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Silkworm disease resistance management: Strategies, mechanisms and emerging approaches

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Abstract

Sericulture, the practice of growing silkworms (*Bombyx mori* L.) for silk production, is an important agro-based industry that supports the livelihoods of millions of farming families throughout Asia, mainly in India, China, Japan and Korea. Silkworms are susceptible to a variety of infections, including viruses, bacteria, fungus, protozoa and nematodes which is one of the most serious risks to sustainable silk production. These illnesses collectively cause yield losses of 15 to 40% of potential silk output each year. The current review summarizes current understanding about cause of silkworm diseases, host immunological responses and the range of management techniques available to sericulturists and researchers. The principal disorders covered include nuclear polyhedrosis virus (BmNPV), cytoplasmic polyhedrosis virus (BmCPV), flacherie caused by bacterial infections and muscardine caused by entomopathogenic fungi, pebrine caused by the microsporidian *Nosema bombycis*, and grasserie as well as the immune mechanisms deployed by silkworms against these pathogens. Management approaches discussed span conventional sanitation practices, chemical and biological control measures, selective breeding for disease resistance, marker-assisted selection, transgenesis, RNA interference, and emerging nanotechnology-based strategies.

Keywords: Silkworm diseases, BmNPV, pebrine, grasserie, disease resistance, sericulture management, biological control, RNA interference, transgenic silkworm

Introduction

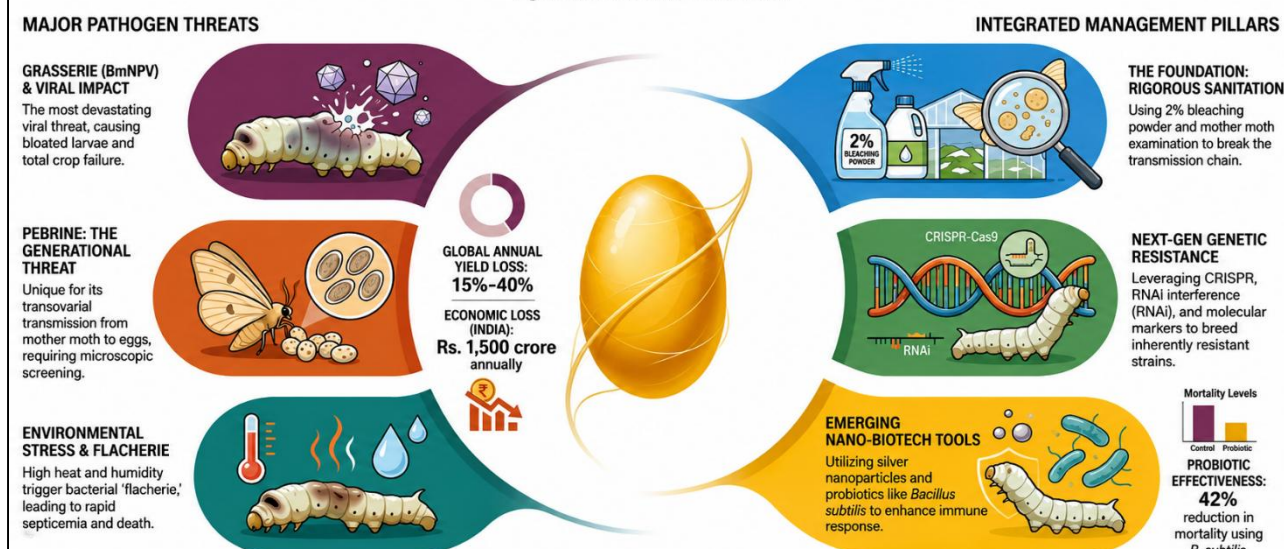
Sericulture has been practiced for more than five thousand years and remains one of the most important agro-industrial activities in several Asian and a few European countries. The sericulture sector in India provides livelihood opportunities to about 9.5 million people engaged in mulberry cultivation, silkworm rearing and silk processing activities Singha *et al.*, (2020) [1]. The silkworm, *Bombyx mori* L. is the principal commercial silk-producing species and its robust cultivation is essential to meet the global demand for natural silk, which continues to command premium prices in international textile markets.

Despite centuries of domestication and refinement of rearing practices, silkworm production remains highly vulnerable to disease outbreaks. Unlike many livestock species where vaccination has drastically reduced mortality and silkworm disease management relies on a more complex evolving combination of sanitation as well as genetic improvement and chemical and biological interventions. (Aruga H, 1994) [2]. Pathogens affecting silkworms encompass all major categories of baculoviruses and cypoviruses among viruses the two important species are *Serratia marcescens* and *Bacillus thuringiensis* among bacteria two important species *Beauveria bassiana* and *Isaria fumosorosea* among fungi two important species are *Nosema bombycis* among protozoa and *Mermis* species among nematodes (Nagaraju & Goldsmith, 2002; Wang *et al.*, 2015) [24, 41].

The economic losses attributed to silkworm diseases are staggering. In India, disease-related losses are estimated at Rs. 1,500 crore annually with nuclear polyhedrosis and pebrine being the most destructive Dandin *et al.*, (2003) [7]. In China, BmNPV epidemics can destroy 20 to 60% of a rearing batch in severe cases Lu *et al.*, (2013) [20].

Safeguarding Silk: Integrated Strategies for Silkworm Disease Management

Silkworm diseases cause significant loss in potential silk output annually, resulting in staggering economic impacts. Sustainable production relies on transitioning from simple sanitation to Integrated Disease Management (ISDM) that combines genetics, biotechnology, and rigorous environmental control.



Major Diseases of Silkworms

Viral Diseases

Viral diseases represent the most economically devastating category of silkworm diseases in which Nuclear polyhedrosis virus disease popularly known as grasserie is caused by *Bombyx mori* nucleopolyhedron virus (BmNPV) a member of the family *Baculoviridae*. The disease derives its name from the bloated yellowish and greasy appearance of infected larvae. BmNPV infects and replicates within the nuclei of various cell types ultimately killing the host larva within a few days post-infection. Polyhedral inclusion bodies (PIBs) released from disintegrating larvae contaminate the rearing environment and serve as the primary route of horizontal transmission (Kamiya & Tatamatsu, 2010; Peng *et al.*, 2010) [14, 28].

Cytoplasmic polyhedrosis virus (CPV) disease or sotto disease is caused by *Bombyx mori* cypovirus (BmCPV), a member of the family *Reoviridae*. Unlike BmNPV, BmCPV replicates within the cytoplasm of midgut epithelial cells. Infected larvae exhibit reduced feeding along with stunted growth and whitish translucent midguts. CPV-infected larvae often survive to the spinning stage but produce thin, low-quality cocoons with poor silk content (Hukuhara & Bonami, 1991) [11]. Infectious flacherie is another viral disease associated with the densovirus BmDNV and is characterized by flaccid limp larvae that stop feeding and die in the later larval instars. Flacherie is frequently compounded by secondary bacterial infections (Seki, 1984) [32].

Bacterial Diseases

Bacterial diseases collectively referred to as flacherie are caused predominantly by *Serratia marcescens*, *Bacillus bombysepticus* and *Streptococcus faecalis*. *Serratia marcescens* is a Gram-negative facultative anaerobe produces prodigiosin and a range of extracellular enzymes that lyse intestinal epithelium leading to rapid septicemia and death. The bacterium is ubiquitous in the rearing environment including mulberry leaves with rearing trays and the exoskeletons of diseased worms Mohan *et al.*, (2011) [32]. Bacterial flacherie is exacerbated by

environmental stresses such as high temperature, high humidity and overfeeding conditions common during summer rearing in tropical countries.

Bacillus thuringiensis (Bt) strains are well-known for their insecticidal properties in pest management but can act as virulent pathogens when silkworms inadvertently ingest Bt-sprayed mulberry leaves. The delta-endotoxin crystals produced by Bt disrupt midgut epithelium integrity, predisposing worms to secondary infections. This has become a significant management challenge in sericulture areas adjacent to cotton and vegetable farms where Bt based bioinsecticides are routinely applied Singh *et al.*, (2010) [33].

Fungal Diseases

Fungal diseases of silkworms are collectively termed muscardine are caused by entomopathogenic fungi that infect larvae through direct cuticular penetration. White muscardine, caused by *Beauveria bassiana* and green muscardine, caused by *Metarhizium anisopliae* and *Isaria fumosorosea* are the most prevalent. The fungal conidia attach to and germinate on the larval cuticle penetrating the integument using enzymatic activity (chitinases, proteases, lipases). Once inside, the fungus proliferates in the hemolymph as hyphal bodies ultimately killing the host and sporulating on the cadaver Inglis *et al.*, (2001) [13]. Muscardine is particularly destructive in humid tropical climates where fungal growth is favored and the mummified cadavers serve as persistent sources of inoculum in rearing environments.

Yellow muscardine caused by *Paecilomyces farinosus* and red muscardine caused by *Sporotrichum densum* have also been documented though they are less prevalent than white and green muscardine. Fungal spores are extraordinarily hardy remaining viable for extended periods on rearing equipment, bamboo trays, and the walls of rearing houses.

Pebrine

Pebrine, caused by the microsporidian obligate intracellular parasite *Nosema bombycis* holds a special place in the history of parasitology as the disease that Louis Pasteur studied in the 1860s, laying the foundations for germ theory.

Pebrine is unique among silkworm diseases in its capacity for transovarial (vertical) transmission from infected female moths to eggs making it the only silkworm pathogen that can be passed from one generation to the next through the egg. This characteristic makes complete eradication from a seed rearing station extremely challenging (Kawakami, 1981) ^[16].

Spores of *Nosema bombyci* ingested orally or inherited transovarially germinate in the midgut and invade multiple tissues including fat body, silk glands, ovaries and epidermis. Heavily infected larvae show characteristic pepper-like brownish-black spots on the integument the origin of the name pebrine. While mildly infected larvae may survive to adulthood cocoon quality is severely compromised and infected moths produce diseased eggs perpetuating the cycle. Secondary microsporidian pathogens including *Vairimorpha bombycis* and *Endoreticulatus bombycis* have also been identified in silkworms Solter *et al.*, (2012) ^[36].

Other Parasites and Abiotic Diseases

Nematode parasites of the genera *Mermis* and *Rhabditis* occasionally infect silkworm larvae in outdoor rearing systems causing internal damage and death. Protozoan infections beyond *Nosema* including those caused by *Gregarina* species have been documented in some rearing regions. Abiotic diseases including chilling injury, scalding (high-temperature injury) and poisoning from pesticide contaminated mulberry leaves collectively add to the burden on silkworm health management and are frequently misdiagnosed as infectious disease leading to inappropriate responses. Ayyasamy *et al.*, (2014) ^[3].

Immune Defense Mechanisms in *Bombyx mori*

Physical and Cellular Barriers

The first line of defense against pathogen entry in silkworms consists of physical barriers including the chitinous cuticle and the peritrophic membrane lining the midgut. The peritrophic membrane composed of chitin fibrils embedded in a protein-glycoprotein matrix physically restricts the passage of large pathogens and pathogen-derived molecules into the hemocoel while allowing nutrient absorption (Lehane, 1997) ^[18]. Disruption of the peritrophic membrane by viral polyhedra or fungal enzymes is a critical early step in successful infection, underscoring its importance as a barrier.

Cellular immunity is mediated by hemocytes circulating and sessile blood cells of several functional classes including plasmatocytes, granulocytes, spherulocytes and oenocytoids. Phagocytosis by granulocytes and plasmatocytes is the primary cellular response to invading bacteria and fungi. Melanization is a rapid nonself-recognition response involving the prophenoloxidase (proPO) activation cascade encapsulates larger pathogens and foreign bodies and is particularly important against nematode and fungal invasion (Hultmark, 2003) ^[12]. Nodule formation hemocytes aggregate around clumps of bacteria effectively removes large bacterial burdens from the hemolymph.

Humoral Immunity

Humoral immunity in silkworms involves the production of a broad array of antimicrobial peptides (AMPs) and proteins by the fat body functional analog of the mammalian liver in response to immune challenge. These include the cecropins,

attacins, defensins, moricins, gloverin, enbocin and lebecin families each with distinct spectra of activity against bacteria and fungi (Tanaka *et al.*, 2008; Zhu *et al.*, (2003) ^[38, 48]. AMP gene expression is regulated primarily through the Toll and immune deficiency (IMD) signaling pathways which sense pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides, peptidoglycans and beta-1,3-glucans through pattern recognition receptors (PRRs) including peptidoglycan recognition proteins (PGRPs) and beta-1,3-glucan recognition proteins (betaGBPs) Tanaka *et al.*, (2008) ^[38].

The prophenoloxidase (proPO) activation system is a critical component of humoral immunity is triggered upon recognition of PAMPs and involves a serine protease cascade culminating in the activation of phenoloxidase which catalyzes the synthesis of melanin and cytotoxic quinones at infection sites. This reaction contributes to wound healing coagulation of hemolymph and killing of pathogens through reactive oxygen species (Cerenius & Soderhall, 2004) ^[6]. Lectins and other opsonins in the hemolymph facilitate recognition and clearance of pathogens by hemocytes.

Antiviral Immunity and RNA Interference

Antiviral defense in insects including silkworms relies heavily on RNA interference (RNAi) pathways particularly the small interfering RNA (siRNA) pathway. Upon viral double-stranded RNA (dsRNA) production during replication the RNase III enzyme Dicer-2 cleaves dsRNA into 21-23 nucleotide siRNAs, which are loaded into the RNA-induced silencing complex (RISC) and guide sequence-specific degradation of viral transcripts (Ding & Voinnet, 2007) ^[8]. The silkworm genome encodes all canonical RNAi pathway components, and silencing of BmNPV and BmCPV replication by RNAi has been demonstrated both in cell culture Singh *et al.*, (2014) ^[24]. The piRNA pathway, involving PIWI-interacting RNAs, also plays a role in antiviral defense in silkworm gonads and potentially somatic tissues.

Conventional Disease Management Strategies

Sanitation and Disinfection

The foundation of all silkworm disease management programs is rigorous sanitation which interrupts the chain of pathogen transmission through the rearing environment. Widely used disinfectants include 2% bleaching powder (calcium hypochlorite), 2% formalin, 5% Vijetha (a commercial silkworm disinfectant based on sodium hypochlorite and organic acids) and lime washing Dandin *et al.*, (2003) ^[7]. Effective disinfection requires thorough cleaning to remove organic matter before chemical application because organic debris inactivates most disinfectants.

Bed disinfectants applied directly on the rearing bed to control bacterial and fungal pathogens include slaked lime (calcium hydroxide) and Labex powder formulations. The Central Silk Board of India has standardized protocols for their application at specific larval stages and intervals. Segregation of diseased larvae and their immediate disposal by burning or deep burial prevents release of infective propagules into the environment. Post-rearing cleanup protocols involving dipping all bamboo equipment in disinfectant solutions and sun-drying are equally important.

Chemical Control

Several chemical compounds have been evaluated for prophylactic and curative use against silkworm diseases. For bacterial flacherie, chemicals like chloramphenicol and tetracycline have been used traditionally but their residues in silk and environmental concerns have substantially limited their continued application. Commercial formulations such as Delfin (*Bacillus thuringiensis*-based) are obviously contraindicated in silkworm rearing areas. Antifungal compounds including boric acid and copper sulphate at sublethal concentrations have been investigated as bed disinfectants against muscardine Singhvi *et al.*, (2001) [35].

Chemical control of pebrine is particularly challenging because *Nosema bombycis* spores are highly resistant to most disinfectants, surviving in dried excreta for years. Fumagillin, an antibiotic derived from *Aspergillus fumigatus*, has been shown to suppress microsporidian spore germination and hyphal proliferation and was used experimentally in some rearing programs. The primary chemical strategy against pebrine therefore remains microscopic examination-based seed selection rather than curative treatment. Sampath kumar *et al.*, (1993) [31].

Microscopical Examination for Pebrine Control

The Pasteur method of pebrine control, involving microscopic examination of female moth body extracts (or egg-laying cloth smears) for *N. bombycis* spores, remains the standard practice in all certified seed rearing programs worldwide. In India, the Central Silk Board mandates that all seed cocoon farms conduct mother moth examination and only crops where less than 0.5% of examined moths test positive for spores are approved for commercial egg production (Narahari & Gopal, 2002) [26]. This labor-intensive but highly effective protocol has largely prevented the entry of pebrine-contaminated seed stocks into commercial production, though the technique requires skilled microscopists and represents a significant bottleneck in high-volume seed production programs.

Biological Control Approaches

Probiotic Bacteria

The concept of manipulating the silkworm gut microbiome to enhance disease resistance has gained considerable attention in recent years. The silkworm gut harbors a relatively simple microbiota compared to vertebrates, dominated by *Enterococcus*, *Lactobacillus*, *Bacillus* and *Sphingomonas* species Wei *et al.*, (2014) [42]. Probiotic supplementation with beneficial bacterial strains, particularly *Bacillus subtilis*, *Lactobacillus plantarum* and *Enterococcus faecium* has been shown to competitively exclude pathogenic bacteria, produce bacteriocins and organic acids that inhibit pathogens and enhance the expression of immune genes (Vidomini *et al.*, 2021; Peng *et al.*, 2020) [39, 29].

Studies by Peng *et al.*, (2020) [29] demonstrated that oral use of *Bacillus subtilis* significantly reduced mortality from *Serratia marcescens* infection in silkworms by up to 42% compared to untreated controls. Similarly, *Lactobacillus* supplementation enhanced haemocyte counts and AMP gene expression in challenged larvae. The practical application of probiotics in commercial silkworm rearing faces challenges related to formulation stability, dose optimization and integration with existing sanitation protocols that may inadvertently eliminate beneficial bacteria.

Entomopathogen-Derived Biocontrol Agents

Entomophagous fungi that are pathogens of silkworm pests such as the mulberry tortrix, *Glyphodes pyloalis* and the mulberry scale insect, *Pseudaulacaspis pentagona* can be used as biological control agents against these insects without direct adverse effects on silkworms, provided application timing and dosage are carefully managed. Preparation of botanical pesticides from neem (*Azadirachta indica*), garlic extract and tulsi (*Ocimum sanctum*) has been explored for surface application on mulberry leaves to reduce pathogen load and repel insect vectors without toxic effects on rearing silkworms Reddy *et al.*, (2010) [30].

Genetic Approaches to Disease Resistance

Conventional Breeding for Disease Resistance

Systematic scientific breeding for disease resistance began in the early twentieth century in Japan with the identification of hybrid vigor in crosses between divergent pure lines. Inter-strain hybridization and backcross breeding programs at National sericulture research institutes in Japan, China, India and Korea have generated commercially viable breeds with improved resistance to BmNPV, bacterial flacherie and muscardine compared to ancestral stocks (Nagaraju, 2002) [23]. The inheritance of disease resistance traits in silkworms is complex and quantitative for most diseases. Resistance to BmNPV is controlled by multiple quantitative trait loci (QTLs) distributed across several chromosomes, with no single major gene conferring complete resistance. Environmental effects, particularly rearing temperature, humidity and nutritional status, significantly modify the expression of resistance phenotypes, complicating selection for stable resistance Nagaraju *et al.*, (2005) [25]. Resistance to *N. bombycis* has a partially heritable component and strains showing reduced susceptibility to pebrine have been identified in biodiversity studies, though the genetic basis remains incompletely characterized.

Molecular Markers and Marker-Assisted Selection

The sequencing of the *Bombyx mori* genome completed to high quality by Chinese and Japanese consortia in 2004 has provided an invaluable resource for identifying genetic markers associated with disease resistance (Mita *et al.*, 2004; Xia *et al.*, 2004) [21, 43]. Random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers have been used to construct high-density genetic maps and identify QTLs for resistance to BmNPV, BmCPV, flacherie and pebrine (Nagaraju *et al.*, 2005; Li *et al.*, 2008) [25, 19].

Marker-assisted selection (MAS) enables breeders to select for resistance-associated alleles without the lengthy and laborious bioassay-based phenotyping required for direct disease resistance evaluation. A prominent example is the identification of SNP markers flanking QTLs for BmNPV resistance on chromosome 17 and chromosome 23, which have been incorporated into breeding programs at the Central Sericultural Research and Training Institute, Mysore, India Banerjee *et al.*, (2012) [4]. However, the limited predictive power of currently mapped QTLs typically explaining only 10 to 30% of phenotypic variance constrains the precision and reliability of MAS for complex resistance traits.

Transgenic Approaches

The development of robust germline transformation systems for *Bombyx mori* using the piggyBac transposon vector by Tamura *et al.*, (2000) ^[37] opened the door to targeted genetic enhancement of disease resistance. Transgenic silkworms expressing dsRNA targeting BmNPV late genes (polyhedrin, gp64, p25) constitutively or under inducible promoters have demonstrated substantially elevated resistance to lethal BmNPV challenge in laboratory assays (Kanginakudru *et al.*, 2007; Singh *et al.*, 2014) ^[15, 34]. The mechanism involves RNAi-mediated silencing of viral transcripts in tissues where the dsRNA transgene is active, reducing viral replication below the threshold of lethal infection.

Transgenic silkworms overexpressing antimicrobial peptides such as defensin and cecropin under heat shock or fat body-specific promoters have shown enhanced survival upon bacterial challenge Yamamoto *et al.*, 2004) ^[45]. Expression of antifungal peptides and lectin-like proteins to bolster resistance against *Beauveria bassiana* has also been reported. Despite promising laboratory results, the commercialization of transgenic silkworm strains faces regulatory hurdles, public acceptability concerns and technical challenges related to maintaining homozygosity of transgene expression across generations of commercial rearing. Li MW *et al.*, 2008) ^[9].

RNA Interference as a Management Tool

Beyond its role as a natural antiviral mechanism, RNAi has been explored as an exogenous management tool for silkworm disease control. Oral delivery of dsRNA targeting essential BmNPV genes in silkworm feed has been shown to significantly reduce viral load and larval mortality in experimental settings Singh *et al.*, (2014) ^[34]. Nanoparticle-encapsulated dsRNA formulations improve stability during transit through the silkworm gut and enhance cellular uptake compared to naked dsRNA, a critical consideration for practical application. The systemic spread of RNAi signals from the midgut to peripheral tissues in *Bombyx mori*, which is less efficient than in some other insects, remains a challenge that researchers are actively addressing through the engineering of chimeric dsRNA constructs with enhanced systemic activity Gu *et al.*, (2014) ^[10].

Microsporidia such as *Nosema bombycis*, which are exclusively intracellular, present particular challenges for RNAi-based control since the parasite resides within host cells and has a compact genome with limited non-essential gene targets. Nevertheless, dsRNA targeting *N. bombycis* spore wall proteins and polar filament assembly genes has shown partial suppression of spore germination and proliferation *in vitro*, and *in vivo* validation studies are ongoing Pan *et al.*, 2018) ^[27]. The application of RNAi against silkworm pathogens requires careful off-target analysis to ensure that silkworm host genes are not inadvertently silenced.

Omics and Genomics Contributions to Disease Resistance Research

High-throughput transcriptomic studies using RNA sequencing have profoundly enhanced understanding of the silkworm immune response to individual pathogens. Comparative transcriptomic analyses of BmNPV-resistant and BmNPV-susceptible strains identified differentially

expressed genes involved in the ubiquitin-proteasome pathway, prophenoloxidase activation and lysosomal digestion as candidate resistance factors Lu *et al.*, (2013) ^[20]. Similarly, transcriptome profiling of *N. bombycis*-infected silkworm tissues revealed the upregulation of autophagy-related genes, stress response pathways and specific AMP genes as part of the host response to microsporidian invasion Xu *et al.*, (2015) ^[44].

Proteomics using two-dimensional gel electrophoresis and mass spectrometry has complemented transcriptomic approaches by identifying differentially expressed haemolymph proteins and fat body proteins in response to pathogen challenge. Immune-responsive proteins identified include phenoloxidase, serine protease inhibitors (serpins), juvenile hormone binding proteins and a range of novel lectins with putative PRR functions Kong *et al.*, (2007) ^[17]. Genome-wide association studies (GWAS) conducted on diverse *Bombyx mori* accessions have begun to pinpoint specific genomic regions associated with field resistance to NPV and to flacherie, providing finer resolution mapping of resistance loci than QTL studies. Zhang *et al.*, (2007) ^[46].

Nanotechnology-Based Disease Management

Nanotechnology offers novel approaches to silkworm disease management. Silver nanoparticles (AgNPs) synthesized by green chemistry methods using plant extracts have demonstrated potent antibacterial activity against *Serratia marcescens* and *Bacillus* species implicated in flacherie, at concentrations that do not adversely affect silkworm larval viability or silk production Vijayakumar *et al.*, (2014) ^[40]. Zinc oxide nanoparticles (ZnONPs) similarly exhibit antibacterial and antifungal activity and have been evaluated as additives to rearing bed disinfectant formulations.

Nanoparticle-mediated delivery of dsRNA and chemical disinfectants addresses several limitations of their free forms. Chitosan nanoparticles and lipid nanoparticles improve the stability of dsRNA against nuclease degradation in the silkworm gut and facilitate endosomal escape and cytoplasmic delivery of the RNA cargo. Carbon nanotube-based formulations have been investigated as carriers for antimicrobial peptides. Bhattacharya *et al.*, (2019) ^[5]. The biosafety of nanomaterials in food-chain animals including silkworms requires careful toxicological evaluation and standardized testing protocols specific to sericulture applications are currently lacking. The translation of nanotechnology-based disease management tools from laboratory proof-of-concept to practical on-farm application will require cost-effective synthesis methods, scalable production and regulatory frameworks tailored to agricultural nanotechnology.

Environmental and Agroecological Management

The rearing environment plays a fundamental role in disease incidence. Temperature, humidity and air circulation within the rearing house directly influence the virulence and replication rates of pathogens and the immune competence of silkworms. High temperature (above 28 °C) and high relative humidity (above 85%) are strongly correlated with increased incidence of bacterial flacherie and fungal muscardine. Optimal rearing condition includes temperature of 24 to 26 degrees Celsius, relative humidity 70 to 80% and good ventilation constitute a powerful disease prevention

measure that is often undervalued relative to chemical interventions. Dandin *et al.*, (2003) ^[7].

Mulberry leaf quality significantly impacts larval immune competence. Leaves from mulberry plants infected with bacterial or fungal leaf pathogens, or contaminated with insecticide residues from neighbouring crops, represent a significant disease risk. Integrated pest management of mulberry pests reducing the need for broad-spectrum insecticide applications that leave residues on leaves is therefore an important component of silkworm disease management. Water-stressed mulberry plants produce leaves with lower moisture content and reduced nutritional quality, predisposing silkworms to increased disease susceptibility. Hence, irrigation management of mulberry gardens directly impacts silkworm health outcomes.

Rearing infrastructure design also matters considerably. Traditional rearing houses with poor ventilation, earthen floors that retain moisture and inadequate separation between different rearing cohorts are associated with higher disease incidence than well-designed modern rearing structures. The adoption of improved rearing technologies such as plastic netting rearing frames that facilitate waste removal and reduce contact between larvae and decomposing excreta, chawki rearing centers providing controlled-environment early instar rearing and mechanized leaf chopping that reduces leaf bruising and pathogen ingress through wounds has contributed to reduced disease incidence in well-managed sericulture programs.

Integrated Disease Management and Extension Approaches

No single disease management strategy is adequate for comprehensive protection of silkworms against the full spectrum of pathogens encountered in commercial rearing. Integrated silkworm disease management (ISDM) synthesizes multiple complementary approaches to achieve durable, cost-effective disease control while minimizing chemical inputs and their associated costs and risks. The ISDM framework, as conceptualized by national sericulture research systems in The use of disease-resistant or tolerant varieties, stringent sanitation and disinfection procedures, ideal rearing conditions that minimize environmental stress, high-quality mulberry leaf management, frequent monitoring and early disease detection and targeted chemical or biological interventions when threshold levels are reached are the six pillars that comprise China and India. Early disease detection is a critical but often neglected component of ISDM. Diagnostic tools enabling rapid, field-applicable detection of BmNPV, BmCPV, *Nosema bombycis* and major bacterial pathogens allow timely decision-making that can prevent a localized outbreak from escalating into a catastrophic crop loss. Conventional diagnostic methods including hemolymph microscopy, gram staining and cultural isolation are adequate for many purposes but are time-consuming and require trained personnel. Rapid immunochromatographic strip tests for BmNPV detection have been developed and validated for field use in China and Japan, offering results in 15 minutes without specialized equipment. Zhao *et al.*, (2017) ^[47]. PCR-based diagnostic kits for *N. bombycis*, BmNPV, and *Serratia marcescens* are commercially available in Japan and are increasingly being adopted in Indian seed production programs.

Technology transfer and extension education are indispensable for translating research findings into on-farm practice. A persistent gap between research recommendations and actual farmer practice remains a major constraint to disease management in smallholder sericulture in South and Southeast Asia. Extension approaches that have demonstrated success include farmer field school models where groups receive hands-on training in disease diagnosis and management, mobile phone-based advisory services providing real-time guidance on disease incidence and management in local languages, demonstration farm networks showcasing best practices, and government-subsidized distribution of certified disinfectants and disease-resistant seed (Gowda & Bhanu, 2018) ^[9].

Challenges and Future Directions

Despite significant advances in understanding silkworm disease biology and management, several challenges limit the effectiveness of current approaches. The emergence of new viral strains with altered virulence, such as the highly virulent BmNPV genotypes identified in recent outbreaks in southern India and Guangdong province, China, underscores the dynamic nature of the host-pathogen arms race and the need for continuous surveillance and strain characterization (Peng *et al.*, 2010; Lu *et al.*, 2013) ^[28, 20]. Multiple pathogen infections in single larvae, co-infections by BmNPV and *Nosema bombycis* or by BmCPV and bacterial flacherie complicate both disease diagnosis and management.

The regulatory landscape for novel disease management tools like transgenic silkworms, RNAi-based products and nanoparticle formulations is evolving but remains complex and inconsistent across countries. India, China and Japan have different regulatory frameworks for genetically modified insects and gaining approval for commercial deployment of transgenic disease-resistant silkworm breeds requires extensive safety data on non-target effects and environmental persistence, which takes years to generate. International harmonization of regulatory standards for biotechnology-based sericulture inputs would significantly accelerate commercialization timelines.

Climate change presents an emerging and largely unquantified threat to silkworm disease management. Shifts in temperature and humidity regimes in sericulture-intensive regions are expected to alter the geographic distribution and seasonal dynamics of silkworm pathogens, potentially extending the epidemiological windows for fungal and bacterial diseases and intensifying outbreaks during periods of thermal stress on silkworms. Adaptation of disease management strategies to climate-altered rearing environments will require coordinated research programs integrating entomopathology, climate science, and sericulture agronomy.

Conclusion

Silkworm disease management is a multidimensional challenge that sits at the intersection of entomology, plant science, microbiology, genetics and agricultural economics. The silkworm industry supports the livelihoods of millions of people globally and supplies one of humanity's most celebrated natural fibers, yet its productivity remains chronically constrained by preventable disease losses. The most pressing need is for the integration of modern molecular diagnostic and breeding tools with the proven foundations of good sanitation and environmental

management. No single technology will suffice; rather, it is the thoughtful and context-appropriate combination of genetic improvement, informed sanitation, biological inputs and farmer education that will drive sustainable reductions in disease-related yield losses. As the genomic and molecular toolkit for silkworm research continues to expand, the prospects for durable, environmentally benign and economically accessible disease resistance management in sericulture are genuinely encouraging. Sustained investment in sericulture disease research and an effective research-to-practice pipeline will be essential to realize this potential and secure the future of the global silk industry.

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Mode (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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