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Thiruvithancode, on 13-10-2021 and presented a paper entitled **Effect of Medicinal Plant Extract**
on the **Bacterias of Bacterial Infection in the Mulberry Silkworm, Bombyx Mori**.....

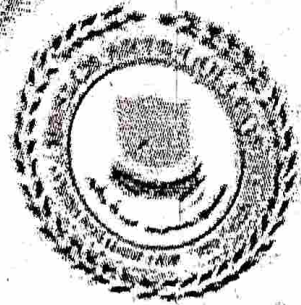
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Theme: **REVELATIONS OF SUBMERGED CONTINENT**

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Date: 13-10-2021



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MUSLIM ARTS COLLEGE, THIRUVITHANCODE

Pin - 629174, Tamil Nadu, India.

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EFFECT OF MEDICINAL PLANT EXTRACT ON THE PATHOGENS OF BACTERIAL INFECTION IN THE MULBERRY SILKWORM, BOMBYX MORI L.

ZOOLOGY-14

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Abstract

Sericulture is an art of rearing silkworm for the production of cocoon which is the raw material for the production of silk. In the present study the diseased silkworms were collected, dissected to separate the contents of midgut. The samples were serially diluted and from which 10^{-7} and 10^{-8} 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 *Streptococcus* sp. and *Staphylococcus* sp. Herbal extracts of *Coccinlagrandis* was found to be more effective antibacterial nature.

Keywords: *Coccinlagrandis*, silkworm, midgut, *Staphylococcus* sp. *Streptococcus* sp.

Introduction:

Bombyxmori L. has an economic importance because of the commercial value of its silk. Therefore several trials for developing the biological processes and improving the qualitative and quantitative characters of silk yield took place (Taha, 2002). Bacterial sheath is a common disease of mulberry silkworm. The aetiology of bacterial diseases is not fully understood because of the multiplicity of bacterial types involved in bacterial infections (Choudhury *et al.*, 2002). Bacterial infection is more prevalent in the silkworm. *B. mori* among the protozoan, bacterial, viral and fungal pathogens and constitutes about 60-70% of total silk crop loss in Japan and India. The bacterial infection rooted to growing silkworm larvae via biotic and abiotic factors such as by transovarial transmission of *B. subtilis* reported earlier and caused mortality due to growing infection in late phase of silkworm larvae and factors such as air, water, dust and improper handling also spread the infections. *B. thuringiensis* also linked to produce endotoxin causing mortality due to damage of gut lining and paralysis in response to starvation reported during several investigations (Selvakumar *et al* 1999)

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 (Priyandharshini *et al.*, 2008). The medicinal plants have the potential to be exploited as

sources of antibiotics against the bacterial infections of both man and plants (Jain *et al.*, 2004).

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). The medicinal plants have the potential to be exploited as sources of antibiotics against the bacterial infections of both man and plants. The medicinal plants have the potential to be exploited as sources of antibiotics against the bacterial infections of both man and plants. The present study was undertaken to find out the possibility of using the extracts of medicinal plant *Coccinlagrandis* for controlling the bacterial pathogens causing septicemia diseases in the mulberry silkworm, *B. mori*.

Materials and methods

Collection of sample and identification of microbial isolates

Healthy and diseased silkworms were collected from Konam Sericulture department, Kanyakumari district, Tamilnadu. The samples were surface sterilized with methanol. After sterilization the silkworms were dissected to collect the midgut. Serial dilution was carried out from which 10^{-7} 10^{-8} dilutions were plated on Nutrient agar medium. (Govindhan *et al.* 1998).

Identification of microbial isolate

Two bacterial colonies were obtained and subjected for identification by various biochemical test, morphological and physiological. (Aneja, 1996)

Herbal Extract Preparation

The powdered herbal products of were obtained from the wildha medical shop, Kanyakumari Ten grams of the powder was weighed and kept in a conical flask washed with water for a few under air tight condition. The content were then stirred for an hour in magnetic stirrer and filtered through a filter paper. These filtrates were evaporated in a rotary evaporator. The extracts were then stored at 4 °C until use. The remaining residue washed made up to required volume using double distilled water (Karthikraj et al., 2014).
Antibacterial activity
Result

The effectiveness of these extracts on the microbial pathogen was then checked by line of inhibition test and turbidimetry analysis. Line of inhibition test was carried out in petriplate on Muller-Hinton agar medium. Different concentrations (10%, 20% and 30%) of the extract were placed in the plate, wells were made as needed. Plates were incubated at 37°C temperature for 2 days. After incubation the zone of inhibition was measured.

Table 1. Morphological and biochemical characters of isolated bacterial species

Physical/chemical characters	Staphylococcus	Streptococcus
Cultural characters	Mucoid colonies	Mucoid with central portion raised colonies
Elevation	convex	convex
Colony margin	Regular	Irregular
Spore	-	+
Shape	circular	Rod
Opacity	opaque	opaque
Gram stain	+	+
Colour	White	Slight pink
Mannitol	+	-
Motility	-	-
Glucose	+	+
Lactose	+	+
Sucrose	+	-
H ₂ S production	-	+
Ona production	-	-
Peptone water	-	+
Simmon water	+	-
Oxidase	+	+
Catalase	+	+

Table 2: Antibacterial activity

Concentration	Zone of inhibition (mm)		
	25µl	50µl	100µl
Control			
(Streptomycin)	11.3±1.0	12.45±0.56	14.65±0.45
Staphylococcus sp.	10.64±0.94	11.34±0.87	12.32±0.58
Streptococcus sp.	9.32±0.46	10.93±0.67	11.7±0.75

The powdered herbal products of were obtained from the Siddha medical shop, Kanyakumari. Ten grams of the powder was weighed out and kept in a conical flask soaked with water for a hrs under air-tight condition. The content were then stirred for an hour in magnet stirrer and filtered through a filter paper. These filtrates were evaporated in a rotary evaporator. The extracts were then stored at 4 °C until use. The resultant residue was then made up to required volume using double distilled water (Karthikalinget *et al.*, 2014).

Antibacterial assay
Result

The effectiveness of these extract on the microbial pathogens was then assayed by Zone of inhibition test and Turbidity analysis. Zone of inhibition test was carried out in petriplates on Muller-Hinton agar medium. Different concentrations (11%, 25% and 51%) of the extracts impregnated discs 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.

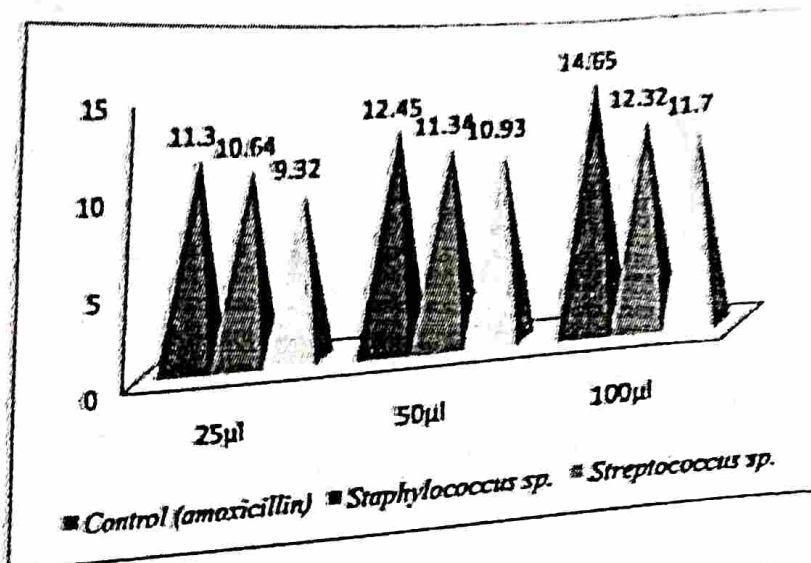
Table 1. Morphological and biochemical characters of isolated bacterial species

Physical/chemical characters	<i>Staphylococcus</i>	<i>Streptococcus</i>
Cultural characters	Mucoid colonies	Mucoid with central portion raised colonies
Elevation	convex	convex
Colony margin	Regular	Entire
Spore	-	+
Shape	circular	Rod
Opacity	opaque	opaque
Grams stain	+	-ive
Colour	White	Slight pink
Mannitol	+	-
Milkly	-	-
Glucose	+	+
Lactose	+	+
Sucrose	+	-
H ₂ S production	-	+
Gas production	-	-
Peptone water	-	+
Simmon water	+	-
Oxidase	+	+
Catalase	+	+

Table 2: Antibacterial activity

Concentration	Zone of inhibition (mm)		
	25µl	50µl	100µl
Control			
(Sirenomycin)	11.3±1.0	12.45±0.56	14.65±0.45
<i>Staphylococcus</i> sp.	10.64±0.94	11.34±0.87	12.32±0.58
<i>Streptococcus</i> sp.	9.32±0.46	10.93±0.67	11.7±0.75

Figure 1:



Results of the biochemical and physiological characterization of the most frequent isolated strains *Staphylococcus* and *Streptococcus* are presented in Table 1. In this study, from the 4th instar larval midguts of infected silkworm larvae two bacteria species were isolated and identified. The species were isolated using Colony morphology such as size, shape, colour and margins of the isolated colonies and gram staining was considered for morphological characterization of bacterial isolates. When the plant extracts were tested against the silkworm pathogen, *Staphylococcus sp.* the maximum antibacterial activity was observed in *Cocciniagrandsis* extract with 20.32 ± 0.58 mm zone of inhibition at 100 µl of aqueous extract. The *Cocciniagrandsis* aqueous plant extract against *Streptococcus sp.* maximum zone of inhibition 21.7 ± 0.75 mm was observed in 100 µl concentration.

Discussion

In the present investigation the pathogenic microbes isolated from the disease affected larvae, two species of bacteria were isolated and species identified. The result are in confirmly with the earlier report of Previous studies reported isolation of bacteria of different genera from diseased silkworm, such as *Staphylococcus albus*, *Staphylococcus aureus* (Priyadharshini et al. 2008) and *Streptococcus faecalis* (Patil 1994), *Bacillus thuringiensis* (Nataraj et al. 1991). (Murugan et al., 1998) who reported a decrease in cocoon weight when pathogenic cultured treatment were tested. The impact was high in silkworm larvae treated with *B. thuringiensis*. (Nishitsutsu et al., 1979).

The rise in temperature and humidity in rearing place leads to dysfunction of alimentary canal which encourages flacherie (Nataraj et al., 2005). The control and prevention of bacterial infection during silkworm rearing helps to increase the silk

productivity by preventing the mortality to a great extent. Various treatments in practice are of synthetic chemicals but no natural product has been commercialized yet (Dilip et al 2014).

Plant extracts at different concentrations showed tendency not only in improving many of the economic characters but also showed antibacterial activity against the bacteria that infected the silkworms. Plants act as the richest source of organic chemicals and phytochemicals influence the life and behaviour of several insects (Eid et al., 1989). Plant extracts are also considered as cheap and abundant source to increase the silk yield. Plant derived medicines have been part of our traditional healthcare in most parts of the world and there has now been an increasing interest in using plants as the sources of agents to fight microbial diseases (Sandhya et al., 2006).

Conclusion:

The pathogenic microbes had caused a breakdown in protein synthetic machinery in the silk gland. Hence, it is imperative to develop management strategies. 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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