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Relative Efficacy of Antibiotics on Larval and Cocoon Parameters of Silkworm Infected with *Bacillus thuringiensis* var*. kurstaki*

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ABSTRACT

Among six antibiotics tested, with "norfloxacin" lowest larval mortality (22.43%), highest larval weight (3.01 g), cocoon weight (1.67 g), shell weight (0.27 g) and shell percentage (15.92%) was noticed. Among different concentrations used at 1500 ppm concentration the larval mortality was lowest (32.82%). Among different methods of application of antibiotics tested, with spraying highest cocoon parameters were reported, and also the larval mortality was reduced.

Key words : Antibiotics, *Bacillus thuringiensis* var. *kurstaki,* Bacterial flacherie, *Bombyx mori,* Sericulture,Silkworm, Norfloxacin

Sericulture is an agrobased industry generating enough employment potential and fetching remunerative returns to the silkworm rearers and other entrepreneurs engaged in various sectors of the industry. It also earns considerable foreign exchange to the county. Though, India is the second largest producer after China, a wide gap in terms of quality, quantity and productivity per unit area of raw silk exists due to various reasons. Among these causes diseases of silkworm are more important.

The chief diseases affecting mulberry silkworm, *Bambyx mori* L. are flacherie, grasserie, muscardine and pebrine (Dasgupta, 1950). Microbial flacherie is caused by both bacteria and viruses. Bacterial flacherie is caused by *Bacillus thuringiensis.* In India, flacherie prevails to the extent of 57.2 per cent among the diseases of silkworm (Samson *et al.,* 1990). This present investigation is to study the relative efficacy of different antibiotics in reducing the diseases incidence and increasing the larval weight.

MATERIAL AND METHODS

The six antibiotics *viz.,* amikacin, chloramphenicol, erychromycin, gentamycin, neomycin, and norfloxacin were tested to know their efficacy in brining down the infection of *Bt* var. *kurstaki* in silkworm. The antibiotics were used at 500, 1000, 1500 ppm concentrations. Mulberry leaves were first swabbed with a piece of cotton dipped in distilled water and then with 70 per cent ethanol to remove all dust and dirt and for surface sterilization. These leaves were smeared with the pathogen and cut into pieces before offering as the first feed to the fifth instar silkworms (PM ´ CSR_2). Later, the worms were fed with the antibiotic treated leaves were treated with different concentrations of antibiotics. In each concentration of antibiotic the leaves were treated by different methods such as leaf dipping, smearing and spraying. Every day the first feeding was given with the antibiotic treated leaves and subsequent feedings with normal leaves. Similarly, a treatment of pathogen inoculated leaves without antibiotics were fed to the worms and they were kept for comparison with three replications.

RESULTS AND DISCUSSION

Larval mortality

The mortality of silkworm larvae differed significantly in different methods of application of antibiotics, highest mortality of 37.01 per cent was noticed when the antibiotics were applied by the method of smearing whereas the larval mortality recorded was only 33.54 per cent when they were applied by the method of spraying (Table 1).

The larval mortality significantly differed with the concentration of the antibiotics applied. The minimum mortality of 32.87 per cent was reported with 1500 ppm concentration. The larval mortality was maximum (37.37%) when 500 ppm concentration was used. Baig *et al.* (1990) reported that streptomycin sulphate, gentamycin, cloxocillin and kanamycin at 0.05 and 0.1 per cent reduced the occurance of grasserie and flacherie significantly compared to normal (untreated check). Similar results were also reported by Samson and Baig (1976). Samson (1987) reported that 100 ppm penicillin was found to be more effective in reducing the incidence of bacterial flacherie Narayanan *et al.* (1973) also found that chloramphenicol and

tetracycline inhibited the growth of *B. thuringiensis* at all concentrations.

The lowest mortality (22.43%) of silkworm was recorded when norfloxacin was applied as against 55.13 per cent mortality recorded in normal (untreated check). The above results were also in conformity with the findings of Sam Devadas (1991), who reported that chloramphenicol was most effective against *Serratia marcescens* and neomycin was found to be least effective.

In the interaction effect between antibiotic treatments, antibiotic concentrations, and method of application, the minimum mortality of 18.67 per cent was noticed when 1500 ppm norfloxacin was applied as spray followed by 1000 ppm norfloxacin as spray (21.33%) and 1500 ppm amikacin as spray (21.33%). The mortality recorded was maximum (43.21%) when 500 ppm erythromycin was applied by smearing of leaves with antibiotic followed by 500 ppm chloramphenicol as leaf dip (42.20%), 100 ppm neomycin applied by smearing (42.13%) and 1000 ppm erythromycin applied by smearing (42.11%).

Larval weight

Larval weight differed significantly with methods of applications of antibiotics. Highest larval weight (2.80 g) was recorded when the antibiotics were applied by the method of spraying but only 2.39 g of larval weight was recorded when they were applied by smearing (Table 2).

Larval weight differed significantly with the concentration antibiotics. Larval weight recorded was maximum (2.81 g) when antibiotics applied at the concentration of 1500 ppm. Minimum larval weight of 2.67 g was noticed with 500 ppm concentration.

Maximum larval weight (3.01 g) was noticed when norfloxacin was applied as against 2.25 g of larval weight recorded in normal (untreated check).

In the interaction effect between antibiotic treatment, antibiotic concentration and method of application, lowest larval weight (2.49 g) was observed when erythromycin applied at 500 ppm concentration, by the method of smearing followed by 500 ppm erythromycin applied by spraying (2.54 g). Highest larval weight of 3.17 g was reported when 1500 ppm norfloxacin was applied by spraying the followed by 1500 ppm amikacin applied by spraying (3.12 g).

Cocoon weight

Highest cocoon weight of 1.55 g was noticed when the antibiotics were applied by the method of spraying. Cocoon weight recorded was lowest (1.50 g) when antibiotics applied by smearing (Table 1), followed by leaf dipping method (1.52 g).

The cocoon weight was maximum (1.57 g) when antibiotics were applied at 1500 ppm concentrations. Lowest cocoon weight of 1.49 g was recorded with 500 ppm concentration. Cocoon weight of 1.51 g was recorded at 1000 ppm concentration. Maximum cocoon weight of 1.67 g was noticed when norfloxacin was applied as against 1.28 g of cocoon weight recorded in normal (untreated check) which was followed by Amikacin (1.64 g) and chloramphenicol (1.58 g). Minimum cocoon weight of 1.49 g was recorded with Gentamycin.

Highest cocoon weight of 1.74 g was reported when 1500 ppm norfloxacin was applied by spraying, whereas cocoon weight recorded in leaf dipping and smearing methods was 1.72g only. Lowest cocoon weight (1.35 g) was noticed when 500 ppm neomycin was applied by smearing. These results were on par with Murthy *et al.,* (1954) and Radha *et al.* (1981).

Shell weight

Shell weight significantly differed with methods of application of antibiotics. When antibiotics applied by the method of spraying maximum shell weight (0.24 g) was recorded. Shell weight was minimum (0.20 g) when antibiotics applied by smearing. Moderate shell weight (0.21 g) was recorded with the method of leaf dipping (Table 4).

Shell weight differed significantly with the concentration of antibiotics. Highest shell weight (0.23 g) was recorded when antibiotics applied at 1500 ppm concentration and shell weight recorded was minimum (0.20 g) at 500 ppm concentration. Among the six treatments, highest shell weight of 0.27 g was noticed with norfloxacin treatment as against 0.16 g recorded in normal (untreated check).

In the interaction effect between antibiotic treatment, antibiotic concentration and method of application, maximum shell weight of 0.29 g was noticed when norfloxacin applied by the method of spraying at the concentration of 1500 ppm. When norfloxacin was applied by leaf dipping and smearing at the concentration of 1500 ppm and amikacin was applied by spraying at 1500 ppm concentration 0.28 g of shell weight was recorded. The shell weight recorded was minimum (0.15 g) when neomycin applied by smearing at the concentration 500 ppm.

Shell percentage

Shell percentage significantly differed with methods of application of antibiotics. Maximum shell

Table 1. Effect of different concentrations and methods of application of antibiotics on larval mortality of silkworm, *(Bombyx mori* L.) infected with *Bacillus thuringiensis*

Treatment	Larval mortality (%)															
	500 ppm				1000 ppm				1500 ppm				Mean of methods			
	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Over all mean
Amikacin	36.45 (37.17)	38.32 (38.23)	33.33 (35.18)	36.03 (36.86)	28.54 (32.27)	33.37 (35.30)	26.67 (31.11)	29.53 (32.89)	24.71 (29.80)	27.25 (31.50)	21.33 (27.49)	24.43 (29.59)	29.90 (33.15)	32.98 (35.00)	27.11 (31.37)	30.00 (33.21)
Chloramphenicol	42.20 (49.51)	40.65 (39.64)	40.00 (39.23)	40.95 (40.79)	34.65 (36.09)	39.48 (38.94)	33.33 (35.24)	35.82 (36.75)	31.89 (34.39)	35.65 (36.69)	29.33 (32.77)	32.29 (34.62)	36.25 (37.05)	38.59 (38.41)	34.22 (35.79)	36.35 (37.11)
Erythromycin	40.52 (39.53)	43.21 (41.09)	38.67 (38.47)	40.80 (39.70)	40.00 (39.23)	42.11 (40.45)	38.06 (38.12)	40.06 (39.27)	39.43 (38.88)	41.37 (40.05)	37.33 (37.64)	39.38 (38.86)	39.98 (39.17)	42.23 (40.51)	38.02 (38.06)	40.08 (39.29)
Gentamycin	32.76 (34.94)	34.25 (35.49)	29.33 (32.77)	32.11 (34.50)	35.32 (30.20)	27.61 (31.69)	25.33 (30.20)	26.09 (30.69)	30.30 (33.40)	33.44 (35.30)	29.33 (32.77)	31.02 (33.82)	29.46 (32.90)	31.77 (34.33)	28.00 (31.95)	29.74 (33.02)
Neomycin	32.01 (34.45)	35.01 (36.27)	30.67 (33.65)	32.56 (34.89)	40.27 (39.41)	42.13 (40.45)	36.00 (36.87)	39.47 (38.91)	26.67 (31.11)	27.00 (31.31)	26.67 (31.11)	26.78 (31.18)	32.98 (35.00)	34.71 (36.09)	31.11 (33.90)	32.93 (35.00)
Norfloxacin	24.00 (29.33)	24.00 (29.33)	24.00 (29.33)	24.00 (29.33	22.00 (27.97)	23.46 (37.17)	21.33 (27.49)	22.26 (28.02)	21.39 (27.56)	23.02 (28.66)	18.67 (25.62)	21.03 (27.27)	22.46 (28.32)	23.49 (29.00)	21.33 (27.49)	22.43 (28.25)
Control (Pathogen alone)	55.12 (47.93)	55.33 (46.89)	55.00 (47.87)	55.15 (47.56)	55.12 (47.93)	55.12 (46.89)	55.33 (46.89)	55.15 (47.56)	55.12 (47.93)	55.33 (46.09)	55.00 (47.89)	55.12 (47.48)	55.12 (47.93)	55.33 (46.09)	55.00 (47.89)	55.15 (47.48)
Mean	37.58 (38.12)	38.68 (38.18)	35.85 (36.69)	37.37 (37.66)	35.13 (36.13)	37.64 (37.50)	37.64 (37.50)	35.48 (36.30)	32.79 (34.72)	34.72 (35.73)	31.09 (33.69)	32.87 (34.69)	35.16 (36.22)	37.01 (37.06)	33.54 (35.20)	

	F-test	SEm±	CD	F-test	SEm±	CD	F-test	SEm±	CD
Effect of method	**	0.2492	0.7049	**	0.0126	0.0356	**	0.0238	0.0674
Effect of treatments	**	0.3807	1.0768	**	0.0192	0.0543	**	0.0364	0.1029
Interaction effect	**	0.6594	1.8651	**	0.0333	0.0941	**	0.0630	0.1782

Table 2. Effect of different concentrations and methods of application of antibiotics on larval weight of silkworm, *(Bombyx mori* L.) infected with *Bacillus thuringiensis*

Treatment	Larval weight (g)															
	500 ppm				1000 ppm				1500 ppm				Mean of methods			
	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Over all mean
Amikacin	2.75	2.73	2.80	2.76	2.95	2.91	3.02	2.96	3.07	3.04	3.12	3.08	2.92	2.89	2.98	2.93
Chloramphenicol	2.73	2.69	2.78	2.73	2.82	2.78	2.87	2.82	2.80	2.76	2.89	2.82	2.78	2.74	2.85	2.79
Erythromycin	2.54	2.49	2.60	2.54	2.64	2.62	2.67	2.64	2.67	2.65	2.70	2.67	2.62	2.59	2.66	2.62
Gentamycin	2.80	2.77	2.84	2.80	2.94	2.89	2.97	2.93	2.97	2.97	3.00	2.98	2.90	2.88	2.94	2.91
Neomycin	2.70	2.67	2.72	2.70	2.74	2.69	2.78	2.74	2.80	2.75	2.85	2.80	2.75	2.70	2.78	2.74
Norfloxacin	2.89	2.86	2.97	2.91	3.05	3.00	3.10	3.05	3.12	2.97	3.17	3.09	3.02	2.94	3.08	3.01
Control (Pathogen alone)	2.24	2.20	2.32	2.25	2.24	2.20	2.32	2.25	2.24	2.20	2.32	2.25	2.24	2.20	2.32	2.25
Mean	2.66	2.63	2.72	2.67	2.59	2.73	2.90	2.74	2.81	2.76	2.86	2.81	2.75	2.39	2.80	

	F-test	SEm±	CD	F-test	SEm±	CD	F-test	SEm±	CD
Effect of method	**	0.0021	0.0089	**	0.0027	0.0096	**	0.0017	0.0015
Effect of treatments	**	0.0019	0.0076	**	0.0022	0.0092	**	0.0026	0.0124
Interaction effect	**	0.0042	0.0121	**	0.0054	0.0204	**	0.0045	0.0278

Table 3. Effect of different concentrations and methods of application of antibiotics on cocoon weight of silkworm, *(Bombyx mori* L.) infected with *Bacillus thuringiensis*

Treatment	Cocoon weight (g)															
	500 ppm				1000 ppm				1500 ppm				Mean of methods			
	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Over all mean
Amikacin	1.59	1.58	1.61	1.59	1.64	1.60	1.66	1.63	1.70	1.69	1.72	1.70	1.64	1.62	1.66	1.64
Chloramphenicol	1.53	1.51	1.56	1.53	1.57	1.56	1.58	1.57	1.63	1.62	1.64	1.63	1.58	1.56	1.59	1.58
Erythromycin	1.56	1.52	1.58	1.53	1.45	1.45	1.50	1.47	1.53	1.53	1.55	1.54	1.52	1.50	1.54	1.52
Gentamycin	1.44	1.44	1.49	1.46	1.45	1.45	1.53	1.49	1.52	1.52	1.54	1.53	1.48	1.47	1.52	1.49
Neomycin	1.41	1.35	1.47	1.41	1.47	1.47	1.52	1.49	1.60	1.58	1.60	1.59	1.49	1.47	1.53	1.50
Norfloxacin	1.61	1.61	1.64	1.62	1.68	1.64	1.70	1.67	1.72	1.72	1.74	1.73	1.67	1.66	1.69	1.67
Control (Pathogen alone)	1.28	1.26	1.30	1.28	1.28	1.26	1.30	1.28	1.28	1.26	1.30	1.28	1.28	1.26	1.30	1.28
Mean	1.49	1.47	1.52	1.49	1.51	1.49	1.54	1.51	1.57	1.56	1.58	1.57	1.52	1.50	1.55	

	F-test	SEm±	CD	F-test	SEm±	CD	F-test	SEm±	CD
Effect of method	**	0.0011	0.0030	**	0.0011	0.0031	**	0.0009	0.0025
Effect of treatments	**	0.0016	0.0046	**	0.0017	0.0047	**	0.0013	0.0038
Interaction effect	**	0.0028	0.0079	**	0.0029	0.0082	**	0.0023	0.0065

Table 4. Effect of different concentrations and methods of application of antibiotics on shell weight of silkworm, *(Bombyx mori* L.) infected with *Bacillus thuringiensis*

Treatment	Shell weight (g)															
	500 ppm				1000 ppm				1500 ppm				Mean of methods			
	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Over all mean
Amikacin	0.23	0.21	0.25	0.23	0.25	0.23	0.27	0.25	0.27	0.26	0.28	0.27	0.25	0.23	0.27	0.25
Chloramphenicol	0.21	0.19	0.24	0.21	0.23	0.22	0.25	0.23	0.25	0.24	0.26	0.25	0.23	0.22	0.25	0.23
Erythromycin	0.20	0.18	0.24	0.21	0.22	0.20	0.24	0.22	0.24	0.23	0.25	0.24	0.22	0.20	0.24	0.22
Gentamycin	0.18	0.16	0.22	0.19	0.20	0.18	0.24	0.21	0.22	0.22	0.24	0.23	0.20	0.19	0.23	0.21
Neomycin	0.17	0.15	0.21	0.18	0.19	0.18	0.23	0.20	0.22	0.20	0.24	0.22	0.19	0.18	0.23	0.20
Norfloxacin	0.24	0.24	0.26	0.25	0.27	0.26	0.28	0.27	0.28	0.28	0.29	0.28	0.26	0.26	0.28	0.27
Control (Pathogen alone)	0.15	0.14	0.18	0.16	0.15	0.14	0.18	0.16	0.15	0.14	0.18	0.16	0.15	0.14	0.18	0.16
Mean	0.20	0.18	0.23	0.20	0.22	0.20	0.24	0.22	0.23	0.22	0.25	0.23	0.21	0.20	0.24	

	F-test	SEm±	CD	F-test	SEm±	CD	F-test	SEm±	CD
Effect of method	**	0.0011	0.0030	**	0.0010	0.0029	**	0.0009	0.0025
Effect of treatments	**	0.0016	0.0046	**	0.0015	0.0044	**	0.0013	0.0038
Interaction effect	**	0.0028	0.0079	**	0.0027	0.0076	**	0.0023	0.0066

Table 5. Effect of different concentrations and methods of application of antibiotics on shell percentage of silkworm, *(Bombyx mori* L.) infected with *Bacillus thuringiensis*

Treatment	Shell percentage (%)															
	500 ppm				1000 ppm				1500 ppm				Mean of methods			
	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Mean	Leaf dipping	Smearing	Spraying	Over all mean
Amikacin	14.47 (22.38)	13.29 (21.39)	15.59 (23.18)	14.42 (22.30)	15.24 (22.95)	14.38 (22.30)	16.26 (23.81)	15.29 (23.02)	15.88 (23.50)	15.38 (23.11)	16.27 (23.03)	15.97 (23.21)	15.20 (22.94)	14.35 (22.27)	16.02 (23.34)	15.19 (22.85)
Chloramphenicol	13.73 (21.72)	12.58 (21.05)	15.38 (23.11)	13.89 (21.89)	14.64 (22.46)	14.10 (22.06)	15.82 (23.42)	14.85 (22.65)	15.34 (23.39)	14.81 (22.63)	15.85 (23.50)	15.33 (23.05)	14.57 (22.40)	13.83 (21.91)	15.68 (23.32)	14.69 (22.54)
Erythromycin	12.82 (20.96)	11.84 (20.09)	15.19 (22.95)	13.28 (21.39)	15.06 (22.87)	13.79 (21.81)	16.00 (23.58)	14.95 (22.75)	15.68 (23.34)	15.03 (22.79)	16.16 (23.66)	15.61 (23.26)	14.52 (22.39)	13.55 (21.56)	15.77 (23.40)	14.61 (22.45)
Gentamycin	12.50 (20.70)	11.11 (19.46)	14.76 (22.63)	12.79 (20.96)	13.51 (21.56)	12.41 (20.62)	15.69 (23.34)	13.87 (21.84)	14.47 (22.38)	14.47 (22.38)	15.58 (23.26)	14.83 (22.67)	13.39 (21.55)	12.66 (20.82)	15.34 (23.08)	13.83 (21.82)
Neomycin	12.05 (20.27)	11.11 (19.46)	14.28 (22.22)	12.48 (20.70)	12.96 (21.05)	12.24 (20.44)	15.13 (22.87)	13.43 (21.45)	13.75 (21.81)	12.65 (20.88)	15.00 (22.79)	13.58 (21.83)	12.91 (21.04)	12.00 (20.26)	14.80 (22.63)	13.24 (21.31)
Norfloxacin	14.91 (22.71)	14.91 (22.71)	15.85 (23.50)	15.22 (22.95)	16.07 (23.66)	15.85 (23.50)	16.47 (23.97)	16.13 (23.71)	16.27 (23.03)	16.27 (23.03)	16.66 (23.34)	16.40 (23.13)	15.75 (23.13)	15.68 (23.08)	16.33 (23.60)	15.92 (23.27)
Control (Pathogen alone)	11.72 (20.00)	11.11 (19.46)	13.85 (21.64)	12.22 (20.44)	11.76 (20.00)	11.11 (19.46)	13.85 (21.64)	12.22 (20.44)	11.72 (20.00)	11.11 (19.46)	13.85 (21.64)	12.22 (20.44)	11.72 (20.00)	11.11 (19.46)	13.85 (21.64)	12.22 (20.44)
Mean	13.17 (21.25)	12.27 (20.52)	14.97 (22.75)	13.47 (21.51)	14.16 (22.08)	13.41 (21.46)	15.60 (23.23)	14.39 (22.26)	14.63 (22.44)	14.24 (22.04)	15.67 (23.03)	14.85 (22.51)	14.02 (21.92)	13.31 (21.34)	15.40 (23.00)	

	F-test	SEm±	CD	F-test	SEm±	CD	F-test	SEm±	CD
Effect of method	**	0.0637	0.1803	**	0.0598	0.1693	**	0.0747	0.2112
Effect of treatments	**	0.0974	0.2754	**	0.0914	0.2585	**	0.1141	0.3226
Interaction effect	**	0.1686	0.4770	**	0.1583	0.4478	**	0.1976	0.5588

percentage of 15.40 per cent was reported when antibiotics applied by the method of spraying. Shell percentage recorded was minimum (13.31%) with smearing method (Table 5).

Shell percentage recorded was highest (14.85%) when antibiotics applied at the concentration of 1500 ppm. Lowest shell percentage (13.47%) was noticed at 500 ppm concentration. Among the treatments highest shell percentage of 15.92 per cent was recorded with norfloxacin followed by amikacin (15.19%), chloramphenicol (14.69%) and erythromycin (14.61%). Shell percentage reported was lowest (13.24%) with neomycin followed by gentamycin (13.83%).

In the interaction effect between antibiotic treatments, antibiotic concentrations and method of application, when 1500 ppm norfloxacin was applied by spraying, shell percentage was maximum (16.66%) which was followed by 1000 ppm norfloxacin applied by spraying (16.47%). Shell percentage of 11.11 was noticed with 500 ppm neomycin and 500 ppm gentamycin when they were applied by smearing. These results were tallying with the findings of Dechu (1995).

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