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In vitro Studies of Efficacy of Different Chemicals for Management of Fungal Disease (White Muscardine) and Bacterial Disease (*Bacillus* and *Staphylococcus*) in Silk Worm *Bombax mori* L.

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A B S T R A C T

Among the silkworm diseases, white muscardine caused by *Beauveria bassiana* is the most common disease. The seven different chemicals *viz.*, BKC (90%), pine Oil (100%), bleach (12%), sodium chlorite (80%), bleaching powder (30%), sanitech (2000ppm) and hydrated lime (80%) were tested and screened against fungus (*beauveria bassiana* and bacteria (*bacillus* and *staphyllococcus*) based on poison food technique with different concentrations such as 0.05%, 0.5%, 1%, 2%, 2.5%, 3%, 5%and 10% with three replications for each chemical concentration along with control. Among all the chemical the BKC (90%) gave better result. all the six concentration *viz.*, 0.5, 1.0, 1.5, 2.5, 5 and 10 were tested against fungus (*beauveria bassiana*) and bacteria (bacillus and staphylococcus). The maximum inhibition was recordrd in 10 % concentration of BKC followed by 5% .The minimum inhibition of radial growth of fungus was recorded in Pine oil i.e. 5% concentration followed by 2.5% .In case of bacterial pathogen 5 and 10% concentration shows maximum inhibition and 2.5% concentration shows partial inhibition. In general, the inhibition of radial growth of fungus increased with increase in concentration of each chemical.

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). The mulberry silkworm, *Bombyx mori* is of great economic importance as a foreign exchange earner for many silk producing countries of the world (Krishnaswami *et al.*, 1992). Silkworm, *Bombyx mori* L. is an important economic lepidopteron insect and utilized for the commercial production of the natural silk fiber "Queen of Textiles". The silkworm *Bombyx mori* is prone to various pests and diseases. Diseases in the silkworm *Bombyx mori* are fairly common in occurrence and are serious in inflicting cocoon crop loss. These can be grouped under four major categories, namely the microsporidian disease, viral diseases, bacterial diseases, and fungal diseases. Among the diseases of silkworm, white muscardine caused by *Beauveria bassiana* inflicts heavy economic loss to the sericulturists in India.

Beauveria bassiana is an entomopathogenic hypomycetes fungus,distributed all over the world.it infects over 100 different insect species coming fromseveral insect orders. In silk worm *bombax mori*, it causes white muscardine disease infecting significant crop loss in all sericulture countries. Muscardine is one of the contagious diseases, which is causing loss of 5-50 per cent in total loss due to diseases (Hanumappa, 1986).

The fungus infects silkworm under favorable condition through the integument,enters hemocoel, and produce hyphal bodies and parasitise various tissues leading to death of the hosts.after death, the fungus grows saprophitically and forms mycellial mass that turns the host body into hard structure. the conidiophores emerges from the host body and produce infectious conidia (Datta *et al.*, 1998).

Symptoms of muscardine includes loss of appetite, sluggishness of the worms, increased rate of dorsal heart beat, inactiveness of worms, larvae become pale in colour, vomiting, diarrhea and decreased elasticity of the larval body. The larval corpse turns pink or reddish in colour due to the secondary invasion.

Several chemical formulations have been developed to prevent the germination of conidia on the integument and its entry into host body (Samson *et al.*, 1986; Sashidharan *et al.*, 1997).

The microbes were gram positive, rod shaped bacteria (*Bacillus* spp.), cocci shaped (*Staphylococcus* spp.) and gram negative (Sengupta *et al.*, 1990).

Materials and Methods

In vitro studies of efficacy of different chemicals for control of fungal disease (white muscardine) and bacterial disease (*bacillus* and *staphylococcus*) in silk worm *bombax mori* L. was carried out during 2015 at the Department of Sericulture, KVK, University of Agricultural Sciences, GKVK, Bangalore.

The diseased silkworms were collected from field, and fungus and bacteria were isolated in the pure form (Govindan *et al.*, 1998). A small bit of mycellium of *Beauveria bassiana* was incorporated into poisonated potato dextrose agar media (PDA) and incubated at 26 ± 1^{0}C for a period of 15 days and was observed for the growth of fungus. The growth of fungus was quantified visually as -: negative; ±: negligible; +: satisfactory growth; ++: good growth; and +++: very good growth. (Plate no. 1 to 3)

For *bacteria* to obtain samples from silk worm, the insect was surface sterilized in 70% ethanol and washed 3 times with sterile distilled water. The insects were then triturated in sterile water blanks using glass rod. The suspension was serially diluted up to 10^{-6} then 0.1 ml of the suspension was inoculated into nutrient agar using the streak plate method and incubated at 26°C for 24 h.

This set up was observed for colony growths and the total number of colonies observed was recorded to determine the standard plate count from 3 replicates. Distinct colonies were subsequently isolated for further analysis, and different chemical concentrations were prepared and added to the Nutrient Agar (NA) medium. A loop of bacterial culture was drawn from culture of *Bacillus sp*. and were streaked on the plates and kept for incubation. Observations were made on the growth after 48 hours quantified visually as ± Partial inhibition, + No inhibition, − Inhibition.

Seven different chemicals *viz.,* BKC (90%), pine Oil (100%), bleach (12%), sodium chlorite (80%), bleaching powder (30%), sanitech (2000ppm) and hydrated lime (80%) were tested and screened them against fungus

(*Beauveria bassiana* and bacteria (*bacillus* and *Staphylococcus*).The *in vitro* screening of the seven chemicals was performed based on poison food technique with different concentrations such as 0.05%, 0.5%, 1%, 2%, 2.5%, 3%, 5%and 10%. There were three replications for each chemical concentration maintained along with control (Table 1).

Results and Discussion

The silkworm showed symptoms of white muscardine, it was inferred that the fungus could be *Beauveria bassiana*, the most common one causing white muscardine in India (Chandrasekaran and Nataraju, 2008).

Efficacy of Seven different chemicals *viz.*, BKC (90%), pine Oil (100%), bleach (12%), sodium chlorite (80%), bleaching powder (30%), sanitech (2000ppm) and hydrated lime (80%) were tested and screened them against fungus (*Beauveria bassiana* and bacteria (*bacillus* and *Staphylococcus*). The *in vitro* screening of the seven chemicals was performed based on poison food technique with different concentrations such as 0.05%, 0.5%, 1%, 1.5, 2%, 2.5%, 3%, 5%and 10%). Three replications for each chemical concentration maintained along with control. The results thus obtained have been presented in table 2a to 8b and depicted.

In table 2a and 2b the different concentration of BKC chemical such as 0.5, 1.0, 2.5, 5.0 and 10% tested among all these concentrations the highest inhibition of radial growth of fungus were recorded in 2.5% (0 cm), 5% (0cm) and 10% (0cm) concentration. The minimum inhibition of radial growth of fungus was recorded in 1.5% (0.36cm), 1% (0.6cm) and 0.5% (0.95cm) concentration. In case of bacterial pathogen 5% and 10% concentration showed maximum inhibition and 0.5, 1.0 and 1.5% concentration showed partial inhibition.

In table 3a and 3b the different concentration of Pine oil were tested in different concentration such as 2.5, 5 and 10% among all these concentrations the highest inhibition of radial growth of fungus were recorded in10% (0cm) concentration. The minimum inhibition of radial growth of fungus was recorded in 5% (0.63cm) followed by 2.5% (0.95cm). In case of bacterial pathogen 5and 10% concentration shows maximum inhibition and 2.5%concentration shows partial inhibition.

In table 4a and 4b the different concentration of Bleach were tested in different concentration such as 0.5, 1, 2.5, 5 and 10% among all these concentrations the highest inhibition of radial growth of fungus were recorded in 10% (0cm), 5%(0cm) and 2.5%(0cm) concentration. The minimum inhibition of radial growth of fungus was recorded in 0.5% (0.63cm) followed by 0.5 %(0.95cm).In case of bacterial pathogen 1, 2.5, 5 and 10% concentration shows maximum inhibition and 0.5%concentration shows partial inhibition.

In table 5a and 5b the different concentration of Sodium chlorite was tested in different concentration such as 1, 2 and 3% among all these concentrations the highest inhibition of radial growth of fungus were recorded in 2% (0cm) followed by3 %(0cm). The minimum inhibition of radial growth of fungus was recorded in 1% (0.95cm). In case of bacterial pathogen 2%and 3% concentration shows maximum inhibition and 1%concentration shows partial inhibition.

In table 6a and 6b the different concentration of Bleaching Powder was tested in different concentration such as 1 and 2% among all these concentrations the highest inhibition of radial growth of fungus were recorded in 2% (0cm).The minimum inhibition of radial growth of fungus was recorded in 1%

(0.95cm).In case of bacterial pathogen 3% concentration shows maximum inhibition and 1%concentration shows partial inhibition (Subba Rao *et al.*, 1992). In table 7a and 7b the 1% concentration of Sanitech was tested and shows complete inhibition of radial growth of fungus (0cm).In case of bacterial pathogen 1% concentration shows complete inhibition.

Table.1 List of chemicals used for *in vitro* evaluation against *Beauveria bassiana*, *Bacillus* and *Staphylococcus*

S NO.	Chemicals	Concentrations (%)				
1	Benzo Conium Chloride (BKC) (90%)	2.5	5	10	-	-
2	Pine oil (100%)	2.5	5	10		
3	Bleach (12%)	0.5	1	2.5	10	-
4	Sodium chlorite (80%)	1	2	3	-	-
5	Bleaching powder (30%)	1	2	-	-	-
6	Sanitech (2000ppm)	0.05	-	-	-	-
7	Hydrated lime (80%)	1	2	-	-	-

Table.2a *In vitro* screening of BKC on *Beauveria bassiana* by poison food technique

DAI	Concentration(%) of chemical (BKC) and growth of fungus			control
	2.5	**5**	**10**	
1	±	±	±	± (0.6mm)
3	±	±	±	+ (1.8mm)
5	±	±	±	+ (2.1mm)
7	±	±	±	++ (3.2mm)
9	±	±	±	++ (3.6mm)
11	±	±	±	+++ (3.9mm)
13	±	±	±	+++ (4.2mm)
15	±	±	±	+++ (4.2mm)

-: negative; ±:negligible; +:satisfactory growth; ++:good growth; and +++:very good growth.

Table.2b *In vitro* screening of BKC on *Bacillus* and *Staphylococcus* by streak plate method

DAI	Concentration(%) of chemical (BKC) and growth of bacteria			control
	2.5	5	10	
1	±	-	-	+
3	±	-	-	+
5	±	-	-	+
7	±	-	-	+
9	±	-	-	+
11	±	-	-	+
13	±	-	-	+
15	±	-	-	+

± Partial inhibition, + No inhibition, − Inhibition

Table.3a *In vitro* screening of pine oil on *Beauveria bassiana* by poison food technique

DAI	Concentration(%) of chemical (BKC) and growth of fungus			control
	2.5	5	10	
1	±	±	±	± (0.6mm)
3	±	±	±	+ (1.8mm)
5	±	±	±	+ (2.1mm)
7	±	±	±	++ (3.2mm)
9	±	±	±	++ (3.6mm)
11	±	±	±	+++ (3.9mm)
13	±	±	±	+++ (4.2mm)
15	±	±	±	+++ (4.2mm)

-: negative; ±:negligible; +: satisfactory growth; ++: good growth; and +++: very good growth.

Table.3b *In vitro* screening of BKC on *Bacillus* and *Staphylococcus* by streak plate method

DAI	Concentration(%) of chemical (pine oil) and growth of bacteria			control
	2.5	5	10	
1	±	-	-	+
3	±	-	-	+
5	±	-	-	+
7	±	-	-	+
9	±	-	-	+
11	±	-	-	+
13	±	-	-	+
15	±	-	-	+

± Partial inhibition, + No inhibition, − Inhibition.

Table.4a *In vitro* screening of bleach on *Beauveria bassiana* by poison food technique

DAI	Concentration(%) of chemical (bleach) and growth of fungus					control (mm)
	0.5	1	2.5	5	10	
1	±	±	±	±	±	± (0.6mm)
3	±	±	±	±	±	+ (1.8mm)
5	±	±	±	±	±	+ (2.1mm)
7	±	±	±	±	±	++ (3.2mm)
9	±	±	±	±	±	++ (3.6mm)
11	±	±	±	±	±	+++ (3.9mm)
13	±	±	±	±	±	+++ (4.2mm)
15	±	±	±	±	±	+++ (4.2mm)

-: negative; ±:negligible; +: satisfactory growth; ++: good growth; and +++: very good growth.

Table.4b *In vitro* screening of bleach on *Bacillus* and *Staphylococcus* by streak plate method

DAI	Concentration(%) of chemical (bleach) and growth of fungus					control
	0.5	1	2.5	5	10	
1	±	-	-	-	-	+
3	±	-	-	-	-	+
5	±	-	-	-	-	+
7	±	-	-	-	-	+
9	±	-	-	-	-	+
11	±	-	-	-	-	+
13	±	-	-	-	-	+
15	±	-	-	-	-	+

± Partial inhibition, + No inhibition, − Inhibition.

Table.5a *In vitro* screening of sodium chlorite on *Beauveria bassiana* by poison food technique

DAI	Concentration(%) of chemical (sodium chlorite) and growth of fungus			control (mm)
	1	2	3	
1	±	±	±	± (0.6mm)
3	±	±	±	+ (1.8mm)
5	±	±	±	+ (2.1mm)
7	±	±	±	++ (3.2mm)
9	±	±	±	++ (3.6mm)
11	±	±	±	+++ (3.9mm)
13	±	±	±	+++ (4.2mm)
15	±	±	±	+++ (4.2mm)

-: negative; ±:negligible; +: satisfactory growth; ++: good growth; and +++: very good growth

Table.5b *In vitro* screening of sodium chlorite on *Bacillus* and *Staphylococcus* by streak plate method

DAI	Concentration(%) of chemical (sodium chlorite) and growth of bacteria			control
	1	**2**	**3**	
1	±	-	-	+
3	±	-	-	+
5	±	-	-	+
7	±	-	-	+
9	±	-	-	+
11	±	-	-	+
13	±	-	-	+
15	±	-	-	+

± Partial inhibition, + No inhibition, − Inhibition

Table.6a *In vitro* screening of bleaching powder on *Beauveria bassiana* by poison food technique

DAI	Concentration(%) of chemical (bleaching powder) and growth of fungus		control (mm)
	1	**2**	
1	±	±	± (0.6mm)
3	±	±	+ (1.8mm)
5	±	±	+ (2.1mm)
7	±	±	++ (3.2mm)
9	±	±	++ (3.6mm)
11	±	±	+++ (3.9mm)
13	±	±	+++ (4.2mm)
15	±	±	+++ (4.2mm)

-: negative; ±:negligible; +: satisfactory growth; ++: good growth; and +++: very good growth

Table.6b *In vitro* screening of bleaching powder on *Bacillus* and *Staphylococcus* by streak plate method

DAI	Concentration(%) of chemical (bleaching powder) and growth of bacteria		control
	1	2	
1	±	-	+
3	±	-	+
5	±	-	+
7	±	-	+
9	±	-	+
11	±	-	+
13	±	-	+
15	±	-	+

± Partial inhibition, + No inhibition, − Inhibition

Table.7a *In vitro* screening of sanitech on *Beauveria bassiana* by poison food technique

DAI	Concentration(%) of chemical (sanitech) and growth of fungus	control (mm)
	1	
1	±	± (0.6mm)
3	±	+ (1.8mm)
5	±	+ (2.1mm)
7	±	++ (3.2mm)
9	±	++ (3.6mm)
11	±	+++ (3.9mm)
13	±	+++ (4.2mm)
15	±	+++ (4.2mm)

-: negative; ±:negligible; +: satisfactory growth; ++: good growth; and +++: very good growth

Table.7b *In vitro* screening of sanitech on *Bacillus* and *Staphylococcus* by streak plate method

DAI	Concentration(%) of chemical (sanitech) and growth of bacteria	control
	1	
1	-	+
3	-	+
5	-	+
7	-	+
9	-	+
11	-	+
13	-	+
15	-	+

± Partial inhibition, + No inhibition, − Inhibition

Table.8a *In vitro* screening of lime on *Beauveria bassiana* by poison food technique

DAI	Concentration(%) of chemical (lime) and growth of fungus		control (mm)
	1	2	
1	±	±	± (0.6mm)
3	±	±	+ (1.8mm)
5	±	±	+ (2.1mm)
7	±	±	++ (3.2mm)
9	±	±	++ (3.6mm)
11	±	±	+++ (3.9mm)
13	±	±	+++ (4.2mm)
15	±	±	+++ (4.2mm)

-: negative; ±:negligible; +: satisfactory growth; ++: good growth; and +++: very good growth

Table.8b *In vitro* screening of lime on *Bacillus* and *Staphylococcus* by streak plate method

DAI	Concentration(%) of chemical (lime) and growth of bacteria		control
	1	2	
1	-	-	+
3	-	-	+
5	-	-	+
7	-	-	+
9	-	-	+
11	-	-	+
13	-	-	+
15	-	-	+

± Partial inhibition, + No inhibition, − Inhibition

Plate.1 Diseased silk worm larvae

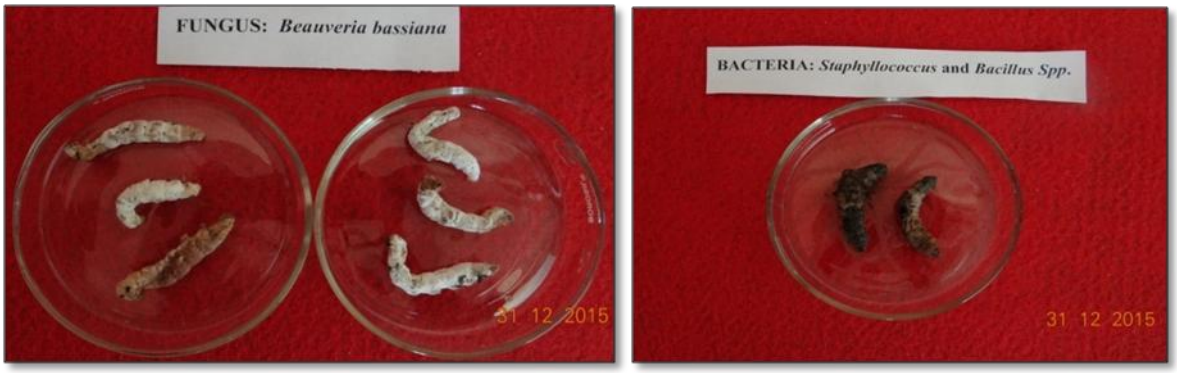


Plate.2 Pure culture of Fungus (*Beauveria bassiana*)

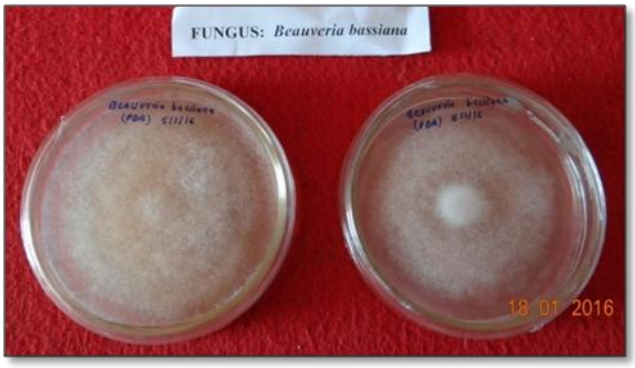
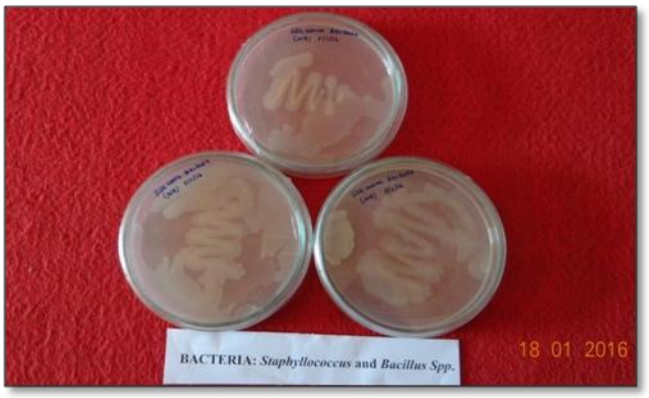


Plate.3 Pure culture of bacteria (*Bacillus* and *Staphylococcus*)



In table 8a and 8b the different concentration of lime was tested in different concentration such as 1 and 2% among all these concentrations the highest inhibition of radial growth of fungus were recorded in both 1 and 2% (0cm).In case of bacterial pathogen both 1 and 2% concentration shows complete inhibition (Rangaswamy *et al.,* 2003).

Among all the chemical the BKC (90%) gave better result. In BKC all the six concentration viz., 0.5, 1.0, 1.5, 2.5, 5 and 10 were tested against fungus (*Beauveria bassiana*) and bacteria (bacillus and staphylococcus) the result sows that when concentration increases the per cent inhibition of pathogen also increases the maximum inhibition was recordrd in 10 % concentration of BKC followed by 5%.

If the diseases are controlled below the economic threshold level then there will be an increase of 25 per cent silk production without any increase in the area under mulberry sericulture (Nagarajan and Radha, 1990).

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