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Note

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Systemic Fungicide Application for the Control of White Muscardine in Silkworm Rearing

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Among silkworm diseases, white muscardine is the most virulent and contagious disease caused by *Beauveria bassiana* and common during winter and rainy seasons. The disease is managed at present by practicing the silkworm body and rearing seat disinfection to prevent the spread of white muscardine during silkworm rearing as the available methods do not cure the silkworms against white muscardine. The use of systemic fungicide was suggested recently to control white muscardine. The present study investigated the practicability of application aspect of systemic fungicide as spray. It is observed that 3 times feeding of systemic fungicide through mulberry leaf, fed immediately after third, fourth moult and on 4th day of final instar as most effective in suppressing the multiplication of the fungus in silkworm. Spraying of the systemic fungicide on mulberry in the rearing house, air-dried and feeding was suggested as suitable application method. Alternately the spraying of the systemic fungicide on mulberry in mulberry garden 6 hrs prior to feeding was also suggested as a method for the control of white muscardine in silkworm rearing.

Key words: Systemic fungicide, Silkworm, White muscardine

Introduction

White muscardine is a fungal disease in silkworm commonly prevalent during winter and rainy seasons in all sericultural countries. The disease is caused by entomo pathogenic fungi, *Beauveria bassiana,* results in a loss of 10 – 40% cocoon crop (Selvakumar *et al.*, 2000) in India. Silkworm body and rearing seat disinfectants such as Labex (Subba Rao *et al.*, 1992), Reshamkeet Oushadh (Baig *et al.*, 1993), Vijetha (Datta *et al.*, 1998), Resham Jyothi (Samson *et al.*, 1998) and Suraksha (Patil *et al.*, 1999) have been suggested for management of disease in silkworm rearing. These approaches though effective had not given fool proof control as the pathogen cause infection within few hours of its coming in contact with the host. Once the pathogen enters the host, cure is not possible with the above suggested methods. To overcome this problem, recently Virendrakumar *et al.* (1998) suggested the use of systemic fungicide and identified Bayleton for the control of white muscardine. Mallikarjuna (1998) have also confirmed the effect of systemic fungicide, Bayleton, through haematological studies. Virendrakumar *et al.* (1998) have suggested the continuous feeding of systemic fungicide for five times once after third moult, fourth moult and 4th day of fifth instar.

In the present study, an attempt was made to reduce the feedings of systemic fungicide so as to make it less laborious for practical utilization of the technology in silkworm rearing to control muscardine disease.

Materials and Methods

Preparation of systemic fungicide solution

Bayleton (25% W. P. Triadiamefon, a triazole compound, Rallip, India, Ltd., India) was used as a systemic fungicide in the present study. 0.1% of Bayleton was prepared by dissolving 1 g of Bayleton fungicide in 1,000 ml of sterilized distilled water.

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Preparation of inoculum (*Beauveria bassiana*) suspension Infective conidia were collected from silkworm mummified due to infection of *Beauveria bassiana*. Five loops of conidia were collected using microbiological inoculation loop and suspended in sterilized distilled water and agitated. The concentration of conidia in the stock solution was determined by Neubauer double haemocytometer and diluted to obtain suspension containing 1×10^7 conidia/ml.

Efficacy of different schedules of post inoculation feeding of Bayleton against white muscardine of silkworm: Silkworm bivoltine hybrid (CSR2 × CSR5) received from Silkworm Breeding Laboratory, CSRTI, Mysore was reared following the standard method up to third moult. Immediately after third moult the larvae were grouped into 6 batches of 200 larvae each for the treatment. All the batches, except the normal control were topically inoculated with the inoculum (20 ml/200 larvae). Eight hrs after the inoculation the systemic fungicide treatments were given as per the following schedule. T1, Feeding systemic fungicide once after 8 hrs of inoculation and then once after fourth moult and once on 4th day of final instar; T2, Feeding systemic fungicide twice after 8 hrs of inoculation and then twice after fourth moult and twice on 4th day of final instar; T3, Feeding systemic fungicide thrice after 8 hrs of inoculation and then thrice after fourth moult and thrice on 4th day of final instar; T4, Feeding systemic fungicide four times after 8 hrs of inoculation and then four times after fourth moult and four times on 4th day of final instar; T5, Feeding systemic fungicide five times after 8 hrs of inoculation and then five times after fourth moult and five times on 4th day of final instar.

Two control batches, one (inoculated control) where the larvae were inoculated and reared without feeding fungicide and another (normal control) reared with neither inoculation nor the treatment. All the treatments and control batches were fed with on normal untreated leaves outside the treatment schedule till spinning.

Comparative efficacy of different feeding schedules of Bayleton sprayed on mulberry plants against white muscardine of silkworm: A specific number of plants in mulberry garden were sprayed using gattor sprayer with 0.1% Bayleton at the rate of 200 ml/kg leaf. Spraying periods also adjusted to the treatment schedule *viz.*, spraying 6, 12, 24 and 48 hrs prior to feeding to silkworms.

Bivoltine hybrid silkworm (CSR2 × CSR5) was reared following the standard method up to third moult and was grouped into batches of 200 larvae each. All the batches except the normal control were inoculated with *Beauveria bassiana* inoculum. The larvae were subjected to treatment T-T4 after 8 hrs of inoculation. The treatments were as follows. T1, Silkworms fed on mulberry 6 hrs after the spraying fungicide in mulberry garden; T2, Silkworms fed on mulberry 12 hrs after the spraying fungicide in mulberry garden; T3, Silkworms fed on mulberry 24 hrs after the spraying fungicide in mulberry garden; T4, Silkworms fed on mulberry 48 hrs after the spraying fungicide in mulberry garden.

In T1 after 8 hrs of inoculation the larvae were fed consecutively three times with 6 hrs after Bayleton sprayed mulberry leaves. It was followed by normal feeding. Again the larvae were fed consecutively three times with 6 hrs after Bayleton sprayed leaves immediately after fourth moult, three feedings. Same treated leaves were fed again 3 times on 4th day of final instar and continued with normal untreated leaves up to spinning. The treatment T2, T3 and T4 is same as T1 except that the fungicide treated leaves were fed 12, 24 and 48 hrs after spray instead of 6 hrs. In fifth batch larvae were inoculated with *B. bassiana* solution (20 ml/200 larvae) and feeding continued with normal untreated leaves up to spinning. This served as inoculated control.

There was normal control with neither inoculation nor treatment. Each treatment and control were represented by three replications of 200 larvae. Every day the larvae were observed and mortality due to white muscardine was recorded till spinning. The data obtained were subjected to statistical analysis of variance (ANOVA) and calculated the standard error and C. D. value (Kwanchai and Arturo, 1984).

Results

The observations on the efficacy of different feeding schedule of Bayleton against white muscardine of silkworm are presented in Table 1. The mortality due to white muscardine was significantly reduced in all treatments (T1 - T5) compared to inoculated control. The mortality in inoculated control batch was 85.33% where as in fungicide treatment batches the mortality was ranged from 31.33 – 64.00%. Among fungicide treated batches, highest mortality (64.00%) was recorded in T1 batch, where one feeding of fungicide was given after third, fourth moult and on 4th day of final instar. As the number of feedings of fungicide increased from one to five the mortality due to muscardine decreased and five feedings as suggested by Virendrakumar *et al.* (1998) reduced the mortality to 31.33%. There was no significant difference between T3 (3 feedings) and T4 (4 feedings) where mortality was 33.00 and 32.33% respectively. Also there was also no significant difference between T4 (4 feedings) and T5 (5 feedings) where mortality was 32.33 and 31.33%

Table 1. Efficacy of different feeding schedule of Bayleton against white muscardine of silkworm

Sl. no.	Treatment	No. of larvae inoculated	Larval mortality due to white muscardine (%)	Larvae survived (%)	% survival increased over inoculated control
1	T1	200	64.00	36.00	25.00
2	T2	200	48.00	52.00	43.75
3	T3	200	33.00	67.00	61.33
4	T4	200	32.33	67.67	62.11
5	T5	200	31.33	68.67	63.28
6	Inoculated control	200	85.33	14.67	
	F- test		**		
	S. E. ±		**1.68**		
	C. D. at 5 %		**5.19**		

**Significant at 1% level.

T1: 1 feeding fungicide treated leaves after third moult, fourth moult and on 4th day of fifth instar silkworms inoculated with *B. bassiana.*

T2: 2 feedings fungicide treated leaves after third moult, fourth moult and on 4th day of fifth instar silkworms inoculated with *B. bassiana.*

T3: 3 feedings fungicide treated leaves after third moult, fourth moult and on 4th day of fifth instar silkworms inoculated with *B. bassiana.*

T4: 4 feedings fungicide treated leaves after third moult, fourth moult and on 4th day of fifth instar silkworms inoculated with *B. bassiana.*

T5: 5 feedings fungicide treated leaves after third moult, fourth moult and on 4th day of fifth instar silkworms inoculated with *B. bassiana.*

Table 2. Comparative efficacy of different feeding schedules of Bayleton sprayed on mulberry plants against white muscardine of silkworm

Sl. no.	Treatment	No. of larvae inoculated	Larval mortality due to white muscardine (%)	Larvae survived (%)	% survival increased over inoculated control
1	T1	200	40.00	60.00	53.12
2	T2	200	43.67	56.33	48.82
3	T3	200	47.00	53.00	44.92
4	T4	200	53.00	47.00	37.89
5	Inoculated control	200	85.33	14.67	
	F - test		**		
	S. E. ±		**1.16**		
	C. D. at 5 %		**3.67**		

**Significant at 1% level.

T1: Fungicide sprayed and exposed to 6 hrs on mulberry plant before feeding.

T2: Fungicide sprayed and exposed to 12 hrs on mulberry plant before feeding.

T3: Fungicide sprayed and exposed to 24 hrs on mulberry plant before feeding.

T4: Fungicide sprayed and exposed to 48 hrs on mulberry plant before feeding.

respectively. The percent increase in larval survivability in treated batches over inoculated control is presented in Table 1. The larval survivability increased from 25.00% (T1) to 63.28% (T5). Considering the economic viability of the technology and minimum variation between treatments T4 and T5, T3 and T4, the treatment T3 was selected for further studies.

The efficacy of fungicide treated mulberry fed after different duration of spray is presented in Table 2. Inoculated silkworm fed with mulberry 6 hrs after the spray of fungicide recorded a mortality of 40.00% while mulberry sprayed with fungicide and fed 12 and 24 hrs after spray recorded mortality of 43.67 and 47.00%. However, the highest mortality (53.00%) was recorded by mulberry fed after 48 hrs spray. The control batch (T5) without fungicide treated batch inoculated with *Beauveria bassiana* recorded 80.00% mortality due to white muscardine. The percent increase in larval survivability in treated batches over inoculated control is presented in Table 2.

Discussion

The results clearly indicated that the systemic fungicide (Bayleton) reduced the mortality due to white muscardine. In the case of inoculated control batch, infection started

soon after the silkworm body was contaminated with the conidia. During the initial stage of infection (1 – 2 days) no external symptoms were noticed, but as the disease progresses the larvae lost their appetite and became in active. The larval body became limp and lost their elasticity and mortality was recorded from fourth day of infection. Just after death the head was soft and resilient, but was covered with conidia with fruiting body appearing from inter segmental membrane, eventually the whole body get covered with powdery conidia and became chalky white and mummified. In the case of fungicide treated batches also the mortality due to white muscardine was started on 4/5th day and the external symptoms of the dead larvae were similar to those of control batch but significant decrease in mortality due to white muscardine was recorded in fungicide treated batches. The present study confirms the report of Virendrakumar *et al.* (1998). It is also in line with observations of Zhou *et al.* (1990) where the curative effect for the white and green muscardine was to the extent of 90% on feeding silkworms with mulberry leaves sprayed 25 to 200 times with Kejiang-1 solution. Sreedhara *et al.* (1991) also reported that feeding mulberry leaves sprayed with different concentrations of triadimefon and uniconazole increased the survival rate of silkworm larvae inoculated with the conidia of *Beauveria bassiana*. The triazole compounds are known to behave as strong systemic fungicides and to control a wide range of economically important diseases caused by fungi in animals and plants (Fletcher *et al.*, 1988; Siegel, 1981). Triazole compounds in fungi interfere with sterol and inhibit ergosterol biosynthesis. Virendrakumar *et al.* (1998) has recommended five feedings of systemic fungicide (Bayleton) sprayed on mulberry leaves inside the rearing house and fed immediately after third molt, fourth molt and 4th day of fifth instar for the control of white muscardine. In the present study results clearly indicated that there was no significant difference in the efficacy of fungicide between 3 feeding and 4 feedings fed batches and 4 feedings and 5 feedings fed batches. Spraying fungicide on mulberry leaves and feeding them timely during 4th instar (immediately after third moult), 5th instar (after fourth moult) and on 4th day of 5th instar has given better survivability.

Silkworm fed with mulberry within 6 hrs of spray of fungicide was effective in control of muscardine. However, the efficacy was reduced by 7% compare to feeding with mulberry immediately after 12 hrs spray with fungicide. As the duration of after spray increases, the efficacy gradually reduced to 56.33% (12 hrs), 53.00% (24 hrs) and 47.00% (48 hrs). The reduction in the efficacy may be due to adverse effect of environmental factor such as sun light on the fungicide.

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