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Application of Systemic Fungicide for Control of White Muscardine in Silkworm *Bombyx mori* L.

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Two systemic fungicides, SF1 (Bavistin, a carbandazim fungicide 50% WP, Rallis India ltd., India) and SF2 (Bayleton 25% WP-Triadiamefon, a Triazole compound, Rallis India Ltd., India) were screened for control of muscardine disease in silkworm, *Bombyx mori*. One and two percent of SF1 and 0.05 and 0.1% of SF2 in aqueous solution were found to be effective in *in vivo* condition for the control of the disease. These fungicides, on feeding through mulberry leaves continuously for two days to 4th and 5th instar silkworm larvae inoculated topically with conidia of *Beauveria bassiana* (4×10^6 conidia/ml) resulted in reduction in mortality due to muscardine by over 90% as against 100% mortality in inoculated control. SF1 at 1% reduced the mortality by 90% in 4th instar and 91% in final instar silkworm while at 2%, the reduction was 92% and 96%, respectively. SF2 at 0.05 and 0.1% concentration reduced the mortality by 82 and 88% during 4th instar and by 88 and 92% during 5th instar, respectively.

Key words: *Beauveria bassiana*, Carbandizim, Systemic fungicide, Triazole, Muscardine

Introduction

Beauveria bassiana is an entomopathogenic hypomycetes fungus, distributed all over the world. It infects over 100 different insect species coming from several insect orders. In silkworm *Bombyx mori*, it causes white muscardine disease inflicting significant crop loss in all sericulture countries. In India, the loss due to the disease is estimated to be 9.05% (Hanumappa, 1986). The fungus infects silkworm under favorable conditions through the integument, enters hemocoel, and produces hyphal bodies and parasitise various tissues leading to death of the host. After death, the fungus grows saprophytically and forms mycelial mass that turns the host body into hard structure. The conidiophore emerges from the host body and produces infectious conidia.

Several fungicide formulations have been developed to prevent the germination of conidia on the integument and its entry into host body (Samson *et al.*, 1986, 1987; Subba Rao *et al.*, 1992; Sashidharan *et al.*, 1997; Datta *et al.*, 1998). These fungicide formulations are ineffective once the conidium penetrates into host body. In view of the limitations in the existing system of management of muscardine, the need for curative measures was felt and some attempts have been made in recent period (Zhou *et al.*, 1990; Sreedharan *et al.*, 1991). This paper presents the results of investigations involving systemic fungicides, leading to the development of curative measure against muscardine in silkworm *B. mori*.

Materials and Methods

Two systemic fungicides *viz.*, SF1 (Bavistin, a carbandazim fungicide 50% WP, Rallis India Ltd., India) and SF2 (Bayleton 25% WP-Triaduamefon, a Triazole compound, Rallis India Ltd., India), were selected based on preliminary studies by the authors and screened them against *Beauveria bassiana*.

The *in vitro* screening of the two fungicides was performed by incorporating the systemic fungicide into sterilised Samsinokova's fungal culture broth to obtain final concentration of 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 1 and 2% of systemic fungicide in broth. A control was maintained having culture broth and a loop of conidia of *B. bassiana*. There were three replications for each fungicide concentration and control. To the broth, a loop of conidia of *B.*

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bassiana was incorporated and incubated at 26 ± 1°C for a period of 8 days and was observed for the growth of fungus. The growth of fungus was quantified visually as - : Negative; ± : Negligible growth; + : Satisfactory growth; ++ : Good growth; and +++: Very good growth.

The *in vivo* screening of the fungicides was conducted by feeding the fungicide following specific schedule and of selected concentrations in distilled water (SF1: 1 and 2%; SF2: 0.05, 0.10 and 0.15%) along with mulberry prior to or after topical inoculation of 4th and 5th instars NB_4D_2 silkworm with the inoculum dosage of conidia of *B. bassiana* (4×10^6/ml) that causes 100% mortality in silkworm. The larvae were reared till spinning in environment controlled room at 26 ± 1°C and 80 – 85% RH. In treatment T1, silkworm of 4th and 5th instars was fed on mulberry sprayed with specific fungicide of specific concentration. The fungicide was sprayed at 80 ml/kg of mulberry leaves air-dried. The fungicide sprayed mulberry leaves was fed continuously for 2 days and then the larvae were subjected to topical inoculation with the conidia of 4 $\times 10^6$/ml. The duration of feeding of fungicide treated larvae was determined based on the preliminary studies by the authors. The rearing of the larvae was continued with leaves having no treatment. In T2, silkworm of 4th and 5th instars was first subjected to topical inoculation with the conidia and after 1 hr, the mulberry leaves sprayed with the specific fungicide of specific concentration was fed continuously for 2 days. In T3, silkworm of 4th and 5th instars were subjected to the treatment as T2 except that the fungicide was fed after 24 hrs instead of 1 hr of topical inoculation. There were appropriate controls *viz.*, Control 1: Silkworm fed with systemic fungicide-SF1 treated leaves, No inoculation; Control 2: Silkworm fed with systemic fungicide-SF2 treated leaves, No inoculation; Control 3: Silkworm fed on untreated leaves and topically inoculated; Control 4: Silkworm fed untreated leaves without inoculation. Each treatment and control had three replications and each replication was of 100 larvae. The larvae were continued to be reared till spinning on untreated mulberry. The mortality due to muscardine was recorded on day-to-day basis.

Results and Discussion

The observations are presented in Table 1, 2 and 3. It is seen from the results of *In vitro* studies, that both the fun-

Table 1. *In vitro* screening of systemic fungicides, SF1 on *Beauveria bassiana*

Days p. i.	Concentration (%) of fungicide and growth of *B. bassiana*								Control
	0.05	0.1	0.15	0.20	0.25	0.50	1.0	2.0	
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	±
3	±	-	-	-	-	-	-	-	+
4	+	±	-	-	-	-	-	-	+
5	+	+	±	-	-	-	-	-	++
6	+	+	+	±	-	-	-	-	++
7	++	+	+	+	±	-	-	-	+++
8	++	++	++	+	+	±	-	-	+++

-, Negative; ±, Negligible growth; +, Satisfactory growth; ++, Good growth; +++, Very good growth.

Table 2. *In vitro* screening of systemic fungicides, SF2 on *Beauveria bassiana*

Days p. i.	Concentration (%) of fungicide and growth of *B. bassiana*								Control
	0.05	0.1	0.15	0.20	0.25	0.50	1.0	2.0	
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	±
3	-	-	-	-	-	-	-	-	+
4	-	-	-	-	-	-	-	-	+
5	-	-	-	-	-	-	-	-	++
6	-	-	-	-	-	-	-	-	++
7	±	-	-	-	-	-	-	-	+++
8	+	±	-	-	-	-	-	-	+++

-, Negative; ±, Negligible growth; +, Satisfactory growth; ++, Good growth; +++, Very good growth.

Table 3. Efficacy of systemic fungicide on the incidence of muscardine in silkworm, *Bombyx mori*

Treatment	4th instar				5th instar			
	SF1		SF2		SF1		SF2	
	1%	2%	0.05%	0.1%	1%	2%	0.05%	0.1%
T1	10.00 (90.00)	8.00 (92.00)	18.00 (82.00)	12.00 (88.00)	9.00 (91.00)	4.00 (96.00)	12.00 (88.00)	8.00 (92.00)
T2	30.00 (70.00)	16.00 (84.00)	25.00 (75.00)	26.00 (74.00)	16.00 (84.00)	10.00 (90.00)	21.00 (79.00)	16.00 (84.00)
T3	40.00 (60.00)	30.00 (70.00)	36.00 (64.00)	34.00 (66.00)	36.00 (64.00)	18.00 (82.00)	38.00 (62.00)	32.00 (68.00)
Cont. 1 (SF1 2%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cont. 2 (SF1 0.1%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cont. 3 (Inoc. cont)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Cont. 4 (Normal control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

SF, Systemic fungicide; SF1, Systemic fungicide - Bavistin; SF2, Systemic fungicide - Beyleton; Inoc. Cont, Inoculated control. Value in parenthesis is percent reduction in muscardine compared to inoculated control.

gicides *viz.*, SF1 inhibited the growth of *B. bassiana* at concentrations of 1 and 2% (Table 1) while SF2 at 0.15% and above (Table 2). These results confirm the earlier report on the efficacy of these fungicides against *B. bassiana* (Krishnaprasad *et al.*, 1978; Sreedharan *et al.*, 1991).

Among the different concentrations tested *in vivo viz.*, 1.00 and 2.00% of SF1 and 0.05, 0.10 and 0.15% of SF2, the SF2 of concentration 0.15% was toxic to silkworm. The larvae fed on mulberry treated with the fungicide SF2 of 0.15% developed mild and typical toxicity symptoms such as vomiting, paralysis and death. The mulberry leaves sprayed with fungicide SF1 of concentration 1 and 2% and SF2 of 0.05 and 0.10% did not cause toxicity. The treatment involving the feeding of specific systemic fungicides in effective and non toxic concentration *viz.*, SF1: 1 and 2% and SF2: 0.05 and 0.1% to silkworm which were topically inoculated with the conidia of *B. bassiana* (4×10^6/ml) resulted in reduction in mortality due to muscardine, compared to inoculated control (Control 3). The mortality in the inoculated control of 4th and 5th instars silkworm was 100% (Table 3).

In T1, SF1 at 1% concentration was effective to reduce the mortality due to muscardine in the 4th and 5th instars silkworm population by 90 and 91% while at 2% the mortality was reduced by 92 and 96%, respectively. Similarly, the SF2 at 0.05% reduced the mortality by 82 and 88% and at 0.1% by 88 and 92%, respectively.

In T2 of 4th and 5th instars silkworm, feeding fungicide after 1 hr of topical inoculation SF1 at 1% concentration reduced the mortality by 70 and 84%, respectively, while at 2% by 84 and 90%. Similarly the SF2 at 0.05% and 0.1% reduced the mortality by 75 and 79% and 74 and 84%, respectively.

In T3 of 4th and 5th instars silkworm, involving feeding the fungicide after 24 hr of topical inoculation with SF1 at 1% and 2%, the mortality was reduced by 60 and 64 and 70 and 82%, respectively. With SF2 at 0.05% and 0.1%, the reduction in mortality was 64 and 62, respectively and 66 and 68%, respectively.

Zhou *et al.* (1990) reported similar result with a cure of above 90% using Kejiang-1 solution against the muscardine disease in silkworm. In the present study also the carbendizim fungicide-SF1 (Bavistin) at 1 – 2%, fed continuously through mulberry leaves, for a period of 2 days to 4th and 5th instars silkworm larvae resulted in the reduction of the disease by 90 – 96%. The Traizole compound-SF2 (Beyleton) at 0.05 – 0.1% concentration also reduced the mortality by 82 – 92%.

These systemic fungicides have been observed to be non-toxic to silkworm as neither of them caused mortality in silkworm at the identified concentrations. The systemic fungicides are generally specific in their activities. Carbendazim fungicides are known to affect the lipid and nuclei acid synthesis as well as nuclear function. Triozole compounds interfere with sterol synthesis and inhibit ergosterol biosynthesis. This inhibition curtails membrane synthesis and subsequent fungal growth (Buchenauer, 1977; Siegal, 1981).

The conidia of *B. bassiana* germinate on the silkworm body integument in 6 8 hrs under favorable conditions. It is essential to dust the fungicide at frequent intervals that results in practical problems. In order to overcome this limitation, the application of systemic fungicide, which is effective against the pathogen in the host system, will be most useful. The two fungicides identified in the present study have reduced the mortality due to muscardine by

over 90% by effectively controlling the infection in the host system and could be an important component in the of integrated management of muscardine in sericulture.

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