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Antimycotic Activity of *Allium Sativum* Against *Beauveria Bassiana*, Pathogenic Fungus of White Muscardine Disease in Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

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White Muscardine is the most common fungal disease of silkworm, *Bombyx mori* L. caused by the pathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin. In the present investigation, an attempt has been made to screen locally available medicinal/ weed plants against *Beauveria bassiana*. Among the plant extracts (PE) tested, 5% aqueous crude extract of the bulb of *Allium sativum* (Garlic) has been found to be most effective against *Beauveria bassiana*. The radial growth of *Beauveria bassiana in vitro* was inhibited to the tune of 54.9% in aqueous extract and 54.4% in ethanolic extract of *Allium sativum* and correspondingly mycelial dry weight gave rise to 110.7 mg and 108.7 mg against 201.7 mg in control 15 days post treatment. Similarly, silkworm larvae topically inoculated with the *Beauveria bassiana* conidia (1.8×10^6/ml) registered survival up to 53.0% against 0.0% in control after treatment with aqueous extract of *Allium sativum*. Simultaneously, as a preventive measure, silkworm larvae were put to rear in conidia contaminated seat paper instantly treated with aqueous extract of *Allium sativum* that also increased survival up to 61.0% against 4.6% in control. It is also observed that the plant extract is absolutely innocuous to silkworm.

Key words: White Muscardine, Botanical, Eco-friendly, *Bombyx mori*

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Introduction

White muscardine is caused by an entomopathogenic fungus called *Beauveria bassiana* and is one of the major threats to the Sericulture industry (Lu Yup and Liu, 1991). In India, loss caused by muscardine varies from 30 - 40% every year and is much more in winter, when temperature is low and humidity is high (Jayaramaiah *et al.*, 1986). In sericulture, for the prevention/ control of murcardine, many chemical formulations have been tested and encouraging results were also achieved (Samson *et al.*, 1986; Baig *et al.*, 1993; Bhattacharya *et al.*, 1995). But, considering the recent eco-friendly approach in research and the delicate nature of the target insect, these chemicals have their own limitations. At the same time, many botanicals have been tried and proved effective against fungal diseases in various agricultural crops (Mahadevan, 1982; Wilson *et al.*, 1997; Bianchi *et al.*, 1997; Purnima Sinha and Saxena, 1999) due to their eco-friendly and target specific nature. But, no effort has been made so far in sericulture to utilize these botanicals for the prevention/ control of fungal diseases except little efforts against Aspergillosis. So, we thought it worthwhile to identify some potential plants from locally available medicinal/ weed plants for the prevention/ control of white muscardine disease of *Bombyx mori* L.

Materials and Methods

Assay of antifungal property of plant extracts

Twenty six locally available medicinal/ weed plants (Table 1) were screened *in vitro* against *Beauveria bassiana*. Both aqueous and ethanolic extracts were prepared

Table 1. Medicinal/ weed plants used for the study

Sl. No.	Name of the Plant	Sl. No.	Name of the Plant
01	*Gingiber* sp.	14	*Elangi* sp.
02	*Cassia* sp.	15	*Eucalyptus* sp.
03	*Curcuma* sp.	16	*Moringa* sp.
04	*Oscimum* sp.	17	*Clerodendron* sp.
05	*Cassia* sp.	18	*Ageratum* sp.
06	*Catheranthus* sp.	19	*Phylanthus* sp.
07	*Ipomia* sp.	20	*Achyranthus* sp.
08	*Tagetes* sp.	21	*Lantana* sp.
09	*Andrographis* sp.	22	*Azadiracta* sp.
10	*Adathoda* sp.	23	*Eupatorium* sp.
11	*Allium* sp.	24	*Boerhavia* sp.
12	*Allium* sp.	25	*Nictanthus* sp.
13	*Polyalthia* sp.	26	*Lawsonia* sp.

from fresh leaves, bulbs and rhizomes using standard method (Natarajan and Lalithakumari, 1987; Chauhan and Singh, 1991). The antifungal activity of the extracts was assayed *in vitro* by 'food poison technique' (Grover and Moore, 1962) using Potato Dextrose Agar (200 g peeled potato, 20 g dextrose and 20 g agar for 1 litre) and Potato Dextrose broth (200 g peeled potato and 20 g dextrose for 1 litre) culture media. The fungus was incubated at 22 ± 2°C and measured radial growth 15 days after inoculation and calculated radial growth inhibition (%). For mycelial dry weight, first mycelia were separated by filtering through Watman filter paper using Millipore vacuum pump and then dried them at 60°C for 24 h in a hot air oven. The experiments were conducted in triplicate and sham controls were also kept in identical condition where instead of plant extract distilled water was added to the medium.

For determination of effective dose, both aqueous and ethanolic extracts of *Allium sativum* were tested *in vitro* at five different dosages (1.0%, 2.0%, 3.0%, 4.0% and 5.0%) against *Beauveria bassiana* and antifungal activity was assayed as mentioned above. Sporicidal activity of 5% aqueous crude extract of *Allium sativum* was also assessed through spore germination (Singh and Singh, 1981).

Bio-assay of plant extract

A popular multivoltine silkworm, M12(W) fed on mulberry (*Morus alba* cultivar S_1) leaves was used for the bioassay and standard rearing techniques were followed (Krishnaswamy, 1978). Efficacy of the plant extract was tested on larvae (1^{st} day 4^{th} instar) artificially inoculated with *Beauveria bassiana* (1.8×10^6 conidia/ml) as well as larvae reared on the seat paper contaminated with pathogen. Plant extract was sprayed ones daily for 4 days on artificially inoculated larvae whereas spray was done on contaminated seat paper 30 m post inoculation as well as on larvae after each moult. Data like survival, cocoon yield and other economic characters were recorded. Data were subjected to ANOVA and means were compared for significant difference.

Results and Discussion

Among the plant extracts tested *in vitro*, both aqueous and ethanolic crude extracts of *Allium sativum* at 5% were significantly ($p<0.01$) reduced the fungal growth thereby showed higher growth inhibition (%) and less mycelial dry weight (mg) than control. The radial growth of the fungus, percent growth inhibition and mycelial dry weight are depicted in Table 2 and Plate 1. Fifteen days post treatment with 5% aqueous and ethanolic crude extracts of *Allium sativum*, fungus showed 54.9% and 54.4% growth inhibition respectively over control. Similarly, fungal dry weight in aqueous and ethanolic extract treated lots was

Table 2. Efficacy of *Allium sativum* as antimycotic agent against *Beauveria bassiana*, fungal pathogen of White Muscardine disease in *Bombyx mori* L. Data represent mean ± SE (n = 15)

Concentration (%)	Radial Growth (mm)		Growth Inhibition (%)		Fungal Dry Weight (mg)	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
1.0	25.5 ± 0.39	24.9 ± 0.49	6.7 ± 1.04	9.1 ± 0.95	164.8 ± 3.1	156.5 ± 6.5
2.0	23.3 ± 0.37	22.0 ± 0.53	14.7 ± 1.07	19.7 ± 1.11	147.0 ± 3.7	133.7 ± 5.1
3.0	20.1 ± 0.41	18.5 ± 0.43	26.6 ± 0.97	32.7 ± 1.21	137.0 ± 3.8	132.0 ± 7.5
4.0	15.3 ± 0.18	14.6 ± 0.27	43.7 ± 1.19	46.2 ± 1.77	128.8 ± 3.1	119.3 ± 4.6
5.0	12.3 ± 0.30	12.3 ± 0.42	54.9 ± 1.48	54.3 ± 2.14	110.7 ± 3.2	108.7 ± 5.1
Control	27.4 ± 0.46		00.0 ± 0.00		201.7 ± 6.0	
CD at 5%						
Extract	0.35		1.19		6.42	
Conc.	0.61		2.07		11.11	

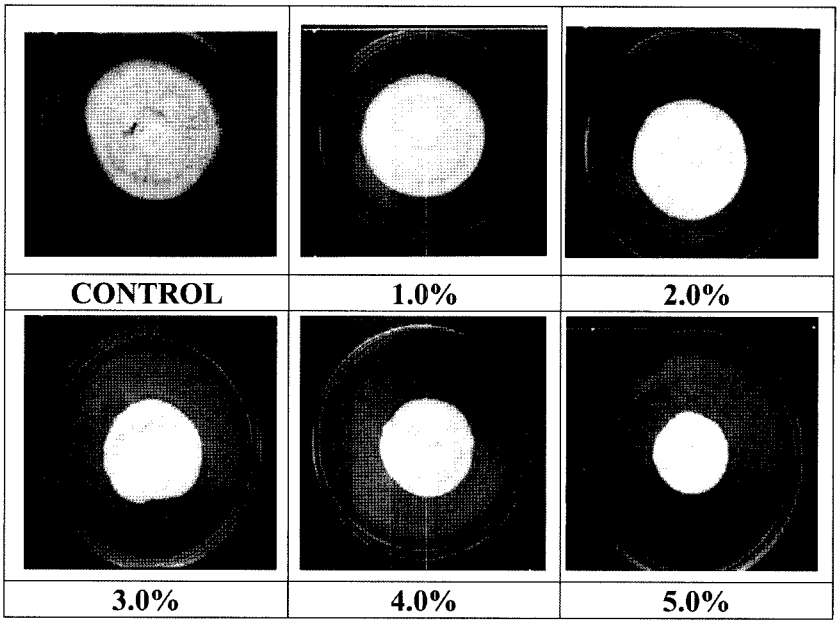

Plate 1. Radial growth of *Beauveria bassiana* 15 days post treatment with different dosages of aqueous crude extracts of *Allium sativum*.

110.7 mg and 108.7 mg respectively against 201.7 mg in control. 5% aqueous extract of *Allium sativum* also completely inhibited the germination of conidia of *Beauveria. bassiana*.

The results indicate that the plant extract contains some antifungal substances, which is responsible for inhibition of fungal growth. Antifungal activity was reported in botanicals like *Lawsonia inermis* (Tripathi *et al.*, 1978; Natarajan and Lalithakumari, 1987), *Allium cepa* and *Allium sativum* (Wilson *et al.*, 1997; Purnima Sinha and Saxena, 1999). Antimycotic activity of saponins from *Madhuca butyracea* Macb. against plant pathogenic fungi was also reported (Lalitha and Venkataraman, 1991). Many fungal inhibitors like Lawsone (2-hydroxy-1, 4-naphthoquinone) in *Lawsonia alba/ inermis* (Lal and Dutt, 1933; Karawaya, *et al.*, 1969), Allicin in *Allium sativum* and catechol and protocatechuric acid in *Allium cepa* (Bianchi *et al.*, 1997; Wilson *et al.*, 1997) were reported, which are in support with our results.

Bio-assay results showed that daily application of extract as spray on artificially inoculated (1.8×10^6 conidia/ml) larvae of M12(W) significantly ($p<0.01$) increased survival, which was 53.1% more than control (0.0%). Larvae treated with plant extract registered significantly higher weight than healthy control, whereas, shell weight (g) and shell (%) were found to be statistically at par (Table 3).

Table 3. Efficacy of *Allium sativum* on silkworm [M12(W)] larvae after artificially inoculated with *Beauveria bassaina* (daily spray)

Treatment	Survival (%)	Single Larval Wt. (g)	Single Cocoon Wt. (g)	Single Shell Wt. (g)	Shell (%)
Plant Extract	53.11	2.476	1.038	0.130	12.579
Infected Control	00.00	-	-	-	-
Healthy Control	83.80	2.369	1.078	0.135	12.515
CD at 5%	2.07	0.09	0.03	NS	NS

Table 4. Efficacy of *Allium sativum* on *Beauveria bassaina* after spray on artificially contaminated seat paper and larvae [M12(W)] after each moult and on 4th day of 5th instar

Treatment	Survival (%)	Single Larval Weight (g)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Plant Extract	61.00	2.020	0.861	0.116	13.493
Infected Control	4.60	2.022	0.874	0.118	13.548
Healthy Control	89.80	2.018	0.896	0.120	13.067
CD at 5%	4.47	NS	NS	0.003	0.39

Table 5. Effect of *Allium sativum* on post cocoon characters and reproductive potential of silkworm [M12 (W)] artificially inoculated with *Beauveria bassiana*

Treatment	NBFL (m)	Denier	Renditta	Raw Silk (%)	RSR (%)	Reelability (%)	Fecundity	Egg Hatching (%)
Plant Extract-02	416	1.56	10.71	9.34	73.68	87.81	338.60	89.70
H. Control	325	1.64	13.63	7.34	64.70	80.65	327.50	91.40

Higher survival (61.0%) was also recorded in larvae reared on fungal conidia contaminated seat paper against 4.6% in untreated control. Plant extract treated lot also recorded significantly higher shell (%) than healthy control. However, larval and cocoon weights did not differ significantly among the treatments (Table 4). Similarly, plant extract treated lot yielded more cocoon yield (ca. 21.0 kg/ 100 Dfls) than untreated control (ca. 1.6 kg/ 100 Dfls). The higher survival in extract treated lots is due to antimycotic activity of the extract especially inhibitory effect of the extract on the spore germination. Inhibitory effect of volatiles of garlic, onion and ginger was reported on the conidial germination of *Erysiphe polygoni* (Singh and Singh, 1981) and zoospores of *Phytophthora drechsleri* (Chauhan and Singh, 1991), which are in conformity with present study.

In general, data on pre and post cocoon characters revealed that plant extract treatment has no adverse effect on the economic characters of *Bombyx mori* L, which are in full agreement with previous studies. After studying the effect of crude seed extract of *Psoralea coryleifolia* L. on economic characters of *Bombyx mori* L. Rajashekhargouda *et al.* (1999) reported significant improvement in larval and cocoon weight. Increase in the weight of larvae, cocoon and shell along with shell ratio was also reported in *Bombyx mori* L. (Patil *et al.*, 1997) and Eri silkworm (Jayaprakash Rao, 1998) due to foliar supplementation of plant extracts. Application of Tulsi and Neem powder at lower concentration not only reduced flacherie but also enhanced economic characters in *Bombyx mori* L. (Sujatha and Rao, 2004).

The reeling performance of the cocoons and reproductive potential of silkworm are depicted in Table 5. The plant extract treated lot showed better filament length, reelability, renditta and fecundity than control, which are in conformity with the previous studies. Higher fecundity was reported after treatment with *Amaranthus spinosus* in Eri silkworm (Jayaprakash Rao, 1998), *Parthenium hysterophorus* treatment in *Dysdercus cingulata* (Gunasekharan and Chelliah, 1984) and *Psoralea coryleifolia* treatment in *Bombyx mori* L. (Rajashekhargouda *et al.*, 1999). It was reported the use of whole plant extract viz., *P. coryleifolia* and *Tribulus terrestris* with JH-mimic activity increased shell (%) and silk yield (Rajashekhargouda, 1991; Rajashekhargouda *et al.*, 1999) in silkworm, *Bombyx mori* L.

The present study indicates that 5% aqueous crude extract of *Allium sativum* can be used effectively as spray for prevention/ control of white muscardine disease in silkworm, *Bombyx mori* L with out any adverse effect on the host insect. Efforts are on for purification and determination active ingredients of the plant extract in collaboration with other research institute.

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