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Botanicals Mediated Reproductive Enhancement in Mulberry Silkworm (*Bombyx mori* L.)

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Ethanol extract of 20 botanicals in 3 concentrations i.e., 2.5%, 5% and 10% were evaluated for their efficacy to improve reproductive performance of mulberry silkworm (*Bombyx mori* L.) through feeding botanical enriched mulberry leaves during 2nd day of 4th age as well as 1st and 3rd day of 5th age larvae. The preliminary screening was done by ranking the botanicals based on influence in most important reproductive contributing parameters such as single cocoon weight, effective rate of rearing, pupation rate, fecundity and recovery of eggs/kg cocoon. Five botanicals *viz.*, *Asparagus recemosus*, *Achyranthes aspera*, *Tribulus terrestris*, *Withania somnifera*, *Parthenium histerophorus* ranked first were further evaluated at 8% 5% and 3% concentrations to confirm their effectiveness and standardize effective concentration. Among the rearing parameters, mature larval weight, shell ratio and number of cocoons/kg were not significantly influenced by the treatments. However, reproductive parameters such as effective rate of rearing (wt), pupation rate (%), fecundity (No.) and egg recovery (g)/kg cocoon were significantly ($P<0.05$) influenced by the botanicals. Higher fecundity and egg recovery were obtained on treatment with Withania somnifera irrespective of concentrations. However at 5% concentration, pupation rate, fecundity as well as egg recovery were found significantly higher than that of other concentrations, confirming its effectiveness for improving reproductive efficiency.

Key words: *Bombyx mori*, reproductive efficiency, botanicals, fecundity, egg recovery.

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Introduction

Botanicals are having immense ability to influence on the metabolic activities of insects. In view of this, in the recent past many attempts have been made to fortify mulberry leaf with botanical extracts so as to improve the mulberry leaf quality and feed efficiency of silkworm, which in turn help to increase cocoon production and silk quality. These studies confirmed effect of many plant extracts on various metabolic activities resulting acceleration of spinning (Shivakumar *et al.*, 1995), increase in larval, cocoon as well as shell weight (Sujatha and Purushothamarao, 2003; Sridevi *et al.*, 2003; Krishnaprasad *et al.*, 2001; Jayaprakaṣhrao, 1998; Patil *et al.*, 1997; Rajasekharagouda, 1991) silk gland weight (Santhosh kumar, 1997) improvement reeling parameters (Mamadapur, 1994), increase in pupal weight, increase in silk content, silk filament length and weight (Sujatha *et al.*, 2003; Patil *et al.*, 1997). All these studies were emphasized to improve rearing parameters of silkworm. Many botanicals are used to improve reproduction in vertebrates (Dastur, 1962; Chopra *et al.*, 1956; Joshi and Magar, 1952) and lower invertebrates (Devi, 1995; Hilda, 1992). However studies on effectiveness of botanicals on reproductive performance of silkworm are scanty. Since the silkworm seed production is a very important area in sericulture, improvement of reproductive efficiency also is imperative to look into. The present work has been undertaken to study efficacy of botanicals to improve the reproductive performance of silkworm so as to improve the silkworm seed production.

Materials and Methods

Twenty locally available botanicals, which are reported to have influenced on the reproductive efficacy in some organisms were selected for the study. The botanicals were collected and cleaned in distilled water and dried at

60°C for 24 h in oven. The pant materials (root, leaf, seed or whole plant) were then powdered and extracted in ethanol using soxhlet apparatus. The extract was then placed below a ceiling fan and evaporated the ethanolic part. The residue is then diluted to 100 ml distilled water, which is taken as mother solution. The extracts were diluted in to 2.5, 5 and 10% concentrations (v/v). The extracts were then sprayed separately on the mulberry leaves (variety V1) harvested from the experimental plot of the laboratory, raised following standard package of practices (Ramakanth *et al.*, 2005) and allowed to drain off the water. The leaves were then fed to the silkworms (race CSR2) once during the 2nd day of 4th instar and 1st and 3rd day of 5th instar larvae. Other rearing practices are followed as per the recommendation (Kawakami, 2001). The leaves sprayed with distilled water served as control. Three replications with 300 worms per replication were kept against each treatment and control. The mature larval weight was taken on 6th day of 5th instar larvae. The ripened worms were transferred in the mountages. After harvesting single cocoon weight and single shell weight were assessed and shell ratio, effective rate of rearing per 10000 larvae were calculated. The experiment was repeated twice and the data were pooled and compiled.

The grainage performances were assessed following standard methods (Angadi and Somireddy, 2002). The harvested cocoons were cut and pupation rate (%) was assessed. The pupae were then sex separated. After emergence of the moth they were allowed for pairing for three hours and 24 h oviposition. The eggs per layings were assessed counting 5 layings selected randomly from replications of each treatment and control. The layings were washed and then loose eggs were made. In order to screen the effective botanical and its concentration, the indexing was done selecting five important reproductive parameters such as single cocoon weight, effective rearing rate, pupation, fecundity and egg recovery per kg of cocoon using the formulae of Mano *et al.*, (1993) and selected five botanicals ranked first for further studies.

The five selected botanicals were further screened for confirming their efficacy. In this experiment, in order to standardize the concentration the effective concentration (5%) in the first screening and two other concentrations (8% and 3%) of each botanical were taken. The other methods were followed as mentioned in the preliminary screening. The experiment was repeated for thrice, the data were pooled and evaluated for two factors ANOVA and means were compared for significant difference.

Table 1. Impact of various botanicals and their concentrations on the reproductive performance of silkworm

Sl. No	Botanicals	Parts used	Score		
			Concentration (%)		
			2.5	5	10
1	*Achyranthes aspera* Linn.	WP	51.89 (17)	57.29 (3)	46.41 (50)
2	*Amaranthes spinosus* Linn.	WP	49.75a (32)	49.67 (34)	40.38 (60)
3	*Argemone mexicana* Linn.	L	50.03 (30)	46.74 (48)	49.26 (37)
4	*Asparagus recimosus* Willd.	L	48.33 (39)	54.67 (8)	49.43 (36)
5	*Bambosa arundinacea* (Retz) Willd.	L	45.13 (53)	46.17 (51)	44.28 (56)
6	*Boerhavia diffusa* Linn.	WP	47.61 (45)	51.39 (23)	51.50 (20)
7	*Cassia sophera* Linn.	L	47.62 (44)	44.66 (54)	51.87 (18)
8	*Cassia tora* Linn.	L	54.11 (10)	53.67 (12)	51.46 (21)
9	*Centella asiatica* (L.) Urban	WH	50.95 (25)	42.94 (58)	36.31 (61)
10	*Cynodon dactylon* Pers.	WP	50.34 (29)	52.69 (14)	49.63 (35)
11	*Lantana indica* Rpxb.	L	50.69 (28)	47.66 (43)	46.53 (49)
12	*Moringa oleifers* Lamk.	L	53.14 (13)	51.60 (19)	44.64 (55)
13	*Macuna pruriens* (L.) D.C.	S	47.53 (47)	49.25 (38)	42.32 (59)
14	*Parthenium hysterophorus* Linn.	L	50.83 (26)	54.15 (9)	50.73 (27)
15	*Phyllanthus niruri* Linn.	WP	44.22 (57)	51.16 (24)	47.97 (41)
16	*Psoralia corylifolia* Linn.	L	53.76 (11)	52.24 (15)	47.47 (42)
17	*Tagetes erecta* Linn.	L	48.06 (40)	46.16 (52)	47.57 (46)
18	*Tribulus terrestris* Linn.	S	56.41 (5)	63.68 (1)	57.10 (4)
19	*Platycladus orientalis* (L.) Franco	L	52.03 (16)	49.73 (33)	51.42 (22)
20	*Withania somnifera* Dun	R	55.80 (7)	63.56 (2)	56.40 (6)
	Control	-	49.78 (31)	–	–

WP-Whole plant, L-Leaf, S-seed, R-root. Figures in parenthesis are ranks of botanicals

Results and Discussion

Preliminary screening

In the preliminary screening based on influence of botanicals and their three concentrations showed varied responses on reproductive contributing parameters. The botanical *T. terrestris* performed well in all the concentrations ranking first with highest score 68.68 at 5% conc. However the same at 2.5% conc. ranked fifth with a score of 56.41 and at 10% conc. ranked fourth with a score of 57.10. Similarly, *W. somnifera* performed equally well at all the concentrations ranking second with a score of 63.56 at 5% conc. However the same at 2.5% conc. ranked seventh with a score of 55.80 and at 10%, it ranked sixth with a score of 56.40. Next to these, *A. aspera* at 5% conc. ranked third with a score of 57.29. Likewise, *A. recemosus* at 5% conc. scored 54.67, ranking eighth and *P. histerophorus* at 5% performed good with a score of 54.15 ranking ninth. However, *C.asiatica* at 10% scored least with last rank. The control however scored 49.78 with 31st rank (Table 1).

Afore mentioned five botanicals performed well were further screened for confirmation of the result and standardization of the effective concentration. Since among the concentrations 5% showed best result in all the cases, it is presumed that the effective concentration is either 5% or any concentration in between 10% or 2.5% therefore, in the second trial 8% and 3% were taken in place of 10% and 2.5%, for evaluation.

Influence selected botanicals on rearing characters

Mature larval weight was higher due to the treatment with 5% *A. apera* followed by *T. terrestris* and *W. somnifera* in same concentration. Nevertheless, none of the treatment was significantly influenced on the mature larval weight (Table 2). Single cocoon weight was significantly higher due to treatment with *A. aspera* and *A. recemosus* irrespective of concentration. However the same was found significantly less in all the concentrations of *P. hystero-*

Table 2. Impact of selected botanicals on the rearing parameters of silkworm

Botanicals	Concentration (%)	10 Mature larval wt. (g)	Single Cocoon wt. (g)	Single Shell wt. (g)	Silk Ratio (%)	Effective rearing rate (ERR) for 10000 larvae	
						(No.)	Wt. (kg)
A. aspera	8	39.43	1.76	0.392	22.15	9533	18.72
	5	40.41	1.75	0.400	22.85	9400	17.73
	3	39.24	1.76	0.400	22.72	9500	18.59
A. recimosus	8	39.05	1.80	0.421	23.33	9400	18.93
	5	40.00	1.79	0.423	23.46	9433	18.40
	3	38.90	1.76	0.384	22.01	9533	18.13
P. hysterophorus	8	39.58	1.69	0.380	22.48	9533	17.57
	5	39.20	1.74	0.371	21.26	9400	17.83
	3	39.30	1.70	0.374	21.76	9500	17.54
T. terrestris	8	39.20	1.62	0.361	22.22	9400	16.58
	5	40.23	1.65	0.372	22.42	9433	17.24
	3	39.47	1.60	0.361	22.50	9433	16.82
W. somnifera	8	39.44	1.65	0.370	22.42	9500	17.96
	5	40.21	1.75	0.396	21.71	9467	18.14
	3	38.67	1.71	0.373	21.63	9467	17.92
Control	–	38.61	1.74	0.380	21.83	9387	18.02
CD ($P < 0.05$)							
Treatments		NS	0.02	0.0075	NS	NS	0.43
Concentration		NS	0.02	0.018	NS	NS	NS
Treat. × Conc.		NS	0.19	0.017	NS	NS	NS

NS: Not significant

phorus and *T. terrestris*. *W. somnifera* significantly increased the cocoon weight only at 5%. There was a significant interaction between the concentration and treatment. The highest cocoon weight was observed in the lot applied with *A. recemosus* at 8% followed by the same at 5% and the least was observed in *T. terrestris* (3%) applied lots. Likewise, single shell weight was significantly higher ($P<0.05$) in *A. aspera* and *A. recemosus* irrespective of their concentrations. However shell weight was not increased significantly by *P. hysterophorus* and *T. terrestris*. *W. somnifera* was found significantly effective at 5% only. Among the concentrations, 5% was found significantly effective than other concentrations. There was significant interaction between the treatment and concentration in respect of shell weight also. Shell weight was significantly less with the application of *A. recemosus* at 3% compared to the same at other concentrations. Similarly *W. somnifera* was effective only at 5%. The shell weight was not varied significantly among concentrations of other botanicals. Nevertheless, none of the botanical was significantly influenced on Silk ratio and effective rearing rate (No.). The effective rearing rate (wt.) was found significantly higher in *A. aspera* at 8% and 3% and no interaction was found between treatment and their concentrations.

Influence selected botanicals on grainage characters

Pupation rate was found significantly higher ($P<0.05$) due to treatment with *W. somnifera* (94.09%) followed by treatment with *T. terrestris* (93.56%). However, among the concentrations 5% and 3% were found significantly higher compared to 8%. The extracts *W. somnifera* (95.67%) and *T. terrestris* (93.67%) at 5% registered significantly higher pupation rate at 5% concentration (Fig. 1). Similarly, average fecundity was significantly higher due to application of extract *W. somnifera* treated lots (543) compared to control (491) Concentration was not significantly influence on the fecundity (Fig. 2). Number of eggs per gram was found significantly lower in *A. aspera* at 8% followed by *A. recimosus* at 3%, all concentrations of *P. hysterophorus* and 5% and 3% of *W. somnifera* compared to control (Fig. 3). Egg recovery was found significantly higher in botanical *W.somnifera* treated lots with highest average egg recovery (56.80 g). The concentration of the botanicals also significantly influenced on the egg recovery with average high egg recovery (51.29 g) in case of 3% and 5% (50.22 g), which was significantly higher than that of 10% concentration (Fig. 4). There was a significant interaction among the treatment and concentration with highest egg recovery in case of treatment *W. somnifera* (60.69 g) at 5% concentration, which was significantly higher than that of egg recovery in obtained at 8% (54.22 g) or 3% (55.51 g).

The botanicals influenced the rearing as well as grain-

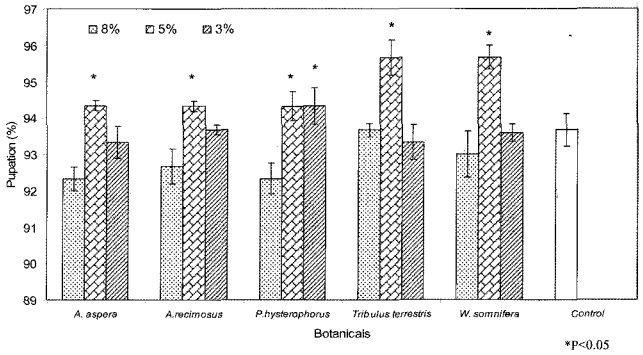

Fig. 1. Influence of botanicals pupation rate of silkworm.

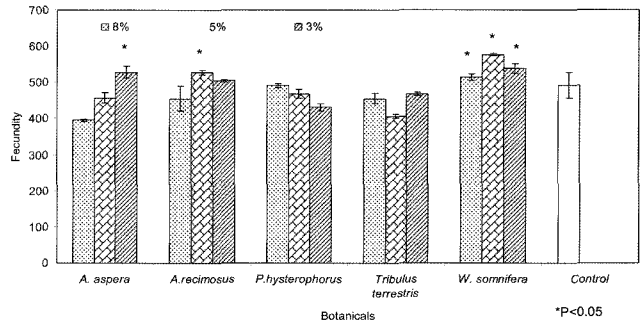

Fig. 2. Influence of botanicals on fecundity of silkworm.

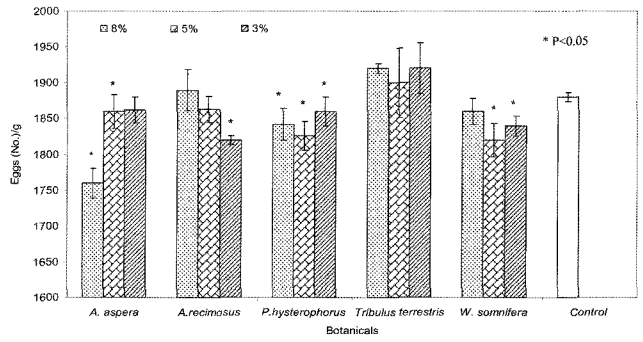

Fig. 3. Influence of botanicals on eggs/gram of silkworm.

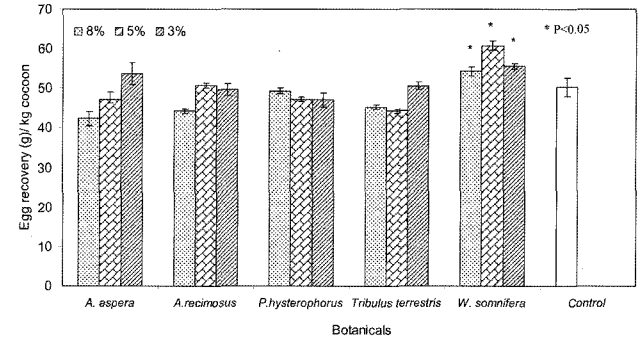

Fig. 4. Influence of botanicals on egg recovery of silkworm.

age characters. Mane *et al.* (1997) opined that some of the commonly available plants with phagostimulant properties should be screened and feed to silkworms along with mulberry leaves, thus supplementation of phagostimulant may increase the appetite with least wastage of leaves and increase over all productivity. Ito, (1961) and Ito *et al.*, (1964) reported many sterols have the feeding stimulants. Jaypaul *et al.* (2003) observed treatment with leaf extracts of certain plants on mulberry leaves can increase the productivity of silkworm with limited food leading to economic gain. In the present study, five botanicals selected form the preliminary screening may be having the phytosterols, which possess the activity to influence the qualitative and quantitative characters of silkworm. Further, Murugan *et al.* (1998) reported that the plant extracts generally show marginal tendency to improve many of the biological characters of silkworm. In the present study, many of the biological characters are improved due to the application of plant extract along with mulberry leaves corroborate with these findings. It is reported (Murugan *et al.*, 1998; Sreedevi *et al.*, 2003) that the medium concentration of the botanicals is highly effective for the improvement of many parameters of silkworm rearing. In the present study also the medium concentration (5%) was found highly effective. The less impact of high as well as low concentration of botanicals may be due to the deleterious action of high or low action of lower dosage. Some of the plants used in this study such as *Achyranthes* sp. (Kim *et al.*, 1997), *A.spinosus* (Jayaprakashrao, 1998) as well as *P. histerophorus* (Hipparagi *et al.*, 2003), *W. somnifera* (Sreedevi *et al.*, 2003) are reported to have positive influence on various quantitative and qualitative characters of silkworm. This study reveals the effectiveness of some of the botanicals to improve reproductive performance of silkworm.

The botanicals identified for its impact on the reproductive characters can be further utilized for improving the seed crop production of silkworm. Use of botanicals in crude form though reflects its effectiveness, the active ingredient of the identified botanicals and their chemical analogues are to be tested for getting better results. Further, the synergistic effects of botanicals which are either effective in reproductive or rearing characters are to be studied.

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