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Evaluation of medicinal plants, *Emblica officinalis*, *Calendula officinalis*, *Cassia fistula*, *Allium sativum* and *Pelargonium hortorum* Extract to grasserie diseased Silk worm *Bombyx mori* L.

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Abstract

The study was undertaken to find out the possibility of using the extract of five medicinal plants such as *Emblica officinalis*, *Calendula officinalis*, *Cassia fistula*, *Allium sativum* and *Pelargonium hortorum* for controlling the viral pathogens causing grasserie disease in the mulberry silkworm *Bombyx mori*. In the present study, the results of toxicity of chloroform extract of botanicals on larval characters of *B.mori viz.*, larval mortality, larval weight, larval duration, cocoon weight, shell weight and shell ratio are reported. The treatment *E.officinalis* (1000ppm) gave lower larval mortality (43 percent) which was on par with *C. officinalis* (1000ppm) which gave (45 percent) and untreated control (57 percent). Higher larval mortality (65 percent) reported in 1000ppm of *A.sativum* treatment. The treatment *E. officinalis* (1000ppm) gave higher cocoon weight (1.03g) which was on par with the untreated control (0.98g) and *C. officinalis* (1000ppm) (1.04g) which was also on par with the untreated control. *A. sativum* (1000ppm) recorded the lowest values of cocoon weight (0.83g).

Keywords: Larval mortality, Silkworm disease, Shell ratio, Cocoon weight.

Introduction

Among the silk producing nation of the world India occupies unique position. Sericulture in India provides a subsidiary occupation to farmers. Sericulture practices have undergone changes to improve productivity. Disease development and mortality are ever present phenomenon in the silk worms as in other living organisms. However, due to continuous rearing of mulberry silkworm became highly susceptible to various diseases which accounts for 30-40% loss in the cocoon yield. Mulberry silkworm, *Bombyx mori* L. is prone to viral, bacterial, fungal and protozoan infections due to its domestication for about 5000 years. Management of silkworm disease is one of the highly important component of successful silkworm rearing in order to obtain a higher yield and quality cocoons. Various methods are available for disease management *viz.*, chemical disinfectants inside chawki rooms are not only hazardous to silkworms but also to human beings employed for the purpose. The use of

plant molecules for management of diseases inside the rearing house is appropriate for the current scenario because of their cost effectiveness and eco-friendly nature. Besides the antimicrobial effects, the growth promoting factors of certain botanicals have been demonstrated by several authors (Rajasekhar Gouda, 1991; Manimegalai and Chandramohan, 2006).

Materials and Methods

Preparation of plant extracts

Leaf materials from collected plants *Emblica officinalis*, *Calendula officinalis*, *Cassia fistula*, *Allium sativum* and *Pelargonium hortorum* were thoroughly washed with running tap water, shade dried at room temperature for two weeks. The dried plant leaves were ground into fine powder and 25g of

the powdered leaves of *E.officinalis*, *C.officinalis*, *C.fistula*, *A.sativam* and *P.hortroum* were mixed with 200 ml chloroform separately and percolated for 24 hours. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples sealed with another filler disc and compressed this was fitted to electronic heating mantle with soxhlet unit filtered with 240 ml of chloroform and temperature 400⁰ C and 600⁰ C was maintained for hexane and chloroform respectively. The unit was regulated with water to give a slow controlled flow of the solvent through the compressed sample. The filtrate was collected in a vacuum evaporator for the evaporation of the solvent. The residue thus obtained was stored at 40⁰ C in air-tight bottles for future use (Ketta et al., 2006). The percent extractive values were calculated by using the formula (Ankita and Kanika Sharma, 2011)

$$\text{Percent extractive} = \frac{\text{weight of the dried extract}}{\text{weight of dried plant material}} \times 100.$$

Silkworm rearing

The chawki worms were purchased from the Amman Chawki centre, Coimbatore, Tamil Nadu, India and the experiments were conducted using the bivoltine double hybrid, DH1 ((CSR6×CSR26) × (CSR2 ×CSR27)) as this is mostly reared by farmers.

Evaluation of medicinal plant extracts against grasserie

An experiment was carried out to find out the effect of medicinal plants to grasserie in silkworm larvae. The extracts (chloroform extract) of medicinal plant in the concentration of 1000 ppm was prepared in distilled water. Mulberry leaves were dipped in the suspensions, shade dried and feed to the larvae. Observations on larval mortality (%), larval weight(g), cocoon weight, shell weight and shell ratio were recorded.

Larval weight

Larval weight was recorded at 5th day of V instar. The weight of 10 randomly selected larvae from each treatment was taken and weighed with an electronic balance and mean was expressed in gram.

Effective rate of rearing (ERR)

On the third day of spinning, the total number of cocoons formed in each treatment was recorded and E.R.R. was worked out based on the number of

healthy worms that's spun the cocoon to the total worms reared. This was expressed in percent. To obtain cocoon weight, after complete spinning of the cocoons, 10 cocoons were randomly selected weighted and the mean was expressed in gram. Similarly, pupal weight was taken from 10 randomly selected by cutting open the cocoons. The pupae were removed and the mean pupal weight was expressed in gram. The shell weight was recorded from 10 randomly selected cocoons by cutting open the cocoons and mean was expressed in gram.

Shell percentage (Shell ratio)

The shell percentage was determined by using the following formula. The mean is expressed in percent

$$\text{Shell percent} = \frac{\text{Shell weight}}{\text{shell ratio}} \times 100$$

Results and Discussion

The results of experiment conducted to evaluate the best botanicals (Chloroform) for the management of brasserie in silkworm *Bombyx mori* in the Laboratory (Tables 1 -7).

Toxicity of aqueous extract of botanicals

Larval mortality

The treatment *E.officinalis* (1000ppm) gave lower larval mortality (43 percent) which on par with *C.officinalis* (1000ppm) which gave (45 percent) and untreated control (57 percent). Higher larval mortality (65 percent) was reported 1000 ppm of *A.sativum* treatment.

Larval weight

The treatment *E.officinalis* (1000ppm) gave higher larval weight (3.13g) when compared with the untreated control (3.15g). This was followed by *C.officinalis* (1000ppm) gave (3.13 g) which was also on par with the untreated control (Table.1). Treated control recorded the lowest value for larval weight (2.9g).

Cocoon weight

The treatment *E. officinalis* (1000ppm) gave higher cocoon weight (2.00 g) ; *C.officinalis* (1000ppm) gave (1.77g) and in the untreated control gave (1.11g). The *A.sativum* (1000ppm) and *C.fistula* recorded the lowest values of cocoon weight (1.07g) (Table.7).

Shell weight

The treatment *E.officinalis* (1000ppm) gave higher shell weight (0.27g) which was on par with *C. officinalis* (1000ppm) (0.26g) and in untreated control gave (0.21g). *C.officinalis* (Table.7) *A.sativum* (1000ppm) shows lowest shell weight (0.17g).

Shell ratio

The treatment *E. officinalis* gave higher shell ratio (23.82 percent). This was par with *C.officinalis* (23.05percent) respectively. The 1000ppm of *A. sativum* was recorded the lowest values of shell weight (18.68 percent).

Table: 1. Resistance to increase NPV treated *B.mori* larvae supplemented with medicinal plants:

NO.	Treatment	Larval Mortality (%)	Larval Weight (g)
T1	<i>Embilica officinalis</i>	3.75 (10.78)	3.13
T2	<i>Cassia fistula</i>	3.55 (11.16)	3.05
T3	<i>Pelargonium hortroum</i>	3.68 (10.42)	3.09
T4	<i>Allium sativum</i>	3.20 (10.53)	3.0
T5	<i>Calendula officinalis</i>	3.65 (11.89)	3.13
T6	Untreated control	3.80 (11.53)	3.15
	CD	3.15	0.05
	SE (d)	1.78	0.16

Figures in the parentheses are sine transformed

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

Table :2 . Resistance to increase NPV treated *B.mori* larvae supplemented with medicinal plants.

No.	Treatment	Concentration	Larval Mortality	
			Larval Mortality (%)	Larval Weight (g)
T1	<i>Embilica officinalis</i>	1000ppm	3.56 ^a (11.53)	3.78 ^a
T2	<i>Cassia fistula</i>	1000ppm	3.56 ^{ab} (11.53)	3.23 ^c
T3	<i>Pelargonium hortroum</i>	1000ppm	3.10 ^{abc} (14.17)	3.27 ^{ab}
T4	<i>Allium sativum</i>	1000ppm	3.03 ^{abc} (14.17)	3.06 ^{ab}
T5	<i>Calendula officinalis</i>	1000ppm	35.00 ^d 89.42	3.76 ^{ab}
T6	Treated control	–	100.00 (89.42)	2.99 ^{abc}
T7	Untreated control	–	3.69 (10.46)	3.80 ^a
	CD	–	3.28	0.54
	SE(d)	–	2.01	0.20

Figures in the parentheses are sine transformed

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

Table :3. Effect of aqueous extract of medicinal plants on larval characters of NPV infected *B. mori*

No.	Treatment	Larval Mortality (%)	Larval Weight (G)
T1	<i>Embilica officinalis</i>	16.25 ^b (23.77)	3.52 ^a
T2	<i>Cassia fistula</i>	22.75 ^c (28.48)	3.29 ^b
T3	<i>Pelargonium hortroum</i>	55.0 ^d (47.87)	3.27 ^b
T4	<i>Allium sativum</i>	57.5 ^d (49.31)	3.22 ^c
T5	<i>Caiendula officinalis</i>	100 ^c (89.42)	3.58 ^{ab}
T6	<i>Bmnpv aione</i>	0.0 ^a (0.57)	-
T7	Untreated Control	17.55 ^a (20.21)	3.6 ^a
	CD	2.22	0.24
	SE(D)	1.0	0.12

Figures in the parentheses are sine transformed

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

Table: 4. Effect of aqueous extract of medicinal plants on larval characters of NPV infected *B. mori*

No.	Treatment	Larval mortality (%)	Larval weight (g)
T1	<i>Embilica officinalis</i>	19.97 ^b (33.19)	3.72 ^a
T2	<i>Cassia fistula</i>	32.15 ^c (34.54)	3.52 ^b
T3	<i>Pelargonium hortroum</i>	43.54	3.42 ^c
T4	<i>Allium sativum</i>	65.07 ^c (53.77)	2.97
T5	<i>Caiendula officinalis</i>	100 ^f (89.42)	3.67 ^a
T6	<i>Bmnpv aione</i>	0.0 ^a (0.57)	-
T7	Untreated Control	-	3.79 ^a
	CD	0.61	0.14
	SE(D)	0.22	0.06

Figures in the parentheses are sine transformed

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

Table 5. Effect of chloroform extract of medicinal plants on larval characters of NPV infected *B.mori*

No.	Treatment	Concentration	Larval Characters	
			Larval Mortality%	Larval Weight(g)
T1	<i>Embilica officinalis</i>	1000ppm	21.13 ^b (26.65)	3.86 ^b
T2	<i>Cassia fistula</i>	1000ppm	19.00 ^c (28.65)	3.66 ^c
T3	<i>Pelargonium hortroum</i>	1000ppm	23.00 (30.00)	3.05 ^d
T4	<i>Allium sativum</i>	1000ppm	18.00 ^d (30.65)	3.04 ^d
T5	<i>Caiendula officinalis</i>	1000ppm	55.00 ^f (47.87)	3.7 ^e
T6	Chloroform alone	-	50.00 ^e (45.00)	3.00 ^{ef}
T7	<i>Bmnpv aione</i>	10 ⁶ pob/ml	70.00 ^h (56.79)	2.90 ^{fg}
T8	Untreated Control	-	65.03 ^g (53.74)	3.96 ^g
	CD	-	0.75	0.14
	SE(D)	-	0.32	0.05

Figures in the parentheses are sine transformed

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

Table 6: Effect of chloroform extract of medicinal plants on larval characters of NPV infected *B.mori*

No.	Treatment	Concentration	Larval characters	
			Larval Mortality (%)	Larval Weight(g)
T1	<i>Embilica officinalis</i>	1000ppm	10.66 ^b (20.84)	3.66 ^a
T2	<i>Cassia fistula</i>	1000ppm	19.66 ^c (26.32)	3.46 ^{bc}
T3	<i>Pelargonium hortroum</i>	1000ppm	20.00 ^{cd} (27.27)	3.39 ^c
T4	<i>Allium sativum</i>	1000ppm	22.66 ^d (29.10)	3.36 ^{cd}
T5	<i>Caiendula officinalis</i>	1000ppm	30.33 (43.47)	3.66 ^{def}
T6	Chloroform alone	1000ppm	40.33 ^e (44.04)	3.40 ^{cde}
T7	<i>Bmnpv aione</i>	-	52.66 (48.82)	2.73 ^f
T8	Untreated Control	-	55.03 (47.87)	3.73 ^{ef}
	CD	-	1.88	0.58
	SE(D)	-	0.09	0.24

Figures in the parentheses are sine transformed

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

Table: 7. Resistances to increase NPV treated *B.mori* larvae for Cocoon character Supplemented with medicinal plants.

No.	Treatment	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)
T1	<i>Embilica officinalis</i>	2.00 ^a	0.30 ^a	15.00 ^a
T2	<i>Cassia fistula</i>	1.75 ^{abc}	0.20 ^{bc}	11.42 ^{ab}
T3	<i>Pelargonium hortroum</i>	1.50 ^{bc}	0.22 ^c	14.66 ^b
T4	<i>Allium sativum</i>	1.45 ^{bc}	0.18 ^b	12.41 ^{ab}
T5	<i>Calendula officinalis</i>	1.77 ^{bc}	0.29 ^b	15.31 ^{ab}
T6	Untreated control	1.11 ^{ab}	0.33 ^a	16.50 ^{ab}
	CD	0.25	0.02	1.16
	SE(d)	0.13	0.01	0.39

Figures in the parenthesis are arc sine transformed values.

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

Discussion

The data of present study revealed that chloroform extracts of *E.officinalis* gave higher economic parameters in silkworm and which was on par with *C.officinalis* and untreated control. Shubha et al. (2007) reported that the fifth instar larvae of PMxCSR₂ fed with *Phyllanthus niruri* (5.00 and 4.21 %), *Tribulus terrestris* (7.00 and 5.39 %), *Withania somnifera* (12.00 and 5.73 %) and *Adhatoda vasica* (13.00 and 11.633 %) registered decreased level of mortality due to control (24.00 and 18.41 %). Similarly, the administration of mulberry leaves fortified with extracts of *W.somnifera*, *Tinospora cordifolia* and *Terminalia arjuna* to silkworms, resulted in increased larval weight compared to control. Sridevi, 2003 and Gayathri (2005) also evidenced that, larval weight was significantly higher in *Euphorba prostrata* (3.286g) and *Centella asiatica* (3.284g) as compared to water (3.248g) and normal control (3.255g). Similar results have been obtained by Murugash (2002), who reported that aqueous extract of *T. procumbens*, *T.terrestris* and *P. hysderophorus* sprayed on silkworm resulted in maximum larval

weight (30.25g/10larvae) compared to control (30.70g/10larvae). Patil et al., (1997) also recorded maximum larval weight of 33.8kg /10 larvae where extra foliation was done with 20 per cent *P. hysterothorus* aqueous leaf extract compared to control (24.47/10 larvae).

Sashindran Nair et al., (1998) proved that polyto hormonal supplementation to larvae of *B.mori* improved the economic parameters like cocoon, shell weights and shell ration. Similar, results were obtained by Bhaskar et al., (2004) who found significantly better cocoon weight over control when *W. somnifera* aqueous extract treated mulberry leaves were fed to silkworms (PMxCSR₂ and CSR₂xCSR₄). The results of present work has been positively correlated with the findings of the Sashindran Nair et al., (1998). Bhaskar et al., (2004) and the chloroform extract of *Emblica officinalis*, *Calendula officinalis*, *Cassia fistula*, *Allium sativum* and *Pelargonium hortroum* are the best botanicals for the management of grasserie in silkworm *Bombyx mori*.

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