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EFFECT OF AQUEOUS SOLUTION OF ANTIBIOTICS NORFLOXACIN ON THE TOTAL PROTEIN CONTENTS IN THE FIFTH INSTAR LARVAE OF SILKWORM, *BOMBYX MORI* (L) (DOUBLE HYBRID RACE).

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ABSTRACT The yield of silk depends on the quality of food material supplied to the larval instars of silkworm, *Bombyx mori* (L). The present attempt deals with utilization of aqueous solution of Norfloxacin for treating the leaves of mulberry, *Morus alba* (L). Two concentrations of Norfloxacin were selected for the attempt. Two concentrations of Norfloxacin include 50 mg per liter (50 ppm) and 100 mg per liter (100 ppm). The solution was prepared by adding powder antibiotic in distilled water and made the required concentration. 400 ml of aqueous solution of Norfloxacin were used to treat 100 grams of leaves of mulberry, *Morus alba* (L) for feeding the group of hundred larval instars for each attempt of feeding. Treated mulberry leaves were fed to the fifth larval instars. The feeding the larvae with treated leaves was carried for first four days. Four feedings were given for each day. For each feeding hundred grams of leaves (for the group of hundred larvae) were used. The larvae fed with untreated control & water treated control leaves were also maintained. Through the use of silk glands; fat bodies and haemolymph, bioassay of total protein was carried out on fifth day. The total protein content of silk glands; fat bodies and haemolymph was found improved 61.519 to 114.667; 79.928 to 90.055 and 30.983 to 31.010 percents respectively through feeding the fifth instars of silkworm, *Bombyx mori* (L) (Double Hybrid Race) leaves of mulberry, *Morus alba* (L) treated with aqueous solution of Norfloxacin antibiotics.

KEYWORDS : Antibiotic Treatment, Norfloxacin, Growth of silk gland, *Bombyx mori* L.

INTRODUCTION

The metabolites of the plants and the life of insect herbivores are closely interlinked. There is orchestrate progression of insect metamorphosis and the titers of juvenile hormone (JH) and moulting hormone (MH) in the body of larval instars of the insect. Bowers, *et al* (1966) opined the relation between the bio-chemical constituents of various parts (like Roots; Stems; Leaves and Fruits) of the plants. Such bio-chemical constituents could have been the factors for the insect growth and insect metamorphosis. There is avoidance of poor quality of food material by the life stages of the insect. The insects deserve capability of selection of right mood material from varieties of herbal food material available for them. The larval instars of of silkworm, *Bombyx mori* (L) are called as monophagous. The larval instars of of silkworm, *Bombyx mori* (L) are feeding exclusively on the leaves of tree of mulberry *Morus alba* (L). The strategy for the getting qualitative silk cocoons, it is needful to fortify either the quality of food (mulberry leaves) or to improve the appetite of larval instars of silkworm, *Bombyx mori* (L). Murugan and George (1992), listed the quality of nutrition (biochemical status of nutrients in the food / Leaves of mulberry, *Morus alba* L); titer or concentration of hormones (hormonal level) in the body and the conditions of climate (environmental conditions) as the key factors responsible for influencing the growth, development & subsequent physiology of body of larval stages of silkworm, silkworm, *Bombyx mori* (L). The energy source material, the functional food get metabolized to convert it into various elements in body of larval instars of the insects like silkworm, *Bombyx mori* (L). For the successful metamorphosis of larval instars into pupa and then into the adult the leaves of mulberry, *Morus alba* (L) serve as the exclusive source of nutrients. Large number of stimulants and the nutrients are present in the leaves of mulberry, *Morus alba* (L) (as if the aim of life mulberry, *Morus alba* L. is the life for larval instars of silkworm, *Bombyx mori* (L) (Ito, 1960,1961; Nayar & Fraenkel, 1962; Ito, *et al*, 1964; Ito & Hyashiya, 1965). The qualitative rate of metamorphosis in larval instars of silkworm, *Bombyx mori* (L) is depend on the quality of the nutrients in the leaves of mulberry, *Morus alba* (L.). The nutrients in the leaves of mulberry, *Morus alba* (L) holds the sole credit of life of silkworm, *Bombyx mori* (L). Therefore, the leaves of mulberry, *Morus alba* (L) forms the physiological foundation for sericulture. The proteins, lipids and carbohydrates are the biochemical constituents of the leaves of mulberry are the mulberry, *Morus alba* (L) (Murali, 1992) & minerals (Subramanyam Reddy, 1992). The diversity of mid gut enzymes in the body of larval instars of silkworm, *Bombyx mori* (L) is the result of influence of biochemical profile of the leaves of mulberry, *Morus alba* (L.). The body tissues of larval instars of silkworm, *Bombyx mori* (L) especially, the fat bodies are meant for the purpose to store the nutrients in the form of proteins; lipids; carbohydrates (glycogen) derived from the leaves of mulberry, *Morus alba* (L).

According to Slansky (1982), the variation in the food consumption in phytophagous insects is because of presence of varied biochemical processes, ultimately for successful adaptations. According to Horie, *et al*. (1963), there is a functional difference between the enzyme activity for digestion by the digestive fluid in mid gut lumen and by the tissue of mid gut itself. The molecular proteins are hydrolyzed into respective peptides and then into the individual amino acid through peptidase enzymes in the mid gut tissue of silkworm, *Bombyx mori* (L). Likewise, the polysaccharides, are digested in the insect gut lumen by digestive fluid & disaccharides and/or trisaccharides get hydrolysed into their constituent monasaccharide sugars mainly in the gut tissue (Horie, 1967). Yamafugi and Yonezawa (1935) reported the analogy of pancreatic lipase in the body of vertebrate animals with the lipase enzyme in the insect mid gut. The very first attempt towards production of the qualitative silk is the improvement in the efficiency of consumption & utilization of food by larval instars of silkworm, *Bombyx mori* (L). On this line, the attempts include: improvement in the quality of mulberry leaves & supplementation of nutrient biocompounds like soya protein; potassium iodide, copper sulphate, other mineral salts, herbal products (or drugs) like digoxin (Vitthalrao & Kulkarni, 2011) kho-go (Desai, *et al*, 2011) and stevia inulin (Shubhangi Pawar, *et al*, 2017). Quality of mulberry leaves get reflected into the quality of the cocoons spun by fifth instar larvae of silkworm, *Bombyx mori* (L). There are reports on Use of soya protein; potassium iodide, copper sulphate, mineral salts, herbal products for improvement of the quality of leaves of mulberry, *Morus alba*. Herbal products are well known for the acceleration of metabolism in the body of larval instars of silkworm, *Bombyx mori* (L). Use of vital stains for treating the mulberry leaves and the feeding the fifth instars of silkworm, *Bombyx mori* (L) was recently reported for the improvement in the levels of total proteins in the silk glands, haemolymph and fat bodies (Vitthalrao Bhimasha Khyade, 2018; Vitthalrao Bhimasha Khyade and Eric Richard Kandel, 2018; Sharad Ganpat Jagtap and Vitthalrao Bhimasha Khyade, 2019; Pragati Prabhakar Doke, *et al*., 2019 and Vitthalrao B. Khyade, 2019).

The chemical substance working against the bacteria like microbials is said to be the antibiotic. The antibiotics are known for fighting against the infection of the bacteria. The medication through the antibiotic is widely used in the treatment and prevention of such infections. The antibiotics either kill bacteria or inhibit their growth. The viruses are not susceptible for the antibiotics. A special group of antibiotics is reserved for the use of animal health. The antibiotics were used to promote the health of animals through efficient digestion. their food more efficiently, The antibiotics were used for maximum benefit from and allow animal to develop into strong and healthy individuals. This is achieved by destroying or inhibiting undesirable bacterial population in the alimentary canal of the animals. The harmful bacterial population in the alimentary canal of animals prevent optimum

absorption of food material (Phillips, 2007). Some of the antibiotics are well recognized as "Growth Stimulating Factors" in the body of animals. Therefore, such type of antibiotics are extensively used to enrich the nutrition of farm and other animals for their increased productivity (Baig, *et al*., 1990).

One of the most effective methods to enrich the silkworm diet is fortification of mulberry leaves. It is possible for upgrade the quality of biochemical parameters in silkworm larvae through the antibiotic supplementation. The larvae of silkworm obtain nutrients from leaves of mulberry to build up body, to sustain their life, to spin cocoons and to prepare for egg production. This type of nutritional requirements in the consumption of food material have direct impact on larval health; weight of cocoon; quantity (and quality) of silk production; pupation trait and reproductive traits. There are many reports on the use of antibiotics in rearing the larval instars of silkworm, *Bombyx mori* (L). Attempts include enrichment of leaves of mulberry with small quantity of different antibiotics before feeding the larval instars of silkworm (Murthy *et al*., 1951; 1954; Shyamala *et al*., 1962, Verma and Atwal, 1963). These attempts are reporting increased the larval weight, growth, fecundity and silk content through feeding of antibiotics along with mulberry leaves. The growth, development and economic parameters of larvae of silkworm are influenced to a great extent by the nutritional content of mulberry leaf (Ahamed, 1994; Shivakumar, 1995). Antibiotics are known to improve the growth of the larvae and to certain extent enhance the silk production (Radha *et al*., 1980). Rahmathulla *et al*. (2003) reported significant improvement in the rearing, larval parameters (larval duration, larval weight, growth index) and cocoon parameters (single cocoon weight, single shell weight and shell ratio) and silk filament parameters (average filament length, non breakable filament length, raw silk recovery percentage, denier, reelability and neatness). Better performance by the silkworm larvae were recorded with the increase of antibiotics concentration. Tayade *et al*. (1988) reported boosting the growth, fecundity and silk contents through oral administration of antibiotics along with mulberry leaves to silkworm larvae. Rai and Devaiah (1988) reported reduction in the incidence of diseases through the oral supplementation of antibiotics through the mulberry leaves.

There is adverse effect of higher or lower temperature and humidity, ventilation and feed on the physiological functions of the silkworms. The higher or lower temperature and humidity, ventilation and feed make the silkworm larvae highly susceptible to diseases. The common microbial diseases of silkworm larvae include: grasserie (viral), flacherie (bacterial), muscardine (fungal) and pebrine (protozoan). The poor quality mulberry leaves will not be able to provide sufficient quantity of essential requirement to the larva for production of anti-microbial factors. The poor quality mulberry leaves result into high rate of multiplication of infectious bacteria and development of flacherie disease (Nataraju *et al*., 2005). The nuclear polyhedrosis virus (NPV) diseases in *Bombyx mori*(L) account for 70-80% of the total loss. It pose the major threat in sericulture (Babu *et al*., 2005). In India the white muscardine disease of silkworm is observed during high humid seasons. Inactiveness; loss of appetite; hardening of the body; growth of mycelia of pathogen and change in the body color are some of the important symptoms of white muscridine disease in silkworm, *Bombyx mori* (L) (Ramanjaneyulu, 1992). *Beauveria bassiana* (Bals.) Vuill is the fungal species known for causing white muscardine disease in silkworm larvae (Seema K. Dongare, *et al*., 2019). The white muscardine disease in silkworm larvae is the most contagious and dreaded disease. It is more common during rainy and winter seasons in India (Chandrasekharan *et al*., 2006). Antibiotics used for clinical purposes have therapeutic effects on silkworms infected with the pathogens (Staphylococcus aureus, Candida albicans) (Hamamoto, *et al*., 2004). Kaito *et al*. (2002) found that when ampicillin (200 µg), oxacillin (200 µg) or vancomycin (200 µg) was injected in the silkworm larvae after injection of the bacterium, at least 90% of the larvae survived for four days. Ganciclovir, foscarnet, vidarabine and ribavirin (antiviral agents) inhibit the proliferation of baculovirus in silkworm body fluid and had therapeutic effects (Orihara *et al*., 2008).

Norfloxacin belongs to the class of fluoroquinolone antibiotics. Norfloxacin is associated with a number of rare serious adverse reactions as well as spontaneous tendon ruptures and irreversible peripheral neuropathy. Tendon problems may manifest long after therapy had been completed and in severe cases may result in lifelong disabilities. There are no reports on use of Norfloxacin antibiotics in sericulture. Therefore, the present attempt was planned.

MATERIAL & METHODS

The attempt was carried out through the steps like: Rearing of larval instars; Norfloxacin solution Preparation; Grouping the Fifth Instared Larvae; Treating the leaves of mulberry with Norfloxacin solution and feeding the larvae; Bioassay of Total Protein Bioassay and Statistical analysis of the data.

(A). Rearing of Larval Instars of Silkworm, *Bombyx mori* (L):

The bivoltne, crossbreed (Double Hybrid) race: [(CSR6 x CSR26)] x [CSR2 x CSR27)] of silkworm, *Bombyx mori* (L) was selected for the attempt on utilization of aqueous solution of antibiotics norfloxacin for quantitative influence on total protein contents. The disease free layings (DFL) (in the form of loose eggs) of selected race of silkworm, *Bombyx mori* (L) were procured through the unit of sericulture at Sheti Farm of Agriculture Development Trust, Malegaon. The disease free laying were processed for black boxing for incubation of individual egg. The first and second instarred larvae the early age larvae or Chawki larvae. The third, fourth and fifth insatrred larvae are the late age larvae. Both, the early and late age larvae were reared in the laboratory of "Dr. APIS" through the methods prescribed by Krishnaswami, *et al* (1978) & explained in earlier attempts by Khyade (2004); Vitthalrao & Kulkarni (2011); Desai, *et al*, (2011) Shubhangi Pawar, *et al* (2017); Ramprakash Verma, *et al* (2018); Pranita Rajendra Vare, *et al* (2018); Manisha Mahendra Nalwade, *et al* (2018); Seema K. Dongare, *et al* (2018) and the others. The appropriate and qualitative leaves of mulberry, *Morus alba* (L) were used for feeding the larvae. The schedule of feeding prescribed by Sharad G. Jagtap (2014) was followed for both early age larvae (First and Second instared larvae) (Chawki) and late age larvae (Third; Fourth and Fifth instared larvae). The fifth instared larvae were selected for the analysis of influence of treating the mulberry leaves with aqueous solution of Norfloxacin on contents of total protein in silkworm, *Bombyx mori* (L).

(B). Preparation of Norfloxacin Aqueous Solution:

Norfloxacin belongs to the class of fluoroquinolone antibiotics. The Norfloxacin powder was procured through the local dealer. Two different concentrations of Norfloxacin solution were prepared, which include: 50 ppm and 100 ppm. For each strength of Norfloxacin, 400 ml of solution was prepared. For 50 ppm strength, 20 mg of Norfloxacin powder was dissolved in 400 ml distilled water. For 100 ppm strength, 40 mg of Norfloxacin powder was dissolved in 400 ml distilled water. The solution was prepared half an hour before the use. 400 ml of aqueous solution of Norfloxacin powder was used for treating 100 grams of fresh mulberry leaves.

(C). Grouping the Fifth Instar Larvae for the schedule of treatment:

Completion of the fourth moult was considered as zero hour age of the fifth instar larvae. Soon after passing the fourth moult, the fifth instared larvae were divided into four groups. Each group of the larvae was with hundred individuals. The groups include: Untreated Control; Water treated Control and two treated groups. The two treated groups of the larvae include: 50 ppm and 100 ppm. 400 ml of aqueous solution of Norfloxacin powder was used for treating 100 grams of fresh mulberry leaves. 100 grams of fresh mulberry leaves were kept immersed in 400 ml of aqueous solution of Norfloxacin powder for half an hour. The mulberry leaf - treatment was carried out for half an hour before feeding the fifth instar larvae of silkworm, *Bombyx mori* (L). The mulberry leaf treatment was carried out separately for each strength of aqueous solution of Norfloxacin powder. After the treatment, mulberry leaves were drained off completely & then used for feeding to the fifth instar larvae of silkworm, *Bombyx mori* (L) in respective groups. The Norfloxacin treated mulberry leaf feeding was carried out for the first four days of fifth in stars.

(D).Treating the leaves of mulberry, *Morus alba* (L) and feeding the fifth instar larvae of silkworm, *Bombyx mori* (L):

Treating the leaves of mulberry, *Morus alba* (L) with aqueous solution of Norfloxac in was carried half an hour before of each feeding to the fifth instar larvae of silkworm, *Bombyx mori* (L). For treating hundred grams of leaves of mulberry, *Morus alba* (L), the volume of aqueous solution of Norfloxac in was four hundred milliliters. Hundred grams of leaves of mulberry, *Morus alba* (L) were used for feeding the group of hundred larval instars of silkworm, *Bombyx mori* (L). Fresh leaves of mulberry, *Morus alba* (L) were weighed. The known volume of solution of fifty milligram per liter and hundred milligram per liter strength was taken in separate glass jar. Known quantity of leaves of

mulberry was kept immersed separately in aqueous solution of Norfloxacin of known strength. The treating the mulberry leaves through immersing in aqueous solution of Norfloxacin was carried out for half an hour before feeding. After the schedule of treatment, the mulberry leaves were drained off completely and then used for feeding to the fifth instar larvae of silkworm, *Bombyx mori* (L) in respective groups. Four feedings for each day for each group were followed. The first feeding was at 5.00 a.m. The second feeding was at 11.00 a.m. The third feeding was at 5.00 p.m. The fourth feeding was at 11.00 p.m. One hundred grams leaves of mulberry, *Morus alba* (L) were used for feeding the group of hundred larvae for each time. Such type of feeding the silkworm larvae with the treated mulberry was carried out for the first four days of fifth instars. The groups of larvae fed with untreated mulberry leaves and water treated mulberry leaves were also maintained as control groups.

(E). Bioassay of Total Proteins from the Salivary Glands; Fat bodies and Haemolymph:

The bioassay of total proteins from silk (salivary glands); fat bodies and haemolymph was carried out on fifth day of fifth instar. Thirty larval instars of fifth stage from each group were selected randomly. Ten larvae were utilized for the bioassay of total protein estimation from silk glands. Ten larvae were utilized for the bioassay of total protein estimation from fat bodies. And remaining ten larvae were used for the bioassay of total protein estimation from haemolymph. The chloroform soaked cotton pads were used for the provision of anaesthetia to the fifth instar larvae of silkworm, *Bombyx mori* (L). Weight of individual larva was recorded. Individual anaesthetized larva was dissected open from dorsal side. Both the silk glands from individual larva were separated. The larval dissection for silk glands and fat bodies was carried in chilled saline (0.9 percent sodium chloride solution). The tissues were blotted separately. The tissues were weighed accurately on electronic balance. Both the tissues (silk glands and fat bodies) were washed separately in ice cold saline. There after, each tissue was blotted. Weight of each tissue was recorded through the use of electronic balance. Each tissue was then processed for fragmentation followed by homogenization in chilled in chilled distilled water. Clean & sterilized morter & pestle were used for tissue homogenization. Each tissue assay sample was processed for keeping at 37°C for twenty four hours in the solution of sodium hydroxide of normal (1.0 N) strength. The homogenate was centrifuged at 1000 rpm for 10 minutes. The supernatant was used as assay sample. The haemolymph was subjected for centrifugation at 1000 rpm for 10 minutes and the supernatant was used as assay sample.

For the purpose of assay sample of hemolymph, individual larva was used. The haemolymph was collected from individual larva of silkworm. Individual larva of silkworm was anaesthetized with the help of placing chloroform soaked cotton pads on the spiracles. Then, larva was processed for surface rinsing with ethanol. With the help of sterile blade, the prologs of individual larval were cut. The haemolymph of individual larva was collected in separate small vials precoated with phenyl thiourea (phenyl thiourea prevent melanization of content). Volume of haemolymph was measured. Each vial was weighed accurately. Weight of empty vial was subtracted to get the weight of haemolymph (mg/ml). The haemolymph was subjected for centrifugation at 1000 rpm for 10 minutes and the supernatant was used as assay sample.

It was stored at – 20^0 C and used for bioassay of total proteins.

Bioassay of total proteins from silk glands and fat bodies was carried in triplicate (for each assay sample three test tubes were taken). 1 ml assay sample was transferred to each test tube. Addition of 5.0 ml Lower's ―C solution was made in each of the test tube mixed well and kept for 15 min to allow the formation of copper protein complex. A blank was also prepared simultaneously. After 15 min, 0.5 ml Folin's phenol regent was added to each tube and mixed well. Then they were allowed to develop colour for 30 min at room temperature. After it, the optical density was recorded at 660 nm on spectrophotometer. The results were replicated three times. The protein concentration of assay sample was calculated by referring the optical density obtained for sample and by using standard graph and expressed in the unit as µg proteins per mg tissue.

(F). Statistical analysis:

Consistency in the results is qualitative parameter in research studies. Therefore, the whole experimentation in the present study was repeated for thrice. The data of all the three attempts was collected and subjected for statistical analysis. The statistical parameters for analysis considered in the study include mean, standard deviation, percent change & significance through student t – test introduced by William Sealy Gosset (a chemist working for the Guinness brewery in Dublin, Ireland. "Student" was his pen name) (https://en. wikipedia. org/wiki/Student%27s_t-test) and explained by Norman & Baily (1955).

RESULTS & DISCUSSION

The results on the contents of total protein in the fifth instar larvae of bivoltine, crossbreed, silk worm, *Bombyx mori* (L) fed with mulberry *Morus alba* (L) (M-5: variety) leaves treated with water solution of Norfloxacin powder are summarized in table 1 and Figure- 1, 2 and 3.

The total protein contents of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, *Bombyx mori* (L) recipients of untreated leaves of mulberry, *Morus alba* (L) (M-5: variety) (untreated control group) in present attempt were found measured 23.147(±1.559); 20.223 (±1.805) and 14.779 (±1.764) units respectively.

The quantitative estimation of total protein of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, *Bombyx mori* (L) recipients of leaves of mulberry, *Morus alba* (L) (M-5: variety), treated with aqueous solution of Norfloxacin powder, with 50 ppm strength in present attempt was found measured 37.387 (±3.259); 36.387 (±3.956) and 19.358 (±1.762) units respectively. In comparison with the control group, there was 61.519; 79.928 and 30.983 percent increase in total proteins respectively in of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, *Bombyx mori* (L) through treating leaves of mulberry, *Morus alba* (L) (M-5: variety) with 25 ppm aqueous solution of Norfloxacin powder.

The total proteins of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, *Bombyx mori* (L) recipients of leaves of mulberry, *Morus alba* (L) (M-5: variety), treated with aqueous solution of Norfloxacin powder, with 100 ppm strength in present attempt were found measured 49.689 (±7.863); 38.435 (±3.678) and 19.362 (±2.205) units respectively. In comparison with the control group, there was 114.667; 90.055 and 31.010 percent increase in total proteins respectively in of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, *Bombyx mori* (L) through treating leaves of mulberry, *Morus alba* (L) (M-5: variety) with 100 ppm aqueous solution of Norfloxacin powder.

Change in the strength of aqueous solution of Norfloxacin powder from 50 ppm to 100 ppm for treating the leaves of mulberry, Morus alba (L) (M-5: variety) and feeding the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) was observed to exert considerable change amount of the total protein of silk glands; fat bodies and haemolymph. Significant increase in the levels of total proteins of silk glands; fat bodies and haemolymph of the fifth instar larvae of silkworm, Bombyx mori (L) fed with mulberry leaves treated with various concentrations of aqueous solution of Norfloxacin powder, eco-friendly formulation may be explained away as due to enhanced break down of contents of mulberry leaves. The Norfloxacin may improve appetite & digestion.

The sole aim of rearing the larval instars of silkworm, Bombyx mori (L) is production of cocoons of qualitatively and quantitatively superior quality. According to Priyadharshini et al., (2008), silkworm larvae are affected by a number of diseases through various biological, chemical, physical, nutritional and environmental causes. The wrong method of rearing, low nutritional status of mulberry leaves and ill health of larvae of silkworm favour the multiplication of pathogen. It contributes for the loss of cocoon crop yield. The Indian sericulture reports annual crop loss through the microbial pathogens in silkworm, Bombyx mori (L). In the poikilotherms, internal temperature varies considerably. The poikilotherm is exactly opposite to that of homeotherm (animal which maintains thermal homeostasis). Silkworm is poikilotherm. It use to respond very quickly to the changes in the environment, particularly to temperature and relative humidity. The condition of higher or lower temperature and humidity, ventilation and quality of food material exert adverse influence on the physiological functions of the silkworm, Bombyx mori (L). The larval

in stars of the silkworm, Bombyx mori (L) become highly susceptible to diseases.

CONCLUSION

The total protein content of silk glands; fat bodies and haemolymph was found improved 61.519 to 114.667; 79.928 to 90.055 and 30.983 to 31.010 percents respectively through feeding the fifth instars of silkworm, *Bombyx mori* (L) (Double Hybrid Race) leaves of mulberry, *Morus alba* (L) treated with aqueous solution of Norfloxacin antibiotics. The Norfloxacin antibiotics use to enhance food consumption and growth through stimulating the metabolic processes within the body of larval instars of silkworm, *Bombyx mori* (L). Norfloxacin antibiotics reduce the occurrence of diseases which causes immense loss to sericulture industry. Antibiotics that are used in clinic to treat infection in mammals showed therapeutic effect in silkworm larvae infected with bacteria, virus or true fungi. The attempt on use of Norfloxacin antibiotics for treating leaves of mulberry, *Morus alba* (L) before feeding the larval instars of silkworm, *Bombyx mori* (L) may provide a convenient and novel way to discover new antibiotics which have not been found using the existing systems of drug screening and pathogen studies.

ACKNOWLEDGEMENT

Twelveth December is birthday of Hon. Sharad Govindrao Pawar (born 12 December 1940). During his long career, he has served as the Chief Minister of Maharashtra on three occasions and held the posts of Minister of Defence and Minister of Agriculture in the Government of India. He leads the society in the Rajya Sabha, the upper chamber of Indian parliament. The present attempt is wishing happy birthday to Hon. Sharad Govindrao Pawar.

Table – 1: Influence of Norfloxacin treated mulberry leaves on Total Proteins in Silk Glands (Salivary Glands); Fat Bodies and Haemolymph the fifth instar larvae of silkworm, *Bombyx mori* (L).

Group Tissue	Untreated Control	Water Treated Control	50 ppm Norfloxacin	100 ppm Norfloxacin
Silk Glands	23.147 (±1.559) 00.000	23.147 (±1.786) 00.000	37.387 (±3.259) 61.519	49.689 (±7.863) 114.667
Fat Bodies	20.223 (±1.805) 00.000	20.223 (±2.514) 00.000	36.387 (±3.956) 79.928	38.435 (±3.678) 90.055
Haemolymph	14.779 (±1.764) 00.000	14.779 (±2.021) 00.000	19.358 (±1.762) 30.983	19.362 (±2.205) 31.010

- Each figure is the mean & three replications.
- Figure in parenthesis with ± sign is the standard deviation.
- Figure below parenthesis is percent change.

* :P<0.05
** :P<0.01
*** :P<0.001

Fig. 1: Influence of Norfloxacin treated mulberry leaves on Total Proteins in Silk Glands (Salivary Glands) the fifth instar larvae of silkworm, *Bombyx mori* (L).

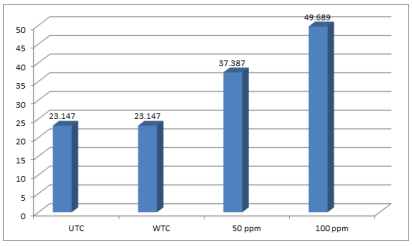


Fig. 2: Influence of Norfloxacin treated mulberry leaves on Total Proteins in Fat Bodies the fifth instar larvae of silkworm, *Bombyx mori* (L).

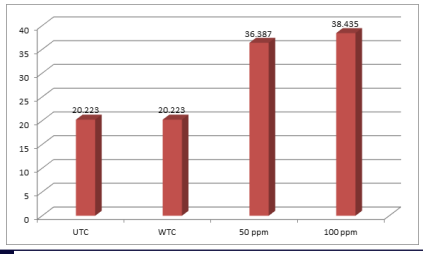
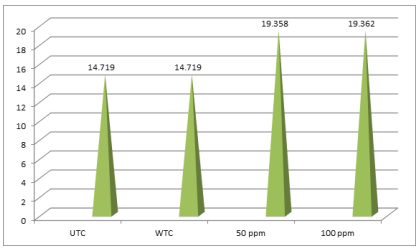


Fig. 3: Influence of Norfloxacin treated mulberry leaves on Total Proteins in Haemolymph the fifth instar larvae of silkworm, *Bombyx mori* (L).



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