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# Effect of *Phyllanthus niruri* on the *Escherichia coli* Infected Silkworm, *Bombyx mori* L.

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The success of silkworm crop depends on many factors. The good quality mulberry leaf, larval spacing, aeration, protection from disease and pests etc. Many silkworm diseases are caused due to the infection of various bacteria, virus, fungus etc. Diseases such as sotto, flacherie and septicemia are caused due to bacterial infection. In the present investigation, flacherie disease is caused by *E. coli.* In this study, the leaf extract containing biologically active ingradients from a medicinal plant, Keelanelli (*Phyllanthus niruri*) was tested for potential in improving the morphology,

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haemocyte count and economic parameters of silkworm, *B. mori.* Haemocyte count was significantly increased in the 5µl *E. coli* inoculated and treated with (6hrs) *P. niruri* to *B. mori* larvae. The economic characters of the silkworm viz., shell weight, cocoon weight and shell ratio were also improved by the topical application of plant extract. This study report suggested that the flacherie disease (*E. coli)* infected silkworm treated with *P. niruri* plant extract provide significant improvement in cocoon parameters.

*Keywords: B. mori; P. niruri; haemocyte count; economic traits.*

## 1. INTRODUCTION

Sericulture industry has great potential to increase the quality and improve the quality of silk fibre. It is rearing domesticated silkworms and the cultivation of mulberry for raw silk production [1]. Silkworm domestication resulting in inbreeding depression and variations in climatic conditions and diseases pose great menace to the sericulture industry [2].

The most prevalent and serious diseases in the silkworm are grasserie, flacherie, muscardine and pebrine caused by the virus, bacteria, fungi and microsporidia respectively [3]. Bacterial flacherie is a common disease of mulberry silkworm. This is a disease of high temperature (above 30°C) and low relative humidity below 80%. It is reported to be responsible for 20-40% of total cocoon loss in Karnataka [4] every year. Bacterial flacherie also known as “Bacterial disease of digestive organ” is caused due to the multiplication of different kinds of bacteria like *Streptococci, Escherichia coli* L.*,* proties group Bacilli in the alimentary canal, disturbing the normal function of the gut, multiply in the digestive tract and destroy the membranous tissue [5]. Antibiotics are widely used in sericulture as a component of bed disinfectants and as the rapeutic applications against bacterial diseases [6]. Antibiotics in silkworm are approved for four different purposes: disease treatment, disease prevention, disease control and for health maintenance or growth promotion [7].

*Ocimum basillicum* L. is an important medicinal plant that contains several antimicrobial compounds [8]. The essential oil of *O.basillicum* contains methyl eugenol, methyl chavicol, monoterpenes and phenylpropanoids which have bactericidal and anti fungal properties. *P.niruri* leaf extracts are one of the organic based management strategies of viral and bacterial diseases of the silkworm [9]. It contains varied range of biomolecules such as alkaloids, phenols, tannins, carbohydrates, proteins and flavonoids having strong antimicrobial property and act against silkworm pathogens [10]. Hence the present investigation was carried out the effect of plant extract such as *P. niruri* on the immunological changes and economic traits in bacterial infected fifth instar *B. mori* was studied.

## 2. MATERIALS AND METHODS

This investigation was carried out on mulberry silkworm, *B. mori*. Disease Free Layings (DFLs) of *B. mori* ($FC_1 \times FC_2$) were obtained from the State Government Sericulture Center at Thenkasi and incubated at 27ºC in ant proof racks at 70-80% humidity. The incubation time was 8 days, during which time, the young caterpillars hatched out. Hatched larvae were transferred to clean bamboo basket (25cm diameter and 5 cm deep) with a scaffolding of paraffin paper after Krishnaswamy, [11]. The second day of fourth instar were selected randomly and grouped into 2 batches for experimental and control was also set up. The experimental silkworms were fed with mulberry leaves smeared with *E. coli* pathogen. The dilution was 5µl and 10µl and it smeared on the mulberry leaves are fed to the silkworms in 1$^{st}$ and 3rd day of larvae. After infection the diseased larvae were treated with *P. niruri* (2ml) plant extract. This extract was sprayed and fed at 6hrs intervals. Haemolymph of infected and treated silkworms were also collected at six hours interval. Each group consisted of 20 silkworms. The control silkworms were maintained with healthy leaves. This same procedure was carried out fifth instar group. All infected and treated silkworms was collected separately and counting of haemocytes was done using the standard method of Jones, [12] and economic traits were recorded after Sonwalker, [13] All the data were analyzed statistically by t-test [14].

## 3. RESULTS AND DISCUSSION

One of the main factors impeding the successful formation of cocoons is the infection of the silkworm colonies by different bacteria, viruses,

fungi, microsporidia, etc. These pathogens are the cause of diseases including grassarie, flacherie, muscardine, and pebrine. microbiological agents and microbiological flacherie are the sources of flacherie disorders, which can be brought on by either bacteria or virues. Flacherie is a specific pathogen that is caused by bacteria.

**Table 1. Amount of haemocyte in the *E. coli* infected *B. mori* larvae**

| Haemocyte | | Pre Haemocyte | Plasma Haemocyte | Granulocytes | Spherulocytes | Oenocytoids |
|---|---|---|---|---|---|---|
| Control | | 53.04±4.06 | 17.00±1.63 | 21.72±1.97 | 23.1±2.06 | 25.3±2.67 |
| IV Instar | 5 | 58.01±4.91 (8.54)* | 26.34±2.38 (35.3)* | 22.60±1.97 (3.88) | 25.12±2.40 (7.77) | 26.80±2.72 (5.59) |
| | 10 | 54.23±4.37 (2.18) | 22.01±1.85 (22.7)* | 20.46±1.85 (-6.14) | 20.00±1.71 (-15.5) | 17.05±1.64 (-48.3) |
| Control | | 53.04±4.06 | 17.00±1.63 | 21.72±1.97 | 23.1±2.06 | 25.3±2.67 |
| V Instar | 5 | 21.20±4.76 (-149) | 15.4±3.67 (-10.3) | 19.8±3.78 (-39.9) | 21.4±2.14 (-7.9) | 11.40±1.07 (-209) |
| | 10 | 19.40±1.80 (-173) | 15.34±3.61 (-10.8) | 17.1±3.90 (-26.9) | 20.3±1.83 (-13.7) | 10.02±0.98 (-252) |

*Percent deviation over control value in parentheses N=20 *Significant*
*All other deviation not significant at P≤0.05 (t-test)*

**Table 2. Enzyme activity of *P. niruri* treated *B. mori* larvae**

| Treatment (hrs) | | Phenoloxidase (µl/min/mg) | Peroxidase (µl/min/mg) | GST-Glutathione Transferase (µl/min/mg) | Esterase (µl/min/mg) |
|---|---|---|---|---|---|
| Control | | 0.30±0.021 | 0.39±0.024 | 0.39±0.031 | 0.58±0.040 |
| 4th stage | 5 µl | 0.35±0.028 (14.28)* | 0.41±0.038 (4.87) | 0.40±0.028 (2.5) | 0.58±0.052 (0) |
| | 10 µl | 0.34±0.025 (11.76)* | 0.39±0.037 (0) | 0.41±0.025 (2.43) | 0.48±0.046 (20.83)* |
| Control | | 0.30±0.021 | 0.39±0.024 | 0.39±0.031 | 0.58±0.040 |
| 5th stage | 5 µl | 0.34±0.031 (11.76)* | 0.41±0.053 (4.87) | 0.41±0.033 (4.87) | 0.61±0.068 (5.06) |
| | 10 µl | 0.33±0.017 (9.09)* | 0.44±0.027 (11.36)* | 0.40±0.026 (2.50) | 0.60±0.041 (3.33) |

*Percent deviation over control value in parentheses N=20 *Significant*
*All other deviation not significant at P≤0.05 (t-test)*

**Table 3. Impact of *E. coli* on the economic traits of *B. mori***

| Haemocyte | | Cocoon weight (mg) | Pupal weight (mg) | Shell weight (mg) | Shell ratio (%) |
|---|---|---|---|---|---|
| Control | | 1320±117.25 | 1110±102.36 | 210±17.35 | 15.90±1.37 |
| IV Instar | 5 | 1230±110.26 (-7.29) | 997±71.88 (-11.3) | 233±21.67 (9.86)* | 18.94±1.68 (16.04)* |
| | 10 | 1210±110.41 (-9.02) | 975±76.07 (-13.77) | 235±21.06 (10.62)* | 19.42±1.29 (18.12)* |
| V Instar | 5 | 1050±95.36 (-25.65) | 850±69.62 (-30.42) | 200±18.73 (-5) | 19.04±1.72 (16.49)* |
| | 10 | 960±80.65 (-37.44) | 801±75.26 (-38.31) | 159±12.79 (-32.02) | 16.56±1.37 (3.98) |

*Percent deviation over control value in parentheses N=20 *Significant*
*All other deviation not significant at P≤0.05 (t-test)*

**Table 4. Economic traits of *P. niruri* treated *B. mori***

| Haemocyte | | Cocoon weight (mg) | Pupal weight (mg) | Shell weight (mg) | Shell ratio (%) |
|---|---|---|---|---|---|
| Control | | 1320±117.25 | 1110±102.36 | 210±17.35 | 15.90±1.37 |
| IV Instar | 5 | 2020±182.27 (34.3)* | 1618±149.75 (30.98)* | 402±42.01 (47.61)* | 19.90±1.68 (20.1)* |
| | 10 | 1360±109.00 (2.92) | 1117±110.52 (0.02) | 243±20.61 (13.56)* | 17.86±1.64 (10.97)* |
| V Instar | 5 | 1660±142.03 (20.4)* | 1380±110.35 (19.44)* | 280±26.09 (24.99)* | 16.86±1.40 (5.69) |
| | 10 | 1670±147.35 (20.65)* | 1400±136.38 (7.14) | 270±18.39 (14.8)* | 16.16±1.17 (1.60) |

*Percent deviation over control value in parentheses N=20 *Significant*
*All other deviation not significant at P≤0.05 (t-test)*

The silkworm, *B. mori,* was found to have flacherie disease in the current experiment. *E. Coli* was the cause of it. The greatest number of prehaemocyte (58.01±4.91) was found in larvae infected with *E. coli* (5µl). The lowest observed value is in oenocyte (17.05± 1.64), 10µl) larvae infected with *E. coli*. There was disagreement with this report [15]. One of the main factors impeding the successful formation of cocoons is the infection of the silkworm colonies by different bacteria, viruses, fungi, microsporidia, etc. These pathogens are the cause of diseases including grassarie, flacherie, muscardine, and pebrine. microbiological agents and microbiological flacherie are the sources of flacherie disorders, which can be brought on by either bacteria or viruses. Flacherie is a specific pathogen that is causedbybacteria.

The silkworm, *B. mori,* was found to have flacherie disease in the current experiment. *E. Coli* was the cause of it. The greatest number of prehaemocyte (58.01±4.91) was found in larvae infected with *E. coli* (5µl). Prohemocytes are involved in larval immune functions like phagocytosis, melanization and encapsulation They can produce many immune proteins like antibacterial peptides [16,17]. The lowest observed value is in oenocyte (17.05 ± 1.64), 10µl) larvae infected with *E. coli*. Bora and Handique (2008) also recorded decreased trend of oenocytes Haemocyte Population Dynamics in Fifth Instar Silkworm *Bombyx mori* L Inoculated with Beauveria bassiana in muga silkworm following Catharanthus treatment. Oenocytes of muga silkworm contain small crystal like structures and electron dense spherules. It has been reported that such crystals in oenocytes contain tyrosine and involved in synthesis of phenoloxidase [18].

The quantity of hemocytes in the hemolymph of *B. mori* larvae fed E. coli during their fifth instar was measured. When *P. niruri* (10µl) plant extract group was applied to fifth instar silkworms, the highest amount of prehaemocyte (21.4 ±2.14) increased. And in the fifth instar larvae treated with *P. niruri* (10µl) after 6 hours, there is a drop in the oenocytoid level (10.02 ± 0.98). Priyadharshini et al., [19] provided support for this report by studying how plant extracts have been shown to be a good substitute for synthetic chemicals due to their antibiotic properties, which are extremely valuable given the ongoing threat of bacterial strains developing resistance to conventional antibiotics.

Table 2 shows the enzyme activity of silkworms infected with *P. niruri* in 5µl and 10µl of each case. Treatment of the fourth instar silkworm larvae with 5µl of E. coli results in a rise in the amount of some enzymes, such as esterase (0.58±0.052). When phenoloxidase (0.34±0.025) is present at a concentration of 10µl in infected silkworms, it is significantly reduced in the fourth instar. Phenoloxidase was responsible for melanization and was present in a latent form, which in turn was activated by a proteinaceous activator upon injury. The regulation mechanism of activation of pro-phenol oxidase which is a zymogen of phenoloxidase plays an important role in defense mechanism [20].

The esterase (0.61±0.068) of *P. niruri* (5µl) treated fifth instar larvae was significantly elevated. These outcomes concur with the findings of Rasool et al. [21]. This may also be

explained by the possibility that allelochemicals and other phenolic compounds work against infections to improve silkworm survival.

Significant differences were found in the cocoon weight, shell weight, and cocoon shell % in the current study. The fourth instar silkworm treated with E. coli showed a significant increase in cocoon weight (1230± 110.26), pupal weight (997± 71.88), and shell ratio (19.42± 1.29) after infection with *E. coli* (5µl).

Significant differences were found in the cocoon weight, shell weight, and cocoon shell % in the current study. The fourth instar silkworm treated with E. coli showed a significant increase in cocoon weight (1230± 110.26), pupal weight (997± 71.88), and shell ratio (19.42± 1.29) after infection with E. coli (5µl). In the group of fifth instar silkworm treated with E. coli, there is a significant rise in the cocoon weight (1050±95.36), pupal weight (850 ±69.62), and shell ratio (16.56±1.37). Prasad et al. [22] have found findings that are similar. According to him, the silkworms that were fed mulberry leaves along with potato leaf extract (from crops that were in the active growth stage) had the highest shell ratio (16.37%), cocoon weight, and larval weight. Additionally, the shell weight and decreased larval mortality and larval duration.

When *P. niruri* (5µl) was applied to 4th instar silkworms for 6 hours, significant changes were seen in their cocoon weight (2020±182.27), pupal weight (1618 ±149.75), and shell ratio (19.90±1.68). When *P. niruri* (10µl) was applied for 6 hours, the group of 5th instar silkworms showed significant increases in their cocoon weight (1670 ± 147.35), pupal weight (1400 ± 136.38), and shell ratio (16.86±1.40).

In yet another study, dusting of ankush vijetha green compounds which are known to contain anti-microbial properties reduced the diseases in silkworm which in turn increased the cocoon yield and cocoon parameters and these results are inconformity with Datta *et al.,* [23]. Similarly, Vijetha dusted batches found more effective in the parameters studied such as larval mortality, cocooning %, single cocoon weight etc., than the Resham keet oushadh dusted batches [24]. The results clearly indicate that the farmers are achieving higher cocoon productivity mainly because of low incidence of diseases and the technology was effectively adopted [25]. Similarily, the *P. niruri* extracts may be helpful in containing falcharie with improvd silkworm health and cocoon characteristics [26-30].

## 4. CONCLUSION

The economic characters of the silkworm viz., shell weight, cocoon weight and shell ratio were also improved by the topical application of plant extract. This study report suggested that the flacherie disease (*E. coli)* infected silkworm treated with *P. niruri* plant extract provide significant improvement in cocoon parameters.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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