ISSN (Print) 0975-685X
ISSN (Online) 0975-864X

J. Appl. Biosci., 52(1): 66-70, June, 2026
doi no. 10.61081/joab/52v1i108

ORIGINAL RESEARCH

Seasonal Influence of Antibiotics and Botanicals on Development, Reproductive Performance and Cocoon Characteristics of B*ombyx mori* l.

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ABSTRACT

The influences of antibiotic supplementation and use of botanical disinfectants on development, reproductive potential and commercial characters of mulberry silkworm, *Bombyx mori* L have been evaluated. The study was carried out for during monsoon, winter and summer seasons over three consecutive years using bivoltine double hybrid race $CSR_2 \times CSR_4$. Treatments consisted of Azithromycin and Ofloxacinas as antibiotics, and botanicals such as neem, tulsi, karanj as disinfectants. Various parameters such as larval weight, mortality, cocoon formation, cocoon weight, shell ratio, denier, fecundity and egg hatching have been evaluated. The results showed significant changes in silkworm performance. Groups supplemented with antibiotics particularly Ofloxacin and Azithromycin, reduced the larval mortality and increased cocoon productivity, whereas treatments with neem and tulsi improved shell ratio and reproductive performance. The results obtained during winter, and summer supported growth and commercial traits as compared to monsoon season. The study suggests that antibiotic supplementation together with selected botanical treatments may improve the silkworm health and cocoon productivity influenced during different climatic conditions.

Keywords: Antibiotics, *Bombyx mori*, botanicals, cocoon, disinfectants, sericulture.

INTRODUCTION

Sericulture is an important agro-based industry; greatly contribute to rural employment through silk production in several Asian countries. The mulberry silkworm, *B. mori* L. is given a great deal of attention due to its excellent silk quality and great economic significance. The growth and productivity of silkworms depend greatly on the environmental factors, quality of silkworm nutrition through mulberry leaves and disease control measures. The silkworm larvae are very vulnerable to a range of bacterial, viral, fungal, and protozoan infections, due to fluctuation in the climatic conditions (Doreswamy *et al*., 2004). Seasonal changes exert considerable impact on survival of larvae, yield of cocoons, and reproductive capacity in different silkworm races. Because of this, disinfection and hygiene with other rearing practices are critical for achieving optimum cocoon production (Swathi *et al*., 2014).

Botanicals used as disinfectants like neem (*Azadirachta indica*), tulsi (*Ocimum* sp.) and karanj (*Pongamia pinnata*) are natural antimicrobial agents and have been studied as green alternatives in sericulture industry. The earlier reports suggested that when antibiotics applied to silkworms through mulberry leaves, the chances of diseases occurrence get decreased and the growth as well as the cocoon yield get enhanced (Baig *et al*., 1990). Similarly, Sheeba *et al.* (2008) observed, feeding antibiotics to larvae also improved the silkworm growth and silk production.

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The effect of disinfectants and antibiotics on silkworms has been evaluated even earlier, but the information on their long-term seasonal effect is very scarce, in Vidarbha's climate. The present work, therefore, was carried out to assess the effect of antibiotics and botanical disinfectants on growth, reproductive and commercial characters of $CSR_2 \times CSR_4$ hybrid race of silkworm, *B. mori* during three different seasons.

MATERIALS AND METHODS

Experimental Design

The present investigation was carried out at the Centre of Sericulture and Bioresource Management Research (CSBR), RTM Nagpur University, Nagpur, Maharashtra, India. The study was conducted for three consecutive years (2022-2024) during monsoon, winter and summer seasons using bivoltine double hybrid race $CSR_2 \times CSR_4$ of silkworm, *B. mori*. Disease free layings (DFLs) were procured from NSSO, Bengaluru and reared under standard rearing conditions.

Rearing Procedure

Silkworm larvae were reared on fresh mulberry leaves of Kanva-2/M5 variety. Newly moulted third instar larvae were separated into different treatment groups T1 to T6 Tcontaining 100 larvae in each treatment and all the treatments were done in triplicate. The rearing conditions have been maintained as described earlier.

The experimental groups included

Disinfectants:

- T1 – Control (RKO treatment): Application of Resham Keet Oushad.
- T2 – *Pongamia pinnata* (karanj): 5 g Karanj leaf powder + 95 g RKO powder.
- T3 – *Ocimum* sp. (tulsi): Dry powder in pure form.
- T4 – *Azadirachta indica* (neem): 10 g neem leaf powder + 90 g RKO powder.

Powders such as RKO, and mixed with other disinfectants prepared as mentioned have been used for dusting during dusting as described for rearing over the larvae during regular rearing and larval moulting at different larval stages.

Antibiotics supplementation:

- T5 - Azithromycin: 2.5 ml Azithromycin OSB diluted with 50mL Distilled water.
- T6 – Ofloxacin: 0.5 ml Ofloxacin OSB diluted with 50 ml Distilled water.

Antibiotic solutions of specified concentrations smeared on fresh mulberry leaves, air dried and fed to third instar larvae daily/alternate days till onset of spinning of cocoon.

The Experiment was repeated in all the three seasons over the three years, and during rearing the following parameters were recorded statistically analysed and compared:

Biological Parameters: Larval weight, larval mortality, Cocoon formation

Commercial Parameters: Cocoon weight, Shell weight, Shell ratio, Denier

Reproductive Parameters: Fecundity, Egg hatching percentage

RESULTS

GROWTH PERFORMANCE

Larval Weight

During monsoon the larval weight was highest with antibiotic, Ofloxacin (2.50 ± 0.02 g) followed by Azithromycin (2.45 ± 0.02 g), while larvae treated with botanicals had larval weight ranged between 2.25 - 2.40 g as compared to 2.10 ± 0.02 g in control (Table 1). In winter, the larval weight increased in all treated groups. In antibiotic treated groups, Ofloxacin led the highest increase to 2.70 ± 0.02 g, followed by Azithromycin 2.65 ± 0.02 g. In botanical treated groups the highest with Tp followed by Kp and Np compared to control group 2.30 ± 0.02 g. Similarly, in summer, the larval weight maximum was found in the Ofloxacin-treated larvae (2.60 ± 0.02 g), followed by the Azithromycin treatment (2.55 ± 0.02 g), and the similar trend observed with botanicals as observed in winter season (Table 1).

Larval Mortality

Larval mortality experienced a wide range alongside treatment and seasons recorded in (Table 1). During the monsoon, the highest larval mortality recorded in control group 15%, which reduced with botanicals between 8 and 12%, while antibiotic resulted the lowest 6 and 7 % with Azithromycin and Ofloxacin respectively (Table 1). A significant reduction in mortality was observed in all the groups during winter, lowest 4% with Azithromycin and Ofloxacin, and still higher 8% with neem powder compared to 7% in control. During summer mortality ranged from 5-6% in all the treated groups, whereas it was highest 9% in control (Table 1).

Cocoon Formation

The successful cocoon formation higher with antibiotics 92-94% followed by botanicals 88-91 and the lowest 84% in control during Monsoon (Table 1). During winter Azithromycin and Ofloxacin antibiotic treatments lead to slight increase in Cocoon formation to 95% and similar trend was noticed with botanicals and control as well (Table 1). The results suggest that Azithromycin and Ofloxacin, lowered

Table 1: Influence of botanicals and antibiotics on seasonal variations in biological traits in *B. mori* (2022-2024).

Seasons	Treatments		Larval weight (g)	Mortality (%)	Cocoon Formation (%)
Monsoon	**Control**		2.1 ± 0.02	15	84
	Botanicals	**Kp**	2.25 ± 0.02	08	91
		Tp	2.3 ± 0.02	11	88
		Np	2.22 ± 0.02	12	88
	Antibiotics	**Az**	2.45 ± 0.02	06	94
		Of	2.5 ± 0.02	07	92
Winter	**Control**		2.3 ± 0.02	07	92
	Botanicals	**Kp**	2.45 ± 0.02	06	94
		Tp	2.5 ± 0.02	07	92
		Np	2.4 ± 0.02	08	91
	Antibiotics	**Az**	2.65 ± 0.02	04	95
		Of	2.7 ± 0.02	04	95
Summer	**Control**		2.2 ± 0.02	09	91
	Botanicals	**Kp**	2.35 ± 0.02	07	92
		Tp	2.4 ± 0.02	06	93
		Np	2.32 ± 0.02	06	93
	Antibiotics	**Az**	2.55 ± 0.02	06	94
		Of	2.6 ± 0.02	05	94

Note: Kp- *P. pinnata*; **Tp**- *Ocimum sp*.;**Np**- *A. indica*; **Az**- Azithromycin; **Of**- Ofloxacin; ± Values represent pooled mean SE.

the larval mortality and increase cocoon formation across all seasons as compared to botanical treatments and the control group (Table 1).

ECONOMIC PERFORMANCE

Cocoon Weight

Cocoon weight recorded during monsoon was 1.450 ± 0.02g and 1.440 ± 0.01g with Azithromycin and Ofloxacin respectively. Among botanicals, neem powder produced the cocoon of higher weight 1.427 ± 0.01g being the highest among 1.337 ± 0.04 g in control (Table 2). During winter, Ofloxacin produced cocoon of 1.450 ± 0.02 g and Azithromycin 1.434 ± 0.03g while botanicals produced weight of 1.397 ± 0.03g, 1.334 ± 0.04g and 1.273 ± 0.03 g with Neem, Karanj and Tulsi powder respectively. The result obtained during summer was similar to the monsoon where antibiotic produced cocons of highest weight similar to Monsoon (Table 2).

Shell Weight

Shell weight was highest 0.293 ± 0.03g and 0.290 ± 0.01 with Ofloxacin and Azithromycin, respectively, larvae treated with Np and Tp was higher than the control but treatment of Kp produced shell of lowest weight. During the winter no significant variations observed among antibiotic and botanical treated larvae compared to control. The result of summer showed the nearly similar shell weight among the treated and control as observed during monsoon season (Table 2).

Shell Ratio

Shell ratio is a ratio between cocoon weight and empty shell (weight without pupae) weight, which depicts the ratio of the silk present in the cocoon. During mansoon shell ratio among all the treatments and in control was in the range of 20% plus and was slightly higher 21.3% with Tp. During winter the shell ratio was higher with Np as compared to other treated and control groups. During summer treatment with Tp produced significantly higher shell percent (22.18%), followed by Kp and Control, whereas antibiotics and Np produced 20% plus (Table 2).

Denier

During monsoon, the highest denier (thickness of the silk filament) was 2.77 produced with Np treatment, followed by Of and Az and the lowest was with Tp (2.39). treated larvae had the highest denier of 2.83 ± 0.11. Winter, prolong larval duration helped to consolidate the denier which was highest with antibiotics and Np treatments when compared with other treated larvae. While during summer only Ox produced filament of significantly high 2.7 fallowed by 2.57 with Kp and the rest produced below 2.35 denier (Table 2).

Table 2: Influence of botanicals and antibiotics on seasonal variations in commercial traits in *B. mori* (2022-2024).

Seasons	Treatments		Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Denier
Monsoon	Control		1.337 ± 0.04	0.272 ± 0.01	20.03 ± 0.42	2.54 ± 0.17
	Botanicals	Kp	1.294 ± 0.01	0.268 ± 0.00	20.70 ± 0.27	2.67 ± 0.15
		Tp	1.390 ± 0.04	0.273 ± 0.02	21.32 ± 0.63	2.39 ± 0.05
		Np	1.427 ± 0.01	0.287 ± 0.00	20.11 ± 0.27	2.77 ± 0.16
	Antibiotics	Az	1.450 ± 0.02	0.290 ± 0.03	20.00 ± 0.22	2.71 ± 0.33
		Of	1.440 ± 0.01	0.293 ± 0.01	20.34 ± 0.28	2.73 ± 0.03
Winter	Control		1.295 ± 0.04	0.274 ± 0.00	20.65 ± 0.14	2.39 ± 0.04
	Botanicals	Kp	1.334 ± 0.04	0.249 ± 0.01	20.80 ± 0.31	2.57 ± 0.05
		Tp	1.273 ± 0.03	0.263 ± 0.02	20.65 ± 0.36	2.58 ± 0.15
		Np	1.397 ± 0.03	0.253 ± 0.02	21.37 ± 0.12	2.72 ± 0.06
	Antibiotics	Az	1.434 ± 0.03	0.287 ± 0.02	20.01 ± 0.12	2.76 ± 0.08
		Of	1.450 ± 0.02	0.271 ± 0.02	20.74 ± 0.17	2.72 ± 0.07
Summer	Control		1.305 ± 0.04	0.244 ± 0.03	21.37 ± 0.31	2.38 ± 0.05
	Botanicals	Kp	1.313 ± 0.01	0.263 ± 0.01	21.09 ± 0.08	2.57 ± 0.21
		Tp	1.419 ± 0.03	0.285 ± 0.02	22.18 ± 0.19	2.33 ± 0.03
		Np	1.354 ± 0.02	0.271 ± 0.01	20.51 ± 0.35	2.35 ± 0.04
	Antibiotics	Az	1.480 ± 0.03	0.298 ± 0.01	20.76 ± 0.13	2.33 ± 0.11
		Of	1.453 ± 0.02	0.291 ± 0.01	20.02 ± 0.12	2.71 ± 0.17

Note: Kp- *P. pinnata*; **Tp**- *Ocimum sp*.;**Np**- *A. indica*; **Az**- Azithromycin; **Of**- Ofloxacin; ± Values represent pooled mean SE.

REPRODUCTIVE PERFORMANCE

Fecundity

In the monsoon season, the adult females of Ofloxacin-treated larvae laid significantly high number of eggs (589 ± 23) than control (494 ± 31), followed by Tp (582 ± 11), Azithromycin (579 ± 15). Eggs produced in others treated groups showed no significant difference as compared to control (Table 3). In the winter season, the adult female of larvae treated with Np produced highest number of eggs (604 ± 4), followed by Tp (600 ± 3) and Azithromycin (599 ± 5). During summer, the females of group treated with Azithromycin produced 586 ± 9 eggs, which was significantly higher than the other groups including control (522 ± 20 eggs) (Table 3).

Egg Hatching

Egg hatching ranged from 95 to 96% in all the treated groups and even in control except Az where hatching was 94% during monsoon. During winter the hatch9ng ranged from 94-96 as observed during monsoon however it was the lowest 91% in control. During summer, hatching was highest 98% with the antibiotics followed by treated groups and the lowest hatching observed in control (Table 3).

DISCUSSION

The present study revealed that, the seasonal change has a significant influence on the biological as well as the commercial performance of *B. mori*. It is quite probable that high larval mortality during the monsoon at least partly, results from the conditions of high humidity, which in turn make the larvae more vulnerable to microbial infections. Corresponding situation of seasonal disease prevalence in silkworm rearing has also been noted by Doreswamy *et al*. (2004).

Achieving antibiotic delivery by way of mulberry leaves has resulted in improved larval survival and cocoon productivity in this experiment. The studies by Baig *et al*. (1990) and Santha *et al*. (2007) confirmed the effectiveness of antibiotics in lowering the incidence of bacterial flacherie and thereby improving cocoon yield of *B. mori*. Supplementation of ampicillin was more effective than streptomycin which reduced the larval mortality and increase larval, cocoon and shell weight (Sridhar *et al.,* 2000). Tayade *et al*. (1988), suggested that antibiotic supplementation is a double-edged sword that not only helps in killing the microbes but also assists in larval growth and silk productivity by enabling better nutrient utilization.

Table 3: Influence of botanicals and antibiotics on seasonal variations in reproductive traits in *B. mori* (2022-2024).

Seasons	Treatments		Fecundity (No.)	Hatching (%)
Monsoon	Control		494 ± 31	95
	Botanicals	Kp	541 ± 29	97
		Tp	582 ± 11	95
		Np	540 ± 31	97
	Antibiotics	Az	579 ± 15	94
		Of	589 ± 23	96
Winter	Control		543 ± 24	91
	Botanicals	Kp	581 ± 7	94
		Tp	600 ± 3	96
		Np	604 ± 4	95
	Antibiotics	Az	599 ± 5	95
		Of	592 ± 6	96
Summer	Control		522 ± 20	94
	Botanicals	Kp	582 ± 9	97
		Tp	566 ± 11	97
		Np	560 ± 23	97
	Antibiotics	Az	586 ± 9	98
		Of	588 ± 2	98

Note: Kp- *P. pinnata*; **Tp**- *Ocimum sp*.;**Np**- *A. indica*; **Az**- Azithromycin; **Of**- Ofloxacin; ± Values represent pooled mean SE.

Botanical supplements like neem and tulsi resulted in better shell ratio and reproductive performance compared to other treatments. Dhirwani *et al*. (2015) also reported similar results where larval mortality was reduced and cocoon traits were improved after the use of plant-based disinfectants. One simple reason for the efficacy of neem and tulsi is their antimicrobial and physiological regulatory properties. The data help to understand seasonal consistency and long-term treatment responses in Vidarbha climate conditions more effectively. The current data propose that combining antibiotics and botanical disinfectants may be a very effective way to improve larval survival, cocoon production and reproductive efficiency in silkworm rearing.

CONCLUSION

In the present study it was found that adding antibiotics and using plant-based disinfectants have a major impact on the biological, reproductive, and commercial characters of the silkworm (*B. mori*) over different seasons. The groups that were given antibiotics showed the least larval mortality plus high cocoon production, whereas, neem and tulsi treatments were the best about shell ratio and reproductive performance.

The comparative study revealed that the silkworm's growth and characteristics of cocoons are impacted by the seasonal conditions mostly. Results also showed that antibiotic use and addition of certain botanicals may promote good health of silkworms, their survival and commercial cocoon production in sericulture under Vidarbha climatic conditions.

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