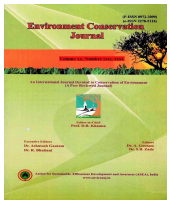
Environment Conservation Journal 24 (3): 54-62, 2023

Journal homepage: https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



Disease incidence, larval parameters and mortality of mulberry silkworm, *Bombyx mori* L. effected by bed disinfectants

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ARTICLE INFO

Received : 12 September 2022
Revised : 13 November 2022
Accepted : 03 January 2023

Available online: 09 April 2023

Key Words:
Bed disinfectant
Bombyx mori
Disease incidence
Larval mortality
Growth parameters

ABSTRACT

With an aim to find out an effective bed disinfectant regulating disease incidence, larval growth parameters and mortality of mulberry silkworm, *Bombyx mori* L. revealed a positive influence on the disease incidence, and larval growth of mulberry silkworm race, (CSR6 × CSR26) × (CSR2 × CSR27). The commercial bed disinfectant, Ankush manifested better result in terms of reduction in disease incidence (1.833 %) and larval parameters (larval duration, 21.888 days; full grown larval weight, 4.596 g; silk gland weight, 0.889 g and 24.181% SGTSI) compared to the control (22.667 days, 3.976 g, 0.817 g and 20.726 %, respectively) followed by Sericillin and Turmeric rhizome powder alone or in combinations. However, a combination of bed disinfectants *viz*., Ankush + Sericillin, Ankush + Turmeric rhizome powder and Sericillin + Turmeric rhizome powder recorded better results compared to Turmeric rhizome powder alone. Though bed disinfectants did not show any significant effect on mortality percentage but Ankush resulted highest reduction of mortality over control (46.933 %). The performance of all the bed disinfectants was found to be better during the late spring season.

Introduction

Mulberry silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) is a domesticated monophagous silkworm and completely reared under indoor condition. China is the leading producer of silk with a production of 53, 359 MT mulberry raw silk (Anon., 2022). Contribution of India is next to China in silk production (33,770 MT). Among the four varieties of silk produced in India, mulberry silk accounted for 70.76 per cent of the total raw silk production. Mulberry silk occupies an important place in Assam's sericulture covering an area of 2,653.00 ha with a total production of about 16 metric tonnes of mulberry silk (Anon., 2021). Various factors like quality silkworm layings, incubation of silkworm eggs, disinfection of rearing house, maintenance of hygiene and rearing environment, quality of mulberry leaf, disease and pest management, mounting etc. are considered to be the most important for healthy and vigorous growth of silkworm and its successive higher production. Moreover, mulberry silkworm is sensitive and susceptible to various diseases caused by microsporidia, bacteria, viruses and fungi (Doreswamy *et al*., 2004) sharing about 30 per cent of total loss and estimated loss due to disease incidence is about 15-20 kg per unit of 100 disease free layings (Selvakumar *et al*. 2002). Silkworm rearing bed or seat always acts as a major source for infection and multiplication with some secondary infection. Hence, proper bed disinfection

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is considered as an essential precautionary measure in mulberry silkworm culture. In mulberry silkworm culture, several workers had already revealed efficacy of different bed disinfectants in preventing contamination, spread and multiplication of various silkworm diseases *viz*., grasserie, flacherie, muscardine and pebrine, which ultimately helps in increasing the larval growth parameters of silkworm and thereby silk production (Singhvi *et al*., 2004). In silkworm rearing, reports can be obtained on indiscriminate use of various bed disinfectants *viz*., Vijetha, Lime, Captan, Dithane M-45, Resham Keet Oushad, Ankush, Vijetha Green, Resham Jyothi, Formalin Chaff and Labex etc. in large quantities (Swathi *et al*., 2014; Shashidhar *et al*., 2018). Thus, selection and proper use of bed disinfectant could help the farmers in achieving the success in mulberry silkworm culture. Therefore, the present study was undertaken with the objective to evaluate existing chemical bed disinfectants and also formulation of new botanical disinfectant alone or in combination to reduce the silkworm disease effectively for good larval growth and quality cocoon production suitable for the prevailing very high relative humidity induced environmental condition of Assam.

Material and Methods

Silkworm rearing

The present investigation to find out the effect of botanical and chemical bed disinfectants on larval characters and disease incidence of mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) was conducted at the Department of Sericulture, Assam Agricultural University, Jorhat during the year 2019-20 under ambient environmental condition. Seeds of double hybrid mulberry silkworm race, (CSR6×CSR26) × (CSR2×CSR27) were collected from Silkworm Seed Production Centre, Central Silk Board, Bangalore and were reared (Fig. 1) separately during the late spring (May-June, 2019-20) and the autumn season (September-October, 2019-20). The newly hatched silkworms were reared on mulberry leaves (variety: S1635) under ambient room temperature and relative humidity following standard rearing technique suggested by Kumar *et al*. (2015).

Treatments and method of use

Altogether 3 (three) bed disinfectants *viz*., Ankush, Sericillin, and Turmeric rhizome powder were selected and utilized @ 3g/sq.ft bed area based on earlier scientific reports (Balavenkatasubbaiah *et al*., 2014), and were tested at various treatment combinations (Table 1) by dusting on the body as well as rearing seat of mulberry silkworm so reared with the help of a muslin cloth. Treatments were repeated once after every moult at 30-40 minutes before the resumption of feed from first to fifth instars. Moreover, one additional dusting with the selected bed disinfectants was done on the 4th day of fifth instar after bed cleaning. Each treatment was replicated three times with 100 numbers of larvae per replication. The data on total larval duration and instarwise larval weight (from 2nd instar onward) were recorded. Silk gland weight, silk gland tissue somatic index were calculated during the fifth instar. Disease incidence and larval mortality were observed during the larval period as a whole. All the treatments alone or in combinations were tested in both the late spring (May-June, 2019-20) and the autumn season (September-October, 2019-20)

Statistical Analysis

The data on different seasonal growth parameters, diseases incidence and mortality percentage were subjected to Analysis of variance (ANOVA) following Completely Randomized Design (CRD) and interaction effects of both season and bed disinfectants were calculated following the method proposed by Sahu and Das (2009).

The percent of Disease Incidence (%) was calculated by the following method of Zagar (2010) as mentioned below –

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased larvae}}{\text{Total number of healthy larvae}} \times 100$$

The per cent of disease reduction was calculated by the following method of Abbott (1925) as mentioned below-

$$\text{Per cent disease reduction (\%)} = \frac{C - T}{C} \times 100$$

Where,
C = Mortality per cent of control batch
T = Mortality per cent of treatment batch

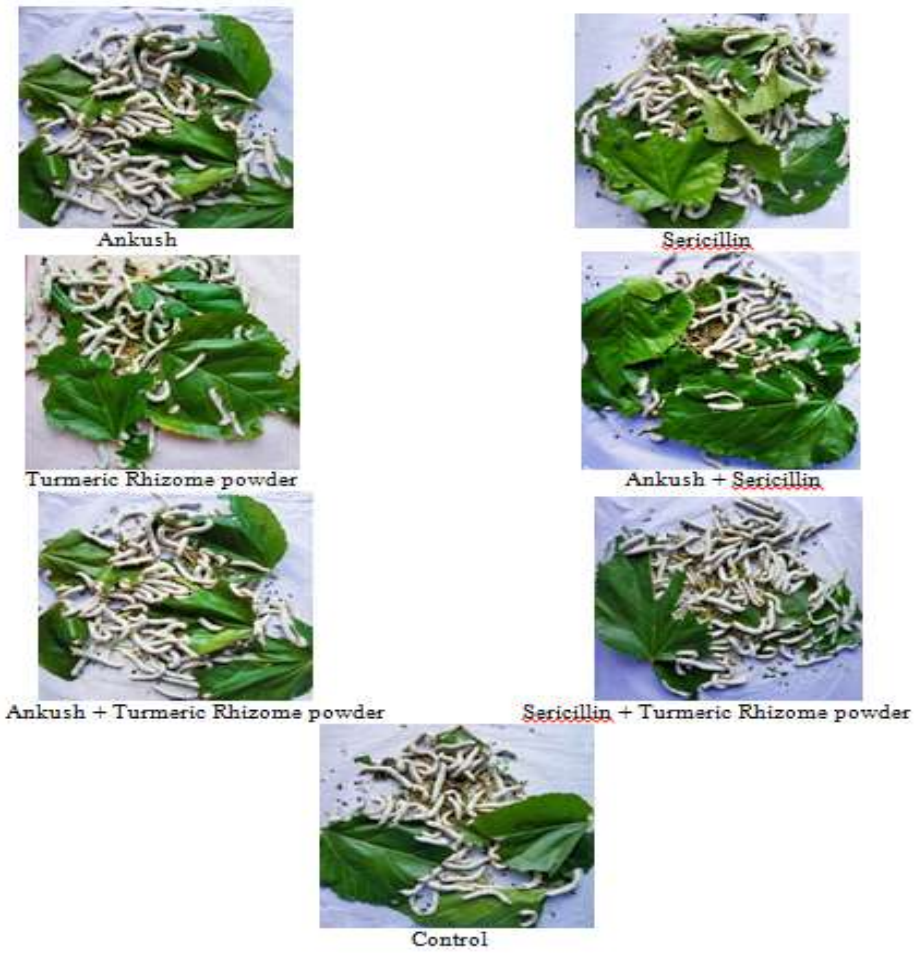

Figure 1: 5th instar mulberry silkworm larvae in different treatments.

Table 1: Treatment details and dosage

Treatment No	Treatment details	Dosage (g/sq.ft bed area)
T_1	Ankush	3
T_2	Sericillin	3
T_3	Turmeric rhizome powder	2
T_4	Ankush + Sericillin	3 (1.5+1.5)
T_5	Ankush + Turmeric rhizome powder	2.5 (1.5+1)
T_6	Sericillin + Turmeric rhizome powder	2.5 (1.5+1)
T_7	Control	No application

The Silk Gland Tissue Somatic Index (SGTSI) expressed in percentage (%) was determined by using the standard formula described by Venkata Rai Reddy and Benchamin (1989) as mentioned below –

$$\text{SGTSI}\ (\%) = \frac{\text{Weight of the silk gland tissue}\ (g)}{\text{Weight of larval body}\ (g)} \times 100$$

Larval mortality (%) was calculated with the data on numbers of larvae died out of total number of larvae brushed, which is represented below –

$$\text{Larval mortality}\ (\%) = \frac{\text{Total number of larvae died}}{\text{Total number of larvae brushed}} \times 100$$

Results and Discussion

Table 2 represents the data on effect of selected bed disinfectants on disease incidence (%) of double hybrid mulberry silkworm and the data revealed a higher disease incidence during autumn season (2.000 to 5.333%) as compared to late spring season (1.667 to 5.000%). The data on disease

Table 2: Effect of bed disinfectants on disease incidence (%) of double hybrid mulberry silkworm race in late spring and autumn season

Treatment	Season		Mean
	Late spring	Autumn	
T_1	1.667	2.000	1.833
T_2	2.000	2.333	2.167
T_3	4.000	4.333	4.167
T_4	2.333	2.667	2.500
T_5	2.667	3.333	3.000
T_6	3.000	4.000	3.500
T_7	5.000	5.333	5.167
Mean	2.952	3.429	
	S. Ed (±)	C.D (5%)	
Season	0.471	NS	
Bed disinfectant	0.882	1.816	
Season × Bed disinfectant	1.247	NS	

Data are mean of three replications
NS = Non Significant

incidence revealed that all the treatments showed better performance over control and season had non-significant effect but bed disinfectants had significant effect on disease incidence of mulberry silkworm larvae. Among the different treatments of bed disinfectants, significantly the lowest disease incidence of 1.833% was recorded with the application of Ankush @ 3 g/sq. feet as compared to 5.167% disease incidence in the control. Mulberry silkworm, being an indoor reared and completely domesticated insect is susceptible to various diseases caused by bacteria, virus, microsporidia and fungus mainly due to fluctuation of environmental conditions during different seasons. The larva after each moult has the newly formed skin which is thin, loose and delicate; and pathogen finds it easy to infect. Bed disinfectant helps to keep the silkworm bed dry during moulting. Further, once the disease occurs, management is difficult due to very short larval life and spreads within a short period of time causing heavy crop loss. There are several research reports of using bed disinfectants as a preventive measure to protect the life of mulberry silkworms. Earlier Reddy and Rao (2009) recorded the higher incidence of diseases in summer and winter seasons and was suppressed by applying disinfectant of 2% bleaching powder in 0.3% slaked lime solution and Ankush@3g/sq. feet in young age silkworms and 5g/ sq. feet in late age silkworms. Shashidhar *et al*. (2018) also reported that application of Ankush Vijetha green and slaked lime powder as per recommended schedule in combination leads to low incidence of silkworm diseases like grasserie, flacherie and muscardine during post rainy and *rabi* season which supports present findings. It was also reported that Sericillin treated larvae showed significantly less (8.4%) incidence of grasserie disease compared to control (13.10%) (Mohanan *et al*. 2009). Manimegalai and Subramaniam (1999) observed that dusting of Turmeric powder + Chalk powder and Vijetha resulted in 63.16 per cent reduction in grasserie infection during summer season. Sujatha *et al*. (2007) showed that turmeric powder at lower concentration resulted in 17.00 % reduction of diseases. In the present investigation, incidence of grasserie and muscardine were mostly observed and their incidences were lowered down by the application of all the bed disinfectants either alone or in combination compared to control might be due to lowering down the humidity. Ankush + Turmeric rhizome powder, Sericillin + Turmeric rhizome powder could not able to suppress the disease incidence as much as Ankush or Sericillin might be because of lower dose (2.5 g/sq.ft) and efficacy of Ankush or Sericillin was more than Turmeric rhizome powder. Among Ankush, Sericillin and Turmeric rhizome powder; Ankush might be more effective to reduce the humidity compared to other two bed disinfectants. Therefore, Ankush could able to lower down the disease incidence more efficiently than Sericillin, Turmeric rhizome powder and Ankush + Sericillin.The data on effect of bed disinfectants on larval duration are presented in Table 3, which revealed a larval duration ranging between 21.933 to 22.733 days in late spring season and 21.833 to 22.600 days in autumn season. All the bed disinfectants had significant effect causing lower larval duration of the tested mulberry silkworms. Significantly the lowest larval duration (21.888 days) was observed with the treatment with Ankush, as compared to the highest of 22.667 days in the control. During the experimentation, the effect of seasons on larval duration was found to be non-significant. Shorter larval duration is preferred for commercial exploitation. The larval duration generally varies with the quality of mulberry leaves and other environmental conditions (Kamili and Masoodi, 2000).

Table 3: Effect of bed disinfectants on larval duration (days) of double hybrid mulberry silkworm race in late spring and autumn season

Treatment	Season		Mean
	Late spring	Autumn	
T_1	21.933	21.833	21.888
T_2	21.967	21.900	21.933
T_3	22.433	22.367	22.400
T_4	22.233	22.167	22.200
T_5	22.267	22.200	22.233
T_6	22.333	22.233	22.283
T_7	22.733	22.600	22.667
Mean	22.271	22.186	
	S. Ed (±)	C.D (5%)	
Season	0.084	NS	
Bed disinfectant	0.156	0.322	
Season × Bed disinfectant	0.221	NS	

Data are mean of three replications
NS = Non Significant

Present findings are in conformity with Thakur (2010) who recorded shortest larval duration (21.91 days) due to application Ankush powder @50, 100, 600, 1250 and 2000 g per 100 dfls at the time of 1st, 2nd, 3rd and 4th day old 5th instar mulberry silkworm larvae, which however found *at par* with other bed disinfectants like Sanitech, Bleaching powder, Slaked lime powder, Neem powder, Serichlor-20, Turmeric powder. Manjunath *et al*. (2020) recorded extended larval duration in 5th instar mulberry silkworm over control with application of turmeric extract but shorter than Amruthaballi, Tulasi and Asparagus. Present results also revealed that Turmeric rhizome powder alone or in combination exhibited longer larval duration compared to all the treatments. However, longest larval duration was recorded in control.The data on effect of different bed disinfectants on larval weight of mulberry silkworm are presented in Table 4 and the data revealed a significant effect with respect to larval weight increase in all the instars was observed across the seasons. It was apparent from the table that all the bed disinfectants recorded significantly higher larval weight over control. A significantly higher larval weight of II, III, IV instar, full grown and matured (0.039g, 0.308g, 0.941g, 4.596g and 3.393g) was observed in treatment with Ankush @ 3 g/sq. feet, which followed by Sericilin @ 3 g/sq. feet, Ankush + Sericillin @ 3 g/sq. feet, Ankush + Turmeric rhizome powder @ 2.5 g/sq. feet, Sericillin + Turmeric rhizome powder @ 2.5 g/sq. feet, Turmeric rhizome powder @ 2.0 g/sq. feet and the lowest of 0.035g, 0.257g, 0.842g, 3.976g and 2.931g in the control. Irrespective of the treatments, late spring reared larvae showed higher larval weight (0.038g, 0.281g, 0.886g, 4.388g and 3.189g) than autumn reared larvae (0.036g, 0.272g, 0.879g, 4.227g and 3.074g) in all the instars. It was revealed from the data of each instar that the larval weight was gradually increased in each of successive instar i.e. from 2nd instar to 5th instar. Sericillin is a mixture of lime, bleaching powder and fungicide whereas Ankush is the mixture of

Table 4: Effect of bed disinfectants on instar wise larval weight (g) of double hybrid mulberry silkworm race in late spring and autumn season

Treatment	2nd Instar			3rd Instar			4th Instar			5th Instar					
										Full Grown weight			Matured weight		
	Late Spring	Autumn	Mean	Late Spring	Autumn	Mean	Late Spring	Autumn	Mean	Late Spring	Autumn	Mean	Late Spring	Autumn	Mean
T_1	0.039	0.038	0.039	0.316	0.300	0.308	0.944	0.939	0.941	4.605	4.587	4.596	3.483	3.303	3.393
T_2	0.039	0.038	0.038	0.295	0.287	0.291	0.925	0.917	0.921	4.534	4.482	4.508	3.375	3.219	3.297
T_3	0.037	0.035	0.036	0.264	0.259	0.262	0.856	0.845	0.850	4.182	4.021	4.102	3.013	2.932	2.973
T_4	0.038	0.036	0.037	0.284	0.273	0.279	0.893	0.882	0.888	4.481	4.279	4.380	3.274	3.185	3.230
T_5	0.038	0.035	0.037	0.275	0.269	0.272	0.873	0.870	0.871	4.471	4.194	4.333	3.140	3.077	3.108
T_6	0.038	0.035	0.037	0.273	0.264	0.268	0.864	0.860	0.862	4.413	4.105	4.259	3.040	2.943	2.991
T_7	0.036	0.033	0.035	0.264	0.250	0.257	0.846	0.839	0.842	4.027	3.924	3.976	3.000	2.861	2.931
Mean	0.038	0.036		0.281	0.272		0.886	0.879		4.388	4.227		3.189	3.074	
		S.Ed(±)	C.D(5%)		S.Ed(±)	C.D(5%)		S.Ed(±)	C.D(5%)		S.Ed(±)	C.D(5%)		S.Ed(±)	C.D(5%)
Season		2.4×10^{-4}	4.8×10^{-4}		0.001	0.001		0.001	0.001		0.026	0.053		0.010	0.021
Bed Disinfectant		4.4×10^{-4}	9.0×10^{-4}		0.001	0.002		0.001	0.002		0.048	0.099		0.019	0.039
Season × Bed Disinfectant		6.2×10^{-4}	1.2×10^{-4}		0.002	0.003		0.002	0.003		0.068	0.139		0.027	0.056

indigenously available eco-friendly chemicals and botanicals.That may be the reason of the better performance of these two bed disinfectants alone or in combination compared to Turmeric rhizome powder alone or in combination which might positively affect the growth and development of silkworm. An increase in larval weight on applying Ankush was also found by Thakur (2010), which might be because of the fact that effective regulation of rearing bed humidity and thereby providing optimum condition for growth of silkworm. Similar results were recorded by several workers by applying different chemical bed disinfectants (Jagannatha, 1996; Swathi *et al*., 2014). However, results of Thakur (2010), Mohanan *et al*. (2009), which corroborate our findings. Karuppasamy *et al*. (2013) stated that mean larval weight of the final instar larvae increased as compared to the control due to Turmeric powder extracts at different concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1% along with sugar solution). Sujatha *et al*. (2007) showed that the higher larval weight was obtained at lower dose of Turmeric powder as compared to higher dose.The growth and development of silk gland depends on the healthy growth of silkworm and is important to the sericulture industry because silk glands are responsible for synthesis of silk protein (Akai, 1983; Sailaja and Sivaprasad, 2010). The Silk Gland Tissue Somatic Index (SGTSI) is the ratio between silk gland weight and the body weight (Vijayakumar *et al*., 2007), which gives us an idea about the potential productivity of silkworms.During our experimentation, season and bed disinfectant were found to be having significant effect on silk gland weight and Silk Gland Tissue Somatic Index (Table 5, 6) of mulberry silkworm larvae. Application of Ankush @ 3 g/sq. feet at late spring season registered higher values compared to other treatments and season. Vallaanchira *et al*. (2013) observed that the higher weight of silk gland could be achieved when larvae were reared under the environmental condition of 25°C and 70% RH. Again, between the seasons, both the parameters exhibited better result in late spring season. Silk gland weight is positively correlated with larval weight (Singh and Kumar, 1996). Higher larval weight in all the instars in Ankush dusted larvae and maximum weight of silk gland in the same observed in the present study confirmed the statement. Application of all the bed disinfectants performed better results over control may be due to disease free environment and vigorous growth of the silkworm. Our experiments on possible effect of bed disinfectants on larval mortality (Table 7) revealed the highest mortality ranging from 3.667 to 6.667 % and 5.000 to 9.667% in late spring and autumn season, respectively.

Table 5: Effect of bed disinfectants on silk gland weight (g) of double hybrid mulberry silkworm race in late spring and autumn season

Treatment	Season		Mean
	Late spring	Autumn	
T_1	0.895	0.883	0.889
T_2	0.883	0.872	0.877
T_3	0.844	0.832	0.838
T_4	0.872	0.861	0.867
T_5	0.859	0.850	0.855
T_6	0.853	0.839	0.846
T_7	0.825	0.809	0.817
Mean	0.861	0.849	
	S. Ed (±)	C.D (5%)	
Season	0.003	0.006	
Bed disinfectant	0.005	0.011	
Season × Bed disinfectant	0.007	NS	

Data are mean of three replications
NS = Non Significant

Table 6: Effect of bed disinfectants on Silk Gland Tissue Somatic Index (SGTSI) (%) of double hybrid mulberry silkworm race in late spring and autumn season

Treatment	Season		Mean
	Late spring	Autumn	
T_1	24.782	23.579	24.181
T_2	24.373	23.261	23.817
T_3	22.390	21.109	21.755
T_4	23.642	22.467	23.055
T_5	23.461	22.346	22.904
T_6	23.402	22.137	22.769
T_7	21.273	20.178	20.726
Mean	23.333	22.154	
	S. Ed (±)	C.D (5%)	
Season	0.005	0.114	
Bed disinfectant	0.104	0.213	
Season × Bed disinfectant	0.147	NS	

Data are mean of three replications
NS = Non Significant

Table 7: Effect of bed disinfectants on larval mortality (%) of double hybrid mulberry silkworm race in late spring and autumn season

Treatment	Season		Mean	Reduction over control
	Late spring	Autumn		
T_1	3.667	5.000	4.334	46.933
T_2	4.333	6.000	5.167	36.733
T_3	6.000	8.000	7.000	14.289
T_4	4.667	6.667	5.667	30.611
T_5	4.667	7.000	5.834	28.566
T_6	5.000	7.667	6.334	22.444
T_7	6.667	9.667	8.167	
Mean	5.000	7.143		
	S.Ed (±)	C.D (5%)		
Season	1.012	2.091		
Bed disinfectant	1.900	NS		
Season ×Bed disinfectant	2.687	NS		

Data are mean of three replications
NS = Non Significant

Control. It was apparent from the table that though the effect of bed disinfectant was non significant, however,the lowest larval mortality (4.334%) was observed ni the treatment with Ankush @3g/Sq. ft. as compared to 8.167% in the conntrol. Irrespective of all the treatments, season had significant effect ($P<0.05$) on larval mortality of mulberry silkworm. Treatment with Ankush recorded maximum (46.933%) reduction of mortality over control and the lowest (14.289%) reduction of mortality was observed in treatments with turmeric rhizome powder @ 2 g/sq. feet. Late spring reared larvae recorded minimum larval mortality (5.000%) while autumn reared larvaerecorded maximum (7.143%). There was no significant effect due to interaction of season and bed disinfectant on larval mortality of mulberry silkworm. Singh (2012) reported that Vijetha dusted batches found more effective in the parameters of larval mortality than the RKO dusted batches in spring season. Dusting of Lime: Paraformaldehyde: Sodium benzonate (94: 3: 3) and Lime: Paraformaldehyde: Benzoic acid (95: 3: 2) mixture on rearing bed resulted in decreased larval mortality (16.5% and 15.4%, respectively) over control (68.30%) (Barman, 1991). Rasool *et al.* (2018) stated that the lowest mortality (55.00%) was registered by Vijetha followed by Lime + Captan + Walnut hull (59.77%), Lime + Cefixime + Turmeric (66.77%), Lime + Turmeric + Bavistin (68.95%) and Lime + Turmeric (72.42%). The disinfectant such as Turmeric + RKO (1:1 ratio), pure Turmeric and RKO produced 12, 16 and 20 per cent larval mortality respectively (Dhirwani *et al*., 2015).

Conclusion

Sericulture industry has a significant contribution to the economy of the developing countries like India and this industry suffers a huge loss due to incidence of different infectious diseases caused by pathogen. In this case, the silkworm bed disinfectants play an important role in preventing the spread of various diseases through secondary contamination. From our present investigation, it could be concluded that all the bed disinfectants showed better results compared to untreated larvae, but the performance of Ankush was better compared to others, which might be due to its constituents as it is a mixture of plant product and non-hazardous chemicals. In future, dissemination of information on possible use of eco-friendly bed disinfectants like Ankush, Sericilin and turmeric at proper dose and method of application to the extension personnel and mulberry silkworm rearers through home visits, field days, discussions, training and demonstrations could help to cater the full benefits in silk industry.

Acknowledgement

Authors are thankful to Assam Agricultural University, Jorhat for providing necessary support to conduct the experiment.

Conflict of interest

The authors declare that they have no conflict of interest.

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