

PROTECTION OF *Bombyx mori* L. LARVAE AGAINST CERTAIN DISEASES USING SOME CHEMICAL DISINFECTANTS

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ABSTRACT

*This study was conducted to evaluate the use of flom fort, potassium sulphonate and dettol compounds as disinfectants and their impacts on the biological characteristics and production of silk in silkworm *Bombyx mori* L artificially infected with bacteria that causes filahari disease.*

Treatments achieved that potassium sulphonate (0.1% and 0.05%) resulted lower mortality rate, the highest percentage of adult emergency, fertility of the female and hatching percentage , and also increased the resulting cocoon weight , silk content ratio, the filament length per cocoon, weight and size of the reeled filament. flom fort (0.1 and 0.05%) increased adult emergence percentage, female fecundity, silk cocoon ratio and the length of reeled filament. while treatment of mulberry leaves with dettol (0.1%) increased the cocoon shell weight.

Key words: *Bombyx mori* l., Larvae, some chemical disinfectants.

INTRODUCTION

The mulberry silkworm diseases are very serious problem that face silkworm rearing as they cause quantitative and qualitative reduction of natural silk crop production.

Therefore, the produced cocoons by survivors have the depleted economic characters. Diseases control can only be affected by prophylactic use of certain disinfectants or antibiotics (Radha *et al.*, 1981; Serbanescu and Pau 1989; EL-Zohairy, M.; Govindan *et al.*, 1990; Saha *et al.*, 1995 ; Sanakal *et al.*, 1996; Dechu *et al.* 1997; Bali *et al.*, 2008; and Saad *et al.*, 2012).

Owing to the economic importance of silkworm, *Bombyx mori* L. as the main source of natural silk the present work was under taken to investigate three chemical compounds as disinfectants on some biological aspects and silk production of the insect.

MATERIALS AND METHODS

This work was carried out during the spring season of 2012 in laboratory of Sericulture Research Department, Sharkiya Province, Plant Protection Research Institute, Agriculture Research Center.

Materials:

- 1-The mulberry silkworm, *Bombyx mori* L.(Giza –K hybrid), obtained from the Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture Giza, Egypt.
- 2- Mulberry leaves, *Morus alba* var. Balady (native).
- 3- Three compounds namely; flume forte (antibiotic: fluoroquinolone group), potassium sulfonate and dettol. Each was used with three concentrations ; (0.1,0.05 and 0.025%).
- 4- Triton X 100 was used as emulsifier agent.

Methods:

Silkworm rearing technique

Eggs of the silkworm *Bombyx mori* L. (Giza –K hybrid) were rearing procedures were achieved under laboratory conditions of 26 ± 2 °c and 70 ± 5 % RH according to technique of Krishnaswami (1978). Chicken egg cartons plates were used as montages for cocoon spinning (Zannoon and Omara 1994). The cocoons were harvested seven days after mounting. Half number of the resulted cocoons was used for the biological studies and after emergence, each couple of moths was impaired in paper sacule for copulation and oviposition. The other half of cocoons were dried in an oven to study the technological characters. The three compounds namely; flume forte, potassium sulfonate and dettol were added to mulberry leaves as disinfectants against the flacherie disease.

Isolation of bacteria:

Bacterial pathogens were collected from diseased black thorax septicemia infected larvae, the diseased larvae were crushed by using mortar and pestle and the solution was filtered. The filtrate was centrifuged at 4500 rpm for 10 minutes. The supernatant was discarded and the pellet was used for bacterial culture after resuspending in distilled water (Aneja, 2003).

Infecting of silkworm larvae with bacteria:

Newly molted 4th instar larvae were divided into two groups; the first was left without infection (control group). The second group was fed on infected mulberry leaves of 20 ml /200 g leaves /100 larvae. The infected group were divided into two subgroups, the first subgroup treated with the

investigated disinfectants and the second subgroup with distilled water. For the diseased and treated groups, the infected leaves were provided to larvae at the first feed on first day and thereafter the larvae were fed with normal leaves. Every treatment and control was included 3 replicates of 100 larvae for each treatment.

Treatment by disinfectants:

Using a small atomizer, clean mulberry leaves were sprayed with different concentrations (0.1, 0.05 and 0.025 %) of disinfectants; flume forte, potassium sulfonate and dettol at the rate of (20 ml /200 gm leaves /100 larvae) and offered to infected groups .Larvae fed on treated mulberry leaves along the 1st days of each 4th and 5th larval instars.

1- Biological aspects:

1.1.Grown larval stage aspects: Grown larval mortality percentage (%), grown larval duration (days),and cocooning percentage (%):

Formation of a good cocoon is important aim of rearing silkworm, so the number of formed cocoons of each tray was recorded and percentage of cocooning was calculated as follows:

$$\text{Cocooning percentage (\%)} = \frac{\text{Total No of larvae at the beginning of the 5th instar /}}{\text{No. of fresh cocoon}} \times 100$$

1.2. Adult stage aspects: Adult emergence percentages, fecundity of female moths (egg / female) and egg fertility percentages of mulberry silkworms were recorded and tabulated.

2. Technological aspects:

2.1. Cocoon indices: Cocoon weight, cocoon shell weight and silk content ratio(%) was calculated according to Tanaka (1964) formula:

$$\text{Silk content ratio (\%)} = \frac{\text{Weight of cocoon shell} \times 100}{\text{Weight of fresh cocoon}}$$

2.2. Reeled silk filament

indices: Silk filament length (m),Silk filament weight (g) and Silk filament size (dn) which was calculated

The size of reeled silk filaments by denier (dn.) was calculated according to the following equation (Tanaka, 1964):

$$\text{Size of silk filament (dn.)} = \frac{\text{Weight of filament (g)} \times 9000}{\text{Length of filament (m)}}$$

Statistical analysis

Collected data were subjected to statistical analysis of variance test according to Sendecor and Cochran (1967) methods using software COSTAT program.

RESULTS AND DISCUSSION

1. Biological aspects:

1.1. Grown larval stage:

1.1.1. Larval mortality percentage (%):

The least percentages of grown larval mortality were recorded (11.50, 19.20 and 26.90 %) for the treatment with 0.1, 0.05 and 0.025 % of potassium sulfonate compound followed by (28.80, 32.70 and 36.50 %) for the treatment with 0.1, 0.05 and 0.025 % of flume forte compound, while the control (without infection) manifest the highest percentage of grown larval mortality recording (52.00 %) as shown in Table 1.

1.1.2. Larval duration of 4th and 5th instars (day):

Larval treatment with 0.1 and % flume forte, potassium sulfonate and dettol exhibited the shortest duration (15.50 days), while the control group elongated the grown larval duration (17 days) as shown in Table 1.

1.1.3. Cocooning percentage:

The highest percent cocooning of silkworm larvae were recorded (and 96.00, 88.00 and 89.00 %) for treatment with 0.1 and 0.05 % potassium sulfonate and 0.1% flume forte, respectively. While, the control group manifested the least cocooning percentage recording (71.00 %) as shown in Table 1.

1.2. Adult Stage:

1.2.1. Adult emergence percentage:

The highest percentages of adult emergence were recorded with larval treatments with 0.1 and 0.05 % concentrations of flume forte, potassium sulfonate and dettol compounds recording 99.60 to 99.90 %. While the control group reduced the adult emergence percentages to 63.25 %. as shown in Table 2.

1.2.2. Female fecundity (eggs/female):

It was found that, the infected silkworm fed on the concentrations 0.1 and 0.05 % potassium sulfonate and dettol compounds increased the female fecundity to (437 to 515 eggs/ female), while the control group resulted the least female fecundity recording (281 eggs/ female) as shown in Table 2.

Table (1): Effect of chemical disinfectants on grown larval mortality percentage, duration and larval duration of mulberry silkworm, *Bombyx mori* L.

Compounds	Conc. (%)	Grown Larval mortality(%)	Grown larval duration(day)	Cocooning percentage(%)
Flume forte	--	28.8	15.50	88.00
	0.05	32.7	15.50	80.00
	0.025	36.5	15.50	81.00
Potassium sulphonate	0.1	11.5	15.50	96.00
	0.05	19.2	16.50	89.00
	0.025	26.9	16.50	87.00
Dettol	0.1	28.8	15.50	82.00
	0.05	36.5	16.50	81.00
	0.025	40.4	16.50	80.00
Control		12.00	17.00	90.00
L.S.D. conc.		1.17249**	0.481**	2.521**

1.2.3. Egg fertility percentages (%):

The highest fertility percentages of eggs laid by female moths were found by the treatments with 0.1 % of flume forte, potassium sulfonate and dettol compounds recording;97.50, 99.30 and 98.50%, respectively. Whereas, the control group recorded the least fertility percentages of eggs laid by female moths(84.00%) as shown in Table 2.

The results are in agreement with Kotby *et al.* (1990) who used papsol and newceresin (powders), formalin and calcium hypochlorite (solutions) as bed-disinfectants on the grown larvae of the silkworm *Bombyx mori* L. Saha *et al.*(1995) and Ray and Senapati used cloxacillin (01%) (2002)). Also, Saad *et al.* (2012) indicated that the two antibiotics, cefotax and enrotryl at 200, 300 and 400 ppm decreased the larval duration and gave the high percentages of cocooning of *Bombyx mori* L. Also , in connection, Megalla (1984) clarified that, the presence of ascorbic acid, yeast or chloramphenical alone or in any combination usually increase the percentage of emerged moth.. Majumdar (1982) stated that, silkworm *Bombyx mori* L. larvae fed on mulberry leaves soaked in water alone or with potassium iodide gave moderate number of deposited eggs laid by females. Ray and Senapati (2002) declared that when

Table (2): Effect of chemical disinfectants on adult emergence, female fecundity and egg fertility of mulberry silkworm, *Bombyx mori* L.

Compounds	Conc. (%)	Adult Emergence (%)	Fecundity (egg/female)	Egg fertility (%)
Flume forte	0.1	99.90	427	97.50
	0.05	99.80	419	96.80
	0.025	94.70	391	91.70
Potassium sulphonate	0.1	99.60	515	99.30
	0.05	99.80	442	95.30
	0.025	94.80	377	91.10
Dettol	0.1	99.90	465	98.50
	0.05	99.70	437	94.20
	0.025	90.40	400	93.50
Control	-----	94.30	497	94.00
L.S.D. Conc.		2.111 **	61.73 **	5.494 **

fed the 5th instar larvae, leaves containing higher nutrient level at 0.1 % Gentamycin (antibiotic) the fecundity percentage of silkworm was increased.

2. Technological studies:

2.1. Cocoon indices:

2.1.1. Fresh cocoon weight (g):

The highest values of fresh cocoon weights were recorded (1.458, 1.436 and 1.440 g) for 0.1 % flume forte, potassium sulfonate compounds and dettol, respectively as shown in Table 3.

2.1.2. Cocoon shell weight:

Flume forte compound and dettol (0.1 %) manifested the highest values of cocoon shell weight recording (0.328 and 0.330 g) compared with (0.262 g) for control group as shown in Table 3.

2.1.3. Silk content ratio:

No significant effect of the three tested compounds appeared as shown in Table 3.

2.2:Reeled filament parameters:

2.2.1. Silk filament length (m):

The treatments 0.1, 0.05 % of flume forte, potassium sulfonate and dettol caused the highest means of silk filament length recording;

Table (3): Effect of chemical disinfectants on cocoon weight, cocoon shell weight and silk content ratio of mulberry silkworm, *Bombyx mori* L

Compounds	Conc. (%)	Cocoon weight (g)	Cocoon shell weight (g)	Silk content ratio (%)
Flume forte	0.1	1.458	0.328	22.47
	0.05	1.392	0.312	22.41
	0.025	1.206	0.306	25.37
Potassium sulphonate	0.1	1.436	0.306	21.31
	0.05	1.122	0.280	24.96
	0.025	1.078	0.270	25.05
Dettol	0.1	1.460	0.330	22.63
	0.05	1.192	0.280	23.49
	0.025	1.128	0.242	21.45
Control	-----	1.282	0.262	20.44
L.S.D.		0.128 **	0.055 **	ns

(1212.90,1118.50 and 1013 m, respectively. The lowest mean of silk filament lengths was recorded with control group (952.45 m) as shown in Table 4.

2.2.2. Silk filament weight (g):

Obtained results cleared that, 0.1 % flume forte compound and potassium sulfonate exhibited the highest value of silk filament weight recording (0.300 and 280 g), respectively. The control recorded (0.233 g) as shown in Table 4.

2.2.3. Silk filament size (dn):

When treated the infected silkworm with 0.1 % flume forte compound and 0.1 % potassium sulfonate the highest silk filaments sizes recorded (2.229, and 2.292 dn., respectively compared to 2.202 dn. for control as shown in Table 4.

Majumdar (1982) mentioned that feeding silkworm, *Bombyx mori* L.larvae on mulberry leaves soaked in water alone or with potassium iodide 25, 50 or 100 μ g/m. increased the weight of silk.. Saad *et al.* (2012) indicated that the two antibiotics (Cefotax and Enrotryl) at concentrations of

Table (4): Effect of chemical disinfectants on filament length, filament weight and filament Size of mulberry silkworm, *Bombyx mori* L.

Compounds	Conc. (%)	Filament length (m.)	Filament weight (g)	Filament Size (dn.)
Flume forte	0.1	1212.90	0.300	2.229
	0.05	1035.70	0.255	2.219
	0.025	965.90	0.198	1.857
Potassium sulphonate	0.1	1118.50	0.280	2.292
	0.05	990.70	0.250	2.276
	0.025	962.30	0.200	1.903
Dettol	0.1	1013.90	0.235	2.109
	0.05	975.60	0.207	2.061
	0.025	922.80	0.195	1.684
Control	----	952.45	0.233	2.202
L.S.D. conc.		161.48**	0.0385 **	0.419 **

(400 ppm) caused significant increase of filament weight, length and size. Sailaja *et al.* (1991) reported that oral treatment with the antibiotic oxy tetracycline increased the cocoon weight of *Bombyx mori* L. west value of cocoon weights recording (0.830 g).

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وقاية يرقات دودة الحرير التوتية من بعض الأمراض باستخدام بعض المطهرات الكيميائية كمطهرات

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أجريت هذه الدراسة لتقييم استخدام المركبات فلوم فورت و بوتاسيوم سلفونيت و الديتول كمواد مطهرة و أثرها علي الصفات البيولوجية و إنتاج الحرير في دودة الحرير التوتية المصابة صناعيا بالبكتيريا المسببة لمرض الفلاشري .
حققت المعاملة بمركب بوتاسيوم سلفونيت بتركيز ٠,١ و ٠,٥ % أقل نسبة موت و أعلى نسبة خروج للحشرات الكاملة و أعلى خصوبة للأنتى و أعلى نسبة فقس للبيض و أيضا زيادة في وزن الشرائق الناتجة و أعلى نسبة حرير في الشرنقة و أطول خيط في الشرنقة و اعلى وزن و حجم للخيط المحلول للشرنقة. يليها المعاملة بتركيز ٠,١ و ٠,٥ % لمركب فلوم فورت الذى أدى إلى زيادة نسب خروج الحشرات الكاملة و زيادة نسبة الحرير في الشرنقة و طول الخيط الحريرى، بينما ادت معاملة أوراق التوت البلدى بمركب الديتول (%) إلى زيادة وزن قشرة الشرنقة.