A new research project proposal

ARP-3630: Development of room and silkworm bed disinfectant through screening of potential chemicals

June 2018 - May 2021
(3 years)

Submitted to -

Central Silk Board
Ministry of Textiles: Government of India
Bangalore

By

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PROFORMA – I

PROFORMA FOR SUBMISSION OF PROJECT PROPOSALS ON RESEARCH AND DEVELOPMENT, PROGRAMME SUPPORT

(To be filled by the applicant)

PART I: GENERAL INFORMATION

1.	**Name of the Institute/ University/ Organisation submitting the Project Proposal**	:	Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore, Murshidabad, W.B.-742101, India.
2.	**Status of the Institute(s)**	:	Govt.
3.	**Name and designation of the Executive Authority of the Institute/University forwarding the application**	:	Dr. Kanika Trivedy, Director
4.	**Project Title**	:	Development of room and silkworm bed disinfectant through screening of potential chemicals
5.	**Category of the Project**	:	Research and Development
6.	**Specific area**	:	Silkworm Pathology
7.	**Duration**	:	June 2018 to May 2021 (3 years)
8.	**Total cost**	:	Rs. 10.0 Lakh
9.	**Is the project Single Institutional or Multiple-Institutional (S/M):**	:	Single - Institutional
10.	**If the project is multi-institutional, please furnish the following: Name of Project Co-ordinator Affiliation & Address**	:	Not applicable
11.	**Project Summary**	:	Since the success of silkworm crop lies to a great extent on the extent of silkworm mortality due to diseases, disinfection is of paramount importance in reducing the possibility of disease incidences. Two types of disinfection is practiced in sericulture – disinfection of rearing room, its surrounding areas and rearing implements before and after each rearing and the second type of disinfection through application of bed disinfectants during the course of rearing. Disinfection before and after each rearing is

			ensured to bring down the pathogen load in the rearing room to minimum before the onset of rearing and also to break the multiplication cycle of the disease causing pathogens from the preceding rearing. However, during rearing, the outbreak of diseases is closely related to the hygienic conditions of the rearing beds. Pathogens are excreted by the silkworm along with the faeces and these contaminate the mulberry leaves. When these mulberry leaves are eaten by the healthy larva, disease spread. So, rearing bed is the main source for propagation of the pathogens through secondary infection and hence the disinfection of rearing bed is also very essential. Silkworm suffers both from infectious and non-infectious diseases. The infectious diseases are caused by different types of pathogens viz. virus, bacteria, fungi and microsporidia which causes bacteriosis, virosis, mycosis and microsporidiosis respectively. Among these diseases maximum mortality is caused by Nuclear Polyhedrosis, a viral disease caused by *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV), a Baculovirus (an occluded virus). The extent of loss due to this disease can go up to 40%. Several room and bed disinfectants have been developed by this Institute and elsewhere. However, of late several reports are being received from farmers' field which suggests a reduction in their efficacy for controlling the silkworm diseases. ***The reason for such reduction in the efficacy of different chemicals may be attributed mainly to development of immunity by different microorganisms (Pepper et al. 2015).*** Hence, through this project it is attempted to develop a room disinfectant and a silkworm bed disinfectant with special emphasis on control of viral diseases.

PART II: PARTICULARS OF INVESTIGATORS

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Chief Executive Authority

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14. **Research Fellow:** 1 (one)

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PART III: TECHNICAL DETAILS OF PROJECT

16.1. Definition of the Problem

Disinfection and hygiene are integral part of silkworm rearing which plays a very important part in elimination of pathogens and thereby reducing pathogen load in the environment before the onset of rearing, during the course of rearing and after completion of the rearing. Although a pathogen free environment is desired before and after each rearing with proper disinfection of rearing room, its appliances/equipments and the surrounding areas, yet the chances of mortality of silkworms due to infectious disease causing pathogens during the course of rearing remains very high, more so during unfavourable conditions. Improper and incomplete disinfection of the rearing room compounds to the problem of silkworm mortality arising during the course of rearing through secondary contamination from increased pathogen load on the rearing trays. Hence, proper disinfection of the rearing room as well as disinfection of the rearing tray through use of bed disinfectants become pronounced, more so during conditions of high temperature and high humidity. In tropical countries like India, almost all the major categories of pathogenic microbes infect and cause disease in silkworm (Rajsekhar *et al.*,1992: Doreswamy *et al.*, 2004). The most common diseases of silkworm are grasserie, caused by a virus; flacherie caused by both virus and bacteria; muscardine caused by fungi; and pebrine, a microsporidian parasitic disease. The diseases prevail throughout the year, and in the tropics they are significantly high (Srivastava and Kumar, 2009). Crop loss from silkworm diseases, either partial or complete, is common

in all the silkworm rearing areas in India, consequently affecting the national plan for silkworm eggs and raw silk production and also reducing personal income. Among the diseases, mortality due to grasserie in comparison to other diseases has always been an impending challenge to sericulture industry, where severe losses up to 55% have been reported (Nataraju *et al.* 1998). Dandin *et al.* (2000) reported the nuclear polyhedrosis prevails throughout the year and found more during summer and rainy season. Nataraju *et al.* (2004) reported silkworm crop loss to the tune of 22 to 43 per cent due to flacherie. Reddy & Rao (2009) have reported mortality due to muscardine around 20-23% in winter. In West Bengal during the commercial crops of 2016-17 silkworm disease incidence of flacherie up to 1.45 %, grasserie 2 - 10.16 %, gattine 2.5 - 12.13 % and muscardine below 1% was reported. During the Seed Crops for the same period, incidence of all the diseases were reported below 1% except grasserie which was up to 2.76% (2016-17 Annual Report, CSR&TI Berhampore; *in press*).

16.2. Origin and Introduction of the problem

Silkworm like any other lepidopteran insects suffer from diseases caused by various group of pathogens viz. virus, bacteria fungi and microsporidia causing virosis, bacteriosis, mycosis and microsporidiosis respectively. Success of the crop depends on proper management and protection of silkworm from these diseases. Diseases are the major yield limiting factors in silkworm crop. There is no curative measure available for these diseases and even though where some are available, those are not economically viable. Hence, a several pronged integrated disease management strategy has been evolved for controlling these diseases. These integrated measures comprises of ensuring a disease free stock, disinfection of rearing room and appliances, application of rearing seat or bed disinfectants, sanitation, personal hygiene, cleaning and or removal of contaminated units, development of disease tolerant breeds/hybrids, regular survey and surveillance of silkworm diseases and control of mulberry pests including alternative pests. Among these measures two major factors which are directly related to the extent of disease incidences are the disinfection of rearing room and rearing appliances, before and after each rearing, and application of chemicals in the form of 'rearing seat/bed disinfectants' during the course of rearing. Proper disinfection of rearing room, its appliances and the surrounding vicinity of the rearing room ensures a pathogen free environment at the beginning of the rearing and controls the same after the completion of rearing, as pathogen loads starts gradually developing once the rearing starts. The pathogen build up, due to transmission of diseases from diseased larva to the healthy larva during the course of rearing is controlled through use of bed disinfectants during the course of rearing. Since, the temperature and humidity plays an important role on the multiplication of pathogens, the importance of room and bed disinfectant(s) becomes more pronounced, more so in the tropics.

Several room and bed disinfectants have been developed by this Institute and elsewhere. However, these disinfectants/bed disinfectants have certain limitations in their usage. Moreover, of late, several reports are being received from farmers' field which suggests a reduction in their efficacy for controlling the silkworm diseases. One possible reason for this reduced efficacy of the disinfectant could be the development of tolerance of the pathogens through repeated use of these chemicals over a period of time. Hence, a possibility may be explored for development of a new room disinfectant and a bed disinfectant which acts strongly against all the pathogens and specially against *Bombyx mori* Nuclear Polyhedrosis Virus (*Bm*NPV) which causes the maximum damage.

16.3. Expected Outcome

This project envisages development of a cost effective and user-friendly a new room disinfectant and a bed disinfectant which acts strongly against all the pathogens and specially against *Bombyx mori* Nuclear Polyhedrosis Virus (*Bm*NPV) which causes the maximum damage.

16.4. Objectives:

- To screen several potential chemicals based on their efficacy of controlling microbial diseases.
- To develop a broad spectrum room disinfectant for eradication of pathogens causing diseases in silkworm
- To develop a silkworm bed disinfectant comprising of compatible disinfectant chemicals having synergistic action in controlling all microbial diseases of silkworm, specially grasserie (nuclear polyhedrosis).

17. Review of status of research and development on the subject

17.1. International Status:

Lots of information is available on the role of several chemicals as disinfectants and several disinfectants have also been developed world wide, as disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. Information on the importance of some important chemicals and their role as disinfectants is given below:

- ***Lime:*** Lime is listed as an effective disinfectant in many national regulations or guidelines (Germany, France, Austria, Switzerland, UK) and is recommended as an *in situ* disinfectant on a regular basis and in case of epidemic outbreaks such as Foot and Mouth disease (Official website of the Deptt. of Veterinary Services, Malaysia), Aujeszky's Disease (Koch and Euler, 1984), and African Swine Fever (FAO Animal Health Manual, 2000). Scientific research conducted in 2007 by the Institut Pasteur de Lille has demonstrated that the H5N1 virus is effectively and rapidly (within 5 minutes at 4°C) inactivated by lime (Deboosere *et al.*2008). This inactivation is due to the pH increase brought about by lime (Turner and Williams, 1999). Lime is effective against a range of bacteria and is, therefore, ideal for use wherever a high standard of hygiene is essential.
- ***Alcohols:*** Although several alcohols have been shown to be effective antimicrobials, ethyl alcohol (ethanol, alcohol), isopropyl alcohol (isopropanol, propan-2-ol) and n-propanol (in particular in Europe) are the most widely used (Morton, 1983). Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal. They are, however, known to inhibit sporulation and spore germination (Yasuda-Yasuki *et al.*, 1978), but this effect is reversible (Trujillo and Laible, 1970). Because of the lack of sporicidal activity, alcohols are not recommended for sterilization but are widely used for both hard-surface disinfection and skin antisepsis.
- ***Aldehydes:***
  1. **Glutaraldehyde** is an important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment and as a fixative in electron microscopy. Glutaraldehyde has a broad spectrum of activity against bacteria and their spores, fungi, and viruses, and a considerable amount of information is now available about the ways whereby these different organisms are

inactivated. Earlier reviews of its mechanisms of action have been published (Gorman and Scott, 1977; Gorman *et al.*, 1980; Power, 1995; Scott and Gorman, 1991). The bactericidal studies of glutaraldehyde demonstrated (Power, 1995) a strong binding of glutaraldehyde to outer layers of organisms such as *E. coli* and *Staphylococcus aureus* (Gorman and Scott, 1977; Hughes and Thurman, 1970; Munton and Russell, 1970, 1971; Munton and Russell, 1973), inhibition of transport in gram-negative bacteria (Gorman and Scott, 1977), inhibition of dehydrogenase activity (Munton and Russell, 1973), inhibition of RNA, DNA, and protein synthesis (McGucken and Woodside, 1973) etc. There are no recent studies of the mechanisms of fungicidal action of glutaraldehyde. Earlier work had suggested that the fungal cell wall was a major target site (Gorman and Scott, 1977; Gorman *et al.*, 1980; Navarro and Monsan, 1976), especially the major wall component, chitin, which is analogous to the peptidoglycan found in bacterial cell walls. Glutaraldehyde is a potent virucidal agent (Favero and Bond, 1991; Kobayashi *et al.,* 1984).

2. **Formaldehyde** (methanal, CH_2O) is a monoaldehyde that exists as a freely water-soluble gas. Formaldehyde solution (formalin) is an aqueous solution containing *ca.*34 to 38% (wt/wt) CH_2O with methanol to delay polymerization. Its clinical use is generally as a disinfectant and sterilant in liquid or in combination with low-temperature steam. Formaldehyde is bactericidal, sporicidal, and virucidal, but it works more slowly than glutaraldehyde (Power,1995; Scott and Gorman, 1991). It interacts with protein (Fraenkel-Conrat *et al.*, 1945, Fraenkel-Conrat and Olcott, 1946), DNA (Fraenkel-Conrat, 1961), and RNA (Fraenkel-Conrat, 1961) *in vitro*. It is difficult to pinpoint accurately the mechanism(s) responsible for formaldehyde-induced microbial inactivation. Clearly, its interactive and cross-linking properties must play a considerable role in this activity.

3. ***o*-Phthalaldehyde** (OPA) is a new type of disinfectant that is claimed to have potent bactericidal and sporicidal activity and has been suggested as a replacement for glutaraldehyde in endoscope disinfection (Alfa and Sitter, 1994). OPA is an aromatic compound with two aldehyde groups. To date, the mechanism of its antimicrobial action has been little studied, but preliminary evidence (Walsh, *et al.,* 1997) suggests an action similar to that of glutaraldehyde. Further investigations are needed to corroborate this opinion.

- ***Biguanides:***

1. **Chlorhexidine** is probably the most widely used biocide in antiseptic products, in particular in handwashing and oral products but also as a disinfectant and preservative. This is due in particular to its broad-spectrum efficacy, substantivity for the skin, and low irritation. Of note, irritability has been described and in many cases may be product specific (Gardner and Gray, 1991; Rosenberg *et al.* 1976). Despite the advantages of chlorhexidine, its activity is pH dependent and is greatly reduced in the presence of organic matter (Russell and May, 1993). Chlorhexidine is a bactericidal agent (Denyer, 1995; Hugo, 1999). Chlorhexidine was claimed by Harold *et al*. (199) to be an inhibitor of both membrane-bound and soluble ATPase as well as of net K^+ uptake in *Enterococcus faecalis*. Although chlorhexidine collapses the membrane potential, it is membrane disruption rather than ATPase inactivation that is associated with its lethal effects (Barett-Bee, 1994, Kuyyakanond and Quesnel, 1992). Work to date suggests that chlorhexidine has a similar effect on the trophozoites of *Acanthameoba castellanii*, with the cysts being less sensitive (Khunkitti *et al,* 1996, 1997,

1998). The antiviral activity of chlorhexidine is variable. Chlorhexidine is not always considered a particularly effective antiviral agent, and its activity is restricted to the lipid-enveloped viruses (Park and Park, 1989).

2. **Alexidine** differs chemically from chlorhexidine in possessing ethylhexyl end groups. Alexidine is more rapidly bactericidal and produces a significantly faster alteration in bactericidal permeability (Chawne and Gilbert, 1989).

➢ ***Halogen releasing agents:***

1. **Chlorine-releasing agents.** Excellent reviews that deal with the chemical, physical, and microbiological properties of chlorine-releasing agents (CRAs) are available (Bloomfield, 1996); Dychdala, 1991). The most important types of CRAs are *sodium hypochlorite*, *chlorine dioxide*, and the N-chloro compounds such as *sodium dichloroisocyanurate* (NaDCC), with *chloramine-T* being used to some extent. Sodium hypochlorite solutions are widely use for hard-surface disinfection (household bleach) and can be used for disinfecting spillages of blood containing human immunodeficiency virus or HBV. NaDCC can also be used for this purpose and has the advantages of providing a higher concentration of available chlorine and being less susceptible to inactivation by organic matter. Although CRAs have been predominantly used as hard-surface disinfectants, novel acidified sodium chlorite (a two-component system of sodium chlorite and mandelic acid) has been described as an effective antiseptic (McDonnell and Russell, 1999). CRAs are highly active oxidizing agents and thereby destroy the cellular activity of proteins (Bloomfield, 1996); potentiation of oxidation may occur at low pH, where the activity of CRAs is maximal, although increased penetration of outer cell layer may be achieved with CRAs in the unionized state. Deleterious effects of CRAs on bacterial DNA that involve the formation of chlorinated derivatives of nucleotide bases have been described (Dennis *et al.* 1979; Dukan and Touati, 1996; Shih and Lederberg, 1996). At 50 mM (2.6 ppm), HOCl completely inhibited the growth of *E. Coli* within 5 min, and DNA synthesis was inhibited by 96% but protein synthesis was inhibited by only 10 to 30%. Because concentrations below 5 mM (260 ppm) did not induce bacterial membrane disruption or extensive protein degradation, it was inferred that DNA synthesis was the sensitive target. In contrast, chlorine dioxide inhibited bacterial protein synthesis (Benarde *et al.*1967). CRAs at higher concentrations are sporicidal (Bloomfield and Arthur, 1992; Russell, 1994, Russell and Day, 1996); this depends on the pH and concentration of available chlorine (Rudolf and Levine, 1941; Russell, 1982). CRAs also possess virucidal activity (Best *et al.* 1974, Bloomfield *et al.* 1990, Dennis *et al.* 1979; Mbithi *et al.*1990, Rubin, 1991, Sattar *et al.*1994; Springthorpe and Satter, 1990).

2. **Iodine and Iodophors.** Although less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal (Gottardi, 1991). Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures (Rutala, 1995). Iodine rapidly penetrates into microorganisms (Chang, 1971) and attacks key groups of proteins (in particular the freesulfur amino acids cysteine and methionine [Gottardi, 1991, 267]), nucleotides, and fatty acids (Apostolov, 1980; Gottardi, 1991), which culminates in cell death (Gottardi, 1991). Less is known about the antiviral action of iodine, but nonlipid viruses and parvoviruses are less sensitive than lipid enveloped viruses (Prince *et al.* 1991). Similarly to bacteria, it is likely that iodine attacks the

surface proteins of enveloped viruses, but they may also destabilize membrane fatty acids by reacting with unsaturated carbon bonds (Springthorpe and Satter, 1990).

3. **Bromine.** Bromine is an excellent primary disinfectant similar to chlorine, but with some advantages. Bromine hydrolyses in water to hypobromous acid (HOBr) and bromide. It is used usually as bromochlorodime-thylhydantoin in swimming pools, spas and cooling towers. Bromamines' disinfection efficacy is much greater than chloramines' (combined chlorine) because their hydrolysis equilibrium in water favors hypobromous acid (HOBr), whereas chloramine hydrolysis produces only a small amount of hypochlorous acid (HOCl). Bromine is also more biocidal at higher pHs than chlorine because HOBr ionizes to a lesser degree than HOCl at pH 8.7.

- ***Silver Compounds:***

1. **Silver nitrate.** The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfydryl, -SH) groups (Belly and Kydd, 1982; Bragg and Rannie, 1974; Fuhrmann and Rothstein, 1968; Furr *et al.* 1994), although other target sites remain a possibility (Richards, 1991, Thurmann and Gerba, 1988). Interaction of Ag^{+} with thiol groups in enzymes and proteins plays an essential role in bacterial inactivation, although other cellular components may be involved. Silver nitrate causes marked inhibition of growth of Cryptococcus neoformans and is deposited in the vacuole and cell wall as granules (Brown and Smith, 1976). Ag^{+} inhibits cell division and damages the cell envelope and contents of P. aeruginosa (Richards *et al.* 1984).

2. **Silver sulfadiazine.** AgSD is essentially a combination of two antibacterial agents, Ag^{+} and sulfadiazine (SD). AgSD has a broad spectrum of activity and, unlike silver nitrate, produces surface and membrane blebs in susceptible (but not resistant) bacteria (Coward *et al.* 1973). AgSD binds to cell components, including DNA (Modak and Fox, 1973, Rosenkranz and Rosenkranz, 1972). Clearly, the precise mechanism of action of AgSD has yet to be solved.

- ***Peroxygens:***

1. **Hydrogen Peroxide.** Hydrogen peroxide (H_2O_2) is a widely used biocide for disinfection, sterilization, and antisepsis. It is a clear, colorless liquid that is commercially available in a variety of concentrations ranging from 3 to 90%. H_2O_2 is considered environmentally friendly, because it can rapidly degrade into the innocuous products water and oxygen. Although pure solutions are generally stable, most contain stabilizers to prevent decomposition. H_2O_2 demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores (Block, 1991). In general, greater activity is seen against gram-positive than gram-negative bacteria; however, the presence of catalase or other peroxidases in these organisms can increase tolerance in the presence of lower concentrations. Higher concentrations of H_2O_2 (10 to 30%) and longer contact times are required for sporicidal activity (Russell, 1991), although this activity is significantly increased in the gaseous phase. H_2O_2 acts as an oxidant by producing hydroxyl free radicals (•OH) which attack essential cell components, including lipids, proteins, and DNA. It has been proposed that exposed sulfhydryl groups and double bonds are particularly targeted (Block, 1991).

2. **Peracetic acid.** It is considered a more potent biocide than hydrogen peroxide, being sporicidal, bactericidal, virucidal, and fungicidal at low concentrations (<0.3%) (Block,

1991). PAA also decomposes to safe by-products (acetic acid and oxygen) but has the added advantages of being free from decomposition by peroxidases, unlike H_2O_2, and remaining active in the presence of organic loads (Lensing and Oei, 1984; Malchesky, 1993). Its main application is as a low-temperature liquid sterilant for medical devices, flexible scopes, and hemodialyzers, but it is also used as an environmental surface sterilant (Crow, 1992, Malchesky, 1993). Similar to H_2O_2, PAA probably denatures proteins and enzymes and increases cell wall permeability by disrupting sulfhydryl (-SH) and sulfur (S-S) bonds (Baldry and Fraser, 1988; Block, 1991).

- ***Phenols:*** Phenolic-type antimicrobial agents have long been used for their antiseptic, disinfectant, or preservative properties, depending on the compound. Pulvertaft and Lumb, 1948 demonstrated that low concentrations of phenols (0.032%, 320 mg/ml) and other (nonphenolic) agents lysed rapidly growing cultures of E. coli, staphylococci, and streptococci and concluded that autolytic enzymes were not involved. The phenolics possess antifungal and antiviral properties. Their antifungal action probably involves damage to the plasma membrane (Russell and Furr, 1996), resulting in leakage of intracellular constituents.

- ***Bis-Phenols:***

  1. **Triclosan** exhibits particular activity against Gram-positive bacteria (Savage, 1971; Vischer and Regos, 1973). Its efficacy against Gram-negative bacteria and yeasts can be significantly enhanced by formulation effects. For example, triclosan in combination with EDTA caused increased permeability of the outer membrane (Leive, 1974). The specific mode of action of triclosan is unknown, but it has been suggested that the primary effects are on the cytoplasmic membrane.

  2. **Hexachlorophene** is another bis-phenol whose mode of action has been extensively studied. The primary action of hexachlorophene, based on studies with *Bacillus megatherium*, is to inhibit the membrane bound part of the electron transport chain. Despite the broad-spectrum efficacy of hexachlorophene, concerns about toxicity (Kimbrough, 1973), in particular in neonates, have meant that its use in antiseptic products has been limited.

- ***Halophenols:*** Chloroxylenol (4-chloro-3,5-dimethylphenol; p-chloro-m-xylenol) is the key halophenol used in antiseptic or disinfectant formulations (Bruch, 1996). Chloroxylenol is bactericidal, but P. Aeruginosa and many molds are highly resistant (Bruch, 1996, Russell and Furr, 1977). Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it would be expected to have an effect on microbial membranes.

- ***Quaternary Ammonium Compounds:*** Cationic agents, as exemplified by quaternary ammonium compounds (QACs), are the most useful antiseptics and disinfectants (Frier, 1971). They are sometimes known as cationic detergents. QACs have been used for a variety of clinical purposes (e.g., preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of noncritical surfaces). In addition to having antimicrobial properties, QACs are also excellent for hard-surface cleaning and deodorization. QACs are membrane active agents (Hugo and Frier, 1969) (i.e., with a target site predominantly at the cytoplasmic (inner) membrane in bacteria or the plasma membrane in yeasts) (Hugo, 1999). There is thus a loss of structural organization and integrity of the cytoplasmic membrane in bacteria, together with other damaging

effects to the bacterial cell (Denyer, 1995). The QAC cetrimide was found (Denyer and Hugo, 1977) to have an effect on the PMF in S. aureus. They are also believed to damage the outer membrane of gram-negative bacteria. The QAC cetylpyridium chloride (CPC) induces the leakage of K1 and pentose material from the yeast S. cerevisiae and induces protoplast lysis as well as interacting with crude cell sap (Hiom *et al.* 1993). The QACs have an effect on lipid, enveloped (including human immunodeficiency virus and HBV) but not non-enveloped viruses (Resnick *et al.* 1986; Springthorpe *et al.* 1986; Springthorpe and Satter, 1990).

- ***Vapor-Phase Sterilants:*** Many heat-sensitive medical devices and surgical supplies can be effectively sterilized by liquid sterilants (in particular glutaraldehyde, PAA, and hydrogen peroxide) or by vaporphase sterilization systems. The most widely used active agents in these "cold" systems are ethylene oxide, formaldehyde, hydrogen peroxide and PAA. As alkylating agents, Ethylene oxide and formaldehyde attack proteins, nucleic acids, and other organic compounds; both are particularly reactive with sulfhydryl and other enzyme- reactive groups. Vapor-phase hydrogen peroxide and PAA are considered more active (as oxidants) at lower concentrations than in the liquid form (Moore and Perkinson, 1979).

- ***Potassium permanganate:*** It is a strong oxidiser and although it can inactivate various bacteria and viruses, it is not used as a primary or secondary disinfectant when applied at commonly used treatment levels. Potassium permanganate levels that may be required to obtain primary or secondary disinfection could be cost prohibitive. The primary mode of pathogen inactivation by potassium permanganate is direct oxidation of cell material or specific enzyme destruction (Webber and Posselt, 1972). In the same fashion, the permanganate ion (MnO_4^-) attacks a wide range of microorganisms such as bacteria, fungi, viruses, and algae.

- ***Sodium hypochlorite phosphate:*** Sodium hypochlorite phosphate, an inclusion complex of trisodium phosphate and sodium hypochlorite, is a dry form of sodium hypochlorite providing germicidal and disinfectant properties as well as alkalinity. It is used in as a bactericide in food and dairy processing and cleanser of medical instruments and scouring. It is used in detergents, automatic dishwasher detergent and laundry soaps.

- ***Glyoxal:*** Glyoxal (Ethanedial 40%) is a liquid at room temperature and it crystallizes at 15 ˚C to form yellow prismatic crystals. It is nonvolatile and is easily biodegradable. It has bactericidal properties similar to that of gluteraldehyde and therefore is often used as an adjunct of these agents in disinfectants. Glyoxal attacks amino groups of proteins, nucleotides and lipids. It is known to react with amino acids like lysine, arginine and guanine forming advanced glycation end products (AGEs). This leads to inactivation of enzymes, disturbance in the cellular metabolism, impaired proteolysis, inhibition of cell proliferation and protein synthesis.

- ***Glucoprotamin:*** It is a new disinfectant based on a conversion product of L-glutamic acid and coco ($C_{12/14}$) alkyl-propylene-1.3-diamine. It has excellent toxicological properties and active *in vitro* against vegetative bacteria, including mycobacteria, fungi and viruses. Some glutaraldehyde resistant strains of *Mycobacterium chelonae* were efficiently killed by glucoprotamin but not by other disinfecting agents. It is non-volatile, easily dissolved in water, non teratogenic, non mutagenic, easily degradable, non-corrosive to metals and compatible with most materials used in health care.

17.2. **National Status**

The terminologies Room disinfectant and Bed disinfectant are used in the field of sericulture only. All the room and bed disinfectants developed so far, are based on those which are already identified as chemicals having anti-microbial/disinfectant activity including some botanicals having anti microbial property. Several room and bed disinfectants have been developed by different sericulture/sericulture related institutes in India having different trade name viz. LABEX, VIJETHA, RESHAM KEET OUSADH, RESHAM JYOTHI, SERICILLIN, ANKUSH, VIJETHA SUPPLEMENT, RAKSHAK etc. as bed disinfectants and SERICHLOR, SANITECH, DECOL, ASTHRA, GHAR SODHON etc. as room disinfectants. All the bed disinfectants are in powder formulation and most of them are having either lime/bleaching powder/kaolin as one of the ingredients.

17.3. **Importance of the proposed project in the context of current status**

- Economic benefit resulting from reduced mortality of silkworms due to silkworm diseases by adopting the developed room and bed disinfectant.

17.4. **Anticipated Products, processes / Technology, Packages / Information or other outcome from the project and their expected utility**

A room disinfectant and a silkworm bed disinfectant for reducing mortality of silkworm due to diseases and thereby giving economic benefit to the stakeholders.

17.5. **Expertise available with proposed investigation group/institution on the subject of the project:**

Name of the Scientists	**Designation**	**Experience**
K. Rahul	Scientist- B	Isolation, cultivation, polyphasic characterization of bacteria. Preparation of pathogenic suspensions (Bacteria, *Bm*NPV) and artificial induction of the same to *Bombyx mori*.

18. **Work Plan**

18.1. Methodology

18.1.1. ***In vitro & in vivo testing of pathogens with selected chemicals/ disinfectants*** **(E-01)**

8-10 chemicals/disinfectants will be screened based on literature survey having potential antimicrobial activity (the chemicals that were reported to be tested and used in sericulture will be avoided while developing the disinfectants). Out of these 4-5 chemicals/disinfectants will again be short listed based on their efficacy study against pathogens causing diseases in silkworms, using the following methodology. Efficacy of selected chemicals on the fungal & bacterial pathogens (*in vitro*) and viral & microsporidian pathogens (*in vivo*) will be studied.

Specific pathogens to be tested:

- ***Virus*** (Bm NPV)
- ***Bacteria*** (*Staphylococcus* sp., *Bacillus* sp. and other spp. of bacteria already identified to be causing bacteriosis)
- ***Fungi*** (*Beauveria bassiana, Aspergillus sp.*)
- ***Microsporidia*** (*Nosema bombycis*)

The antimicrobial efficiency of the chemicals/disinfectant on **bacterial and fungal** pathogens will be studied employing the following tests: ***Quantitative Suspension Tests*, *Practical Test (Surface Disinfection Test) and In use Test***

For **viral and microsporidian** pathogens, they will be exposed to different concentrations/duration of the selected chemicals and inactivation of the same will be assessed *in vivo*.

18.1.2. ***Development of room disinfectant & study of its efficacy*** **(E-02)**

The short listed chemicals/disinfectants exhibiting positive results will be employed to develop a room disinfectant based on their compatibility and efficacy. Estimation of pathogen load in the rearing room before and after application of room disinfectant will be done to assess the efficacy of the developed room disinfectant. However, only those chemicals which are reported to be in use without causing any adverse effect on the human health and environment, in permissible quantity, will be considered for the study.

18.1.3. ***Development of silkworm body disinfectant with chemicals of proven efficacy having synergistic activity in controlling silkworm diseases, specially grasserie*** **(E-03)**

Those chemicals showing positive results in inactivating/killing pathogens causing silkworm diseases will be tested for their toxic effect on silkworm before being taken into consideration for development of the disinfectant. Chemicals/disinfectants found safe for silkworm will be considered for formulation of a broad spectrum bed disinfectant.

18.1.4. ***Efficacy study of the developed bed disinfectant*** **(E-05)**

Suspensions of purified bacterial, viral, microsporidian and fungal pathogens of different doses will be inoculated to healthy silkworm larva employing methodology of Sugimori *et al*., 1990, Biabani *et al*., 2005, Pachiappan *et al*., 2009, Sato & Watanabe, 1980, Hughes & Wood, 1981 and Rajitha & Savithri, 2015. These carrier larvae will be kept along with healthy larvae and developed bed disinfectant will be dusted at different periods of interval combination to assess the efficacy of the disinfectant at a particular schedule of application. Assessment of efficacy of the disinfectant will be judged based on the comparison of ERR of healthy control and treatments.

18.1.5. ***Comparative study of the developed disinfectant with available room and bed disinfectants*** **(E-05)**

Comparative efficacy study of the developed disinfectant with those of available room disinfectants (SANITECH, ASTRA and GHAR SODHON) and bed disinfectants (LABEX, SERICILLIN, ANKUSH, VIJETHA) available in the market will be undertaken during two unfavourable seasons.

18.1.6. *Calculation of economics of the technology, if developed* (E-06)

Benefit Cost Ratio of the technology/product, if developed, will be calculated to assess the feasibility of the same in the farmers' field.

18.2. Organization of Work Elements

Name of Scientists/JRF	Desig.	Time	Organization of work elements
K. Rahul	Sci-B	40 %	Development of silkworm body disinfectant with chemicals of proven efficacy having synergistic activity in controlling silkworm diseases, specially grasserie Efficacy study of the developed bed disinfectant
JRF	JRF	60%	*In vitro* and *in vivo* testing of pathogens with selected chemicals/ disinfectants Development of room disinfectant & study of its efficacy Comparative study of the developed disinfectant with available room and bed disinfectants Calculation of economics of the technology developed & preparation of final report

18.3. Proprietary / Patented items, if any, expected to be used for this Project

Room disinfectants: SANITECH, ASTRA, GHAR SODHON and Bed disinfectants: LABEX, SERICILLIN, ANKUSH and VIJETHA

18.4. Suggested plan of action for utilization of the expected outcome from the project

The developed room and bed disinfectant will be validated at the Institute/RSRS/REC/DoT (Seri) Farm and farmers' level before its release for adoption at farmers' level.

18.5. Time Schedule of activities giving milestones

#	Organization of work/Milestone /Activity	Period of study (Tentative)	
		Starting	Completion
1	Procurement of equipment and chemicals; **E-01:** *In vitro* & *in vivo* testing of pathogens with selected chemicals/ disinfectants	June, 2018	January, 2019
2	**E-02:** Development of room disinfectant & study of its efficacy	February, 2019	September, 2019
3	**E-03:** Development of silkworm body disinfectant with chemicals of proven efficacy having synergistic activity in controlling silkworm diseases, specially grasserie	October, 2019	May, 2020
4	**E-04:** Efficacy study of the developed bed disinfectant	June, 2020	November 2020

5	**E-05:** Comparative study of the developed disinfectant with available room and bed disinfectants	December, 2020	March, 2021
6	**E-06:** Calculation of economics of the technology developed (**E-06**) & preparation of final report	April,2021	May, 2021

18.6. Project Implementing Agency / Agencies

Name of the agency	Address of the agency	Proposed Research Aspects	Proposed Amount	Cost Sharing %
Central Silk Board, Bangalore	BTM Layout Madiwala Bangalore	Silkworm Crop protection	**10.0 lakh**	100 %

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PART-IV: BUDGET PARTICULARS

19. BUDGET (in Lakhs): [In case of multi-institutional projects, the budget details should be provided separately for each of the Institute]

(Fig. in lacs)

A. Non-Recurring (e.g. equipments, accessories, etc.)* **[Total – 3.0]**

#	Item	1st Year	2nd Year	3rd Year	Total
1.	Air Sampler	3.0	-	-	3.0
	Subtotal A	**3.0**	-	-	**3.0**

B. Recurring **[Total – 7.0]**

#	Designation/Items	No.	Consolidated Emolument			
			1st Year	2nd Year	3rd Year	Total
B.1 Manpower						
1	Junior Research Fellow (Proposed)	1 No.	1.6	1.6	1.8	5.0
	Subtotal B1		**1.6**	**1.6**	**1.8**	**5.0**
B.2 Consumables						
1	Chemicals, disinfectants, media components, reagents, etc.		0.5	0.5	-	1.0
	Subtotal B2		**0.5**	**0.5**	**-**	**1.0**
B.3 Other Items						
1	Contingency		0.4	0.3	0.3	1.0
	Subtotal B3		**0.4**	**0.3**	**0.3**	**1.0**

Sub-total of B (B1+B2+B3)				**7.0**
Grand total (A+B)				**10.0**

Total cost of the project Rs: 10.0 lakh

PART-V: EXISTING FACILITIES

20. Available equipment and accessories to be utilized for the project:

CSR&TI, Berhampore (SWPathology Section)

#	Name of the instruments	Make	Source	Year of procurement
1	Spectrophotometer	Lasany	CSB	2018
2	Gel documentation system	Biorad	CSB	2017
3	Incubation Shaker	Remi	CSB	2017
4	PCR	Eppendorf	CSB	2017
5	Refrigerated Centrifuge	Remi	CSB	2017
6	-20 Deep freezer	Remi	CSB	2016
7	Laminar air flow	Digitech	CSB	2016
8	BOD incubator	Indosati	NSSO	2016
9	Sartorius water purification system	Germany, 61316 RO and 611 DI	CSB	2007
10	Table-top Centrifuge	Remi	CSB	2006
11	Stereoscopic Binocular Compound Microscope	Wild Heerburgg M8, Make - Leitz	CSB	1993
12	Leitz Diaplan Phase Contrast Microscope	Germany	CSB	1991
13	Refrigerator	LG	CSB	-
14	Autoclave	Spac & services	CSB	-
15	Electronic balance	Anamed	CSB	-

PART VI: DECLARATION/CERTIFICATION

It is certified that

a) The research work proposed in the project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b) The same project has not been submitted to any other agencies for financial support.

c) The emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute / university or as per the Ministry of science & technology guidelines (Annexure – III).

d) Necessary provision for the project will be made in the Institute in anticipation of the sanction of the scheme/project.

e) If the project involves the utilization of genetically engineered organism, it is agreed that we will ensure that an application will be submitted through our institutional bio-safety committee and we will declare that while conducting experiments, the bio-safety committee will declare that while conducting experiments, the bio-safety guidelines of the Department of Biotechnology would be followed in toto.

f) If the project involves field trials / experiments / exchange of specimens etc. we will ensure that ethical clearances would be taken from the concerned ethical committees / competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.

g) It is agreed by us that any research outcome or intellectual property right(s) on the invention (s) arising out of the Project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance. Department of Expenditure, as contained in Annexure - V.

h) We agree to accept the terms and conditions as enclosed in Annexure - IV. The same is signed and enclosed.

i) The Institute agrees that the equipment, the basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator throughout the duration of the project.

j) The Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Executive Authority of Institute
(with Seal and Date)

Signature of Principal Investigator
(K.Rahul)
Scientist-B
Date: