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In-Vitro Efficacy of Medicinal Botanical and Different Groups of Bacteria Associated with Flacherie Disease of Silkworm (*Bombyx mori* L.)

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Abstract

The *in-vitro* effect of different medicinal botanicals on flacherie disease causing bacteria showed significant effect on inhibition zone. The statistical data revealed that the inhibition zone of *Bacillus* sp. (hemolymph) was found to be significant on first and second day of observation. However, among the botanicals the minimum inhibition zone of 6.45 mm and maximum of 11.08 mm were recorded for (T_5) *Solanum nigrum* and (T_2) *Aegle marmelos*, respectively. Further, concentration of the botanical extracts also recorded significant results. With respect to zone of inhibition, minimum and maximum of 5.86 and 6.23 mm; 6.82 and 7.28 mm were recorded for first and second day at 1: 3 and 1 : 1 concentration, respectively compared to control. The same trend was niticed on inhibition zone of *Bacillus* sp. (midgut) on different medicinal plant extracts as a reflected in experimental data.
Key words : Bacteria group, Flacherie disease, Silkworm.

Among many constraints that influence the success of cocoon production, the occurrence of diseases is the prime one. Mulberry silkworm in prone to several infectious diseases like muscardine, flacherie, grasserie and pebrine. In silkworm bacterial flacherie is caused by the many bacteria, like *Bacillus* sp., *Pseudomonas* sp. (Hemolymph,) and *Bacillus* sp., *Staphylococcus* sp. and *Streptococcus* sp. (midgut). The *in-vitro* and *in-vivo* effect of medicinal plant extracts on bacterial flacherie causing organisms is due to certain chemical substances present in them. Similarly in recent days many plants are being extensively used in sericulture to increase the silk productivity in terms of quantity and quality of *B. mori* L.

Methods

Culturing of Bacteria

Bacterial flacherie causing organisms were isolated from diseased worms and cultured on nutrient agar (NA) plates were inoculated at 37 C for three days. Further, the bacterial colonies that are developed on the culture plates pierced and purified by routine methods. Pathogenicity of the individual bacteria was confirmed by principle of Koch's postulates.

Preparation of Plant Extracts

The extracts from different plants were prepared following the procedure adopted by Krishna Prasad et al. (1). The tender leaves of medicinal botanicals were collected from Sanjeevini Vatika (Herbal Garden), Division of Horticulture, UAS, Bangalore. The required quantity of fresh leaves of each plant was harvested and surface sterilized with 70% ethyl alcohol, then washed with sterile distilled water. Later, they were taken in pestle and mortar separately and 10 ml of sterile distilled water was added to 1 g of leaf for maceration. The extract was squeezed through double layered muslin cloth, and then extract collected was used as stock solution. Further, the same was diluted by using sterile distilled water to achieve different concentrations i. e. 1 : 1, 1 : 3 proportions.

Inhibition Zone Method

Sterilized Whatman No. 1 filter paper discs of 5 mm diameter were dipped in botanical extracts for 1 minute and drained by the edges of petriplate, then placed at the center of the petriplate. Three replications were maintained for each treatment; further control (distilled water) was used for comparing the other treatments. The plates were incubated for 48 hours at room temperature.The diameter of the inhibition zone of the bacteria by various botanicals was measured (mm). The extracts which inhibited the bacterial growth

Table 1. *In-vitro* effect of botanical extracts on zone of inhibition (mm) of the *Bacillus* spp. (hemolymph). * Figures in the parentheses are angular transformed values.

Botanical extracts	Zone of inhibition (mm) 1st day 1 : 1	1 : 3	Mean	2nd day 1 : 1	1 : 3	Mean
Adathoda vasica	8.50 (2.99)	7.47 (2.82)	7.98 (2.91)	9.17 (3.10)	7.83 (2.88)	8.50 (2.99)
Aegle mormelos	11.70 (3.49)	9.03 (3.07)	10.38 (3.28)	12.50 (3.60)	9.67 (3.10)	11.08 (3.39)
Ocimum sanctum	6.83 (2.70)	6.40 (2.62)	6.62 (2.66)	7.33 (2.79)	6.67 (2.67)	7.00 (2.73)
Phyllanthus niruri	7.43 (2.81)	6.63 (2.66)	7.03 (2.74)	8.00 (2.91)	7.00 (2.73)	7.50 (2.82)
Solanum nigrum	6.50 (2.64)	5.57 (2.46)	6.03 (2.55)	7.00 (2.73)	5.90 (2.52)	6.45 (2.63)
Tinospora cardifolia	7.20 (2.77)	6.53 (2.65)	6.87 (2.71)	7.50 (2.82)	7.00 (2.73)	7.25 (2.78)
Tylophora indica	6.47 (2.63)	5.43 (2.43)	5.95 (2.53)	6.83 (2.70)	5.90 (2.52)	6.37 (2.61)
Withania somifera	6.73 (2.68)	5.63 (2.47)	6.18 (2.58)	7.17 (2.76)	6.17 (2.58)	6.67 (2.67)
Control	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Mean	6.82 (2.60)	5.86 (2.43)		7.28 (2.68)	6.23 (2.50)	
Test of significance	Botanical extracts	Concentrations		Botanical extracts	Concentrations	
F test	*	*		*	*	
SE ±	0.263 (0.045)	0.124 (0.021)		0.291 (0.048)	0.137 (0.022)	
CD 5%	1.012 (0.174)	0.477 (0.082)		1.121 (0.186)	0.528 (0.087)	

effectively were used for *in-vivo* studies.

Results and Discussion

All the botanicals were tested for inhibition zone of the bacteria, *Bacillus* sp. (hemolymph) of which *Aegle marmelos* leaf extract showed maximum inhibition zone of (11.08 mm) while that of *Tinospora cordifolia* (6.37 mm) followed by *Solanum nigrum* (6.45 mm), *Withania somnifera* (6.67 mm), *Ocimum sanctum* (7.00 mm) and *Adathoda vasica* (8.50 mm) among days of observation. Second day of observation recorded maximum of 7.28 and minimum of 6.23 on contrary to 6.82 and 5.86 mm zone of inhibition were noticed for 1 : 1 and 1 : 3 botanical proportions respectively, compared to control (Table 1). These results are comparable with earlier findings (2) that reported the antimicrobial activity of basil against gram positive (*Bacillus* sp.) and gram negative bacteria (*Pseudomona* sp.). Mandal et al. (2) reported *in vitro* antibacterial efficacy of asparagus on *Staphylococcus* sp. Antibacterial/antimicrobial activity of the basil finds support by earlier reports (3—6).

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