Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 6 (11): 805-812, 2011
ISSN 1816-4935 / DOI: 10.3923/jm.2011.805.812

Efficacy of Systemic Fungicides for Control of White Muscardine in Tasar Silkworm, *Antheraea mylitta* D.

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ABSTRACT

Penicillium citrinum causes white muscardine in tasar silkworm and significantly damage cocoon production at farmer's field. Four systemic fungicides Bavistin, a carbandazim fungicide (AK 1), Bayleton, a triazole compound (AK 2), Dithane M-45, a mancozeb fungicide (AK 3) and Thiram, a dimethyl dithiocarbamate (AK 4) were tested for efficacy to control white muscardine in, *Antheraea mylitta* D. One and two percent of AK 1 and AK 3, 0.15 to 2% of AK 2 and 2% of AK 4 in aqueous solution were found to be effective in *in vitro* condition for the control of muscardine. These fungicides on feeding through the *T. arjuna* leaves continuously for two days in 5th instar larvae inoculated topically with conidia of *Penicillium citrinum* (4×10^6 conidia mL^{-1}) resulted in reduction in mortality due to muscardine by 49-94% as against 100% mortality in inoculated control (control 6). AK 1 reduced the mortality by 93 and 94% in the final instar silkworm at 1 and 2%, respectively. AK 2 at 0.05 and 0.1% concentration reduced the mortality by 85 and 87%, respectively. In case of AK 3 and AK 4 at 1% concentration reduced the mortality by 88 and 69%, whereas at 2% reduced mortality by 90 and 75%, respectively. No mortality was noticed in the controls 1 to 4 which indicated the non toxicity at the particular concentrations. Bavistin (AK 1) and Dithane M-45 (AKP 3) at 1 and 2% concentrations were more effective among tested systemic fungicides in suppressing muscardine in tasar silkworm.

Key words: *Penicillium citrinum*, systemic fungicides, muscarsdine, *in vitro*, *in vivo*, *Anteraea mylitta* D

INTRODUCTION

White muscardine in tasar silkworm is caused by the infection of *Penicillium citrinum* distributed all over the world infecting significant crop loss in all tasarculture countries. The incidence of muscardine is noticed particularly in silkworm rearing during winter and rainy seasons (Verweij *et al*., 1999). This fungus grows saprophytically and forms mycelia mass that turns the host body and produces infectious conidia.

Prevention is better than cure is most suitable proverb for tasar silkworm diseases because there are no curative methods for the silkworm diseases including white muscardine. Therefore, when the tasar silkworms are reared under natural conditions it is essential to disinfect the surroundings thoroughly and maintain cleanliness. Several fungicidal formulations have been developed to prevent the germination of conidia on the integument and its entry into host body in *Bombyx mori* L. (Sasidharan *et al*., 1997; Datta *et al*., 1998). Kumar *et al*. (2002) studied the application

of systemic fungicides for control of white muscardine in *Bobyx mori* L. Several attempts were made to develop specific measures against white muscardine. Different fungicides and chemicals (Rangaswamy *et al*., 2003), botanicals (Sharma *et al*., 2002; Sasidharan *et al*., 2000; Chandrasekharan, 2009; Bimal, 2006; Kumar *et al*., 2009) and bed disinfectants (Chandrasekharan *et al*., 2004) were evaluated against *B. bassiana* by different workers. Integrated technology and curative methods for controlling white muscardine disease were reported by Nataraju *et al*. (2002), Kumar *et al*. (2002) and Datta *et al*. (2003), respectively.

in vitro testing of disinfectants to control the growth of fungi was carried out by Trivedy *et al*. (2011), Boughalleb *et al*. (2006), Pakdaman *et al*. (2002) and Shah *et al*. (2006). Probiotic application for silkworm disease management was reported recently by Subramanian *et al*. (2009), application of compounds from marine halophytes by Kumar *et al*. (2009) and dichloromethane and methanol (1:1) extract of seaweed brown algae, *Turbinaria conoides* against *Beauveria bassiana* by Kumari *et al*. (2011).

In contrast to the research carried out on fungal diseases in *Bombyx mori* L. not much work has been published so far on pathological aspects of tasar silkworm (Reddy *et al*., 2008, 2009a, b, 2010). Especially, the research findings and literature on efficacy of systemic fungicides against white muscardine in tasar silkworm are not available. Hence, *in vitro* and *in vivo* inactivation study of the systemic fungicides was carried out against *Penicillium citrinum* to find out most suitable fungicide to control white muscardine in tasar silkworm.

MATERIALS AND METHODS

The study was conducted during July, 2009 to March, 2011 in Central Tasar Research and Training Institute, Ranchi, India. Four systemic fuzicides viz. Bavistin, a carbandazim fungicide (AK 1), Bayleton, a triazole compound (AK 2), Dithane M-45, a mancozeb fungicide (AK 3) and Thiram, a dimethyl dithiocarbamate (AK 4) procured from Rallis India Ltd., India were selected to study efficacy to control white muscardine in, *Antheraea mylitta* D.

Chemicals: All chemicals were analytical grade and potato dextrose agar was procured from the Himedia Laboratories, Calcutta, India.

Fungal culture collection: *Penicillium citrinum* was obtained from tasar silkworm infected with white muscardine and maintained on Potato Dextrose Agar (PDA) slants. These slants were sub-cultured in petridishes prior to testing for *in vitro* and *in vivo*.

Study design: There were appropriate controls viz., Control 1: Tasar silkworm fed with systemic fungicide AK 1 treated leaves, no inoculation; Control 2: Tasar silkworm fed with systemic fungicide AK 2 treated leaves, no inoculation; Control 3: Tasar silkworm fed with systemic fungicide AK 3 treated leaves, no inoculation; Control 4: Tasar silkworm fed with systemic fungicide AK 4 treated leaves, no inoculation; Control 5: Silkworm fed untreated leaves without inoculation. Control 6: Silkworm fed on untreated leaves and topical inoculated. 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 leaves. The mortality due to muscardine was recorded on day to day basis.

***In vitro* bioassay:** *In vitro* testing of the four fungicides was performed by incorporating the systemic fungicide into sterilized ready-made potato dextrose agar medium 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 loop of conidia of *Penicillium citrinum*. There were three replications for each fungicide concentration and control. To the broth, a loop of conidia of *Penicillium citrinum* was inoculated and incubated at 26±1°C for a period of 8 days and was observed for the growth of the fungus. The growth of the fungus was quantified visually as -: negative; ±: Negligible growth; +: Satisfactory growth; ++: Good growth and+++: Very good growth.

***In vivo* bioassay:** The most effective concentrations of the systemic fungicides in *in vitro* were evaluated for bioassay studies on tasar silkworm. The *in vivo* efficacy of the fungicides was conducted by feeding the fungicide following specific schedule and of selected concentrations in distilled water (AK 1, AK 3 and AK 4 @ 1 and 2%; AK 2 @ 0.05 and 0.10%) along with *T. arjuna* prior to or after tropical inoculation of 5th instar Daba B.V with the inoculated dose of the conidia of *Penicillium citrinum* (4×10^6 mL^{-1}) that causes 100 % mortality in tasar silkworm. The larvae were reared in indoor till spinning.

In treatment T1, silkworm of 5th instar was fed on T. arjuna sprayed with specific fungicide of specific concentration. The fungicide was sprayed at 80 mL kg^{-1} of *T. arjuna* leaves air-dried. The fungicide sprayed mulberry leaves was fed continuously for two days then the larvae were subjected to topical inoculation with the conidia of 4×10^6 mL^{-1}. The rearing of larvae was continued with leaves having no treatment. In T2, silkworm of 5th instar was first subjected to topical inoculation with the conidia and after 1 h, the mulberry leaves sprayed with the specific fungicide of specific concentration was fed continuously for 2 days. In T3, silkworm of 5th instar was subjected to the treatment as T2 except that the fungicide was fed after 24 h instead of 1 h of topical inoculation.

RESULTS

***In vitro* bioassay:** In case of AK 1 at 0.05% concentration on 4 day of post inoculation negligible growth of fungi was observed and on 8th day good growth was observed (Table 1). As the concentration of the fungicide is increased fungal growth was decreased. The 0.5% of the AK 1 has shown negligible growth of the fungus on 8th day of post inoculation. The concentration from 1-2% of the AK 1 fungicide had not shown growth of the fungus even on the 8th day of post inoculation. In the case of control, fungus growth started on 2nd day of the inoculation. As the days passed the growth was abundant and very good growth was observed on 7th and 8th day of inoculation.

The systemic fungicide AK 2 had shown very good results against growth of fungus. At 0.05% AK 2 started showing negligible growth of fungus and reached satisfactory growth on the 7th and 8th day of post inoculation. At 0.1% negligible growth of the fungus was observed on 8th day and at concentration from 0.15-2.0% the growth was not found even on the 8th day of post inoculation (Table 2).

In case of AK 3 at 0.05% concentration the growth of fungus was negligible on 3rd day while very good growth was observed on the 8th day of post inoculation (Table 3). The concentration of the fungicide between 0.1 to 0.5% fungal growth was decreased. The concentration from 1-2% of the AK 3 fungicide had not shown growth of the fungus even on the 8th day of post inoculation.

In case of AK 4 at 0.05 to 0.2% concentration on 3rd day of post inoculation, growth of fungi was started with negligible amount. On the 8th day of post inoculation, very good and good growth of fungus was observed in the concentration between 0.05-0.1 and 0.15-0.2%, respectively (Table 4).

Table 1: *In vitro* screening of systemic fungicide, Bavistin (AK 1) on *Penicillium citrinum*

	Concentration (%) of fungicide and growth of *Penicillium citrinum*								
Days post inoculation	0.05	0.1	0.15	0.2	0.25	0.5	1	2	Control
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 fungicide, Bayleton (AK 2) on *Penicillium citrinum*

	Concentration (%) of fungicide and growth of *Penicillium citrinum*								
Days post 1 inoculation	0.05	0.1	0.15	0.2	0.25	0.5	1	2	Control
1	-	-	-	-	-	-	-	-	
2	-	-	-	-	-	-	-	-	±
3	-	-	-	-	-	-	-	-	+
4	-	-	-	-	-	-	-	-	+
5	-	-	-	-	-	-	-	-	++
6	-	-	-	-	-	-	-	-	++
7	±	-	-	-	-	-	-	-	+++
8	+	±	-	-	-	-	-	-	+++

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

Table 3: *In vitro* screening of systemic fungicide, Dithane M-45 (AK 3) on *Penicillium citrinum*

	Concentration (%) of fungicide and growth of *Penicillium citrinum*								
Days post inoculation	0.05	0.1	0.15	0.2	0.25	0.5	1	2	Control
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	±
3	±	-	-	-	-	-	-	-	+
4	+	±	-	-	-	-	-	-	+
5	+	+	±	-	-	-	-	-	++
6	+	+	+	±	-	-	-	-	++
7	++	+	+	+	±	±	-	-	+++
8	+++	++	++	+	+	±	-	-	+++

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

AK 4 fungicide even at 1% concentration, the fungus growth was observed but at 2% concentration no growth was observed.

***In vivo* bioassay:** The observations are presented in Table 5. In the treatment 1 (T1), the fungicide AK 1 at 1 and 2% concentration was effective in reducing the mortality due to muscardine in 5th instar silkworm population by 93 and 94%, respectively and occupied first place.

Table 4: *In vitro* screening of systemic fungicide, Thiram (AK 4) on *Penicillium citrinum*

	Concentration (%) of fungicide and growth of *Penicillium citrinum*								
Days post inoculation	0.05	0.1	0.15	0.2	0.25	0.5	1	2	Control
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	±
3	±	±	±	±	-	-	-	-	+
4	+	+	+	±	-	-	-	-	+
5	+	+	+	+	±	-	-	-	++
6	+	+	+	+	+	±	-	-	++
7	++	++	++	+	+	+	±	-	+++
8	+++	+++	++	++	++	+	+	-	+++

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

Table 5: Efficacy of systemic fungicides on the incidence of white muscardine in tasar silkworm, *A. mylitta* D

	5th Instar							
	AK 1		AK 2		AK 3		AK 4	
Treatment	1%	2%	0.05%	0.1%	1%	2%	1%	2%
T1	7.00	6.00	15.00	13.00	12.00	10.00	31.00	25.00
	(93.00)	(94.00)	(85.00)	(87.00)	(88.00)	(90.00)	(69.00)	(75.00)
T2	25.00	12.00	40.00	26.00	31.00	19.00	51.00	42.00
	(75.00)	(88.00)	(60.00)	(74.00)	(69.00)	(81.00)	(49.00)	(58.00)
T3	37.00	25.00	33.00	30.00	40.00	31.00	45.00	42.00
	(63.00)	(75.00)	(67.00)	(70.00)	(60.00)	(69.00)	(55.00)	(58.00)
Cont. 1 (AK1, 2%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cont. 2 (AK 2, 0.1%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cont. 3 (AK 3, 2%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cont. 4 (AK 4, 2%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cont. 5 (Normal control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cont. 6 (Inoc. Cont)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

AK 1- Bavistin; AK 2 - Beyleton; AK 3 - Dithane M-45; AK 4 - Thiram; Inoc. Cont- Inoculated Control. Value in parenthesis is percent reduction in muscardine compared to inoculated control

The second and third places were occupied by AK 3 with 88 and 90% at 1 and 2% concentration and the SF 2 with 85 and 87% at 0.05 and 0.1%, respectively. High mortality was observed in the case of AK 4 with 31 and 25% at 1 and 2% concentrations, respectively.

In T2 of 5th instar silkworm, feeding fungicide after 1 h of topical inoculation of AK 1 at 1 and 2% concentration reduced the mortality by 75 and 88%, respectively. Reduction of the mortality due to the virosis in case of AK 3 is 69 (at 1% conc.) and 81% (at 2% conc.) whereas in case of AK 2 mortality was 60% (at 0.05% conc.) and 74% (at 0.1% conc.). The least reduction percent was noticed in AK 4 with 49 (at 1% conc.) and 58% (at 2% conc.).

In T3 of 5th instar silkworm, involving feeding the fungicide after 24 h of topical inoculation, AK 1 at 1 and 2%, the mortality was reduced by 63 and 75%, respectively. AK 2 at 0.005 and 0.1%, the reduction in mortality was 67 and 70%, respectively. AK 3 and AK 4 at 1% the mortality was reduced by 60 and 55% whereas at 2% the reduction was 69 and 58%, respectively.

In the case of controls form 1 to 5, no mortality was observed but in control 6, mortality due to AmCPV was 100%.

DISCUSSION

It is witnessed from the results of *in vitro* studies, the fungicides viz. AK 1 and AK 3 inhibited the growth of *Penicillium citrinum* at concentrations of 1 and 2 %, while AK 2 and AK 4 at 0.15% and above and at 2%, respectively. These results confirm the earlier reports on the efficacy of some fungicides against *B. bassiana* in *B. mori* L. (Siddaramaiah and Prasad, 1987; Sreedhara *et al.*, 1991; Kumar *et al.*, 2002). Among the different concentrations tested *in vivo* viz., 1 and 2% (AK 1, AK 3, AK 4) and 0.05, 0.10 and 0.15% (AK 2), the AK 2 of concentration 0.15% was toxic to silkworm. The larvae fed on *T. arjuna* treated with the fungicide AK 2 of 0.15% developed mild and typical toxicity symptoms of such as vomiting, paralysis and death. The *T. arjuna* leaves sprayed with fungicide AK 1, AK 3 and AK 4 of concentration 1 and 2 % and AK 2 of 0.05 and 0.10% did not cause toxicity. The treatment involving the feeding of specific systemic fungicide effective and non-toxic concentration viz., AK 1, AK 3 and AK 4: 1 and 2% and AK 2: 0.05 and 0.1% to silkworm which were topically inoculated with the conidia of *Penicillium citrinum* (4×10^6 mL^{-1}) resulted in reduction in mortality due to the muscardine, compared to inoculated control.

Bavistin (AK 1), Dithane M-45 (AK 3) and Thiram (AK 4) at 1-2%, fed continuously through mulberry leaves, for a period of 2 days to 5th instar silkworm larvae resulted in the reduction of the disease by 93-94, 88-90 and 69-75%, respectively. The Traizole compound Beyleton (AK 2) at 0.05-0.1% concentration also reduced the mortality by 82-92%. Kumar *et al.* (2002) reported similar result with a cure of above 90% using Bavistin and Bayleton solution against the muscardine disease in mulberry silkworm. The present results also confirm the results of Zhou *et al.* (1990), who have used Kejiang-1 solution for the containment of white muscardine in *B. mori* L.

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 nucleic acid synthesis as well as nuclear function. Trizole compounds interfere with sterol synthesis and inhibit ergosterol biosynthesis and subsequent fungal growth (Buchenauer, 1977; Siegel, 1981). The Carbonates fungicides like Mancozeb and Thiram affects the function of membrane biosynthesis.

The conidia of *Penicillium citrinum* germinate on the silkworm body integument in 68 h 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 four fungicides which are tested for their efficacy in the present study have reduced the mortality due to muscardine in between 49-94%.

CONCLUSION

Bavistin (AK 1) and Dithane M-45 (AK 3) at 1 and 2% concentrations were fund more effective among tested systemic fungicides in suppressing muscardine in tasar silkworm and could be an important component in the integrated management of muscardine in tasar sericulture.

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