

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(4): 402-407
www.biochemjournal.com
 Received: 02-01-2026
 Accepted: 05-02-2026

Seetharamulu J
 Andhra Pradesh State
 Sericulture Research and
 Development Institute
 (APSSRDI) Kirikera,
 Hindupur, Sri Sathya Sai
 District, Andhra Pradesh,
 India

Thrilekha D
 Andhra Pradesh State
 Sericulture Research and
 Development Institute
 (APSSRDI) Kirikera,
 Hindupur, Sri Sathya Sai
 District, Andhra Pradesh,
 India

Madhavi K
 Andhra Pradesh State
 Sericulture Research and
 Development Institute
 (APSSRDI) Kirikera,
 Hindupur, Sri Sathya Sai
 District, Andhra Pradesh,
 India

Vijaya Kumar DR
 R. V. Seri Agro Vet, Malur,
 Kolar, Karnataka, India

Corresponding Author:
Seetharamulu J
 Andhra Pradesh State
 Sericulture Research and
 Development Institute
 (APSSRDI) Kirikera,
 Hindupur, Sri Sathya Sai
 District, Andhra Pradesh,
 India

Studies on the efficacy of Germitol - A general room disinfectant in sericulture industry

Seetharamulu J, Thrilekha D, Madhavi K and Vijaya Kumar DR

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4f.8156>

Abstract

This study evaluates the efficacy of Germitol, a phenol-based disinfectant, in controlling silkworm pathogens and enhancing productivity in sericulture. Both laboratory and field assessments were conducted to test Germitol's germicidal effectiveness against major silkworm pathogens. *In vitro* test demonstrated a significant reduction in colony-forming units (CFUs) of pathogens, with Germitol achieving over 98% reduction in *Bacillus thuringiensis* and 96% in *Beauveria bassiana* and *In vivo* bioassay studies indicated lower mortality and improved survivability in Germitol treated batches. Further, field trials were conducted with the farmers in Sri Sathya district of Andhra Pradesh and Chikkaballapur, Kolar districts of Karnataka. These results shows the improved cocoon yield with quality cocoons in Germitol treated batches and less larval mortality, higher larval weight and cocoon yield in the germitol treated silkworm rearing houses when compared against traditional disinfectants. Further, in-house evaluation resulted in, significant improvement in cocoon weight, shell weight and cocoon yield per 100 Disease-Free Layings (DFLs). The low toxicity and broad-spectrum biocidal properties of Germitol signifies a promising alternative to conventional disinfectants, with potential for large-scale adoption to enhance productivity and reduce disease incidence in commercial sericulture.

Keywords: *Bombyx mori*, disinfectant, disinfection efficacy, Germitol, hygiene, *In vivo*, *In vitro*, pathogenic microbes, silkworm diseases

Introduction

The mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), is vulnerable to several diseases that pose significant challenges in achieving optimal cocoon yield and productivity. Some of the most common diseases affecting silkworms include Nuclear polyhedrosis, Bacterial and Viral Flacherie, Muscardine and Pebrine. Nuclear polyhedrosis is caused by *Bombyx mori* Nucleopolyhedrovirus (*BmNPV*). Viral flacherie is triggered by multiple viruses, including *Bombyx mori* infectious flacherie virus (*BmIFV*), *Bombyx mori* densovirus (*BmDNV*) and *Bombyx mori* cytoplasmic polyhedrosis virus (*BmCPV*). Bacterial flacherie caused by bacteria such as *Bacillus thuringiensis*, *Serratia marcescens*, *Streptococcus* and *Staphylococcus* species. Muscardine, a fungal disease caused by *Beauveria bassiana* and Pebrine caused by *Nosema bombycis*, a microsporidian pathogen. These silkworm diseases occurs in all major silk-producing countries, with a reported loss of 15-20 kg of cocoons per 100 dfls, accounting for up to 25% of total production losses (Selvakumar *et al.*, 2002) [17]. Sometimes, it has been noticed that most of the farmers have lost their complete crop due to viral diseases.

Pathogens can spread through various means into the rearing environment, including contaminated air, mulberry leaves, rearing equipment and direct contact with infected silkworms or through the rearing personnel leading to disease outbreaks and substantial economic losses (Baig *et al.*, 1990) [2]. Effective disease control is essential for increasing silk production by up to 25% without expanding the mulberry cultivation area (Nagarajan and Radha, 1999) [12]. Despite strict rearing techniques, controlling diseases remains crucial for sustainable sericulture practices.

At present, no effective curative methods are available to manage silkworm diseases, making preventive and prophylactic measures are the most reliable approach for disease control. Hence, disinfection and hygiene are integral parts of successful silkworm rearing. Proper disinfection of the silkworm rearing room and its appliances plays a crucial role in minimizing pathogen load and preventing disease outbreaks thereby ensuring healthy cocoon

production. Various disinfectants *viz.*, Formalin (Kagawa, 1980), Bleaching powder (Balavenkatasubbaiah *et al.*, 1994 a,b) [4], Iodine compounds (Kawakami, 1970; Venkata Reddy *et al.*, 1990) [10], Calcium hydroxide (Ishiwata & Zhou, 1968) [8], Chlorine dioxide (Sanitech) in combination with lime (Balavenkatasubbaiah *et al.*, 1999; 2006) [3], Astra (Venugopal *et al.*, 2012) [21], Decol (Santha *et al.*, 2012) [16] have been used in sericulture industry for disinfection of rearing houses and appliances. However, many of these disinfectants have certain limitations, such as being corrosive, carcinogenic or causing irritation to the eyes and throat of the users, particularly for sericulture farmers.

Germitol, a new spray disinfectant developed by RV Seri Agro Vet in Malur, Kolar district, Karnataka has been introduced for disinfecting rearing houses, surrounding areas and equipments. This phenol-based disinfectant is reported to be effective against a broad spectrum of silkworm pathogens. This broad-spectrum disinfectant offers promising potential for silkworm rearing due to its non-corrosiveness and cost-effective properties. Germitol is formulated as a synergistic blend of multiple active compounds, based on selected phenolic derivatives. In addition to its antimicrobial efficacy, it is designed to be less toxic than traditional phenol-based disinfectants. When used at the recommended concentration, Germitol remains effective across a range of temperatures while minimizing environmental impact. This study aims to evaluate the efficacy of Germitol in reducing microbial contamination in the silkworm rearing environment and to assess its impact on silkworm health and cocoon yield.

Material and Methods

This study was conducted at the Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI) in Hindupur, Andhra Pradesh. Germitol was evaluated under laboratory and field conditions to assess its efficacy against silkworm pathogens and its impact on silkworm productivity through a series of *in vitro*, *in vivo* and field trials. The germicidal activity of Germitol was tested *in vitro* against *Bacillus thuringiensis* (1×10^8 spores/ml) and *Beauveria bassiana* (1×10^8 conidia/ml). In each case, pathogen suspensions were centrifuged at 5000 rpm for 10 minutes and the resulting pellets were exposed to a 1% Germitol solution for 30 minutes at room temperature. After treatment, the pathogens were centrifuged again and the pellets were resuspended in 1 ml of sterilized distilled water. Viability was assessed by inoculating the treated pathogens onto Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. The plates were incubated at 28-30 °C for 24-48 hours for bacteria and at 25-28 °C for 48-72 hours for fungi (Anonymous, 2019). A control group, consisting of untreated pathogens, was processed in parallel for comparison. The number of Colony Forming Units per millilitre (CFU/ml) was calculated using the formula:

$$\text{CFU/ml} = \frac{\text{Number of colonies counted} \times \text{Dilution factor}}{\text{Volume plated (ml)}}$$

The percentage reduction in microbial load was calculated using the following formula:

$$\text{Reduction (\%)} = \frac{\text{CFU in control} - \text{CFU in treated}}{\text{CFU in control}} \times 100$$

The *in-vivo* germicidal efficacy of Germitol was evaluated under simulated silkworm rearing conditions. Four major silkworm pathogens were selected for the study *viz.*, *BmNPV*, *BmCPV*, *Bacillus thuringiensis* and *Beauveria bassiana*. Pathogen suspensions were prepared using standardized concentrations of 1×10^7 POBs/ml for *BmNPV*, 1×10^7 polyhedra/ml for *BmCPV*, 1×10^8 spores/ml for *B. thuringiensis* and 1×10^7 conidia/ml for *B. bassiana*. The rearing room was artificially contaminated by uniformly spraying the pathogen suspensions uniformly over the rearing bed, walls, racks and surrounding equipment. After allowing the pathogens to settle for 30 minutes, the contaminated surfaces were sprayed thoroughly with a 1% Germitol solution and left to dry completely before initiating rearing. Following disinfection, healthy third instar larvae of the FC1 × FC2 hybrid were introduced into the treated environment and reared under standard environmental conditions. Each treatment was replicated three times with 100 larvae per replication. A separate control group was maintained in an identically contaminated rearing room without the application of Germitol. Larvae were observed daily for ten days to monitor the disease incidence and larval mortality.

To further assess the effectiveness of Germitol, FC1 × FC2 reared under three conditions *viz.*, disinfection of rearing room and appliances with 1% Germitol (1 liter of Germitol in 99 liters of water, applied at 150 ml per square foot or 1.5 liters per square meter) as per the recommendation of the product developer. Disinfection using bleaching powder (2.5% concentration) and an untreated control group for comparison (table 1). Each treatment had three replications with 5 dfls per replicate. Silkworm rearing was conducted following the standard rearing practices (Dandin and Giridhar, 2014) and V1 mulberry leaves were fed to silkworm till spinning. Rearing and cocoon parameters *viz.*, larval mortality (%), fifth instar duration (h), fifth instar larval weight (g), cocoon yield by number (per 10,000 larvae), cocoon yield by weight (kg/per 10,000 larvae), pupation rate(%), single cocoon weight, shell weight (g) and shell ratio (%) were recorded.

Table 1: Treatment details

T ₁	Rearing room was disinfected with 1% Germitol solution.
T ₂	Rearing room treated with standard disinfectant Bleaching powder (control)
T ₃	No disinfection treatment (untreated control for baseline pathogen load comparison).

The efficacy of Germitol was also evaluated at farmers rearing houses in Sri Sathya district of Andhra Pradesh and Chikkaballapur, Kolar districts of Karnataka. Rearing houses were disinfected with a 1% Germitol solution and observations were recorded on cocoon yield and other parameters. A total of 20 silkworm cocoon crops, involving 4108 dfls with different silkworm breeds/hybrids (CSR2, CB, FC2, FC1 and FC1 × FC2) were assessed. Further, an in-house evaluation was conducted over five silkworm rearing crops involving 125 DFLs and the rearing performance *viz.*, single cocoon weight (g), single shell weight (g), shell ratio (%), yield/100 dfls (kg) was recorded. All laboratory and field experiments were conducted under standardized environmental conditions. Data was analysed using a completely randomized design (CRD) with OPSTAT statistical software.

Results and Discussion

Germitol proved significant germicidal efficacy under *in vitro* and *in vivo* against major silkworm pathogens. *In vitro* testing revealed that exposure to 1% Germitol solution for 30 minutes significantly reduced the number of colony-forming units (CFU) viz., 98.83 per cent in *B. thuringiensis*

and 96.91 per cent in *B. bassiana* (fig. 1). Control plates showed normal colony growth, confirming the viability of untreated pathogens. The near-complete reduction of bacterial and fungal colonies on the treated plates confirms the efficacy of Germitol.

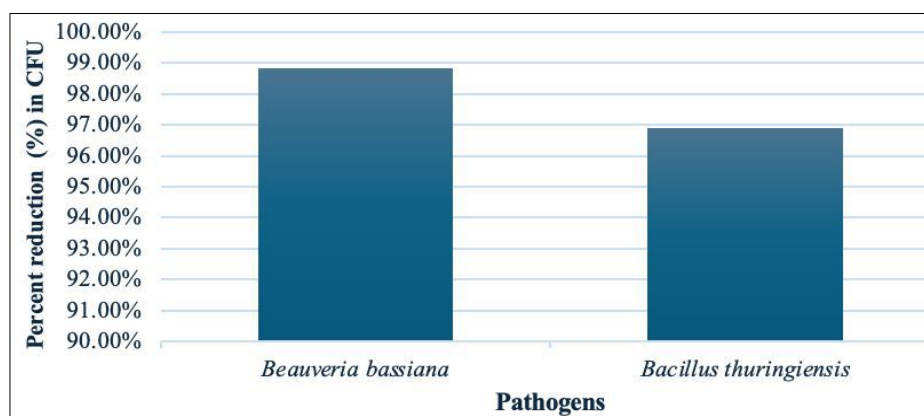


Fig 1: Per cent reduction of Pathogenic CFU after Germitol Treatment (*In vitro*)

The *in-vivo* evaluation of Germitol under simulated rearing conditions revealed a significant reduction in disease incidence and larval mortality compared to the untreated control. The average cumulative mortality of larvae over the ten day observation period was markedly lower in the Germitol-treated group across all pathogen challenges. In the Germitol-treated batches, the mean larval mortality due to *Bm*NPV, *Bm*CPV, *Bacillus thuringiensis* and *Beauveria bassiana* was recorded as 7.95%, 7.72%, 6.13% and 6.45%, respectively (table 2). In contrast, the corresponding mortality in the control group without disinfection was

significantly higher, with values of 72.43%, 68.91%, 64.71% and 63.17%, respectively.

Among the four pathogens, *Bm* NPV caused the highest larval mortality in the control group, whereas *Bacillus thuringiensis* showed relatively lower mortality. Germitol treatment resulted in more than 85% reduction in mortality across all tested pathogens, indicating its broad-spectrum efficacy. No abnormal larval behaviour was observed in the Germitol-treated groups, suggesting the absence of any adverse effects of the disinfectant on silkworm health and development.

Table 2: Impact of Germitol on silkworm larval mortality under Pathogen-Contaminated rearing conditions (*In vivo* Bioassay)

Pathogen	Mean Larval Mortality (%) (Germitol Treated)	Mean Larval Mortality (%) (Control)	Percent reduction over control (%)
<i>Bm</i> NPV	7.95	72.43	89.02
<i>Bm</i> CPV	7.72	68.91	88.79
<i>Bacillus thuringiensis</i>	6.13	64.71	90.52
<i>Beauveria bassiana</i>	6.45	63.17	89.78
F test ±	*	*	-
S.Em	0.049	0.676	-
CD@5	0.153	2.107	-
CV (%)	1.387	2.009	-

* = Significant at 5%

The results from the room disinfection trials revealed significant improvement in the performance of silkworms reared in Germitol treated (T_1) silkworm rearing room across all key parameters (table 3). Larval mortality in the Germitol treated group was significantly lower (4.80%) compared to the bleaching powder (9.71%) and the untreated control group (25.95%). Although the fifth instar duration showed no significant difference among the treatments, it was shortest in the Germitol treated group (149.11 hours) compared to the bleaching powder (155.92 hours) and control groups (153.81 hours). The shorter duration in the Germitol treated group reveals more efficient growth and development, likely due to reduced disease stress and improved hygiene in the rearing environment. Germitol treated group also had the highest fifth instar larval weight (57.21 g), followed by bleaching powder (52.38 g) and control group (49.29 g), which contributed to higher

single cocoon weight of 2.23 g, 2.02 g and 1.88 g, respectively. The Germitol-treated group recorded the highest cocoon yield per 10,000 larvae (9,611.39 cocoons with a total weight of 21.94 kg) which was notably higher than the bleaching powder treatment (9,439.27 cocoons weighing 20.02 kg) and the control group, which produced 7,595.23 cocoons with a weight of 14.64 kg. The higher cocoon yield in the Germitol treated group is due to the lower larval mortality and healthier larval growth, both of which can be attributed to better pathogen control in the rearing environment. The pupation rate was 95.20% in the Germitol treated group, compared to 92.28% in the bleaching powder and 73.68% in the control group. Shell weight and shell ratio were also highest in the Germitol treated group (0.45 g and 22.07%) compared to the bleaching powder (0.43 g and 20.09%) and the control group (0.38 g and 18.84%).

Table 3: Silkworm rearing performance in the Double Hybrid, FC1 x FC2 treated with Germitol and control batches under standard rearing conditions

Parameters	Larval mortality (%)	Fifth Instar duration (h)	Fifth Instar larval weight (g)	Pupation rate (%)	Cocoon yield by number (per 10,000 larvae)	Cocoon yield by weight (kg/per 10,000 larvae)	Single cocoon weight	Shell weight (g)	Shell Ratio (%)
Treatments									
T ₁ (Germitol)	4.80	149.11	57.21	95.20	9611.39	21.94	2.23	0.49	22.07
T ₂ (Bleaching Powder)	9.71	155.92	52.38	92.28	9439.27	20.02	2.02	0.41	20.29
T ₃ (Untreated)	25.95	153.81	49.29	73.68	7595.23	14.64	1.88	0.35	18.61
F test ±	*	NA	*	*	*	*	*	*	*
S.Em	0.162	2.96	0.930	0.801	111.530	0.184	0.037	0.007	0.268
CD@5%	0.571	NA	3.28	2.825	393.448	0.650	0.132	0.025	0.945
CV (%)	2.08	3.355	3.041	1.593	2.175	1.692	3.171	2.970	2.283

* = Significant at 5%; NA: Not analysed.

The promising results in Germitol-treated batches across all parameters highlight its effectiveness in maintaining a pathogen-free rearing environment, reducing larval mortality and enhancing productivity. The bleaching powder treatment (T₂) showed moderate efficacy with slightly lower yield and higher disease incidence. The poor performance of the untreated control group (T₃) was likely due to a high pathogen load, adversely affecting silkworm health and cocoon production.

Field trials conducted at farmer's rearing houses also showed promising results, confirming the laboratory

findings with Germitol treated farmers rearing houses showing consistent improvements in cocoon yield and achieved higher market rates per kilogram of cocoons (table 4), resulting in successful and consistent cocoon crop harvests. The data suggest that Germitol is a promising disinfectant for large-scale use, providing enhanced productivity and pathogen control. The significant reduction in disease incidence in large-scale field trials reinforces the laboratory and small-scale findings, establishing Germitol as an effective disinfectant for commercial sericulture operations.

Table 4: Efficacy studies of Germitol at the farmers rearing houses

Sl.No.	Village/District/State	Name of the hybrid	No. of dfls. Reared	Cocoon yield (kg)	Rate/kg cocoons (Rs.)
	Nagondahalli, Kolar/Karnataka	CSR2	150	127	1300
	Thoraganadoddi, Kolar/Karnataka		150	132	1300
	Channakalla halli, Kolar/Karnataka		250	221	1300
	Pulagamphalli, Kadiri, AP		100	84	1300
	Chikkahasala, Kolar/Karnataka		300	248	1300
	Hindupur, Sri Sathya Sai/Andhra Pradesh	Crossbreed (PM×CSR2)	100	74	450
	Nagondahalli, Kolar/Karnataka		150	110	445
	Channakal, Kolar/Karnataka		220	161	422
	Dimbachamanahalli, Kolar/Karnataka		250	184	595
	Mudavadi, Kolar/Karnataka		80	60	603
	Roddam, Sri Sathya Sai/ Andhra Pradesh	FC1	150	121	1300
	Nayakarahalli, Kolar/Karnataka		150	118	1300
	Kammasandra, Kolar, Karnataka		150	124	1300
	Thornahalli, Kolar/ Karnataka		150	130	1300
	Santehalli, Kolar/Karnataka		150	116	1300
	Yalehalli, Chikkaballapura/ Karnataka	FC2	150	112	1300
	Madakasira, Sri Sathya Sai/Andhra Pradesh		150	122	1300
	Kaivara, Chikkaballapura/ Karnataka		150	128	1300
	Segehalli, Chikkaballapura/ Karnataka		150	24	1300
	Y. Hunasenahalli, Chikkaballapura/ Karnataka		150	120	1300
	Vakkaleri, Kolar/Karnataka	FC1 × FC2	300	278	595
	Penukonda, Sri Sathya Sai/Andhra Pradesh		220	195	630
	Holur, Kolar/Karnataka		100	90	650
	Handiganala, Chikkaballapura/ Karnataka		150	136	550
	Kurbur, Chikkaballapura/ Karnataka		88	75	640

The results from the In-house evaluation further supported Germitol effectiveness in maintaining hygiene and preventing disease outbreaks over multiple rearing cycles. The data over 5 rearing crops is presented in table 5. Over 125 dfls reared, the mean cocoon weight of double hybrid is 2.33g, shell weight is 0.52g with a shell ratio of 22.39 %.

The cocoon yield per 100 dfls recorded an average of 91.77 kg, which is higher compared to traditional disinfectants. These findings indicate that Germitol is effective in eliminating pathogens from silkworm rearing surfaces and equipment, aligning with earlier research on the effectiveness of phenol-based disinfectants in sericulture.

(Santha *et al.*, 2012; Samson *et al.*, 1995) [16, 15]. The antimicrobial properties of phenolic compounds have been documented by various researchers (Klarmann & Shternov,

1936; Rettger *et al.*, 1929; Read & Miller, 1932; Terleckyj & Axler, 1987), reinforcing the results observed in this study.

Table 5: In-house evaluation of Germitol on the Performance of FC1 × FC2

Crop No.	No. of dfls reared	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)	Yield/100 dfls (kg)
1.	25	2.45	0.56	22.86	91.08
2.	25	2.09	0.44	21.05	89.97
3.	25	2.38	0.51	21.43	90.93
4.	25	2.60	0.62	23.85	94.74
5.	25	2.11	0.48	22.75	92.14
Total/Average	125	2.33	0.52	22.39	91.77

Conclusion

The study demonstrated that Germitol is an effective disinfectant for controlling silkworm pathogens under both laboratory and field conditions. With its broad-spectrum biocidal properties, low toxicity and cost-effectiveness when used in recommended concentration, it stands out as a best alternative to traditional disinfectants like bleaching powder. By significantly reducing microbial contamination, it improved larval health and cocoon yield, making it a valuable addition to sericulture practices. Field trials confirmed Germitol practical applicability in commercial sericulture, proving it to be a reliable disinfectant that helps farmers maintain better hygiene standards and reduce disease-related losses. Its successful commercialization for large-scale production further underscores its potential to enhance productivity in the industry.

Acknowledgment

The authors are thankful to RV Seri Agro Vet, Malur, Kolar district for their financial support in facilitating the evaluation of Germitol efficacy and also thank farmers of Andhra Pradesh and Karnataka for their kind cooperation during the study period.

Authors Contribution

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Disclaimer (artificial intelligence)

Author(s) hereby declares that no generative AI technologies such as large language models (ChatGPT, Copilot, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Declaration

The authors declare that they have no conflict of interest.

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