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EFFICACY OF THE DISINFECTANT 'DISFECT-S' AGAINST PATHOGENS OF THE SILKWORM, *Bombyx mori*

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ABSTRACT

In-vitro efficacy tests of Disfect-S (Poly alkyl monohydric phenol) on the viability of infective propagules of silkworm disease at varying concentrations of 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 2.0% & 3.0% were carried out. Efficacy of 'Disfect' - S as surface sterilant of silkworm eggs was tested at concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 1.0% with treatment durations of 1 min, 2 min, 5 min, 10 min and 20 min. The viability and the infectivity of *Nosema bombycis* spores was inactivated at 0.4% with 5 min. of treatment duration and the infectivity of conidia of *Beauveria bassiana* was completely arrested at 0.8% with 5 min. duration whereas Nuclear polyhedra virus (BmNPV) were completely inactivated at 0.8% for 10 minutes treatment duration. Disfect-S was highly effective as surface sterilant of silkworm eggs at 0.4% with 2 min. duration treatment without any deleterious effect on hatching. The present study gives an higher scope for Disfect-S as potential commercial disinfectant in sericulture.

KEYWORDS : Silk worm pathogens, Disinfectants

The silkworm, *Bombyx mori* L is susceptible to infection by different pathogens which incite diseases *viz*., pebrine, grasserie, cytoplasmic polyhedrosis, infectious flacherie, densonucleosis and muscardines causing mortality in the larval and pupal stages. Evolving or identifying potential disinfectants capable of inactivating the disease causing pathogens and enhancing cocoon yield is of prime importance to the industry. A formulation of bleaching powder with lime has been reported to be successful in controlling grasserie and muscardine diseases of silkworm (Subba Rao *et al*., 1992). Presently 2 per cent solution of formalin or 5 per cent solution of bleaching powder (having a chlorine content of 30% or above) is widely used as spray for disinfection purpose. Formalin has been known to be hazardous to health and therefore, need to be used with adequate protective measures. It is fully effective only under air-tight condition. Bleaching powder is more safer to use, but improper storage deteriorates its quality, besides it is highly corrosive to metallic installations. Under

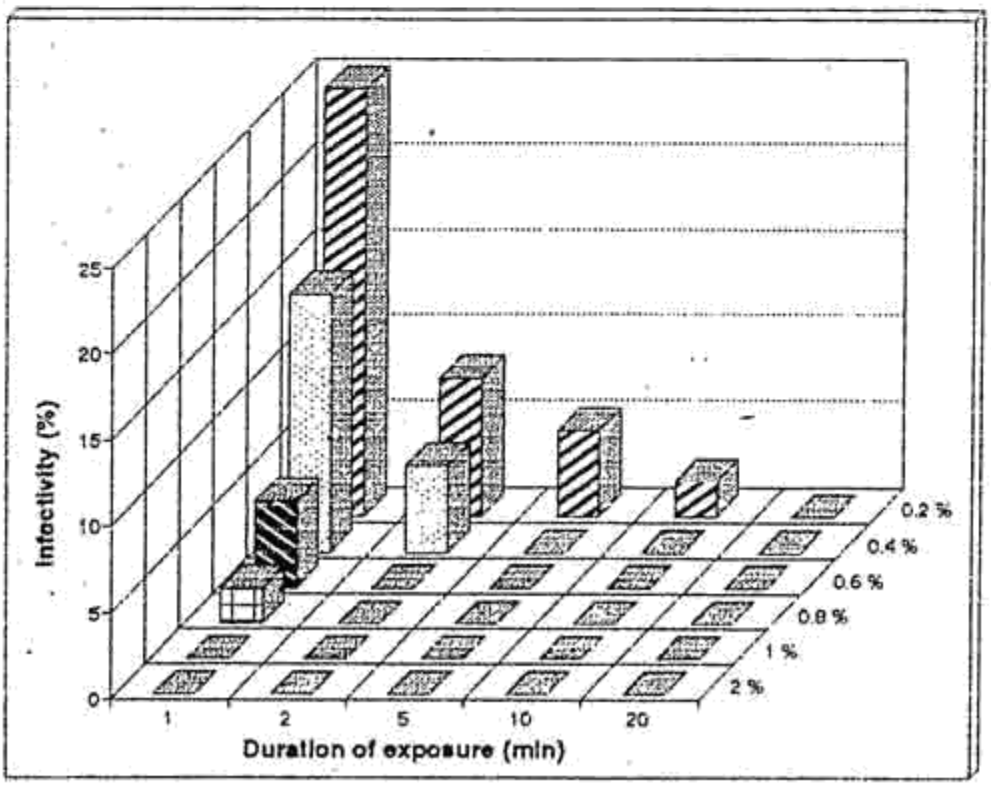

Fig. 1a: Infectiviy of *N.bombycis* treated with Disfect-S in Silkworms.

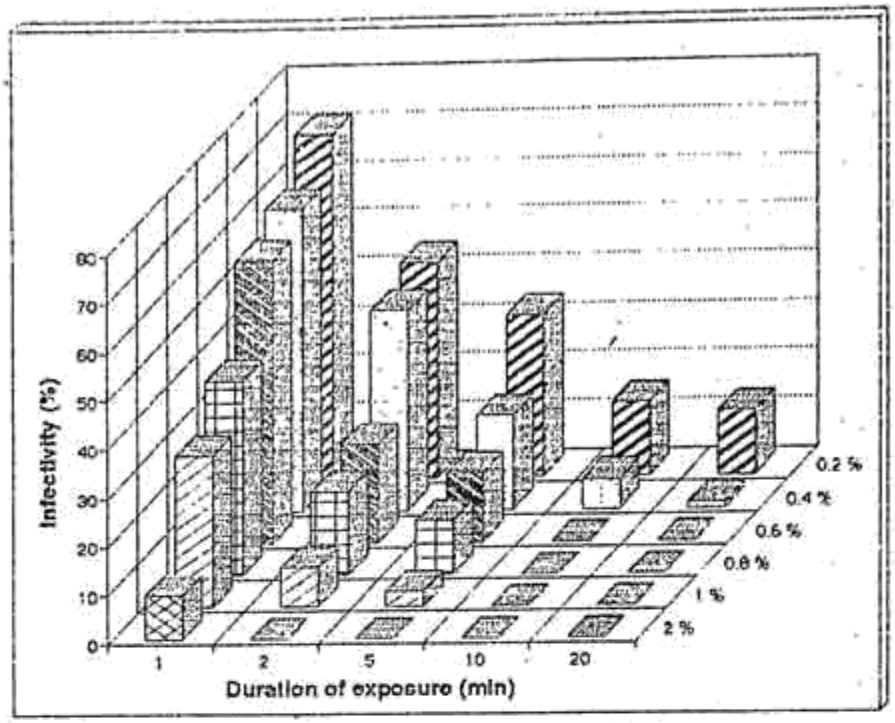

Fig.1b. Infectivity of BmNPV treated with Disfect -S in Silkworms.

these circumstances, alternate disinfectants which do not pose such problems will have better prospects. The present study was conducted with this objective using the new polyphenolic disinfectant, 'Disfect-S' manufactured and marketed by ESKAYEF for its *in-vitro* efficacy against some important disease causing pathogens of the silkworm, *B mori*. The efficacy of the disinfectants as a surface sterilant against surface contamination of silkworm eggs by *Nosema bombycis* has also been investigated.

MATERIALS AND METHODS

Preparation of disinfectant

The commercially available disinfectant, 'Disfect-S' is diluted with water in volumetric

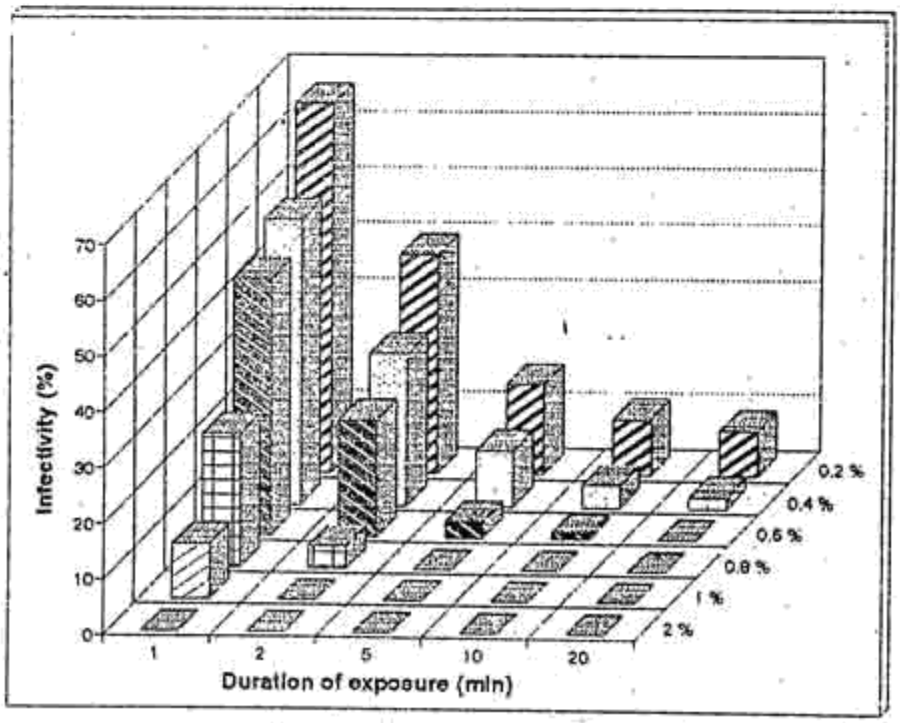

Fig.1c. Infectivity of *B.bassiana* treated with Disfect-S in Silkworms.

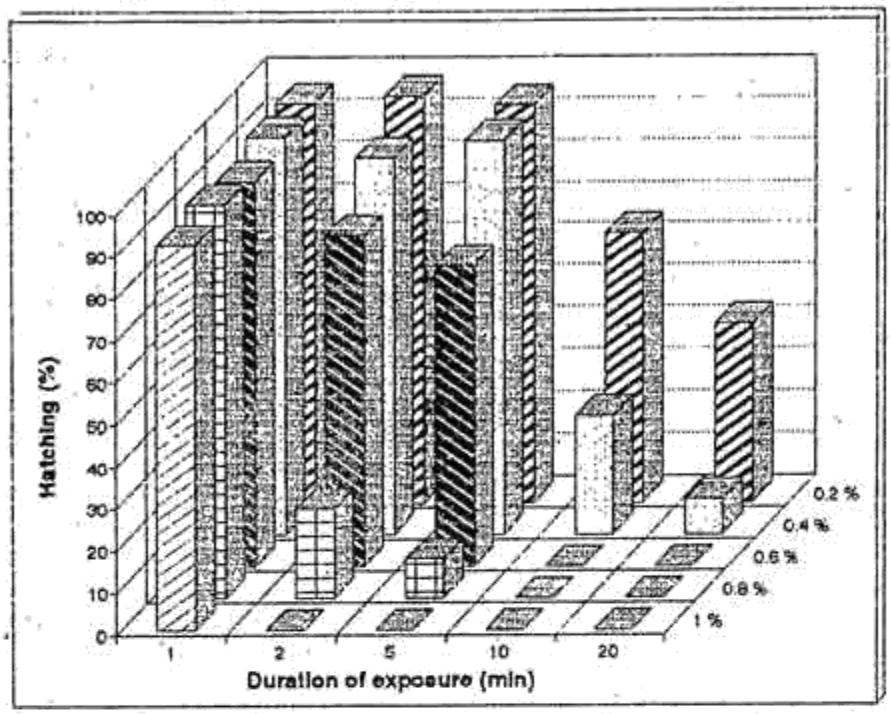

Fig.2. Disfect-S as surface sterilant against *N.bombycis.*

flasks to prepare 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 2.0% and 3.0% solutions of the disinfectant.

In vitro efficacy tests against silkworm pathogens

Nosema bombycis (Pebrine)

The spores of *N bombycis* were extracted from pebrine infected silkworms and purified (Fujiwara, 1993). The spore suspension was maintained in sterile distilled water and the concentration was adjusted to $1x10^{10}$ spores/ml by enumerating the spore concentration using an improved Neubaur haemocytometer. In six eppondorf tubes 0.1ml of spore suspension was taken and 0.4 ml of different concentration (0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 2.0% and 3.0%) of 'Disfect-S' was added to the spore suspension. The mixture was shaken well and left for 1,2,5,10 and 20 min. After the specific time intervals, the tubes were centrifuged and disinfectant was removed. The sedimented spores were washed twice in sterile distilled water and sedimented by repeated centrifugation. The spore concentration was then adjusted to $1x10^{7}$ spores/ml and orally inoculated (by smearing over fresh mulberry leaves) to neonate larvae of silkworm NB_4D_2 race. Thirty larvae in each of the four replications were kept. The larvae were reared in the laboratory till third instar under optimum conditions (25±1 °C and 75-80% RH) according to the procedure described by Krishnaswami *et al.*, (1973). Prior to third moult, individual larva was crushed and microscopically examined for the presence of pebrine spores to ascertain infection.

BmNPV (Grasserie)

The nuclear polyhedra were isolated and purified from grasserie infected silkworm and the concentration was adjusted to 1×10^{10} polyhedra/ml suspension. The test was conducted in the similar manner as done for pebrine.

Beauveria bassiana (Muscardine)

The assay was done using the conidia of *B bassiana.* The inoculation of the treated conidia was done percutaneously by spraying conidial suspension on to larvae out of II moult at the rate of $1x10^{7}$ conidia/ml and the incidence of muscardine was assessed till cocooning.

Testing as surface sterilant to overcome surface contamination of silkworm eggs

Healthy silkworm layings (sheet eggs) were contaminated with *N.bombycis* spores suspension containing $1x10^{10}$ spores/ml by smearing the spore suspension on the eggs. The smeared layings were dried in shade and dipped in different concentrations of the disinfectant solutions (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) for different durations

(1,2.5,10 and 20 minutes). The treated layings were dried and incubated under optimum conditions (25±1 °C and 80- 85% RH) in a rearing tray for hatching. Hatched larvae from each treatment were brushed separately, reared till 3rd moult and were examined microscopically for *N.bombycis* infection to assess the efficacy of the disinfectant as a surface sterilant. Separate control was maintained, in which the layings after smearing with pebrine spores were dipped in water.

RESULTS AND DISCUSSION

The effect of exposure of the pathogens *N.bombycis* spores, Nuclear polyhedra of BmNPV and conidia of *B.bassiana* for varying durations to different concentrations of 'Disfect-S' was assessed by testing the spore viability through bio-assay. The results are presented in Table 1,2. Analysis of variance of the results indicated that the infectivity significantly decreased as the duration of exposure and concentration increased in respect of Pebrine, Nuclear Polyhedrosis and Muscardine. The decrease in infectivity due to the interaction effect of duration of exposure and concentration of the three tested silkworm pathogens are presented in Fig.1a, 1b and 1c. Pebrine spores were completely inactiviated at 0.4 per cent of 'Disfect-S' when exposed for 5 min or at 0.6 per cent when exposed

Table 1. Infectivity of *Nosema bombycis* spore, nucler polyhedra (BmNPV) and *Beauveria bassiana* conidia treated with 'Disfect-S' in silkworm.

Duration of Exposure	Incidence of Disease	Different concentration of 'Disfect-S' (%)						
(Mins.)		0.2%	0.4%	0.6%	0.8%	1.0%	2.0%	3.0%
1	Pebrine	25.00 ±7.14	15.00 ±1.73	5.00 ±1.73	2.00 ±2.00	N.I.	N.I.	N.I.
	Nuclearpolyhedrosis	71.00 ±3.32	62.00 ±10.77	58.00 ±10.00	40.00 ±9.38	31.00 ±3.32	9.00 ±1.73	N.I.
	Muscardine	66.00 ±4.47	51.00 ±10.31	46.00 ±3.46	24.00 ±4.89	10.00 ±2.00	N.I. N.I.	N.I. N.I.
2	Pebrine	8.00 ±2.83	5.00 ±1.73	N.I.	N.I.	N.I.	N.I.	N.I
	Nuclearpolyhedrosis	49.00 ±7.14	41.00 ±9.95	20.00 ±2.83	17.00 ±6.56	8.00 ±4.89	N.I. N.I.	N.I. N.I.
	Muscardine	39.00 ±4.36	27.00 ±4.36	21.00 ±3.32	4.00 ±2.83	N.I.	N.I.	N.I.
5	Pebrine	5.00 ±3.32	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
	Nuclearpolyhedrosis	33.00 ±6.56	19.00 ±3.32	14.00 ±3.46	11.00 ±7.68	3.00 ±3.32	N.I.	N.I.
	Muscardine	16.00 ±2.83	10.00 ±4.47	3.00 ±1.73	N.I.	N.I.	N.I.	N.I.
10	Pebrine	2.00 ±2.00	N.I	N.I.	N.I.	N.I.	N.I.	N.I.
	Nuclearpolyhedrosis	15.00 ±3.32	6.00 ±6.00	5.00 ±5.19	N.I.	N.I.	N.I.	N.I.
	Nuscardine	10.00 ±1.89	4.00 ±2.83	N.I.	N.I.	N.I.	N.I	N.I
20	Pebrine	N.I	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
	Nuclearpolyhedrosis	13.00 ±7.14	1.00 ±1.73	N.I.	N.I.	N.I.	N.I.	N.I
	Muscardine	8.00 ±2.83	2.00 ±2.00	N.I.	N.I.	N.I.	N.I.	N.I
Control	Pebrine		100.00					
	Nuclearpolyhedrosis		100.00					
	Muscardine		100.00					

(Values are mean ± S.D.) N.I. : No infection.

for 2 min. In the case of Nuclear polyhedra, 0.8 per cent of the compound was effective in completely inactivating the polyhedra inclusion bodies (PIBs)/viruses when exposed for 10 min or 2 per cent for 2 min. The effect of the disinfectant on conidia of *B. bassiana* was highly appreciable and 0.8 per cent. 'Disfect-S' completely inactivated the conidia when exposed for 5 min.

The observations on the efficacy of the disinfectant as surface sterilant of silkworm eggs and its effect on hatchability and intensity of infection are furnished in Table 3,4. The analysis of variance revealed that the hatchability decreased significantly due to interaction effects of duration of exposure and concentration. It may be observed from the Fig.2 that hatchability decreased significantly with the 5 minutes duration of exposure and 0.6 per cent concentration of 'Disfect-S. Treatment of surface contaminated eggs with 'Disfect - 0.4 per cent for2 min. duration has eliminated the surface contamination completely with no adverse effect on egg hatchability.

The three silkworm pathogens used in these efficacy tests cause a great deal of concern to silkworm rearers very often. Disinfectants like formalin (Golanski, 1959, 1961; Ignoffo and Dutky,1963; Ignoffo and Garcia, 1968; Vail *et al*., 1968) has been extensively tested against several pathogenic microbes including Nuclear Polyhedrosis virus of silkworm. The present study has revealed that 0.4 per cent of 'Disfect-S' with treatment duration of 5 min. has completely checked the infectivity of *N. bombycis*, and the infectivity of *B. bassiana* was arrested by 0.8 per cent of the compound when treated for 5 mins. However, PIBs were inactivated at the same concentration only when exposed for 10 mins. The wide spectral efficacy of 'Disfect-S' could be attributed to its chemical activity leading to coagulation and protein coat denaturation, cytoplasmic leakage and cell lysis, as is normally observed in the case of phenolic compounds (Prindle, 1993). Investigations on comparative efficacy of different phenolic disinfectants have been conducted on several pathogenic microbes since long and positive results were recorded (Hegna, 1977). Formalin and bleaching powder though are disinfectants par excellence in sericulture, they have certain major disadvantages with regard to efficacy and human health. The

Table 2. Anova table for efficacy test of 'Disfect-S' against pebrine (*Nosema bombycis*), Nuclear Polyhedrosis (BmNPV) and Muscardine (*Beauveria bassiana*) of silkworm, *Bombyx mori* L.

Source of variation	DF	Mean sum of squares		
		Pebrine	Nuclear Polyhedrosis	Muscardine
Duration	4	215.60**	6303.83**	3551.89**
Concentration	6	185.03**	3268.46**	2316.38**
Replication	3	1.03	18.29	5.03
Duration x Concentration	24	62.60**	442.36**	439.62*
Residual	72	3.88	29.92	12.14

** Significant at $P \leq 0.01$

Table 3. Effect of 'Disfect-S' as surface sterilant against *Nosema bombycis* contaminated silkworm eggs on hatchability and intensity of infection

Duration of Exposure (min.)	0.2% Conc.	Intensity of Infect.	0.4% Conc.	Intensity of Infect.	0.6% Conc.	Intensity of Infect.	0.8% Conc.	Intensity of Infect.	1.0% Conc.	Intensity of Infect.
1	95.3±2.26	±	94.8±0.88	±	91.8±1.54	-	93.9±0.18	-	91.5±0.53	-
2	97.0±0.37	±	89.9±2.15	-	78.8±3.98	-	21.3±1.92	-	0	-
5	94.8±1.97	-	93.8±2.46	-	71.4±2.03	-	9.2±0.92	-	0	-
10	64.1±2.81	-	28.0±1.54	-	0	-	0	-	0	-
20	42.1±3.47	-	8.2±0.55	-	0	-	0	-	0	-
Control (with spores)	94.2±1.65	±								

±: 1-3 spores/microscopic field

-: No spores

Hatching percentage values are given as mean ± S.D.

Table 4. Anova table for efficacy test of 'Disfect-S' as surface sterilent against *Nosema bombycis* contaminated silkworm eggs on hatchability.

Source of variation	DF	Mean sum of squares Hatching
Exposure	4	22467.32**
Concentration	4	12855.24**
Replication	3	10.20
Exposure x Concentration	16	1960.30**
Residual	48	1746.12

** Significant at $P \leq 0.01$.

results obtained in the present investigation may be therefore relevant and the compound 'Disfect-S' can be a potential commercial disinfectant in the field of sericulture.

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CORRELATION AND PATH ANALYSIS IN PEARL MILLET (*Pennisetum glaucum*)

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ABSTRACT

Genotypic and phenotypic correlations were computed in a collection of twelve diverse male sterile lines, five restorers and the resulting sixty hybrids of pearl millet (*Pennisetum glaucum*(L.) R. Br.). Grain yield per plant showed high positive and significant correlation with plant height, ear length, ear girth and total number of tillers. The path coefficient analysis indicated the highest positive direct effect on grain yield per plant was exhibited by number of productive tillers followed by ear girth. Plant height, ear length and 1000 grain weight and positive indirect effects through ear girth and the total number of tillers had indirect effect through the number of productive tillers on grain yield.

KEY WORDS: Pearl Millet, Correlation, Path analysis

Yield is a complex dependent character. It depends upon a number of independent component characters which may contribute directly or indirectly. The knowledge on correlation helps in determining the component characters of complex entity whereas, the path coefficient analysis provides an effective means of partitioning direct and indirect causes of association. It permits a critical look to recognise the special forces acting to produce a given correlation and its relative importance. Hence a study was undertaken in pearl millet parents and hybrids to study the correlation and path analysis among the grain yield and its components.