The Pharma Innovation Journal 2021; SP-10(3): 150-154

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(3): 150-154

www.thepharmajournal.com
Received: 08-01-2021
Accepted: 11-02-2021

Iqra Rafiq
College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Srinagar, Jammu & Kashmir, India

ZI Buhroo
College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Srinagar, Jammu & Kashmir, India

KA Sahaf
College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Srinagar, Jammu & Kashmir, India

NA Ganie
College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Srinagar, Jammu & Kashmir, India

MF Baqual
College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Srinagar, Jammu & Kashmir, India

Corresponding Author:
Iqra Rafiq
College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Srinagar, Jammu & Kashmir, India

Role of antibiotics with reference to growth and development of silkworm (*Bombyx mori* L.)

Iqra Rafiq, ZI Buhroo, KA Sahaf, NA Ganie and MF Baqual

Abstract
In the sericulture industry, antibiotics are commonly used to keep worms healthy and disease-free. Apart from utility in managing disease in silkworms, antibiotics are widely used in the sericulture industry as a component of synthetic diets for silkworm rearing or as an ingredient in bed disinfectant formulation. Antibiotic administration has shown attractive outcomes in reaping superior crop harvest and controlling the silkworm disease. The antibiotics supplemented mulberry leaves enhance the growth and development of silkworm. Besides this silkworm models offer a more economic means of testing the effectiveness of antibiotics. Silkworms are used as models to quantitatively evaluate the therapeutic effects of some antibiotics. The connection among antibiotics and silkworm is additionally supported by the current uses of silkworm in drug screening and pathogen studies systems due to its short larval period and morally acknowledged model. So, the focus of the current review is on the use of antibiotics as economic tools for treatment, prevention programmes and for production enhancement.

Keywords: Antibiotics, *Bombyx mori* L, development, growth, silkworm

1. Introduction
Silkworm *Bombyx mori*, a typical representative of lepidoptera insects, is of great agricultural and economic importance. Having short generation time, clear genetic background and rich genetic resources, silkworms have been widely used in various life science studies. Currently, the application of silkworm model has gradually emerged in the field of life science such as in antipathogenic drug screening. Sericulture is a multi-disciplinary cottage industry that includes mulberry plant cultivation, silkworm rearing, and cocoon reeling for silk yarn production. Silkworm has an economic significance due to commercial value of its silk.
Silkworm is highly susceptible to microbial diseases that affect quality and quantity of silk, thereby causing huge economical losses. Insects have developed efficient innate immune system to fight against microbial pathogens. The innate immune system plays a crucial biological role in the limitation of microbial infections. So, various antimicrobial agents are being used for controlling silkworm diseases and to improve the production of silk and among those the most frequently advocated medications in modern scientific era are antibiotics.
An antibiotic is a concoction substance, produced by micro-organism, life forms, which has the ability to repress the development of and even to annihilate different microorganisms. The activity of an antibiotic against micro organisms is particular in nature, a few life forms being influenced and others somewhat, each antibiotic is thus characterized by a specific antimicrobial spectrum. The antibiotics supplemented mulberry leaves enhance the growth and development of silkworm. Oral supplementation of anti-infection agents along with mulberry leaves to sound silkworm helps in the development, fertility and silk contents and decrease the frequencies of diseases [1]. Antibiotics in silkworm are approved for four different purposes: disease treatment, disease prevention, disease control and for health maintenance or growth promotion [2].

2. Effect of antibiotics on growth indices
The varied use of antibiotics has reduced the likelihood of spreading the infection to other, made a major contribution by assisting in the treatment of existing disease conditions, by aiding in the disease prevention measures that utilize subtherapeutic prevention of diseases that are predictable during various antibiotic doses and are designed to protect different phases of the animal's life, and by enhancing the performance during the time that they are most susceptible. When antibiotics are administered to the silkworm, there is a change in the metabolism of nitrogen to increase body weight and silk production [3]. Chloromycetin and

glycine in single dose and in combination, for the nourishment of the silkworm yields about 6% more silk than control [4]. There was an improvement of single cocoon weight by the administration of Chloramphenicol and Tetracycline to silkworm [5]. Increased cocoon weight has been reported on treatment with Amikasin 1000 ppm and Tetracycline 1000ppm [6]. In a study conducted with injection of ampicillin (200 µg), oxacillin (200 µg) or vancomycin (200µg), at least 90% of the larvae survived for 4 days after injecting the treatment [7]. Antibiotic agents play a significant role in conferring immunity to silkworms and accordingly expedite the effective rate of rearing [8]. Common assumptions for why antimicrobials increase growth include: (1) reducing colonization of intestinal bacteria and inhibiting the growth of pathogenic microorganisms; (2) decreasing the thickness of mucous membrane, leading to more absorption of nutrients; (3) directly neutralizing the host immune response; (4) and shifting to more energy efficient conversion. All of these modes of action manipulate host intestinal flora, intestinal physiology, and immune system which contributing to the prevention of disease and changes in microbiome equilibrium [9]. Antibiotic supplemented or fortified feed to silkworms resulted in heavier caterpillars with increase in nitrogen metabolism [10]. Antibiotics are also known to promote larval growth and to increase the production of silk to some degree as the growth of silkworms and their economic characteristics are significantly influenced by the nutritional quality of mulberry leaves [11]. Antibiotics, like Penicillin, Ampicillin and Streptomycin were found to be successful in decreasing the mortality of silkworms by 23-25% [12]. Administration of antibiotic (Norfloxacin) administered feed has significantly improved higher silk gland, larval, shell and cocoon weight in treated batches [13].

3. Effect of antibiotics on feeding behaviour

Antibiotics make a valuable contribution to animal agriculture when used as economic tools to improve feed efficiency as antibiotics have been reported to play a vital role in improvement of disease resistance and growth enhancement in farm animals and insects. The efficiency of feed utilisation on the basis of the digested leaf between the controls and chloromycetin treated larvae, has shown about 25% increase in the feed utilisation [14]. There is a debate while studying the mechanism of action of antibiotics in relation to biomass accumulation and higher productivity in sericulture as whether antibiotics are completely effective by their antibacterial properties or by favourably influencing silkworm physiology and by improving feed efficiency or activating enzymes or by hormonal action [15]. A detailed study conducted on the impact of various antibiotics (amoxicillin, oxytetracycline and doxycycline) on physiological parameters of fifth instar larvae and reported that the most improved results for food utilization, food use and food edibility of fifth instar larvae was recorded at 5% concentration [16].

4. Effect of antibiotics on intestinal microbiota

The method of administration of antibiotics varies depending on the desired outcome of the treatment. The dosage, duration, mode of administration, and withdrawal times are some of the considerations when administering antimicrobials to insects. The beneficial effect of antibiotics has been due to their activity in conditioning intestinal flora, their potential role as growth factors, their biological efficiency in increasing feed conversion to body weight and their potential activity for disease control [17]. It has been revealed that supplementation with chloromycetin revamps the oxygen absorption from the silkworm gut by regulating the intestinal flora of the larvae [18]. In a study it has been reported that beneficial effects of antibiotics are due to modulation of gut micro-flora and influence on mucosal immunity or through altering enzymatic activities which has been extensively studied in humans, animals and many insects [19]. A study on antibiotic (Norfloxacin revealed that the use of antibiotic treated batches in food was comparable to control. However, the nutritional indices, such as digesta, edibility and reference ratio, were altogether higher in the batches treated with antibiotics [20]. Thus, in the antibiotic treated batches, the nutritional proficiency parameters, such as the productivity of ingested food into the larvae, cocoon and shell, were absolutely higher. Antibiotic intake (such as norfloxacin) can promote greater assimilation and conversion of food, leading to an increase in the body weight and digestibility of silkworm [21].

5. Antibiotics and their therapeutic effects in relation to silkworm diseases

In India considerable fluctuations occur in the nutritional value and composition of the mulberry leaves depending on factors such as weather, pests, diseases and agricultural practices which have an immense impact on growth and development of silkworm which in turn results in crop loss [22]. Crop loss due to diseases is also a common scenario in sericulture. Fortification of mulberry leaves by using supplementary nutrients and feeding to the silkworm is a constructive modern technique to increase economic value of cocoons besides offering disease resistance. Mulberry leaves sprayed with antibiotics have been found to have a better influence on development in silkworm. Administration of Tetracycline increased the resistance against *Bacillus thurigiensis* and lowered lethality in silkworm and cabbage looper [23]. Oral supplementation of antibiotics with mulberry leaves resulted in the increased growth and silk production and it has been reported that the useful activity of the antibiotics can be ascribed to enhance action of anti-infection agents which diminishes fundamentally the occurrence of flacherie and grasserie [1]. The impact of various antibiotic agents provided to silkworm *B. mori* larvae at 0.05% and 0.1% and it has been found that there was decrease in disease (grasserie and flacherie) in contrasted to control [24].

In a study it has been reported that Rifampicin proved to be significantly effective in reducing mortality of NPV infected worms, followed by Chloramphenicol [25]. Supplementation of ampicillin was more effective than streptomycin by reducing the larval mortality to 39% and also increased the larval, cocoon and shell weights [26]. Antibiotics used for clinical purposes have therapeutic effects on silkworms that are infected with the pathogens *Staphylococcus aureus*, *Candida albicans* [27]. Antibiotics like Penicillin, Ampicillin and Streptomycin have been found to be successful in decreasing the mortality of silkworms by 23-25% [12]. Antibiotic feed supplementation not only show prophylactic steps to deter bacterial diseases, but the nourishment and economic parameters are further upgraded in *B. mori* [28]. It has been reported that in silkworm body fluid, antibiotics such as Ganciclovir, foscarnet, vidarabine and ribavirin, inhibit baculovirus replication and thus had therapeutic effects [29]. Mulberry leaves supplemented with oflaxacin, acyclovir and griesovin along with feed have been discovered to be best in expanding the survival rate as well as forestalling the event of

grasserie, flacherie and muscardine diseases [30]. *In vitro* streptomycin sulfate use @1000ppm successfully controll bacterial diseases upto 52.37% in silkworm [31].

6. Antibiotics and their effects on silkworm haemocytes

Insect blood or haemolymph is better defined as the circulating intracellular fluid filling the cavity of the body or haemocoel while bathing different tissues. Haemocytes play multiple important roles during insect growth and development. Haemocytes in the haemolymph of the insects assume a significant function in the protection mechanism. There is growing evidence that diverse classes of antibiotics have immunomodulatory effects, in addition to their antimicrobial activity [32]. Balavenkatasubbaiah *et al.* (2001) contemplated the haemocytes in various types of silkworm, *B. mori* and their progressions during the reformist of BmNPV. The influence of feeding frequency on the total and differential haemocyte count in silkworms breeds have revealed that the quantity of haemocytes is enormously influenced by age, stage and physiological status of an insect [33]. Haemolymph plays an important role in the inherent immunity response, which is induced when bacteria invade the body of the silkworm [34]. Antimicrobial studies in relation to haemolymph and haemocytes in silkworm (*B. mori*) have reported that after injection of the larvae with soluble peptidoglycan, the anti-bacterial activity of silkworm haemolymph increased in parallel with cecropin activity [35].

7. Antibiotic screening using silkworms

The conventional approach of antibiotic screening clearly requires remodeling. To overcome the problems associated with conventional screening methods, silkworm has been used as an animal model to evaluate the therapeutic effects of antibiotics. In this review, *we discuss the advantages of the silkworm model for antimicrobial drug development. The silkworm model is technically convenient, ethically acceptable and fast as larval period is short, and can be used on a wider scale in the study of pathogens and drugs [36]. With recent developments, the silkworm now has a sophisticated genetic modification system and can thus be used to establish disease models to screen out different compounds and analyze physiologic processes. It has been found that a model compound is metabolized in silkworms by the cytochrome P450 enzyme, follows the metabolic pathway via the conjugation reaction, and exhibits the similar pharmacokinetics as in mammals [37]. It has been demonstrated that the general non-specific transport of molecules through paracellular routes is comparable between the mammalian intestine and the silkworm midgut [1, 38]. Further more silkworms have been utilized to study pathogenic bacterial toxins [39]; evaluate the target specificity of antibacterial agents [40]. Identify novel S. aureus virulence genes [41, 42]; and identify novel probiotic bacteria that promote survival during P. aeruginosa infection [43]; Candida tropicalis [27]; Candida albicans [27]. By utilizing the silkworm infection model, novel antimicrobial agents-Lysocin E [44], nosokomycins [45], and ASP2397 [46] has been successfully identified.

8. Conclusion

This review represented an attempt to comprehensively investigate the effects of antibiotics on the silkworm. Antibiotic administration increases silk yield and also play a significant role in conferring immunity to silkworms and accordingly expedite the effective rate of rearing. Our results demonstrated that antibiotics can significantly affect the feeding behaviour and intestinal flora of silkworm. These evidences show that antibiotics exert substantial effects on the silkworm physiology. Mulberry leaves sprayed with antibiotics have been found to have a better influence on development in silkworm by increasing the resistance against pathogens and lowered lethality in silkworm. Silkworm has also been found to be useful as an animal model to evaluate the therapeutic effects of antibiotics.

9. Acknowledgement

The first author sincerely acknowledges the support provided by Associate Dean, CoTS, SKUAST-K, Mirgund and Head Division of Silk Product Sciences, CoTS, SKUAST-K, Mirgund for providing the required facilities in pursuit of this research program.

10. Competing Interest

The authors declare no conflict of interest in the publication of this manuscript.

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