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PHAGOSTIMULANT ACTIVITY OF SOME BOTANICALS TO *BOMBYX MORI* LINN

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ABSTRACT

Administration of mulberry leaves treated with methanol extracts of five botanicals and medicinal plant oils indicated a slight phagostimulatory activity in *M.azedarach* leaves at 0.5% (C value=1.02) to fifth instar larvae of *Bombyx mori*. Among the medicinal plant oils, the oils from *Psoralea corylifolia* (C value=1.29 and 1.16), *Asparagus racemosus* (C value=1.02 and 1.12), *Juglans regia* (C value=1.35 and 1.17), *Santalum album* (C value=1.06 and 1.04), *Trigonella foenum-graecum* (C value=1.05 and 1.03) at 1 and 2% respectively were preferred while *Lageneria vulgaris* (C value =1.05) was preferred by the worm at 2%.Thes botanicals could further be tested for their effect on economic parameters of *B. mori*.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* feeds exclusively on mulberry leaves. The mulberry leaf quality is considered as a sole factor governing the production of good cocoon crop. Improvement in the nutritional quality of mulberry leaves can enhance the efficiency of cocoon and seed production in silkworm. Many approaches have been tried to augment productivity and cocoon quality. Fortification of mulberry leaves is one of the approaches, wherein the nutritional quality can be improved and superior quality silk can be achieved. Fortification of mulberry leaves with plant products including medicinal plant extracts and oils alter the physiology of the silkworm. Various botanicals are known to influence the silkworms by increasing either food consumption or biomass, thereby enhancing cocoon yield (Maribashetty *et al.*, 2010). Studies have been conducted wherein the ayurvedic and homoeopathic preparations *viz.,* alfalfa, mahadraksharista and kalmegh,(dasmularishta) and amlaki (rasayan) have shown a biotonic effect and improved the rearing standards of silkworm (Laskar and Datta, 2000; Datta and Prasad, 1999 and Datta and Gupta, 2002). Earlier studies on plant essential oils have shown their phagostimulant and growth promoting effects on silkworm (Dubey and Srivastava, 2004., Shahin *et al.*, 2013).The present work is an attempt to evaluate the phagostimulant properties of some plant oils and medicinal plant extracts on the final instar larvae of the mulberry silkworm, *B.mori* race [Nx(SK6xSK7)].

MATERIALS AND METHODS

Rearing of *B. mori*

Multi x bivoltine chawki reared worms [race:N×(SK_6×SK_7)] were obtained from Government Sericulture Station, Haldwani, District Nainital (Uttarakhand). A rolling culture of silkworm was maintained on soft and tender leaves of mulberry variety Chak Majra (CM) (preferably 3rd and 4th leaves from the top). The worms were kept in wooden rearing trays (size: 3¢ × 2¢ × 3¢¢). In order to maintain proper humidity in the rearing trays, two layers of wet filter paper (ordinary lab quality) were placed on the bottom which were further covered with a layer of paraffin paper as and when required. Trays were covered with muslin cloth. Fresh leaves were provided every day and proper hygienic conditions were maintained in the room.

Preparation of medicinal plant extracts

The phagoactivity of five medicinal plant extracts viz. *Ageratum conyzoides*, *Anethum graveolens, Lantana camara*, *Melia azedarach* and *Pteris excelsa* were evaluated against the 24d (fifth instar) old worms by no-choice feeding bioassay technique (Isman *et al*., 1990., Srivastava and Proksch, 1990, 1991). The fresh plant parts were washed in running tap water and dried in the shade for a week. The dried plants were weighed and macerated in an electric grinder into a fine paste, completely dipped in methanol in separate flasks, and kept at room temperature. After 3-4 days, the extract was filtered through Whatmann filter paper and centrifuged at 5000rpm for 10 minutes. The supernatant was evaporated under vacuum (Kurucheve *et al*., 1997).The crude powder obtained after evaporation of the supernatant was kept in sealed glass vials and stored in the refrigerator. A test solution (on dry wt/

Table 1: Effect of five medicinal plant extracts (methanol powder) on feeding behaviour of 24d old worms of the mulberry silkworm, *Bombyx mori* Linn. [Race: N×(SK6×SK7)]

Plant species	Part used	MLAC (cm^2)/ larva(Conc.) 0.5%	1.0 %	Feeding inhibition (%) 0.5%	1.0%	Preference index 0.5%	1.0%	Antifeedant category 0.5%	1.0 %
Ageratum conyzoides	Flower Head	0.37 ± 0.26^{a}	0.11 ± 0.06^{a}	92.59	97.46	0.09	0.02	Extremely antifeedant	Extremely antifeedant
	Leaves	4.12 ± 1.43^{b}	1.96 ± 1.65^{ab}	31.09	60.82	0.69	0.40	Moderately antifeedant	Strongly antifeedant
Anethum graveolens	Leaves	5.29 ± 0.14^{bc}	6.37 ± 0.57^{b}	19.17	10.01	0.80	0.89	Slightly antifeedant	Slightly antifeedant
Lantana camara	Fruit	6.38 ± 0.88^{bcd}	5.12 ± 2.08^{bc}	10.63	20.93	0.89	0.84	Slightly antifeedant	Slightly antifeedant
	Leaves	1.05 ± 0.51^{a}	1.48 ± 1.40^{ab}	77.27	57.32	0.23	0.31	Extremely antifeedant	Strongly antifeedant
Melia azedarach	Leaves	7.29 ± 1.39^{cd}	8.25 ± 0.84^{c}	4.00	-2.50	0.96	1.02	Slightly antifeedant	Preferred
Pteris excelsa	Leaves	5.46 ± 0.253^{bc}	4.00 ± 2.87^{bc}	18.18	32.20	0.82	0.67	Slightly antifeedant	Moderately antifeedant
Control (Water)	-	7.80 ± 0.34^{cd}	7.80 ± 0.34^{c}	-	-	1.00	1.00	-	-
SEM(±)		0.81	1.51	-	-	-	-	-	-
CD at 1%		3.35	6.26	-	-	-	-	-	-
CD at 5%		2.43	4.59	-	-	-	-	-	-
F-value		**	*	-	-	-	-	-	-

Means followed by common letters do not differ significantly by DMRT (p=0.05%); MLAC= Mean leaf area consumed, Mean ± SD (Standard Deviation); NAntifeedant category following Kogan and Goeden, (1970) *A .conyzoides* with still lower C values (0.02 -0.09) was put in antifeedant category (0.1-0.25); * = significant; ** = highly significant

volume basis) was prepared for each extract by dissolving appropriate amount of powder in definite volume of water. Mulberry leaves were utilized to treat and feed the worms during the experimentation. The fresh and mature leaves were plucked, thoroughly washed and dried with the help of filter paper and the leaf discs (area = 5 × 5cm^2) were cut from them. Two conc. (0.5 and 1.0%, dry wt. basis) of each of the methanol powder were prepared in the hot water. The leaf discs were later dipped in the extracts for approx. 4 to 5 minutes and air dried for a while. The leaf discs were kept in centre of pre sterilized corning glass petridishes (dia. 9cm) containing an inner lining of moist filter paper. All the treatments were replicated three times along with control. Prestarved (3h) and freshly moulted worms of uniform size were released in each petridish (n=2) and were allowed to feed until more than 75% leaf discs were eaten away in control.

Plant essential oils

Phagostimulatory activity of eleven plant oils viz. *Asparagus racemosus*, *Celastrus paniculatus*, *Juglans regia*, *Myristica fragrans, Psoralea corylifolia, Cinnamomum tamala, Ellettaria cardamom*, *Hedychium spicatum*, *Lagenaria vulgaris*, *Santalum album* and *Trigonella foenum-graecum* were screened against 22 and 23d old worms of *B. mori* by no-choice feeding bioassay method. Plant oils used in the experiment were obtained from general grocery store, Haldwani, Nainital. Two concentrations (1% and 2%) of each of the plant oils were prepared in acetone. Same procedure as in the case of plant extracts was followed for the conduct of the experiment.

Observations

The observations were recorded on leaf area consumed with the help of graph paper. The calculations were made on feeding percentage (Singh and Pant, 1980), feeding inhibition (Purwar and Srivastava, 2003) and preference index (Kogan and Goeden, 1970).The index measures the relative amount of feeding on 2 species of plants present in the arena in a 0 to +2 scale. A value of 1 indicates that feeding on test plant was equivalent to the feeding on the standard. A, C -value > 1 indicates a preference for the test plant and a C- value < 1 indicates less acceptance to the test plant **.**

On the basis of C- values, the experimental plant extracts were assigned categories as under

Category (C-value)

1. Extremely antifeedant plant extracts	:	0.1-0.25
2. Strongly antifeedant plant extracts	:	0.26-0. 50
3. Moderately antifeedant plant extracts	:	0.51-0.75
4. Slightly antifeedant plant extracts	:	0.76-0.99
5. Preferred plant extracts	:	e”1

RESULTS AND DISCUSSION

All the plant extracts showed a considerable reduction in feeding over control (7.80cm^2) against *B.mori* at both concentrations except *M.azedarach* leaf 1% (8.25cm^2) which caused insignificant increase over control (7.80cm^2). At 0.5 and 1% feeding was significantly lowered in *A.conyzoides* flower head (0.37 and 0.11cm^2), *A.conyzoides* leaves (4.12

Table 2: Effect of five medicinal plant oils on feeding behaviour of 22 d old worms of the mulberry silkworm, *Bombyx mori* Linn. [Race: N × (SK6 × SK7)]

Plant species Scientific name	MLAC (cm^2)/larva(Conc.)		Feeding inhibition (%)		Preference index		Antifeedant category	
	1.0%	2.0%	1.0%	2.0%	1.0%	2.0%	1.0%	2.0%
Asparagus racemosus	4.19 ± 1.37^{bc}	5.12 ± 2.20^{bc}	21.61	11.93	1.02	1.12	Preferred	Preferred
Celastrus paniculatus	0.16 ± 0.04^{a}	0.17 ± 0.09^{a}	96.66	94.68	0.07	0.08	Extremely antifeedant	Extremely antifeedant
Juglans regia	8.37 ± 0.28^{d}	5.64 ± 0.58^{c}	-12.47	7.17	1.35	1.17	Preferred	Preferred
Myristica fragrans	2.6 ± 0.13^{ab}	0.81 ± 0.34^{ab}	42.92	77.82	0.79	0.34	Slightly antifeedant	Strongly antifeedant
Psoralea corylifolia	7.27 ± 0.98^{d}	5.52 ± 2.23^{c}	-5.47	-12.41	1.29	1.16	Preferred	Preferred
Control (Acetone)	6.51 ± 1.25^{cd}	6.51 ± 1.25^{c}	-	-	1.00	1.00	-	-
SEM(±)	0.86	1.40	-	-	-	-	-	-
CD at 1%	3.75	6.06	-	-	-	-	-	-
CD at 5%	2.67	4.32	-	-	-	-	-	-
F value	**	*	-	-	-	-	-	-

Means followed by common letters do not differ significantly by DMRT (p = 0.05%); MLAC = Mean leaf area consumed, Mean ± SD (Standard Deviation); Antifeedant category following Kogan and Goeden (1970) *C.paniculatus* with still lower C values (0.07 -0.08) was put in antifeedant category (0.1-0.25); * = Significant; ** = highly significant

Table 3: Effect of six medicinal plant oils on feeding behaviour of 23 d old worms of the mulberry silkworm, *Bombyx mori* Linn. [race: N × (SK6 × SK7)]

Plant species	MLAC (cm^2)/larva (Conc.)		Feeding inhibition (%)		Preference index		Antifeedant category	
	1.0%	2.0%	1.0%	2.0%	1.0%	2.0%	1.0%	2.0%
Cinnamomum tamala	9.42 ± 1.23^{b}	0.78 ± 0.39^{a}	4.49	85.83	0.14	0.95	Slightly antifeedant	Extremely antifeedant
Elettaria cardmum	10.12 ± 0.88^{b}	5.76 ± 3.43^{b}	0.92	28.28	0.71	0.99	Slightly antifeedant	Moderately antifeedant
Hedychium spicatum	4.11 ± 2.07^{a}	0.59 ± 0.57^{a}	42.92	89.05	0.10	0.57	Moderately antifeedant	Extremely antifeedant
Lagenaria vulgaris	8.92 ± 1.18^{b}	11.49 ± 0.25^{c}	7.18	-5.44	1.05	0.92	Slightly antifeedant	Preferred
Santalum album	11.78 ± 0.53^{b}	11.20 ± 0.15^{c}	-6.65	-4.15	1.04	1.06	Preferred	Preferred
Trigonella foenum-graecum	11.53 ± 0.22^{b}	10.97 ± 0.78^{c}	-5.61	-3.09	1.03	1.05	Preferred	Preferred
Control (Acetone)	10.31 ± 0.39^{b}	10.31 ± 0.39^{c}	-	-	1.00	1.00	-	-
SEM(±)	1.10	1.36	-	-	-	-	-	-
CD at 1%	4.63	4.63	-	-	-	-	-	-
CD at 5%	3.33	3.33	-	-	-	-	-	-
F value	**	**	-	-	-	-	-	-

Means followed by common letters do not differ significantly by DMRT (p = 0.05%); MLAC = Mean leaf area consumed, Mean ± SD (Standard; Deviation); Antifeedant category following Kogan and Goeden, (1970) ** = Highly significant

and 1.96cm²) and *L.camara* leaves (1.05 and 1.48cm²) respectively in comparison of control. Lowest feeding inhibition was exerted by *L.camara* fruits10.63 and 20.93% at 0.5 and 1% respectively. *M.azedarach* leaves105.76 and 93.46% respectively enhanced feeding over control. On the basis of preference index *A.conyzoides* flower head at 1.0 and 0.5% with C-value of 0.02 and 0.09, was placed above 'extremely antifeedant', *A.conyzoides* (0.40) and *L.camara* (0.31) leaves in 'moderately antifeedant,'*A.graveolens* (0.89) and *L.camara* fruits (0.84) in 'slightly antifeedant' and *M.azedarach* (1.02) in 'preferred'(>1) categories at 1%.

The oils from *P.corylifolia* 1 and 2% (MLAC = 7.27 and 5.52cm²), *A.racemosus* (4.19 and 5.12cm²), *J. regia* (8.37 and 5.64cm²) *L.vulgaris* 2% (11.49cm²), *S.album* (11.78 and 11.20cm²) and *T.foenum-graecum* (11.53 and 10.97cm²) and at 1 and 2% respectively increased feeding, although non-significant.

Phagodeterrent activities were exhibited by *C.tamala* (85.83%), *H.spicatum* (89.05%) and *E.cardomum* (28.28%) at 2% and *C.paniculatus* (96.66 and 94.68%) and *M.fragrans* (42.92 and 77.82%) at 1 and 2% respectively. The preference index (C-value) showed *A.racemosus* (1.02 and 1.12), *J.regia* (1.35 and 1.17) and *P.corylifolia* (1.29 and 1.16), *S.album* (1.06 and 1.04), *T.foenum-graecum* (1.05 and 1.03) were 'preferred' by *B.mori* at 1 and 2% while *L.vulgaris* (1.05) was preferred by silkworm at 2%. *C.paniculatus* (0.07 and 0.08) at 1 and 2% was placed above the category 'extremely antifeedant' showing highly detrimental antifeedance response. *M. fragrans* (0.34) was 'strongly antifeedant' at 2 % and 'slightly antifeedant' (0.79) at 1% . *C.tamala* (0.95), *E.cardomum* (0.99) and *L.vulgaris* (0.92) in 'slightly antifeedant' and *H.spicatum* (0.57) in 'moderately antifeedant' at 1% conc. At 2% conc., *C.tamala* (0.14) and *H.spicatum* (0.10) were 'extremely antifeedant' while *E.cardomum* (0.71) was 'moderately antifeedant'.

The results indicated that only *M.azedarach* leaves had phagostimulant activity towards *B.mori* . A comparison of antifeedant activity between *L.camara* fruits and leaves indicated strong activity in leaves as compared to fruits. In support of these observations we cite the findings of the Kulkarni *et al.* (1997), Siddiqui *et al.* (2001) and Desmukh *et al.* (2011) who are of the opinion that the toxicity in leaves may be due to the presence of active groups like lantadene, lantoniside, linaroside and carminic acid. *A.conyzoides* flower head and *L.camara* leaves are known for their insecticidal and feeding deterrent activities against many insect species (Vyas and Mulchandani, 1980., Saxena and Saxena, 1992., Srivastava and Proksch, 1993., Pari *et al.*,1998., Gbolale *et al.*,1999., Parugrug and Roxas, 2008, Dua *et al.*,2010, Deshmukh *et al.*,2011 and Baidoo and Adam, 2012). Such activities have been observed in this experiment against *B. mori* too. *M.azedarach* leaves are also known to contain toxic constituents (Bohnenstengel *et al.*, 1997 and 1999), but in our experiment at 0.5% and 1%

conc. considerable deterrent activity has not been observed against 24d old worms of *B.mori* rather non significant feeding phagostimulatory activity has been noticed at 1%. It is therefore, inferred that the deterrent activity could be observed at still high concentrations.Many medicinal plant species at conc. 0.1 and 0.5% are reported to improve the growth, development and economic parameters of *B.mori*. For example Sridevi et *al*. (2004) observed an improvement with *Withania sominifera* extract recording significantly higher pupation (97%), moth emergence (98%), fecundity (6.03 eggs/dfl) and hatching percentage (94%) in *B. mori* hybrid CSR×CSR4. *Sauropus androgynus* and *Phyllanthus niruri* decreased the grainage parameters. Dubey and Srivastava (2004) reported enhanced feeding over control in lavender, citronella, mentha and lemon grass oils against final instar of B.mori (race NB4D2x SH6) in closed desiccators at 2, 4 and 10ul/l of space.

Amongst the eleven medicinal plant oils tested only the oils from *S.album, P.corylifolia, J.regia, A.racemosus, L.vulgaris and T.foenum-graecum* showed feeding preference towards *B.mori*.These oils could be further studied for their effect on cocoon parameters of *B.mori*.

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