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Impact of certain herbal extracts against bacterial disease of silkworm *Bombyx mori* L

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Abstract
Sericulture is an art of rearing silkworm for the production of cocoon which is the raw marterial for the production of silk. Silk is the product of *Bombyx mori* a silkworm found in different zones, temperature and tropical and in different voultinisms. In the present study the diseased silkworms were collected, dissected to separate the contents of foregut, midgut and hindgut. The samples were serially diluted and from which 10^{-6} and 10^{-7} dilutions were plated on to nutrient agar medium. Each observed colony was purified and subjected to identification procedure such as morphological, physiological and biochemical tests. As the result the organisms identified were *Pseudomonas fluorescence, Bacillus cereus, Klebsiella pneumonia,* and *Escherichia coli.* The three fungal species also isolated from the gut of the diseased silkworm such as *Aspergillus flavus, A.tamarii* and *Penicillium sps.* Studies were carried out invitro to assess efficacy of some herbal extracts for observations made during this study revealed that the aqueous extracts of five herbs such as, *Eclipta prostrata, Phyllanthus niruri, Punica granatum*, *Acalypha indica and Cannamomum zeylenica* are effective against these microbes causing flacherie and muscardine disease in silkworm control was maintained. Of the five herbal extracts acalypha indica and cannamomum zeylenica was found to be more effective antibacterial nature than the other, and *Eclipta prostrate* and Phyllanthus niruri was found more effective antifungal property than the other extracts. The present study recommends, plant (herbal) extracts as the effective antibacterial as well as antifungal agent to utilize sericulture operations.

Keywords: *Bombyx mori*, biochemical, flacherie, muscardine

Introduction

Sericulture is an art of rearing silkworm for the production of cocoon which is the raw material for the production of silk. Silk is the most cherished of all textiles even today, along with a wide variety of man-made fibre of inimitable excellence as "Queen of Textiles" (Shelagh, 2004, Welford, 1969) [9]. There are more than twenty countries in which silkworms are reared for commercial purposes. Sericulture has become one of the most important cottage industries in a number of countries like china, Japan, India, Korea, Brazil, Russia, Italy and France. Today china and India are the two main producers, together manufacturing more than 60% of the world population each year. India is the second largest producers of silk production around 20,000 M tons.

The term "sericulture" is used to denote both the industry concerned with silkworm rearing and the science which provides the technical basis for the industries. Archeological and Bibliographical evidences show that the sericulture was practiced in china about 2500 BC (Gnanaraj, 2011) [11]. One of the major constraints in silk production is the diseases in silkworm rearing. Silkworm *Bombyx mori* is domesticated for silk production and are reared in colonial forms. A code of conduct for rearing silkworm is practiced to ensure survival of silkworm and cocooning (Sakthivel, 2012) [12].

Mulberry (Morus sp) the traditional feed for leaf yield and quality in many environments and is spread throughout the world. Mulberry leaves are highly palatable and digestible (70-90%) for herbivorous animals and can also be for to monegastrics, protein content in the leaves and young stems, with a good essential amino acid profile, varies form 15-28 recent depending on the variety, minerals content is high and no antinutritional factors or tonic compounds have been identified. The mulberry silkworm, *Bombyx mori* is of great economic importance as a foreign exchange earner for many silk producing countries of the world. (Krishna swami *et al*., 1992) [17]. All the major pathogenic microbes cause disease in silkworm and the most common among them are nuclear polyhedrosis, bacterial and viral flacherie, muscardine and pebrine.

The efficacy of antibiotics against bacterial pathogens of B. mori has been proved already by several authors (Manimegalai and Chandramohan, 2005) [13]. Though bacterial infection is well managed by antibiotics, the ability of bacteria to aquire resistance to drugs makes it ineffective within a short duration and hence attempts are being made for the use of plant compounds especially the crude aqueous extracts of plants against silkworm bacterial pathogens (Priyadharshini *et al*., 2008) [14].

Medicinal and aromatic plants constitute a major source of natural organic compounds widely used in human health care. These plants produce many compounds as secondary metabolites that have no apparent metabolic, physiologic and structural role in the producer, but often have effects on other organisms. In many cases they are believed to function as biochemical defence (Jain *et al.,* 2004) [10]. The medicinal plants have the potential to be exploited as sources of antibiotics against the bacterial infections of both man and plants.

Recently, Murugan *et al.,* 1998 [1] studied the growth of enhancing effects of some botanical on silkworm which have potential medicinal value (kirthikar and Basu 1981) [5]. The present study was undertaken to find out the possibility of using the extracts of five medicinal plants such *as Eclipta prostrata, Phyllanthus niruri, Punica granatum, Acalypha indica* and *Cannamomum zeylenica* for controlling the

bacterial and fungal pathogens causing flacherie and muscardine diseases in the mulberry silkworm, *Bombyx mori*.

Materials and Methods

Collection of sample and identification of microbial isolates

Healthy and diseased silkworms were collected from V.M chatram, Tirunelvali, Tamilnadu. The samples were surface sterilized with methanol. After sterilization the silkworms were dissected to collect the contents. Serial dilution was carried out from which 10^{-7},10^{-8} dilutions were plated on Nutrient agar medium, Rose Bengal agar medium and Saboroud dextrose agar medium in which healthy silkworm as the control and the plates were incubated for 48 hrs at 37 °C (Govindhan *et al*. 1998) [7].

Identification of microbial isolate

Four bacterial colonies were obtained and subjected for identification by various biochemical test morphological and physiological. (Aneja k.k 1996) [15] From the gut region three fungal species isolated and identified with the help of computerized identificate system in Arvind Eye Hospital laboratory, Tirunelveli.

Herbal Extract Preparation

The powdered herbal products of *Eclipta prostrate, Phyllanthus niruri, Punica granatum*, acalypha indica and *Cannamomum zeylenica* were obtained from the siddha medical shop, Tirunelveli. Ten grams of the powder was weighed out and kept in a conical flask soaked with acetone for 6 hrs under air tight condition. The content were then stirred for an hour in magnet stirrer and filtered through a filter paper. The residual extract was collected in a flask and the solvent was allowed to evaporate at room temperature. The extracts were then stored at 4 ^{0}C until use. The resultant residue wasthen made up to required volume using double distilled water (Karthikairaj *et al*., 2014). Similarly, the aqueous extract of the herbal powder was collected using distilled water.

Antimicrobial test

Sensitivity to herbal extracts was tested for selected pathogens isolated from diseased worms. The powdered plant material was extracted (Aqueous extract), the air dried Nutrient agar, Rose Bengal agar and Saboroud dextrose agar plates were taken and 0.1 ml test organisms were swabbed. Different concentration (10%, 25% and 50%) of the extracts impregnated disc were placed in the plate, sterile water was used as control. Plates were incubated at room temperature for 2 days. After incubation the zone of inhibition was measured.

Result

Four colonies isolated and identified using biochemical tests were tabulated in table 1 fungal species also isolated, in a sample of 20 diseased worms about 18 had *Aspergillus sps.* This indicates that Aspergillosis is a predominant fungal organism affecting *Bombyx mori*. In order to control the bacterial and fungal infection the sensitivity of isolated microbes from the silkworm was tested against different plant (herbal) extracts of different concentrations (10%, 25%, and 50%) Table 2 and 3.

Discussion

In the present investigation the pathogenic microbes isolated from the disease affected larvae, four species of bacteria were isolated and 3 species of fungi also isolated and identified. The result are in confirmly with the earlier report of (Murugan *et al.,* 1998) [1] who reported a decrease in cocoon weight when pathogenic cultured treatment were tested. The impact was high in silkworm larvae treated with B. thuriensis. (Nishiit sutsuj *et al.,* 1979) [4], Endo *et al.,* 1979 [4]. Yungen and Bharthi (2001) [3] also reported that certain bacteria like *S. marcescens* were found to infect the haemocoel causing the pathological changes in the blood. The major fact responsible for bacterial flacherie was the rearing conditions. The rise in temperature and humidity in rearing place leads to dysfunction of alimentary canal which encourages flacherie (Nataraju *et al*., 2005) [8]. Herbal medicine that could eliminate the fungal pathogens were studied. As the plant Cassytha capillaries is reported as an antifungal agent (Ranjit singh *et al.,* 2004) [16] it was tried against the fungal pathogen isolated from *Bombyx mori*. The growth and development of silkworm in influenced constantly by factors operating within and outside of the body. (Murugan *et al.,* 1998) [1]. Shymala *et al.,* (1956) [2] reported that silkworms reared on mulberry leaves sprayed with water alone consumed. The antimicrobial properties of bacterial isolates to five herbal extracts was in order, *Acalypha indica, Cannamomum zeylenica, Punica granatum, Eclipta prostrate and phyllanthus niruri*. The antimicrobial properties of fungal to four plants (herbal) extracts was in order to *Eclipta prostrate and Phyllanthus niruri, Acalipha indica, Cannamomum zeylenica and Punica granatum.*

The present study reported that differential sensitivity of bacterial and fungal to herbal extreact will reduce the pathogenicity of microbes in the silkworm *Bombyx mori* L. are likely to throw much light on the possibility of using such extracts as a prophylactic measure during silkworm rearing to improve silk production and protecting the cocoon crops against the microbes.

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