



Research Article

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## Assessment of General Disinfectants against Mulberry Silkworm (*Bombyx mori* L.) Diseases and Its Impact on FC<sub>1</sub>×FC<sub>2</sub> Bivoltine Double Hybrid at Farmer's Field Level

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### ABSTRACT

Silkworms are susceptible to a range of pathogenic diseases, which are primarily caused by protozoan, fungal, bacterial and viral agents. These infections can significantly impact the health and productivity of the silkworm population, thereby affecting silk cocoon quality and yield. Prevention of these diseases is very important to get stable cocoon crops. Many general disinfectants are used to disinfect the silkworm rearing house at farmers as a precautionary measure. In the present study, assessment of widely accepted Astra, Sanitech, Bleaching powder and Formalin general disinfectants was conducted against mulberry silkworm diseases and its impact on FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid rearing at farmer's field level. The results revealed that, Bleaching powder solution sprayed rearing house batch has shown best results in minimum larval duration (22.06 days), 10 matured larval weight (45.3 g), highest shell ratio (22.62%), highest yield/ 100 DFLs (78 kg) and rate kg<sup>-1</sup> cocoons (Rs. 513.00). Astra, Sanitech and Formalin sprayed silkworm rearing batches occupies second, third and fourth places, respectively. The clean water sprayed rearing batches have shown very poor cocoon qualitative and quantitative parameters. The bleaching powder sprayed could able to suppress diseases intensity and maintained healthy environment because of which the silkworms were healthy. Based on the perusal of the results obtained, bleaching powder is suggested for the disinfection of the rearing houses.

### How to Cite:

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### INTRODUCTION

Diseases are characterised as abnormalities in an organism's normal behaviour and physiological processes, usually brought on by pathogenic agents.

Every disease has its own unique signs and symptoms. Similarly, protozoa, fungus, bacteria, and viruses can cause a variety of diseases in silkworms.

Since these illnesses have a substantial financial impact on the sericulture sector, it is imperative that efficient preventive and control measures be put in place in order to reduce losses and maintain output (Sisodia *et al.*, 2019). The diseases found in silkworms are either chronic or acute in nature depending on the pathogens involved. Silkworm larvae may contract an infection from mulberry leaves or other sources during the rearing phase (Baig *et al.*, 1993). In all the main silk-producing nations, there is a high incidence of infections brought on by different pathogens and subsequent crop losses as a result of illnesses. These losses are anticipated to be between 15 and 20 kg per unit of 100 disease-free layings, which makes up almost 30% of the entire output loss (Rasool *et al.*, 2018). The crop must be effectively protected from disease-causing pathogens in order to succeed the silkworm rearing (Manochaya and Kumar, 2010) and commercial success of any breed depends on its tolerance to diseases under adverse conditions (Anonymous, 2013). The larvae show signs of sluggishness and stop eating in the early stages of illness (Chairman *et al.*, 2012). Numerous reports abound that describe instances of bacterial Grasserie and Flacherie (Sen *et al.*, 2003; Kaewwises, 2006). Pathogens are known to induce various biochemical and physiological changes in insect tissues, contributing to an estimated loss of 30-40% in productivity. Research has established that multivoltine breeds exhibit greater resistance to these pathogens compared to bivoltine breeds (Anonymous, 1973; Sen *et al.*, 1997; Samson, 1998; Malik *et al.*, 2006; Venugopal *et al.*, 2012). As there are no foolproof curative measures, these diseases are best prevented through proper disinfection of rearing houses, surroundings, rearing appliances and sterilization of larval surface and rearing bed, by application of various bed disinfectants. Disinfection is a critical practice in silkworm rearing, aimed at eliminating disease-causing pathogens from the rearing environment. This process involves the application of chemical disinfectants through spraying prior to the commencement of rearing, as well as the dusting of disinfectants onto the rearing beds during the rearing process. Various

disinfectants are utilized in differing concentrations and the quantity to be applied varies based on factors such as the floor area, height and type of rearing house, as well as the specific rearing method employed. Numerous disinfectants, including Formalin, Bleaching powder, Chlorine dioxide (Sanitech), Decol and Astra, are available for use in disinfecting rearing houses. In this present study, assessment of certain general disinfectants against mulberry silkworm diseases and its impact on FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid at farmer's field level was aimed.

## MATERIALS AND METHODS

### *Place of the Study*

A traditional sericulture village namely, Yenumuladoddi of Kundurpi mandal (Lat. 14.2833° N and Long. 77.0333° E) belonging to Kalyandurg Cluster Promotion Programme (CPP) Mega cluster where all technologies of sericulture are disseminated through the Extension Communication Programmes and Farmers Skill Training (FST) under Capacity Building Programme by Central Silk Board. The Yenumuladoddi village has the mulberry gardens to the extent of 100 acres and 50 silkworm rearing sheds. Five farmers in the village were selected whose rearing houses were almost similar in 50'×20'×16' measurements along with 10 feet verandah in L-shape and well maintained two acres of V<sub>1</sub> mulberry bush gardens.

### *Source and Silkworm Hybrid*

250 DFLs chawki silkworm larvae of FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid per each farmer were procured from same private CRC, Ligadahalli village and distributed to the selected 5 farmers on the same day in the month of June, 2022.

### *General Disinfectants Selected for the Study*

T<sub>1</sub> (Astra): Astra is a user-friendly compound developed by the Central Sericultural Research and Training Institute, part of the Central Silk Board in Mysore. This disinfectant is specifically designed for use in silkworm rearing houses and their surroundings. As a chlorine-based spray disinfectant,

Astra demonstrates effectiveness against a wide range of silkworm pathogens, even at low concentrations of 0.05% (Venugopal *et al.*, 2012).

*Preparation of 0.05% solution:* 50 g of Astra powder is dissolved in 100 liters of water. To guarantee total breakdown of the powder and the release of chlorine from the solution, the resultant mixture is thoroughly agitated for 5-10 minutes and then left to stand for 1 hour. This solution can be used to disinfect the raising house and related rearing equipment after it is prepared.

T<sub>2</sub> (*Sanitech*): It is a stabilized chlorine dioxide (20,000 ppm) with activator crystals. Gaseous chlorine dioxide is known to be one of the most effective antibacterial agents. Because of its well-established effectiveness as a hard surface disinfectant, it's a great option for maintaining hygienic conditions in a variety of settings. It has been stabilized into solution form which is very effective disinfectant against pathogens causing diseases in silkworms. Like oxygen, chlorine dioxide is soluble in water and exists in solution as a gas. It works *via* chemical oxidation, just like ozone and doesn't produce any by-products that are chlorinated organic. The efficacy of stabilised chlorine dioxide is maintained throughout a broad pH range. In comparison to chlorine, this type of chlorine dioxide is thought to be a better disinfectant since it is more stable, about 2.5 times more effective than chlorine, and 50 times more effective than hypochlorites. It is a biotoxic agent causing the membrane damage. It reacts with nucleotide and amino acid moieties of the germs. Recommendation of Sanitech solution spray is 140 ml ft<sup>-2</sup> or 1.5 l m<sup>-2</sup> floor area.

T<sub>3</sub> (*Bleaching powder*): Bleaching powder is a mixture of calcium hypochlorite, basic chloride, calcium hydroxide and free slaked lime. The active ingredient is hypochlorite. Chlorine dioxide is a white, amorphous powder that smells strongly like chlorine. To effectively disinfect, a composition with a 30% active chlorine level must be used. Hypochlorite in combination with weak acid (CO<sub>2</sub> in air) and water produces hypochlorous acid and hydrochloric acid. Hypochlorous acid is unstable

and resolves into hydrochloric acid and nascent oxygen. The oxidation reaction by nascent oxygen is germicidal. Hydrochloric acid also aids in the release of chlorine, which is also germicidal. Chlorine alters the membrane permeability of cells allowing the macromolecules such as proteins and nucleic acids to leak out. The main component of chlorinated lime, Calcium hydroxide is germicidal which dissolves the polyhedron protein to release and disintegrate virions. Because bleaching powder works best in damp environments, it's critical to completely saturate the surfaces of walls and equipment with the solution to provide the best possible disinfection.

To make 100 litres of a 3% bleaching powder solution, first we make a paste using 3 kg of bleaching powder and a modest amount of water. Then we stir well after adding this paste to the leftover water. After letting the mixture settle for 10 minutes, we use the supernatant to disinfect objects.

T<sub>4</sub> (*Formalin*): Formaldehyde in water is formalin and is known as formic aldehyde, methyl aldehyde, ethylene oxide, oxomethane and oxymethylene. It is a colourless gas and usually available as a 36% solution in water and methanol. It is clear and colourless solution with irritating odour, pungent and suffocating. Formaldehyde, the active ingredient in formalin solution is a strong reducing agent. It forms formic acid utilizing the oxygen from the cells leading to the death of the germ. Formalin is effective exclusively in rearing houses that can be sealed airtight, at high humidity (70%) and temperature of above 20 °C. The gaseous nature of the disinfectant demands a closed condition for a longer period. It was till recently most used disinfectant in sericulture but its use is limited as it is reported to be a carcinogen and is classified as hazardous chemical. Formaldehyde is highly effective at 2% concentration.

The amount of formalin required to prepare a particular volume of a 2% formalin solution is calculated by dividing the total intended volume of the solution by 18. To reach the required 2% concentration, we add water at a ratio of 17 parts to

1 part formalin. As an alternative, we might mix 17 parts water to 1 part formalin.

T<sub>5</sub> (Water treatment): Cleaning of the rearing house, Verandha and rearing house surroundings were cleaned with clean water without disinfectant and it is treated as control.

**Note:** Solution required for cleaning the rearing house, verandah and all around the rearing house. Disinfection solutions of required concentrations were prepared as illustrated in the table 1.

*Schedule of disinfection:* Three times the disinfection was scheduled and performed.

*First time:* After immediate completion of the rearing house.

*Second time:* 10 days prior to commencement of the rearing house.

*Third time:* 3 days before commencement of the rearing house.

**Observations Recorded**

*Weight of Mature Larvae (g)*

On the day prior to spinning, ten fully developed larvae were randomly selected from each shoot and weighed.

**Table 1:** The details of various general disinfectants taken for the study

Treatment	Concentration of the disinfectant	Solution required	Disinfectant required	Cost/ 1 disinfection	Cost/ 3 disinfections
T <sub>1</sub> : Astra	0.05% solution	400 liters	400 liters of water is combined with 200 g of Astra powder	Rs. 400.00	Rs. 1,200.00
T <sub>2</sub> : Sanitech	2.50% + activator	400 liters	10 liters of Sanitech and 1 kg of activator	Rs. 1,100.00	Rs. 3,300.00
T <sub>3</sub> : Bleaching powder	3.00%	400 liters	12 kg of bleaching powder	Rs. 360.00	Rs. 1,080.00
T <sub>4</sub> : Formalin	2.00%	400 liters	24 liters of formalin	Rs. 1,200.00	Rs. 3,600.00
T <sub>5</sub> : Control water treatment	Fresh water	400 liters	NA	NA	NA

*Duration of the Fifth Instar Larva (hours)*

The duration of the larval stage was recorded from the onset of the first instar until the point at which 50% of the silkworms' commenced spinning.

*Cocoon Weight (g)*

Approximately 10 cocoons were randomly selected from each shoot on the sixth day after spinning, and their individual weights were recorded. The average weight of the cocoons was subsequently calculated.

*Shell Weight (g)*

For shell weight determination, 10 cocoons were randomly selected from each shoot, and the weight of the cocoon shells was recorded following the removal of the pupae from the cocoons.

*Shell Ratio (%)*

The shell ratio was estimated using the formula that relates the shell weight to the corresponding cocoon weight,

$$\text{Shell Ratio} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

*Yield/ 100 DFLs (kg)*

Yield per 100 DFLs was calculated based on the following formula,

$$\text{Yield/ 100 DFLs (kg)} = \frac{\text{Total weight of the cocoons harvested}}{\text{No. of DFLs brushed}}$$

### Diseases Identification

Diseased larvae were collected from different selected rearing batches during the fifth instar and identified the particular disease based on morphological symptoms.

## RESULTS AND DISCUSSION

### Total Larval Duration of Silkworms Reared in Rearing Houses Disinfected with Different General Disinfectants

Minimum larval duration (22.06 days) was observed in that batch where the disinfection of the rearing house is carried with T<sub>3</sub>: Bleaching powder solution, followed by T<sub>1</sub>: Astra treated rearing house batches

(22.18 days) and maximum larval duration (24.12 days) was observed in that batch where only clean water sprayed rearing house (Control) (Table 2). Surapwar *et al.* (2019) used bed disinfectants like Vijetha, Labex and Ankush have shown influence on larval duration. It can be assumed that, the influence of disinfectants on larval duration may be because of orosius feeding pattern in good healthy environment and obtained maturation of spinning at timely. In contrast to this, unhealthy environment might have reduced the eating pattern resulted little higher larval duration for their maturation. Similar trends were observed by Swathi *et al.* (2014) and Jawale and Tayade (1987), when the bed was treated with certain bed disinfectants.

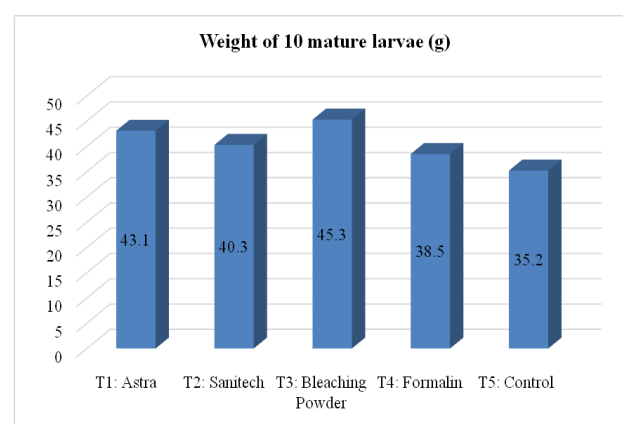
**Table 2:** Impact of various general disinfectants on the rearing performance of FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid of mulberry silkworm (*Bombyx mori* L.) at farmers' level

Treatments	Weight of 10 mature larvae (g)	Mean larval duration (in days-hours)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Yield/ 100 DFLs (kg)	Rate kg <sup>-1</sup> (Rs.)
T <sub>1</sub> : Astra	43.1	22.18	1.85	0.40	21.62	73	506
T <sub>2</sub> : Sanitech	40.3	23.12	1.78	0.36	18.75	69	472
T <sub>3</sub> : Bleaching Powder	45.3	22.06	1.92	0.38	22.62	78	513
T <sub>4</sub> : Formalin	38.5	23.20	1.68	0.34	20.24	70	468
T <sub>5</sub> : Control	35.2	24.12	1.34	0.24	17.91	47	423

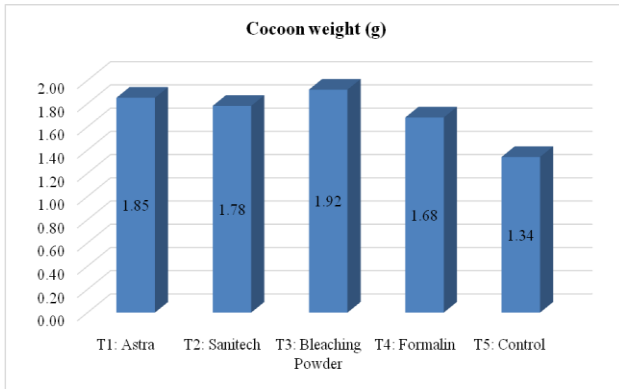
### 5<sup>th</sup> Instars Larval Weight of FC<sub>1</sub>×FC<sub>2</sub> Bivoltine Double Hybrid Silkworms Reared in Rearing Houses Disinfected with Different General Disinfectants

The results showed that significantly higher 10 larval weight (45.3 g) of fifth instar of double hybrid (FC<sub>1</sub>×FC<sub>2</sub>) was observed in that batch where the disinfection of the rearing house was carried with T<sub>3</sub>: Bleaching powder solution, followed by T<sub>1</sub>: Astra treated rearing house batches (43.1 g) and minimum 10 larval weight (35.2 g) was observed in that batch where only clean water sprayed rearing house (Control) (Table 2 and Figure 1). However, slight improvement was observed in larval weight in those batches where the rearing houses disinfected with Astra, Sanitech and Formalin general disinfectants. Sivaprakasam (1999), Anonymous

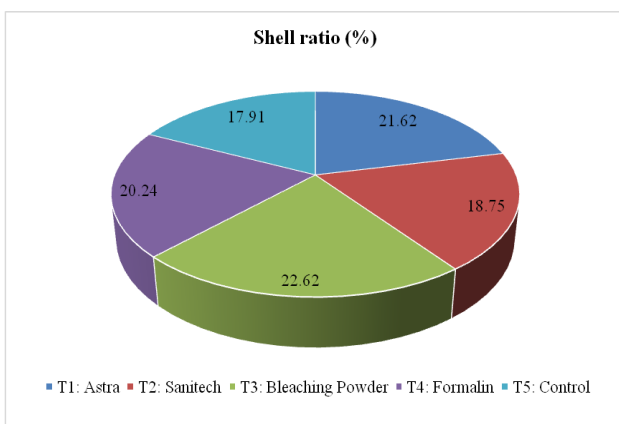
(2002) and Sujatha *et al.* (2007) were also noticed increase in larval weight with larvae treated with bed disinfectants.



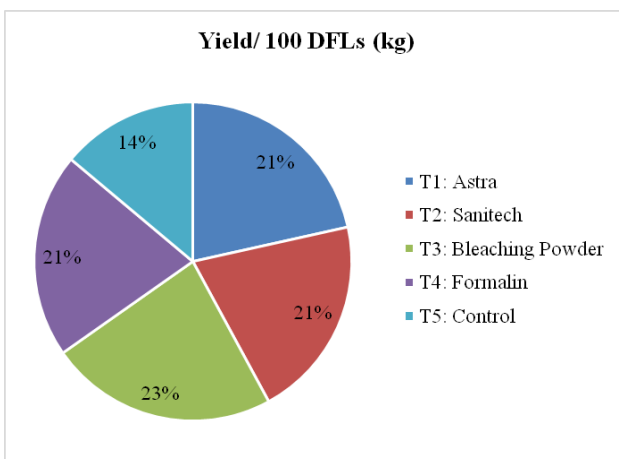
**Figure 1:** Weight of FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid 10 mature larvae



**Figure 2:** FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid single cocoon weight in grams



**Figure 3:** FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid cocoon shell ratio under the different treatments



**Figure 4:** Yield/ 100 DFLs of FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid

### Cocoon Weight

The effects of different general disinfectants in double hybrid mulberry silkworm race (FC<sub>1</sub>×FC<sub>2</sub>)

are illustrated in table 1 and figure 2. The results unveiled that the highest weight of the cocoon (1.92 g) was observed in that batch where the disinfection of the rearing house was carried with T<sub>3</sub>: Bleaching Powder solution, followed by T<sub>1</sub>: Astra treated rearing house batches (1.85 g) and minimum cocoon weight (1.85 g) was observed in that batch where only clean water sprayed rearing house (Control). Manimegalai and Subramaniam (2000) and Sivaprakasam (1999) also noticed increase in single cocoon weight when they treated the silkworm rearing batches with certain recommended bed disinfectants and the same trend was noticed here in this experiment also.

### Cocoon Shell Ratio

The results revealed that, the highest shell ratio (22.62%) was observed in that batch where the disinfection of the rearing house was carried with T<sub>3</sub>: Bleaching Powder solution, followed by T<sub>1</sub>: Astra treated rearing house batches (21.62%) and minimum SR% (17.91%) was observed in that batch where only clean water sprayed rearing house (Control) (Table 2 and Figure 3). Manimegalai and Subramaniam (2000) and Sivaprakasam (1999) also noticed increase in SR percentage when they treated the silkworm rearing batches with certain recommended bed disinfectants.

### Yield/ 100 DFLs (kg)

The effects of various general disinfectants on yield of double hybrid mulberry silkworm race (FC<sub>1</sub>×FC<sub>2</sub>) are illustrated in table 2 and figure 4. The results unveiled that the highest yield/ 100 DFLs (kg) (78 kg) was observed in that batch where the disinfection of the rearing house was carried with T<sub>3</sub>: Bleaching powder solution, followed by T<sub>1</sub>: Astra treated rearing house batches (73 kg) and minimum yield per 100 DFLs was noticed in that batch where only clean water sprayed rearing house (Control). Yield/ 100 DFLs is very important commercial parameter when batches obtain highest yield definitely there will be reduction in diseases incidence which directly affects the income the farmer.

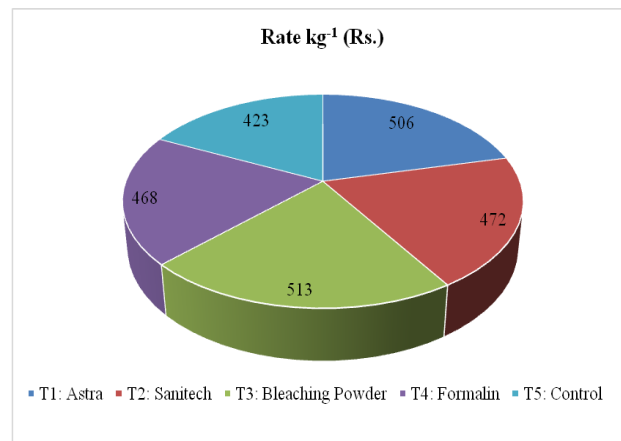
### Rate kg<sup>-1</sup> Cocoons in the Cocoon Market

The rate kg<sup>-1</sup> cocoons was observed high in the case of treatment T<sub>3</sub> with Rs. 513.00, followed by T<sub>1</sub> with Rs. 506.00, T<sub>2</sub> with Rs. 472.00 and least price was observed in the case of control batches with the rate of Rs. 423.00 kg<sup>-1</sup> of cocoons (Figure 5), it is because of inferior quality of cocoons which has suffered with presence of grasserie, viral and bacterial flacherie diseases which has affected the quantitative as well as qualitative cocoon parameters.

### Disease Symptoms Shown in Different Treatments

The five farmers rearing houses were disinfected with different commercially exploited general disinfectants like Astra, Sanitech, Bleaching powder and Formalin, in all the rearing batches different diseases like grasserie, viral and bacterial flacherie

were noticed in mulberry silkworms when they obtained to 5<sup>th</sup> instar (Table 3), but the intensity of the disease incidence was vastly varied to one another treatments.



**Figure 5:** FC<sub>1</sub>×FC<sub>2</sub> bivoltine double hybrid cocoon rate kg<sup>-1</sup> (Rs.)

**Table 3:** Disease symptoms shown in different treatments

Treatments	Grasserie	Viral Flacherie	Bacterial Flacherie	Muscardine	Pebrine
T <sub>1</sub> : Astra	Yes	No	No	No	No
T <sub>2</sub> : Sanitech	No	Yes	No	No	No
T <sub>3</sub> : Bleaching powder	No	No	No	No	No
T <sub>4</sub> : Formalin	Yes	No	Yes	No	No
T <sub>5</sub> : Control	Yes	Yes	Yes	No	No

T<sub>1</sub>: Astra disinfected rearing house silkworm batch was found certain silkworm showing the symptoms like inter segmental bulging, turbid white milky haemolymph oozing out when the larvae are ruptured, which are characteristic symptoms of grasserie disease (Kaewwises, 2006). Exact number and disease intensity can't be calculated because of high quantity of 250 DFLs rearing but it has shown adverse impact on cocoon yield/ 100 DFLs and rate of the 1 kg cocoons when the cocoons were sold in cocoon market.

T<sub>2</sub>: Sanitech disinfected rearing house silkworm batch was found certain silkworm showing only viral flacherie but remaining silkworm diseases were not found during the 5<sup>th</sup> instar.

T<sub>3</sub>: Bleaching powder solution disinfected rearing house silkworm batch didn't find any silkworm

showing any kind of diseases symptoms hence, these batches have shown higher single cocoon weight, shell weight, shell ration, yield per 100 DFLs which indicated the high quality resulting higher cocoon price.

T<sub>4</sub>: Formalin solution disinfected rearing house silkworm batch have shown the symptoms of grasserie and bacterial flacherie. These treatment batches has shown low qualitative and quantitative cocoon parameters next to the control, it may be because of improper disinfection with formalin since, this disinfectant is pungent and limitations to be effective in windows and doors in open conditions.

T<sub>5</sub>: Clean water spray rearing house silkworm batch have shown grasserie, viral and bacterial flacherie

diseases symptom in rearing and affected largely on cocoon yield and cocoon rate.

Muscadine and Pebrine diseases were not found in any above mentioned treatments including control; it is because of the season, this experiment was carried out in the month of June, 2022. Hence, low temperature and high humidity could not find during this season in this experimented Yenumaldoddy village. So, Muscadine disease silkworms were not found, at the same time Pebrine disease was also not noticed which confirms strict examination of Mother moth examination for the detection of Pebrine disease at grainage level.

## CONCLUSION

The disinfection is an essential part of silkworm rearing for successful cocoon crop. Among all the general disinfectants namely Astra, Sanitech, Bleaching powder, Formalin taken for the study Bleaching Powder have shown very good results. The bleaching power sprayed could able to suppress diseases intensity and maintained healthy environment because of which the silkworms are healthy and feed orosiously resulting high in cocoon parameters, high cocoon yield and high rates also at the same time the amount spent on disinfection with the bleaching powder solution is less compared with the other general disinfectants tested. Bleaching powder is available in almost all villages and easy to procure. The bleaching powder spray causes corrosive to the iron-shoots in the rearing house but it can be managed with one moth prior painting of the shoot before usage will solve the corrosive of the shoots. Based on the perusal of the results obtained, Bleaching powder is suggested for the sanitization of the rearing houses.

## Conflict of Interest

The authors declare no conflict of interest.

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