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Effect of UV Radiation on Physiological Performance of Silkworm *Bombyx mori*

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ABSTRACT: Silkworms (*Bombyx mori*) are poikilothermic organisms and are highly sensitive to environmental stressors, including ultraviolet (UV) radiation. This study investigates the impact of UV exposure on various morphological and economic traits of bivoltine silkworm breeds under different exposure durations (2, 5, and 10 minutes). To assess these effects, a two-way factorial design was implemented, considering both silkworm breed and UV exposure time as independent variables. The study examined key quantitative traits such as hatching percentage, cocoon weight, shell weight, pupation rate, and survivability. Statistical analysis using two-way ANOVA revealed highly significant effects (at the 0.1% level) of breed, UV exposure time, and their interactions on all physiological traits, highlighting breed-specific responses to UV-induced stress. Among the tested breeds, WB1HH exhibited relatively higher resilience, showing minimal reductions in performance and even improvements in larval weight and size. In contrast, CSR4 was the most susceptible, experiencing substantial declines in fecundity, survivability, and silk production traits. These findings emphasize the importance of breed selection and optimized rearing strategies to mitigate the negative effects of UV radiation on silkworm performance. The study provides valuable insights for improving silkworm management, particularly in regions with high UV exposure, contributing to sustainable sericulture practices.

Keywords: Silkworm (*Bombyx mori*), UV radiation, physiological traits, sericulture, mutation.

INTRODUCTION

Silkworms (*Bombyx mori*) are economically significant insects that play a crucial role in sericulture and silk production worldwide. India has strong sericulture potential but needs improved technology for high-quality silk production which offer better stability and survival under Indian conditions. However, challenges like climate, disease control, high equipment costs, labour shortages, and inefficient cocoon trading hinder production (Gautam *et al.*, 2022). The physiological performance of silkworms is influenced by various environmental factors, genetic traits, and stress conditions, which collectively affect their growth, survival, and silk yield. Among the factors, temperature is crucial for silkworm growth and cocoon quality thus maintaining optimal conditions enhance post cocoon parameters for superior silk production (Sudan *et al.*, 2023). Among environmental challenges, ultraviolet (UV) radiation is a key abiotic factor that has gained increasing attention due to its potential effects on living organisms, including silkworms. UV radiation emits three distinct wavelengths namely UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm) which possess unique mutagenic properties (Pfeifer, 2020). UV radiation can cause DNA damage either directly and indirectly. UVB and UVC create thymine dimer which leads to base pair deletion (Cadet and Douki 2018). The exposure of silkworms to UV radiation is becoming increasingly relevant due to changes in global environmental conditions, such as ozone layer depletion, which has led to heightened UV levels reaching the Earth's surface. UV radiation can negatively affect vital physiological traits of silkworms, such as hatching percentage, larval growth, silk gland development, and silk production Faruki and Kundu (2005). Hosagoudar and Manjunatha (2011) also explore the effects of picosecond UV laser exposure on the silkworm *Bombyx mori*, focusing on morphological, biochemical, and biological changes. While investigating the effect of UV-light exposure (20 W, 40 cm) in two *Bombyx mori* popular bivoltine hybrids, 30-minute exposure showed no significant effects while 60 and 120 minutes led to reduced silk gland weight and decreased levels of total protein, free amino acids, RNA, and carbohydrates, indicating impaired biosynthetic activity Bharathi and Yungen (2008). UV irradiation also led to decreased band densities in both spider dragline and silkworm silk, indicating protein degradation. However, the degradation rate of silkworm silk was 1.7 times higher than that of spider dragline, suggesting superior UV resistance in *N. clavata* silk

(Matsuhira *et al.,* 2013). When UV radiation (366 nm) was applied to 5-day-old *Bombyx mori* (Urboshi-1) pupae for 1, 5, and 10 minutes, it significantly affects 16 economic traits. Three lines (U1-DW, U1-LW, and U10-LD) were isolated in the R1 generation based on cocoon shape and size. These traits were retained in the R2 generation, with improved rearing performance over the control. The R3 generation evaluation indicated that U1-LW and U10-LD lines were commercially promising, with U10-LD also exhibiting the lowest larval mortality (Ali *et al.,* 2012). However, the degree of impact varies depending on the silkworm breed, as genetic diversity plays a critical role in determining stress tolerance. While certain breeds exhibit resistant to UV-induced stress, others may be more vulnerable, leading to significant reductions in silk yield and economic returns. There are several silkworm mutant breeds were developed in past such as Apodal (ap), this mutant has degraded thoracic legs Chen *et al.* (2016). Lemon (lem), during moulting this mutant express yellow body color Meng *et al.* (2009). Non-moulting dwarf (nm-d), this mutant has difficulty in purine synthesis Fujii *et al.* (2021).

Despite the importance of understanding UV stress on silkworm physiology, there is limited research on the combined effects of UV exposure and genetic variation among silkworm breeds. To address this gap, the present study investigates the effects of UV exposure on physiological traits across three silkworm breeds and aims to identify potential breed-specific adaptations and provide insights for improving silkworm rearing practices in UV-stressed environments. These findings will contribute to enhancing silk production efficiency and sustainability in the face of changing environmental conditions.

MATERIALS AND METHODS

Experimental Evaluation of UV Exposure on Physiological Traits of *Bombyx mori.* This experiment was conducted at the Central Sericultural Research and Training Institute (CSRTI), Berhampore, during June–July 2023, to assess the effects of UV-C radiation on the physiological traits of three bivoltine silkworm breeds (*Bombyx mori* L.). The study utilized disease-free layings (DFLs) of three parental strains, namely CSR4, SK7HH, and WB1HH, as experimental subjects.

Experimental Design & UV Irradiation Protocol. The experiment consisted of four treatment groups: 0 min (control), 2 min, 5 min, and 10 min of UV exposure, with each treatment replicated three times. A germicidal UV-C lamp (GE15T8, 254 nm) served as the irradiation source. The experimental setup ensured uniform exposure by placing the test larvae in 15 cm diameter Petri dishes, positioned 12 cm below the lamp under controlled conditions. A two-way factorial design was employed, considering Breed and UV exposure duration as the independent variables.

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Rearing & Environmental Conditions. Following irradiation, larvae were reared on fresh S1635 mulberry leaves following the standard silkworm rearing protocol (Krishnaswami, 1978). All rearing was conducted under regulated temperature and humidity conditions, ensuring optimal larval growth.

Treatment Application & Developmental Stage Consideration. To comprehensively evaluate the physiological response to UV-C stress, irradiation was applied to:

1. Newly hatched larvae (prior to first instar feeding).
2. Post-moulting larvae at each successive instar (I–V instars).

Control groups were maintained under identical conditions without UV exposure. This study aimed to elucidate the impact of UV radiation on silkworm physiology, contributing to an understanding of stress tolerance mechanisms in different bivoltine breeds.

Data collection. Total data on ten quantitative traits were recorded separately on replication wise for both control and each treatment batches. The traits such as hatching percentage, fecundity, larvae weight(g), larvae size(cm), effective rate of rearing by number (ERRN), single cocoon weight(g), single shell weight(g), shell percentage, pupation rate and survival percentage were recorded. Pupation rate (%) measured by the live pupa present inside the cocoon during metamorphosis of larva into pupa expressed as a percentage. The Cocoon weight (g) was average single cocoon weight in grams for 10 male and 10 female cocoons chosen randomly on the 6th or 7th day of spinning. Shell weight (g) calculated by average single cocoon shell weight in grams of 10 male and 10 female cocoons shell chosen randomly. The shells used were the same cocoons used for the cocoon weight determination. Shell ratio (%) indicate total quantity of silk available from a single cocoon was expressed as a percentage using the following equation. (Single cocoon shell weight (g)/single cocoon weight (g)) × 100.

For statistical analysis a two-way analysis of variance (ANOVA), box plot and heat map were performed using R-4.4.2 package to analyse and visualize the effects of breed and UV exposure time and their interaction on each physiological trait.

RESULTS AND DISCUSSION

Descriptive Statistics and Breed-Specific Trends

Descriptive statistics, including means, standard deviations (SD), and coefficient of variance (%CV), were calculated for each trait across different UV exposure times (Table 1). Earlier studies by Tazima (1978); Faruki (2004) reported that the highest mean hatching percentage was observed under 2 minutes of UV exposure (89.73 ± 3.65%), while the lowest was under 10 minutes (82.85 ± 12.12%). Similarly, survival percentage declined significantly from 89.67% (SD = 1.25) at 0 minutes (control) to 64.50% (SD = 17.74) at 10 minutes. Across all breeds, prolonged UV exposure led to a consistent reduction in SSW, SCW, ERRN, pupation rate, and shell percentage, with WB1HH exhibiting the least reduction, indicating superior stress tolerance. Future research should focus on elucidating the genetic and physiological mechanisms underlying UV tolerance in these breeds, as well as exploring potential interventions to mitigate UV-induced stress in silkworm populations (Lee & Park 2020). Apart from the comparative data between the breeds, a noticeable difference was also observed between the control and treated batches (Fig. 3).

Statistical Analysis. The results of the two-way ANOVA highlight the significant effects of Breed and UV Exposure Time (UET) on all physiological traits of silkworms in the Table 2. The interaction effects indicate that the impact of UV exposure varies significantly among breeds, demonstrating breed-specific resilience and vulnerability to UV stress indicating differences in genetic adaptation and tolerance to UV-induced stress (Singh *et al.*, 2020). Overall, UV exposure negatively affected all physiological traits, with the strongest reductions observed in fecundity, pupation rate, single cocoon weight (SCW), and survival percentage (Survival%). Significant differences among breeds were observed for all traits. These findings align with previous studies indicating that UV radiation can induce physiological stress, negatively affecting silkworm growth, development, and reproductive capacity (Kumar *et al.*, 2018).

The consistently high median and narrow interquartile range (IQR) observed in WB1HH for SCW, pupation rate, and larval weight suggest that this breed has greater adaptability to UV exposure and environmental stress. This makes WB1HH a potential candidate for breeding programs in stress-prone environments (Zhang *et al.*, 2021). The stability in WB1HH's physiological traits may be attributed to its genetic makeup, which potentially includes adaptive mechanisms that mitigate UV-induced damage.

In contrast, the lower median and variability for larval size, effective rate of rearing by number (ERRN), and fecundity in CSR4 indicate moderate sensitivity to UV exposure. The narrower IQR of CSR4 suggests a uniform response to stress, albeit at a slightly reduced success rate compared to WB1HH. This consistency may be beneficial in controlled breeding programs where predictability of traits is essential (Chen *et al.*, 2019).

The larger median and wider distribution of larval sizes and SCW in SK7HH suggest that this breed has greater growth potential under the experimental conditions. However, the high variability in these traits indicates that environmental or genetic factors may significantly influence individual outcomes. The presence of single outliers in SK7HH for larval weight, single shell weight (SSW), and SCW, and in CSR4 for larval size and fecundity highlights the variability in individual responses within the breed, possibly due to minor environmental inconsistencies or genetic variations that influence trait expression (Ghosh *et al.*, 2022). The observed breed-specific differences in UV stress response highlight the importance of targeted breeding strategies for improving silkworm resilience.

Effects of UV Exposure Time (UET). The independent factor UET significantly affected all traits. Notable effects were observed for:

- **SSW: F (3,24)** = 1155.1, $p < 0.001$
- **SCW: F (3,24)** = 8018.59, $p < 0.001$
- **Shell percentage**: F (3,24) = 139, $p < 0.001$
- **Survival rate:** F (3,24) = 4707.03, $p < 0.001$
- **Pupation rate:** F (3,24) = 3316, $p < 0.001$

As UV exposure increased, a progressive decline was observed in SSW, SCW, shell percentage, survival rate, and pupation rate. Notably, fecundity in the M1 generation was reduced by 80–85% compared to the M0 (parental stock), suggesting a strong UV-induced reproductive decline.

Additionally, significant interaction effects between UET and Breed were detected for all traits, indicating that the impact of UV exposure was breed-dependent. The most pronounced interactions were observed for SSW, SCW, pupation rate, fecundity, and mortality, where WB1HH exhibited greater UV tolerance, whereas larval size and larval weight showed minimal variation under short to prolonged UV exposure.

Box Plot Analysis of Breed-Specific Variation. Box plots were generated to visualize the distribution and variability of physiological traits across silkworm breeds (Fig. 1). Key observations include:

1. Hatching Percentage:

- CSR4: Median ~85%, IQR: 75%–90%; one outlier (50%) suggests high sensitivity.
- SK7HH: Median ~80%, IQR: 75%–85%; lower variability than CSR4.
- WB1HH: Median ~90%, IQR: 88%–92%; highest and most stable performance.

2. Larval Size and Weight:

- SK7HH exhibited the highest median larval size (5 cm), followed by WB1HH (4.75 cm) and CSR4 (4.5 cm).
- WB1HH had the highest median larval weight (22 g), indicating superior growth.

3. Effective Rate of Rearing by Number (ERRN):

- WB1HH exhibited the highest median ERRN (~7000), indicating better survival and development.
- CSR4 showed the lowest ERRN (~5500), with SK7HH falling in between (~6000).
- SK7HH displayed the largest IQR (4800–7500), indicating high variability.

4. SSW, SCW, Pupation Rate, and Survival%:

- WB1HH exhibited the highest median values, with a minimal IQR, indicating stable performance under UV stress.
- CSR4 had the lowest median values, with notable variability, indicating higher sensitivity.

5. Fecundity:

• SK7HH exhibited the highest median fecundity, whereas CSR4 showed the lowest, reflecting reproductive fitness differences across breeds.

Heatmap Analysis of UV-Induced Stress Responses. A heatmap (Fig. 2) was generated to visualize breed-specific and time-dependent variations in physiological traits under UV stress conditions. The colour intensity reflects the magnitude of change, revealing key trends. Traits such as fecundity, survival%, pupation rate, SSW, SCW, and larval weight showed consistent declines with prolonged UV exposure (2, 5, and 10 minutes). This suggests that prolonged UV irradiation imposes physiological stress, leading to impaired reproductive and developmental parameters. These findings are consistent with prior research demonstrating the detrimental effects of UV stress on silkworms, particularly in reducing survival and reproductive efficiency (Kotari *et al.*, 2002). Hatching percentage and larval size remained relatively stable, indicating possible UV resistance. Fecundity showed a drastic reduction in M1 (~85% in CSR4 and SK7HH, ~80% in WB1HH) compared to M0, consistent with earlier reports (Datta and Roy 1976; Sengupta *et al.*, 1977).

Breed-Specific Responses to UV Exposure. In the present investigation CSR4 showed the most pronounced sensitivity, particularly in SSW, fecundity, and pupation rate, with values decreasing significantly over time, indicating high vulnerability while SK7HH exhibited moderate adaptability, with hatching percentage, larval size, and fecundity showing slight reductions under prolonged exposure. Bivoltine breed WB1HH demonstrated the least variation across traits, particularly in ERRN, SSW, and hatching percentage, suggesting UV resistance with potential implications for ecological adaptability.

UV Exposure Duration and Physiological Adjustment. As observed by Karthi *et al.* (2014), Short-term UV exposure (2 min) resulted in subtle changes in SCW, survival%, pupation rate, and ERRN, indicating an initial physiological adjustment phase. Prolonged exposure (5–10 min) led to significant reductions in fecundity, pupation rate, survival%, and SSW, particularly in CSR4 and SK7HH, demonstrating a cumulative stress effect. The present findings suggest that silkworm breed significantly influenced all measured traits, consistent with earlier reports by Hasan *et al.* (1998); Singh *et al.* (1990). Among the breeds, WB1HH exhibited superior performance in single shell weight (SSW), single cocoon weight (SCW), and fecundity, while SK7HH demonstrated higher survival and a greater effective rate of rearing by number (ERRN), indicating lower mortality.

Table 1: Descriptive statistics of traits under different UV exposure time.

BREED	UET	HA%	FEC	LW	LS	SCW	SSW	SR	PR	ERRN	SV%
WB1HH	0	92.46	412	23.45	4.66	1.685	0.255	19.18	89.89	9100	91
SK7HH	0	88.96	397	21.95	5.06	1.498	0.205	13.68	97.78	9000	90
CSR4	0	85.24	401	18.71	4.56	1.488	0.289	19.42	86.11	8800	88
	Mean	88.89	403	21.37	4.76	1.56	0.25	17.43	91.26	8966.67	89.67
	SD	2.95	6.34	1.98	0.22	0.09	0.03	2.65	4.86	124.72	1.25
	CV%	3.32	1.57	9.26	4.54	5.82	13.82	15.21	5.33	1.39	1.39
WB1HH	2	90.09	299	22.83	5	1.377	0.251	18.2	89.89	7600	76
SK7HH	2	85.09	300	21.38	5.13	1.161	0.218	18.78	84.29	7000	70
CSR4	2	94.01	144	18.49	4.5	1.18	0.197	16.69	75.41	6100	61
	Mean	89.73	247.67	20.90	4.88	1.24	0.22	17.89	83.20	6900.00	69.00
	SD	3.65	73.30	1.80	0.27	0.10	0.02	0.88	5.96	616.44	6.16
	CV%	4.07	29.60	8.63	5.57	7.88	10.01	4.92	7.17	8.93	8.93
WB1HH	5	81.14	194	21.47	4.46	1.256	0.226	17.99	74.24	6600	66
SK7HH	5	76.29	252	21.36	4.5	1.181	0.203	17.19	59.18	4900	49
CSR4	5	88.24	102	17.09	4.4	1.125	0.164	14.58	44	5000	50
	Mean	81.89	182.67	19.97	4.45	1.19	0.20	16.59	59.14	5500.00	55.00
	SD	4.91	61.76	2.04	0.04	0.05	0.03	1.46	12.35	778.89	7.79
	CV%	5.99	33.81	10.21	0.92	4.52	12.95	8.78	20.87	14.16	14.16
WB1HH	10	87.93	74	22.08	4.36	1.249	0.206	16.49	60.42	4800	48
SK7HH	10	78.26	59	20.2	4.4	1.012	0.169	16.7	46.34	4100	41
CSR4	10	46.49	55	14.57	4.13	1.012	0.16	15.81	43.18	4400	44
	Mean	82.85	224.08	20.30	4.60	1.27	0.21	17.06	70.89	6450.00	64.50
	SD	12.12	132.05	2.50	0.30	0.20	0.04	1.71	18.77	1774.12	17.74
	CV%	14.62	58.93	12.30	6.45	15.53	17.65	10.00	26.48	27.51	27.51

N.B. UET: UV Exposure Time, HA%: Hatching%, FEC: Fecundity, SCW: Single Cocoon Weight (g), SSW: Single Shell Weight (g), SR: Shell Percentage, PR: Pupation Percentage, ERRN: Effective Rate of Rearing by Number, SV%: Survival Percentage.

Table 2: Two-way Analysis of variance of ten physiological traits.

Traits	Source	DF	MS	F-value	P-value
Hatching%	Breed	2	260.5	5396	< 2e-16 ***
	UET	3	676.3	14010	< 2e-16 ***
	Breed: UET	6	449.7	9317	< 2e-16 ***
	Residuals	24			
Fecundity	Breed	2	20181	3243	< 2e-16 ***
	UET	3	180421	28996	< 2e-16 ***
	Breed: UET	6	7039	1131	< 2e-16 ***
	Residuals	24	6		
Larvae wt. (g)	Breed	2	87.68	10027.5	< 2e-16 ***
	UET	3	9.79	1119.9	< 2e-16 ***
	Breed: UET	6	2.35	268.6	< 2e-16 ***
	Residuals	24	0.01		
Larvae Size(cm)	Breed	2	0.4076	806.1	< 2e-16 ***
	UET	3	0.6655	1316.3	< 2e-16 ***
	Breed: UET	6	0.0669	132.4	< 2e-16 ***
	Residuals	24	0.0005		
SCW(g)	Breed	2	0.1272	2885.14	< 2e-16 ***
	UET	3	0.3535	8018.59	< 2e-16 ***
	Breed: UET	6	0.0042	94.94	1.61e-15 ***
	Residuals	24	0.0000		
SSW(g)	Breed	2	0.004824	691.9	< 2e-16 ***
	UET	3	0.008054	1155.1	< 2e-16 ***
	Breed: UET	6	0.002396	343.7	< 2e-16 ***
	Residuals	24	0.000007		
SR	Breed	2	8.077	267.2	< 2e-16 ***
	UET	3	4.201	139.0	2.66e-15 ***
	Breed: UET	6	11.505	380.6	< 2e-16 ***
	Residuals	24	0.030		
PR	Breed	2	836	8093	< 2e-16 ***
	UET	3	3425	33160	< 2e-16 ***
	Breed: UET	6	139	1342	< 2e-16 ***
	Residuals	24	0		
ERRN	Breed	2	3283890	117.48	4.01e-13 ***
	UET	3	35881505	1283.67	< 2e-16 ***
	Breed: UET	6	676687	24.21	4.54e-09 ***
	Residuals	24	27952		
SV%	Breed	2	330	457.19	< 2e-16 ***
	UET	3	3400	4707.03	< 2e-16 ***
	Breed: UET	6	61	84.83	5.81e-15 ***
	Residuals	24	1		

N.B. *** 0.1% level of significance, DF: Degrees of Freedom, MS: Mean Sum of Square, UET: UV Exposure Time, SCW: Single Cocoon Weight (g), SSW: Single Shell Weight (g), SR: Shell Percentage, PR: Pupation Percentage, ERRN: Effective Rate of Rearing by Number, SV%: Survival Percentage.

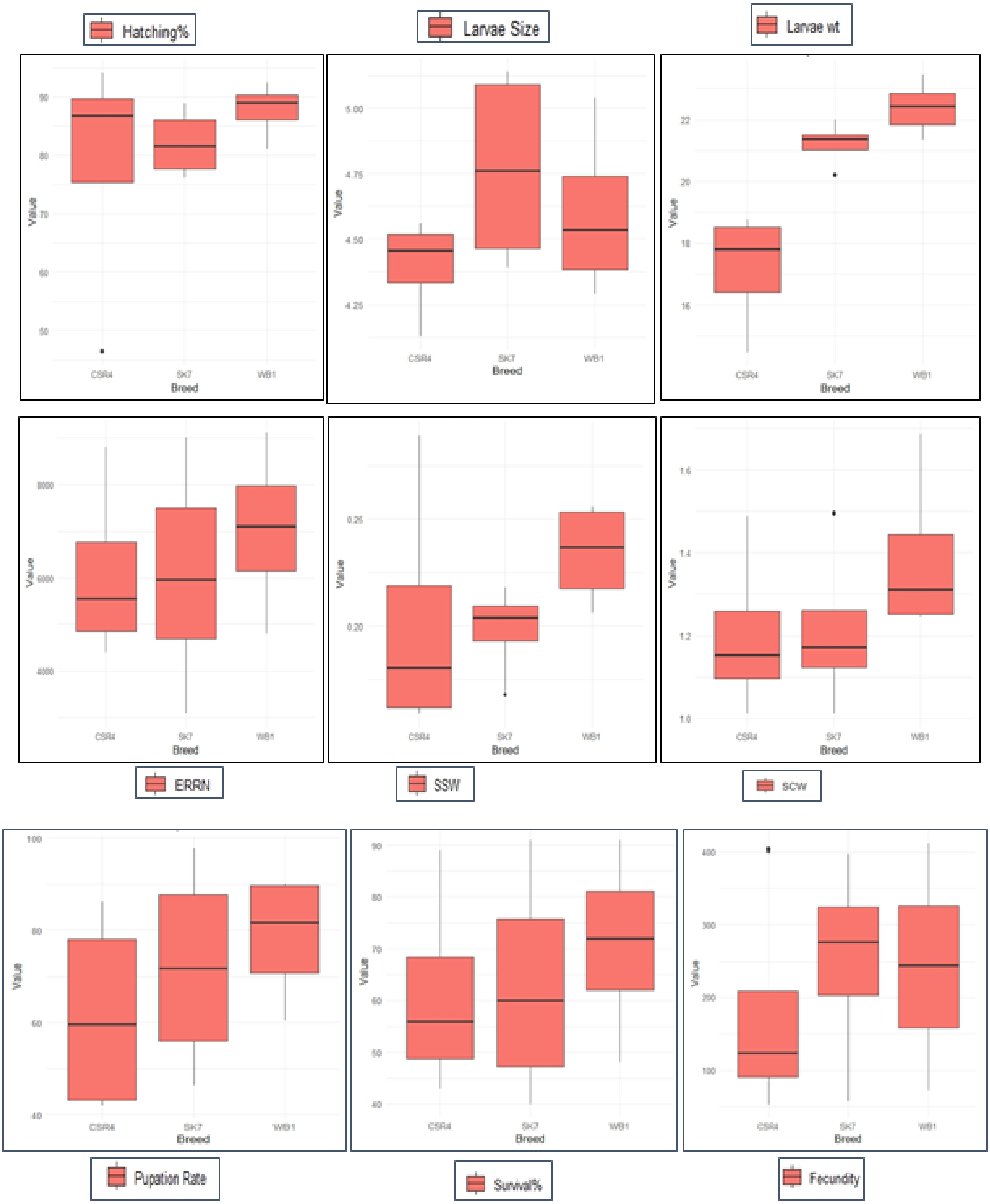

Fig. 1. Box plot showing distribution of physiological traits across breed.

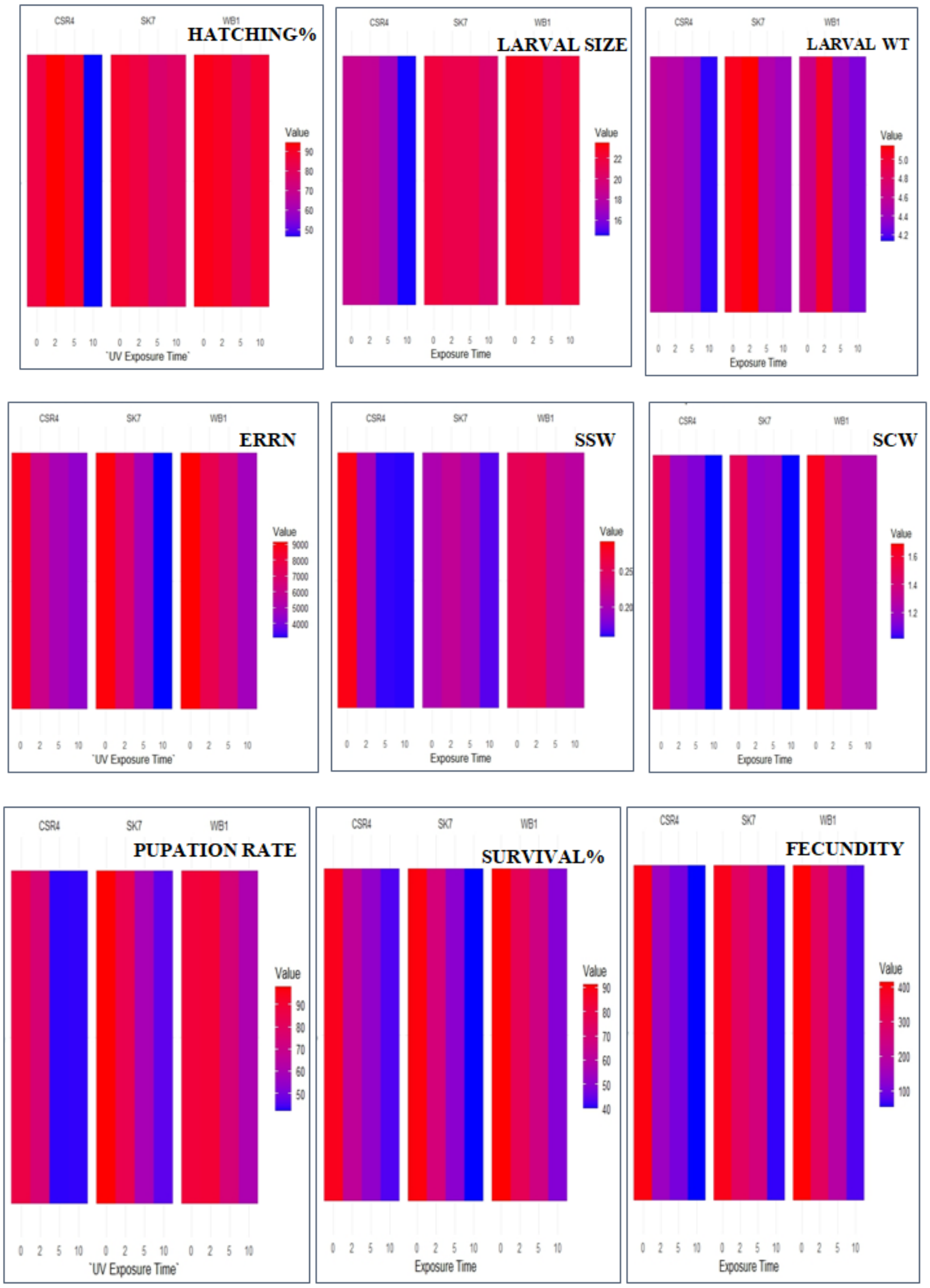

Fig. 2. Heatmap of UV exposure time on physiological traits across silkworm breed.

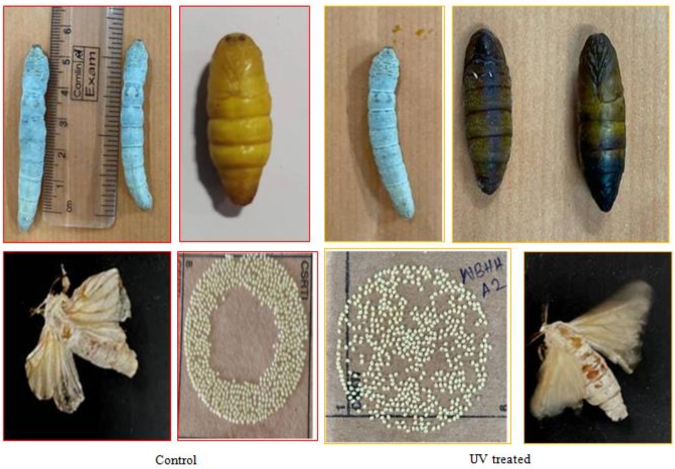

Fig. 3. Comparison of physiological traits between control and UV treated.

CONCLUSIONS

This study highlights the significant impact of UV exposure on silkworm physiological traits, with variations observed across different breeds. Prolonged UV exposure resulted in reductions in hatching percentage, larval weight, silk cocoon weight, fecundity, and survival percentage, underscoring its detrimental effects on silkworm productivity. Among the tested breeds, WB1HH exhibited greater tolerance to UV stress, while CSR4 and SK7HH were more adversely affected. These findings emphasize the importance of selecting UV-resistant breeds and implementing protective measures during silkworm rearing to mitigate the adverse effects of UV radiation. Such strategies will be crucial for ensuring sustainable silk production in changing environmental conditions.

FUTURE SCOPE

With the changing environmental condition, s**i**lkworm breeds which can resist the present environmental factors like high temperature, humidity including Ultraviolet radiation can uplift the sericultural activities benefitting the sio economics factors of the sericulture farmers.

Author contributions: Dr. Raviraj VS and Ms. Y Surjalata Devi conceived and designed the analysis; Y Surjalata Devi and V. Lalremruatpuia performed the experimental rearing and collected the initial data; Ms. Y Surjalata Devi and Mr. Anowar Hossain wrote the first draft of the manuscript; Dr. Raviraj VS and Anowar Hossain edited the draft and performed statistical analysis. All authors read and approved the final manuscript.

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Conflict of Interest. None.

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