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Assessment of botanicals extract on grasserie disease of mulberry silkworm, *Bombyx mori* L.

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Abstract
The grasserie of silkworm is a devastating serious disease to mulberry silkworm *Bombyx mori* L. which causes considerable economic loss to sericulture industry. The usage of chemicals as room and bed disinfectants for the management of diseases are reported to be carcinogenic, non-biodegradable and posing potential threat to environment. The botanicals possessing antimicrobial and antiviral activities can be used in silkworm rearing which are non-toxic, non-hazardous and eco-friendly in nature. Hence attempts were made to manage the disease through effective botanicals. The chloroform leaf extract of *Psoralea corylifolia* (1000 ppm) was found to be most effective against *Bm*NPV with the larval mortality value 19.23 per cent followed by *Phyllanthus niruri* (1000 ppm) (24.45%). The cocoon weight, shell weight and shell ratio of *P. corylifolia* (1000 ppm) treated larvae showed the values of 2.08 g, 0.39 g and 18.75 per cent respectively. *Bm*NPV treated silkworm larvae recorded highest filament length in the treatment of chloroform extract of *P. corylifolia* (1000 ppm) when applied. The filament length of *P. corylifolia* (1000 ppm) (967.00 m) was on par with untreated control (987.33 m) and *P. corylifolia* (500 ppm) (946.33 m). *Emblica offcinalis* (500 ppm) recorded lowest value of filament length (868.00 m).

Keywords: Grasserie, *P. corylifolia, P. niruri, E. officinalis*

Introduction
The pathogenic microorganisms are responsible for causing infectious diseases of silkworm. *Bombyx mor*i, the mulberry silkworm, is known to be susceptible to bacterial, viral, fungal, and protozoan infections. Due to the serious outbreak of grasserie disease in silkworm, Indian sericulture is often experiencing complete crop failure. In India, this disease in silkworm has been attributed to a 30–70 per cent reduction in silk output (Chandrasekharan *et al.*, 2006a; Manimegalai *et al.*, 2010) [3, 8]. In general, viral diseases account for 70 per cent total loss to silkworm crop. Among the viruses, *Bombyx mori* nuclear polyhedrosis virus (*Bm*NPV) has caused the highest damage to silkworm in tropical regions (Sivaprasad *et al.*, 2003; Biabani *et al.*, 2005) [3, 2]. *Bm*NPV infection alone leads to 64 per cent drop in cocoon yield in India especially in major silkworm growing states. The prevalence of grasserie disease has been reported more in summer (55%) followed by winter (42%) and rainy seasons (33%). One of the most significant and important factors in the success of commercial sericulture is the management of silkworm diseases. To achieve a high quality and steady yield of cocoon, initially pathogen load and pathogenicity should be reduced and then larval health is to be improved by boosting disease resistance (Singh *et al.*, 2003) [12]. The plants possessing antimicrobial and antiviral activities can be used in silkworm rearing which are non-toxic, non-hazardous and eco-friendly in nature. The bio-molecules present in botanicals such as flavonoids, terpenoids, alkaloids and phenols are found to possess antimicrobial activity and fight against the viral pathogens could be exploited for the eco-friendly disease management in silkworm.

Materials and Methods
Experiments were conducted to evaluate chloroform extract of different botanicals, *viz., Emblica officinalis, Phyllanthus niruri* and *Psoralea corylifolia* on *Bm*NPV infected mulberry silkworm.

Soxhlet apparatus for preparation of botanicals extracts
Soxhlet apparatus was used for extraction purpose. 25 g of the powdered leaves of botanicals, *viz., E. officinalis, P. niruri* and *P. corylifolia* were weighed separately into 250 ml of chloroform and percolated overnight.

The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples, sealed with another filter disc and compressed. This was fitted to electric heating mantle with soxhlet unit, filled with 250 ml chloroform and a temperature of 60 ºC was maintained for 6 hours. The unit was regulated with water to give a slow controlled flow of the solvent through the compressed sample. After 6 hours, the filtrate was collected in a rained bottom flask. The residual extract was collected in a flask and transferred to a rotary flask vacuum evaporator for evaporation of the solvent. The residue thus obtained were weighed and dissolved in equal volume of solvents (W/V) to get a working stock solution and stored at 4 °C in airtight bottles for future use (Kéita *et al.*, 2001) [4].

Treatment details

The viral suspension with the concentration of 10^6 POBs/ml was prepared in distilled water and fresh mulberry leaves were dipped in the viral suspension, shade dried and fed to the larvae. The worms treated with *Bm*NPV alone served as treated control, and untreated control were also maintained. The *Bm*NPV viral suspension, diluted to the appropriate concentration was given as the first feed to the third instar larvae. The botanical treated leaves were fed to silkworm larvae on the next day as first feed and then after fed with normal mulberry leaves. Treatments were administered twice, once on the second day of third instar and the other on the first day of fourth instar. The worms fed with *Bm*NPV alone served as treated control. Untreated controls were also maintained.

Statistical analysis

The data collected in various experiments were statistically analyzed using Completely Randomized Resign (CRD). The data were transformed to either *arc sine* (Angular transformation) or square root transformation for the purpose of analysis wherever needed. Latin square design (LSD) was applied for comparing the treatment means (Rangaswami, 2010) [10].

Result and Discussion

Management of silkworm diseases has become one of the important criteria for the success of commercial sericulture. In order to fetch stable and highest cocoon yield, it is mandatory to take necessary efforts to reduce pathogen load and pathogenicity and to strengthen the health of the larva by increasing their disease resistance capacity. Plants has specialized biochemical capabilities to synthesize and accumulate a vast array of primary and secondary Phytoconstituents useful for plant itself as a protecting mechanism against stress factors. These phytocompounds have made useful for living organisms for instance, as spices and medicines (Akinmoladun *et al.*, 2007) [1]. Hence, botanicals were administered to evaluate the disease resistance of silkworm to grasserie disease.

The results of effects of chloroform extract of botanicals, *viz., E. officinalis*, *P. niruri* and *P. corylifolia* used on mulberry silkworm *B.mori* treated with *Bm*NPV for evaluating the botanical extracts for antiviral property is described below. The larval mortality was greatly influenced by the mulberry leaves treated with botanicals during rearing. Mortality of *Bm*NPV infected larvae was lowest in *P. corylifolia* (1000 ppm) (19.23%) followed by *P. corylifolia* (500 ppm) (21.60%), *Phyllathus niruri* (1000 ppm) (24.45%). The untreated control larvae recorded highest larval weight (4.34g) followed by *P. corylifolia* (1000 ppm) (3.83 g), *P. corylifolia* (500 ppm) (3.65 g), *P. niruri* (1000 ppm) (3.41 g). *Bm*NPV treated larvae gave lowest larval duration in the treatment where chloroform extracts of *P. corylifolia* (1000 ppm) was applied (Table 1). Similarly, Manimegalai *et al.* (2000) [7] reported that the larva treated with *P. corylifolia* attained highest weight of 3.96 g and it was on par with Vijetha which recorded 3.94 g followed by *C. longa* powder + chalk powder and *T. terrestris*. Cocoon weight of 1.48 g was obtained in Vijetha and *T. terrestris* treatment and also reported that the maximum shell ratio was obtained in *C. longa* powder + chalk powder (15.27%) followed by *P. corylifolia* (14.74%). Interestingly, results have been reported by Mahalingam *et al.* (2010) [6] who reported that the larval weight, cocoon weight, shell weight and shell ratio of 3.68 g, 1.74 g, 0.323 g and 18.56% were registered in TNAU Seridust + *Psoralea* extract treated silkworm larvae while untreated control recorded 3.45 g, 1.53 g, 0.277 g and 18.10% respectively. The effect of botanicals on larval parameters of mulberry silkworm (PM x CSR2) was studied by Shubha (2005) [11] and revealed that, *Psoralea corylifolia* followed by *Phyllanthus niruri* at 1:3 concentration enhanced larval weight of fifth instar and mature larval weight, ERR and moth emergence.

Table 1: Effect of chloroform extract of select botanicals on larval characters of *Bm*NPV infected *B. mori*

No.	Treatment	Concentration (ppm)	Larval mortality (%)	Larval weight (g)	Larval duration (hrs)
T_1	*Emblica officinalis*	1000	47.34^e	2.94^e	235.00^a
T_2	*Emblica officinalis*	500	48.90^e	2.63^f	235.00^a
T_3	*Phyllanthus niruri*	1000	24.45^{cd}	3.41^{cd}	226.00^{ab}
T_4	*Phyllanthus niruri*	500	27.38^d	3.27^d	224.00^{abc}
T_5	*Psoralea corylifolia*	1000	19.23^b	3.83^b	209.00^{bc}
T_6	*Psoralea corylifolia*	500	21.60^c	3.65^{bc}	217.00^{abc}
T_7	Treated control (*Bm*NPV)	10^6 POB/ml	86.90^f	1.79^g	236.00^a
T_8	Untreated control	-	2.0^a	4.34^a	203.00^c
	S.Ed		2.30	0.14	10.53
	CD (0.05)		4.88	0.30	22.31

In a column means followed by a common letter(s) are not significantly different by LSD (0.05).

In this study, the untreated control larvae gained maximum cocoon weight of 2.39 g which was followed by *Bm*NPV treated larvae on influence with chloroform extract of *P. corylifolia* (1000 ppm) (2.08 g), *P. corylifolia* (500 ppm) (1.84 g). The lowest cocoon weight was recorded in *E. officinalis* (500 ppm) treated larvae (1.28 g). The untreated control larvae recorded highest shell weight with the value 0.46 g. This was followed by *P. corylifolia* (1000 ppm) treated larvae with the value 0.39 g. The highest shell ratio was recorded in the treatment where chloroform extract of *P. corylifolia* (1000 ppm) was given. The shell ratio of *P. corylifolia* (1000 ppm) (18.75%) and the untreated control (19.24%) was on par with each other (Table 2). Similarly, Latha *et al.* (2011) [5] reported that aqueous extract of different medicinal plants, *viz., A. vasica, B. spectabilis, P. niruri, T. arjuna* and *Pongamia glabra* feed to silkworm larvae through mulberry leaves once during 4th and 5th instars of PM × CSR2 revealed the positive response of cocoon weight, shell weight, shell ratio and silk productivity. However, *P. niruri* recorded highest cocoon weights and shell weights during 4th and 5th instars (1.59 g and 1.65 g; 0.264 g and 0.284 g) compared to control cocoon weight and shell weight (1.64 g and 1.66g; 0.299 g and 0.310 g).

Table 2: Effect of chloroform extract of select botanicals on cocoon characters of *Bm*NPV infected *B. mori*

No.	Treatment	Concentration (ppm)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
T_1	*Emblica officinalis*	1000	1.40^{f}	0.19^{ef}	13.57^{d}
T_2	*Emblica officinalis*	500	1.28^{g}	0.17^{f}	13.28^{d}
T_3	*Phyllanthus niruri*	1000	1.69^{d}	0.25^{d}	14.79^{c}
T_4	*Phyllanthus niruri*	500	1.54^{e}	0.21^{e}	13.63^{d}
T_5	*Psoralea corylifolia*	1000	2.08^{b}	0.39^{b}	18.75^{a}
T_6	*Psoralea corylifolia*	500	1.84^{c}	0.32^{c}	17.39^{b}
T_7	Treated control (*Bm*NPV)	10^6 POB/ml	1.18^{h}	0.12^{g}	10.16^{e}
T_8	Untreated control	-	2.39^{a}	0.46^{a}	19.24^{a}
	S.Ed		0.04	0.01	0.53
	CD (0.05)		0.09	0.03	1.12

In a column means followed by a common letter(s) are not significantly different by LSD (0.05).

The filament length of *P. corylifolia* (1000 ppm) (967.00 m) was on par with untreated control (987.33 m) and *P. corylifolia* (500 ppm) (946.33 m). Highest filament weight was observed in untreated control (0.32 g) which was on par with *P. corylifolia* (1000 ppm) and (500 ppm) with the values 0.31 g and 0.30 g respectively. The untreated control recorded filament denier with value 2.91 which was followed by *P. corylifolia* (1000 ppm) and (500 ppm) with the values (2.88 and 2.85) which were on par with each other (Table 3). Similar results were recorded by Mavilashaw (2013) [9] on silkworms treated with *Bm*NPV and medicinal plants extract yielded filament length, filament weight and filament denier. During winter season, chloroform extract of *R. officinalis* (1000 ppm) recorded highest Filament length (974.75 m), filament weight (0.30 g) and filament denier (2.84) on *Bm*NPV infected larvae.

Table 3: Effect of chloroform extract of select botanicals on post cocoon characters of *Bm*NPV infected *B. mori*

No.	Treatment	Concentration (ppm)	Filament length (m)	Filament weight (g)	Denier
T_1	*Emblica officinalis*	1000	882.00^{c}	0.25^{de}	2.55^{cd}
T_2	*Emblica officinalis*	500	868.00^{bc}	0.24^{e}	2.48^{d}
T_3	*Phyllanthus niruri*	1000	925.00^{abc}	0.29^{bc}	2.82^{ab}
T_4	*Phyllanthus niruri*	500	903.00^{c}	0.27^{cd}	2.69^{bc}
T_5	*Psoralea corylifolia*	1000	967.00^{ab}	0.31^{ab}	2.88^{ab}
T_6	*Psoralea corylifolia*	500	946.33^{abc}	0.30^{ab}	2.85^{ab}
T_7	Treated control (*Bm*NPV)	10^6 POB/ml	632.00^{d}	0.13^{f}	1.85^{e}
T_8	Untreated control	-	987.33^{a}	0.32^{a}	2.91^{a}
	S.Ed		38.63	0.01	0.09
	CD (0.05)		81.93	0.02	0.20

In a column means followed by a common letter(s) are not significantly different by LSD (0.05).

Conclusion

Botanicals are also becoming more crucial in sericulture, especially in the management of silkworm infection. The compounds present in the botanicals possess both antimicrobial and antiviral properties which can be employed through different modes of application in silkworm rearing. They can indirectly aid in reducing further outbreak of *Bm*NPV and also involved in promoting growth of silkworm. In this study, experiment were conducted using *Bm*NPV treated third instar larvae of bivoltine double hybrid DH 1 to assess the antiviral activities of select botanicals, *viz.*, *E. officinalis*, *P. niruri* and *P. corylifolia*. It was found that chloroform leaf extract of *P. corylifolia* (1000 ppm) significantly reduced the larval mortality of silkworm and also improved the economic parameters such as larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight and denier.

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