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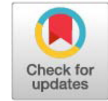
Effects of Antibiotic Treated Mulberry Leaves Feeding on Larval Growth, Cocoon Production, and Gut Bacteria of Silkworm



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Authors' Contribution
AM and MH conceived the idea, designed, and organized project. MH supervised the work, analyzed data and revised the manuscript. AN, GD and SI helped in data analysis and write up. MH, KA, SB and SHS helped in data analysis and manuscript revisions.

Key words
Bacterial isolation, Levofloxacin, Susceptibility, *Bombyx mori*, Sericulture

ABSTRACT

The effect of different concentrations of Levofloxacin and Cefixime on bacterial profile, larval growth, and cocoon production of silkworms was evaluated by rearing under controlled conditions of temperature, relative humidity, and photoperiod (25 ± 1 °C, 75 ± 5 % and 16:08 h of light to darkness ratio, respectively). The larvae were fed mulberry leaves treated with two antibiotics i.e., Levofloxacin and Cefixime each with three concentrations (10, 20, and 30 ppm) during 3rd, 4th and 5th larval instars. The experiment was laid out in the Completely Randomized Design (CRD) and replicated thrice. The significant variations in larval length (cm) and larval body weight in 3rd, 4th and 5th instar larvae were recorded ($p < 0.05$). The antibiotic supplementation also affected cocoon weight and cocoon shell weight positively. Maximum larval body length and body weight were observed in both antibiotics at 20 ppm concentrations. Four surface bacterial strains (*Bacillus cereus*, *Bacillus* sp., *Bacillus pumilus* and *Actinobacterium* sp.) and three gut bacterial strains (*Bacillus licheniformis*, *Actinobacterium* sp., and *Bacillus pumilus*) associated with silkworm were isolated. These strains were identified based on 16S rRNA gene sequencing and were highly susceptible to Levofloxacin but showed resistance to Cefixime. The results demonstrated that both Levofloxacin and Cefixime enhanced silkworm growth, commercial traits and make them less susceptible to diseases when larvae were fed leaves treated with 20 ppm concentration.

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INTRODUCTION

The sericulture industry has benefitted mankind for centuries by providing natural animal silk (Holland *et al*., 2019; Karthik *et al*., 2015) of great quality like lightweight, natural sheen, an inherent affinity for dyes, drape, resilience, high absorbance, and vibrant color (Altman *et al*., 2003; Reddy, 2009; Karthik *et al*., 2015; Babu, 2018). This industry has great importance as an allied sector of agriculture (Neelaboina *et al*., 2018) and contributes to the rural economy in addition to its associated role in the ecosystem (Zhou *et al*., 2003; Neelaboina *et al*., 2018; Manjunath *et al*., 2020).

Continuous domestication of silkworm results in inbreeding depression that drastically lowers immunity and adds in the susceptibility to diseases (Adel *et al*., 2015). Silkworm disease prevalence results from several biological (nutritional, biological) or environmental factors (temperature and humidity) (Sakthivel *et al*., 2012). Commercial and biological features silkworm are prone to natural degradation (Adel *et al*., 2015). Continuous improvement in rearing technology and disease management is requisite for successful crop harvesting on sustainable basis. However, these two parameters have been remained an issued for making sericulture as an emerging industry despite its global importance for its silk production (Shah *et al*., 2007; Wurm, 2003; Hakimi *et al*., 2007).

During last decade, bacterial flacherie has become a major threat to silkworm larvae during rearing (Stockland, 2017; Gani *et al*., 2017; Chairman *et al*., 2012; Saad *et al*., 2019; Gore *et al*., 2014), contributing about 70% loss to sericulture annually (Manimegalai, 2003). Bacterial flacherie incur an estimated loss of 30-40% to cocoon crops (Anantha, 2011). In another study, the most prevalent silkworm disease was the flacherie (47.9 %) followed by grasserie (42.6 %), and then kenchu (9.5 %) (Manimegalai, 2003).

Sericulturists suggest management of bacterial flacherie is key to successful silk crop production

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(Yuan *et al*., 2006; Gani *et al*., 2017). Bacterial disease management in silkworms has been achieved by exposing larvae to different antibiotics with encouraging results (Hamamoto *et al*., 2004a; Javaid *et al*., 2021; Rafiq *et al*., 2021a). Antibiotics are used in the sericulture industry as an element of bed sterilizers and therapeutic impact in bacterial diseases management (Venkatesh and Srivastava, 2010; Srivastava and Kumar, 2009; Mahmoud and Taha, 2012; Subramanian *et al*., 2010). Such antibiotics include penicillin, ampicillin, tetracycline, erythromycin, and chloramphenicol that showed promising results in bacterial disease management of silkworm larvae Subramanian *et al*., 2010; Venkatesh and Srivastava, 2010; Ashok and Ramakrishna, 2014; Prakash and Puttaraju, 2006; Kaito *et al*., 2011; Kaito and Sekimizu, 2007; Meng *et al*., 2017). Silkworm being domesticated has specific bacterial microbiota associated with their gut which has not been evaluated for the impact of antibiotics in Pakistan. This study highlighted the effect of antibiotic-treated mulberry leaves on bacteria associated with silkworm larval gut. Additionally, the larval growth, and cocoon production was also assessed.

MATERIALS AND METHODS

Stock culture preparation

The disease-free silkworm eggs were obtained from the Sericulture Research Laboratory, Lahore. All equipment, rearing room and food were cleaned before shifting the larvae, the rearing room was disinfected with a 4% formalin solution and was kept airtight for 24 h (Ito, 1969; Krishnaswami, 1978). The eggs were incubated in hatching plates at 25 ± 1 °C temperature and 75 ± 5% relative humidity (Hussain *et al*., 2011). Temperature and humidity were monitored by thermo- hygrometer (HM 16) to monitor.

Larval rearing

Larval rearing was carried out at temperature of 25 ± 1 °C, 75 ± 5% relative humidity (RH), and photoperiod of *12* h of light:*12* h of dark (Rahmathulla, 2012). Three different concentrations (10, 20, 30 ppm) of two analytical grade antibiotics were sprayed on fresh mulberry leaves which were then chopped and fed to the larvae. The feeding schedule for larval rearing comprised of 3 times a day during 1st two instars and 04 times a day during 3rd, 4th and 5th instar (Hussain *et al*., 2011). Antibiotics used in this experiment were Cefixime ($C_{16}H_{15}N_5O_7S_2$) and Levofloxacin ($C_{18}H_{20}FN_3O_4$).

Experimental design

In the present work, the effect of three different concentrations (10, 20, 30 ppm) of Levafloxacian and Cefixime treated mulberry leaves were studied on larval growth, cocoon production, and gut bacteria associated with silkworms. The research work was carried out in Complete Randomized Design (CRD) and replicated thrice. At the beginning of the 3rd instar, the larvae were divided into 21 groups, each replicate containing 30 larvae. For bacterial isolation, 4th and 5th instar silkworm larvae were randomly sampled from each replicate and were stored in disinfected airtight sealed vials at -18°C in the refrigerator.

Data on larval growth and cocoons

At the end of 3rd, 4th and 5th instar, larval weight (g) and larval length (cm) were measured by using electronic balance, and measuring tape, respectively. Larval weight (g) was measured by taking mean values of randomly selected five larvae from each replication of all treatment groups. Larval length (cm) was taken as mean of randomly selected five larvae (Zulfiqar *et al*., 2022).

Cocoon weight (g) was recorded on 7th day of spinning and shell weight (g) was calculated by removing pupae from the same cocoons which were used to calculate cocoon weight and shell weight by following formulae:

$$\text{Cocoon weight (g)} = \frac{\text{weight (g) of 5 female cocoons} + \text{weight (g) of 5 male cocoons}}{10}$$

$$\text{Cocoon shell weight (g)} = \text{Cocoon weight} - \text{pupal weight}$$

Isolation of bacteria

Bacteria associated with silkworm surface and gut were isolated as follows:

To obtain bacterial samples from the surface of silkworm larvae, the refrigerated larvae were sterilized by dipping in 2% sodium hypochlorite (5% of reagent grade) for three to five minutes, then washed with autoclaved distilled water (Ayoade *et al*., 2014; Adel *et al*., 2015). The suspension was then serially diluted up to six times (Adel *et al*., 2015). Then 0.1 mL suspension was inoculated with the help of a micropipette onto a nutrient agar plate and incubated for 24 h at 37°C (Ayoade *et al*., 2014). Distinct colonies (based on color and shape) were then isolated and streaked for further analysis.

For the isolation of silkworm gut bacteria, dissection of previously surface-sterilized silkworm larvae was carried out. Surface sterilized larvae were placed on a dissection tray which were sterilized with 95% ethanol (Ayoade *et al*., 2014). The larvae were fixed with fine pins in a dorsoventral position on a dissection board. An incision was made carefully with a pair of sterilized blades and scissors through the dorsal midline from posterior to anterior end and the digestive tract was extracted. The

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extracted gut was then ground and deposited in 200 mL saline solution in a sterilized Eppendorf tube (Ayoade *et al*., 2014). The suspension was then serially diluted up to 6 times. Then 0.1 mL suspension was inoculated with the help of a micropipette onto a nutrient agar plate and incubated for 24 h at 37°C (Ayoade *et al*., 2014). Distinct colonies (based on color and shape) were then isolated and streaked for further analysis.

Antimicrobial susceptibility

Antibiotic sensitivity was measured by using analytical grade antibiotic susceptibility discs. Nutrient agar plates were prepared and 50µl of nutrient broth containing bacterial isolates was spread on them separately. Two different antibiotic disks levofloxacin and cefixime were placed with the help of sterilized forceps on the surface of inoculated plates. Plates were then incubated for 24 h at 37°C (Bauer *et al*., 1966). The appearance of the transparent area around the antibiotic disk was taken as an indicator of sensitivity while the growth of colonies around the discs showed the resistance of isolates. Diameter of zone of bacterial growth inhibition was measured in millimeters (mm) with the help of a scale (Kabir *et al*., 2013; Aditi *et al*., 2017).

PCR amplification of 16S rRNA sequence

The genomic DNA of bacterial strains was extracted using a Dream Taq™ Green PCR Master Mix which was stored at -18°C until used. Universal primers were employed for the molecular characterization of bacterial strains. The forward primer was 16F (5'-GAGTTTGATCCTGGCTCAG-3') and the reverse primer was 1510R (5' –GGCTACCTTGTTACGA- 3') (Satokari *et al*., 2001; Leitch *et al*., 2007; Carroll *et al*., 1999). PCR amplification was performed in a total volume of 25µl containing 12.5µl of Master Mix, 1µl of Reverse Primer, 1µl of forwarding Primer, 5.5µl of dd H_2O, and 5µl of DNA template. PCR was performed in a thermo-cycler with successfully optimized PCR conditions. The thermal cycle comprised of incubation at 95 °C for 5 min followed by 35 cycles, each of denaturation at 95 °C for 45 sec, annealing at 50 °C for 30 sec and extension at 72 °C for 1 min. Final extension was done at 72 °C for 5 min.

The amplified products (~1,500 bp) were subjected to 2% agarose gel electrophoresis and sequenced directly in the Center of Excellence In Molecular Biology (CEMB), Lahore. The similarity of the sequences was compared using the NCBI BLAST database (http://www.ncbi.nlm.nih.gov/Blast.cgi). CHROMAS 2 was used for the editing of FASTA files while CLUSTALW was used for sequence alignment. Mega X software (Hall, 2005) was used to prepare phylogenetic trees for each strain. Phylogenetic trees were constructed using the Neighbor-joining tree method based on the Tamura-Nei model (Tamura and Nei, 1993). Furthermore, antibiotic sensitivity was also measured by using analytical-grade antibiotic susceptibility discs.

Data collection and analysis

To determine significance, the data were subjected to ANOVA. While the treatment means were compared by Tukey's post hoc test.

RESULTS

Larval length (cm)

ANOVA results of larval length during the rearing of larvae from 3rd to 5th instar at the end of each instar showed significant differences ($p < 0.05$). Maximum larval length was recorded in $T_{(Lev\text{-}20ppm)}$ (3.37cm) during 3rd instar whereas lowest larval length was observed in $T_{(control)}$ (2.53cm) (Fig. 1A). The larval lengths recorded at the end of the 4th instar showed significant differences between means obtained from larvae reared under different treatment groups ($p < 0.05$). Mean maximum larval length was found $T_{5(Lev\text{-}20ppm)}$ (3.9cm) whereas minimum length in $T_{0(control)}$ (3.47cm) (Fig. 1B). Body length in 5th instar larvae demonstrated significant differences in means between treatment groups. Mean larval length was observed maximum in $T_{5(Lev\text{-}20ppm)}$ (7.08cm) and lowest in $T_{0(control)}$ (6.37cm) (Fig. 1C).

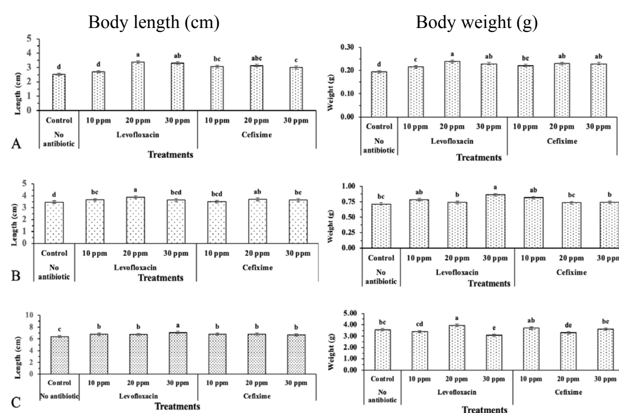

Fig. 1. Body length (cm) and body weight (g) of 3rd (A), 4th (B) and 5th (C) Wister larvae of silkworm.

Larval weight (g)

Mean larval weight recorded at the end of 3rd, 4th, and 5th instar larvae showed significant differences between treatment groups ($p < 0.05$). The larval weight recorded at the end of 3rd instar showed maximum larval weight (0.239g) in $T_{(Ce\text{-}30ppm)}$ whereas minimum larval weight

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(0.194g) was observed in $T_{(control)}$ (Fig. 1A).

The larval weight recorded at the end of 4th instar showed significant differences in mean larval weight between treatments ($p < 0.05$). Maximum larval weight (0.865g) was observed in $T_{5(Ce\text{-}20ppm)}$, and minimum larval weight (0.715g) was recorded in $T_{0(control)}$ (Fig. 1B). The larval weight (g) recorded at the end of 5th instar showed significant differences in means ($p < 0.05$). Maximum larval wight (3.90g) was recorded in $T_{5(Lev\text{-}20ppm)}$ followed by $T_{2(Ce\text{-}20ppm)}$ (3.710g). While minimum larval weight (3.47g) was recorded in $T_{0(control)}$ (Fig. 1C).

Cocoon weight (g)

Cocoon weight (g) showed significant difference in mean values among different treatment groups ($p < 0.05$). Maximum weight was observed in $T_{5(Ce\text{-}20ppm)}$ followed by $T_{2(Lev\text{-}20ppm)}$ (Fig. 2A).

Shell weight (g)

Shell weight (g) recorded at the end of spinning after 5th instar demonstrated significant variations in means in different treatment groups ($p < 0.05$) (Fig. 2B).

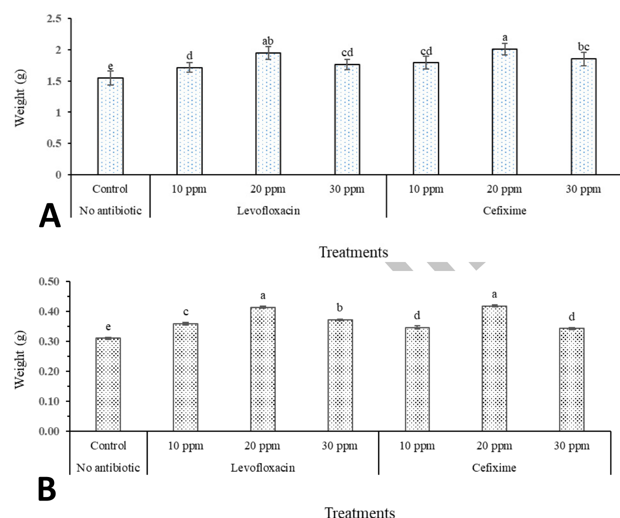

Fig. 2. Effect of different concentrations of treatment groups on cocoon weight (A) and shell weight (B) of silkworm larvae.

Isolation of bacteria

A total of seven bacterial strains were isolated from silkworm out of which four were from the body surface, and three were isolated from the gut of 4th and 5th instar silkworm larvae. These isolates were classified based on color, colony shape, and morphological properties (Table I).

Table I. Morphological characterization of bacterial isolates from *B. mori* (in the column two under the title strains, the codes represent: The first letter shows that whether the strain was isolated from the gut (G) or surface (S), while the next two characters symbolize the isolate's color and shape).

S. No.	Strains	Colony, morphology and color
1.	SYR	Surface yellow round
2.	SGA	Surface green amorphous
3.	SYCR	Surface yellowish creamy round
4.	SWAE	Surface white amorphous elevated
5.	GRW	Gut round white
6.	GPW	Gut pure white
7.	GWSP	Gut white spreading pure

Table II. Isolation of bacterial strains from silkworm in different antibiotic concentrations of Levofloxacin and cefixime. (note: "+" indicate presence of bacterial strains and "-".

Bacterial strains	Cefixime (Ce)			Levofloxacin (Lev)			Control
	10 ppm	20 ppm	30 ppm	10 ppm	20 ppm	30 ppm	
SYR	+	−	+	-	-	-	+
SGA	-	-	-	+	-	-	+
SYCR	-	-	-	+	+	-	+
SWAE	+	-	-	-	-	-	+
GRW	-	-	-	-	+	-	+
GPW	-	-	+	-	-	-	+
GWSP	-	+	-	-	-	-	+

Moreover, each antibiotic concentration has a different effect on bacterial strains (Table II). For this reason, the presence of each bacterial strain in different antibiotic concentrations was also recorded to measure the effect of each concentration on bacteria (Table II).

Antibiotic susceptibility measurement

Antibiotic sensitivity was measured by using analytical-grade antibiotic susceptibility discs. The diameter of the zone of bacterial growth inhibition was measured in millimeters (mm) with the help of a scale. Antibiotic susceptibility measurement demonstrated that bacterial strains were resistant to cefixime but sensitive to levofloxacin. The multiple antibiotic resistance (MAR) index value was greater than 0.2 that indicated a high-risk source of contamination (Table III).

Table III. Antibiotics zone diameter (mm) and MAR index.

S. No.	Strains	Cefixime Ce (5μg)	Levofloxacin Lev (5μg)	MAR index
1	SYR3	R	16.50	0.5
2	SGA11	R	13.75	0.5
3	SYCR12	R	15.00	0.5
4	SWAE15	R	15.25	0.5
5	GRW	R	16.00	0.5
6	GPW	R	17.50	0.5
7	GWSP	R	15.00	0.5

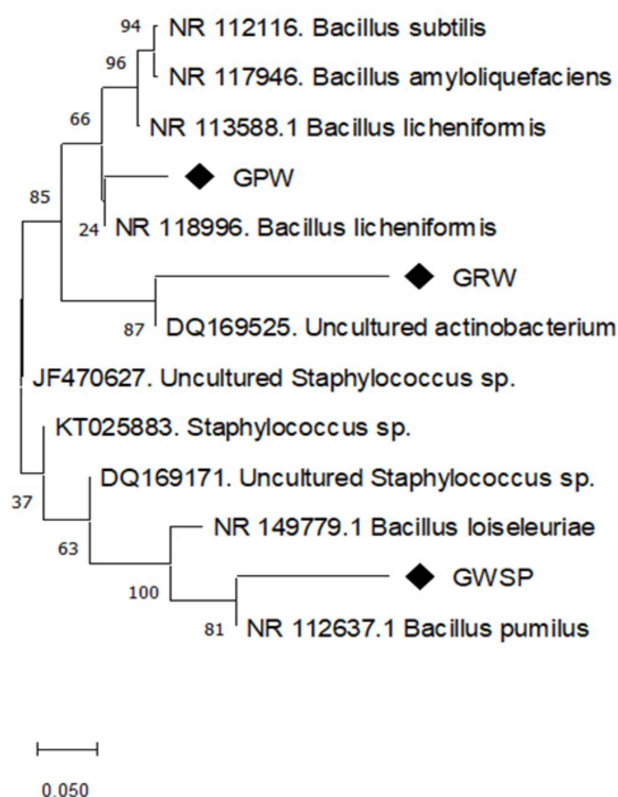

Fig. 3. Phylogram of gut bacterial strains.

Phylogenetic analysis

The phylogenetic tree and 16S rRNA gene sequence analysis showed that GPW, GRW, GWSP, SYR, SWCR, SGA, and SYCR had sequence homology with *Bacillus licheniformis* (24%), *Actinobacterium* sp. (87%), *Bacillus pumilus* (81%), *Bacillus cereus* (100%), *Bacillus* sp. (99%), *Bacillus pumilus* (46%) and *Actinobacterium* sp. (76%), respectively (Figs. 3 and 4).

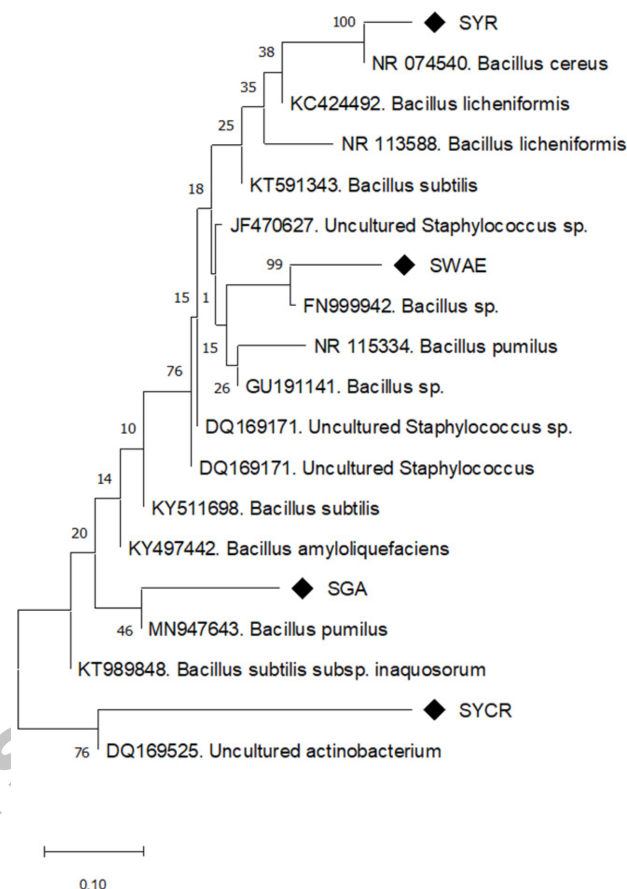

Fig. 4. Phylogram of surface bacterial strains.

DISCUSSION

Impact of different antibiotics on the development and cocoon production of silkworm has been evaluated and established in some studies (Javaid *et al*., 2021; Rafiq *et al*., 2021b). Antibiotic administration significantly enhanced the biological and commercial traits of silkworm (Rahmathulla *et al*., 2003; Hamamoto *et al*., 2004b; Adel *et al*., 2015; Shyamala *et al*., 2013; Savithri, 2007; Rahmathulla and Nayak, 2017; Kushelaf *et al*., 2014; Mahdi *et al*., 2017b; Mahmoud and Taha, 2012). In our study, three concentrations of each levofloxacin and cefixime showed significant effects on larval growth, cocoon production, and bacteria associated with silkworms. Both antibiotics are of broad spectrum and are proven to work against a wide range of Gram-positive and Gram-negative bacteria (Anderson and Perry, 2008; Hurst *et al*., 2002; Brogden and Campoli-Richards, 1989). Antibiotics improved growth of silkworm with increased concentrations (Rafiq *et al*., 2021b). Comparatively higher mean values of larval length and larval weight in

the antibiotic treatment is indicative of improved cocoon production (Rafiq *et al*., 2021b).

Similarly, improved cocoon weight and cocoon shell was recovered from antibiotic treated groups. While concentration within antibiotic treatment resulted in statistically significant differences. Such results were reported in earlier studies where antibiotic concentrations showed variations in the response of larval performance for cocoon production (Rafiq *et al*., 2022). Higher larval body length and body weight improvement may be attributed to higher efficiency of food ingestion, approximate digestibility and resistance to diseases (Kaito *et al*., 2002; Kushelaf *et al*., 2014; Savithri, 2007; Nayak, 2006; Shyamala *et al*., 2013; Mahdi *et al*., 2017; Mahmoud and Taha, 2012; Venkatesh and Srivastava, 2010; Kumar and Srivastava, 2020; Li *et al*., 2010). In both antibiotics, the concentration of varying degree of impact on larval growth and cocoon production. While the concentration of 20 ppm in both antibiotics has more pronounced impact than other two concentrations (10 ppm and 30 ppm) more effective and significantly enhanced larval length, larval weight, cocoon and shell weight, and shell percentage as compared to control. Such findings were reported in some studies highlighting the effect of antibiotics and their varying concentrations (Rasool *et al*., 2018; Saranya *et al*., 2019; Mahdi *et al*., 2017; Rahmathulla and Nayak, 2017).

Through phylogenetic analysis, isolated gut bacterial strains were identified as *Bacillus licheniformis* (GPW), *Actinobacterium* sp. (GRW) and *Bacillus pumilus* (GWSP) (Fig. 3). These results were supported by other studies reporting these species associated with silkworm (Adel *et al*., 2015; Chen *et al*., 2018, 2020; Dong *et al*., 2018; Liu *et al*., 2018; Yeruva *et al*., 2019). However, surface bacterial strains were identified as *Bacillus cereus* (SYR), *Bacillus* sp. (SWCR), *Bacillus pumilus* (SGA) and *Actinobacterium* sp. (SYCR) (Fig. 4). The results were supported in earlier studies in which these species were isolated and identified (Sakthivel *et al*., 2012; Dong *et al*., 2017, 2018; Liu *et al*., 2018; Chen *et al*., 2018, 2020; Adel *et al*., 2015; Li *et al*., 2015, 2019; Yeruva *et al*., 2019).

From all the isolated strains, *Bacillus cereus* is known as the highly threatening hemolytic strain (Li *et al*., 2015, 2019; Pandiarajan *et al*., 2011; Dong *et al*., 2017). While *Bacillus pumilus, Actinobacterium* sp., and *Bacillus licheniformis* are silkworm gut associated strains. They play a vital role in silkworm health as they enhance the development and growth of larvae and reduced larval mortality (Mala and Vijila, 2018; Yeruva *et al*., 2019; Bhuyan *et al*., 2018). *Bacillus pumilus* has antiviral activity against silkworm nucleopolyhedrovirus *in vitro* (Liu *et al*., 2018). *Bacillus licheniformis* produces protease inhibitors that provides resistance against entomopathogenic fungi (*Beauveria bassiana*) (Li *et al*., 2012). While *Actinobacterium* sp. provides resistance against the toxic effects of insecticides (Chen *et al*., 2020).

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CONCLUSION

The impact of different levels (10, 20, and 30 ppm) of levofloxacin and Cefixime enhanced larval growth performance and cocoon production. The use of Levofloxacin and Cefixime (20 ppm) can be used to in sericulture to enhance larval growth and cocoon production of silkworm.

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Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Adel, A., Abou, E., Abdelaleim, Y. and Kariman, M., 2015. Isolation and identification of some bacteria causing infections in silkworm (*Bombyx mori* L.). *Int. J. Curr. Res. Biosci. Pl. Biol.,* **2**: 69-74.

Aditi, F.Y., Rahman, S.S. and Hossain, M.M., 2017. A study on the microbiological status of mineral drinking water. *Open Microbiol. J.,* **11:** 31–44. https://doi.org/10.2174/1874285801711010031

Altman, G.H., Diaz, F., Jakuba, C., Calabro, T., Horan, R.L., Chen, J., Lu, H., Richmond, J. and Kaplan, D.L., 2003. Silk-based biomaterials. *Biomaterials,* **24:** 401-416. https://doi.org/10.1016/S0142-9612(02)00353-8

Anantha, S.G., 2011. *Studies on the management of flacherie in the mulberry silkworm* Bombyx mori *L.* PhD, Manonmaniam Sundaranar University.

Anderson, V.R. and Perry, C.M., 2008. Levofloxacin. *Drugs,* **68:** 535-565. https://doi.org/10.2165/00003495-200868040-00011

Ashok, K.H. and Ramakrishna, S., 2014. Effect of antibiotics on varied density of septicemia causing bacteria of silkworm. *Int. J. Emerg. Technol. Comput. Appl. Sci.,* **3:** 317-320.

Ayoade, F., Oyejide, N.E. and Fayemi, S.O., 2014. Isolation, identification, antibiogram and

characterization of bacterial pathogens of the silkworm, *Bombyx mori* in South-West Nigeria. *J. biol. Sci.,* **14:** 387-402. https://doi.org/10.3923/jbs.2014.425.430

Babu, K.M., 2018. *Silk: Processing, properties and applications*, Woodhead Publishing.

Bauer, A., Kirby, W., Sherris, J.C. and Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. clin. Pathol.,* **45:** 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bhuyan, P.M., Sandilya, S.P., Nath, P.K., Gandotra, S., Subramanian, S., Kardong, D. and Gogoi, D.K., 2018. Optimization and characterization of extracellular cellulase produced by *Bacillus pumilus* MGB05 isolated from midgut of muga silkworm (*Antheraea assamensis* Helfer). *J. Asia-Pac. Ent.,* **21:** 1171-1181. https://doi.org/10.1016/j.aspen.2018.08.004

Brogden, R.N. and Campoli-Richards, D.M., 1989. Cefixime. *Drugs,* **38:** 524-550. https://doi.org/10.2165/00003495-198938040-00004

Carroll, N.M., Adamson, P. and Okhravi, N., 1999. Elimination of bacterial DNA from TaqDNA polymerases by restriction endonuclease digestion. *J. clin. Microbiol.,* **37:** 3402-3404. https://doi.org/10.1128/JCM.37.10.3402-3404.1999

Chairman, K., Singh, A.R., Amalarani, G., Padmalatha, C. and Alagumuthu, G., 2012. Effect of marine extracts on the microbial pathogens causing flacherie in the mulberry silkworm, *Bombyx mori* L. *Asian Pac. J. Trop. Biomed.,* **2:** S1858-S1861. https://doi.org/10.1016/S2221-1691(12)60508-5

Chen, B., Du, K., Sun, C., Vimalanathan, A., Liang, X., Li, Y., Wang, B., Lu, X., Li, L. and Shao, Y., 2018. Gut bacterial and fungal communities of the domesticated silkworm (*Bombyx mori*) and wild mulberry-feeding relatives. *ISME J.,* **12:** 2252-2262. https://doi.org/10.1038/s41396-018-0174-1

Chen, B., Zhang, N., Xie, S., Zhang, X., He, J., Muhammad, A., Sun, C., Lu, X. and Shao, Y. 2020. Gut bacteria of the silkworm *Bombyx mori* facilitate host resistance against the toxic effects of organophosphate insecticides. *Environ. Int.,* **143:** 105886. https://doi.org/10.1016/j.envint.2020.105886

Dong, H.L., Zhang, S.X., Chen, Z.H., Tao, H., Li, X., Qiu, J.F., Cui, W.Z., Sima, Y.H., Cui, W.Z. and Xu, S.Q., 2018. Differences in gut microbiota between silkworms (*Bombyx mori*) reared on fresh mulberry (*Morus alba* var. *multicaulis*) leaves or an artificial diet. *RSC Adv.,* **8:** 26188-26200. https://doi.org/10.1039/C8RA04627A

Dong, X., Lü, P., Cao, W., Zhang, C., Zhu, F., Meng, X., Nie, Z., Lu, S. and Chen, K., 2017. Study of the toxicity of *Bacillus cereus* on silkworm (*Bombyx mori*) and the relevant proteome. *Invertebr. Surviv. J.,* **14:** 129-139.

Gani, M., Chouhan, S., Babulal, R.G., Khan, G., Kumar, N.B., Saini, P. and Ghosh, M., 2017. *Bombyx mori* nucleopolyhedrovirus (BmBPV): its impact on silkworm rearing and management strategies. *J. Biol. Contr.,* **31:** 189-193. https://doi.org/10.18311/jbc/2017/16269

Gore, D., Rai, M. and Shinde, G., 2014. Effect of Amla extract on bacterial infection in the Silkworm, *Bombyx mori* L. *J. Pharm. Res.,* **8:** 679-680.

Hakimi, O., Knight, D.P., Vollrath, F. and Vadgama, P. 2007. Spider and mulberry silkworm silks as compatible biomaterials. *Composites B Eng.,* **38:** 324-337. https://doi.org/10.1016/j.compositesb.2006.06.012

Hall, B.G., 2005. Comparison of the accuracies of several phylogenetic methods using protein and DNA sequences. *Mol. Biol. Evol.,* **22:** 792-802. https://doi.org/10.1093/molbev/msi066

Hamamoto, H., Kurokawa, K., Kaito, C., Kamura, K., Manitra Razanajatovo, I., Kusuhara, H., Santa, T. and Sekimizu, K., 2004. Quantitative evaluation of the therapeutic effects of antibiotics using silkworms infected with human pathogenic microorganisms. *Antimicrob. Agents Chemother.,* **48:** 774-779. https://doi.org/10.1128/AAC.48.3.774-779.2004

Holland, C., Numata, K., Rnjak□Kovacina, J. and Seib, F.P., 2019. The biomedical use of silk: Past, present, future. *Adv. Healthc. Mater.,* **8:** 1800465. https://doi.org/10.1002/adhm.201800465

Hurst, M., Lamb, H.M., Scott, L.J. and Figgitt, D.P., 2002. Levofloxacin. *Drugs,* **62:** 2127-2167. https://doi.org/10.2165/00003495-200262140-00013

Hussain, M., Khan, S.A., Naeem, M. and Nasir, M.F., 2011. Effect of rearing temperature and humidity on fecundity and fertility of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Pakistan J. Zool.,* **43:** 979-985.

Ito, T., 1969. Rearing of the silkworm under aseptic conditions. *Japan Agric. Res. Quart.,* **4:** 33-35.

Javaid, A., Hussain, M., Aftab, K., Malik, M., Umar, M. and Iqbal, T., 2021. Isolation and characterization of bacteria associated with silkworm gut under antibiotic-treated larval feeding. *Braz. J. Biol.,* **84:** e249664. https://doi.org/10.1590/1519-6984.249664

Kabir, S.L., Amin, M.M. and Hossain, K.M., 2013. Identification and antimicrobial susceptibility of

Online First Article

Salmonella species isolated from washing and rinsed water of broilers in pluck shops. *Int. J. Anim. Vet. Adv.,* **5:** 1-8. https://doi.org/10.19026/ijava.5.5569

Kaito, C. and Sekimizu, K., 2007. A silkworm model of pathogenic bacterial infection. *Drug Discov. Ther.,* **1:** 89-93.

Kaito, C., Akimitsu, N., Watanabe, H. and Sekimizu, K., 2002. Silkworm larvae as an animal model of bacterial infection pathogenic to humans. *Microb. Pathogen.,* **32:** 183-190. https://doi.org/10.1006/mpat.2002.0494

Kaito, C., Usui, K., Kyuma, T. and Sekimizu, K., 2011. Isolation of mammalian pathogenic bacteria using silkworms. *Drug Discov. Therapeut.,* **5:** 66-70. https://doi.org/10.5582/ddt.2011.v5.2.66

Karthik, T., Rathinamoorthy, R. and Ganesan, P., 2015. *Sustainable luxury natural fibers production, properties, and prospects*. Handbook of Sustainable Luxury Textiles and Fashion. Springer. https://doi.org/10.1007/978-981-287-633-1_4

Krishnaswami, S., 1978. *New technology of silkworm.*

Kumar, R.V. and Srivastava, D., 2020. *Silkworm: A unique creature for natural products*. Natural materials and products from insects: Chemistry and applications. Springer.

Kushelaf, N., El-Salehein, A. and Rashed, A., 2014. Protection of *Bombyx mori* L. larvae against certain diseases using some chemical disinfectants. *J. Prod. Dev.,* **19:** 253-262. https://doi.org/10.21608/jpd.2014.42659

Leitch, E.C.M., Walker, A.W., Duncan, S.H., Holtrop, G. and Flint, H.J., 2007. Selective colonization of insoluble substrates by human faecal bacteria. *Environ. Microbiol.,* **9:** 667-679. https://doi.org/10.1111/j.1462-2920.2006.01186.x

Li, B., Su, T., Chen, X., Liu, B., Zhu, B., Fang, Y., Qiu, W. and Xie, G., 2010. Effect of chitosan solution on the bacterial septicemia disease of *Bombyx mori* (Lepidoptera: Bombycidae) caused by *Serratia marcescens*. *Appl. Ent. Zool.,* **45:** 145-152. https://doi.org/10.1303/aez.2010.145

Li, G., Shi, M., Zhao, S., Long, Y. and Zhu, Y. 2019. Toxicity response of silkworm intestine to *Bacillus cereus* SW7-1 pathogen. *Sci. Total Environ.,* **692:** 1282-1290. https://doi.org/10.1016/j.scitotenv.2019.07.349

Li, G.N., Xia, X.J., Zhao, H.H., Sendegeya, P. and Zhu, Y. 2015. Identification and characterization of *Bacillus cereus* SW7-1 in *Bombyx mori* (Lepidoptera: Bombycidae). *J. Insect Sci.,* **15:** 136. https://doi.org/10.1093/jisesa/iev121

Li, Y., Zhao, P., Liu, S., Dong, Z., Chen, J., Xiang, Z. and Xia, Q., 2012. A novel protease inhibitor in *Bombyx mori* is involved in defense against *Beauveria bassiana*. *Insect Biochem. mol. Biol.,* **42:** 766-775. https://doi.org/10.1016/j.ibmb.2012.07.004

Liu, R., Wang, W., Liu, X., Lu, Y., Xiang, T., Zhou, W. and Wan, Y., 2018. Characterization of a lipase from the silkworm intestinal bacterium *Bacillus pumilus* with antiviral activity against *Bombyx mori* (Lepidoptera: Bombycidae) nucleopolyhedrovirus *in vitro*. *J. Insect Sci.,* **18:** 3. https://doi.org/10.1093/jisesa/iey111

Mahdi, S.H., Rokonuzzaman, M., Uddin, M.A. and Ahsan, M.K., 2017. The effects of amoxicillin, oxytetracyclin and doxycyclin on the growth and development of silkworm, *Bombyx mori* L. *J. Ent. Zool. Stud.,* **5:** 1316-1321.

Mahmoud, S.M. and Taha, R.H., 2012. Antibiotic (Gentamicin) Impact on Bacterial Flacherrie Disease of Silkworm, *Bombyx mori* L. *Egypt. Acad. J. biol. Sci. A, Ent.,* **5:** 55-63. https://doi.org/10.21608/eajbsa.2012.14792

Mala, M. and Vijila, K., 2018. Beneficial effects of *Bacillus licheniformis* and *Bacillus niabensis* on growth and economic characteristics of silkworm, *Bombyx mori* L. *Int. J. chem. Stud.,* **6:** 1750-1754.

Manimegalai, S., 2003. *Bacterial pathogens of silkworm* Bombyx mori *L. and their management.* PhD, Tamil Nadu Agricultural University.

Manjunath, R., Kumar, A. and Kumar, K.A., 2020. Utilisation of sericulture waste by employing possible approaches. In: *Contaminants in agriculture*. Springer. https://doi.org/10.1007/978-3-030-41552-5_19

Meng, X., Zhu, F. and Chen, K., 2017. Silkworm: A promising model organism in life science. *J. Insect Sci.,* **17:** 97.

Nayak, P., 2006. Influence of antibiotic on feed conversion efficiency of mulberry silkworm (*Bombyx mori* L.). *Anim. Biol.,* **56:** 13-22. https://doi.org/10.1163/157075606775904731

Neelaboina, B.K., Khan, G.A., Gani, M., Ahmad, S., Ahmad, M.N. and Ghosh, M., 2018. Exploration of sericulture in unexplored region of Jammu and Kashmir. *J. Ent. Zool. Stud.,* **6:** 1922-1925.

Pandiarajan, J., Cathrin, B.P., Pratheep, T. and Krishnan, M., 2011. Defense role of the cocoon in the silk worm *Bombyx mori* L. *Rapid Commun. Mass Spectromet.,* **25:** 3203-3206. https://doi.org/10.1002/rcm.5213

Prakash, B. and Puttaraju, H., 2006. Tetracycline: Boon for silkworm and bane for uziflies. *Indian Silk,* **44:**

Online First Article

9-10.

Rafiq, I., Buhroo, Z.I., Sahaf, K.A., Ganie, N.A. and Mir, S.A., 2022. Impact of antibiotic administration on the growth and development of silkworm *Bombyx mori* L. *Int. J. agric. Sci.,* **18:** 480-483. https://doi.org/10.15740/HAS/IJAS/18.1/480-483

Rafiq, I., Buhroo, Z., Sahaf, K., Ganie, N. and Baqual, M., 2021a. Role of antibiotics with reference to growth and development of silkworm (*Bombyx mori* L.). *Pharma Innov. J.,* **10**: 150-154.

Rafiq, I., Buhroo, Z., Sahaf, K., Padder, B., Ganie, N., Mir, S., Baqual, M., Nagoo, S.A. and Ashraf, S., 2021b. Effect of antibiotics on the rearing performance of silkworm *Bombyx mori* L. *Int. J. Curr. Microbiol. appl. Sci.,* **10:** 1-7. https://doi.org/10.9734/cjast/2020/v39i4831274

Rahmathulla, V., 2012. Management of climatic factors for successful silkworm (*Bombyx mori* L.) crop and higher silk production: A review. *Psyche,* **2012**. https://doi.org/10.1155/2012/121234

Rahmathulla, V. and Nayak, P., 2017. Effect of antibiotic administration on growth and development of silk gland in mulberry silkworm (*Bombyx mori* L.). *Munis Ent. Zool.,* **12:** 41-49.

Rahmathulla, V., Nayak, P., Vindya, G., Himantharaj, M. and Rajan, R., 2003. Effect of antibiotic (norfloxacin) administration on commercial characters of new bivoltine and cross breed hybrid silkworm (*Bombyx mori*). *Int. J. Ind. Ent.,* **7:** 191-195.

Rasool, S., Ganie, N., Younus Wani, M., Dar, K., Khan, I. and Mehraj, S., 2018. Evaluation of a suitable silkworm bed disinfectant against silkworm diseases and survivability under temperate conditions of Kashmir. *Int. J. chem. Stud.,* **6:** 1571-1574.

Reddy, R.M., 2009. Innovative and multidirectional applications of natural fibre, silk. A review. *Acad. J. Ent.,* **2:** 71-75.

Saad, M.S., Elyamani, E.M. and Helaly, W.M., 2019. Controlling of bacterial and fungal diseases that contaminating mulberry silkworm, *Bombyx mori* by using some plant extracts. *Bull. natl. Res. Centre,* **43:** 1-9. https://doi.org/10.1186/s42269-019-0218-3

Sakthivel, S., Angaleswari, C. and Mahalingam, P., 2012. Isolation and identification of bacteria responsible for flacherie in silkworms. *J. Microbiol. Biotechnol. Res.,* **2:** 913-915.

Saranya, M., Krishnamoorthy, S., Balachandar, D. and Murugesh, K., 2019. Antibiotic sensitivity pattern of the isolated lactic acid bacteria from silkworm (*Bombyx mori* L.) gut. *Innov. Farm.,* **4:** 140-143.

Satokari, R.M., Vaughan, E.E., Akkermans, A.D., Saarela, M. and de Vos, W.M., 2001. Bifidobacterial diversity in human feces detected by genus-specific PCR and denaturing gradient gel electrophoresis. *Appl. environ. Microbiol.,* **67:** 504-513. https://doi.org/10.1128/AEM.67.2.504-513.2001

Savithri, G., 2007. *In vivo* effects of antibiotics on silkworm *Bombyx mori* L Infected with *Bacillus coagulans*. *Int. J. Ind. Ent.,* **15:** 9-16.

Shah, S.I.A., Khan, I.A., Hussain, Z., Shah, M., Usman, A. and Sadozai, A., 2007. Studying the performance of silkworm, B*ombyx mori* L. races fed with different mulberry varieties. *Sarhad J. Agric.,* **23:** 1079-1084.

Shyamala, M.M., Murthy, M.V. and Bhat, J., 2013. Effect of chloromycetin on feed utilisation by the silkworm *Bombyx mori* L. *J. Indian Inst. Sci.,* **38:** 177.

Srivastava, A. and Kumar, R.V., 2009. Efficacy of three antibiotics on reduction of mortality rate in mulberry silkworm (*Bombyx mori* L.) in the monsoon season of Lucknow. *Trends Biosci.,* **2:** 33-35.

Stockland, E., 2017. Patriotic natural history and sericulture in the French Enlightenment (1730–1780). *Arch. Natl. Hist.,* **44:** 1-18. https://doi.org/10.3366/anh.2017.0410

Subramanian, S., Mohanraj, P., Sivakumar, S. and Muthuswami, M., 2010. Influence of antibiotics and probiotics on endogenous gut micro flora in silkworm *Bombyx mori*. *Sericologia,* **50:** 333-342.

Tamura, K. and Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.,* **10:** 512-526.

Venkatesh, K. and Srivastava, A., 2010. Relevance of antibiotics with reference to sericulture industry. *Int. J. Sci. Nat.,* **1:** 97-100.

Wurm, F.M., 2003. Human therapeutic proteins from silkworms. *Nat. Biotechnol.,* **21:** 34-35. https://doi.org/10.1038/nbt0103-34

Yeruva, T., Vankadara, S., Ramasamy, S. and Lingaiah, K., 2019. Identification of potential probiotics in the midgut of mulberry silkworm, *Bombyx mori* through metagenomic approach. *Probiot. Antimicrob. Proteins***,** pp. 1-6. https://doi.org/10.1007/s12602-019-09580-3

Yuan, Z., Lan, X.Q., Yang, T., Xiao, J. and Zhou, Z., 2006. Investigation and analysis of the bacteria community in silkworm intestine. *Wei sheng wu xue bao (Acta microbiol. Sin.,*) **46:** 285-291.

Zhou, L., Chen, X., Shao, Z., Zhou, P., Knight, D.P.

Online First Article

and Vollrath, F., 2003. Copper in the silk formation process of *Bombyx mori* silkworm. *FEBS Lett.,* **554:** 337-341. https://doi.org/10.1016/S0014-5793(03)01184-0

Zulfiqar, S., Hussain, M., Aftab, K., Malik, M.F., Khursheed, R., Liaqat, S. and Kanwal, M., 2022. Comparative performance of three silkworm races fed on local mulberry against biological and commercial parameters. *Pakistan J. Zool.,* **54:** 2865-2869. https://doi.org/10.17582/journal.pjz/20190414180419

Online First Article