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In Vitro Screening of Plant Products against Fungal Growth of *Beauveria bassiana*

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Among silkworm diseases, white muscardine is the most virulent and contagious disease caused by the fungus, *Beauveria bassiana*. In the present study, 30 aqueous extracts of plant products were tested *in vitro* against *Beauveria bassiana*. Of them, Garlic *(Allium sativum)* bulb, Ginger *(Zingiber officinale)* rhizome, Turmeric *(Curcuma longa)* rhizome, Cardamom *(Elettaria cardamomum)* seeds, Safed AK *(Calotropis procera)* leaves, Walnut *(Juglans regia)* epicarp and Pumba chalan *(Rheum australe)* root were found effective in inhibiting the conidial germination and vegetative mycelial growth of *Beauveria bassiana* at 1% concentration in 30 minutes duration. Safed Ak *(Calotropis procera)* and Walnut *(Juglans regia)* were found effective at 0.5% - 1.0% in 30 minutes duration.

Key words: Plant products, *Beauveria bassiana*, *in vitro* effects.

Several factors influence the silk cocoon yield, of them silkworm diseases are important. Diseases cause extensive damage to silk cocoon crop and loss to sericulturists as it results in reduced cocoon yield or failure to harvest cocoon. Among silkworm diseases, white muscardine is the most common, virulent and contagious disease caused by *Beauveria bassiana*. The conidia of *Beauveria bassiana* infect silkworm on contact with integument and infection takes place through a direct penetration of germ tube. Several chemicals (Samson and Mummigatti, 1979a; Balavenkatasubbaiah *et al.,* 1994), fungicides (Krishnaprasad *et al.,* 1978; Samson and Mummigatti, 1979b. Siddaramaiah *et al.,* 1979; Sohaf *et al.,* 1994) have been screened *in vitro* against *Beauveria bassiana*. Different bed disinfectant formulations (Subba Rao *et al.,* 1992; Baig *et al.,* 1993; Datta *et al.,*1998) were also reported to kill the conidia on the silkworm body before germination and prevent white muscardine disease in silkworm rearing. But farmers are reluctant to use these chemicals/ fungicides/disinfectants mainly because of economic constraint and also felt that excessive use of these chemicals/fungicides/ disinfectants may be hazardous to human and environment.

There are several reports on the efficacy of extracts from several plants in prevention/control of different diseases in human and animals. These were observed to be eco-friendly and non-hazardous. The presence of naturally occurring anti-fungal compounds in plants have been recognized and tested against a wide range of pathogenic fungi infecting several commercially important crops (Singh and Dwivedi, 1990; Mohanty *et al.,* 1995; Rajappan *et al.,* 1997). Recently some plant products with anti-viral and anti-fungal activity have been reported for prevention/suppression of nuclear polyhedrosis and muscardine disease in silkworm, (Virendrakumar *et al.,* 1998; Krishnaprasad *et al.,* 1978). In the present study extracts from several plants were screened *in vitro* against *Beauveria bassiana*. The identified plant extracts may be useful for prevention/suppression of white muscardine disease in silkworm rearing.

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Materials and Methods

In vitro screening of plant products against fungal growth of Beauveria bassiana

Aqueous extracts from 30 plants mentioned in Table 1 were screened *in vitro* against *Beauveria bassiana.* One gram of each was separately homogenized in 100 ml of sterilized distilled water using pestle and mortar to obtain 1% aqueous extract.

In order to determine the effect of each plant extract on the conidia of *Beauveria bassiana,* one ml of conidial suspension ($1x10^7$ conidia/ml) was centrifuged at 5,000 rpm for 5 minutes and the pellet was suspended in 1ml of 1% of plant extract and incubated at 25±1°C for 30 minutes. The extract was diluted with 5ml of distilled water and centrifuged at 5,000 rpm and the supernatant was discarded and the pellet was washed in sterilized distilled water twice by centrifugation. Finally the pellet was suspended in 1 ml of sterilized distilled water.

To determine the effect of treatment on the viability of conidia, 5 ml each of Samsinokova's corn steep liquor media was taken in test tubes and

sterilized. To the sterilized media, 100µl of treated conidia with plant extract was added and incubated at room temperature (25±1°C and 85±5% R.H.) for 10 days and observed visually for the vegetative growth of fungus. The observation of vegetative growth was recorded as positive (+) and no growth as negative (-). Inoculated control batch was also maintained whereas conidia were not treated with plant extracts. Three replications were maintained for each plant extract and control batches.

Evaluation of different concentrations of plant extracts against fungal growth of Beauveria bassiana

The plant product extracts, that permitted no vegetative growth of mycelium, were further tested at lower concentrations. The identified plant extracts were prepared at 0.3, 0.5, 0.8 and 1.0 % in distilled water as mentioned above. Individually, the effects of different concentrations of plant extract were tested following the method described above except that the conidia were suspended in different concentrat ions of plant extracts. Inoculated control batch was also maintained with similar procedure but conidia were not treated with plant extracts.

Determination of effective duration for promising plant extracts against the fungal growth of Beauveria bassiana

The plant product extracts found promising were further tested to determine minimum effective duration at selected concentrations. The conidial suspension was treated with plant product extracts for different durations viz., 10, 20 and 30 minutes. After specific duration, the conidia were washed by repeated centrifugation and suspended in growth media as described earlier.

Results and Discussion

The observations on screening of 30 plant product extracts against *Beauveria bassiana* resulted in identification of seven plant products being effective against *Beauveria bassiana* (Table 1) at 1 % concentration. Seven plant product extracts *viz.,* bulb of Garlic *(Allium sativum)*, rhizome of Ginger *(Zingiber officinale)*, rhizome of Turmeric *(Curcuma longa)*, seeds of Cardamom, *(Elettaria cardamomum)*, leaves of Safed Ak *(Calotropis procera)*, epicarp of Walnut, *(Juglans regia)* and root of Pumba chalan *(Rheum australe)* were found effective in inhibiting conidial germination *in vitro* and mycelial growth of *Beauveria bassiana.*

Table 1. Screening of plant extracts effective against the growth of *Beauveria bassiana*

Sl. No.	Plant		Plant part used	Growth of fungus
	Vernacular name	Scientific Name		
1	Black pepper	*Piper nigrum*	Seed	+
2	Onion	*Allium cepa*	Bulb	+
3	Garlic	*Allum sativum*	Bulb	-
4	Ginger	*Zingiber officinale*	Rhizome	-
5	Turmeric	*Curcuma longa*	Rhizome	-
6	Lemon grass	*Cymbopogon citratus*	Leaves	+
7	Hibiscus	*Hibiscus furcatus*	Leaves	+
8	Curry leaves	*Murraya koenigii*	Leaves	+
9	Tulsi	*Ocimum sanctum*	Leaves	+
10	Mulberry leaf	*Morus alba (V1)*	Leaves	+
11	Coriander	*Coriandrum sativum*	Leaves	+
12	Datura	*Datura stramonium*	Leaves	+
13	Parthenium	*Parthenium hysterophorus*	Leaves	+
14	Mint/pudina	*Mentha longifolia*	Leaves	+
15	Cinnamon	*Cinnamomum zeylanicum*	Bark	+
16	Cardamom	*Elettaria cardamomum*	Seed	
17	Safed Ak	*Calotropis procera*	Leaves	
18	Clove	*Syzygium aromaticum*	Flower	+
19	Croton	*Croton tiglium*	Leaves	+
20	Lajwanti (Touch me not)	*Mimosa pudica*	Leaves	+
21	Nut grass	*Cyperus rotundus*	Root	+
22	Apamarg	*Achyranthus aspera*	Leaves	+
23	Phyllanthus	*Phyllanthus niruri*	Leaves	+
24	Costus	*Saussurea costus*	Root	+
25	Sazmool	*Lavetera cashmeriana*	Root	+
26	Walnut	*Juglans regia*	Epicarp	
27	Gandasoi	*Marrubium vulgare*	Dried Shoot	+
28	Gowzaban	*Arnebia benthamii*	Dried Shoot	+
29	Pumba chalan	*Rheum australe*	Root	
30	Tethwan	*Artemisia absinthum*	Dried Shoot	+
31	Inoculated control			+

Treatment concentration : 1% aqueous extract ; Treatment duration : 30 min;
+ = Growth of fungus ; - = No growth of fungus

All the seven plant part extracts were effective in inhibiting the conidial germination and mycelial growth of *Beauveria bassiana* (Table 2). Garlic, Ginger, Turmeric, Cardamom and Pumba chalan were found effective only at 1 % concentration whereas at lower concentrations viz., 0.3, 0.5, 0.8 % they were not found effective in inhibiting the conidial germination and mycelial growth of *Beauveria bassiana*, at exposure duration of 30 min. The plant extracts of Safed Ak and Walnut were found effective even at 0.5, 0.8 and 1.0 % concentration at exposure duration of 30 min.

Table 2. *In vitro* screening of different concentrations of selected plant extracts against *Beauveria bassiana*

Sl. No.	Name	Concentration (%)	Growth of fungus
1	*Garlic, Allium sativum*	0.3%, 0.5%, 0.8%, 1.0%	+ + + -
2	Ginger, *Zingiber officinale*	0.3%, 0.5%, 0.8%, 1.0%	+ + + -
3	Turmeric, *Curcuma longa*	0.3%, 0.5%, 0.8%, 1.0%	+ + + -
4	Cardamom, *Elettaria cardamomum*	0.3%, 0.5%, 0.8%, 1.0%	+ + + -
5	Safed Ak, *Calotropis procera*	0.3%, 0.5%, 0.8%,1.0%	+ - - -
6	Walnut, *Juglans regia*	0.3%, 0.5%, 0.8%,1.0%	+ - - -
7	Pumba chalan, *Rheum australe*	0.3%, 0.5%, 0.8%,1.0%	+ + + -
8	Inoculated control		+

Treatment duration : 30 min;
+ = Growth of fungus ; - = No growth of fungus

Five plant product extracts viz., Garlic, Ginger, Turmeric, Cardamom and Pumba chalan at 1 % concentration and two plant part extracts *viz.,* Safed Ak and Walnut at 0.5-1.0% concentration were tested against *Beauveria bassiana* at different time durations (Table 3). Garlic, Ginger, Turmeric, Cardamom and Pumba chalan were found effective only at 30 min time exposure duration, whereas at 10 and 20 min durations they did inhibit the conidial germination and mycelial growth. Whereas Safed Ak and Walnut at 0.5, 0.8 and 1.0% concentration were found to be effective only at 30 min durations in inhibiting the conidial germination and mycelial growth, where as 10 and 20 min durations they were not found to be effective.

The results obtained from the screening of plant product extracts for anti-fungal activity against Beauveria bassiana is encouraging. The identified plant product homogenates were effective at 0.5 - 1.0% at 30 min exposure period. These observations will pave way for utilizing the plant products for the management of fungal disease in silkworm and other economically important insects. Jagannathan and Narasimhan (1988) screened 66 plant product extracts against two foliar pathogens of finger millet *viz., Pyricularia grisea* and *Helminthosporium nodulosum*. Rajendran et al. (1998) reported anti-bacterial activity of ginger against *Staphylococcus aureus*. In silkworm, Sivaprakasam and Rabindra (1996) demonstrated that the leaf extracts of *Psoralea corylifolia* possessed anti-viral property against BmNPV infection. Virendrakumar *et al.* (1998) identified eight plants having anti-BmNPV activity. Singh *et al.* (2002) screened 10 plant product extracts *in vitro* against *Aspergillus flavus* and *Aspergillus tamari*. The importance of utilization of eco-friendly products having natural and biological sources have been realized in recent years. The

Table 3. *In vitro* screening of selected plant extracts against *Beauveria bassiana* at different exposure times

Plant	Concentration (%)	Duration (min)	Growth of fungus
Garlic, *Allium sativum*	1.0%	10	+
		20	+
		30	-
Ginger, *Zingiber officinale*	1.0%	10	+
		20	+
		30	-
Turmeric, *Curcuma longa*	1.0%	10	+
		20	+
		30	-
Cardamom, *Elettaria cardamomum*	1.0%	10	+
		20	+
		30	-
Pumba chalan, *Rheum australe*	1.0%	10	+
		20	+
		30	-
Safed Ak, *Calotropis procera*	0.5%	10	+
		20	+
		30	-
	0.8%	10	+
		20	+
		30	-
	1.0%	10	+
		20	+
		30	-
Walnut, *Juglans regia*	0.5%	10	+
		20	+
		30	-
	0.8%	10	+
		20	+
		30	-
	1.0%	10	+
		20	+
		30	-
Inoculated control		10	+
		20	+
		30	-

+ = Growth of fungus ;- = No growth of fungus

present study has identified plants having anti-fungal activity against *Beauveria bassiana*. However, the active anti-fungal principles in these plant extracts need to be identified and formulated. Delivery mechanism and their effect on economic parameters of silkworm have also to be studied in detail.

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