Environment & Ecology 27 (2A) : 841—844, 2009
 ISSN 0970-0420

Evaluation of Antibacterial Efficacy of Certain Botanicals Against Bacterial Pathogen *Klebsiella cloacae* of Silkworm *Bombyx mori* L.

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Abstract

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 pathogen *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 *Klebsiella cloacae* both *in vitro* and *in vivo* studies.
Key words : *Bombyx mori* L., Botanicals, *Klebsiella cloacae.*

Among many constraints that influence the success of cocoon production, the occurrence of diseases is the prime one. The major diseases affecting mulberry silkworm, *B. mori* L. are muscardine, flacherie, grasserie and pebrine. Bacterial flacherie is one of the serious diseases of silkworms causing cocoon crop loss to the tune of 40% (1) and 47.9% (2). This disease is locally known as Sappe meaning sluggishness which is characteristic symptom of this disease.Antibiotics of chemical origin such as erythromycin, kanamycin, streptomycin, terramycin 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 (3). Singh et al. (4) reported the antibacterial activity in rhizome extracts of *Curcuma longa* against gram positive and gram negative bacteria. Chattopadhyay et al. (5) found that the curcumin and the oil fraction of turmeric suppressed the growth of *Streptococcus, Staphylococcus* and *Lactobacillus.* Antimicrobial activity of basil was reported by Suppakal et al. (6) and aqueous extract of *Emblica officinalis* by Geer Mohammed Ishag et al. (7).

Methods

Extraction Procedure of Botanicals Extract

Botanicals like thizomes of turmeric (*Curcuma longa*) and leaves of amla (*Phyllanthus emblica*), asparagus (*Asparagus racemosus*), bael (*Aegle marmelos*), boerhavia (*Boerhavia diffusa*), garlic (*Allium sativum*) and basil (*Ocimum basilicum*) 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—60 C) and the unit was regulated to give a slow controlled flow of the solvent through 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.

Table 1. Effect of botanicals on *in vitro* growth of *Klebsiella cloacae* by streak plate method. ± Partial inhibition, + No. inhibition, – Inhibition.

Botanicals	Growth of *K. cloacae* at different concentration 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	+	+	+	–	–	–	–

The final product was the crude extract (8).

In Vitro Studies on the Effect of Botanicals

Streak Plate Method. Botanicals at 1, 2, 3, 4 and 5% concentrations were prepared and added to the nutrient agar medium. A loop of bacterial culture was drawn from culture of *Klebsiella cloacae,* and was 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 1% of bacterial culture *Klebsiella cloacae.* The plates were incubated at 37 C for 24 hours. The observations were recorded for the inhibition zone exhibited by different botanicals (4).

In Vivo Studies on the Effect of Botanicals

Preparation of Cells from Bacterial Culture. Twenty four hour old cultures of *Klebsiella cloacae* at 1% 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 10,000 rpm for 10 minutes and the supernatant was discarded. The cells 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.

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

	Botanicals	Inhibition zone (mm) Dose (ppm) 5,000	10,000	20,000	30,000
1	Basil	+	+	7.0	8.0
2	Bael	+	+	6.0	7.0

Feeding Method. Bacterial suspension at 10^7 cells/ml of *Klebsiella cloacae* was measured in Neubauer hemocytometer 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 sometime. Thirty third instar larvae (PM × CSR2 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 3% 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 recorded, shell ratio and ERR per cent were computed.

Results and Discussion

In Vitro Effect of Botanicals Against Bacterial Strain Klebsiella cloacae

Asparagus and basil, amla and boerhavia, basil and bael at concentration of 20,000 ppm performed significantly with higher antibacterial activity against *Klebsiella cloacae* by streak plate and disc assays (Tables 1 and 2). This corroborates with the findings of Salmah et al. (9) who reported the antimicrobial

Table 3. *In vivo* effect of botanicals on the larval weight and survival of *B. mori* exposed to *Klebsiella cloacae.* Figures in parentheses arc sine transformed values. In a column, means followed by same letters (s) are not significantly different (*P*=0.05).

Treatments	Larval weight (g)	Mortality (%)	ERR (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
1. Turmeric	3.3 f	33.0 (35.06) g	60 (50.77) g	1.32 c	0.22 d	16.20 ab
2. Amla	3.4 ef	20.0 (26.57) d	72 (58.45) d	1.58 b	0.30 a	17.54 a
3. Asparagus	3.5 de	25.5 (30.33) f	67 (54.94) f	1.62 b	0.25 c	15.43 b
4. Bael	3.8 b	17.7 (24.88) c	74 (59.34) c	1.71 ab	0.30 a	17.54 a
5. Boerhavia	3.6 cd	22.2 (28.11) e	70 (56.79) e	1.64 b	0.25 c	15.85 ab
6. Garlic	3.1 g	37.7 (37.88) h	56 (48.45) h	1.25 cd	0.20 d	15.60 b
7. Basil	3.7 bc	15.5 (23.18) b	76 (60.07) b	1.68 ab	0.28 b	16.66 ab
8. Treated control	2.8 h	73.0 (58.69) i	24 (29.33) i	1.13 d	0.12 e	10.61 c
9. Untreated control	4.1 a	10.0 (18.43) a	90 (71.57) a	1.85 a	0.31 a	16.75 ab
SE	0.0816	0.8189	0.8165	0.0816	0.0082	0.8193
CD (0.05)	0.1715	1.7205	1.7154	0.1715	0.0172	1.7212

activity of basil against gram positive and gram negative bacteria and Mandal et al. (10) reported *in vitro* antibacterial efficacy of asparagus against *Staphylococcus* sp. Antibacterial/antimicrobial activity of the basil also finds support from the reports of Rajangam et al. (11). Vijulan Harris and Chinnusamy (12) have also mentioned about the antimicrobial activity of amla.

In Vivo Effect of Botanicals on Bacterial Pathogen Klebsiella cloacae

The bael and basil showed higher efficiency against *K. cloacae.* The antimicrobial activity of basil was gains also reported by Manonmani et al. (13). The economic parameters and survivability were increased. Hence that highest larval weight (3.8 g and 3.7 g), cocoon weight (1.71 g and 1.68 g), shell weight (0.30 g and 0.28 g), shell ratio (17.54 and 16.66%), ERR (74 and 76%) and lowest mortality (17.7 and 15.55%), respectively were recorded (Table 3). The performance of better economic characters over the treated control itself is an evidence of the efficiency of the botanical extracts of bael and basil against *K. cloacae. Thuja orientalis* was found to be effective against *Bacillus thuringiensis* in managing flacherie (14).

The increase in larval weight and cocoon parameters due to administration of plant products was demonstrated by Rajashekhar Gouda (15) for *Psoralea corylifolia L.* and *Tribulus terrestris* L. *Lantana camera.* L. and *Clerodendron inermae* by Mamadapur, (16).

Conclusion

Both *in vitro* and *in vivo* studies conducted to evaluate the antibacterial efficacy of certain botanicals revealed that Asparagus (*Asparagus racemosus*) and basil (*Ocimum basicilum*), amla (*Phyllanthus emblica*) and boerhavia (*Boerhavia diffusa*), basil (*Ocimum basicilum*) and bael (*Aegle marmelos*) at concentration of 20,000 ppm showed higher antibacterial activity against *Klebsiella cloacae* bacterial pathogens of silkworm *Bombyx mori* L.

References

1. Chitra C., N. G. K. Karanth and V. N. Vasantharajan. 1975. Disease of the mulberry silkworm, *Bombyx mori* L. J. Sci. Ind. Res. 34 : 386—401.
2. Savanurmath C. J., S. Basavarajappa, S. B. Hinchigeri, S. S. Ingalhalli, K. K. Singh and R. D. Sanakal. 1992. Relative incidence of silkworm viral diseaes in agroclimatic zones of northern Karnataka, India. Nat. Conf. on mulberry sericulture research, 10—11 Dec, CSR & RI, Mysore, India, pp. 123.
3. Cavallito C. and J. H. Bailey. 1994. Allicin, the antibacterial principle of *Allium sativum* isolation physical properties and antibacterial action. J. Am. Chem. Soc. 66 : 24.
4. Singh R., C. B. Ramesh, Mridula and K. L. Pratibha. 2002. Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria. Curr. Sci. 83 : 737—740.
5. I. Chattopadhyay, K. Biswas, U. Bandyopadhyay and R. Banerjee. 2004. Turmeric and curcumin : Biological actions and medicinal applications. Curr. Sci. 87 : 44—47.
6. Suppakl P., J. Millz, K. Sonneveld and S. W. Bigger.

2003. Antimicrobial properties of basil and its possible application in food packaging. J. Agric. and Food Chem. 51 : 3197—3207.

7. Geer Mohammad Ishaq S., B. Zia-ul-Arifeen Dilawer, G. Ahmad, S. Moinuddin, A. Ahmad and K. Devi. 2005. Hypotensive potential of aqueous extract of Emblica officinalis on anaesthetized dogs. J.K. Practitioner 12 : 213—215.
8. Keita S., S. B. A. Umoetok and J. G. Smith. 2006. The insecticidal activity of petroleum ether extracts of *Hyptis suaveolens* Poit. (Labiatae) seeds on *Plutella xylostella* (L.) (Lepidoptera : Ponomeutidae). Agric. J. 1 : 11—13.
9. Salmah A. A., Mahmood and K. Sidik. 2005. Synergistic effects of alcoholic extract of sweet basil (*Ocimum basilicum* L.) leaves and honey on cutaneous wound healing in rats. Int. J. of Mole. Med. and Adv. Sci. 1 : 220—224.
10. Mandal S. C., A. Nandy, M. Pal and B. P. Saha. 2000. Evaluation of antibacterial activity of *Aspragus racemosus* wild root. Phycother Res. 14 : 118—119.
11. Rajangam J., S. Murugesan, M. Hemalatha, K. R. Rajadurai and S. Anbu. 2006. Evaluation of genetic resourced in *Ocimum* species for crop improvement. Int. Conf. on globalization of traditional, complementary and alternative systems of medicine, 16—18 Mar 2006, Hort. Coll. and Res. Inst., Tamil Nadu Agric. Univ., Coimbatore, India, pp. 99.
12. Vijulan Harris C. and C. Chinnusamy. 2006. Traditional medicinal value addition in amla (*Emblica officinalis*). Int. Conf. on globalization of traditional, complementary and alternative systems of medicine, 16—18 Mar 2006, Hort. Coll. and Res. Inst., Tamil Nadu Agric. Univ., Coimbatore, India, pp. 217.
13. Manonmani K., M. Jeyasekhar and R. Jagannathan. 2006. Identification of bacterial proteins from the leaf extracts of selected herbs. Int. Conf. on globalization of traditional, complementary and alternative systems of medicine, 16—18 Mar, Hort. Coll. and Res. Inst., Tamil Nadu Agric. Univ., Coimbatore, India, pp. 152.
14. Manimegalai S. and N. Chandramohan. 2005. Botanicals for the management of bacterial flacherie of silkworm, *Bombyx mori* L. Sericologia 45 : 51—58.
15. Rajasekhar Gouda R. 1991. Studies on methods to increase silk yield of *Bombyx mori* L. Doctoral thesis, Tamil Nadu Agric. Univ., Coimbatore, Inda. pp. 242.
16. Mamadapur B. B. 1994. Botanicals with IGR activity on *Bombyx mori* L. (Lepidoptera : Bombycidae). M.Sc (Ag.) thesis, Univ. Agric. Sci., Dharwad, India.