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The Impact of Juvenile and Molting Hormone Analogues on Silk Quality of Silkworm, *Bombyx mori L.* Fed on Mulberry Leaf and Artificial Diet

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The impact of juvenile hormone analogue, methoprene, molting hormone analogue, MH–III (phytoecdysone) and combination of methoprene and MH–III on silk quality was studied in two bivoltine hybrids viz., Jufang (Chinese)xChenxin(Japanese) and ChenxinxJufang during autumn season.

The filament length, non-breakable filament length and reelability percentage were increased over control when treated with methoprene, MH–III and combination of methoprene and MH–III. The filament weight was increased in the batches treated with methoprene, and combination of methoprene and MH–III. But the administration of MH–III showed significant decrease in the filament weight over control. The denier and size deviation were decreased significantly in the treated batches over control. The results suggest that improvement in reeling parameter lead to high grade silk which has more demand in the international market.

The effect of JH and MH–III showed increase in post-cocoon parameters in silkworm fed on mulberry leaf when compared to silkworm fed on artificial diet. The post-cocoon parameters were found to be superior in bivoltine hybrid ChenxinxJufang when compared to Jufang xChenxin. The application of insect hormone analogues has stimulatory effect on silk quality which has much significance in the reeling industry.

Key words: silkworm, Bombyx mori L., methoprene, MH–III, denier, reelability

The domesticated mulberry silkworm, *Bombyx mori L* has been the target of intensive scientific study because of its industrial importance. The growth of silkworm especially that of the larval stage, which mainly depend on ecdysone and juvenile hormone has also been studied . Ecdysone is secreted from the prothoracic gland and JH produced from corpora allata are most directly concenced with silkworm growth, while the former induces molting, the latter helps to remain juvenile characters in their life cycle. The process of molting and metamorphosis characteristic to growth and development in insect is regulated by circulating hormones like prothoracicotropic hormone (PTTH), juvenile hormone and ecdysterone (Wigglesworth 1965). The set pattern of insect development can be altered to a certain extent by exogenous administration of the mimics or analogues of these circulating hormones (Sakurai 1983).

The synthesized JH active substances were studied for utilization of rearing (Akai & Kobayashi 1971, Otaki et al. 1971) and methoprene was one of the JH analogues, which was disseminated as an

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agricultural chemical for increase of cocoon shell weight. In the practical application of combined juvenile hormone analogue and ecdysteroids, the occurrence of non-cocooning larvae could be prevented and silk production was increased(Chou & Lu 1980). The effects of ecdysteroids on the silk production, Diapause and reproduction of silkworm have been applied in sericulture (Guo 1989). Several attempts were made in the application of MH in silk production (Shigematsu et al. 1974, Dai et al. 1986, 1992, and Osamu, Makoto 1996).

Hence, in the present study, an attempt has been made to study the effects of juvenile and molting hormone analogues on the silk quality in two bivoltine hybrids of silkworm, *Bombyx mori L* fed on mulberry leaf and artificial diet.

Material and Methods

Experimental animals

The disease free layings of two popular bivoltine hybrid silkworm races 1.Jufang (Chinese) X Chenxin (Japanese) and 2.Chenxin (Japanese) × Jufang (Chinese) were procured from the Sericultural Department of Zhejiang Agricultural Bureau. The silkworms were reared on fresh mulberry leaf and on artificial diet at temperature of 24-28°C, RH-70-85% and 12L:12D photoperiod. After fourth molt, the larvae were separated into three groups. Each group consists of three replications each of 250 larvae for each treatment.

Application of juvenile hormone analogue (methoprene)

Juvenile hormone analogue, methoprene was dissolved in acetone and sprayed on the silkworm body at the rate of 1.5µg/larva on third day of fifth instar and the batches of larvae are fed on fresh mulberry leaf and artificial diet.

Application of molting hormone analogue (MH–III)

MH–III, a phytoecdysone extracted from *Radix achyranthes* (The Organic Chemistry, Institute of CAS,).MH–III was dissolved in water and sprayed on the mulberry leaf at the rate of 2µg/larva on fourth and fifth day of fifth instar period. MH–III was added to the artificial diet and fed to silkworm larvae on fourth and fifth day of fifth instar period.

Application of combination of methoprene and MH–III

Juvenile hormone analogue, methoprene was dissolved in acetone and sprayed on silkworm body on third day of fifth instar at the rate of 1.5µg/larva. For these larvae, the mulberry leaf fortified with MH–III was given at the rate of 2µg/larva on fourth and fifth day of fifth instar period.

The filament length, filament weight, non-breakable filament length, denier, reelability and size deviation were investigated in control and experimental bivoltine hybrids of silkworm, *Bombyx mori L* fed on mulberry leaf and artificial diet.

Results

Tables 1 and 2 showed the control and experimental bivoltine hybrids of silkworm, Bombyx mori L fed on mulberry leaf and artificial diet. Results revealed the extent of changes in reeling parameters, such as filament length, filament weight, non-breakable filament length, denier, reelability and size deviation.

Filament length

Jufang×Chenxin: The filament was increased significantly in the batches treated with methoprene (16.65%) and combination of methoprene and MH–III (9.92%) over control when fed on mulberry leaf, but there was a marginal increase in the filament length when treated with MH–III (1.42%).

When the larvae fed on artificial diet, a marginal decrease was observed in the filament length in the batches treated with methoprene (13.13%) with MH–III (1.19%) and combination of methoprene and MH–III (9.31%) as compared to larvae fed on mulberry leaf.

Chenxin×Jufang: The filament length was increased significantly over in the batches treated with methoprene (16.72%) and combination of methoprene and MH–III (10.38%) over control when fed on mulberry leaf, but there was a marginal increase noticed in the length of silk filament when treated with MH–III (2.51%).

When the larvae fed on artificial diet, a marginal decrease was observed in the filament length in the batches treated with methoprene (14.45%), with MH–III (1.26%) and combination of methoprene and MH–III (9.40%) as compared to larvae fed on mulberry leaf.

The filament length was found to be slightly longer in hybrid, Chenxin×Jufang when compared to Jufang×Chenxin.

Filament weight

Jufang×Chenxin: The filament weight increased significantly over in the batches treated with methoprene (8.33%) and non-significant change was noticed in the combination of methoprene and MH–III (2.08%) over control, when fed on mulberry leaf, but there was a significant decrease in the weight of filament when treated with MH–III (10.00%).

Table 1. Changes in reeling parameters in control and experimental silkworm, Bombyx mori L fed on mulberry leaf.

		Jufang (Chinese) x Chenxin (Japanese)				Chenxin (Japanese) x Jufang (Chinese)			
			Experimental				Experimental		
S. No.	Parameter	Control	Methoprene	MH–III	Methoprene and MH–III	Control	Methoprene	MH–III	Methoprene and MH–III
1	Filament length (m)	847.00	988.00	859.00	931.00	915.00	1068.00	928.00	1010.00
		±15.83	±22.85	±12.86	±10.84	±15.83	±12.69	±10.64	±21.69
			+16.65	+1.42	+9.92		+16.72	+2.51	+10.38
			P<0.001	NS	P<0.001		P<0.001	NS	P<0.001
2	Filament weight (gm)	0.240	0.260	0.216	0.245	0.251	0.272	0.220	0.252
		±0.023	±0.023	±0.020	±0.021	±0.024	±0.025	±0.021	±0.022
			+8.33	-10.00	+2.08		+8.37	-12.35	+0.40
			P<0.001	P<0.001	NS		P<0.001	P<0.001	NS
3	Non-breakable filament length (m)	720.00	866.00	789.00	822.00	791.00	960.00	876.00	920.00
		±0.12.6	±16.82	±16.35	±18.23	±18.59	±11.63	±13.38	±10.37
		5	+20.28	+9.58	+14.17		+21.37	+10.74	+16.31
			P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
4	Denier (d)	2.55	2.37	2.26	2.32	2.47	2.29	2.11	2.25
		±0.23	±0.22	±0.20	±0.22	±0.21	±0.18	±0.19	±0.19
			-7.06	-11.37	-9.02		-7.29	-14.5	-8.91
			P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
5	Reelability (%)	85.00	87.65	91.85	88.29	86.44	89.89	93.39	91.08
6	Size deviation (d)	0.356	0.29	0.39	0.30	0.32	0.26	0.29	0.28
		±0.032	±0.028	±0.028	±0.045	±0.049	±0.026	±0.028	±0.026
			-17.140	-8.57	-14.29		-18.750	-9.380	-14.290
			P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001

Values are the mean of 10 individual observations. Mean ±S.D., "+" and "-" indicate percent increase and decrease over control respectively, "P" denotes the statistical significance, "NS"-non-significant.

Table 2. Changes in reeling parameters in control and experimental silkworm, Bombyx mori L fed on artificial diet.

		Chenxin (Japanese) x Jufang (Chinese)				Jufang (Chinese) x Chenxin (Japanese)			
			Experimental				Experimental		
S. No.	Parameter	Control	Methoprene	MH–III	Methoprene and MH–III	Control	Methoprene	MH–III	Methoprene and MH–III
1	Filament length (m)	872.00	998.00	883.00	954.00	838.00	948.00	848.00	916.00
		±18.23	±20.36	±10.52	±14.64	±16.84	±11.36	±14.87	±10.34
			+14.450	+1.26	+9.40		+13.13	+1.19	+9.31
			P<0.001	NS	P<0.001		P<0.001	NS	P<0.001
2	Filament weight (gm)	0.245	0.263	0.216	0.248	0.235	0.253	0.213	0.238
		±0.023	±0.024	±0.019	±0.023	±0.021	±0.021	±0.019	±0.019
			+7.350	-11.84	+1.22		+7.66	-9.36	+1.28
			P<0.001	P<0.001	NS		P<0.001	P<0.001	NS
3	Non-breakable filament length (m)	752.00	891.00	818.00	868.00	708.00	826.00	766.00	801.00
		±12.84	±13.83	±10.46	±16.15	±12.53	±11.07	±16.69	±10.13
			+18.480	+8.78	+15.43		+16.67	+8.91	+13.14
			P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
4	Denier (d)	2.53	2.37	2.20	2.34	2.52	2.40	2.26	2.34
		±0.24	±0.21	±0.20	±0.22	±0.24	±0.22	±0.20	±0.23
			-6.32	-13.04	-7.51		-9.75	-5.51	-7.63
			P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001
5	Reelability (%)	86.24	89.28	92.64	90.98	84.49	87.13	90.33	87.45
6	Size deviation (d)	0.35	0.28	0.31	0.30	0.38	0.31	0.3	0.32
		±0.051	±0.018	±0.046	±0.04	±0.035	±0.030	±0.030	±0.031
			-20.0	-11.43	-14.29		-18.42	-10.53	-15.79
			P<0.001	P<0.001	P<0.001		P<0.001	P<0.001	P<0.001

Values are the mean of 10 individual observations. Mean ±S.D., "+" and "-" indicate percent increase and decrease over control respectively, "P" denotes the statistical significance, "NS"-non-significant.

When the larvae fed on artificial diet, a marginal decrease was observed in the filament weight in the batches treated with methoprene (7.66%) and combination of methoprene and MH-III (1.28%) as compared to larvae fed on mulberry leaf. But there was a significant decrease in the weight of filament when treated with MH-III (9.36%).

ChenxinxJufang: The filament weight was increased significantly in the batches treated with methoprene(8.37%) and non-significant change was noticed in the combination of methoprene and MH-III (0.40%) over control when fed on mulberry leaf, but there was a significant decrease(12.35%) noticed in the filament weight when treated with MH-III (12.35%).

When the larvae fed on artificial diet, a marginal decrease was observed in the filament weight in the batches treated with methoprene(7.35%) and non-significant change was noticed in the combination of methoprene and MH-III (1.22%) as compared to larvae fed on mulberry leaf.

The filament weight was found to be slightly more in bivoltine hybrid, ChenxinxJufang when compared to JufangxChenxin, decreased with methoprene and combination of methoprene and MH-III. The percent decrease in filament weight was more in ChenxinxJufang than in JufangxChenxin, treated with MH-III.

Non-breakable filament length

JufangxChenxin: The non-breakable filament length was increased significantly in the batches treated with methoprene(20.28%), with MH-III (9.58%) and with combination of methoprene and MH-III (14.17%) over control when fed on mulberry leaf.

When the larvae fed on artificial diet, a marginal decrease was observed in the non-breakable filament length in the batches treated with metho-prene(16.67%), with MH-III (8.19%) and combination of methoprene and MH-III (13.14%) as compared to larvae fed on mulberry leaf.

ChenxinxJufang: The non-breakable filament length was increased significantly in the batches treated with methoprene(21.37%), with MH-III (10.79%) and with combination of methoprene and MH-III (16.31%) when fed on mulberry leaf.

When the larvae fed on artificial diet, a marginal decrease was observed in the non-breakable filament length in the batches treated with metho-prene(18.48%), with MH-III (8.78%) and combination of methoprene and MH-III (15.43%) as compared to larvae fed on mulberry leaf.

The non-breakable filament length was found to be more in bivoltine hybrid, ChenxinxJufang when compared to JufangxChenxin.

Denier

JufangxChenxin: The filament thickness was decreased significantly in the batches treated with methoprene(2.37d), with MH-III (2.26d) and with combination of methoprene and MH-III (2.32d) over control(2.55d) when fed on mulberry leaf.

The filament thickness was decreased significantly in the batches treated with methoprene(2.40d), with MH-III (2.26d) and the combination of methoprene and MH-III (2.34d) over control(2.52d) when fed on artificial diet.

ChenxinxJufang: The filament thickness was decreased significantly in the batches treated with methoprene (2.29d), with MH-III (2.11d) and with combination of methoprene and MH-III (2.25d) over control (2.97d) when fed on mulberry leaf.

The filament thickness was decreased significantly in the batches treated with methoprene (2.37d), with MH-III (2.20d) and the combination of methoprene and MH-III (2.34d) over control (2.53d) when fed on artificial diet.

Reelability

JufangxChenxin: The reelability was increased in the batches treated with methoprene (87.65%), with MH-III (90.85%) and with combination of methoprene and MH-III (88.29%) over control (85.00%) when fed on mulberry leaf.

The reelability percentage was increased in the batches treated with methoprene (87.13%), with MH-III (90.33%) and the combination of methoprene and MH-III (87.45%) over control(84.49%) when fed on artificial diet.

ChenxinxJufang: The reelability percentage was increased in the batches treated with methoprene (89.89%), with MH-III (93.39%) and with combination of methoprene and MH-III (91.08%) over control (86.44%) when fed on mulberry leaf.

The reelability percentage was increased in the batches treated with methoprene (89.28%), with MH-III (92.64%) and the combination of methoprene and MH-III (90.98%) over control (86.24%) when fed on artificial diet.

The reelability percentage was found to be more in ChenxinxJufang when compared to JufangxChenxin.

Size deviation

JufangxChenxin: The size deviation was decreased significantly in the batches treated with methoprene (0.45d), with MH-III (0.50d) and with combination of methoprene and MH-III (0.48d) over control (0.56d) when fed on mulberry leaf.

The size deviation was decreased significantly in the batches treated with methoprene(0.46d), with MH-III (0.51d) and the combination of methoprene and MH-III (0.49d) over control (0.58d) when fed on artificial diet.

ChenxinxJufang: The size deviation was decreased significantly in the batches treated with methoprene (0.41d), with MH–III (0.46d) and with combination of methoprene and MH–III (0.44d) over control (0.52d) when fed on mulberry leaf.

The size deviation was decreased significantly in the batches treated with methoprene (0.44d), with MH–III (0.49d) and the combination of methoprene and MH–III (0.49d) over control (0.55d) when fed on artificial diet.

Discussion

The results of reeling parameters suggest that the quality of silk is found to be superior which has much economic importance in the reeling industry. Increase in filament length, non-breakable filament length, reelability percentage, fine silk filament with less size deviation are the most important commercial characters in the improvement of silk quality and yield.

It is a well-known fact that larval molting is determined by the interaction of JH secreted by corpora allata and MH secreted by prothoracic gland activated by the brain hormone (PTTH). The larval-pupal and pupal-adult transformations are determined mainly by the function of MH. MH has been shown to regulate many important functions such as morphogenesis (Kajiura & Yamashita 1992, Tanaka & Takeda 1993), the metabolism of nucleic acids (Logan et al.1975) and protein synthesis (Chen 1985, Dai et al. 1985, Morohoshi et al. 1972).

JH mimics have direct stimulatory effect on protein synthesis of silk gland (Kajiura & Yamashita 1989). Dai Yujin et al. (1986) reported that MH could regulate protein synthesis of silkworm. The effect of phytoecdysones on the growth of silkworm has been reported and found to increase in cocoon yield (Kobayashi 1978). The best results of silk production were obtained by spraying JH analogues on body of silkworm and then phytoecdysteroids on mulberry leaf to feed the 5th instar larvae (Chou & Lu 1980).

The stimulating capacity of insect hormone on various post-cocoon characters contributing to silk yield may be attributed to the synthesis of proteins and nucleic acids in the silkworm. The increase in fibroin content leads to superior quality of silk which has more export value.

Conclusion

The application of insect hormone analogues has stimulatory effect on cocoon production and silk quality, which has much economic importance in reeling industry.

The economic importance of silkworms has been attracting the attention of biologists to explore various intricate mechanisms of the action of insect hormones. Effect of insect hormone analogues showed significant development in terms of productivity and quality right from the cocoon production to processing of silk. The action of insect hormone analogues stands as an example of the metabolic flexibility and intrinsic adaptability, where in the basal metabolism of the insect was enhanced to a maximum extent. Basal metabolism was indirectly correlated to life span of insects. Changes in the physiological or molecular level might result in active biosynthesis of silk proteins in silk gland. Since the research work in insect hormone analogues involves the improvement of silk industry, it has research and development importance.

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