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Evaluation of Antibacterial Efficacy of Certain Botanicals Against Bacterial Pathogen *Bacillus* sp. of Silkworm, *Bombyx mori* L.

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An *in vitro* and *in vivo* studies were conducted to evaluate the antibacterial efficacy of certain botanicals viz., rhizomes of turmeric (*Curcuma longa)* and leaves of amla (*Phyllanthus emblica*), asparagus (*Asparagus racemosus*), bael (*Aegle marmelos*), boerhavia (*Boerhavia diffusa)*, garlic (*Allium sativum)* and basil (*Oscimum basicilum*) against bacterial pathogens *viz., Staphylococcus* sp., *Bacillus* sp. and *Klebsiella cloacae,* of silkworm, *Bombyx mori.* Asparagus and basil, amla and boerhavia, basil and bael at concentration of 20,000 ppm showed higher antibacterial activity against *Staphylococcus* sp., *Bacillus* sp., *K. cloacae* respectively, both i*n vitro* and *in vivo* studies.

Key words: *Bombyx mori* L., Botanicals and *Bacillus* sp.

Introduction

Success of sericulture depends on proper management and protection of silkworm crops from diseases. Four silkworm diseases are very common in India *viz.,* grasserie (viral), flacherie (bacterial), muscardine (fungal) and pebrine (protozoan). Of these diseases, bacterial flacherie is one of the serious diseases of silkworm causing cocoon crop loss to the tune of 70 per cent as reported by Sidhu and Singh (1968); 30 to 40 per cent by Chitra *et al.* (1975); 47.9 per cent by Savanurmath *et al.* (1992).

Antibiotics of chemical origin such as erythromycin, kanamycin, streptomycin, terramycin, *etc.,* have been used to suppress bacterial flacherie especially the bacterial disease of digestive origin. But prolonged exposure to these chemicals may lead to development of resistance in silkworms, in the long run. Hence in the present study, possibility of utilizing certain botanicals of medicinal value, especially those antimicrobial/antibacterial properties was probed.

The antibacterial action of garlic was mainly due to allicin and was first demonstrated by Cavallito and Bailey (1994). Lantic acid isolated from *Lantana camera* L. had broad spectrum antibacterial activity against *Bacillus cereus* (Saleh *et al.,* 1999). Abo and Ashidi (1999) reported the antimicrobial activity of *Boerhavia diffusa* against gram positive bacteria. Mandal *et al.* (2000) evaluated antibacterial activity of *Asparagus racemosus* (wild root) against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas* sp. Singh *et al.* (2002) reported the antibacterial activity in rhizome extracts of *Curcuma longa* against gram positive and gram negative bacteria.

Antimicrobial activity of basil was reported by Suppakul *et al.* (2003). The essential oils from *Oscimum* species was predominantly associated with the main constituents linalool and methyl chavicol which were responsible for bactericidal effect on gram-positive and gram-negative bacteria (Lachowiez *et al.,* 1998). Geer *et al.* (2005) reported the antibacterial activity of aqueous extract of *Emblica officinalis.* Manimegalai and Chandramohan (2005) reported the efficiency of *Thuja orientalis L.* at 10,000 ppm and *Curcuma domestica V.* at 40,000 ppm against *B. thuringinesis.*

Materials and Methods

Extraction procedure of botanicals extract

Botanicals like rhizomes of turmeric (*Curcuma longa)*

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and leaves of amla (*Phyllanthus emblica*), asparagus (*Asparagus racemosus*), bael (*Aegle marmelos*), boerhavia (*Boerhavia diffusa*), garlic (*Allium sativum)* and basil (*Oscimum basicilum*) were ground to fine powder using a domestic mixie. The soxahalet extraction instrument was used in the extraction procedure. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground sample, sealed with another filter disc and compressed. This was then filled to the soxahalet unit, filled with 70 ml of petroleum ether (40-60ºC) and the unit was regulated to give a slow controlled flow of the solvent through the compressed sample. The filtrate was collected in a rained bottom flask, which was transferred to a rotary evaporator and placed over a lukewarm water bath to evaporate the petroleum ether. The final product was the crude extract (Keita *et al.,* 2006).

***In vitro* studies on the effect of botanicals**

Streak plate method

Botanicals at one, two, three, four and five percent concentrations were prepared and added to the Nutrient Agar (NA) medium. A loop of bacterial culture was drawn from culture of *Bacillus* sp. and were streaked on the plates and kept for incubation. Observations were made on the growth after 48 hours.

Disc diffusion method

Discs of 5.5 mm diameter were prepared using Whatman No.1 filter paper and sterilized by autoclaving at 15 1b pressure for 20 minutes. The discs were then impregnated with different concentrations of botanicals, air dried and placed on the NA surface already seeded with one per cent of bacterial cultures *Bacillus* sp. The plates were incubated at 37°C for 24 hours. The observations were recorded for the inhibition zone exhibited by different botanicals (Singh *et al.,* 2002).

Preparation of cells from bacterial culture

Twenty four hour old cultures of *Bacillus* sp. at one per cent concentration were inoculated into Nutrient agar broth and kept in a shaker for incubation for about 24-48 hours. The bacterial growth was recorded in broth cultures by observing its turbidity. The bacterial broth cultures were centrifuged at 10000 rpm for 10 minutes and the supernatant was discarded. The cell pellets were further washed with alkaline phosphate buffer for 10 minutes to maintain the pH. The washed cell pellets were resuspended in sterile distilled water.

***In vivo* studies on the effect of botanicals**

Bacterial suspension of *Bacillus* sp. were measured in Neubauer haemocytometer and used for bioassay studies. Mulberry leaves were freshly collected, dipped in bacterial suspension of 10^7 cells/ml and the leaves were allowed to shade dry for some time. Thirty third instar larvae (PM × CSR_2 silkworm race) after second moult were fed with bacteria treated leaves. The treatments were replicated thrice. Observations on mortality were recorded 24 and 48 h after treatment, by counting the number of dead insects.

The treated leaves were provided during the first feed on first day and thereafter the larvae were provided with normal leaves. On next day, the leaves were treated with the botanical extracts of turmeric, amla, asparagus, bael, boerhavia, garlic and basil at the rate of three per cent concentration and fed to the worms. Fresh leaves were dipped in extracts and allowed to dry for some time before feeding it to silkworms. Administration of botanicals was done twice, once on the second day of third instar and the other on the first day of fourth instar. Observations on larval mortality, larval weight, cocoon weight and shell weight were recorded. Further using the data obtained, shell ratio and ERR per cent were computed.

Results and Discussion

***In vitro* effect of botanicals against bacterial strain *Bacillus* sp.**

Asparagus and basil, amla and boerhavia, basil and bael at concentration of 20,000 ppm performed significantly with higher antibacterial activity against *Bacillus* sp., by streak plate and disc assays (Tables 1 and 2). This corroborates with the findings of Salmah *et al.* (2005) who reported the antimicrobial activity of basil against gram positive and gram negative bacteria. Antibacterial/antimicrobial activity of the basil also finds support from the reports of Manonmani *et al*. (2006).

The present investigation showed the effectiveness of amla and boerhavia against *Bacillus* sp. Similar effect of amla extract against *Bacillus* sp. was reported by Geer *et al.* (2005) which was found to be in line with our findings. Vijulan and Chinnusamy (2006) have also mentioned about the antimicrobial activity of amla.

***In vivo* effect of botanicals on bacterial pathogen *Bacillus* sp.**

Under *in vivo* conditions, amla and boerhavia were found to be effective in managing flacherie with a survivability of 75 and 74 per cent respectively against. *Bacillus* sp. Amla and boerhavia botanical extracts also showed effectiveness on larval weight (3.91 g and 3.70 g), cocoon weight (1.76 and 1.60 g), shell weight (0.30 and 0.29 g) and shell ratio (17.04 and 18.12 per cent) respectively

Table 1. Effect of botanicals on in vitro growth of *Bacillus* sp. by streak plate method

Sl. No.	Botanicals	Growth of *Bacillus* sp. at different concentrations of botanicals (ppm)						
		1000	5000	10,000	20,000	30,000	40,000	50,000
1.	Turmeric	+	+	+	+	+	+	+
2.	Amla	+	+	+	–	–	–	–
3.	Asparagus	+	+	+	±	±	±	±
4.	Bael	+	+	+	+	+	±	±
5.	Boerhavia	+	+	+	–	–	–	–
6.	Garlic	+	+	+	+	+	+	+
7.	Basil	+	+	+	+	+	+	±

± Partial inhibition, + No inhibition, – Inhibition.

Table 2. *In vitro* effect of botanicals on the growth of *Bacillus* sp. by the disc diffusion method

Sl. No.	Botanicals	Inhibition zone (mm)			
		Dose (ppm)			
		5,000	10,000	20,000	30,000
1	Amla	+	+	7.0	9.0
2	Boerhavia	+	+	6.0	7.0

(Tables 3). The larval weight, cocoon weight, shell weight, shell ratio in treated control were 2.60 g, 1.15 g, 0.15 g and 13.04 percent respectively which are comparatively lower than in those treated with botanicals. Antimicrobial activity of *Boerhavia diffusa* has also been mentioned in the reports of Prajeesh *et al.* (2006). *Thuja orientalis* was found to be effective against *Bacillus thuringiensis* in managing flacherie (Manimegalai and Chandramohan, 2005).

The increase in larval weight and cocoon parameters due to administration of plant products were demonstrated by Rajashekhar, (1991) for *Psoralea corylifolia L* and *Tribulus terrestris* L. *Lantana camera*. L and *Clerodendron inermae* (Mamadapur, 1994).

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Table 3. *In vivo* effect of botanicals on the larval weight and survival of *B. mori* exposed to *Bacillus* sp.

Sl. No.	Treatments	Larval weight (g)	Mortality (%)	ERR (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
1.	Turmeric	2.72 f	24.4 (29.60) e	68.00 (55.55) e	1.35 de	0.23 de	17.03 ab
2.	Amla	3.91 b	16.6 (24.04) b	75.00 (60.00) b	1.76 ab	0.30 ab	17.04 ab
3.	Asparagus	3.63 cd	20.0 (26.57) c	72.00 (58.05) c	1.56 c	0.26 c	16.66 ab
4.	Bael	3.52 d	22.2 (28.11) d	70.00 (56.79) d	1.54 c	0.24 d	15.58 c
5.	Boerhavia	3.70 c	17.7 (24.88) b	74.00(59.34) b	1.60 bc	0.29 b	18.12 ab
6.	Garlic	2.44 g	44.4 (41.78) g	50.00 (45.00) g	1.22 ef	0.17 f	13.93 cd
7.	Basil	3.31 e	26.6 (31.05) f	66.00 (54.33) f	1.52 cd	0.22 e	14.47 cd
8.	Treated control	2.60 fg	72.0 (58.50) h	25.00 (30.00) h	1.15 f	0.15 g	13.04 d
9.	Untreated control	4.10 a	10.0 (18.43) a	90 (71.57) a	1.85 a	0.31 a	16.75 ab
	SEd	**0.816**	**0.8165**	**0.8165**	**0.0816**	**0.008**	**0.8165**
	CD (0.05)	**0.1715**	**1.7154**	**1.7154**	**0.1715**	**0.0172**	**1.7154**

Figures in parentheses arc sine transformed values (per cent values must converted into arc sine for accuracy and easy calculation)
In a column, means followed by same letters(s) are not significantly different (p=0.05).

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