



## Efficacy of fungicide 'Kavach' against *Beauveria bassiana* L. in silkworm *Bombyx mori* L.

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**Abstract:** Silkworm *Bombyx mori* L. is prone to be attacked by pathogen and more notably *Beauveria bassiana* L. Bed disinfectant Kavach was inoculated in different dosages (0.2%-1.6%) to IV and V instar silkworms of both bivoltine and cross breeds. Potency of disinfectant was assessed for the parameters such as survivability, larval duration, physiological, chemo and bio-assay tests. It was revealed that, bivoltine (NB<sub>4</sub>D<sub>2</sub>) silkworms were highly susceptible to diseases compared to cross breeds (PMxNB<sub>4</sub>D<sub>2</sub>). In bivoltine silkworms, survivability was found to be 61.15% at 1.6% of Kavach, when dusted twice during IV and V instar and crossbred silkworms exhibited better resistance of 63.10% with the same treatments. Kavach treated silkworms showed decreased larval duration compared to control worms. Crossbreed silkworms were capable of maintaining high level of soluble proteins in spite of infection on 3<sup>rd</sup> day (17.40%), 4<sup>th</sup> day (20.50%) and 5<sup>th</sup> day (21.55%) whereas in bivoltine silkworms soluble protein level was brought down on 3<sup>rd</sup> day (19.30%), 4<sup>th</sup> day (22.40%) and 5<sup>th</sup> day (23.40%). Total soluble sugars varied from third day till fifth day in both the races. Kavach dusted twice at 1% proved to be very useful in the improvement of various commercial cocoons characters.

**Keywords:** *Beauveria bassiana*, *Bombyx mori*, Commercial characters, Kavach, Silkworm

### INTRODUCTION

Sericulture is an applied branch of science, multi-disciplinary in nature and is rural oriented, practiced more prominently in the tropical countries (Singhvi *et al.*, 1996; Seidavi *et al.*, 2005; Dandin, 2008; Nagaraju, 2008; Ahmed and Rajan, 2011; Anitha, 2011). Mulberry silkworm *B. mori* is of great economic importance for many silk producing countries in the world (Krishnaswami *et al.*, 1992). Karnataka is the hub of sericulture activities, pioneer in silk production and occupies a prime position in India's total silk production. Karnataka is blessed with salubrious environmental conditions and farmers hailing from different strata of society have been practicing sericulture throughout the year since time immemorial and this makes it highly susceptible to pathogens and hence occurrence of diseases is the rule of nature (Samson *et al.*, 1998).

Silkworm is prone to attacked by several diseases due to biotic and abiotic factors with considerable toll. Four silkworm diseases namely Grasserie (viral), Flacherie (bacterial), Muscardine (fungal) and Pebrine (protozoan) are common in China and India. These diseases caused heavy damage to silkworm crops in the past and in India more than 40 percent of crop

losses still occur due to these diseases (Veeranna, 1999). Due to domestication of silkworms since several decades, silkworms are most susceptible to different diseases caused by various pathogens. White muscardine is an infectious disease caused by pathogenic fungi *B. bassiana* in all seasons and regions in India (Chandrasekaran and Nataraju, 2008). The intensity of the disease is very high in winter and rainy seasons. Several investigators have made an attempt to prevent the occurrence of this disease by using different concentrations of fungicide (Subba Rao *et al.*, 1992; Baig *et al.*, 1993; Isaiarasu *et al.*, 2011). Keeping the above in view, main objective of the present investigation is to prevent/control *Beauveria bassiana*, increase silkworm cocoon yield and to improve the commercial characters of cocoon using Kavach as a fungicide.

### MATERIALS AND METHODS

**Collection, isolation and culture of fungus:** Fungal spores collected from white muscardine infected silkworm cadavers and Photo Dextrose Agar (PDA) is used as media (Pant *et al.*, 2002) for fungal spores culture. Efficiency of the fungicide *invitro* will be

tested against *B. bassiana* at different concentrations viz., 0.05%, 0.125% and 0.25% (Chinnaswamy and Devaiah, 1986). Whatman No. 40 filter paper discs were dipped in the above concentration of fungicides and placed in petri plates containing spore load of one lakh spore/cm<sup>3</sup> of the PDA. Two discs per plate were placed equidistant, replicated thrice. Zones of inhibition were measured after six days. To test the rearing efficiency, two silkworm races namely, cross breed (PMxNB<sub>4</sub>D<sub>2</sub>) and bivoltine (NB<sub>4</sub>D<sub>2</sub>) were used. Disease free layings obtained from National Silkworm Seed Project (NSSP), Bangalore were used. Rearing experiments were conducted by adopting appropriate cellular rearing techniques (Krishnaswami *et al.*, 1970; Krishnaswami, 1978; 1990; Benchamin and Nagaraj, 1987). Spore suspension was prepared and spore counts were done by haemocytometer. Silkworms out of third moult were inoculated with spores through topical application using automiser (Patil, 1993; Patil *et al.*, 2001). Different concentrations of Kavach ranging from 0.2%-3.2% were prepared for the topical application of silkworm with Kaolin as a base material. Kavach applied at concentration of 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.2%, 1.4% and 1.6%. Different concentrations of Kavach were applied topically on inoculated worms at the following frequencies.

- Dusting once in an instar during IV and V instars.
- Dusting twice in an instar during IV and V instars with a duration of 24 hours.
- Dusting thrice in an instar during IV and V instars with a duration of 24 hours.

In each case, the first dusting was carried out after six hours of inoculation. Silkworm reared without any inoculation and without any topical application of Kavach constituted control I and silkworm inoculated without any topical application of Kavach constituted control II. As 100% mortality was recorded in control batch II, first one was considered as control. Physiological parameters viz., food ingested, efficiency percentage of conversion of digested food and growth rate were calculated by adopting standard procedure (Waldbauer, 1968). Total soluble proteins, total soluble sugars in haemolymph were estimated according to standard procedures. Proteins were measured by Lowry *et al.* (1951) method using Folin-phenol reagent. Total sugars were estimated by Plummer (1971) method using anthrone reagent. Larval duration and various commercial characteristics of cocoon were calculated using standard procedures (Sonwalkar, 1991).

**Data analysis:** One-way analysis of variance was used to test the significance of differences between mean values of independent observations. Comparisons were performed with WINSTAT statistical package to find significant differences between the different treatments.

## RESULTS AND DISCUSSION

Results revealed the effect of topical application of Kavach on survivability, larval duration and commercial characteristics of bivoltine and cross breed silkworms inoculated with *B. bassiana* dusted (once, twice and thrice) during IV and V instars. Effect of fungicide Kavach at different concentrations on inoculated bivoltine and crossbreed silkworms resulted in lowering the incidence of infection at different rates. Treatment of larvae with Kavach during IV and V instars resulted in lowest fungal infection in both breeds with the given spore load. It is noticed that bivoltine silkworms had higher infection than crossbreeds.

**Survivability:** Different dosages of fungicide revealed difference in survival percentage of silkworms with the increase in concentration of fungicide in both bivoltine and crossbreed silkworm races. Least mortality was noticed at higher concentrations of fungicide and highest mortality at lower concentration of fungicide. Survivability was 61.15% at 1.6% when Kavach dusted twice during IV and V instars in bivoltine silkworms and in crossbreed silkworms it was 63.10% with same treatment conditions (Tables 1 and 2). Chinnaswamy (1983) and Anitha Peter (1989) have reported that, bivoltine races (NB<sub>4</sub>D<sub>2</sub>, NB<sub>7</sub>, NB<sub>18</sub>, KA) are highly susceptible compared to multivoltine (PM and HM). Soaf *et al.* (1994) have studied the comparative efficacy of a few fungicides and showed that Captan at 3% treatment was most effective followed by Foltaf 3%, which showed a mortality of 2.22% and 2.44% respectively due to infection of *B. bassiana*.

**Larval duration:** Larval stage is an active feeding part in the life cycle of *B. mori* and larval age considerably varied between control and fungicide treated worms (Figs.1 and 2). Kavach treated worms showed decreased larval duration (4hours to 24hours) compared to control worms. Silkworms maintained in control groups behaved normally and they were active and the movements are well coordinated without any disturbances during feeding. But, silkworms treated with lethal dosages of fungicide exhibited contractures, causing worms to roll over one another lose structure of skin and appearance of black spots on the body surface, omitting of gastric fluid and irregular excretion. Present results were in conformity with the findings of Devaiah *et al.* (1983), Subba Rao *et al.* (1992) and Baig *et al.* (1993). Fungicides inhibit acetyl cholinesterase activity in animals. Inhibition of acetyl cholinesterase in central nervous system blocks the transfer of impulse synapse has been set to be the major factor in death of animals (Soaf *et al.*, 1994). Pant *et al.* (2002) opined that, lepidopteron larvae had more or less continuous feeders shortening of feed periods in larvae and decrease in final body weight.

**Biochemical analysis:** Due to infection of *B. bassiana* there is decline in haemolymph pH in bivoltine

**Table 1.** Larval survivability, larval duration and commercial characteristics of bivoltine silkworms dusted twice with Kavach.

Dosage (%)	Larval survivability (%)	Larval duration days-hrs)	Average larval weight (g)	Average cocoon weight (g)	Average shell weight (g)	Shell weight (%)	Filament length (m)	Denier	Renditta
0.2	17.31	28-18	4.04	1.70	0.240	14.33	861	2.15	9.67
0.4	21.25	28-14	4.00	1.70	0.256	15.04	932	2.18	8.85
0.6	29.35	28-12	4.13	1.81	0.281	15.49	1033	2.27	8.67
0.8	37.40	28-10	4.62	1.90	0.314	16.19	1165	2.19	7.87
1.0	46.50	28-08	5.42	2.06	0.338	16.65	1282	2.37	7.29
1.2	53.65	28-04	4.85	1.91	0.318	16.47	1181	2.27	7.68
1.4	56.25	28-00	4.31	1.85	0.293	15.76	1070	2.07	8.34
1.6	61.15	27-22	4.00	1.63	0.245	14.97	865	2.19	9.21
Control I	86.00	28-22	5.47	2.07	0.342	16.79	1287	2.38	7.20
Control II	--	--	--	--	--	--	--	--	--
SE(±)	3.12	0.285	0.159	0.043	0.013	0.294	5.315	0.090	0.289
CD @ 5%	7.04	0.612	0.414	0.143	0.035	0.766	53.177	0.235	0.754

**Table 2.** Larval survivability, larval duration and commercial characteristics of cross breed silkworms dusted twice with Kavach.

Dosage (%)	Larval survivability (%)	Larval duration days-hrs)	Average larval weight (g)	Average cocoon weight (g)	Average shell weight (g)	Shell weight (%)	Filament length (m)	Denier	Renditta
0.2	20.15	30;07	2.91	1.20	0.197	15.75	695	1.97	10.77
0.4	25.64	30;04	3.03	1.25	0.199	15.87	756	1.90	10.06
0.6	31.54	30;00	3.31	1.36	0.219	16.04	803	1.90	10.32
0.8	39.30	29;21	3.95	1.45	0.242	16.25	897	2.14	9.71
1.0	47.50	29;17	4.37	1.59	0.276	16.94	960	2.53	8.80
1.2	51.15	29;14	3.92	1.48	0.237	15.99	879	2.16	9.82
1.4	57.43	29;10	3.54	1.44	0.225	15.65	825	1.96	10.70
1.6	63.10	29;05	3.37	1.39	0.215	15.51	763	1.88	10.65
Control I	88.00	30;10	4.38	1.60	0.280	16.97	970	2.55	8.75
Control II	--	--	--	--	--	--	--	--	--
SE(±)	2.85	4.04	0.141	0.036	0.007	0.147	9.865	0.094	0.445
CD @ 5%	6.87	0.467	0.368	0.091	0.018	0.384	24.041	0.246	1.161

**Table 3.** pH, soluble proteins and soluble sugars in the haemolymph of bivoltine silkworms inoculated and dusted twice with Kavach.

Dosage	pH			Soluble proteins			Soluble sugars		
	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
0.4	7.17	6.74	6.39	18.85	20.57	21.16	32.95	16.40	17.30
1.0	7.12	6.80	6.47	19.15	22.20	22.74	33.80	15.60	16.95
1.6	7.04	6.86	6.55	19.30	22.40	23.40	34.25	14.00	16.30
Control	6.95	6.78	6.67	19.70	23.35	24.65	35.90	13.30	15.10
SE(±)	0.110	0.106	0.102	0.299	0.313	0.299	0.533	0.377	0.451
CD@5%	0.289	0.278	0.267	0.784	0.820	0.782	1.395	0.985	1.180

**Table 4.** pH, soluble proteins and soluble sugars in the haemolymph of cross breed silkworms inoculated and dusted twice with Kavach.

Dosage	pH			Soluble proteins			Soluble sugars		
	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
0.4	7.12	6.44	6.20	16.55	19.10	19.70	29.80	16.00	16.60
1.0	7.09	6.50	6.25	17.25	20.15	20.35	30.60	16.60	15.85
1.6	7.04	6.54	6.31	17.40	20.50	21.55	31.60	14.00	15.05
Control	6.85	6.69	6.50	17.75	21.60	22.65	32.55	13.20	14.10
SE(±)	0.105	0.101	0.098	0.268	0.289	0.268	0.502	0.357	0.428
CD@5%	0.275	0.264	0.254	0.701	0.755	0.700	1.314	0.934	1.119

**Table 5.** Food ingestion of IV and V instar bivoltine (NB<sub>4</sub>D<sub>2</sub>) silkworms treated with Kavach dusted twice.

Dosage	IV instar			V instar			
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
0.4	5.60	5.90	6.95	6.50	11.70	28.70	23.45
1.0	5.85	6.25	7.35	7.45	12.40	30.15	24.95
1.6	5.30	5.60	6.45	6.20	11.50	28.35	23.00
Control	6.20	6.50	7.55	7.85	12.50	30.35	24.90
SE(±)	0.092	0.104	0.116	0.119	0.191	0.470	0.451
CD @ 5%	0.241	0.273	0.304	0.312	0.501	1.229	1.180

**Table 6.** Food conversion of IV and V instar bivoltine (NB<sub>4</sub>D<sub>2</sub>) silkworms treated with Kavach dusted twice.

Dosage	IV instar			V instar			
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
0.4	4.45	4.90	5.25	4.55	8.85	17.60	16.65
1.0	5.70	6.00	6.25	5.70	9.95	22.60	19.85
1.6	4.20	4.60	5.10	4.40	8.70	17.25	17.00
Control	5.95	6.25	6.70	5.85	10.40	22.75	20.30
SE(±)	0.087	0.071	0.095	0.073	0.156	0.305	0.295
CD @ 5%	0.227	0.185	0.249	0.192	0.407	0.798	0.772

(NB<sub>4</sub>D<sub>2</sub>) and crossbreed (PMxNB<sub>4</sub>D<sub>2</sub>). In both the races, haemolymph pH reached an acidic state indicating infection by fungus which sets in academia. Further, increased accumulation of metabolites in haemolymph of infected larvae may contribute some extent for declining of pH (Kusunoki and Watanabe, 1982). Results indicate that, due to infection by *B. bassiana* total soluble protein content is affected considerably and level was brought down in bivoltine silkworms (NB<sub>4</sub>D<sub>2</sub>) whereas crossbreed silkworms

(PMxNB<sub>4</sub>D<sub>2</sub>) are capable of maintaining high level of soluble protein contents in spite of infection. It is concluded that, infection by *B. bassiana* induces hypoproteinemia condition in infected larval haemolymph. Reduction in haemolymph protein may be a consequence of metabolism of protein and amino acid of larval haemolymph by developing pathogen and therefore haemolymph is diluted as far as protein content is concerned. Secondly, reduction in haemolymph is due to starvation (Tables 3 and 4). In



**Fig. 1.** Healthy and infected IV instar Cross Breed ( $PMxNB_4D_2$ ) silkworms.



**Fig. 2.** Healthy and infected IV instar Bivoltine ( $NB_4D_2$ ) silkworms.

**Table 7.** Efficiency of conversion of IV and V instar bivoltine ( $NB_4D_2$ ) silkworms treated with Kavach dusted twice.

Dosage	IV instar			V instar			
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
0.4	22.80	27.00	33.90	30.60	41.30	74.10	41.95
1.0	24.15	29.10	35.65	33.10	43.10	49.10	44.00
1.6	21.90	27.30	32.15	29.90	41.00	46.30	41.25
Control	24.60	29.20	36.00	33.25	43.50	49.20	44.40
SE(±)	0.364	0.439	0.538	0.807	0.659	0.748	0.669
CD @ 5%	0.953	1.148	1.406	2.109	1.723	1.955	1.752

**Table 8.** Growth rate of IV and V instar bivoltine ( $NB_4D_2$ ) silkworms treated with Kavach dusted twice.

Dosage	IV instar			V instar			
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
0.4	29.10	32.80	36.91	26.90	33.25	54.00	44.85
1.0	31.00	34.30	38.90	29.00	35.00	57.00	45.70
1.6	28.40	31.80	35.90	25.95	33.10	54.08	43.15
Control	31.50	34.50	39.05	29.50	35.60	57.60	47.00
SE(±)	0.468	0.520	0.588	0.590	0.534	0.683	0.631
CD @ 5%	1.224	1.361	1.538	1.544	1.397	1.786	1.649

both the races, total soluble sugar increases linearly from third day till fifth day in infected races. In control worms, sugar level considerably decreased in healthy larvae from third day till fifth day. Analysis of variation is increasing in total soluble sugar is due to degradation of carbohydrate by developing pathogen and end product of carbohydrate metabolism are liberated as per the report of Raghavaiah and Jayaramaiah (1989).

**Physiology of silkworm:** Kavach treated silkworm races, showed variable quantity of food ingested during IV and V instars. Kavach (dusted twice) treated bivoltine silkworm larvae indicated maximum food ingestion on third day (7.35g) of IV instar and fourth day (30.15g) of V instar at 1.0%. Noticeable decreases in food intake at lethal doses as well as a moderate increase in food intake at sub-lethal doses were observed. It is clear from the results that, sub-lethal doses of fungicide seem to stimulate larvae to consume more food. Lower doses of fungicide may also act as

attractants to silkworms for consuming more food. At lethal doses, same fungicide may have acted as repellent, resulting in less food consumption. At low concentrations, fungicide is expected to be extracted in faeces and urine within 24hours, where as at higher concentrations, it gets metabolized and joins main stream of haemolymph (Kusunoki and Watanabe, 1982). Bivoltine silkworm larvae in both IV and V instars treated with Kavach at 1% showed maximum food conversion (22.60%), efficiency of conversion of digested food was found to be highest (49.10%) and maximum growth rate (57.00%) on fourth day of V instar at 1% (Tables 5-8). Present findings are in conformity with Ueda and Lizuka (1962) who demonstrated that, silkworms were more sensitive to environmental conditions and fungicides. They considered rate of ingestion and conversion efficiency as better parameters of metabolic rates. Secretion of digestive enzyme is dependent on quantum of food ingested into body because the presence of food may

act as a stimulus for secretion of enzymes. Study of the pattern of distribution of food in digestive tract of *B. mori* has led to conclusion that, silkworms feed continuously. Larval growth percentage depends on nutrients absorbed by intestine, consumed directly for silkworms life and other parts are presented in larval body to increase weight and body volume, thus larval growth takes place. Kavach dusted twice at 1% proved to be very useful and various commercial characters like cocoon weight, shell weight, shell percentage, filament length, renditta and denier showed beneficial results over the control worms (Tables 1 and 2). Isaiarasu *et al.* (2011) revealed that, aqueous and alcoholic crude extracts of three herbs such as *A. indica*, *O. sanctum* and *T. procumbens* are effective against microbes causing flacherie and muscardine diseases in silkworm. Alcoholic extracts were generally more effective than aqueous extracts that helps in disease resistance and silk production. Kumari *et al.* (2011) aimed at investigation effect of dichloromethane and methanol (1:1) extract of seaweed brown algae, *T. conoides* for its antifungal activity against *B. bassiana*. Three different concentrations of this algal extract were tested against *B. bassiana* infected silkworm larvae and effective concentration was evaluated between 1000 to 1500 µg mL<sup>-1</sup> of the algal extracts. It was established that, with the application of these algal extract about 75%-85% of larval mortality due to *B. bassiana* infection was controlled without affecting other qualitative and quantitative traits. Chavan *et al.* (2011) reported that, use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Aqueous extracts of *A. mexicana*, *T. arjuna*, *S. cumini* and *A. squamosa* were tested for antifungal activity *in vitro* and study revealed that, aqueous extract of *A. mexicana* shows effective results on *B. bassiana* infected *B. mori* larvae as compared to other botanicals. However it is clear that, aqueous plant extracts showed decreased mortality with effective rate of rearing. Kumar *et al.* (2011) demonstrated that, four systemic fungicides Bavistin, Bayleton, Dithane M<sub>45</sub> and Thiram were tested for efficacy to control white muscardine in *A. mylitta* D. Bavistin and Dithane M<sub>45</sub> with 1%-2%, Bayleton 0.15%-2% and Thiram 2% concentrations were found more effective among tested systemic fungicides in suppressing muscardine in tasar silkworm.

## Conclusion

Present study concluded that, application of Kavach a potent fungicide encounter the pathogen by forming a thin layer on silkworm *B. mori* body and deactivates the function of *B. bassiana* fungus. It has been observed from the investigation that, moderate dosage (1%) of Kavach could prevent worms from the incidence of muscardine. Food ingestion, digestion and conversion rate is greatly enhanced at 1% Kavach

application. Cross breed silkworms showed better resistance compared to bivoltine silkworms and dusting of Kavach twice during IV and V instar exhibited better commercial characters in both the silkworm races.

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