韓蠶學誌 40(2), 150~157(1998)
Korean J. Seric. Sci.
<論 文>

# Effect of Anti-Juvenile Hormone Analogue (AJH) Treatments on the Silk Filament Properties of the Silkworm, *Bombyx mori L.*

**Moeinnodin Mavvajpour, Do Gyu Bae, Hae Ryong Sohn and Kwang Youl Seol***

*Natural Fiber Science, Kyungpook National University, Taegu 702-701, Korea*
**National Sericulture and Entomology Research Institute, RDA, Suwon, 441-100, Korea*

## ABSTRACT

**This study was carried out to define the effects of anti-juvenile hormone analogue(AJH) treatment at different silkworm larval stages on some silk filament properties. It was revealed that the treatments at the 1st and 2nd days of the 3rd instar as well as the 1st day of the 4th instar resulted to trimolters induction without lethal effect. The trimolters induced by treatment at the 1st day of the 4th instar showed the shortest larval duration and the lowest single cocoon weight, cocoon shell ratio, cocoon size, single filament length and denier of filament compared with those of the control, while the trimolters induced by the 3rd-instar-treatment showed intermediate values. All treatments showed no significant differences from the aspect of degumming ratio, elongation rate and crystallinity index of fibroin. However the silk filament obtained from the treatment at the 1st day of the 4th instar showed the highest tenacity which was revealed to be related to the better uniformity of filament by the means of microscopic photographing. FT-IR spectroscopy of the silk fibroin showed a sharp and strong peak at the position of 794.72 $cm^{-1}$ in all AJH-treated samples regardless of trimolters induction, which was absent in control.**

**Key words : Silkworm, Anti-Juvenile Hormone, Silk property**

## INTRODUCTION

Different aspects of the effect of anti-juvenile hormone analogues(AJH) on silkworm have been investigated. Akai *et al.*(1984) attempted to control cocoon production using both JH and AJH. In this way they produced small cocoons with thiner filament by AJH treatment compared with control. Kiuchi *et al.*(1986) showed the highest effect of time dependent application of AJH at the early stage of the 3rd and 4th instar for trimolters induction from the tetramolters strains. Tsukada and co-workers (1986) concluded that the size of cocoon filament of trimolters induced by AJH treatment at the 3rd instar was half of that for control with a uniform size along the filament. Yamashita *et al.*(1987) studied the effect of an AJH compound(KK-42) as a potent anti-ecdysteroid agent and concluded that KK-42 acted directly on the prothoracic gland to inhibit ecdysone synthesis and the pattern of ecdysteroid level in hemolymph was completely changed from that of the larval molting cycle to that of the metamorphosis. Consequently Akai and Mauchamp (1989) investigated the suppressive effect of KK-42 on the JH level in homolymph of Bombyx larvae. They demonstrated that the treated larvae had no JHs for at least 6 days. These larvae also had no ecdysteroid during near the same period. So they concluded that the hormonal condition responsible for induction of precocious metamorphosis is maintaining low level of both JH and ecdysteroid for 5~6 days. They particularly noted that JH1 and JH2 normally increase before the molting stage and then decrease to zero level until day 1 of the 5th instar, but these levels are completely inhibited by KK-42 treatment. Kang and Seol(1994) attempted to develope the chemical synthesis and application techniques of several imidazole compounds in order to produce

fine silk fibre by the means of bioassay observation as well as actual proof at farm scale and introduced AJH No. 7+8 as an effective agent comparable to KK-42.

Nevertheless in this experiment it was tried to study the effect of the time implementation of AJH on chemical and physical properties of silk filament. In this regard beside the surveying of bio-physiological characters such as larval duration, single cocoon and shell weight, cocoon size as well as filature properties such as length and size of silk yarn and degumming rate, more detailes of filament properties have been cosidered to study by the means of experimental instruments.

## MATERIALS AND METHODS

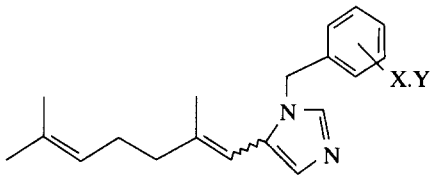
### 1. Materials

For this study about 10 batches of hibernated eggs of F1 hybrid silkworm (CS120×7409) were used.

The chemical formulation of AJH agent used for this study was 1-(4-Fluorobenzyl)-5-[(E)-2,6-dimethyl-1,5-heptadienyl] imidazole 20%

(Z)

whose chemical structure is as follow :




X=CF3, OCF3, F etc.

Marseilles soap (commercial grade)

Sodium carbonate (G.R.)

Potassium bromide (G.R.)

### 2. Methods

Totally 6 treatments including one control, each in 3 replications started after the 2nd molting by using same concentration of AJH solution(500 times diluted). The applying times were as below:

control : no AJH administrating(A)

3rd instar : 1st(B) and 2nd(C) day

4th instar : 1st(D), 2nd(E) and 3rd(F) day

In each treatment the administrating duration was 48 hours and each replication contained 200 silkworm larvae. In order to have only one-way classification on experiment to face the AJH effect exclusively, Completely Randomized Design (C.R.D.) was employed.

1) Cocoon characters were calculated after grading them to good, middle, low and double cocoons 7days after cocooning in each treatment, only using 20 males and 20 females within good cocoons class in each replication.

However the pupation rate was necessary to be calculated within all cocoons harvested from every replication as follow :

$$\text{Pupation rate} = \frac{\text{No. of healthy pupae}}{\text{corrected No. of the 2nd molted larvae}} \times 100$$

2) The following method has been employed to measure the degumming rate using 5 gr of cocoon shell in each replication :

(1) marseilles soap 15%(o.w.f.)+sodium carbonate 10%(o.w.f.) liquid ratio 1 : 50, 90~95°C for 1 hour

(2) rinsing with sodium carbonate(1gr/litre), 60°C for 20 min

(3) rinsing with warm D.W.(60°C)

(4) rinsing with cold D.W., drying(70°C) and weighing

3) The length of cocoon filment was determined using automatic single cocoon reeling machine using the cooking bath temperature from 100 to 60°C, subsequently the reeled filaments was used for measuring their size as below :

$$\text{Single filament size(d)} = \frac{\text{dried filament weight} \times 1.11\text{(gr)}}{\text{lengthoffilament(m)}} \times 9{,}000$$

4) For measuring the physical properties of raw silk filament including elongation rate(%) and tenacity(g/d) using Shimadzu autograph(AGS-500A model) the following method was used :

No. of samples : 5 replications were chosen randomly within each treatment and 3 samples of each replication were prepared.

Length of samples : 20 mm useful length of raw silk filament for measurement was prepared by using

scaled paper frame.

Experiment conditions: test speed 30 mm/min.,chart speed 200 mm/min.

5) One sample of raw silk filament from each treatment was chosen randomly in order to microscopic photographing with the same magnification of 125 by the means of Nickon microscopic camera and Sony video color printer. The thinest and thickest parts of each sample were then measured visually and their ratio was calculated as the uniformity of filament.

6) Chemical structure of silk yarn was surveyed after determining the filature properties of good cocoons such as length and size of filament and degumming rate, using FT-IR spectroscopy. One sample of silk fibroin(degummed silk) from such treatment was used for instrumental analysis. The IR spectra were recorded with a Mattson instruments Inc. Galaxy 7020A in the range 4000~400 $cm^{-1}$ on KBr.

## RESULTS AND DISCUSSION

1) Effects of AJH treatments at different larval stages on the larval/cocoon characters

(1) Larval duration

The average of larval duration of each treatment and control are summerized in Table 1 and shown schematically in Fig. 1.

According to the Table 1, it is clear that the larvae of AJH treatment are induced from tetramolters to trimolters at the 1st and the 2nd day of the 3rd instar as well as the 1st day of the 4th instar. In this study the effect of hormone at the above mentioned times was completely strong and resulted to almost 100% of changing in molting character with elimination of one instar. However from the aspect of marking color

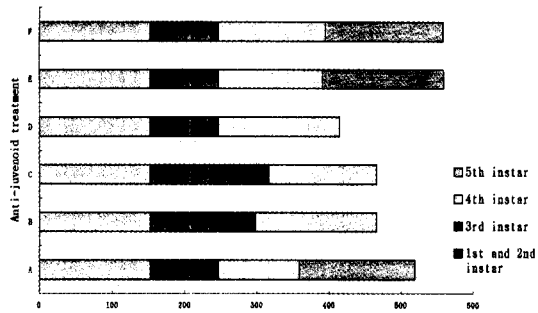



**Fig. 1.** *Effect of time dependent treatments by AJH at the 3rd and 4th instar on larval duration. A: Control, B: Treatment at the 1st day of 3rd instar, C: Treatment at the 2nd day of 3rd instar, D: Treatment at the 1st day of 4th instar, E: Treatment at the 2nd day of 4th instar and F: Treatment at the 3rd day of 4th instar*

and body shape, treated larvae showed differences at their final stage due to the application time of hormone. When treatment was carried out at the 3rd instar, the 4th stage derived larvae were resembled to the characteristic 5th instar larvae with complete larval marking and they prolonged the 3rd instar by about 2~3 days compared with the control. The ultimate stage of these larvae however showed almost the same duration with the 5th instar of the control tetramolters. Therefore it can be concluded that the AJH treatment at the 3rd instar would be resulted to conjunction of normal 3rd and 4th instars as the penultimate stage of induced trimolters with the last stage(4th instar) in similar pattern with the 5th instar of tetramolters larvae.

Meanwhile the treated larvae at the 1st day of the 4th instar showed no differences of body shape compared with ordinary 4th stage silkworm larvae and directly produced cocoon with prolonging of 4th stage by about 3 days compared with the penultimate

**Table 1.** *Differences of larval duration by AJH treatments*

| Treatment \ Duration | 1st&2nd instar days hr. | 3rd instar days hr. | 4th instar days hr. | 5th instar days hr. | Total days hr. |
|---|---|---|---|---|---|
| Control(A) | 6 08 | 3 22 | 4 17 | 6 16 | 21 15 |
| 1st day of 3rd instar(B) | 6 08 | 6 02 | 7 00 | – – | 19 10 |
| 2nd day of 3rd instar(C) | 6 08 | 6 20 | 6 06 | – – | 19 10 |
| 1st day of 4th instar(D) | 6 08 | 3 22 | 7 01 | – – | 17 07 |
| 2nd day of 4th instar(E) | 6 08 | 3 22 | 6 01 | 7 00 | 23 07 |
| 3rd day of 4th instar(F) | 6 08 | 3 22 | 6 05 | 6 19 | 23 06 |

stage of control and elimination of the final stage(5th instar). On the other hand hormone treatment at the 2nd and/or the 3rd day of the 4th instar prolonged the total larval duration without trimolters induction. This is because of the increment of the 4th instar by less than 2 days compared with the control. Keeping in mind that AJH acts directly on prothoracic gland to inhibit ecdysteroid synthesis, treatment after the 2nd day of the 5th instar while prothoracic gland already started its activity seems to be late to block the effect of molting hormone completely.

Also absence of JHs due to the effect of AJH which will be happened 2 or 3 days after application, may changes the hormonal balance between JH/ecdysteroid to that of responsible for larval-larval molting at the late stage of the 4th instar. Furthermore all of the treated larvae at the 2nd day of the 4th instar after completion of the 5th stage feeding and maturation were not able to spin cocoon, remained as unpupated larvae and finally died.

This phenomenon which already had been reported by T. Oshiki *et al.*(1989), for induction of larval-pupal intermediates by single application of terpenoid imidazole, also involved the treated larvae at the 3rd day of the 4th instar in a less extent which reduced the pupation rate down to 55.2%. They showed that the lack of cocooning ability was due to an obstruction at the central part of silk gland. Thus, according to the results obtained in this observation the 2nd day of the 4th instar can be considered as a critical day, which before that AJH would lead to precocious larvae and fine silk production.

Nevertheless to make any accurate judgement will be remained unconfirm and demands more studying considering the endocrine activities inside the silkworm body as well as revealing the probable physiological/anatomical disturbances which made the silkworm larvae unable to secrete the silk protein already accumulated in their silk glands. However as a result of loss of cocoon spinning in treatment E, it is crossed out of the further statistical analysis in this report.

**Table 2.** *Effect of AJH treatment on pupation rate*

| Treatment | Pupation rate |
|---|---|
| Control(A) | 93.5 |
| 1st day of 3rd instar(B) | 92.8 |
| 2nd day of 3rd instar(C) | 93.8 |
| 1st day of 4th instar(D) | 97.7 |
| 3rd day of 4th instar(F) | 55.2 |
| Fs | 50.8** |

**significant at 1% level

(2) Pupation rate(%) :

The data of pupation rate of all treatments excluding treatment at the 2nd day of the 4th instar are shown in Table 2.

The test of significance by the means of Duncan's new multiple range test(data are not shown) revealed that all treatments except treatment F showed high performance of trimolters induction without adverse effect on pupation rate. Therefore similarly to the results obtained from the other studies, application of AJH in order to induce trimolters can be employed at the early stage of the 3rd and/or 4th instar.

(3) Cocoon characters

The effects of treatments on cocoon characters including percentage of good cocoon, cocoon size, single cocoon weight and cocoon shell ratio are summerized in Table 3.

As it is clear from the Table 3, the trimolters

**Table 3.** *Effect of AJH treatment on the cocoon characters*

| Treatment \ Trait | Percentage of good cocoon % | Cocoon size No./*l* | Single cocoon weight g | Cocoon shell ratio % |
|---|---|---|---|---|
| A | 75.2 | 66 | 2.35 | 24.0 |
| B | 86.9 | 99 | 1.52 | 21.6 |
| C | 91.3 | 104 | 1.50 | 21.9 |
| D | 95.4 | 195 | 0.79 | 14.6 |
| F | 72.3 | 70 | 2.33 | 22.7 |
| Fs | 20.0** | 2118** | 824.10** | 604.0** |

**significant at 1% level

induced by the AJH treatment at the 1st day of the 4th instar showed the lowest single cocoon weight and shell ratio as well as the smallest size of cocoon compared with those of the control. The data obtained from the 3rd-instar-treatment presented intermediate values. Considering that the pattern of trimolters induction by the AJH treatment at the 3rd or 4th instar is different, it can be presumed that the lowest values obtained from the 4th-instar-treatment is related to the fact of elimination of the 5th stage which the highest growth rate of silk gland happens. Meanwhile application of AJH at the 3rd instar would prolong the penultimate stage by the means of conjunction of the 3rd and 4th instars in treated larvae(Fig. 1). Thus the derived trimolters would face their ultimate stage(4th instar) with similar pattern to the normal 5th-instar larvae.

On the other hand the highest values for good cocoon percentage produced by trimolters specially in treatment D seem to be related to the size of the cocoon which is remarkablely smaller than tetra-molters. Since the spinning space for every larva was same, the bigger the body and cocoon size, the higher the number of printed and double cocoons which leaded to lower rate of good cocoon.

2) Effects on the Silk Filament Properties by AJH Treatment

The effects of AJH application at the larval stage on some filament properties are summerized in Table 4.

According to the obatained results, all treatments showed no significant differences from the aspect of degumming rate and filament elongation statistically. On the other word the same proportion of fibroin to sericin can be seen in all samples due to the same degumming rate. It logically leads to this conclusion that the growth pattern of different parts of silk gland responsible for synthesis and secretion of different components in silk filament by induced trimolters are same with those of the teramolters but in a smaller size. The distribution of mean values for the length of cocoon filament and filament size, however, obeyed the same fashion as those of the cocoon charaters. Accordingly it can be suggested that the AJH treatment not only decreases the filament thickness through the trimolters induction, but also the denier of the filament can be determined by proper time regulation of treatment on the basis of the demand.

High significant difference also can be observed between the treatments for the tenacity of filament, which the highest value belongs to the silk filament obtained from the treatment at the 1st day of the 4th instar.

3) Uniformity of Filament

The data related to the filament tenacity showed a different pattern within the treatments. As already mentioned from the degumming point of view no considerable differences could be seen among the treatments. Further more from the aspect of crystallinity ratio of silk fibroin all treatments showed same behaviour, which will be dicussed later. Therefore the only possible reason to explain the different tenacities may be laid in the uniformity of filaments. In order to clear this supposition, micros-copic photos of silk filaments from each treatment were prepared(Fig. 2).

Keeping in mind that normally the breakage of filament would happen initially at the thinest point, the ratio of the thinest part to the thickest part for each treatment as the uniformity index was calculated

**Table 4.** *Effects of AJH treatment on silk filament*

| Trait / Treatment | Degumming rate % | Length of the filament m | Filament size d | Elongation % | Tenacity g/d |
|---|---|---|---|---|---|
| A | 26.9 | 1555.4 | 3.1 | 21.0 | 3.8 |
| B | 27.4 | 1354.9 | 2.1 | 28.7 | 4.3 |
| C | 27.4 | 1280.5 | 2.2 | 25.3 | 3.5 |
| D | 27.2 | 916.7 | 1.1 | 24.5 | 5.7 |
| F | 27.4 | 1399.0 | 3.1 | 26.5 | 4.4 |
| Fs | 2.2 | 111.7** | 270.1** | 1.0 | 4.7** |

**significant at 1% level

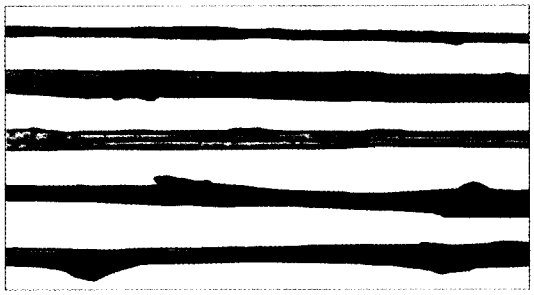


**Fig. 2.** *Comparison microscopic photos of silk filament of each treatment, D, F, B, A and C up to down respectively.*

**Table 5.** *Uniformity of single filament of treatments*

| Treatment | Uniformity (%) | Tenacity (g/d) | Thin part ratio(mm/d) |
|---|---|---|---|
| F | 75.00 | 4.402 | 4.56 |
| D | 74.28 | 5.646 | 4.76 |
| B | 66.67 | 4.256 | 3.83 |
| C | 46.67 | 3.520 | 3.17 |
| A | 44.44 | 3.764 | 2.75 |

(Table 5).

According to the Table 5, the differences among the uniformity of the filaments are remarkablely high. In order to make a comparison basis between tenacity and uniformity, the thin part ratio to filament denier was measured, which shows good agreement between two mentioned parameters. So it can be suggested that the silk produced by AJH treatment induced trimolters is with the higher uniformity along the filament and accordingly presents higher tenacity in spite of similar degumming ratio and crystallinity index.

4) Infrared Spectroscopy

Beside the all physical changes due to AJH application within silk filament which have been discussed already, in order to investigate any chemical differences FT-IR analysing has been employed. The related spectrum for silk fibroin produced by control and treated silkworm larvae are shown in Fig. 3.

Comparison of the spectra(Fig. 3) clearly presents a sharp and strong peak at 794.72 $cm^{-1}$ position in all treatments which is completely absent in control. Even the treatment at the 3rd day of the 4th instar (treatment F) which showed no differences with control for almost all characters, this peak is

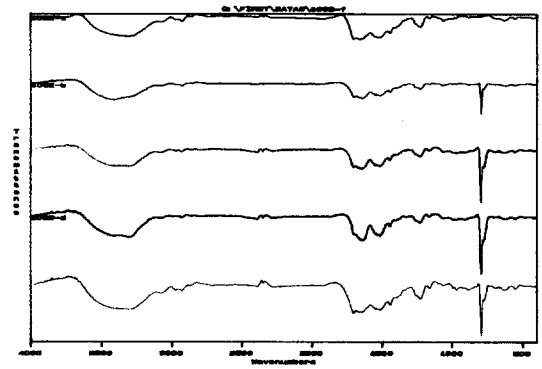


**Fig. 3.** *Comparison of IR spectra of silk fibroin from all treatments A, B, C, D and F from up to down.*

**Table 6.** *Calculated indices of fibroin crystallinity for each treatment*

| Treatment | Percentage of crystallinity(%) |
|---|---|
| A | 65.0 |
| B | 63.2 |
| C | 68.8 |
| D | 64.8 |
| F | 62.3 |

presented. This peak probably related to the bending vibration of aromatic C-H bond and can be seen in all treatments irrespective to the effect of the AJH agent which contains and aromatic C-H bond in its chemical structure. So the peak probably related to the presence of AJH in silkworm body and subsequently in silk gland. But the real nature and related chemical bond to this peak are not clear and need more studying to be revealed.

Furthermore, using each spectrum at the points of 1235 $cm^{-1}$ and 1265 $cm^{-1}$ the crystallinity index for each treatment could be calculated quantitatively. The related data are summerized in Table 6.

Comparison of the indices shows no significant differences among the treatments from the aspect of percentage of crystallinity.

## CONCLUSION

This study was undertaken to clarify the effects of anti-juvenile hormone analogue(AJH) treatments for silkworm on some properties of silk filament. Accordingly it can be suggested that:

1) Dietary application of AJH at the 1st and/or 2nd day of 3rd instar as well as the 1st day of 4th instar for 48 hours will lead to precocious metamorphosis larvae at the level of near 100% without any harm to pupation rate.

2) The cocoon produced by derived trimolters were smaller in size with lower shell rate compared with that of control. Treatment at the 1st day of 4th instar resulted to the lowest single cocoon weight and shell ratio(0.79g and 14.58%), while that of the 3rd-instar-treatments laid between control(2.35g and 24.01%) and the 4th-instar-treatment

3) All treatments showed no significant differences for the sericin to fibroin ratio in degumming process.

4) Length of filament as well as filament size cosiderablely decreased by AJH treatment which the lowest one obtained from the 4th-instar-treatment(916. 73m and 1.05d) as compared with control(1555.41 m and 3.09d).

5) All treatments showed same filament elongation rate while the data of filament tenacity showed to be significantly different. The highest tenacity had been observed in the 4th-instar-treatment(5.65g/d) compared with that of control (3.76g/d).

6) The microscopic photography of silk filaments from each treatment revealed that the trimolters induced by AJH treatment at the 1st day of the 4th instar showed the highest uniformity along their filament compared with control which resulted to the highest tenacity.

7) FT-IR analysis spectra presented a sharp and strong peak at the position of 794.72$cm$-1 in all AJH treated samples which was completely absent in the control spectrum. The related bond to this peak demands more studying to be revealed. Moreover according to quantitative measurment using the spectra at the positions of 1265 and 1235 $cm^{-1}$, the crystallinity indices of all treatments had been observed in a same fashion.

## 적 요

항유약호르몬 활성물질이 누에의 형질과 견사의 성질에 미치는 영향을 알고자 3령 1일, 2일 및 4령 1일, 2일, 3일부터 항유약호르몬을 뽕잎에 뿌려 2일간 처리하여 누에에 먹여 다음과 같은 결과를 통계처리에 의해 얻었다.

3면성 발현구는 3령 1일, 2일 및 4령 1일 처리에서 100%이었다. 그러나 4령 3일구는 모두 4면성이었고 화용비율은 대단히 낮아 55%이었으며 4령 2일구도 모두 4면성이 발현되었으나 영견, 화용되지 못하였다.

3령 1일, 2일 및 4령 1일 처리에 있어서, 유충경과는 3령 1일과 2일구는 같았고, 4령 1일구가 가장 짧았다. 3령기간은 3령 2일구가 길었고, 4령기간은 3령 1일구가 길었다. 4령 1일구는 3면성을 발현한 처리구 중에서 4령기간이 가장 길었다.

단견중과 견층비율은 3령 1일과 3령 2일구가 무겁고 높았으며, 4령 1일구가 가장 가볍고 낮았다. 1 $l$ 과수에 있어서는 4령 1일구가 가장 많았고, 3령 2일, 3령 1일 순으로 적었다.

4령 3일구는 대조구를 비교하면 경과일수는 4령과 5령기간이 길었다. 단견중과 1 $l$과수는 같은 수치를 보였고, 견층비율은 대조구가 높았다.

모든 처리구들은 대조구와 함께 같은 정도의 연감율을 보였고, 섬유장과 섬도에 있어서는 4령 1일째 처리(916.73m와 1.05d)가 AJH 무처리구(1555.41m와 3.09d)에 비해 감소되었으며, 3령에 처리한 것은 이들 수치의 중간 정도로 나타났다. 또한 신도에 있어서는 처리구와 대조구 사이에 큰 차이점이 인정되지 않았으며, 4령 1일째 처리한 것의 견섬유가 가장 높은 강도를 나타냈다. 각 실험 및 대조구 견사의 현미경 사진을 보면, 4령 1일째 AJH 처리한 것에 의해 유도된 3면잠이 대조구와 비교하여 더 높은 균일성을 보였고, 그 결과 강도도 높게 나타났다.

IR spectra 분석에서는 처리간의 결정화도 차이가 인정되지 않았으나, 대조구에서 관찰할 수 없었던 794.72 $cm^{-1}$에서의 날카롭고 강한 피크가 모든 AJH 처리구에서 나타났다.

## REFERENCES

Akai H and B. Mauchamp(1089) Suppressive effects of an imidazole derivative, KK-42 on JH level in hemolymph of *Bombyx larvae. J. seri. sci. Jpn.* **58**: 73~74.

Akai H., K. Kimura, M. Kiuchi and A. Shibukawa (1984) Effect of anti-juvenoid treatment on cocoon and cocoon filaments in *Bombyx mori. J. seri. sci. Jpn.* **53**: 545~546.

Kang, S.K. and K.Y. Seol(1994) Chemical synthesis of imidazole analogues and treatment technique for the production of fine silk fiber. Report Agr. Sp. Sub. RDA 1994: 11~37.

Kiuchi M., O. Shimizu, K. Kimura and H. Akai(1987)

Variation of the susceptibility to a imidazole compound, SSP-11, for the induction of precocious metamorphosis among the various strains of *Bombyx mori*. *J. seric. sci. Jpn.* **56**: 171~172.

Tsukada M., K. Kimura, A. Shibukava, A. Shimazaki and H. Akai(1987) Effect of treatment with juvenile hormone analoge and anti-juvenoid on the size of the cocoon filament. *J. seric. sci. Jpn.* **56**: 231~234.

Yamashita O., K. Kadono-Okuda, E. Kuwano and M. Eto(1987) An imidazole compound as a potent anti-ecdysteroid in an insect, *Agri. Bio. Chem.* **51**(8): 2295~2297.