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Antijuvenoid Action of Terpenoid Imidazole Compound on Larval - Pupal - Adult Development of Silkworm, *Bombyx mori* L.

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Precocious metamorphosis was induced in two popular bivoltine breeds i.e. YB and NB_4D_2 by an imidazole compound having anti-juvenile hormone activity. The chemical was administered by feeding treatment with mulberry leaf to freshly moulted (0–6 hrs) IV instar larvae. The dose of the chemical was found to be breed specific being 650 ppm for YB and 500 ppm for NB_4D_2. The chemical caused complete skipping up of the fifth instar larvae which is most susceptible to diseases. But IV instar was somewhat prolonged. As a result the total larval period was reduced by 4–5 days. However, some of the rearing and reeling parameters like cocoon yield, cocoon weight, shell weight, shell %, filament length and fecundity were reduced for that particular generation only. The effective rate of rearing (ERR %) was significantly increased in trimoulters during the most unfavourable August - September seed crop season. Number of cocoons / kg and number of male moth were significantly higher in trimoulters. These may be useful for preparation of multi × bi hybrid seed. Fine denier was also found in trimoulter cocoon which is the cause of getting fine silk filament from trimoulter cocoons. Normal mating behaviour and emergence pattern was recorded in trimoulters. Bivoltine trimoulter males also showed competence for mating with multivoltine females. These results suggest the possibility of getting trimoulter males during hot and humid seasons when rearing of bivoltine is almost impossible particularly in Eastern and North Eastern India.

Key words: *Bombyx mori*, Induction, Trimoulter, Imidazole compound, Larval period, Rearing parameters, Reeling parameters

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Introduction

In holometabolous insects larval life is maintained by the presence of juvenile hormone in the haemolymph and other tissues. When its level declines below a certain threshold, the appearance of ecdysteroid causes metamorphosis (Riddiford, 1980). The process of moulting and metamorphosis characteristic to larval growth and development in insects are controlled by circulating hormones like Prothoracicotropic Hormone (PTTH), Juvenile Hormone (JH) and Ecdysterone (Wigglesworth, 1985). The set pattern of the insect development can be altered by manipulating environmental conditions or introducing chemical compounds leading either to precocious metamorphosis or supernumerary larval moult (Sakurai 1983). Chemical induction of precocious metamorphosis in insects has been reported for a variety of natural and synthetic compounds like Kojic acid and its related compounds (Murakoshi & Ichimoto, 1972). In silkworm *Bombyx mori*, precocious metamorphosis could be induced by manipulating external factors such as temperature and moisture (Iwashita, 1963). Some chemicals with anti- JH activity have recently been demonstrated to be useful for sericulture from the standpoints of producing fine denier cocoon filament (Akai *et al.* 1984; Gu *et al.*, 1988).

The biological activity of some Imidazole compounds such as 1-citronelly-5-phenylimidazole (KK-22) was at first found to induce precocious metamorphosis in the penultimate instar larvae of the silkworm, *Bombyx mori* when applied at the early stages of that instar (Kuwano *et al.*, 1983). The Imidazole derivative KK-42 (1-benzyl-5[(E)-2, 6-dimethyl-1, 5-heptadienyl}) has been found to be effective in inducing precocious metamorphosis in *Bombyx mori* when applied to the early fourth instars either topically or in their diet (Kuwano *et al.*, 1985, Okuda *et al.*, 1987). Thus, these compounds were assumed to be a kind of anti- juvenile hormone which inhibits the juvenile hormone synthesis in corpus allatum (Asano *et al.*, 1984; Kuwano *et al.*, 1983, 1985).

It is reported that the Imidazole compounds (antijuvenoids) KK-42 and SSP-11 decrease the larval duration, fecundity and induce trimoultism (Kuwano & Eto, 1983; Kuwano *et al.*, 1985; Akai *et al.*, 1984). There is a positive correlation between body and cocoon weights and filament size under the influence of SSP-11 (Kataoka *et al.*, 1985). The silkworm age responses to Imidazole were reported by Akai *et al.*, (1984) and Kiuchi *et al.*, (1985).

Despite an abundance of mulberry leaves for food in parts of Eastern India, the rearing of bivoltine is very difficult particularly during April to October i.e. dry & wet summer. Most of the larvae in advanced stage are attacked by micro-pathogenic diseases. Hence rearing of multivoltine × bivoltine hybrid becomes imperative during this season in commercial sectors. To produce such multivoltine × bivoltine hybrid eggs, bivoltine parent males is very essential. An attempt has been made to produce bivoltine male during adverse season by inducing trimoulter. No information is available from Indian works on the effects of this imidazole compound on the tropical silkworm breeds of *Bombyx mori*, though a little work on this compound has been done in Japan on bivoltine silkworm breeds reared on artificial diet for producing fine filament only. This justifies the present attempt of using the said chemical for induction of trimoulters and to find out impact of these compounds on economic characters of *B. mori* for further utilization of these compounds in large scale. Hence, studies were carried out using this compound to determine: i) the optimum dose for induction of precocious pupation in bivoltine worms, ii) rearing performance of the treated bivoltine worms, iii) effect on the larval and cocoon parameters iv) effect on the reproductive performance of the resultant trimoulter bivoltine males crossed with tetramoulter multivoltine females for the preparation of multi × bi hybrids eggs.

Materials and Methods

Two popular silkworm bivoltine breeds of *Bombyx mori* L viz., YB (Yellow Bivoltine) and NB_4D_2 (White Bivoltine) were used as experimental material and reared with the standard schedule of rearing (Krishnaswami, 1986). The silkworms were reared with S-1 variety mulberry leaves from hatching to 3rd moult. Freshly moulted 4th instar larvae were taken from stock culture maintained at room temperature of 25°C - 28°C and relative humidity of 80 - 85%. Three replications for each treatment with 200 larvae per replication were kept. The experiment was conducted during September-October, December-January & February-March seed crop seasons for consecutive two years.

Imidazole compound was dissolved in distilled water to make different concentrations ranging between 300 to 700 ppm. Fresh mulberry leaves after weighing were dipped into respective concentrations of solutions for 15 minutes in order to facilitate soaking of the chemicals. Then the mulberry leaves were kept on plastic rearing tray for 25 - 30 minutes for air drying and fed to the 4th instar freshly moulted silkworm larvae (0 - 6 hours). The treated mulberry leaves were fed to the silkworms for 48 hours (8 feeding at the interval of 6 hours). Larval mortality and rate of precocious pupation were evaluated for each concentration. In this way, an optimum dose for the each breed was determined. In control lots, fresh mulberry leaves were dipped for 15 minutes in distilled water without chemical and air dried for 25 - 30 minutes and fed to the silkworms for 48 hours (8 feeding) and reared simultaneously.

The data on larval duration, larval weight, yield/10,000 larvae both by number & weight, cocoon weight, shell weight, filament length, denier, sex ratio, emergence percentage and grainage behavior were recorded and analyzed statistically.

In order to evaluate the reproductive potentials, induced trimoulter males were crossed with untreated multivoltine females. Reproductive performance was studied. Mating behavior, fecundity and capacity of the trimoulter males crossed with untreated tetramoulter multivoltine (Nistari) female to produce fertile eggs, and the subsequent rearing performance were studied and compared with control.

Results

Dose determination

Different concentrations (300 - 700 ppm) of the chemical were tested on the two popular bivoltine silkworm breeds i.e. YB and NB_4D_2 and found that the dose of chemical is breed specific. i.e. 650 ppm for YB breed and 500 ppm for NB_4D_2 inducing 84.18% and 76.38% trimoulting respectively. The standard dose of the chemical for inducing trimoulting was also confirmed through cross verification keeping 3 replications per dose with 1000 larvae per replication (Figs. 1, 2).

Induction of precocious metamorphosis

Precocious metamorphosis was induced by feeding mulberry leaves containing the imidazole compound to the freshly moulted 4th instar larvae. The YB and NB_4D_2

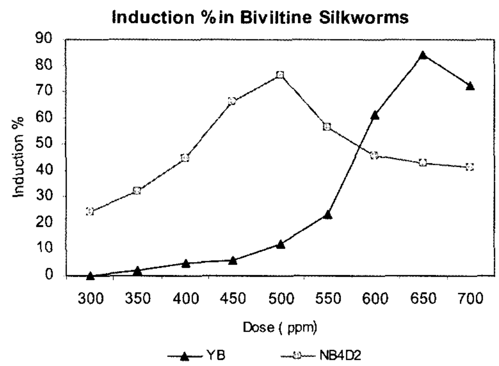

Fig. 1. Standardization of dose of the chemical.

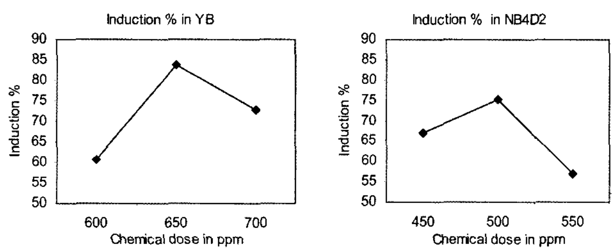

Fig. 2. Confirmation of the dose of the chemical for inducing trimoulting In YB & NB_4D_2

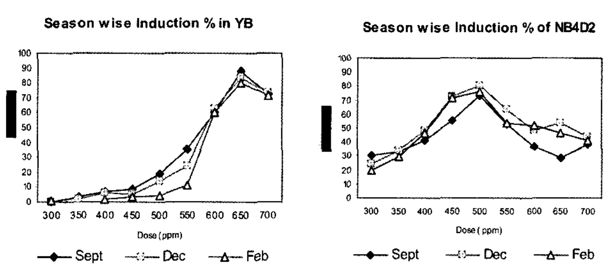

Fig. 3. Induction percentage in YB & NB_4D_2 during different seasons.

bivoltine breeds used in the experiment are genetically tetramoulters. Their final larval stage is the 5th instar, followed by spinning a cocoon and pupating in it. Induction of precocious metamorphosis was found dose dependent (Fig. 3).

When mulberry leaves dipped in the anti-JH emulsion were fed to 4th instar larvae for 48 hours immediately after the 3rd moulting, their larval period at 4th instar was prolonged by about 2 - 3 days as compared to that of the control. These larvae started to spin as trimoulter larvae and produced smaller cocoons than the control lot (tetramoulter).

Effect on the larval developmental period

Actually with the application of chemical on freshly moulted 4th instar larvae, the worms committed to pupate within the same instar without any further ecdysis. The larval duration of 4th instar was prolonged by 2 - 3 days and the 5th instar was completely skipped by applying the chemical. As a result the total larval period was significantly reduced in both the bivoltine breeds i.e. YB & NB_4D_2 when compared to control (Table 1) and most of the larvae started spinning as trimoulter and produced smaller cocoons compared to control (Plate 1).

In YB, almost all 4th instar larvae at 650 ppm while entered into precocious metamorphosis without ecdysis to the subsequent 5th instar, although some ecdysis to the 5th instar was observed with the higher and lower doses. The similar observation was also made in NB_4D_2 when treated with 500 ppm.

Table 1. Effect of induction of trimoulting on larval period of bivoltine silkworm

Season	Larval Period (Days)							
	I – III Instar		IV instar		V instar		Total	
	Tri	Con	Tri	Con	Tri	Con	Tri	Con
				YB				
Sept.	12	12	7.0	5.0	--	6.0	19.0	23.0
Dec.	13	13	7.5	5.0	--	6.5	20.5	24.5
Feb.	12	12	7.5	4.5	--	7.0	19.5	23.5
				NB_4D_2				
Sept.	13	13	7.0	5.0	--	6.5	20.0	24.5
Dec.	14	14	7.5	5.0	--	7.0	21.5	26.0
Feb.	12	12	7.5	5.0	--	7.0	19.5	24.0

Tri = Trimoulter; Con = Control

Effect on the body growth

Body weight : The larval, pupal as well as adult weight

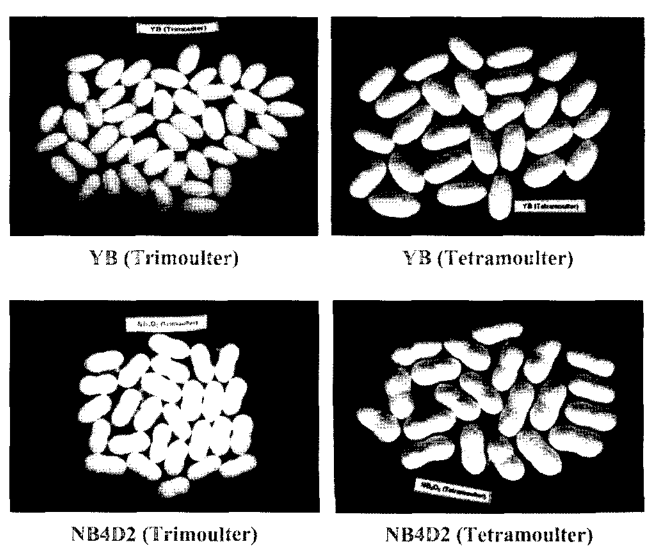

Plate 1. Trimoulter and Tetramoulter cocoon of YB & NB_4D_2

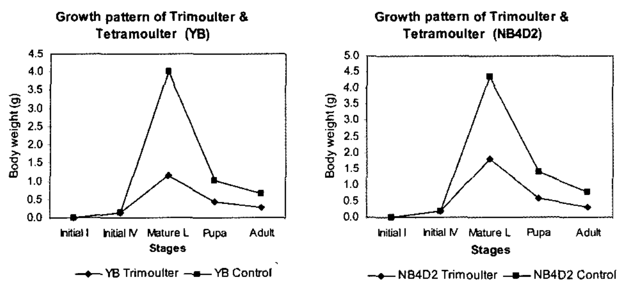

Fig. 4. Growth pattern of trimoulter & tetramoulter YB & NB_4D_2.

significantly decreased in all the treated larvae with the chemical in both the bivoltine breeds - YB & NB_4D_2 when compared to the control (Fig. 4). The result agrees with the findings of Kuwano *et al.* (1985), Akai *et al.*(1984) and Trivedy *et al.*(1994).

Effect on the economic parameters

Yield of cocoon : The yield of trimoulter cocoon by number (trimoulterisation response) varied depending on the breed and season of rearing. The maximum yield by number was recorded during February both in YB (8700) and NB_4D_2 (8502). There was a significant difference between the trimoulter and tetramoulter in respect of yield/10,000 larvae by number during September in both the bivoltine breeds. But in all the seasons there was significant difference in respect of yield/10,000 larvae (by wt.) in both the bivoltine breeds (Table 2).

Single Cocoon weight (SCW) : The cocoon weight of induced trimoulter lots was significantly decreased in both the bivoltine breeds in all the three seasons compared to controls. Average maximum single cocoon weight (0.524 g) was recorded during February in case of YB and 0.744 g in NB_4D_2 trimoulter lots (Table 2).

Number of cocoon per kilogram (Chitt) : The number of cocoons per kilogram in trimoulter lots was significantly higher than the tetramoulter control in all the three seasons tested in both the bivoltines. The numbers being 2032, 1995 & 1906 in trimoulted YB against 650, 660 & 636 in tetramoulter YB during September, December & February respectively. In case of NB_4D_2 it was 1400, 1460 & 1342 in trimoulted NB_4D_2 as against 600, 587 & 620 in tetramoulted NB_4D_2 during the same crop seasons (Table 2).

Cocoon shell percentage (SR%) : Cocoon shell percentage was significantly lower in trimoulter in comparison to tetramoulter in all the three seasons in both the bivoltine breeds. In YB the shell percentage was 13.79, 11.77 &

Table 2. Rearing performance of induced trimoulting and tetramoulting Bivoltine breeds

Season	Treatment	Yield / 10000 larvae		No. of cocoon / kg	SCW (g)	Shell %	FL (m)	Denier
		No.	Wt.					
Silkworm Breed: YB,								
	Tri	8400	4.265	2032	0.493	13.79	430	1.09
Sept.	Con.	820	1.390	650	1.543	20.73	692	2.51
	t-test	S	S	S	S	S	S	S
	Tri	8100	4.150	1995	0.501	11.77	390	1.02
Dec.	Con.	8205	12.45	660	1.510	19.86	661	2.64
	t-test	NS	S	S	S	S	S	S
	Tri	8720	4.569	1906	0.524	12.02	412	1.06
Feb.	Con.	8811	13.75	636	1.569	19.12	677	2.44
	t-test	NS	S	S	S	S	S	S
Silkworm Breed: NB_4D_2								
	Tri	7153	5.082	1400	0.713	14.16	477	1.06
Sept.	Con.	78	0.137	600	1.681	20.91	782	2.13
	t-test	S	S	S	S	S	S	S
	Tri	8212	5.839	1460	0.685	13.43	500	1.17
Dec	Con.	8304	14.657	587	1.695	20.05	707	2.18
	t-test	NS	S	S	S	S	S	S
	Tri	8502	6.588	1342	0.744	14.91	483	1.11
Feb.	Con.	8663	14.000	620	1.624	19.81	714	2.34
	t-test	NS	S	S	S	S	S	S

SCW = Single Cocoon Wt; FL = Filament Length
'S'= Significant at 5% level ; NS = Non significant

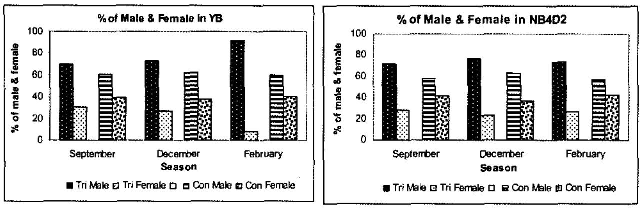

Fig. 5. Male & Female percentage in Trimoulter & Tetramoulter YB & NB_4D_2.

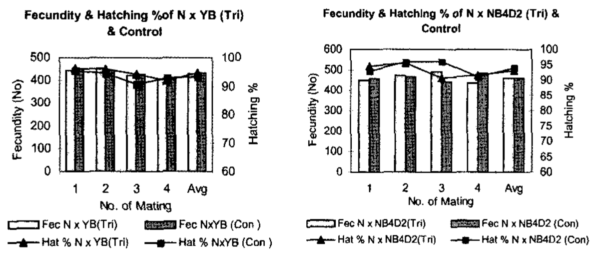

Fig. 6. Fecundity and hatching % in N × YB (Tri) & control and N × NB_4D_2 (Tri) & control.

12.02 in trimoulter against 20.73, 19.86 & 19.12 in tetramoulter control during September, December & February respectively. In case of NB_4D_2 it was 14.16, 13.43 & 14.91 in trimoulter against 20.90, 20.05 & 19.81 in tetramoulter control during the same crop seasons (Table 2).

Effect on the cocoon quality

Filament length and Denier : Filament length of the trimoulter cocoon of both YB and NB_4D_2 breeds were significantly reduced compared to control lots. The denier was also significantly reduced in both the trimoulter bivoltine breeds compared to control lots. The denier ranged between 1.02 to 1.09 and 1.06 to 1.17 during September, December & February crop season in trimoulted YB & NB_4D_2 respectively. Filament length ranged between 390 to 412 m and 477 to 500 m in trimoulted YB & NB_4D_2 respectively (Table 2).

Effect on other commercial parameter

Effect on Sex ratio : Interestingly, it was observed that male percentage was more than the counter part female in the treated lots in both the bivoltine breeds compared to tetramoulter when treated with the chemical. In the trimoulter bivoltines maximum male % (91.66%) was reported during February in YB breed and similar observation (76.95%) was noticed during December in NB_4D_2 breed (Fig. 5).

Effect on grainage behaviour

Emergence pattern : Normal emergence pattern was found in trimoulter cocoons in both the bivoltine breeds like tetramoulter lots.

Mating behavior : The trimoulter males of both the bivoltine breeds were crossed with the normal multivoltine females for production of multi × bi hybrid laying. Mating behaviour of trimoulter males with normal tetramoulter females was found normal (Figs. 6, 8) in spite of weight of the trimoulter males and that was confirmed upto 4 times mating.

Fecundity & hatching % : Fecundity of the trimoulter was found lower than that of tetramoulter. But no significant difference was observed in hatching percentage among the trimoulter and tetramoulter lot. In case of YB fecundity was 164, 201 & 196 in trimoulter against 455, 463 & 487 in tetramoulter control during September, December & February respectively. In NB4D2 it was 194, 222 & 203 in trimoulter against 492, 538 & 523 during September, December & February respectively.

In case of YB hatching percentage ranged between 92.53 - 96.12% in trimoulter against 94.61 - 97.21% in tetramoulter control. Where as in NB_4D_2 hatching percentage varied between 94.23 - 96.38 against 92.19 - 93.41% in tetramoulter control (Fig. 7).

The multi × bi hybrids dfls of both YB and NB4D2 were prepared and tested in the laboratory as well as field level for consecutive two years, the rearing performance revealed that the economic characters of the treated lot (tetra female × tri male) were at par with the control lots (tetra female × tetra male) in both the bivoltine breeds.

So it can be concluded that multi × bi hybrid dfls prepared by using trimoulter bivoltine male can safely be utilized for better cocoon production at commercial level.

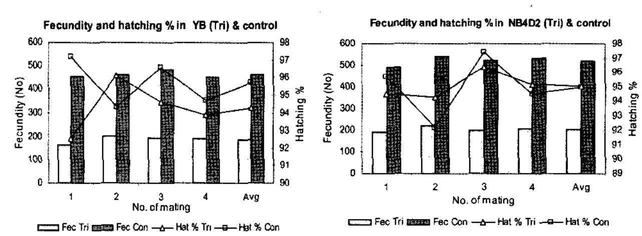

Fig. 7. Fecundity and hatching % in YB (Tri) & control and NB_4D_2 (Tri) & control.

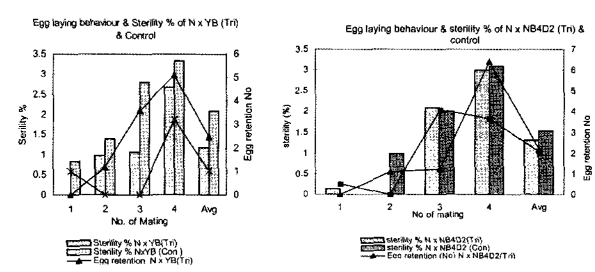

Fig. 8. Effect of trimoulting on egg laying behaviour of YB & NB_4D_2.

Discussion

The chemical induction of precocious metamorphosis in silkworms has been demonstrated by dietary application of kojic acid (Murakoshi, 1972), its related compounds (Murakoshi and Ichimoto, 1972), abietic acid derivatives (Murakoshi *et al.,* 1975), and quinolone alkaloids (Murakoshi *et al.,* 1977), imidazole derivatives (Akai *et al.,* 1984; Kiuchi *et al.,* 1985) and these chemicals strongly inhibit the ecdysteriod levels in haemolymph (Kadono-Okuda *et al.,* 1987; Kiuchi and Akai, 1988). However, no compound administered by per-oral application of this chemical has yet been reported in tropical condition in India.

The results indicate 76.15% induction in NB_4D_2 with 500 ppm and 84% induction in YB with 650 ppm of this chemical. The pupation rate was recorded 80 - 84% and 82 - 86% in NB_4D_2 and YB respectively. The doses of 300 to 700 ppm of this chemical caused the induction of precocious silkworms in both YB and NB_4D_2 bivoltine breeds. These facts suggested that the chemical have some anti-JH action. Similar finding was made by Takako Miyajima *et al.,*2001. Asano *et al.,*(1984), who suggested that KK-22 may also have some anti feeding effect on silkworms in addition to its anti- JH action. A dose of 400 ppm of SSP-11 has been found to induce precocious metamorphosis (Akai *et al.,* 1984; Kuwano *et al.,* 1983). It has been reported to induce precocious metamorphosis in silkworm by application of KK-42 (Banerjee & Deb, 1999; Kuwano *et al.,* 1988; Akai and Mauchamp, 1989; Gu *et al.,* 1992).

In order to induce trimoulting in the tetramoulter strain, the said chemical was used with the mulberry leaves and fed to 4^{th} instar larvae for 48 hours immediately after the 3^{rd} moulting. Their feeding period was prolonged by 2 - 3 days in the 4^{th} instar and 5^{th} stage was completely skipped off. As a result total larval period was reduced by 4 - 5 days compared to that of the control. The said chemical treatment might have upset the hormone levels in the 4^{th} instar and resulted in the production of trimoulter cocoons. Similar results were observed in the silkworm treated with KK-42 (Trivedy *et al.*, 1994: Banerjee and Deb, 1999; with SSP-11 (Kiuchi *et al.*, 1985; Akai *et al.*, 1984). They also reported that precocious metamorphosis in *Bombyx mori* can clearly be induced by treatment of Imidazole derivatives, and that these chemicals strongly inhibit the ecdysteriod levels in haemolymph (Kadono-Okuda *et al.,*1987; Kiuchi and Akai,1988).

The larval development period was shortened by the anti-JH action resulting in smaller matured larvae followed by smaller cocoon and miniature pupae. Emerged adults from the pupae of trimoulter were about 7/10 normal size, and all could mate and laid viable eggs. This also directly reduces the food consumption as well as the labour. The decrease in the larval weight might be attributed to the reduction in the larval duration. Akai *et al.,* (1984) recorded similar observation in SSP-11 treated silkworms. The behaviour of freshly emerged moths obtained from trimoulters was similar to that of controls. Furthermore, the moths, though smaller were as viable as the controls, as also observed by Kuwano *et al.,*1983; Banerjee & Deb 1999.

Significant reduction (60%) in fecundity was observed in treated than the tetramoulters which agrees with the observation of Kimura *et al.*(1986). As the juvenile hormone plays an important role in oogenesis, its inhibition by the AJH may be the reason of the reduced fecundity. But the trimoulter males, in this study, were in no way inferior to the tetramoulter males as breeding partner which corroborates the findings of Banerjee and Deb (1999).

The yield/10,000 larvae by number in control have been increased from 820 to that of 8400 in treated lot in case of YB breed and from 78 to 7153 increased in case of NB_4D_2 breed due to application of the chemical during adverse season (September). The improvement observed in yield/ 10,000 larvae (by number) in trimoulters during September seed crop (unfavourable season) can be ascribed due to the decrease of disease incidence as it was possible to reduce the exposure of larvae to extreme climatic condition by reducing larval period. He and He (1994) have argued that the physiques of the induced trimoulters are stronger than the control tetramoulter. Treatment with this chemical during adverse season (September) reduces the larval mortality. This is one of the reasons for better survivability. These results are in conformity with the observation of Lu Xuefang and Li Ronggi (1987), Akai (1988), Xue and Li (1987), Xue (1992) Xue *et al.* 1990;1994, Banerjee (1998) who recorded a lower incidence of disease and hence a reduced mortality in induced trimoulters. They have argued that induced trimoulters are less susceptible to diseases due to skipping off the disease prone fifth larval instars. The authors recorded a better result in survival during adverse season like September but no significant differences were observed in survival during favourable season like December & February.

Though the filament size of the induced trimoulters cocoons are less (1.02 - 1.17) in comparison to the control (2.13 - 2.64), the length: size ratio are higher in case of the former which indicates that the decreasing rate of the filament size along the length must be lower in the induced trimoulters cocoon resulting in the formation of a filament of such uniform denier with less size deviation. So, apart from its use as a parental component for the production of multi × bi hybrids in West Bengal during adverse seasons, the superfine silk filament may have a very useful contribution in the field of medical textiles or other industrial textiles. Miyajima *et al.*,(2001) have also expressed similar observation. Reductions in silk filament size in both the breeds are in agreement with the observation of Hiromu Akai *et al.* (1984,1986), Koto Katoka *et al.*(1985) and Tsukuda *et al.* (1987).

The mechanism of precocious metamorphosis by KK-42 suggested by Gu and Chow (1996,1997) are : i) the titer of ecdysteriods in the haemolymph decreases due to inhibition of biosynthesis in the prothoracic glands, ii) a decrease in ecdysteriod titer inactivates JH biosynthesis in the corpora allata, and iii) this decrease in JH titer then induces the precocious metamorphosis. It appears that the effect of the chemical is of similar type.

Staal *et al.*, (1981) reported that the mechanisms of anti-JH action in silkworm can be i) interference with the neural and humoral regulation of corpora allata, ii) interference with JH biosynthesis in corpora allata, and iii) interference with JH transport and JH receptor. These facts suggest that the present test chemical, might have epoxidation inhibition to juvenile hormones in corpora allata of the insects.

The cocoon weight have been reduced by about 32-34% of the cocoon weights of tetramoulter control of YB breed and about 40-45% reduction in cocoon weights from the control lot of NB4D2 breed. This is obvious due to less food intake during short larval duration of trimoulters following imidazole treatment. Imidazole induced reduction of cocoon weight corroborates the earlier finding of Staal (1985), Kanda *et al.*, (1986) Akai et at., (1986) Kiuchi *et al.*, (1986) He and He (1994) Himantharaj *et al.*, (1996) and Banerjee and Deb (1999).

Kanda *et al.*, 1986 reported that the cocoons obtained by the treatment of KK-42 were fairly small but shapes of the cocoons were uniform with high percentage of reelable cocoons. In *Bombyx mori* the accumulated food in larval body weight is related to food intake (Ding Nong *et al.*, 1991) and finally to storage protein concentration in larval haemolymph. This is also influenced by nutrition (Nagata and Kobayashi, 1990). Lower food consumption by induced trimoulters due to skipping off of the usual fifth instars resulting reduced cocoon weight. Matsumura and Takeuchi, (1950) reported that the fifth instars alone consumes about 87% of the total food requirement. Though this chemical prolong the duration of 4th instar by 3-5 days over the duration of control larvae, the total food consumption is much less than the tetramoulter larvae. Hence, this chemical certainly put a stress on normal development of larvae forcing them to the neurohormonal response leading to precocious pupation, provided the larva has attained the critical weight as attainment of larval critical weight is a prerequisite for the induction of trimoulters (Asano *et al.*, 1987).

The cocoon shell weight have been 20% of the control weight in case of YB breed and 30% weight of the control lot of NB4D2.. These results are in conformity with the observation of Kanda *et al.*, (1986), Kiuchi *et al.*, (1986) and Himanthraj *et al.*, (1996).

In this finding, only 36-43% egg production was noted in trimoulters lots in comparison to control lots. Kawaguchi *et al.*,(1993) also reported that the egg production in treated batches of KK-42 was only 40% than their control counterparts.

Considering the advantages of rearing *B.mori* larvae through application of imidazoles it appears that better rearing management is possible due to the following reasons : a) it shortens the duration of larval span by 4-5 days thus saving labour and leaf; b) due to the skipping off of the 5th larval instar, incidence of diseases are less resulting in better survival of larvae and good pupation rate, and c) moths of trimoulters are more active compared to those of tetramoulter with less percentage of unfertilized eggs (Xue *et al.*, 1990, 1994; Xue 1992; He and He, 1994). Better performances in terms of oviposition rate, number of fertilized eggs, less unlaid eggs, mating behavior of moths, activeness of male moths were also recorded from induced trimoulter batches during preparation of F1 hybrid eggs using trimoulters. The hatchability of eggs is almost 100 percent. So production of F1 hybrid eggs utilizing induced trimoulters has been recommended (Xue 1992; Xue *et al.*, 1994). He and He (1994) advocated the use of Imidazole compounds for rearing of *B.mori* during unfavourable seasons both in commercial and laboratory level for better rearing results. The authors argued that due to the use of the Imidazoles neither the genotype nor the combining ability of the silkworm breeds is affected. All induced trimoulters returned to tetramoulter if imidazoles are not used even after rearing as induced trimoulter upto F5 generation which corroborates the finding of He and He, 1994.

In the tropical plains, particularly in West Bengal, the rearing of bivoltine breeds of *B.mori* is a serious problem specially during season with high temperature and high humidity. Most of the advanced stages of larvae are attacked by micro pathogenic diseases. Hence rearing of hybrid of multivoltine and bivoltine becomes imperative during this season in commercial sectors. To produce such multivoltine x bivoltine hybrid eggs the use of induced trimoulter bivoltine males may be promising. This justifies the present attempt of using Imidazoles for the induction of trimoulters and to find out the impact of these compounds on economic and reproductive characters of *B.mori* for further utilization of these compounds in large scale.

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