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Silk gland Protease activity under the influence of Juvenile hormone analogues during spring and summer seasons

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Abstract

This study investigated the effects of three juvenile hormone analogues (JHAs)-Methoprene, Fenoxycarb, and Bukchiol-on the protease activity of silk glands in the domestic silkworm (*Bombyx mori* L.) through experiments conducted during the spring and autumn rearing seasons. The study examined the influence of JHA type, application concentration, and timing of administration during the fifth larval instar on silk gland protease activity, expressed as μM tyrosine/mg protein. In the spring trial, the highest protease activity (65 μM) was observed in larvae treated with 0.1 μL of Methoprene applied 48 hours after the onset of the fifth instar, followed closely by the corresponding Bukchiol treatment, which recorded 63 μM . In contrast, the lowest activity (46 μM) occurred in larvae treated with 1.0 μL of Fenoxycarb applied 96 hours into the fifth instar. A similar pattern was recorded during the autumn experiment, with the respective protease activity values of 64, 57, and 46 μM for the Methoprene, Bukchiol, and Fenoxycarb treatments. Overall, the findings demonstrate that Methoprene was the most effective JHA in enhancing silk gland protease activity, followed by Bukchiol, whereas Fenoxycarb exhibited the least stimulatory effect. The results further indicate that the type of juvenile hormone analogue, its concentration, and the timing of application are the principal factors influencing protease activity in the silk glands of *Bombyx mori*.

Keywords: *Bombyx mori*; juvenile hormone analogues (jhas); methoprene; fenoxycarb; bukchiol; silk gland protease activity

Introduction

Agriculture is the core industry that sustains the livelihoods of nearly 70% of India's rural population. Combined with the World Bank's conclusion that the global poor are overwhelmingly concentrated in rural areas, the core challenge facing India's agricultural development has become increasingly prominent with only 2.4% of the world's total land area, the country bears the pressure of meeting the food supply needs of the world's second most populous nation. This paper sorts out five core drivers of the continuous decline in India's agricultural GDP. If this sluggish growth trend cannot be reversed, large-scale hunger and malnutrition will inevitably break out. To solve this predicament, this paper proposes a core solution of promoting interdisciplinary income-boosting industries. Among these sectors, sericulture stands out as a feasible path to help farm households achieve stable income growth, thanks to its unique advantages of being suitable for female workers, delivering quick returns and having low entry barriers. This paper further narrows its research focus to the sericulture industry in Jammu and Kashmir, where the sector has a long development history and has long served as a core pillar of the regional economy. This solid historical foundation of the industry is confirmed by multiple sources: the records of historical scholar Mirza Mohammad Haider in his work Tarikhi Rashide, and the historical fact that ancient king Sultan Zain-ul-Abideen proactively promoted advanced breeding technologies to develop sericulture. Local official archives also document an early cross-border trade record of Kashmir supplying 25,000 ounces of silkworm eggs to Europe in 1855.

On this basis, the paper proceeds to sort out the academic research background of sericulture output improvement: the positive effect of insect growth regulators on silk production has

long attracted academic attention. After Karl Slama and Williams discovered the “Paper Factor” in 1966, relevant research has advanced continuously and the growth-regulating function of juvenile hormones has been gradually clarified. At present, synthetic juvenile hormones have been applied to the output enhancement process in the sericulture industry. A 1988^[4] study by Yilmaz and Omurga clearly established that juvenile hormone is an exclusive hormone that only exists in insects, and its core function is to regulate the development and metamorphosis of insects. Since then, the academic community has synthesized a series of juvenile hormone analogs based on the structure and function of this hormone, among which three compounds methoprene, hydroprene and fenoxycarb have been adopted for commercial use.

Drawing on the research findings of a 2013 study by Devillers and a 2011 study by Goncu and Parlak, fenoxycarb's carbamate structure has no anticholinesterase activity and produces no neurotoxicity. It only binds to juvenile hormone receptors and cannot be degraded by juvenile hormone esterase. When applied at high doses, it disrupts insect metamorphosis and triggers abnormal larval development. On this basis, this study sets out to investigate

the effects of juvenile hormone analogs on the protease activity in the silk glands of the silkworm (*Bombyx mori* L.) across spring and autumn seasons.

Results

Silk gland protease activity (μ M of tyrosine/mg of protein) during Spring

This study conducted experiments to detect protease activity in the silk glands of domestic silkworms (*Bombyx mori* L.) during the springs of 2021 and 2022. The unit for the tested indicator is μ M of tyrosine/mg of protein. Core results are presented upfront, while data from all other non-core treatment groups are categorized in Table-1. The highest activity, 65 μ moles was recorded in the treatment group where 0.1 μ l of the juvenile hormone analog Methoprene was injected at 48 hours into the fifth instar and this value was statistically significantly better than that of all tested groups. The second-highest activity, 63 μ moles was measured in the group injected with 0.1 μ l of Bukchiol at the same time point. The lowest activity, 46 μ moles was found in the treatment group injected with 1.0 μ l of Fenoxycarb at 96 hours into the fifth instar.

Table 1: Effect of juvenile hormone analogue application on silk gland protease activity (μ M of tyrosine/mg of protein) of silkworm, *Bombyx mori* L. during spring (data pooled over same seasons)

JH Time	J1: Methoprene					Sub Mean	J2: Fenoxycarb					Sub Mean	J3: Bukchiol					Sub Mean	Mean
	Co	C1	C2	C3	C4		Co	C1	C2	C3	C4		Co	C1	C2	C3	C4		
A1	46	48	53	55	54	51	46	48	55	56	55	52	46	48	60	62	59	55	52.67
A2	46	48	64	65	63	57	46	48	57	58	57	53.2	46	48	62	63	60	56	55.4
A3	46	48	61	62	60	55	46	48	58	58	56	53.2	46	48	59	59	57	53	53.73
A4	46	48	51	52	49	49	46	48	48	49	46	47.4	46	48	49	49	45	47.4	47.93
Mean	46	48	57	58	56	53	46	48	56	57	55	51.45	46	48	56	57	55	52.85	
Factor Mean	C0 :46						C1 : 48						C2 :56						
													C3 : 57						
													C4:55						

Juvenile Hormone (J): 0.081 C0: Normal A1: 24hrs

Timings (T): 0.094C1: Alcohol A2: 48hrs

C.D ($p \leq 0.05$) Concentration (C): 0.010 C2: 0.01 μ l A3 : 72hrs

J $\times$ C: 0.017 C3: 0.1 μ lA4: 96hrs

C $\times$ T: 0.02 C4: 1 μ l

J $\times$ C $\times$ T: 0.035

Silk gland protease activity (μ M of tyrosine/mg of protein) during Autumn

This study conducted physiological experiments on autumn-reared silkworms (*Bombyx mori* L.). We measured the protease activity of silkworm silk glands (unit: μ M tyrosine/mg protein) under treatments with different juvenile hormone analogs and used statistical analysis to sort out activity differences across groups. The group with the highest activity, the 5th instar 48h treatment group

administered 0.1 μ l methoprene recorded an activity level of 64 μ moles, which was statistically significantly superior to all other treatment groups. The second-highest activity, 57 μ M tyrosine/mg protein was observed in the 5th instar 48h treatment group that received 0.1 μ l bukchiol. The lowest activity, 46 μ moles, was found in the 5th instar 96h treatment group administered 1.0 μ l fenoxycarb. Data for all other treatment groups not listed individually are included in Table 2.

Table 2: Effect of juvenile hormone analogue application on silk gland protease activity (μ M of tyrosine/mg of protein) of silkworm, *Bombyx mori* L. during autumn season

JH Time	J1: Methoprene					Sub Mean	J2: Fenoxycarb					Sub Mean	J3: Bukchiol					Sub Mean	Mean
	Co	C1	C2	C3	C4		Co	C1	C2	C3	C4		Co	C1	C2	C3	C4		
A1	45.5	47	53	54	52	50.3	45.5	46.50	54	56	55	51.4	45.5	46	54	56	55	51.61	51.10
A2	45.5	48	63	64	63	56.8	45.5	47	56	57	56	52.31	45.5	47	56	58	56	52.64	53.91
A3	45.5	47	60	61	59	54.6	45.5	46	55	56	55.5	52.07	45.5	47	57	56	55	52.53	53.06
A4	45.5	47	50	52	50	49.09	45.5	46	47.90	49	46	46.88	45.5	46	47	49	46	47.06	47.67
Mean	45	47	56	57	56	52.69	45	46	53	54	53	50.66	45	46	54	55	53	50.96	
Factor Mean	C0 : 45.5						C1 : 46.91						C2 : 54.80						
													C3 :55.89						
													C4 : 52.09						

Juvenile Hormone (J): 0.012 C0: Normal A1: 24hrs

Timings (T): 0.014 C1: Alcohol A2: 48hrs

C.D ($p \leq 0.05$) Concentration (C): 0.014C2: 0.01 μ l A3: 72hrs

J $\times$ C: 0.022 C3: 0.1 μ l A4: 96hrs

C $\times$ T: 0.025 C4: 1 μ l

J $\times$ C $\times$ T: 0.030

Discussion

The juvenile hormone analogue methoprene (0.1µl) showed the highest silk gland protease activity of 65µmoles during the spring at 48 hours of the fifth instar, which was statistically higher than the other treatment combinations under investigation. At 96 hours of the fifth instar, the juvenile hormone analogue Fenoxycarb (1.0µl) showed the lowest value of silk gland protease activity, 46 µmoles. After 48 hours after the start of the fifth instar, Bukchiol, the second-best of the three juvenile hormone analogues, showed silk gland protease activity of 63µmoles when given at a concentration (0.1µl).

During autumn greatest silk gland protease activity of 64 µmoles was obtained in case of juvenile hormone analogue, Methoprene (0.1µl) at 48th hour of 5th instar which was statistically greater than rest of the treatment combinations under study. The least silk gland protease activity of 46 µmoles was found in case of juvenile hormone analogue, Fenoxycarb (1.0µl) at 96 hours of 5th instar. In terms of this metric, Bukchiol was determined to be the second-best juvenile hormone analogue with a silk gland protease activity of 57 µM of tyrosine per mg of protein when injected at a concentration of 0.1µl after 48 hours of initiation of 5th instar. The hormone analogue's function in maintaining active growth, postponing silk gland cell senescence and promoting enzymatic processes involved in silk protein metabolism may be the cause of the increased protease activity in the silk gland in the treatment receiving methoprene. Methoprene increases the activity of proteases in silkworm *Bombyx mori* L., indicating a general increase in oxidative metabolism, according to Mamatha *et al.* (2008) [2]. Therefore, it is warranted in the current investigation to stimulate the juvenile hormone analogue molecule (methoprene) on different traits of the silkworm *Bombyx mori* L. that contribute to high-quality silk yield.

Summary

The maximum silk gland protease activity was recorded at 65 µmoles in the spring and 64 µmoles in the fall when methoprene was applied at a rate of 0.1 µl per larva (in 10 µl solution) 48 hours after the fifth instar stage. This therapy had a robust and stable benefit over seasons, consistently outperforming all other combinations assessed.

Conclusion

Methoprene at 0.1 µl applied at 48 hours of the 5th instar is the most effective treatment for enhancing silk gland protease activity in *Bombyx mori*. Its consistent performance in both spring and autumn suggests that optimal timing and dosage of juvenile hormone analogues play a crucial role in improving enzymatic activity, which may ultimately contribute to better silk gland function and productivity.

Conflict of interest

The authors have no conflict of interest and Nothing to disclose

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