

Assessment of two disinfectants in the rearing of *Bombyx mori* and their effects on larval performance and cocoon yield

Ayandokun, A. E.*, Ete, J. A., Babayemi, I. S., Aina-Oduntan, O. A.

Department of Forest Conservation and Protection
Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria

*Corresponding Author (E-mail: gbadeayans@yahoo.com)

ABSTRACT

This study assessed the use of ethanol and sodium hypochlorite as alternatives to formalin as disinfectant in the rearing of silkworm. Disease free silkworm eggs (EJ1 x EC1) were incubated for 10 days and brushed into rearing trays after hatching and treated individually with 3% formalin, 70% ethanol, 0.5% Sodium hypochlorite and a control with no disinfectant. Each tray had 100 worms and the trays were arranged in a Completely Randomized Design replicated 3 times. The insects were fed with mulberry leaves (Variety S14) daily until cocooning. The results showed that the percentage larval mortality was not significantly different among the disinfectants throughout the moulting stages; however, the lowest percentage larval mortality was recorded in trays treated with 70% ethanol. The weight of matured larva, total cocoon weight and single shell weight were not significantly different. The highest single cocoon weight (0.98 ± 0.01 g) and single pupal weight (0.8 ± 0.02 g) observed in the control were not significantly different from the other treatments. The highest effective rate of rearing (84%) was recorded in both formalin and Sodium hypochlorite at $P < 0.05$. This study shows that both 70% ethanol and 0.5% Sodium hypochlorite could be used as alternative disinfectants for silkworm rearing.

Keyword: Silkworm, disinfectant, mulberry, larval mortality, rearing

INTRODUCTION

Silkworm larvae, *Bombyx mori* (L.) is an economic insect that converts leaf protein to silk. The industrial and commercial use of silk, its historical and economic importance of production and its application all over the world contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology. Silk, the 'Queen of textiles' remains unchallenged in fibre industry which could be considered as

a nature's gift for human kind. Mulberry silk is the most important type of all silk and contributes to as much as 95% of the world's silk production (ETSPF, 2007).

One major challenge in silkworm rearing is its susceptibility to a number of diseases (Doreswamy *et al.*, 2004). The cocoon loss due to diseases in India is estimated to be about 15-20 Kg per unit of 100 disease free layings (dfls) which accounts for about 30% of total loss (Selvakumar *et al.*, 2002).

Information on investigations related to disease prevalence in silkworm in different parts of the country in different seasons is highly helpful to monitor, prevent and control (Babu *et al.*, 2009).

The high prevalence of diseases in sericultural areas may be due to persistence of the pathogen at high concentrations in the silkworm-rearing environment. The pathogens are not easily destroyed and can persist for long periods under congenial conditions (Kanika *et al.*, 2011). Most diseases of silkworm are non-curable due to shorter larval period and associated with shorter incubation period of pathogen. And the pathogens easily accumulate and spread in the rearing environment through different routes (Kanika *et al.*, 2011).

Reddy and Rao (2009) surveyed and recorded silkworm diseases viz: Grasserie, Flacheria, Pebrine and Muscardine, white muscardine, green muscardine, yellow muscardine, aspergillosis caused by *Beauveria bassiana*, *Spicaria prasina* (*Nomuraea riyeli*), *Metarhizium anisopliae*, *Paecilomyces farinosus*, *Aspergillus flavus*, *A. oryza*, *A. ochraceus*, *A. tamari* etc. (Beena, 1996; Manochaya and Kumar, 2010).

In order to record profit, disinfection becomes an integral part of healthy and successful silkworm rearing. It is important to state that the use of bed disinfectant aims at the total destruction of disease causing pathogens and not to harm silkworm. Several diseases caused by bacteria, viruses, fungi and protozoa causing flacherie, grasserie, muscardine and pebrine diseases respectively affect the silkworm. The spores of the pathogens, especially those of fungi are light and can easily be drifted by air current resulting in easy spread of diseases. It is best to prevent silkworm diseases than curing them (Swathi *et al.*, 2014). This is

achieved by adoption of proper and effective methods of disinfection and stepwise maintenance of hygiene during rearing.

Among the most commonly used bed disinfectants by the farmers are active lime, hydrated lime, bundth power, vijetha, Captan, Dithane M-45, RKO, Ankush, Vijetha Supplement, Rashamjyothi, Labex and formalin (Swathi *et al.*, 2014) which are being used in large quantities without any justification. Also Okpara *et al.* (2011) recommended UV and chlorination disinfection methods. Therefore, when the silkworms are reared under natural conditions it is essential to disinfect the surroundings thoroughly and maintain cleanliness.

Silkworm diseases are major constraint in realizing full potentiality of crop yield in sericulture (Kumar and Naik, 2011), to combat this, certain general bed disinfectants are available in the market like, formalin, bleaching powder, chlorine dioxide among others but as good and effective these disinfectants are in there action, they tend to have certain disadvantages like corrosiveness, non-acceptable odour, irritation to eyes and throat, carcinogenicity and user non friendliness, hence the need to investigate into the use of other disinfectant that are safe and also efficient. Therefore this study aimed to assess and compare influence of three bed disinfectants on the performance of silkworm.

MATERIALS AND METHODS

The study was conducted at Entomology Section of the Department of Forest Conservation and Protection of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Southwest, Nigeria. The silkworm rearing room was swept and washed with

detergent, the equipment used for rearing were washed as well prior to rearing. The rearing of the insect was carried out under the hygro-thermic condition $28 \pm 1^\circ\text{C}$ and $88.3 \pm 3.4\%$ RH (Krishnaswami *et al.*, 1978). Disease free silkworm eggs (EJ1 x EC1) procured from Ekiti State Sericulture Project was incubated for 10 days. The hatched larva were brushed into well prepared tray and fed with succulent and nutritious mulberry leaves (Variety S14) cut into approximately 1 cm x 1 cm which was increased later with age of the insect until when leaves were fed to the insect whole. At the end of 1st moulting, the larvae were distributed into 3 replications at the rate of 100 insects/tray. The larvae were fed three times a day 8 am, 12 pm and 4 pm throughout the period of rearing (Adeduntan, 2013).

Three bed disinfectants (3% formalin, 70% ethanol, 0.5% Sodium hypochlorite and no disinfectant.) were used to determine the silkworm performance. The bed disinfectants were applied with foam pad soaked with each of the disinfectant and water for control before brushing. The treatments were replicated three times and arranged in completely randomized design. Bed cleaning was carried out as occasion demanded during rearing. After feeding the insects for 23 days the matured 5th instar larvae were mounted on plastic mountage for cocoon spinning and the cocoons were harvested six days after mounting.

Data were collected on larva mortality, 5th instar matured larval weight, number of insect mounted, single cocoon weight, single shell weight, single pupal weight, total cocoon weight, number of cocoon formed, floss weight and effective rate of rearing. The data collected were subjected to analysis of variation (ANOVA) using SAS 9.1 software package and means were separated using Duncan New Multiple Range Test.

RESULTS

The study assessed and compared two disinfectants (ethanol and Sodium hypochlorite) to investigate alternatives to the use of formalin.

Table 1 shows mortality index of the insects at different larval stages. The highest larval mortality was recorded in trays where no disinfectant was used at the end of 2nd, 3rd and 4th moulting with the number of dead larvae increasing from 15 to 22 while the least larval mortality was observed in trays disinfected with ethanol where the number of dead larva ranged from 5 to 12 all through the insect larval stages followed by the trays treated with formalin. There was no significant difference in the larval mortality across the insect larval stage across the four treatments.

Table 1: Effect of formalin, ethanol and sodium hypochlorite as disinfectants on silkworm larval mortality across the moulting stages

Treatment	Larva mortality after 2 nd second moulting	Larva mortality after 3 rd moulting	Larva mortality after 4 th moulting
Formalin	8.67±4.18 ^a	12.33±3.28 ^a	12.33±4.09 ^a
Ethanol	5.33±1.45 ^a	10.67±2.96 ^a	12.33±3.71 ^a
NaoCl	7.67±4.98 ^a	13±5.5 ^a	14±5.13 ^a
No disinfectant	14.67±5.21 ^a	21.67±5.84 ^a	22.33±5.67 ^a

Means with the same alphabets are not significantly different at $p < 0.05$

From Table 2, the silkworm 5th instar matured larval weight was highest in trays with no disinfectant which was closely followed by the ones in ethanol treated trays while the lowest larval weight was obtained from trays treated with formalin. Fifth instar matured larval weight across the treatments were not significantly different. The number

of insect mounted was highest in ethanol treated trays followed by the ones in formalin treated trays while the lowest number was found in trays without any disinfectant. No significant difference across the treatments in terms of number of cocoon formed was observed, however, the highest record was found in ethanol treated trays.

Table 2: Effect of formalin, ethanol and sodium hypochlorite as disinfectants on silkworm and 5th instar larval weight, number of insect mounted and number of cocoon formed

Treatment	5 th instar larval weight (g)	Number of insect mounted	Number of cocoon formed
Formalin	2.41±0.08 ^a	84.33±2.60 ^a	75.67±8.35 ^a
Ethanol	2.59±0.12 ^a	86.33±4.09 ^a	76±7.09 ^a
NaoCl	2.48±0.05 ^a	84.33±4.98 ^a	72.33±2.85 ^a
No disinfectant	2.63±0.01 ^a	72±9.54 ^a	61.33±11.85 ^a

Means with the same alphabets are not significantly different at $p < 0.05$

Parameters on the formed cocoons are presented in Table 3. For single cocoon weight, there was no significant difference among the treatments. However, the trays without disinfectant did better than others while the lowest cocoon weight was from formalin treated trays. Total cocoon weight did not record any significant difference among the treatments, however, the total cocoon weight was highest in ethanol treated trays and the least total cocoon weight was recorded in trays without disinfectant.

The single pupal weight in trays without disinfectant was higher but not significantly

different from the ones in ethanol treated trays. The trays without disinfectant gave the highest single shell weight and the least single shell weight was obtained from sodium hypochlorite treated trays.

Although the effective rate of rearing of the insect is highest in formalin treated trays (84.3%) and sodium hypochlorite treated trays (84.3%) followed by ethanol treated trays (83%), while the effective rate of rearing is lowest in trays without disinfectant (72%).

Table 3: Effect of formalin, ethanol and sodium hypochlorite as disinfectants on silkworm single cocoon weight, total cocoon weight, single pupal weight, single shell weight, floss weight and Effective rate of rearing

Treatment	Single cocoon weight (g)	Total cocoon weight (g)	Single pupal weight (g)	Single shell weight (g)	Floss weight (g)	Effective rate of rearing (%)
Formalin	0.80±0.01 ^b	61.2±4.83 ^a	0.67±0.01 ^b	0.14±0.01 ^a	1.1±0.21 ^a	84.3
Ethanol	0.89±0.06 ^{ab}	62.57±2.43 ^a	0.74±0.06 ^{ab}	0.15±0.00 ^a	0.77±0.03 ^{ab}	83
NaoCl	0.9±0 ^{ab}	58.4±1.8 ^a	0.74±0.01 ^{ab}	0.11±0.05 ^a	0.57±0.07 ^b	84.3
No disinfectant	0.98±0.01 ^a	57±9.4 ^a	0.8±0.02 ^a	0.18±0.01 ^a	0.7±0.15 ^{ab}	72

Means with the same alphabets are not significantly different at $p < 0.05$

DISCUSSION

From this study, it was clear that mortality rate of the insect was being dictated by the use or non-use of disinfectant as the mortality rate was highest in trays with no disinfectant where it was 22% at the completion of the insect larval stage. While it was just 12% in ethanol treated trays. The

reason for this can be attributed to the fact that ethanol when used as disinfectant engenders dehydration of protein and the enzyme to deactivate and prevent bacteria growing (James *et al.*, 1999). The efficacy of the 70% ethanol as a disinfectant is also in consonance with the findings of Pendlington (2001) and Rozalski (1996) that ethanol demonstrates the strongest

disinfecting effect in a concentration of 70% in water.

The results of this study also showed a higher larval weight in insect from the trays where no disinfectant was used as compared to where it was used. This is however in disagreement with findings of Swathi *et al.* (2014) who reported that the use of disinfectant like hydrated lime with bundh powder in high temperature enhances matured larval weight.

Also the number of insect mounted was higher in disinfected trays using ethanol, sodium hypochlorite and formalin though the highest being found in ethanol treated tray than non-disinfected trays. This implies a higher survival of the worms when bed disinfectant was used than when not used and subsequently suggests that the disinfectants (ethanol, Sodium hypochlorite and formalin in that order) might have stimulated the enzyme activity which influences the biochemical contents of the haemolymph of the silkworm which is attributable to the survival. This results are in partial agreement with El-Sayed *et al.* (1990) who stated that disinfectants increased the amount of haemolymph protein of larval instars and in agreement with those of Ghada (2009,2012) evaluated the effects of mulberry leaves supplemented with the botanicals oil on silkworm.

The findings of this study also showed highest total cocoon weight from the insects in the trays where ethanol was used while it was lowest where no disinfectant was used. This indicates a positive effect of ethanol as the best on enhanced cocoon parameters and this is in agreement with findings of Shivashankar (2003) when hydrated lime was used as disinfectant. This probably reveals the influence of ethanol, formalin

and Sodium hypochlorite in that order having hormonal effect on silkworm.

The single pupal weight and shell weight were highest in trays without disinfectant though not significantly different from those of ethanol treated tray. This however negates the findings of Swathi *et al.* (2014) that in all cases where no disinfectant was used, shell weight and pupal weight were lowest.

During silkworm rearing, the effective rate of rearing has often been taken to be an important parameter for judging mulberry variety in respect of rearing performance (Radhakrishnan and Periasamy, 1986). The ERR in the trays with bed disinfectant ranged from 83% to 84.3%, this indicates an encouraging rearing performance from the insect irrespective of the type of disinfectant used and this subsequently engendered good rearing performance.

CONCLUSION

In conclusion, the assessment of different silkworm performance parameters like mortality, larval weight, number of insect mounted, total cocoon weight and effective rate of rearing showed that ethanol and Sodium hypochlorite in that order were better than formalin as disinfectant during silkworm rearing and are therefore recommended as bed disinfectant in place of formalin.

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