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Toxicological effect of medicinal plant extracts used against mulberry powdery mildew on growth, development of silkworm (*Bombyx mori* L.), cocoon and silk quality parameter

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Abstract
The different medicinal plant extracts applied against mulberry powdery mildew were tested for their effect on growth and development of silkworm, cocoon and silk quality parameters. It was found that when treated leaves were fed one day after treatment highest larval mortality (25.00%) was recorded in *Azadirachta indica* extract at 15 percent but it was very low in case of *Zingiber officinale* (4.99%). At same concentration, the larval death steadily decreased in all the treatments except *Azadirachta indica* with15.02 percent, when fed two days later, whereas, no mortality noticed in case of *Zingiber officinale* at two days after treatment. All the plant extracts tested had no toxic effect at three days after treatment. Maximum larval weight (29.42g), larval length (5.37cm), cocoon weight (12.87g), pupal weight (9.46g), shell weight (3.41g), shell ratio (26.49%) and maximum cocoon yield (450.45g) was observed in *Zingiber officinale* at 15% which was significantly superior over control treatment in all parameters. The increased larval weight in *Zingiber officinale* treated silkworms might be due to stimulatory effect of ginger on protein synthesis in the silk gland during larval period. The silk quality viz., filament length of 884.02 m and the denier was finer (1.83%) in silkworm larvae fed with *Zingiber officinale* fortified mulberry leaves was better compared to all other treatments followed by *Lantana camara* and *Acorus calamus*.

Keywords: Toxicity, *Azadirachta indica*, *Zingiber officinale*, *Allium cepa*, *Lantana camara*

Introduction
Silk worm (*Bombyx mori* L.) host Mulberry (*Morus alba*) is a perennial plant belongs to the family *Moraceae*. It is cultivated in both tropical and temperate countries of the world. Though mulberry cultivation is practiced in various climates in India, it is extensively grown in the tropical zone covering Karnataka, Andhra Pradesh and Tamil Nadu states with about 90 percent of area where, most of the sericulture industry is concentrated. In the sub-tropical zone, West Bengal, Himachal Pradesh and the north eastern states have major areas under mulberry cultivation (Dutta, S. K., 2011) [4]. Powdery mildew is caused by an obligate biotrophic ascomycete fungus, *Phyllactinia corylea* (Syn. *P. guttata* and *P. moricola*) throughout the world (Chattopadhyay *et al*., 2011) [2]. The mulberry powdery mildew disease is managed by spraying fungicides. But the fungicide residues on the mulberry leaves may be detrimental to silkworms when fed with such leaves. Therefore safe period of more than 15 days need to be followed, thus affecting the availability of safe leaves for the worms. To circumvent such problems, an alternate method of control of disease needs to be explored. The medicinal plant extracts can be used as an alternative strategy to manage the mulberry powdery mildew as it considered safe and eco friendly. Since the work done and information on the residual toxicity of medicinal plant extracts on silkworms is inadequate, an investigation on the effect of residues of medicinal plant extract on silkworm health and cocoon quality and weight were undertaken.

Material and methods
Preparation of extracts of medicinal plants
Different medicinal plants viz: Neem leaf (*Azadirachta indica*), Onion bulb (*Allium cepa*), Ginger rhizome (*Zingiber officinale*), Sweet sledge dried root (*Acorus calamus*), Garlic bulb (*Allium sativum*) and Lantana leaf (*Lantana camara*) were collected and aqueous extracts

prepared. 500g of plant material were taken and cut into small pieces. The sample was put into waring blender containing 500ml sterilized distilled water at a ratio 1:1 (water: plant material). The sample was spun at low speed for 10-15 minutes in a waring blender till the material formed to fine texture. The blended material was then squeezed through a sterilized muslin cloth so as to get a crude liquid extract. The crude extract was filtered through Whatman no 1 filter paper. The filterate was further diluted to 5, 10 and 15 percent concentrations of the aqueous extracts and sprayed uniformly on the mulberry leaves before occurrence of the powdery mildew disease.

The leaves were harvested and fed to the silkworms on first, second and third day after spray to know the residual effect of the plant extracts on growth and development of silkworm and cocoon and silk qualities.

Results and discussion

Investigations were conducted to know the effect of plant extracts on silkworm, which were used to control powdery mildew of mulberry by feeding them with treated leaves. The 3rd instar larva was fed with mulberry leaves supplemented with plant extracts and assessed for the effect of plant extracts on growth and development of silkworm, cocoon and silk quality parameters.

Effect of medicinal plant extracts on larval mortality

From the present findings, it was observed that the maximum larval mortality (25.00%) was recorded in *Azadirachta indica* at 15 percent but it was very low in case of *Zingiber officinale* (4.99%) at 15 percent concentration at one day after treatment. Later, the larval death was steadily decreased in all the treatments with increase in waiting period, except *Azadirachta indica* with15.02 percent larval mortality. No larval mortality was observed with *Zingiber officinale* at higher concentrations at two days after treatment. All the plant extracts tested had no toxic effect at three days after treatment (Table 1).

Effect of medicinal plant extracts on larval weight and length

The growth parameters of silkworm *viz.*, larval weight and larval length was recorded in which the maximum larval weight (29.42g) and larval length (5.37cm) was observed in silkworm larvae fed with rhizome extract of *Zingiber officinale* at 15 percent followed by *Lantana camara* with 26.08g and 5.03 cm at 10 percent and *Acorus calamus* (25.63g and 5.02cm) during 5th instar 7th day at higher concentration, which was more or less equal to untreated control with 27.39g and 4.96cm (Table 2). The reason for increasing larval weight in *Zingiber officinale* treated silkworms might be due to stimulatory effect of ginger on protein synthesis in the silk gland during larval period. This was in support with Sujatha and Sammaiah (2007) [9] who reported that *Cucurma longa* had stimulated the protein synthesis in the silk gland influences the significant increase in larval weight and cocoon parameters during the larval period leading to increase in silk production.

Similarly, increase in the larval weight as a result of supplementation of mulberry leaves with aqueous extracts or dust formulation of medicinal botanicals plants has been reported by many researchers (Gobena and Bhaskar 2015; Dubey and Srivastava, 2005) [6, 3].

Effect of medicinal plant extracts on cocoon parameters

The effects of plant extracts on cocoon parameters like cocoon weight, pupal weight, shell weight was recorded, shell ratio and cocoon yield per disease free laying (DFL) was calculated. The results from the present study revealed that the cocoon weight was found to be highest (12.87g) in *Zinger officinale* treated mulberry leaves fed to silkworm larvae at 15 percent concentration over control (12.41g) followed by *Acorus calamus* (11.81g) treated mulberry leaves fed to silkworm larvae and 10 percent concentration of *Lantana camara* treated mulberry leaves fed to silkworm showed 11.71g of cocoon weight (Table 3). The pupal weight was higher (9.46g) in case of *Zingiber officinale* over control (9.44g), *Acorus calamus* (8.82g) and *Lantana camara* (8.71g) at 15 and 10 percent concentration respectively. The maximum shell weight (3.41g) was noticed in *Zingiber officinale*, which was significantly higher than control (2.97g) and in *Acorus calamus* (2.99g), *Lantana camara* (3.00g) at 10 percent concentration. *Azadirachta indica* at 15 per concentration recorded minimum shell weight (2.20g). The shell ratio was maximum (26.49%) in case of *Zingiber officinale* at 15 percent and the next best was observed in *Lantana camara* with 25.61% at 10 percent concentration followed by *Acorus calamus* of (25.31%) at 15 per concentration (Table 3). The maximum cocoon yield of 450.45g was observed in *Zingiber officinale* at 15% which was significantly superior over control treatment (435.02g). The second best treatment with 413.35g was observed in *Acorus calamus* at 15% followed by *Lantana camara* (409.85g) at 10%. The minimum cocoon yield of 354.90g was recorded in *Azadirachta indica* at 15%. The other plant extracts showed significant improvement in cocoon traits which could be due to improved appetite of silkworms becoming stronger and biochemical constituents of the plant extracts, increased nutritional efficiency of feed which was utilized by worms. The influence of the nutrients in the feed supplied which was probably attributed to the stimulatory effect of the botanicals on protein synthesis in the silk gland during larval period has increased the cocoon weight, pupal weight, shell weight, cocoon shell ratio and cocoon yield from different medicinal plant botanicals administered to mulberry leaves and fed to silkworms was reported by Bhaskar *et al*. (2004) [1], Sridevi *et al*. (2004) [8] and Gayathri *et al*. (2006) [5].

Effect of medicinal plant extracts on silk quality

The quality of the silk was assessed by the filament length, filament weight and denier. Hence, these parameters were used as preliminary strategies in present study during reeling of cocoons. The results from present experiment revealed that the filament length was maximum in case of *Zingiber officinale* (884.02m) at 15 percent concentration as compared to control treatment (841.05m). The next best was observed in *Lantana camara* with 811.15 m at 10 percent and in *Acorus calamus* (808.00 m) at 15 percent concentration (Table 4). This might be due to more shell content of cocoon in *Zingiber officinale* followed by *Lantana camara* and *Acorus calamus.*

The lowest filament length was noticed in *Azadirachta indica* (741.15m). The filament weight was maximum (0.23g) in *Allium cepa* followed by untreated control, *Allium sativum* and *Azadirachta indica* with 0.20g at higher concentrations in all plant extracts except *Zingiber officinale* in which at lower concentration the filament weight was more (0.20g) but in higher concentrations it was 0.18g. This implies that the filament thickness is fine in case of *Zingiber officinale* at 15

percent concentration. The denier was finer (1.83%) in silkworm larvae fed with *Zingiber officinale* fortified mulberry leaves at higher concentration (Table 4). It might be due to bioavailability of certain nutrients to the worms from the plant extracts which might have enhanced the protein synthesis particularly the fibroin synthesis in the posterior silk gland, which in turn might have influenced the filament denier. Similar results were reported by Gobena and Bhaskar (2015) [6] who described that feeding of fortified leaves with of leaf extracts of *Psoralea coryleifolia* and *Phyllanthus niruri* improved filament length and decreased filament denier.

Other than *Zingiber officinale*, *Lantana camara* also showed the better performance on silkworm growth, cocoon characters and silk quality traits in the present investigation. This finding in present study are in agreement with Santosh kumar *et al*. (2000) who reported that, dust formulation of *L. camara* and *Clerodendron inermae* fortified mulberry leaves resulted in increased silk filament length, filament weight, shell ratio and lowest denier when fed to silkworm larvae.

The present investigation revealed that the silkworms reared on mulberry leaves sprayed with plant extracts exhibited significant differences with respect to growth, cocoon and silk quality. Amongst the plant extracted tested, *Zingiber officinale* followed by *Lantana camara* and *Acorus calamus* had favourably influenced the growth, development of silkworm and cocoon and silk quality. Hence, they could be exploited in the management of mulberry powdery mildew disease, as it is safe and ecofriendly.

Table 1: Effect of medicinal plant extracts on larval mortality of silkworm (*Bombyx mori* L.)

Tr.	Plant species	Conc. (%)	Larval mortality (%)		
			1 DAT	2 DAT	3 DAT
T_1	*Azadirachta indica*	5	20.00 (26.56)	5.64 (13.74)	0.00 (0.00)
T_2	*Azadirachta indica*	10	21.00 (27.27)	10.01 (18.44)	0.00 (0.00)
T_3	*Azadirachta indica*	15	25.00 (30.00)	15.02 (22.80)	0.00 (0.00)
T_4	*Allium cepa*	5	9.98 (18.41)	4.36 (12.05)	0.00 (0.00)
T_5	*Allium cepa*	10	19.95 (26.53)	5.68 (13.79)	0.00 (0.00)
T_6	*Allium cepa*	15	25.00 (30.00)	14.78 (22.61)	0.00 (0.00)
T_7	*Zingiber officinale*	5	4.47 (12.20)	0.00 (0.00)	0.00 (0.00)
T_8	*Zingiber officinale*	10	4.57 (12.34)	0.00 (0.00)	0.00 (0.00)
T_9	*Zingiber officinale*	15	4.99 (12.91)	1.01 (5.77)	0.00 (0.00)
T_{10}	*Acorus calamus*	5	6.21 (14.43)	4.91 (12.80)	0.00 (0.00)
T_{11}	*Acorus calamus*	10	14.76 (22.59)	9.98 (18.41)	0.00 (0.00)
T_{12}	*Acorus calamus*	15	22.42 (28.26)	9.99 (18.42)	0.00 (0.00)
T_{13}	*Allium sativum*	5	5.65 (13.75)	4.92 (12.81)	0.00 (0.00)
T_{14}	*Allium sativum*	10	15.29 (23.02)	4.95 (12.85)	0.00 (0.00)
T_{15}	*Allium sativum*	15	22.48 (28.30)	9.99 (18.42)	0.00 (0.00)
T_{16}	*Lantana camara*	5	5.05 (12.98)	0.00 (0.00)	0.00 (0.00)
T_{17}	*Lantana camara*	10	19.49 (26.19)	8.75 (17.20)	0.00 (0.00)
T_{18}	*Lantana camara*	15	20.00 (26.56)	9.59 (18.04)	0.00 (0.00)
T_{19}	Control (Water spray)	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S. Em ±			0.33	0.23	-
CD @ P=0.05			0.95	0.66	-

DAT= Days after treatments; Figures in then parenthesis are Arc sine transformed values; Tr = Treatment

Table 2: Effect of medicinal plant extracts on larval weight and larval length of silkworm (*Bombyx mori* L.)

Tr.	Plant species	Conc.	Average larval weight (g) at 5th instar			Average larval length (cm) at 5th instar		
			5th day	6th day	7th day	5th day	6th day	7th day
T_1	*Azadirachta indica*	5	11.67	14.70	20.20	3.93	4.07	4.62
T_2	*Azadirachta indica*	10	6.70	9.97	12.25	3.17	3.75	4.23
T_3	*Azadirachta indica*	15	5.23	5.91	7.93	2.63	3.48	3.87
T_4	*Allium cepa*	5	14.66	19.08	23.43	3.98	4.48	4.67
T_5	*Allium cepa*	10	15.57	19.73	25.27	3.96	4.49	5.01
T_6	*Allium cepa*	15	14.72	19.10	24.30	3.74	4.26	4.78
T_7	*Zingiber officinale*	5	14.30	19.27	24.80	4.60	4.67	5.08
T_8	*Zingiber officinale*	10	14.80	21.88	27.27	4.68	4.70	5.22
T_9	*Zingiber officinale*	15	16.99	21.95	29.42	4.70	4.77	5.37
T_{10}	*Acorus calamus*	5	14.05	18.41	20.85	3.84	4.27	5.00
T_{11}	*Acorus calamus*	10	15.30	20.00	21.80	4.07	4.38	5.00
T_{12}	*Acorus calamus*	15	15.60	20.92	25.63	4.29	4.49	5.02
T_{13}	*Allium sativum*	5	15.47	18.10	24.17	4.22	4.41	5.02
T_{14}	*Allium sativum*	10	14.33	20.32	24.57	4.19	4.47	5.08
T_{15}	*Allium sativum*	15	13.82	18.67	24.85	4.10	4.27	4.95
T_{16}	*Lantana camara*	5	14.43	20.83	22.05	4.01	4.46	4.89
T_{17}	*Lantana camara*	10	16.77	20.88	26.80	4.27	4.55	5.03
T_{18}	*Lantana camara*	15	13.43	18.33	21.60	3.94	4.34	4.74
T_{19}	Control (Water spray)	-	15.47	21.58	27.39	4.67	4.72	4.96
S.Em±			0.19	0.11	0.22	0.09	0.10	0.14
CD @ 5%			0.54	0.33	0.62	0.27	0.28	0.39

Tr =Treatment;

Table 3: Effect of medicinal plant extracts on cocoon parameters of silkworm (*Bombyx mori* L.)

Tr	Plant species	Conc. (%)	Average Pupal weight (g)	Average Cocoon weight (g)	Average Shell weight (g)	Average Shell ratio (%)	Cocoon yield/ 1dfl (g)
T_1	*Azadirachta indica*	5	8.09	10.37	2.28	21.98 (27.95)	362.95
T_2	*Azadirachta indica*	10	8.01	10.26	2.25	21.92 (27.91)	359.10
T_3	*Azadirachta indica*	15	7.94	10.14	2.20	21.69 (27.75)	354.90
T_4	*Allium cepa*	5	8.67	11.53	2.86	24.80 (29.86)	403.55
T_5	*Allium cepa*	10	8.55	11.37	2.82	24.80 (29.86)	397.95
T_6	*Allium cepa*	15	8.42	11.08	2.66	24.00 (29.33)	387.80
T_7	*Zingiber officinale*	5	9.01	12.03	3.02	25.10 (30.06)	421.05
T_8	*Zingiber officinale*	10	9.02	12.41	3.21	25.38 (30.25)	434.35
T_9	*Zingiber officinale*	15	9.46	12.87	3.41	26.49 (30.97)	450.45
T_{10}	*Acorus calamus*	5	8.53	11.17	2.64	23.63 (29.08)	389.65
T_{11}	*Acorus calamus*	10	8.55	11.28	2.71	24.06 (29.37)	394.80
T_{12}	*Acorus calamus*	15	8.82	11.81	2.99	25.31 (30.20)	413.35
T_{13}	*Allium sativum*	5	8.23	10.97	2.74	24.97 (29.98)	383.95
T_{14}	*Allium sativum*	10	8.17	10.64	2.47	23.21 (28.80)	372.40
T_{15}	*Allium sativum*	15	8.13	10.41	2.28	21.90 (27.90)	364.35
T_{16}	*Lantana camara*	5	8.62	11.52	2.80	24.30 (29.53)	403.20
T_{17}	*Lantana camara*	10	8.71	11.71	3.00	25.61 (30.40)	409.85
T_{18}	*Lantana camara*	15	8.59	11.33	2.74	24.18 (29.45)	396.55
T_{19}	Control (Water spray)	-	9.44	12.41	2.97	23.93 (29.28)	435.02
S.Em±			0.07	0.07	0.09	0.40	0.27
CD @ P=0.05			0.20	0.21	0.25	1.16	0.77

Tr = Treatment; Figures in then parenthesis are Arc sine transformed values

Table 4: Effect of medicinal plant extracts on silk quality traits of silkworm (*Bombyx mori* L.)

Tr.	Plant species	Conc. (%)	Average filament length (m)	Average filament weight (g)	Denier (%)
T_1	*Azadirachta indica*	5	662.63	0.18	2.43
T_2	*Azadirachta indica*	10	743.18	0.21	2.57
T_3	*Azadirachta indica*	15	741.15	0.20	2.76
T_4	*Allium cepa*	5	746.55	0.21	2.48
T_5	*Allium cepa*	10	802.58	0.23	2.61
T_6	*Allium cepa*	15	748.58	0.23	2.78
T_7	*Zingiber officinale*	5	796.72	0.20	2.25
T_8	*Zingiber officinale*	10	811.57	0.18	2.02
T_9	*Zingiber officinale*	15	884.02	0.18	1.92
T_{10}	*Acorus calamus*	5	720.45	0.17	2.13
T_{11}	*Acorus calamus*	10	743.85	0.23	2.47
T_{12}	*Acorus calamus*	15	808.00	0.21	2.34
T_{13}	*Allium sativum*	5	743.85	0.17	2.11
T_{14}	*Allium sativum*	10	777.82	0.20	2.28
T_{15}	*Allium sativum*	15	747.68	0.20	2.41
T_{16}	*Lantana camara*	5	765.00	0.19	2.18
T_{17}	*Lantana camara*	10	811.15	0.19	2.13
T_{18}	*Lantana camara*	15	711.90	0.18	2.27
T_{19}	Control (Water spray)	-	841.05	0.20	2.14
S.Em±			16.69	0.01	0.17
CD @ P=0.05			47.78	0.04	0.48

Tr = Treatment

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