvae in each mass of rice seedlings was counted and the percent repellency was calculated according to the following formula.

$$\text{Repellency}(\%) = \frac{U-T}{U+T} \times 100$$

U=Number of larvae in untreated seedlings.
T=Number of larvae in treated seedlings.

Protective effectiveness test to the rice stem borer:

The aceton solutions of test compounds were poured on the surface of water in Wagner pots (5×10^{-3} are) with the young rice plants. Two days after treatment, the hatched larvae were inoculated on the plant.

Eleven days after infestation, the number of the survival larvae inside the rice plants was counted and percent survival was calculated. Each test was replicated three times.

Residual effectiveness test:

It was the same method as protective effectiveness test except infesting 5, 9 and 14 days after treatment.

Insecticidal activity test to the boring larvae in the rice plants:

The potted young rice plants were infested with the hatched larvae and test compounds solution were applied to them with a spray gun 7 days after infestation. Five days after application, the number of the survival larvae was counted. Each test was replicated three times.

Ovicidal activity test:

The rice stem borer eggs were dipped in the emulsion of test compounds and the number of unhatched eggs were counted 7 days after treatment. Test was conducted at 25°C and three replications.

Synthesis of some Galecron derivatives:

As shown in Fig. 1, the formamidines were prepared from *N,N*-dimethylformamide (I) and the corresponding anilines (II) by the action of phosphorus oxychloride. Physical constants and analytical data for the compounds prepared are shown in Table 1. Detail of a preparation method is described below.

N-(4-Chlorophenyl)*N'*,*N'*-dimethylformamidine 23.1 grams of phosphorus oxychloride dissolved in 40m*l* of dry benzene was added to 36.5g of *N,N*-dimethylformamide in 50m*l* of dry benzene with stirring at 20-25°C. After completing addition, the reaction mixture was stirred at room temperature for an additional 30 minutes. Moreover, 16g of 4-chloroaniline dissolved in 80m*l* of dry benzene was added into a mixture

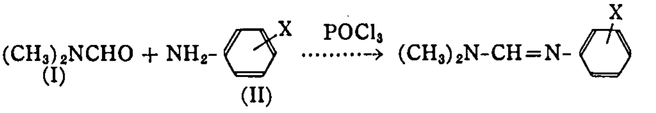
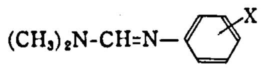


Fig. 1. Synthetic scheme of the formamidines.
X: Some phenyl substituents as shown in Table 1.

Table 1. Physical constants and analytical date of synthetic compounds.



Code No.	X	Yield (%)	Appearance	bp°C/mmHg (mp°C)	nD[20] (Solvent of recrystallization)
I	H	98	Coloress oil	85-90/0.9	1.5959
II	2-CH_3	84	Pale yellow oil	70-80/0.05	1.5796
III	4-Cl	62	Coloress oil	102/0.03	1.6017
IV	2-Cl	83	Pale yellow oil	95-100/0.05	1.6065
V	4-$COCH_3$	35	Pale yellow prism	(89-90)	(*n*-Hexane)

at 30-35°C. After this addition, the reaction mixture was stirred at 30-35°C for additional 3 hrs. The produced precipitates were filtered, and treated with 500m*l* of 2N sodium hydroxide under cooling. The oily parts were extracted with 150m*l* of benzene. The benzene layer was washed with several portions of water and the benzene was removed *in vacuo* after drying by anhydrous sodium carbonate. The residual part was distilled at 102°C (0.03 mmHg), yielding 14 g of coloress transparent oil.

Anticholinesterase activity analysis:

Anticholinesterase activity was analyzed by Hestrin's colorimetric method as modified by Kojima *et al.*[3] The analysis was examined at PH 7.4 and the homogenate of final instar larvae of the rice stem borer was used as enzyme sources. All colorimetric determinations were based on average of 2 replications for each sample.

Antialiesterase activity analysis:

It was analyzed in the same method as anticholinesterase activity analysis except using methyl-*n*-butyrate as substrate.[5]

Inhibition test of melanin formation:

An acetone solution of test compound was poured into a mortar and the acetone was evaporated. Five m*l* of distilled water containing 5 final instar larvae of the rice stem borer was poured into the mortar and was homogenated. After filtering the homogenate through cotton gauze, the filtrate was transfered into a test tube. A few minutes later, untreated filtrate colored from milk-white to dark violet but the filtrate treated with phenyl thiourea (2×10^{-5}M) didn't. The dark violet pigment is owing to the melanin formation. Inhibition activity of the former was determined as 0% and the later was as 100%. Inhibition activity of the test compound was evaluated with unaided eye in comparison with them.

Results and discussion

Direct insecticidal activity to 3rd-instar larvae:

Galecron was very inferior to Sumithion (Table 2). However, the rice seedlings treated with Galecron was less feeded as compared with untreated one.

Therefore, the repellent activity was examined.

Repellent activity to 1st-instar larvae:

Galecron gave the high repellent activity even in low concentration of 5ppm as shown in Table 3. And then, a following test was carried out to know how this compound show the high protective effectiveness against the rice stem borer.

Protective effectiveness of Galecron to the rice stem borer larvae by the application on paddy water:

Table 2. Direct insecticidal activity of Galecron to 3rd-instar larvae of the rice stem borer.

Compound	LC_{50} (ppm)	LC_{95} (ppm)
Galecron	>2000	
Sumithion	0.83	1.4

Table 3. Repellent activity of Galecron to lst-instar larvae of the rice stem borer.

Compound	Concentration (ppm)	Percent Repellency
Galecron	50	80.7
	5	90.6

Table 4. Protective effectiveness of Galecron to 1st-instar larvae of the rice stem borer by the application on paddy water.

Compound	Dosage (a.i.)	Correct percent* survival
Galecron	120g/10are	0
	240	0
	480	0
γ-BHC	120	0

* Correct percent survival (C.P.S.) $= \frac{\text{P.S. of treated plot}}{\text{P.S. of untreated plot}} \times 100$

Percent survival (P.S.) $= \frac{\text{No. of survival larvae}}{\text{No. of infested larvae}} \times 100$

Active ingredient 120, 240 and 480g per 10 are of Galecron did not completely allow survival of the rice stem borer larvae (Table 4). Namely, Galecron was as highly effective as γ-BHC.

Residual effectiveness:

Even on 14 days after treatment with Galecron, all of infested larvae were not seen inside plants (Table 5.) It demonstrated that Galecron had long lasting residual activity.

Insecticidal activity of sprayed Galecron to the rice stem borer larvae inside stems of the rice plants:

Galecron was slightly inferior to Sumithion at concentration of 0.01%, but superior at concentration of 0.001%. This result indicated that the effect of Galecron is active even at low concentration. Such an excellent activity of Galecron could not be expected from the low direct insecticidal activity as shown in Table 2.

Main differences of experimental condition between Table 2. and 6. were depended on an observation time after treatment and instar of tested insects, namely the former was 3 days and 3rd-instar larvae, the later was 5 days and 2nd-instar larvae. It suggests that effectiveness of Galecron decreases with development of the larvae and the appearance of activity becomes delay. In addition to these causes, there may be an activation of Galecron in the young rice plants.

Ovicidal activity:

As Galecron shows excellent ovicidal activity to some spider mites, the activity to the rice stem borer eggs was examined. Galecron showed low activity to 1 day old eggs, but high activity to 4 day-old eggs (Table 7.).

Protective effectiveness to γ-BHC-resistant rice stem borer:

Heretofore, γ-BHC-granule formulation has been widely used for the rice stem borer control.

Kasai *et al.* reported the appearance of the low sensitive rice stem borer to γ-BHC in 1965.[6]

Table 5. Residual protective effectiveness of Galecron to 1st-instar larvae by the application on paddy water.

Compounds	Dosage (a.i.)	Correct percent survival obtained by infesting on indicated day after treatment.		
		5	9	14 day
Galecron	120 g/10 are	0	0	0
γ-BHC	120	0	0	0

Table 6. Insecticidal activity of sprayed Galecron to the rice stem borer larvae in the stems of rice plants.

Compounds	Conc. (%)	Correct percent survival
Galecron	0.03	0
	0.01	12.4
	0.003	9.3
	0.001	6.3
Sumithion	0.03	0
	0.01	0
	0.003	6.3
	0.001	58.6

Table 7. Ovicidal activity of Galecron to the eggs of the rice stem borer.

Compounds	Day ages	Conc. (%)	Percent mortality
Galecron	0-1	0.1	15.3
	3-4	0.1	85.9
		0.01	99.6
EPN	0-1	0.1	100
	3-4	0.1	97.3
		0.01	85.6

Therefore, protective effectiveness of Galecron to γ-BHC-resistant rice stem borer was examined. The tested resistant larvae were about five times more resistant than the sensitive to γ-BHC. Galecron was however rather effective to the resistant than to the sensitive (Table.8). Dittrich reported that the toxic action of Galecron was negatively correlated with organo phosphorus insecticide resistance in a strain of two spotted spider mite.[7] It seems that Galecron has an interesting action to a resistant pest.

Effectiveness of some Galecron derivatives:

The results shown above indicated that Galecron is highly effective for the control of the rice stem borer. In order to find the more effective compound to the rice stem borer than Galecron, some Galecron derivatives were synthesized and their effectiveness to the rice stem borer was tested. Compound I without phenyl substituent was quitely noneffective and each compound with the substituent, 2-CH_3, 4-Cl, 2-Cl and 4-$COCH_3$ showed respectively a little effectiveness but they were entirely inferior to Galecron (Table 9.).

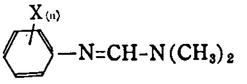
Anticholinesterase activity:

It is common knowledge that the mode of action of organophosphorus and carbamate insecticides is closely associated with the inhibition

Table 8. Protective effectiveness of Galecron to γ-BHC-resistant rice stem borer larvae by the application on paddy water.

Compounds	Strain	Dosage (a.i.)	Correct percent survival
γ-BHC	Susceptible	100g/10 are	2.8
		20	38.9
	Resistant	100	48.7
		20	94.9
Galecron	Susceptible	100	0.0
		20	25.0
	Resistant	100	0.0
		20	0.0

Table 9. Protective effectiveness of some Galecron derivatives to the rice stem borer by the application on paddy water.



Compounds	X	Dosage (a.i.)	Correct percent survival
I	H	400g/10 are	100
II	2-CH_3	400	49.1
III	4-Cl	400	33.4
IV	2-Cl	400	42.8
V	4-$COCH_3$	400	42.8
Galecron	2-CH_3, 4-Cl	90	0
Diazinon		100	0

Table 10. In vitro inhibition of ChE and Ali-E of the rice stem borer larvae by Galecron.

Compounds	Conc. (Mol)	ChE percent inhibition	Ali-E percent inhibition
	5×10^{-3}	28.1	0
Galecron	5×10^{-5}	1.0	0
	5×10^{-7}	0	0
	5×10^{-5}	100	100
DDVP	5×10^{-7}	43.2	100
	5×10^{-9}	0	38.0

of acetylcholine esterase.[8] In order to guess the mode of action of Galecron, anticholinesterase activity was assayed. The result was shown in Table 10. DDVP gave 43.2 percent inhibition at the concentration 5×10^{-7}M, while Galecron scarcely inhibited the acetylcholinesterase of the rice stem borer larvae at 5×10^{-3}M.

Dittrich suggested that the mode of action of Galecron is not based on inhibition of cholinesterase which was shown in comparative toxicological and enzymatic experiments.[1]

Antialiesterase activity:

Van Asperen demonstrated that an aliphatic esterase was present in the thorax of house fly and was strongly inhibited by DDVP.[9] Galecron showed an excellent repellent activity is caused by antifeeding properties. The aliesterase activity is high in the thorax of the insects.[10] Therefore, the antialiesterase activity of Galecron was measured by colorimetric method. Optimum pH value for aliesterase activity of the rice stem borer larvae was about 7.4 (Fig 2). Although DDVP completely inhibited the aliphatic esterase of the rice stem borer larvae, Galecron never inhibited even at the concentration 5×10^{-3}M (Table 10). This result suggested that the mode of action of Galecron is not related with the inhibition of aliphatic esterase.

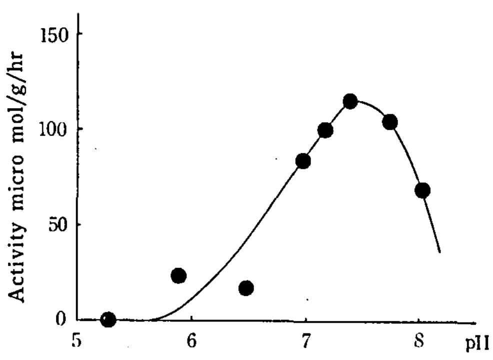


Fig. 2. Activity-pH curve for the methyl-n-butyrate hydrolysis by the Ali-E of the rice stem borer larvae.

In vitro inhibition activity of melanin formation:

It is commonly known that the black pigment, which is produced when an insect body fluid is exposed to the air, is melanin. And the melanin formation was strongly inhibited by the phenylthiourea which is negatively correlated with the

Table 11. In vitro inhibition of melanin formation of the rice stem borer larvae by Galecron.

Compounds	Conc. (Mol)	Percent inhibition
Galecron	2×10^{-3}	0
DDVP	2×10^{-3}	0
Padan	2×10^{-4}	100
	2×10^{-5}	0
phenyl thiourea	2×10^{-4}	100
	2×10^{-5}	100

* Padan: 1, 3-bis(carbamoylthio)-2-(*N, N*-dimethylamino)propane hydrochloride.

DDT-resistance in *Drosophila*. The inhibition of melanin formation may be effect on the physiological action of insect. So, the inhibition activity for Galecron was assayed. Phenylthiourea at the concentration 2×10^{-5}M and Cartap at 2×10^{-4}M gave the values of 100 percent inhibition. But Galecron little inhibited the melanin formation at the concentration 2×10^{-3}M (Table 11). The result indicated that the mode of action Galecron is not associated with the inhibition of melanin formation.

Summary

Some biological activities of Galecron to the rice stem borer larvae were tested. Results obtained are summarized as follows.

(1) The direct insecticidal activity was very low but the repellent activity and the ovicidal activity were excellent.

(2) The high protective effectiveness to the rice stem borer was found with long lasting residual activity by the application into paddy water.

(3) The effectiveness was a little more active to γ-BHC resistant rice stem borer than the susceptible by the application into paddy water.

(4) By the spray method, Galecron showed the same effectiveness as Sumithion against the rice stem borer larvae in the stems of rice plants.

(5) Some derivatives of Galecron were synthesized and evaluated effectiveness to the rice stem borer. But all of them were inferior to Galecron.

(6) Galecron did not inhibit not only the cholinesterase and aliesterase but also the melanin formation of the rice stem borer larvae.

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