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Use of Plant Extracts for the Control of Flacherie Disease in Silkworm, *Bombyx mori* L. (Lepidoptera: Bombicidae)

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Abstract: Sericulture is a unique field of agriculture where silkworms are reared on an extensive scale to produce the fine material of clothing. In this study, the leaf extracts along with biologically active principles from three medicinal plants such as Pakarkai (*Momordia charendia*), Thulasi (*Ocimum sanctum*) and Nilavembu (*Andrographis paniculata*) were tested for their potential in improving silk production. The incidence of diseases at the time of silkworm rearing severely reduces the production of silk. Among the various diseases, bacterial flacherie is very common in our area and has been reported to be caused by bacteria like *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Serratia* and *E. coli.* This study was carried out to find out the possibilities for controlling the diseases in *Bombyx mori* using the extracts of above medicinal plants. The infected haemolymph of *B. mori* was collected and the pathogenic bacteria which caused flacherie disease were isolated and cultured in nutrient agar plates. Antibacterial activity of the medicinal plant extracts were tested in Muller Hinton agar plates. The antibacterial activity of medicinal plant extracts were tested in both aqueous and alcoholic extracts with the concentration of 50, 100 and 150 µl. The sensitivity rate was much effective in both aqueous and alcoholic extracts of *A. paniculata*, when compared to *M. charentia* and *O. sanctum* and it can be used for the control of flacherie disease and thereby improve the silk production.

Key words: Sericulture · *Bacillus* · Medicinal plants

INTRODUCTION

Cultivation of silkworm for the production of fiber is called as "Sericulture". Silk is considered to be 'Queen of Fiber which is proteinecious in nature. Archeological and Bibliographical evidences show that the sericulture was practiced in China about 2500 BC [1]. In 12th century BC, it expanded outside China as Mulberry seed and silkworm egg were smuggled out. During 1920-30's world silk yield exceeds 60, 000 - 70,000 tons, while in 1950 - 60's, it decreased to 30, 000 tons. After 1970, showed rapid development and has become the vocation of small agricultural families in popular developing countries like, China, India, Vietnam and Thailand. Current annual production of silk is about 4, 96, 000 tons. India is 2nd largest producer of silk and the fact that nearly 6 million Indian's are involved in sericulture [2]. The losses occur mainly during the final stages of silkworm rearing resulting considerable energy and money loss. Bacteria such as *Streptococcus faccalis*, *Streptococcus liquifacions*, *Staphylococcus acire, Staphylococcus epidermidis* and *Bacillus* sp. are commonly reported to cause flacherie in silkworm. The mulberry silkworm, *Bombyx mori* L. known for producing silk cocoons are affected by viral, fungal and protozoan pathogens among which bacterial pathogens independently cause cocoon loss to the tune of 75 per cent [3]. The efficacy of antibiotics against bacterial pathogens of *B. mori* was proved [4]. The extracts of *Tridax procumbens* possess antiseptic, insecticidal and parasiticidal properties [5]. Perhaps a good understanding of the disease, its mode of transmission and control of the microbial pathogens causing silkworm diseases using plant extracts could be another possibility of improving silk productivity by reducing the incidence of silkworm diseases. To identify locally available herbs that can be exploited for use in sericulture practice. To assess the antibiotic potential of the herbal extracts on the microbial pathogens of the silkworm.

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MATERIALS AND METHODS

The addresses of farmers practicing sericulture in Srivilliputtur region were obtained from the cocoon market of Tamilnadu state Sericulture department of Sericulture in Srivilliputtur. The information on the incidence of diseases was collected by visiting some of these farmers while they were rearing silkworm. The diseased and normal silkworms at their fifth instar were collected from the sericulture unit from nearby villages. They were transported quickly to the laboratory under cool conditions and sorted out. Their haemolymph was then collected separately in a pre-cooled centrifuge tube. It was then centrifuged at 3000 rpm for 15 minutes in order to separate the haemocytes and the clean supernatant comprising only the haemolymph is transferred to the storage tube. The haemolymph from the diseased and normal silkworms were thus collected separately and kept stored at -20°C until analysis.

The powdered herbal products of *Andrographis paniculata* (Nilavembu), *Ocimum sanctum* (Thulasi) and *Momordica charantia* (Bitter guard) were obtained from the Siddha medical shop. Ten grams of the powder was weighed out and suspended in 50 ml of distilled water in a clean conical flask. The contents were kept agitated for one hour in a magnetic stirrer. Then the extract was filtered using a clean burette containing glass wool and tissue paper as the filters [5]. The extraction was continued with the addition of another 50 ml of distilled water in to the burette until clear filtrate trickled down. The filtered extract was again agitated and concentrated using magnetic stirrer cum heater and made up to a known volume of 100 ml with distilled water. Likewise, alcoholic extract was prepared from 10 grams of herbal powder and kept stored in the freezer until use. As a result 1 ml of the concentrate contained the extract from 100 mg of the herbal powder.

The microbial analysis included the isolation and culture of the microbes using streak plate method [6]. The morphological characterization of the microbes in the culture was then carried out from their shape and motility by Gram stain method and Hanging drop method. The effectiveness of the herbal extracts prepared was then carried out by Turbidimetry analysis of the culture in a nutrient broth both before and after the addition of the herbal extracts at varying quantities. Similarly the efficacy of the herbal extracts on the microbial pathogen causing the falcherie disease in the silkworm, *B. mori* was also assessed using another simple technique with the use of filter paper discs impregnated with the herbal extracts and estimating the zone of inhibition.

RESULTS AND DISCUSSION

The information collected from the total of 9 farmers practicing sericulture in nearby villages belonging to Srivilliputtur Taluk of Virdhunagar District gave an idea about the incidence of silkworm diseases in this area (Table 1). Almost all the farms showed the prevalence of the flacherie disease while muscardine and grasserie were noticed in only two farms that to a minimum extent. Data made available from various sources on the incidence of silkworm diseases in tropical regions put the average annual loss to be around 30 per cent. If the diseases are controlled below the economic threshold level then there will be an increase of 25 per cent silk production without any increase in the area under mulberry sericulture [7]. Aqueous and alcoholic extracts of *Tridax procumbens* are comparatively much effective against the bacterial and fungal pathogens causing diseases in the silkworm *B. mori.* The changes in the optical density of the nutrient broth inoculated with the bacterial culture prepared from the haemolymph of the diseased silkworm with the addition of herbal extracts were noted to find out the susceptibility of the bacterial culture to the effect of the herbal extracts both with the increase in concentration [8]. The inventory thus revealed that flacherie is the most common silkworm disease affecting the sericulture operation in this region. Alcoholic extracts of *Andrographis paniculata* showed increasing in anti-microbial property than the aqueous extract of *Andrographis paniculata.* Concentration of 50µl, 100µl and 150µl of aqueous extract of *Andrographis paniculata* was used to control the microbes in the culture plate. The maximum resistant was observed in 150µl zone of incubation (11mm) followed by 100µl zone of incubation (10mm) and 50µl(8mm). Concentration of 50µl, 100µl and 150µl of alcoholic extract of *Andrographis paniculata* was used to control the microbes in the culture plate. The maximum resistant was observed in 150µl zone of incubation (12.1mm) followed by 100µl zone of incubation (11.3mm) and 50µl zone of incubation (7.3mm) Table 2 and 3. The quantity and quality of dietary protein diet has long been considered to be important in the insect growth. The mulberry silkworm, *B. mori* L. has been domesticated for over 4000 years and this domestication

Table 1: Observation on the incidence of decreases in the some sericulture farms near Sivakasi

S.No	Name of the farmer	Location of the farm	Mulberry variety cultivated	Silkworm disease noticed
1.	Sudha Nursery	Krishnakovil	MR-2	Flacherie,
2.	Gowndar thoopu	Poovani	MR-2	Flacherie,
3.	Rengasamy	Krishnakovil	MR-2	Flacherie,
4.	Cinnathambi	Ramalingapurm	MR-2	Flacherie,
5.	Gurusamy	Ramalingapurm	MR-2	Flacherie,
6.	Martin	Nachirpatti	MR-2	Flacherie, Muscardine
7.	Govindammal	Ramalingapurm	MR-2	Flacherie, Muscardine
8.	Nallasivam	Ramalingapurm	MR-2	Flacherie, Grassarie
9.	Perumal	Ramalingapurm	MR-2	Flacherie, Grassarie

Table 2: Zone of inhibition observed with the increasing concentrations (50 µl, 100 µl and 150 µl) of aqueous extracts of *Momardica charantia*, *Ocimum sanctum* and *Andrographis paniculata.*

	Concentrations (µl) Zone inhibition (mm)		
Name of the Plant	50	100	150
Momardica charentia	7.33±0.577	7.67±0.115	8.83±0.0577
Ocimum sanctum	7.17±0.0577	7.33±0.0577	8.3±0.0577
Andrographis paniculata	8.83±0.0577	9.77±0.0577	10.73±0.0577

Table 3: Zone of inhibition observed with the increasing concentrations (50µl, 100µl and 150µl) of alcoholic extracts of *Momardica charantia*, *Ocimum sanctum* and *Andrographis paniculata,*

	Concentrations (µl) Zone inhibition (mm)		
Name of the Plant	50	100	150
Momardica charentia	7.83±0.0577	8.33±0.0577	9.43±0.0577
Ocimum sanctum	6.77±0.0577	8.13±0.0577	8.93±0.0577
Andrographis paniculata	9.33±0.0577	11.27±0.0577	12.13±0.0577

and continuous rearing of silkworm has resulted in loss of certain wild characters including their tolerance to microbial infections, as a result the silkworm becomes most susceptible to infection by major pathogenic groups leading to diseases and crop losses at farmer's level [9]. Dietary supplementation of the leaf, flower and pod extracts of *Moringa oleifera* elicited varied responses in the final instar larvae of *B.mori* [10]. Turbidametric analysis carried out with the help of colorimeter showed lesser turbidity in the medium containing 1ml of plant extract with increasing concentration showed greater inhibiting and decreased vice versa among the alcoholic and aqueous extract of *Andrographis paniculata.* Disinfection and maintenance of hygiene during rearing are the essential factors for preventing of silkworm diseases. Silkworm extrudes pathogens in the rearing environment to form the source for the spread of diseases. This study was only to find out the possibilities to control the flacherie disease in the silkworm using the aqueous and alcoholic extract of *A. paniculata*, *O. sanctum* and *M. charantia.*

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