Environment & Ecology 27 (2) : 627—629, 2009
 ISSN 0970-0420

In -Vivo Efficacy of Botanicals on Cocoon Parameters of PM × CSR_2 Infected with *Bacillus* sp. (Hemolymph)

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Abstract

Administration of medicinal botanical extracts on third and fourth instar larvae of PM × CSR_2 infected with 10^{-1},10^{-2} and 10^{-3} dilution of *Bacillus* sp. (hemolymph) resulted in reducing bacterial flacherie disease. However, *Aegle marmelos* extract sprayed lots recorded maximum cocoon weight (1.34 and 1.34 g) and pupal weight (1.12 and 1.12 g) at 1 : 1 and 1 : 3 proportions respectively. *Tinospora cardifolia* extract sprayed lots recorded minimum cocoon weight (1.28 and 1.28 g) and pupal weight (1.09 and 1.08 g) at 1 : 1 and 1: 3 proportions respectively as compare to control. The same trend was noticed on silkworm rearing and economic parameters of PM × CSR_2 when treated with other medicinal plant extracts.

Key words : Botanicals, Cocoon parameters, PM × CSR_2, *Bacillus* sp.

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). The relative incidence of bacterial flacherie was 57.22% in Karnataka (3). This disease is locally known as Sappe meaning sluggishness which is characteristic symptom of this disease. Recently many attempts have been made on the use of plant extracts to combat microbial infections thus the use of botanicals in controlling this disease is limited,therefore the present study was undertaken involving different botanicals in the management of disease.

Methods

The third and fourth instar larvae of PM × CSR_2 were maintained in the laboratory based on the standard techniques. The leaves of *Adathoda vasica* (Adusoge), *Aegle marmelos* (Bilvapatre), *Phyllanthus niruri* (Kirunelli), and *Tinospora cardifolia* (Amruthaballi) leaves were collected separately and washed in running water, surface sterilized with 70% alchohol, then washed with sterile distilled water. Further, a known quantity of leaves was crushed (1 : 10 proportions) using an electrically operated grinder by adding a known quantity of water on weight/volume basis. The extract was squeezed through double layered sterile muslin cloth. The collected extract was used as stock solution. Further, it was diluted by using sterile distilled water to make different proportions, i.e 1 : 1 and 1 : 3 dilutions.

Culturing of Bacteria

Bacterial species like *Bacillus* sp., *Pseudomonas* sp. (hemolymph); *Bacillus* sp. *Staphylococcus* sp. and *Streptococcus* sp. (Midgut) were isolated from diseased worms; they were cultured on nutrient broth (NA) plates and incubated for three days at room temperature (25—28C) and humidity (80—85%).

Inoculation of Silkworms

Inoculation of silkworms was done on the third instar first day, fourth instar first day i. e. immediately after second and third moult, respectively. The spore dilution of 10^{-1}, 10^{-2} and 10^{-3} of *Bacillus* spp. (hemolymph) were administered separately to worms at the rate of 4 ml per 75 larvae by using automizer.

Application of Botanical Extracts

After 30 minutes of inoculation with bacterial spores *Bacillus* sp. (hemolymph) each botanical ex-

Table 1. *In-vivo* effect of botanical extracts on the cocoon parameters of PM × CSR_2 administered with *Bacillus* spp. (hemolymph). NS–Non-significant.

	Cocoon weight (g) Botanical proportions							
	1 : 1				1 : 3			
Botanical extracts	Bacterial spore dilution 10^{-1}	10^{-2}	10^{-3}	Mean	1^{-1}	10^{-2}	10^{-3}	Mean
Aegle marmelos	1.33	1.39	1.29	1.34	1.34	1.31	1.36	1.34
Adathoda vasica	1.31	1.33	1.29	1.31	1.39	1.26	1.32	1.32
Phyllanthus niruri	1.29	1.36	1.24	1.29	1.23	1.29	1.31	1.29
Tinospora cardifolia	1.24	1.34	1.26	1.28	1.30	1.25	1.29	1.28
Resham jyoti	1.23	1.29	1.28	1.27	1.26	1.30	1.23	1.26
Control	1.30	1.21	1.23	1.25	1.35	1.21	1.22	1.22
Mean	1.28	1.32	1.29	1.29	1.31	1.27	1.29	1.28
Test of significance	Botanical extracts			Proportions		Bacterial spore dilutions		
F test	*			NS		NS		
SE ±	0.017			0.010		0.012		
CD 5%	0.065			–		–		

Table 1. Continued.

	Pupal weight (g) Botanical proportions							
	1 : 1				1 : 3			
Botanical extracts	Bacterial spore dilution 10^{-1}	10^{-2}	10^{-3}	Mean	10^{-1}	10^{-2}	10^{-3}	Mean
Aegle marmelos	1.10	1.17	1.09	1.12	1.15	1.10	1.13	1.12
Adathoda vasica	1.12	1.12	1.09	1.11	1.18	1.05	1.11	1.11
Phyllanthus niruri	1.10	1.14	1.06	1.10	1.04	1.08	1.12	1.09
Tinospora cardifolia	1.04	1.14	1.07	1.09	1.10	1.06	1.10	1.08
Resham jyoti	1.05	1.08	1.09	1.09	1.06	1.08	1.05	1.06
Control	1.10	1.05	1.04	1.06	1.18	1.05	1.02	1.05
Mean	1.10	1.12	1.09	1.09	1.10	1.07	1.09	1.08
Test of significance	Botanical extracts			Proportions		Bacterial spore dilutions		
F test	NS			NS		NS		
SE ±	0.062			0.035		0.044		
CD 5%	–			–		–		

tract of (1 :1 and 1 : 3 proportions) was administered to third and fourth instar worms of PM× CSR_2 using automizer at the rate of 5 ml /75 larvae. The control lot was also maintained without botanical treatments. In each treatment three replications were maintained (25 larvae/replication). The observations were made on cocoon weight and pupal weight. The data were analyzed statistically by using three way factorial CRD (4).

Results and Discussion

Among various plant extracts tested, spraying of *Aegle marmelos* extract on larvae of *B. mori* L. improved the cocoon parameters (Table 1) . However *Aegle marmelos* extract sprayed on worms inoculated with 10^{-1}, 10^{-2} and 10^{-3} bacterial spore dilution recorded maximum cocoon weight (1.34 and 1.34 g) and minimum of (1.28 and 1.28 g) with 1 : 1 and 1 : 3 proportions of *Tinospora cardifolia* extract compared to control (0.00). The pupal weight was also more (1.12 and 1.12 g) for *Aegle marmelos* extract sprayed lots than other treatments.

Among the proportions tested, 1 : 1 proportion of the botanical treatment recorded better results than 3 proportion in both the cocoon parameters, followed by 10^{-3} (1.29 g) and 10^{-1} (1.28 g) at 1 : 1 proportion and 10^{-3} (1.29 g) and 10^{-2} (1.27 g) at 1 : 3 proportion. On the other hand, the next order of botanical treatments

which were found to be effective against *Bacillus* sp. (hemolymph) were *Adathoda vasica* (1.31 g), *Phyllanthus niruri* (1.29 g) *Tinospora cardifolia* (1.28 g) and the same trend was found even in 1 : 3 proportion of botanical treatment for cocooon weight (1.32, 1.31 and 1.29 g) respectively. Further, the effect of botanicals on pupal weight also registered significant results with administration of different dilutions of bacterial spores and botanical proportion. These results are comparable with earlier reports(1) on the application of *Curcuma longa* stem extract on fourth instar larvae that resulted in higher cocoon weight. Further, Mahesha (5) showed that the increased cocoon weight in both the hybrids ($CSR_2 \times CSR_4$ and $PM \times CSR_2$) was due to the application of medicinal plant extracts of *Withania somnifera*, *T. arjuna* and *Tinospora cardifolia* (1.97, 1.88 and 1.87 g; 2.06, 2.00 and 2.00 g respectively) compared to control (1.64 and 1.74 g). Samson (3) observed that *Tridax procumbens* 40%, *Lantana camara* 30% and *Parthenium hysterophorous* 30% recorded significantly maximum pupal weight (12.32, 12.31 and 12.33 g/10 respectively). Savanurmath et al. (2) also showed the larvae resulting from supplementation of *Parthenium hysterophorous* and *Tridax procumbens* extracts grew vigorously resulting in highest pupal weight of 16.55 and 16.73 g in NB_{18} and 15.11 and 14.83 g in $PM \times NB_{18}$ breed.

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