Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 81-86 (2022)

DOI Number: 10.5958/0974-4541.2022.00043.1

Effect of medicinal plants on cocoon parameters of PM×CSR2 inoculated with *Bm*NPV and *Staphylococcus sciuri*

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ABSTRACT: The silkworm *Bombyx mori* L. is an economically important insect and is prone to many diseases, including flacherie. To manage this disease, chemical based bed disinfectants are being used. These bed disinfectants leave residual toxicity in the rearing bed and inside the rearing house. Given these constraints, using plant extracts and their biomolecules was found to be an alternative to manage the grasserie and flacherie diseases. The leaves of *Adhatoda vasica* and *Phyllanthus niruri* contain many secondary metabolites that possess antimicrobial properties against a wide range of pathogens. The aqueous and methanolic leaf extracts of *A. vasica* and *P. niruri* administered to fourth and fifth instar batches of silkworm (PM×CSR2) recorded the maximum cocoon weight (1.94 and 1.81 g) shell weight (0.326 and 0.298 g), pupal weight (1.58 and 1.46 g) and shell ratio (17.65 and 16.69 %) in methanolic extracts of *P. niruri,* followed by *A. vasica,* aqueous extract of *P. niruri* and *A. vasica.* Whereas the inoculated (*Bm*NPV and *S. sciuri*) batches also recorded the same trend of observations (1.56 and 1.64., 0.255 and 0.271., 1.24 and 1.32 g., 16.21 and 16.05 %) in both fourth and fifth instar silkworms compared to their controls.

Keywords: Silkworms, PM×CSR2, *Adhatoda vasica, Phyllanthus niruri,* cocoon parameters

INTRODUCTION

The silkworm *Bombyx mori* L. is affected by many diseases, among which, flacherie is one of the severe diseases of silkworms caused by bacteria and viruses, with disease incidence of 57.22 per cent of all other diseases of the silkworm in Karnataka (Chopade *et al*., 2021). The flacherie refers to flaccidity in the larva, and the affected silkworm become feeble, lethargic and possess transparent cephalo-thoracic regions. As a result, the larvae vomit gut juice and extrude soft faeces with higher water content (Shah, 2016). The incidence of flacherie was higher during summer, followed by the rainy season and low during winter. The bacterial flacherie of silkworms is caused by different groups of bacteria: *Streptococcus faecalis, Staphylococcus aureus, Serratia* sp. and *Bacillus* sp. (Sugun, 2000). To manage these diseases, chemical based bed disinfectants are being used. Applying chemical disinfectants and their formulations for controlling flacherie leaves residual toxicity in rearing beds and houses. Given these constraints, biomolecules derived from botanical sources could be found as an alternative to treat the grasserie and flacherie diseases. Several botanicals revealed they are effective in silkworm rearing and disease management (Manimegalai and Chandramohan, 2006).

Recent interest has shifted to use of safer and natural botanicals to manage pathogens in sericulture. In this regard, medicinal plants with active constituents that show antimicrobial activity against microorganisms should be exploited to develop disinfectants against a wide range of pathogens (Madhusudhan *et al.,* 2018). The leaves of *Adhatoda vasica* and *Phyllanthus niruri* contain many secondary metabolites. They have been reported to have antiviral (Serkedjieva, 2004; Yasuhara- Bell *et al*. 2010) and antibacterial properties (Choudhary *et al.* 2017).

MATERIALS AND METHODS

Collection and sample preparation

The leaves of medicinal plants *Adhatoda vasica* (Adusoge) and *Phyllanthus niruri* (Kirunelli) were collected from 'Sanjeevini Vatika' (Herbal Garden), Department of Horticulture, UAS, GKVK, Bengaluru. The required quantity of fresh leaves of each plant was harvested and surface sterilized with 70 per cent ethyl alcohol, then washed with sterile distilled water and shade dried. The shade-dried plant samples were then slowly powdered in an electric blender, sieved, and stored in desiccators (Krishnaprasad *et al.*, 1979).

Preparation of plant extract

The aqueous and methanolic extracts of *A. vasica* and *P. niruri* were prepared by the soxhlet extraction method (Ajanal *et al*., 2012) using distilled water and methanol as solvents. Ten grams of leaf powder from both medicinal plants were taken in 250 ml of solvents (Distilled water and methanol), and extracts were prepared (10:250 g/ml). Later, by using a rotary evaporator, these extracts were reduced to 100 ml. The extracts were evaluated on

fourth and fifth instar larvae of PM×CSR2 (Kolar Gold) against *Bm*NPV and *Staphylococcus sciuri*.

Inoculation of silkworms with bacterial isolate

Inoculation of silkworms was done on the fourth and fifth instar first day, *i.e*., immediately after the third and fourth moult. The bacterial stock was prepared, from which 10^{-7} dilution (2.33×10^{9} CFU/ml) was prepared using 9 ml distilled water. The newly moulted fourth and fifth instar larvae were starved for 6 hours and distributed in trays. Each treatment contained three replications (50 larvae/replication). The suspension of haemolymph bacteria was smeared on mulberry leaves and fed to silkworms at 1 ml/50 larvae.

Inoculation of silkworms with *Bm*NPV

The serial dilution (10^{-9}) of *Bm*NPV suspension (6.75×10^{4}) was prepared using distilled water. After the complete feeding of mulberry leaves smeared with bacteria, the larvae were fed with a mulberry leaf smeared with 1.00 ml of diluted PIBs suspension. Three batches were kept as a control in which larvae were fed with leaves smeared with distilled water, the second batch was a leaf with methanol, and the third batch was only mulberry leaves without any application. All the larvae of each treatment and control were fed on fresh mulberry leaves till spinning.

Application of botanical extracts

After 30 minutes of inoculation with PIBs, each botanical extract (1:3) was smeared on leaves and fed to silkworms at 1 ml/50 larvae (Fig. 1). The control batches were fed with distilled water and methanol-sprayed leaves.

Fig 1. Mulberry leaves smeared with leaf extracts before administration

RESULTS AND DISCUSSION

A significant difference was observed between the healthy and inoculated batches, whereas the results were on par with the three controls. The interaction effect between plant extracts and the health of silkworms in both the instars administered showed non-significant results (Table 1). The aqueous and methanolic leaf extracts of *A. vasica* and *P. niruri* were administered to the fourth and fifth instar batches of silkworm (PM×CSR2). The data on cocoon weight registered maximum in methanolic extract of both *P. niruri* (1.94 and 1.81 g/cocoon*)* and *A. vasica* (1.89 and 1.78 g) followed by aqueous extracts (1.81 and 1.72., 1.78 and 1.69 g), respectively. Further, the *Bm*NPV and *S. sciuri* inoculation to silkworms followed by botanical extract administration have recorded the cocoon weight of 1.47, 1.54, 1.48 and 1.56 g in the fourth and 1.52, 1.60, 1.53 and 1.64 g in fifth instar silkworms administered with aqueous and methanolic extracts of *A. vasica* and *P. niruri.* Furthermore, the control batches *viz*., distilled water, methanol and absolute control recorded higher cocoon weight in healthy (1.67, 1.58 and 1.57g in the fourth instar; 1.67, 1.62 and 1.62 g in the fifth instar) compared to inoculated silkworms (1.44, 1.46 and 1.37 g in fourth instar; 1.46, 1.50 and 1.45 g in fifth instar).

Significant results were recorded for shell weight over the control in plant extracts administered in batches of fourth and fifth instars (Table 2). The methanolic extract of *P. niruri* recorded the highest shell weight in healthy and inoculated batches of the fourth (0.326 and 0.255 g) and fifth instar (0.298 and 0.271 g) compared to the methanolic extract of *A. vasica* (0.301 and 0.251., 0.289 and 0.259 g), aqueous extract of *P. niruri* (0.293 and 0.234., 0.278 and 0.247 g) and *A. vasica* (0.291and 0.229., 0.273 and 0.239 g). The fourth instar treated silkworm batch recorded significantly higher shell weight (0.260, 0.276, 0.264 and 0.291 g) than the fifth instar batches (0.256, 0.274, 0.263 and 0.284 g). The control batches *viz.*, distilled water control (0.259 and 0.217, 0.257 and 0.209 g), methanolic control (0.251 and 0.209., 0.250 and 0.210 g), and absolute control (0.249 and 0.206., 0.257 and 0.209 g) recorded significantly lesser shell weight compared to botanical treated batches of both healthy and infected silkworms (Table 2). The interaction effect between plant extracts and the health of silkworms (healthy and infected) was found non-significant.

The effect of administering medicinal plant extracts to healthy and inoculated batches of fourth and fifth instar PM×CSR2 on pupal weight was assessed, and recorded significant results. The pupal weight of 1.58, 1.51, 1.45 and 1.45 g was recorded and found non-significant in the

Table 1. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on cocoon weight (g) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
***A. vasica* -Aqueous**	1.78	1.47	1.63	1.69	1.52	1.61
A. vasica-Methanol	1.89	1.54	1.71	1.78	1.60	1.69
P. niruri-Aqueous	1.81	1.48	1.64	1.72	1.53	1.63
P. niruri-Methanol	1.94	1.56	1.75	1.81	1.64	1.72
Distilled water control	1.67	1.44	1.55	1.67	1.46	1.56
Methanol control	1.58	1.46	1.52	1.62	1.50	1.56
Absolute control	1.57	1.37	1.47	1.62	1.45	1.54
Mean	1.75	1.47	1.61	1.70	1.53	1.62
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	*	*	NS	*	*	NS
S.Em ±	0.05	0.02	0.06	0.04	0.02	0.06
CD at 5 % level	0.13	0.07	0.19	0.12	0.06	0.17

*Significant at 5 % level, NS: Non-significant; A: Plant extracts, B: Health of silkworm

Table 2. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on shell weight (g) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
A. vasica - Aqueous	0.291	0.229	0.260	0.273	0.239	0.256
A. vasica- Methanol	0.301	0.251	0.276	0.289	0.259	0.274
P. niruri-Aqueous	0.293	0.234	0.264	0.278	0.247	0.263
P. niruri-Methanol	0.326	0.255	0.291	0.298	0.271	0.284
Distilled water control	0.259	0.217	0.238	0.257	0.209	0.233
Methanol control	0.251	0.209	0.230	0.250	0.210	0.230
Absolute control	0.249	0.206	0.228	0.257	0.209	0.233
Mean	0.282	0.229	0.255	0.272	0.235	0.253
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	*	*	NS	*	*	NS
S.Em ±	0.005	0.003	0.007	0.009	0.005	0.012
CD at 5 % level	0.015	0.008	0.021	0.025	0.013	0.035

*Significant at 5 % level, NS: Non-significant; A: Plant extracts, B: Health of silkworm

fourth instar healthy silkworm batch administered with methanolic extract of *P. niruri, A. vasica,* aqueous extract of *P. niruri* and *A. vasica,* respectively. The trend was the same in the fifth instar healthy batch, which recorded 1.46, 1.44, 1.39 and 1.37 g of pupal weight compared to their controls.

Further, in the pathogen (*Bm*NPV and *S. sciuri*) inoculated batches, significant results were found for the maximum pupal weight of 1.24, 1.22, 1.19 and 1.17 g in methanolic extract of *P. niruri, A. vasica,* aqueous extract of *P. niruri* and *A. vasica* of fourth instar. The trend was same in the fifth instar inoculated batch (1.32, 1.30, 1.24 and 1.22 g). Among the three control batches maintained for healthy and inoculated batches of both the instars, the healthy silkworms recorded maximum pupal weight (1.37, 1.34 and 1.33., 1.35, 1.32and 1.31 g) compared to inoculated batches (1.14, 1.18 and 1.11., 1.16, 1.17 and1.17 g) in distilled water, methanol and absolute control, respectively (Table 3).

The *in-vivo* effect of botanical extracts and their additive effect on *Bm*NPV and *S. sciuri* inoculation to the fourth and fifth instar of PM×CSR2 registered non-significant results for shell ratio (Table 4). The highest shell ratio of 17.65 and 16.21., 16.69 and 16.05 per cent

Table 3. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on pupal weight (g) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
A. vasica -Aqueous	1.45	1.17	1.31	1.37	1.22	1.30
A. vasica-Methanol	1.51	1.22	1.36	1.44	1.30	1.37
P. niruri-Aqueous	1.45	1.19	1.32	1.39	1.24	1.31
P. niruri-Methanol	1.58	1.24	1.41	1.46	1.32	1.39
Distilled water control	1.37	1.14	1.25	1.35	1.16	1.26
Methanol control	1.34	1.18	1.28	1.32	1.17	1.24
Absolute control	1.33	1.11	1.22	1.31	1.17	1.24
Mean	1.44	1.18	1.31	1.38	1.23	1.30
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	NS	*	NS	NS	*	NS
S.Em ±	0.04	0.02	0.06	0.03	0.02	0.05
CD at 5 % level	0.12	0.06	0.17	0.10	0.05	0.14

*Significant at 5 % level, NS: Non-significant; A: Plant extracts, B: Health of silkworm.

in the methanolic extract of *P. niruri*, followed by *A. vasica* (17.47 and 16.11., 16.64 and 16.00 %). However, the aqueous extract of *P. niruri* (17.18 and 16.09., 16.63 and 15.98 %) and *A. vasica* (16.57 and 16.01., 16.43 and 15.96 %) recorded comparatively less shell percentage in healthy and inoculated batches of both fourth and fifth instars. Between the instars, the fourth instar treated batches found a maximum shell ratio (16.93, 16.64, 16.79 and 16.29 %) compared to the fifth instar (16.37, 16.30, 16.32 and 16.19 %).

Similar results were observed by Rudroju *et al*. (2017), who studied the effect of leaf extracts of *Trichosanthes cucumerina* L. on the cocoon parameters of the flacherie-infected silkworm. The methanolic extract of the leaf showed the highest cocoon characteristics *viz*., cocoon weight (1.94±0.11g), shell weight (0.39± 0.01g) and pupal weight (1.54±0.08g) over the control (1.63±0.02 g). The study on the efficacy of nine different medicinal plant extracts for managing late larval flacherie of silkworm (PM×CSR2) and cocoon parameters was carried out by Manjunatha *et al.* (2020). Among nine medicinal plant extracts administered, *Phyllanthus niruri* was found effective by enhancing the cocoon parameters *viz.,* cocoon weight (10.51 g/10 cocoons), shell weight (1.610 g/10 cocoon shells), pupal weight (8.90 g/10 pupae) and shell ratio (16.46 %) as reflected in the present study.

The aqueous extract of *Ziziphus jujuba* L. was fortified to fifth instar PM×CSR2 larvae (Sunil and Chandrashekhar, 2016) and recorded maximum cocoon weight (1.766, 1.531 and 1.723 g/cocoon), shell weight (0.309, 0.264 and 0.33 g/shell), pupal weight (1.459, 1.267 and 1.393 g/pupa) and shell ratio (17.65, 17.29 and 19.37 %) at 1:2, 1:4 and 1:8 concentrations compared to control (1.322, 0.221, 1.101 g and 16.80 %). Further, the ethanolic extract of *Ocimum sanctum* (2 %) was administered to fifth instar silkworms (PM×CSR2) and recorded cocoon parameters *viz*., cocoon weight, shell weight, pupal weight and cocoon shell ratio (Devi and Bai, 2015), which was found similar with the present study where the cocoon weight, shell weight, pupal weight and cocoon shell ratio were found more in botanical administered silkworm batches compared to healthy and infected controls because of the presence of biomolecules which acts as antimicrobial agents against virus and bacteria in infected batches. In contrast, the biomolecules exhibited an additive effect in healthy batches by supplying extra protein molecules for silk synthesis.

Sisodia and Gaherwal (2019) recorded the effect of amla plant extract on *Bacillus subtilis* infected silkworm and found increased cocoon shell weight (0.178±1.56 g/shell) compared to control (0.16 ±1.40 g/shell). Further, Chavan and Bhawane (2016) also studied the effect of ethanolic plant extract on *Bm*NPV infection and cocoon parameters of pure Mysore and CSR2 silkworm breeds. *Curcuma longa* recorded maximum cocoon weight (995.20 mg/cocoon) and shell weight (147.50 mg/shell) in CSR2. In contrast, in PM, the maximum cocoon weight (931.3 mg/cocoon) was recorded in *Bougainvillea spectabilis,* with shell weight (222.0 mg/shell) in *A. Mexicana,* which is in line with the present findings. Kuntamalla *et al.* (2015) recorded

Table 4. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on shell ratio (%) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
A. vasica -Aqueous	16.57	16.01	16.29	16.43	15.96	16.19
A. vasica-Methanol	17.47	16.11	16.79	16.64	16.00	16.32
P. niruri-Aqueous	17.18	16.09	16.64	16.63	15.98	16.30
P. niruri-Methanol	17.65	16.21	16.93	16.69	16.05	16.37
Distilled water control	16.86	15.62	16.24	16.14	15.92	16.03
Methanol control	16.94	15.61	16.28	16.30	15.84	16.07
Absolute control	16.74	14.99	15.87	16.08	15.82	15.95
Mean	17.06	15.81	16.43	16.42	15.94	16.18
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	NS	NS	NS	NS	NS	NS
S.Em ±	0.63	0.34	0.89	0.48	0.26	0.68
CD at 5 % level	1.83	0.98	2.59	1.40	0.75	1.98

NS: Non-significant; A: Medicinal plants, B: Health of silkworm

higher single cocoon weight (1.571 g), shell weight (0.258 g), pupal weight (1.316 g) and silk ratio (15.97 %) in 3 per cent concentration of aqueous leaf extract of *O. sanctum* compared to other concentrations (1, 2 and 4 %).

The effect of botanical extract of turmeric, amla, asparagus, bael, berhavia, garlic and basil on the shell ratio of silkworms infected with *Bacillus* sp. revealed that the silkworms treated with Boerhavia leaf extract recorded a maximum shell ratio (18.12 %) compared to amla leaf extract (17.04 %) (Priyadharshini *et al*. 2009). The increase in cocoon parameters may be due to phytochemical constituents such as steroids, alkaloids and flavonoids that inhibit the gut microorganisms which compete with the host for nutrients. The ingredients have stimulated the synthesis of silk proteins and nucleic acids, which there by increase silk content.

CONCLUSION

The plant extracts administered to silkworms were inhibited the pathogen multiplication and recorded maximum cocoon weight, shell weight, pupal weight and shell ratio in methanolic extracts of *P. niruri* followed by *A. vasica*, aqueous extract of *P. niruri* and *A. vasica* administered healthy and pathogen inoculated (*Bm*NPV and *S. sciuri*) silkworms. The reduced pathogenicity and increased cocoon parameters were due to the presence of many secondary metabolites which possess antimicrobial property against wide range of bacteria and virus.

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MS Received: 30 November 2022
MS Accepted : 29 December 2022