韓應昆誌 33(2) : 88~95 (1994)
Korean J. Appl. Entomol.

Studies on the Toxicity of Insect Growth Regulators against the Fall Webworm (*Hyphantria cunea* Drury) and the Rice Stem Borer (*Chilo suppressalis* Walker) II. Comparisons in Enzyme Activities

미국흰불나방과 이화명나방에 대한 昆蟲發育沮害劑의 毒性硏究 II. 酵素의 活性 比較

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ABSTRACT In comparisons of the activities of amylase, invertase, chitinase and esterase on the 5th instar larvae of the fall webworm (*Hyphantria cunea* Drury) by the leaf dipping method and on the 3rd instar larvae of the rice stem borer (*Chilo suppressalis* Walker) by the rice stem dipping method at LC_{50} values of IRGs.

In the fall webworm, after treatment of IGRs, the enzyme activities of amylase and invertase became the lower than that of untreated control. The chlorfluazuron showed the lowest activity among the tested IGRs. The chitinase activity increased in IGRs treatment, as compared with untreated control. The esterase isozyme pattern was detected by the 2 bands of Est-$\alpha^1\beta^a$ and Est-$\alpha^2\beta^b$ and was not found the differences in tested IGRs. In the rice stem borer, the enzyme actvities of amylase, invertase and chitinase showed a similar tendency with that of the fall webworm. However, the activities of amylase and invertase showed about 7~10 times lower than that of the fall webworm and the chitinase activity also showed about 3~4 times lower than them. The esterase isozyme pattern was detected by the 4 bands of Est-α^1, Est-β^a, Est-α^2 and Est-α^3. The pyriproxyfen was detected by the strong activity of Est-α^1 as compared with the other IGRs and the chlorfluazuron and tebufenozide were detected by the strong activities of Est-β^a but the diflubenzuron was not detected.

KEY WORDS IGR, Fall webworm, Rice stem borer, Enzyme activity, Esterase isozyme pattern

초 록 침적법에 의해 결정된 IGRs의 LC_{50} 수준(ppm)에 대한 미국흰불나방(*Hyphantria cunea* Drury) 5령충과 이화명나방(*Chilo suppressalis* Walker) 3령충의 amylase, invertase, chitinase와 esterase의 활성 비교 결과, 미국흰불나방은 IGRs 처리 후 amylase와 invertase의 활성은 무처리 보다 낮았고 chlorfluazuron 처리에서는 가장 낮은 활성을 보였으며 chitinase의 활성은 무처리와 비교해서 IGRs처리 후 증가되었고 esterase pattern은 2가지(Est-$\alpha^1\beta^a$ and Est-$\alpha^2\beta^b$)로 분리되었으나 처리간 차이가 없었으며, 이화명나방에서는 amylase, invertase와 chitinase의 활성은 미국흰불나방과 비슷한 경향을 보였으나, amylase와 invertase의 활성은 미국흰불나방보다 7~10배 낮았고 chitinase의 활성도 미국흰불나방 보다 3~4배 낮았다. Esterase pattern은 4가지(Est-α^1, Est-β^a, Est-α^2 and Est-α^3)로 분리되었고 무처리 대비 pyriproxyfen은 Est-α^1에서 chlorfluazuron와 tebufenozide은 Est-β^a에서 강한 활성을 나타내었으나 diflubenzuron은 차이가 없었다.

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Synthesis and secretion of digestive emzymes in insect appeared to be regulated by three possible mechanisms. neural mechanism, a direct stimulus from the nervous pathway; hormonal mechanism, some factors in the meal trigger the endocrine gland to release a hormone into the hemolymph, which, in turn, stimulate the secretory cells of the gut to synthesize and release the digestive enzymes; secretogogue mechanism, a chemical in the food stimulates the secretory cells of the midgut triggering them to secrete appropriate quantity of enzyme (Christopher & Mathavan 1985).

The activity of digestive enzymes depends mostly on the presence of a respective substrate in the food Trehalose plays a significant role in the supply of energy to an insect and the activity of trehalose might serve as an indicator of energy reserves resulting from availability of carbohydrates nutrients Invertase and amylase activities could, therefore, be used as additional parameters for assessing the availability of glucose, trehalose and glycogen (Ishaaya & Swirski 1976)

Chitin is an amino-sugar polysaccharide that serves as a supporting element in extracellular structures, notably in exoskeletons of insects. Synthesis of chitin involves concerted multifaceted cellular activities starting from biotransformations of simple metabolites and culminating in the emergence of a polymer to be extruded outside the cell membrances and the process of chitin formation involves an orderly sequence of complicated cellular events (Vaneck 1978, Cohen 1987). Active catalytic units assembled in cell membranes polymerize N-acetyl-D-glucosamine (GlcNAc) molecules into extracellular chitin chains. The imediate substrate for polymerization, 5'-uridine diphospho-N-acetyl-D-glucosamine (UDP-GlcNAc), is an end metabolite of a cascade of cytoplasmic biochemical transformations that starts from the disaccharide trehalose or from glucose (Cohen 1987).

The hormone of brain that regulates molting was known by Kopec (1922) as a prothoracictrophic hormone (PTTH), and stimulates ecdysone secretion with the prothoracic gland. The molt of an insect, one of the early events, is the detachment of the epidermal cells from the old cuticle, an event referred to as apolysis and one of the last events is ecdysis; the molting insect may degrade, absorb and re-utilize up to 90% of the old cuticle under the presence of enzymes in molting fluid as protease and chitinase secreted from the epidermal cell of epidermal tissue in integument of insect (Chang 1993). The chitinase activity in molting fluid of integument for molting of insect reported a similar cross relationship between chitinase level and molting (Bade & Stinson 1978, Vaneck 1978).

Diflubenzuron, one of the representative of IGRs, was investigated by Guyer & Neumann 1988, Hajjar & Casida 1978, Ishaaya & Acher 1977, Ishaaya & Degheele 1988 and Ivie & Wright 1978 The effects and the mechanism of it as chitin synthesis inhibitors against the various insects, but mainly against lepidoptera were investigated. Recently, in studies on the N-acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis* by IGRs, the potency inhibiting the incorporation of [^{14}C] GluNAc into the cultured integument by Nakagawa *et al.* (1992a, b) was evaluated to be a physiological parameter for the structure-activity study and Oikawa *et al* (1993) reported the enhancement of N-acetylglucosamine by the aforementioned it.

Cyromazine, one of the IGRs, caused epidermal cells of third instar *Lucilia cuprina* larvae to invade the cuticle and produce necrotic lesions where as diflubenzuron inhibits chitin synthesis by Binnington *et al* (1987) and cyromazin does not act by inhibiting chitin formation on same insects but the primary mode of action of cyromazine was suggested by via the hormone systems (Friedel *et al.* 1988). However, Kotze & Reynolds (1990) reported that the primary effect of cyromazine caused rapid changes in mechanical properties of cuticle

Esterase, in general, was known to be distributed broadly in insect body and to be investigated in the strains of susceptible and resistant to insecticides. The aim of our experiment was investigating the differences of isozyme patterns against the survived larvae after treatment at rate of LC_{50} values with IGRs.

The IGRs referred to as the third generation pesticides, developed rapidly in developed countries and

were investigated widely about the mode of actions. In these points, after investigation of insecticidal activity against the various instar stages of fall webworm (FWB) and rice stem borer (RSB) reported by Lee *et al.* (1994), the experimental results of amylase, invertase, chitinase and esterase activities against the servived larvae of 5th instar of FWB and 3rd instar of RSB after treatment of IGRs at LC_{50} values were reported in this paper

MATERIALS AND METHODS

The preparations of enzyme assay solution to measure emzyme activity were shown at indicated steps as follow: the first, the latter half of 5th survived instar larvae of the fall webworm (*Hyphantria cunea* Drury) and 3rd survived instar larvae of the rice stem borer (*Chilo suppressalis* Walker) with IGRs treatment after the rate of LC_{50} values was homogenized with glass bars in centrifuge cell in 500 μl or 50 μl of 0.01 M sodium phosphate buffer (pH 6.0) per each servived larva, respectively. After centrifugation for 5 min with 5,000 rpm at 4℃, only the supernatant of the 1st centrifugal solution was spun further for 20 min. with 15,000 rpm and the enzyme assay solution was collected with micropippette from the supernatant.

The contents of IGRs tested and the levels of LC_{50} values were determined by the results of mortality activities on different instars (Table 1).

The invertase activity was measured with the methods recommended by Christopher & Mathavan (1985) and Ishaaya & Swirski (1976). The assay solution mixed with 0.05 ml of enzyme solution of 0.2 ml of 0.02 M sodium phosphate buffer (pH 6.0) and with 0.2 ml of 1% sucrose solution of 0.02 M sodium phosphate buffer (pH 6.0) was reacted in water bath (Grant Instrument Ltd, SS40 200/min.) for 30 min. at 37℃. After that, added 1.6 ml of 3,5-dinitrosalicylic acid (DNS reagent) to the solution. The assay solution was boiled in water bath for 5 min and followed by immediate cooling in an ice bath. The activity was determined in extinction units (I) with an absorbance at 550 nm with spectrophotometer (Cecil Instrument).

The measuremental method of amylase activity was followed the methods of invertase analysis by Christopher & Mathavan (1985) and Ishaaya & Swirski (1976) and was showed the same procedures as the invertase method except differences of the initial assay solution which was mixed with 0.05 ml of enzyme solution of 0.2 ml of 0.02 M sodium phosphate buffer (pH 6.0) and with 0.1 ml of 2% starch solution of 0.02 M sodium phosphate beffer (pH 6.0).

The preparation of enzyme solution to measure chitinase activity was followed the method mentioned above with the only change in the buffer solution: 0.2 M acetate buffer (pH 5.1) instead of 0.02 M sodium phosphate buffer (pH 6.0).

The chitinase activity was measured as recommended by Kim *et al.* (1993) and Yun & Kim

Table 1. Chemicals used and LC_{50} values (ppm) of the those in experiment

Common name	Trade name	Chemical name	Purity(%)	LC_{50}*	
				FW	RSB
Chlorfluazuron	Atabron	1-[3,5-dichloro-4-(3-chlor-o-5-trifluoromethyl-2-pyndyloxy)phenyl]-3-(2,6-difluorobenzoyl)urea	97.3	23.6	91.7
Diflubenzuron	Dimilin	1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea	91.4	19.4	424.5
Pyriproxyfen	Sumilarv	2-[1-methyl-2-(phenoxyphenoxy) ethoxyl pyridine	98.1	88.3	711.7
Tebufenozide	Mimic	N-tert-butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide	95.0	39.5	35.4

*LC_{50} values indicated the results of the previous reports at indicated instar larvae of each tested insects; FW: Fall Webworm; RSB. Rice Stem Borer.

(1985). The assay solution was mixed with 0.2 ml of enzyme solution, with 0.1 ml of 0.2 M acetate buffer (pH 5.1) and with 0.2 ml of colloidal chitin, following the method of Bemiller (1965), and incubated for 10 min. at 40℃ and after that, added 1 ml of DNS reagent. The assay solution was boiled in water bath for 5 min. and added with 8 ml of distilled water. Then, the assay solution was stored in ice bath and determined the extinction units(E) with an absorbance of 640nm with spectrophotometer (Cecil Instrument) The replication number was 6 in all of this experiments.

The electrophoresis was carried out with the thin agarose electrophoresis method by Ohba (1970) for investigation of esterase isozyme patterns. The enzyme solution for studies of electrophoresis used the same procedures as the solution mentioned above The 2 μl of enzyme assay solution with micropipette was dropped in applicator plate and lodged in 0.8% agarose gel plate with applicator comb. The gel plate was set up with 1.5 mA per cm for 3 hours after embedding it for 10 min. The gel plate was sprayed with substrate solutions which was mixed with 2.5% α-naphthyl acetate and with 0.5% β-naphthyl acetate with reagent acetone and then, the gel plate was reacted with substrate at 38℃ in incubator (Precision Incubator) for 20 min. The gel plate was stained by spraying the solution of 0.5% aqueous α-Fast Blue BB salt at 38℃ in incubator for 20 min. and then, destained with tap water for 24 hours and dried with dry oven (Precision Oven) at 38℃

RESULTS AND DISCUSSIONS

The experimental results of amylase, invertase and chitinase activities on the 5th survival instar larvae of the fall webworm after treatment at LC_{50} values of the IGRs by the leaf-dipping method were shown in Table 2.

Enzyme activities of amylase and invertase after treated with IGRs showed the lower activity than that of untreated control. In the case of amylase, enzyme activities of chlorfluazuron, tebufenozide, pyriproxyfen and diflubenzuron decreased to 6.76, 6.97, 7.20 and 10.96, as compared with 11.01 of control activity, respectively. In the case of invertase,

Table 2. Comparisons of amylase, invertase and chitinase activities on the 5th survival instar larvae of the fall webworm (*Hyphantria cunea* Drury) after treatment at LC_{50} values of the IGRs by the leaf dipping method

IGRs	Enzyme activity		
	Amylase	Invertase	Chitinase
Control	11.01± 2.19*	9.43± 2.01	7.10± 2.89
Chlorfluazuron	6.76± 0.67	6.91± 0.38	9.30± 0.54
Diflubenzuron	10.96± 1.43	8.30± 0.30	10.70± 0.98
Priproxyfen	7.20± 1.39	8.36± 0.37	12.17± 0.41
Tebufenozide	6.97± 0.23	8.70± 0.17	11.33± 1.82

*Mean and standard deviation of six replications; Enzyme activity (Amylase and Invertase): μg of reducing sugar/min/whole body, (Chitinase), μg of hydrolyzed chitin/min/whole body.

enzyme activites of chlorfluazuron, diflubenzuron, pyripoxyfen and tebufenzide decreased to 6.91, 8.30, 8.36 and 8.70 as compared with 9.43 of the control activity, respectively. The chlorfluazuron showed the lowest activity among the treated IGRs both amylase and invertase. This results corresponded with the results of Christopher & Mathavan 1985. The activity of the amylase with developmental stages of the fall webworm (Chung *et al* 1986) increased dramatically over the period of early larval stage to last larval stage at which ingests food most actively but the trend decreased immediately after pupation. Christopher & Mathavan (1985) reported that food intake was thought to be a factor of paramount importance in regulation of digestive enzyme secretion in insects and the activities of amylase and invertase showed a direct correlation to the food consumption. For instance, larvae receiving 100% or *Ad libitum* food, liberated as much as 460 mg /glucose/g gut/hour and those receiving 75, 50 and 25% rations released 400, 296 and 164 mg glucose/ g gut/hour, respectively. Starved larvae, however, showed a little activity (40 mg glucose/g gut/hour). Values for invertase activity 3, 8, 10 and 18 mg glucose/gut/hour in 25, 50, 75 and 100% rationed individuals, respectively. Therefore, the quantity of food ingested significantly affects the digestive enzyme activity. In those points, the low enzyme activities of amylase and invertase after treatment

of IGRs as compared with the levels of untreated control were caused by less food ingestions after exposures of IGRs (data not presented). In the other point of view, the lowest activity of chlorfluazuron which was not directly compared to all of the tested IGRs in this experiment showed by reasons of the longer half-lives then the diflubenzuron (Guyer & Neumann 1988).

The enzyme activities of trehalase, invertase and amylase with diets containing 5.0 ppm of diflubenzuron against the 4th instar larvae of *Tribolium castaneum* showed in a reduction of about 32, 27 and 17%, respectively (Ishaaya & Acher 1977). They also reported that a dose-dependent decrease in the activity of the enzymes was obtained with the increase in diflubenzuron concentration (Ishaaya & Swirski 1976). The low enzyme activities of amylase and invertase after treatment of IGRs on survival of the 5th instar larvae of the fall webworm as compared with that of untreated control accorded well with previous reporters (Christopher & Mathavan 1985, Guyer & Neumann 1988, Ishaaya & Swirski 1976, Ishaaya & Acher 1977).

Enzyme activities of chitinase in the Table 2 showed the results in opposition to those of amylase and invertase activities. The chitinase activities of pyriproxyfen, tebufenozide, deflubenzuron and chlorfluazuron increased 12.17, 11.33, 10.70 and 9.30 as compared with 7.10 of untreated control, respectively. In general, IGRs typically caused the death of immature stages by inducing abnormalities during larvae and pupal development and inhibited the chitinase activity for chitin synthesis in molting fluid. In the other generals, if the enzyme activities of amylase and invertase were disturbed by the IGRs, the IGRs might hamper not only the supply of glucose needed for chitin build-up in carbohydrate metabolism but also the chitinase activity reduced in molting fluid and in insect body. However, Ishaay & Casia (1974) reported that an increased activity of chitinase which was observed in housefly larvae after feeding with diflubenzuron may further accentuate the inhibition of chitin build-up. It was suggested that the disruption of chitin build-up may result from an alteration in the level of hormones, especially that of ecdyson through the inhibition of ecdysone-metabolizing enzymes by diflubenzuron (Yu & Terriere 1977).

Table 3. Comparisons of amylase, invertase and chitinase activities on the 3rd survival instar larvae of the rice stem borer (*Chilo suppressalis* Walker) after treatment at LC_{50} values of the IGRs by the rice stem dipping method

IGRs	Enzyme activity		
	Amylase	Invertase	Chitinase
Control	1.22± 0 33*	1 14± 0.10	2.72± 0 66
Chlorfluazuron	0 90± 0.03	1.13± 0.07	3 13± 0 64
Diflubenzuron	0 97± 0.05	0.99± 0.05	3 13± 0 96
Pyriproxyfen	0.91± 0.05	1 08± 0.03	3 67± 0.89
Tebufenozide	0 99± 0 04	1.00± 0.04	3 00± 0.55

*Mean and standard deviation of six replications; Enzyme activity (Amylase and Invertase): μg of reducing sugar/min/whole body., (Chitinase): μg of hydrolyzed chitin/min /whole body.

Enzyme ativities of amylase, invertase and chitinase on the 3rd survival instar larvae of the rice stem borer after treatment at LC_{50} values of the IGR by the rice stem dipping method were shown as indicated in Table 3.

All the experimental results on rice stem borer as indicated in Table 3, showed a similar tendency with those of the fall webworm (see Table 2). In general, the differences between the fall webworm and the rice stem borer known pupae and old larvae of over-wintering stages, Arctiidae and Pyralidae of Lepidoptera, 160 host plants of difoliated trees and main rice plants, respectively The activities of amylase and invertase with the rice stem borer were lower about 7~10 times than those of the fall webworm and the activity of chitinase was also lower about 3~4 times than the results of two experiments (Table 2 and 3). Amylase and invertase activities in lepidoptera reported by Christopher & Mathavan (1985) showed in relation to the developmental stage and the food availability and in positive correlation between food intake and enzyme activity. The enzyme activity was influenced with importance of these enzymes in food digestion and energy supply and strongly affected by various host plants (Ishaaya & Swirski 1976) In general, the nature of the enzymes secreted depends mostly on the nature of the

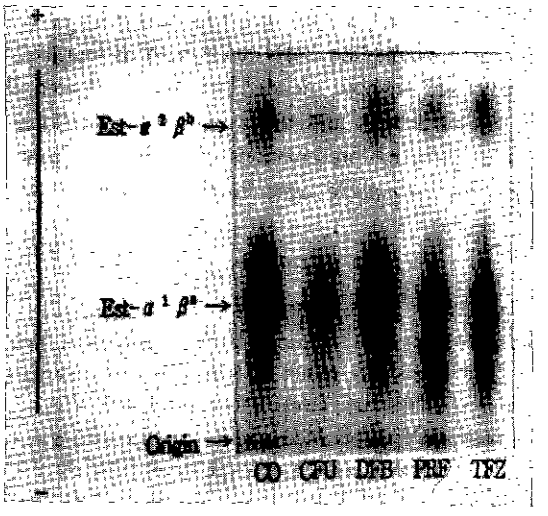

Fig. 1. Zymogram patterns of fall webworm (*Hyphantria cunea* Drury) after treatment at LC_{50} values of IGRs seperated by thin agarose gel electrophoresis method. CO. Control CFU: Chlorfluazuron DEB: Diflubenzuron PRF Pynproxyfen TFZ· Tebufenozide.

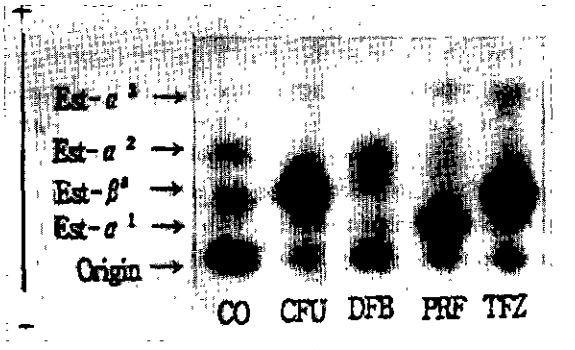

Fig. 2. Zymogram patterns of rice stem borer (*Chilo suppressalis* Walker) after treatment at LC_{50} values of IGRs seperated by thin agarose gel electrophoresis method CO: Control CFU: Chlorfluazuron DEB: Diflubenzuron PRF: Pyriproxyfen TFZ: Tebufenozide

meal: whereas herbivorous insects secreted more carbohydrases, carnivorous insects secreted predominantly proteases. The other reasons, by Ishaaya & Swirski (1976), the enzyme activities (trehalase) in scales reared on potato sprouts increased about 3.5 and 4.0 folds than obtained in scales reared on oleander and on citrus plants, respectively. The results of *Hyphantria cunea* (Chung *et al.* 1986) and *Pieris rapae* (Yun & Kim 1985) showed the increase of enzyme activity according to developmental larval stages. Kim *et al.* (1993) reported that chitinase activity at LC_{50} and LC_{95} values of pyripoxyfen for 7 days in 3rd instar larvae of housefly showed the various differences on local strains. The chitinase activity induced the intact or deproteined cuticle by incubation with enzymatically active molting fluid an acquisition of endogenous chitinase activity by the chitin of intact cuticle required several steps as enzymatic reactions by Bade & Stinson (1978). The activity of chitinases by Singh & Vardanis (1984) increased during pupation, showed a maximum in the middle of the pupal period. Even though, the times of observation in this experiment were not the moulting stages, the major factors of mortality after treatment of the IGRs at latter half days of tested larval stages were reported, in general, the inhibitions of chitin synthesis during the moulting procedures. In results of this experiment, the chitinase activity of the servived larvae against the tested larvae after treatment of LC_{50} values with IGRs showed the differences at the tested larvae and the treated IGRs. However, the mortality mechanisms after treatment of the IGRs should be investigated in various research fields in a futures.

Esterase, in general, was known to be distributed broadly in insect body and the main factors of inseticide resistance. Esterase isozyme patterns of the fall webworm (5th instar larvae) and the rice stem borer (3rd instar larvae) after treatment at LC_{50} values of the IGRs by thin agarose gel electrophoresis method showed in Fig. 1 and Fig 2, respectively.

The fall webworm, as indicated in Fig. 1, was detected by the two bands of Est-$\alpha^1\beta^a$ and Est-$\alpha^2\beta^b$ and chlorfluazuron showed the low esterase isozyme patterns as compared with those of untreated control. However, the difference was not found in the other IGRs. Even though the results of this experiment were not directly compared with the other's reporters, isozyme patterns or activities of esterase in housefly (Ugaki *et al.* 1983) and *Culex pipiens pallens* (Bonning & Hemingway 1991) were investigated in the strains of susceptible and resistant to insecticides. Fortunately, the housefly strain fed on artificial diet mixed with pyriproxyfen LC_{50} had very high activity of -Est-α^1 early stages and the activity

of Est-α^1 was very strong in late stages (Kim *et al.* 1993). The amylase band with gut of *Hyphantria cunea* seperated by acrylamide gel electrophoresis by Chung *et al.* (1986) was detected three isozyme bands in the upper regions of gel. The aim of our experiment was investigating the differences of isozyme patterns after treatment with IGRs. The chlorfluazuron showed the lower activities of amylase and invertase and also the lower levels of chitinase activity (see in Table 2). The results of this electrophoresis accorded well with the activities of enzymes.

The rice stem borer as indicated in Fig. 2 was detected by the four bands of Est-α^1, Est-β^a, Est-α^2 and Est-α^3. The pyriproxyfen was detected by the strong activity of Est-α^1 as compared with the other IGRs and the chlorfluazuron and the tebufenozide were detected by the strong activities of Est-β^a but the diflubenzuron was not detected. The results of this experiment were not directly elucidated and compared with the results of enzyme activity (see in Table 3). Recently, in mode of action of IGRs against the rice stem borer, the diflubenzuron was elucidated the inhibition of N-acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis* Walker (Nakagawa *et al.* 1992a, b) and the dibenzoyl hydrazine and the molting hormone enhanced the N-acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis*. The isozyme patterns of esterases, generally, showed the differences according to the developmental stages of the insects and were affected by the host plant including the exposure of insecticides.

In conclusions, The activities of amylase and invertase became the lower than that of untreated control on servived larvae of FW (5th instar) and RSB (3rd instar) and chitinase increased in IGRs treatment. The enzyme activities in RSB showed a similar tendency with that of the FW. However, the RSB showed about 7~10 times lower than that of the FW in activities of amylase and invertase and showed 3~4 times lower than the FW in chitinase activity. Esterase isozyme patterns of the FW and the RSB were detected 2 and 4 bands, respectively. Although, the FW was not found the differences in tested IGRs, the pyriproxyfen showed the strong activity of Est-α1 and the chlorfluazuron and tebufenozide showed strong activities of Est-β^a in RSB. In these points, the IGRs referred to as low toxicity pesticides, anyhow, needed to be investigated the mode of actions and the expanded applications of major pests for preservations of healthy environments and sustainable agricultural ecosystems.

REFERENCES

Bade. M. L. & A. Stinson 1978. Digestive of article chitin during the moult of *Manduca sexta* (Lepidoptera· Sphingidae). *Insect Biochem.* **9** 221-231.

Bemiller, J. Y. 1965. Methods in carbohydrates chemistry. *Academic press* **5**: 103-105.

Binnington, K. C., A. Retnakaran, S. Stone & P. Skelly. 1987 Studies on cyromazine and diflubenzuron in the sheep blowflt, *Lucilia cuprina*: Inhibition of vertebrate and bacterial dihydrofolate reductase by cyromazine. *Pesti. Biochem. Physiol* **27**: 201-210.

Bonning, B. C & J. Hemingway. 1991. The efficacy of acetylcholinesterase in organophosphorus and carbamate resistance in *Culex pipiens* L. from Italy. *Pesti. Biochem. Physiol* **40**: 143-148

Chang, E. S. 1993 Comparative endocrinology of molting and reproduction Insects and Crustaceans. *Ann. Rev. Entomol.* **38**. 161-180.

Christopher, M. S M. & S Mathavan. 1985. Regulation of digestive enzyme activity in the larval of *Catopsilea crocale* (Lepidoptera). *J Insect Physiol.* **31**(3): 217-221.

Chung, H R, W D Han & H R Kim 1986. Study on amylase in digestive tract of *Hyphantria cunea* Drury. *Ent Res. Bulltin* **12** 49-56

Cohen, E. 1987 Chitinbiochemistry: Synthesis and inhibition. *Ann. Rev. Entomol.* **32**: 71-93.

Friedel, T, D. F. Hales & D. Birch. 1988 Cyromazine-induced effects on the larval cuticle of the sheep blowfly, *Lucilia cuprina*: Ultrastructual evidence for a possible mode of action *Pesti Biochem. Physiol* **31**. 99-107.

Guyer, W & R. Neumann 1988, Activity and fate of chlorfluazuron and diflubenzuron in the larval of *Spodoptera littoralis* and *Heliothis virescens*. *Pesti. Biochem Physiol* **30**: 166-177.

Hajjar, N. P. & J. E Casida. 1978. Insecticidal benzoylphenyl ureas: structure activity relationship as chitin synthesis inhibitors *Science* **200** 30(June): 1499-15 00

Ishaaya, I. & K. R. S. Acher. 1977. Effects of diflubenzuron on growth and carbohydrate hydrolase of *Tribolium castaneum*. *Phytoparasitica* **5**(3). 149-158.

Ishaaya, I. & J. E. Casia. 1974. Dietary TH6040 alters composition and enzyme activity of house fly larval cuticle. *Pesti. Biochem Physiol.* **4**: 484-490

Ishaaya, I. & D. Degheele. 1988. Properties and toxicological significance of diflubenzuron hydrolase activity in *Spodoptera littoralis* Larvae. *Pesti. Biochem. Physiol.* **32**: 180-187.

Ishaaya, I & E. Swirski. 1976. Trehalase, invertase and amylase activities in the black scale, *Saissetia oleal* and their relation to host adaptability. *J Insect Physiol.* **22**· 1025-1029.

Ivie, G. W. & J. E. Wright. 1978. Fate of diflubenzuron in the stable fly and housefly. *J. Agric. Food. Chem.* **26**(1): 90-94.

Kim, J. W., H. R. Lee, I. H. Lee, Y. K. Lee & J. S. Choi. 1993. Studies on toxicological mechanism and development of applied technology with non-pollutant pesticides(IGR) against the medical insects. *J. Agr. Sci.* Chungbuk Not'l. Uni. **10**(2): 126-144.

Kopec, S. 1922. Studies on the necessity of the brain for the inception of insect metamorphosis. *Biol. Bull. Woods. Hole. Mass.* **42**: 323-342.

Kotze, A. C. & S. E Reynolds. 1990. Mechanical properties of the cuticle of *Manduca sextan* larvae treated with cyromazine. *Pesti. Biochemi. Physiol.* **38**: 267-272.

Lee, I H. H. R Lee & J W. Kim 1994. Studies on the toxicity of insect growth regulators against the fall webworm (*Hyphantria cunea* Drury) amd the rice stemborer (*Chilo suppressalis* Walker). *Korean J. Appli. Entomol.* **33**(2):

Nakagawa, Y., M. Matsutani. N. Kurihara, K. Nishimura & T. Fujita 1992a. Inhibition of N-acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis* by diflubenzuron. *Pesti. Biochem. Physiol.* **42**: 242-247.

Nakagawa, Y., M. Matsutani, N. Kurihara, K. Nishimura & T. Fujita. 1992b. Quantitative structure-activity studies of benzoylphenylurea larvicide. viii. Inhibition of N- acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis* Walker by diflubenzuron. *Pesti. Biochem Physiol.* **43**: 141-151.

Ohba, S 1970. Drosophila. Information Service **46**: 56-57

Oikawa, N., Y. Nakagawa, Y. Soya, K. Nishinura, N. Kurihara, T. Ueno & T. Fukita. 1993. Enhancement of N-acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis* by molting hormone and dibenzoylhydrazine insecticides. *Pesti. Biochem. Physiol.* **47**: 165-170.

Singh, G. J. P. & A. Vardanis. 1984. Chitinase in the housefly, *Musca domestica*, pattern of activity in the life cycle and preliminary characterization. *Insect Biochemi.* **14**(2): 215-218.

Ugaki, N., T. Abe, J. I. Fukami & T. Shono. 1983. Electrophoretic analysis of nonspecific esterases and acetylcholinesterases from the house fly *Musca domestica* L. (Diptera:Muscidae), with reference to organophosphorus insecticide resistance *Jap. J. Appl. Ent. Zool.* **18**(4): 447-455.

Vaneck, W H. 1978. Mode of action of two benzoylphenyl urea as inhibitors of chitin synthesis in insects *Insect. Biochemi.* **27**: 71-75.

Yu, S. J. & L. C. Terriere. 1977 Ecdysone metabolism by soluble enzymes from three species of diptera and its inhibition by the insect growth regulator TH6040 *Pesti. Biochemi. Physiol.* 7: 48-55

Yun. C. Y & H. R Kim. 1985. Chitinolytic enzymes in integument of the last instar larva to the pupa of *Pieris rapae* L. *The Kor. J. of Entomology.* **15**(2): 53-56

(Received March 25, 1994)