Efficacy of some herbal extracts on microbes causing flacherie disease in mulberry silkworm, *Bombyx mori* L.

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ABSTRACT

Sericulture is an ideal enterprise which fits into the socio-economic fabric of India. Improvements in the quantity of silk cocoons and quality of silk depend on the quality of mulberry leaves given to the silkworm and can be achieved by the supplementation of mulberry leaves with extra nutrients. However, the mulberry silkworm, *Bombyx mori* L. is prone to infection of several microbial pathogens resulting heavy loss in the silk output. Among the major diseases of the silkworm, bacterial flacherie have been found to be very common in our region and investigation of the haemolymph collected from the diseased silkworm revealed that the cultured colony was that of *Staphylococcus sp.* according to its morphological characters. Assessment of the antibacterial activity of some herbal extracts prepared from *Acalypha indica, Leucas aspera* and *Ocimum sanctum* for the containment of these microbes was then made in our laboratory. It was observed that both aqueous and alcoholic extracts of these herbs proved to be effective against *Staphylococcus sp.* infecting mulberry silkworm *Bombyx mori* L. In this context, the alcoholic extracts produced maximum zone of inhibition against these microbes in the culture plate than the aqueous extracts and that the extracts of *Leucas aspera* are very effective against these microbial pathogens followed by the extracts of *Ocimum sanctum* and *Acalypha indica*. It is thus inferred that such effects of herbal extracts could be exploited to control microbial pathogens at the time of silkworm rearing and to get improved silk production.

MS History: 10.10.2013 (Received)-11.05.2014 (Revised)-03.06.2014 (Accepted)

Key words: Sericulture, Medicinal plants, Microbial pathogens and Flacherie disease.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L., known for the production of silk cocoons are generally affected by viral, fungal and protozoan pathogens among which bacterial pathogens alone cause cocoon loss to the tune of about 75 per cent (Sidhu and Singh, 1968). The efficacy of antibiotics against bacterial pathogens of *B. mori* has been proved already by several authors (Manimegalai and Chandramohan, 2005). Though bacterial infection is well managed by antibiotics, the ability of bacteria to acquire 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). India has a very rich floral diversity yet this potential is not tapped to the fullest extent. Researchers have come out with different sources and classes of bioactive compounds extracted from plants mostly to heal several human ailments (Thirumalaisamy *et al.*, 2009). In the present study, the efficacy of the extracts of three common herbs in controlling the microbial pathogens causing flacherie disease in mulberry silkworm was assessed to identify a simple and effective solution for the control of silkworm diseases.

MATERIALS AND METHODS

Acalypha indica (Kuppaimeni), *Leucas aspera* (Thumbai) and *Ocimum sanctum* (Thulsi) have been selected as the test plants for this study. Leaves of these plants were collected individually from our college campus ($9^{o;}$ 28' N and$77^{o;}$ 48' E at 106 MSL) during the month of November, 2012. The collected leaf samples were washed in running tap water, rinsed with sterile distilled water and shade dried. The shade dried plant samples were then powdered in electric blender at slow speed, sieved and kept stored in desiccators. Known quantity (10g) of this herbal powder was taken in a conical flask and kept soaked for 6 hrs in acetone under air tight condition. The contents were the 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°C until use. The resultant residue was then made up to required volume using double distilled water. Similarly, the aqueous extract of the herbal powder was collected using distilled water.

A visit was made to six sericulture farms in nearby villages to assess the incidence of diseases in the silkworm reared over there. Diseased silkworms were sampled from these farms and brought to the laboratory in separate sterile polythene bags. Haemolymph of the diseased silkworms was then collected in pre-chilled sterile eppendorffs and kept under refrigeration. The microbial analysis included the isolation and culture of the microbes using spread plate method (Khatune, 2000). The morphological characterization of the microbes in the culture was then carried out from their shape by Gram staining method (NCCLS, 2000).

Well Diffusion Analysis

Screening of antibacterial activity of the herbal extracts was performed by well diffusion technique. For this, the Mueller Hinton agar plates were seeded with 0.1 ml of the standardized inoculums of each test organism. The inoculums were spread evenly over plate with sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 6 mm diameter was used to cut uniform wells on the surface of the MHA and 50,100 and 150 µl of each leaf extract was introduced in the well. Respective solvent was used as control. The inoculated plates were incubated at 37°C for 24 hours and the zone of inhibition was measured to the nearest millimeter.

Turbidimetry Analysis

Turbidimetry analysis was also carried out for the plant extracts (Palaombo and Semple, 2001). For this, the standard nutrient broth was prepared and its optical density was read in the spectrophotometer at 550 nm as the initial and 10 ml of the nutrient broth was taken in a clean test tube, inoculated with the bacterial culture isolated from the haemolymph of the diseased silkworm using inoculation loop and a control without bacterial culture was maintained. Similarly, Mueller Hinton broth was taken in different test tubes and was inoculated with bacterial culture. All these test tubes were incubated at 37^{o}C for overnight. They were taken out for the experiment and different volumes of the herbal extract was added into the cultures followed by the addition of distilled water to maintain the volume equal in all test tubes. The test tubes were kept in a shaker for a while and the optical density of the cultures was measured in the spectrophotometer at 550 nm. They were then kept incubated at 37^{o}C and the optical density was measured again at different time intervals. The decrease in the optical density of the culture was taken as an indication of the effectiveness of the herbal extracts against the growth of the bacterial pathogens.

RESULTS AND DISCUSSION

The information collected from the total of 6 farmers practicing sericulture in nearby villages belonging to Srivilliputtur Taluk of Virudhunagar District in this State gave an idea about the incidence of silkworm disease in this area (Table 1).

Table 1. Observation on the incidence of diseases in the sericulture farms in nearby villages

Name of the Farmer	Location	Predominant disease noticed
Rukmani	Atthikulum	Flacherie
Malar	Krishnankovil	Flacherie
Rajesh	Kaariapatti	Flacherie
Sarguru	Ramalingapuram	Flacherie
Murugan	O. Kovilpatti	Flacherie
Navaneethakrishnan	Acchamthavilthan	Flacherie

Diseases of the silkworm, *Bombyx mori* seriously affect their cocoon production (Watanabe, 1986). Disease incidence occur in all larval instars but more commonly in the 4th and 5th instars during all seasons and cause 20-50% cocoon crop losses in India (Khurad *et al.*, 2004). The survey made for this study revealed that the bacterial diseases collectively called as flacherie are more prevalent in silkworms reared in this area. Further, the prime organism causing the flacherie disease at the time of this survey was identified as *Staphylococcus sp.* Taumanoff and Vago, 1951 and Chitra *et al*., 1973.

Table 2. Zone inhibition formed by the aqueous extracts of *Acalypha indica, Leucas aspera* and *Ocimum sanctum* against the silkworm pathogen, *Staphylococcus sp.*

Plant Name	Concentration of aqueous extracts (µl) Zone Inhibition (mm)					
	50(µl)		100(µl)		150(µl)	
	Circumference (mm)	Area (mm^2)	Circumference (mm)	Area (mm^2)	Circumference (mm)	Area (mm^2)
A.indica	15.8 ± 2.3	39.8 ± 11.3	24.0 ± 4.8	92.4 ± 33.1	28.8 ± 4.8	133.2 ± 42.8
L. aspera	28.3 ± 6.1	131. 3 ± 56.6	33.3 ±1.7	176.7±17.7	37.1±1.2	218.7±14.7
O.sanctum	19.3±4.1	58.2 ± 25.3	24.7±3.3	96.6±26.7	29.3±4.48	137.1±41.3
***Staphylococcus* sp.**						
A.indica	21.2±2.6	69.1±17.4	28.1 ±4.6	121.9±41.1	29.5±4.5	123.9±42.1
L. aspera	32.2 ±2.9	162.0 ±28.5	36.1±2.3	204.7±28.6	39.1±1.3	241.5±15.5
O.sanctum	28.4±7.9	131.8±75.5	30.8±3.2	149.6±29.5	32.2±3.1	164.4±33.1

had also reported the incidence of flacherie because of this microbe. Three herbal extracts when tested through well diffusion techniques for their efficacy to contain the microbes showed varied zone of inhibition with the silkworm pathogen *Staphylococcus* sp. at different concentrations. The results obtained from three different concentrations of the aqueous and acetone extracts are given Table 2 and 3 respectively. The circumference and area of the zone inhibition recorded was higher with the acetone extract of *L. aspera* followed by the *O.sanctum* and *A.indica*. The acetone extracts exerted comparatively more antibacterial activity than their aqueous counterparts. The aqueous and acetone extracts of *L. aspera* were found to be more effective against the microbial pathogens, when compared to that from *A. indica* and *O. sanctum*.

Similar growth inhibition zone assays done earlier using herbal extracts on *B. mori* larvae had

suggested that herbal extracts show antimicrobial activity against some pathogenic microorganisms including Gram positive bacteria such as *S. aureus* and *B. subtilis* and Gram-negative bacteria such as *E. coli* (Whitt and Salyers, 2002; Sivakumar *et al*., 2012). In this context, Lachowiez *et al*. (1998) had observed that the essential oils from *Ocimum* species were predominantly associated with the main constituent's linalool and methyl chavicol and that these were responsible for bactericidal effect on gram-positive and gram-negative bacteria.

From the turbidity analysis it was noticed that the extracts of *L. aspera* keep the growth and multiplication of the bacterial inoculums under much control than the extracts of *A. indica* and *O.sanctum* (Table 3). Over the last 20 years, a large number of plant species have been evaluated for their antimicrobial activity and many natural compounds derived from plant and their crude extracts have been proved to be protective against the toxicity of many chemicals (Shyamprasad *et al*., 2002; Sandhya *et al*., 2006). Chloroform bark extracts of *Thuja orientalis* proved effective against *Staphylococcus aureus* and *Bacillus thuringiensis* infecting *B. mori*. Similarly, chloroform leaf extract of *Aegle marmelos* was effecting in managing *B. thuringiensis* might due to the presence of antibacterial compounds stigmast- 3- 5n-ol in leaf extract of *A. marmelos* and phenanthrene carboxylic acid in the bark extract of *T. orientalis* (Manimegalai *et al*., 2010).

Table 3. Turbidity assay on the effect of the extracts of *Acalypha indica, Leucas aspera* and *Ocimum sanctum* against the silkworm pathogen, *Staphylococcus* sp.

Name of the plant extract	Optical Density measured at 550 nm				
	Time (Hours)	Control	1ml	2ml	3ml
Acalypha indica-Aqueous	0	1.28	0.82	0.81	0.63
	1	1.33	0.97	0.84	0.66
	2	1.41	1.03	0.90	0.72
	3	1.44	1.12	0.94	0.86
	4	1.53	1.20	1.01	0.88
Acalypha indica-Acetone	0	1.28	0.80	0.78	0.57
	1	1.33	0.92	0.80	0.59
	2	1.41	0.98	0.83	0.66
	3	1.44	1.05	0.85	0.70
	4	1.53	1.08	0.90	0.79
Leucas aspera-Aqueous	0	1.28	0.82	0.67	0.56
	1	1.33	0.84	0.69	0.59
	2	1.41	0.97	0.73	0.66
	3	1.44	0.99	0.81	0.68
	4	1.53	1.08	0.98	0.74
Leucas aspera- Acetone	0	1.28	0.73	0.57	0.49
	1	1.33	0.77	0.43	0.45
	2	1.41	0.83	0.58	0.39
	3	1.44	0.87	0.71	0.52
	4	1.53	1.01	0.93	0.73
Ocimum sanctum-Aqueous	0	1.28	0.88	0.83	0.50
	1	1.33	0.92	0.89	0.58
	2	1.41	1.06	1.06	1.18
	3	1.44	1.09	1.17	1.28
	4	1.53	1.18	1.21	1.34
Ocimum sanctum- Acetone	0	1.28	0.87	0.81	0.42
	1	1.33	0.99	0.93	0.45
	2	1.41	1.07	1.13	1.17
	3	1.44	1.13	1.24	1.29
	4	1.53	1.23	1.27	1.32

Among the three extracts used in the present study, the extracts of *A.indica* were reported to show antimicrobial activity against four bacterial and fungal strains that is comparable to the effect of antibiotic and antifungal drugs (Somchit *et al*., 2010) and *O.sanctum* (Kishore *et al*., 1982).

The present study that attempted to assess the influence of herbal extracts on the microbial pathogens causing silkworm diseases convincingly reveal that the extracts of *L. aspera* is superior to the extracts of the two plants mentioned earlier in its antimicrobial activity. In the light of the above, it is inferred that screening of herbal extracts and their efficient use while rearing silkworm could control the incidence of silkworm diseases and thereby improve silk production.

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