

## SCREENING AND IDENTIFICATION OF SILKWORM BREEDS OF *BOMBYX MORI* L. FOR THEIR RESISTANT AND SUSCEPTIBLE AGAINST WHITE MUSCARDINE

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**ABSTRACT:** Silkworm breeds are highly unpredictable and pathogens are the main cause of diseases in silkworm rearing, diseases are more prevalent in winter and rainy seasons. The major silkworm diseases in India are grasserie, flacherie, muscardine and pebrine. Susceptibility of silkworm breeds mainly due to influence of environmental and nutritional factors. The disease resistance mechanism of a genotype has immense value, since the disease resistance results in stability of crop performance and increase in productivity. Fungal diseases are recognized as muscardine or mycoses. Silkworm attacked by more than dozen genera of fungi and white muscardine caused by entomopathogenic fungi of *Beauveria bassiana*. In Karnataka, white muscardine is named as Sunnakaddi or Sunnakattu roga and Chuna-Kete in West Bengal. The Italian names Calcino, Agostino Bassi, Italian entomologist discover the name of muscardine in 1835. White muscardine disease is rampant in all sericultural areas during winter and rainy seasons, as the humidity increases with the decreases in temperature there is possibility in increase in the spread of muscardine disease in silkworm rearing. The present study was conducted systematically to screen the multivoltine and bivoltine silkworm breeds of *Bombyx mori* L. and higher dose of conidial *B. bassiana* concentration  $1 \times 10^7$  suspension was used for identify the resistant and susceptible against white muscardine disease. Out of 27 silkworm breeds, the multivoltine breed 2000H and bivoltine CSR6 exhibited relatively more resistant and susceptible breeds. The detail procedure and screening techniques, results and discussion have been dealt in the paper.

**KEY WORDS:** *B. bassiana*, *Bombyx mori* L., humidity, multivoltine and bivoltine breeds, temperature, white muscardine disease.

The silkworm, *Bombyx mori* L. is a delicate and sensitive, completely domesticated insect animal and classic model organisms for lepidoptera. Silkworm rearing is a vital aspect, which in turn decides the quality of cocoon production. The success of cocoon production depends on disease management. Diseases are prevalent throughout the year incidence exhibit differs between winter, rainy seasons and humidity is considered as one of the crucial factor. Silkworm *Bombyx mori* L. is susceptible to various diseases caused by different microbial pathogens such as Bacteria, Fungus, Protozoan and Virus. The environmental factors of temperature and humidity largely determine the growth of the silkworm and success of a rearing reported by (Kenten, 1955; Tazima, 1978). Identification of resistant silkworm breed against particular disease plays major task, silkworm breeds show difference in their susceptibility to infectious various pathogen and pathogenic sensitivity in silkworm varies greatly depending upon larval age, moulting, metamorphosis and rearing condition. But fundamental resistance is determined by genetically. India has a long traditional and experience in the production and utilization of silk. The production of raw silk is about 15, 236 MT (Anonymus, 1999a), which earns a foreign exchange of Rs. 1,086 corers (Anonymus, 1999b). The annual crop losses due to silkworm

diseases are to an extent of 35-40%, out of 5 to 6 cocoon crops and every year two crops are lost completely or partially due to silkworm diseases (Patil et al., 1993). Silkworm races have long been observed to be susceptible to different diseases caused by various micro-organisms (Yokoyama, 1962; Steinhaus, 1963). All the known silkworm diseases are endemic and periodically one disease or the other occurs on an epizootic scale (Samson, 1987). Muscardine is the most virulent and contagious disease caused by fungi, it prevails in all sericultural countries (Steinhaus, 1949). In 1974 and 1975 Karnataka an estimated that, cocoon crop loss worth Rs. 3.5 crores was white muscardine disease (Anonymus, 1975). The severity of the disease is very high under conditions of incomplete disinfections and hygiene (Prasad, 1999).

White muscardine caused by *Beauveria bassiana* (Bals.) Vuille. is the most devastating silkworm disease. The Karnataka, which is the major silk producing state in the country and climatic condition in the tropics, plays a congenial role for the incidence and easy spread of fungal diseases. The spread of muscardine is due to high humidity and low temperature (Samson et al., 1990; Anonymus, 1992). Exploitation of the resistant/tolerant silkworm breeds towards different diseases causing pathogens is a better option for managing the crop loss due to diseases. Certain stress factors have been identified to be most crucial influencing the disease development in silkworm rearing. (Nataraju & Datta, 1995) worked on muscardine in silkworm and reported that, it could be prevented by reducing the humidity in silkworm rearing bed and use a quality bed disinfectant.

Silkworms have adapted to a temperature of  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and humidity  $75 \pm 1^{\circ}\text{C}$  and any increase or decrease in temperature and humidity causes susceptibility in silkworm. (Steinhaus, 1958; Watanabe, 1964) reported that, temperature acting as one of the stress factors. Crowding exerts a stress on the members of the population and also influences the incidence of the diseases. Success of silkworm crop is determined by nutrient quality and its maturity of mulberry leaf and generally bivoltine silkworms are comparatively more susceptible to multivoltine breeds. There are many reports available in literature regarding susceptibility and tolerance of different races to various diseases of silkworm (Tanada, 1967; Liu Shi Xian, 1984; Samson, 1987; Chinnaswamy; Devaiah, 1984; Nataraju, 1995; Sudhakara et al., 2008; Chandrasekharan & Nataraju, 2008). In view of the above, the present attempt has been made to understand the screening and identification of resistance and susceptibility status of silkworm breeds against fungal white muscardine disease.

## MATERIAL AND METHODS

The present study was undertaken to screen and identify the resistance and susceptibility status of different silkworm breeds against white muscardine disease of *Beauveria bassiana*. Twenty seven available productive silkworm breeds of *Bombyx mori* L. were received from the germ plasm bank of multivoltine and bivoltine breeding laboratory, CSRTI, Mysore, and were screened three times. Out of twenty seven silkworm breeds, 15 multivoltine silkworm breeds namely, ND5, NDV6, NP1, L14, 2000H, 96A, Diazo, MAD, BL24, BL69, PM, AGL3, AGL5, 96E, ND7. And 12 bivoltine breeds namely, CSR6, CSR53, CSR51, CSR5, CSR2, CSR16, CSR19, CSR50, CSR27, CS26, CS52 and CSR202(SL) were utilized for conducting experiments.

### Preparation of PDA and inoculum of *Beauveria bassiana* dilutions:

Potato Dextrose Agar medium was used for isolation of fungal pathogen from silkworm mummified dead larva of muscardine. Fungus was cultured and

purified by monohyphal tip method, under aseptic condition of Laminar Air Flow Chamber and burner was flamed till completion of whole experiments. The inoculum was prepared by a fresh conidium of fungus, which was harvested by scrapping the surface of pure PDA 14 days old culture in sterilized distilled water and drop of Tween-20. The conidial suspension was prepared and stock was again diluted in sterilized distilled water to get required high concentration of  $1 \times 10^7$  dose. The stock inoculum was quantified by following standard procedure of Neubauer haemocytometer and counting the conidia followed by (Cantwell, 1973). Silkworm layings of different breeds were collected, incubated and hatched larvae were brushed and standard rearing was followed up to 2<sup>nd</sup> moult. Experimental inoculation was done on newly ecdysed II<sup>nd</sup> moult out of 1st day 3<sup>rd</sup> instar larvae. The suspension/concentration of infective dose  $1 \times 10^7$  conidial stock/1ml (10000l)/100 larvae were inoculated per cutaneous, sprayed on the body of larvae and two feeding was maintained/day with mulberry leaves, wet paper folds kept inside the rearing trays for increase the humidity in rearing bed in the treatment/inoculated batches. And larvae were reared under temperature at  $25^\circ\text{C} \pm 1^\circ\text{C}$  and relative high humidity 90 to 95%. Three replicates of 100 larvae were maintained separately in each breed. Silkworm breeds were reared in plastic trays with blue polythine sheets. The rearing was continued up to 10 days after inoculation. Progressive mortality due to white muscardine disease was observed in silkworm larvae, mortality and survival rate recorded daily in both multivoltine and bivoltine breeds up to 10 days.

The cumulative mortality and survival rate % from the data obtained in three consecutive trials of the screening data indicates the 15 multivoltine and 12 bivoltine silkworm breeds in response to fungi pathogen of *B.bassiana* have been given in the Table 1. and 2.

The mortality was calculated as per the formula given below.

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae in batch}}{\text{Total number of larvae in the batch}} \times 100$$

## RESULTS AND DISCUSSION

The study of resistant and susceptibility status of different silkworm breeds results indicated that, all the tested multi and bivoltine breeds are found to be differs in their susceptible. Most of the breeds are susceptible to disease as compared to resistant. Only two breeds exhibited resistant against muscardine and 52 to 54% mortality values was recorded in multivoltine breeds of 2000H and 96A. As well as higher mortality values of 69 to 71% was noticed in four bivoltine breeds. Usually mortality rate varies in different breeds based on their genetic variability and susceptibility varies according to stage of the silkworm. Newly moultout /ecdysed larvae are more susceptible to diseases was noticed, the infection was observed from the 2<sup>nd</sup> day and rate of mortality started after 5<sup>th</sup> day of inoculation. Maximum mortality was found in 5<sup>th</sup> and 6<sup>th</sup> day and later gradually decreases. Survival batch was continued up to cocooning. 100% mortality was noticed at pupal stage. The percentage of larval mortality and survival data were recorded in silkworm breeds. Most of the breeds showed difference in their mortality and survival.

The data was statically pooled for identifying the resistant and susceptible breed based on their mortality and survival percentage. Among the 15 multivoltine silkworm breeds, 2000H and out of 12 bivoltine breeds, CSR6 were

found to be more resistant and susceptible against white muscardine disease and scored average mortality rate 52.3 and 70.6 % respectively. However, maximum survival rate recorded 46.7 to 47.7% in between 96A and 2000H, the rate was gradually decreased 38.7 to 33.4 % as compared to other multivoltine breeds. As well as minimum survival 29.4% was noticed in CSR6 and it ranged up to 37.7% in bivoltine breeds.

The variation in the susceptibility in silkworm breeds to *Beauveria bassiana* is genetically determined by two major genes responsible for muscardine infection in silkworm. One is 'mus' gene located in the 11<sup>th</sup> chromosome and the other is 'cal' gene located in the 7<sup>th</sup> chromosome (Shimada, 1999). Similarly, this muscardine genes susceptibility may also be related to several polygenes. Early instars/young age silkworms are more susceptible to infection by microbial pathogen and it decreased with larval ageing from first to fourth instars (Aruga & Watanabe, 1964; Kobaara et al., 1967).

In India most of the farmers are unable to follow complete disease management practices due to their poor socio economical problems. Under these conditions, resistance breeds are better options. Although the disease resistance for fungi *B. bassiana* of white muscardine is controlled by polygene. The susceptibility of silkworm to different pathogens is a polygenic character (Aratake, 1973b). The susceptibility to muscardine disease varies from different silkworm breeds (Aratake, 1961). The genetic materials having strong disease resistance can form the substantial basis for breeding disease resistance. The genotypes with disease resistance gene always have better chance to survive. The resistant and susceptibility percentage ratio was calculated between multivoltine and bivoltine silkworm breeds. Three trials average % values of mortality and survival rate given in the Table 1. and 2. And Indo-Stat comparison of SD. data at 5 % level has been presented in the Table 3. and 4.

Success in sericulture industry involves two significant aspects namely prevention to disease and breeding of high yielding and disease tolerant silkworm breeds. Available genetic stock of silkworm breeds were screened systematically for their resistant and susceptibility to white muscardine. The fungal disease is influenced by several environmental conditions, such as temperature, relative humidity and light (Alves, 1988).

Identifying resistant breed will provide the genetic base for further improving productivity of high yielding genotype through various breeding programme. Screening of silkworm breed against particular pathogen possibility of covering their isolation and purification infectivity and evaluation. Screening of genotype to various diseases and identification of resistant races for various diseases has been achieved in both China and Japan (Xian, 1984).

In 1987 (Kuberappa and Jayaramaiah) have reported that, high humidity and low temperature are most congenial for the growth of *Beauveria bassiana*, which cause white muscardine disease in silkworm. (Fargues et al., 1997) stated that, *B. bassiana* can develop with in a wide range of temperatures, but in rearing bed temperature may not be playing a crucial role for diseases. Hat and MacLeod in 1955, identified that, germination of *B. bassiana* conidial development at relative humidities above 94 % . Developing a disease resistant breeds and their genetically determined difference in silkworm has been studied by (Tanada & Kaya, 1993). In 1997, Sen et al., reported that, to develop disease resistant/tolerant breeds, the genetic mechanism underlying it should be properly understood so as to effectively transgress the tolerance in to productive breeds. (Watanabe, 1965) studied the disease resistance regard to CPV, based on genetics and IFV by (Funada, 1968). Also DNA by (Watanabe & Maeda, 1981; Eguchi et al., 1986). In

India breeding for disease resistance has received great attention. Understanding the principle and its techniques of screening of silkworm breeds against particular pathogens will form the basis and benefit to the sericulturist as well as farmer.

The study revealed that, ten days mortality data was ranged from 52.3 to 66.6% in multivoltine breeds. Similarly, it was 62.3 to 70.6% found in bivoltine breeds. Larvae suffering from white muscardine do not show any external difference to healthy ones at the primary stage but as the day progressed all the breeds succumbed to the infection. Mortality was started on 5<sup>th</sup> day after inoculation, and mortality rate was recorded another 5 days for identify the relatively resistance and susceptible to white muscardine. Most of the silkworm breeds are differ in their susceptibility and only two breeds are showed more resistant level towards white muscardine. Results clearly showed silkworms are sensitive, and there may be different genetic mechanism responsible for their resistant and susceptible to fungi *Beauveria bassiana* in silkworm breeds. High mortality and low survival may be due to low level of defense mechanism, this makes silkworm weak and more susceptible to diseases in silkworm rearing. But over all results confirmed that, multivoltine 2000H and bivoltine CSR6 breeds were identified as relatively resistant and most susceptible. The Indo-Stat comparison statement of Anova L.S.D. mortality data analysis at 5 % value was revealed that, multivoltine breeds are comparatively higher significant than bivoltine breeds. Resistant breed could be utilized for the development of diseases resistant breed for developing hybrids. Further work will be carried out studies on biochemical, molecular and histopathological aspects of the resistant and susceptible breed to understand the mechanism of muscardine disease in silkworm.

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Table 1. Screening multivoltine silkworm breeds of *Bombyx mori*, L. (dose  $1 \times 10^7$  /ml/100 larvae).

Sl No	Multivoltine Breeds	Total No. of larvae treated in each trail	Average % cumulative mortality due to white muscardine			3trials total average Mortality %	3trials total average Survival %
			1 <sup>st</sup> trail	2 <sup>nd</sup> trail	3 <sup>rd</sup> trial		
1	ND5	300	60	63	67	63.3	36.7
2	NDV6	300	65	60	68	64.3	35.7
3	NP1	300	64	67	69	66.6	33.4
4	L14	300	64	65	60	63	37
5	2000H	300	52	50	55	52.3 **	47.7 **
6	96A	300	51	55	54	53.3	46.7
7	Diazo	300	65	60	67	64	36
8	MAD	300	64	68	63	65	35
9	BL24	300	65	67	64	65.3	34.7
10	BL69	300	63	64	61	62.6	37.4
11	PM	300	62	63	60	61.6	38.4
12	AGL3	300	60	62	65	62.3	37.7
13	AGL5	300	63	66	61	63.3	36.7
14	96E	300	62	65	64	63.6	36.4
15	ND7	300	59	64	61	61.3	38.7

\*\* Indicated highly resistant breed.

Table 2. Screening bivoltine silkworm breeds of *Bombyx mori*, L. (dose  $1 \times 10^7$ /ml/100 larvae).

Sl No	Bivoltine Breeds	Total No. of larvae treated in each trail	Average%ocumulative mortality due to white muscardine			3 trails total average Mortality %	3 trails total average Survival % rate
			1 <sup>st</sup> trail	2 <sup>nd</sup> trail	3 <sup>rd</sup> trail		
1	CSR6	300	70	72	70	70.6*	29.4*
2	CSR53	300	59	64	63	62.3	37.7
3	CSR51	300	68	70	71	69.6	30.4
4	CSR5	300	68	71	69	69.3	30.7
5	CSR2	300	66	68	72	68.6	31.4
6	CSR16	300	60	65	71	65.3	34.7
7	CSR19	300	65	68	70	67.6	32.4
8	CSR50	300	72	65	69	68.6	31.4
9	CSR27	300	71	70	68	69.6	30.4
10	CSR26	300	64	67	69	66.6	33.4
11	CSR52	300	61	64	67	64	36
12	CSR202 (SL)	300	68	65	63	65.3	34.7

\* Indicates highly susceptible breed.

Table 3. Mean mortality in Multivoltine breeds SD, SE and CV comparisons at 5 % level.

Sl No	Multivoltine Breeds	Mean $\pm$ SD	S.E	C.V
1	ND5	63.33 $\pm$ 3.51	2.028	3.201
2	NDV6	64.33 $\pm$ 4.04	2.333	3.627
3	NP1	66.67 $\pm$ 2.52	1.453	2.179
4	L14	63.00 $\pm$ 2.65	1.528	2.425
5	2000H	52.33 $\pm$ 2.52	1.453	2.776
6	96A	53.33 $\pm$ 2.08	1.202	2.253
7	Diazo	64.00 $\pm$ 3.61	2.082	3.253
8	MAD	65.00 $\pm$ 2.65	1.528	2.350
9	BL24	65.33 $\pm$ 1.53	0.882	1.350
10	BL69	62.67 $\pm$ 1.53	0.882	1.407
11	PM	61.67 $\pm$ 1.53	0.882	1.430
12	AGL3	62.33 $\pm$ 2.52	1.453	2.331
13	AGL5	63.33 $\pm$ 2.52	1.453	2.294
14	96E	63.67 $\pm$ 1.53	0.882	1.385
15	ND7	61.33 $\pm$ 2.52	1.453	2.369

Table 4. Mean mortality in Bivoltine breeds SD, SE and CV comparisons at 5 % level.

Sl No	Bivoltine breeds	Mean $\pm$ SD	S.E	C.V
1	CSR6	70.67 $\pm$ 1.16	0.667	0.943
2	CSR53	62.30 $\pm$ 2.65	1.528	2.464
3	CSR51	69.67 $\pm$ 1.53	0.882	1.266
4	CSR5	69.33 $\pm$ 1.53	0.882	1.272
5	CSR2	68.67 $\pm$ 3.06	1.764	2.569
6	CSR16	65.33 $\pm$ 5.51	3.180	4.867
7	CSR19	67.67 $\pm$ 2.52	1.453	2.147
8	CSR50	68.67 $\pm$ 3.51	2.028	2.953
9	CSR27	69.67 $\pm$ 1.52	0.882	1.266
10	CSR26	66.67 $\pm$ 2.52	1.453	2.179
11	CSR52	64.00 $\pm$ 3.00	1.732	2.706
12	CSR202(SL)	65.33 $\pm$ 2.52	1.453	2.224

Comparison	Std. Error	S.E. Diff	t value 5%	C.V. %	C. Diff
Multivoltine	1.67	2.30	2.06	4.18	4.75
Bivoltine	1.50	2.12	2.04	4.17	4.33