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[Research]

Bio-Economic Changes Due to Long Time Treatment of Carbendazim on Mulberry Silkworm (*Bombyx mori* L.)

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ABSTRACT
The effect of fungicides on silkworm larvae was analyzed with regard to the fact that these beneficial insects are usually affected by fungicides, Daily feeding on 1 and 2g/liter of carbendazim, a systemic fungicide, did not have significant effects on larval and pupal mortality. However the weight of the treated larvae showed considerable weight decrease up to 37% weight.. All the economic traits of male and female adults treated with fungicide decreased but this decrease is not significant in cocoon shell weight and hatching percentage. The comparison of means using Tukey test at 5% confidence level did not show any effect related to the concentration

Keywords: Carbendazim, Fungicide, Residual toxicity, Silkworm, *Bombyx mori*

INTRODUCTION

Environmental pollutants, like pesticides, have been found to be destructive on different aspects of life. Silkworms, as beneficial insects, are no exception. Due to this many problems have appeared in sericulture as a result of the pesticide applications to cultivations, especially when mulberry trees grow next to cultivated plants. Many studies that have focused on the effect of insecticides on *B. mori* deal with toxicity, retardation of development and growth, fecundity, morta-lity, food utilization and economic parameters (Kuribayashi, 1988; Kumar *et al.*, 1992; Vassarmidaki *et al.*, 2000; Vyjayanthi and Subramanyam, 2002). However some of these studies, focused on the effect of fungicide residue on silkworm growth and per-formances (Dutta *et al.*, 2003).

Sik *et al.*, (1976) reported that more than 1.4% of yield reduction in sericulture is due to the side effect of pesticide application. 49.4% was due to the application of different pesticides in rice fields, 21.2% in fruit gardens and 12.3% in olericulture.

In sericulture fungicides are applied for to two dimensions of work, to heal the fungal diseases of mulberry trees and to prevent and control fungal disease of silkworm such as muscardine (Kuberappa and Jayaramaiah, 1988). Hence finding appropriate fungicides with high efficiency in disease control and low side effects on silkworms is very important.

Gunasekhar *et al.*, (1995) showed that the two sprays of foltaf (captafol) or kavach (chloro-thalonil) at 0.2% reduced mulberry leaf rust severity by up to 50% and increased leaf yield by 28%. Treated leaves could be used for feeding silkworms as early as the first day after spraying, without any toxic effect.

Also Lin and Tzeng (1994) reported that alpha-DL-difluoromethylornithine (DFMO) treatment showed no deleterious effect on mulberry plants and treated plant parts were safe to use as feed for the silkworms even immediately after application.

Carbendazim was recommended to control muscardine and mulberry powdery mildew. This fungicide belongs to the class benzimidazole. Azole fungicides, such as triazoles and imidazoles, are molecules that inhibit ergosterol biosynthesis by preventing the cytochrome P-450 dependent 14 alpha-demethylation of lanosterol. They can also inhibit the cytochrome P-450-dependent metabolism of both endogenous and exoge-nous compounds (Snegaroff and Bach, 1989)

Vandame and Belzunces (1998) demons-trated that sub-lethal doses of the triazole

Table 1. The effect of carbendazim on pre-cocoon parameters of silkworm

Concentration (g/liter)	Larval Mortality (%)	5 Larval weight (g)			Pupa Mortality (%)
		Day4 Instar4	Day5 Instar5	Day7 Instar5	
1	3.33 a	3.246 b	13.163 b	16.340 b	7.40 a
2	12.0 a	3.080 b	12.990 b	16.400 b	8.00 a
Control	5.33 a	3.900 a	18.360 a	26.343 a	2.33 a
F-value	1.13 ns	24.78 **	57.1 **	736.2 **	0.75 ns
C.V.	107.1	4.42	4.69	1.86	105.44

Means with the same letter in columns are not significantly different at P>0.05

and imidazole fungicides elicit hypothermia in honey bees treated and kept at an ambient temperature of 22°C. It is hypothesized that certain fungicides, which inhibit the biosynthesis of sterols, alter the sterol composition of the leaves making it difficult or impossible for the insect to form moulting hormones, thus inhibiting cocoon spinning (Suss *et al*., 1992).

Therefore, the present investigation deals with the effects of long term application of systemic fungicide, carbendazim, on silkworm yield and biological performance. Because carben-dazim has recently entered sericulture, it is necessary to study the different side effects of this fungicide on silkworms.

MATERIALS AND METHODS

The eggs of bivoltine hybrid silkworm (103×104), obtained from Iran Sericultural Research Center (Rasht, Iran), were reared in the laboratory with a standard rearing technique (Lim et al. 1990) under 25°C with RH 75±5% and photoperiod 16L:8D. The larvae were fed with mulberry leaves of Ichenoise variety up to the last instar. In this research 1 and 2 g/lit concentrations of systemic fungicide, carbendazim, WP 50% was used as treatments. Fungicide solution was made from its commercial form, Bavistin®.

The Fourth instar larvae were divided into 3 experimental groups, including control and fungicide treatments. Each group consisted of 300 larvae with three replications. Fresh mulberry leaves were soaked in each concentration for 10 minutes and then dried in air for 20 minutes. The contaminated leaves were fed to silkworm from 4th to 5th instar, once a day.

After the treatments, the larval and cocoon parameters were recorded. The weight of the larvae was determined by weighing 5 larvae on different days of the fourth and fifth instars in each replication. Cocoon weight, cocoon shell weight and pupal weight were determined by using the standard technique in sericulture that was described by Etebari *et al*. (2004). The female moths were dissected 48 hrs after oviposition and the number of remaining eggs in ovary was counted.

Collected data were subjected to the statistical analysis of variance test to find out the least significant difference between the parameters of the normal control and treated groups. For every analysis of variance the Tukey test in SAS software was used (SAS, 1997).

RESULT

Pre-cocoon Parameters:

There are no significant differences between treatments and control in larval mortality (Table 1), The percentage of mortality in 2 g/lit of carbendazim was 12% and 5.3% in control. Larval weight was affected by fungicide but the treatments were not effected significantly. The highest difference of larval weight was reported on 7th day of the fifth instar. The weight of 5 control larvae was 26.34 g on this day while in 1 and 2 g/lit treatments it was measured at 16.34 and 16.40 g, respectively.

Post-cocoon Parameters:

Leaves contaminated with carbenda-zim affected cocoon traits and considerable difference was observed between control and two treatments (Table 2). This effect was seen in both sexes and the weight of male cocoon in 1 and 2 g/lit concentrations was 1.70 and 1.73g, respectively and 1.98 g in control. The cocoon weight of females in 1 and 2g/l concentrations showed 13.9 and 12.6% decrease, respectively. Nevertheless cocoon shell weight did not show consider-able difference (Table 2).

Reproductive Traits:

The number of eggs decreased significantly when larvae fed on mulberry leaves contaminated with 2 g/lit fungicide whereas with the mean of 669.0, it outlined more than

Table 2. The effect of carbendazim on post-cocoon parameters of silkworm

Concentration (g/liter)	The Weight of Mail			The Weight of Female		
	Cocoon	Pupa	Cocoon Shell	Cocoon	Pupa	Cocoon Shell
1	1.705 b	1.287 b	0.417 a	2.000 b	1.539 b	0.461 a
2	1.731 b	1.276 b	0.454 a	2.029 b	1.609 b	0.419 a
Control	1.982 a	1.502 a	0.470 a	2.371 a	1.889 a	0.483 a
F-value	31.64 **	19.38 *	1.68 ns	84.82 **	26.99**	2.28 ns
C.V.	2.61	3.71	8.11	1.81	3.68	8.12

Means with the same letter in columns are not significantly different at P>0.05

Table 3. The effect of carbendazim on reproductive parameters of silkworm

Concentration (g/liter)	No. Eggs	No. Remained Eggs in ovary	No. Unfertilized Eggs	Hatchability (%)
1	676.00 ab	14.33 a	27.00 a	86.85 a
2	669.00 b	27.66 a	36.33 a	89.08 a
Control	721.67 a	11.83 a	23.30 a	95.34 a
F-value	6.33 *	1.48 ns	0.98 ns	2.08 ns
C.V.	2.85	67.1	4.59	5.85

Means with the same letter in columns are not significantly different at P>0.05

7.2% decrease (Table 3). The mean number of eggs in 1 g/lit treatment was 676. The eggs in the ovary after the dissection of the female showed that there are no significant differences between the groups (Table 3). Although the number of unfertile-zed eggs varied between 23.3 to 36.3 it had no significant difference .

Carbendazim did not have any effect on egg hatching, although it decreased these parameters to some extentl. Hatching percentage was 86.85 and 89.08 % in 1 and 2g/lit concentrations while in control this was measured at 95.3 %. No significant differences were however observed in the different concentrations studied.

DISCUSSION

In the present study prolonged treatment of carbendazim caused the decrease of cocoon and pupal weight in both sexes but it did not have considerable effects on cocoon shell weight.

The fungicides Dithane M-45 (mancozeb) and carbendazim were tested for the control of *Beauveria bassiana* and their effects on *Bombyx mori* (Kuberappa and Jayaramaiah, 1988). It was reported that dusting the 5th-instar larvae with 2.0% mancozeb or 0.2% Bavistin daily for 5 days was most effective in controlling the fungus and also increased cocoon weight. Dusting was more effective than leaf dipping or spraying (Kuberappa and Jayaramaiah, 1988).

In a recent study, larvae fed on leaves contaminated with pesticide residue for 11 days. Therefore, the application period had considerable effects on the results obtained. This must be kept in mind when using fungicide on mulberry trees in the field. Siddaramaiah and Hegde (1989) have reported that carbendazim was not toxic to *B. mori* as its residual toxicity only lasted for 2 days. Sharma and Govindaiah (1991) tested the residual toxicity of carbendazim on silk-worms and reported that the leaves could be used for silkworm rearing one week after spraying? The larval stage, where the insect is affected by fungicide, affects the insects responses to this stress.

The effects of treating the 3rd or 4th instar larvae of the silkworm *Bombyx mori* with a fungicide of the imidazole group, 1-[N-(4-chloro-2-trifluoromethylphenyl) propoxyace-timidoyl] imidazole, on moulting were determined in laboratory tests by Seki (1985). Cocoons were smaller with treatment of the 4th instar larvae than with that of the 3rd-instar larvae. The number of eggs in the moths treated with 2g/lit carbendazim decreased but other reproductive traits of silkworm did not show significant difference.

Pai *et al.* (1991) investigated the genetic effects of apron 35 SD (metalaxyl) on the male and female germinal cells of *B. mori*. The fungicide was injected into the wing bud region of the 5th day male and female pupae. The resulting adults were mated with

untreated adults and the females of both crosses were allowed to oviposit. When males were treated, the chemical induced a significant amount of dominant lethals. The crosses involving the treated female produced a large percentage of unfertilized eggs. The percentages of dominant lethals and unfertilized eggs increased with increase in concentration of the fungicide.

Larval feeding on mulberry leaves contaminated with carbendazim residue decreased many economic and biological characteristics of silkworm although it did not have considerable effect on cocoon shell weight. Therefore, taking into consideration the period of usage, larval stage and the concentration of fungicide were the most important factors in decreasing thedestructive effects of this fungicide.

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