

Review Article

Mixed Infections in the Silkworm *Bombyx mori* L.: Pathogen Interactions, Disease Dynamics and Implications for Cocoon Productivity

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Article Information:

Received: 06 December 2025 | Revised: 08 January 2026 | Accepted: 09 January 2026 | Published: January 10, 2026

Cite this article:

Pramod, K., Prakruthi S. N., Varun M. S., Mahesh, C. M., Murthy. S. (2026). Mixed Infections in the Silkworm *Bombyx mori* L.: Pathogen Interactions, Disease Dynamics and Implications for Cocoon Productivity *Public Health Open Journal*.

11(S1):32–46. DOI: 10.17140/PHOJ.11.S1.32

Abstract

Sericulture represents an important agro-based industry that supports the livelihood of millions of farmers worldwide, particularly in Asian countries such as India and China. The domesticated silkworm *Bombyx mori* is highly susceptible to a wide range of pathogens, including viruses, bacteria, fungi and protozoa, which significantly affect cocoon yield and silk quality. Among these disease conditions, mixed infections involving two or more pathogens have emerged as a critical challenge in silkworm rearing systems. Mixed infections frequently occur under field conditions due to environmental contamination, poor rearing hygiene and the simultaneous presence of multiple pathogenic microorganisms. These infections often exhibit complex interactions such as synergism, antagonism or competitive inhibition, which can alter disease severity and host immune responses. Studies have demonstrated that combined infections involving pathogens such as *B. mori* nucleopolyhedrovirus, cytoplasmic polyhedrosis virus, infectious flacherie virus and various bacterial species can significantly increase larval mortality and reduce cocoon productivity compared with single infections. The pathogenesis of mixed infections involves disruption of the silkworm midgut epithelium, impairment of nutrient absorption, immune suppression and systemic spread of pathogens within the host. Consequently, infected larvae exhibit reduced growth, prolonged larval duration and impaired silk gland development, ultimately leading to substantial economic losses in sericulture. Understanding the mechanisms underlying mixed pathogen interactions is therefore essential for developing effective disease management strategies. This review synthesizes current knowledge on the occurrence, mechanisms, and consequences of mixed infections in *Bombyx mori*. It also highlights host immune responses, pathogen interactions, and their impact on silkworm physiology and productivity. Finally, the review discusses emerging research directions, including molecular diagnostics, genomics-based resistance breeding and integrated disease management approaches that could mitigate the impact of mixed infections and promote sustainable sericulture production.

Keywords: *Bombyx mori*, mixed infection, flacherie, viral diseases, bacterial pathogens, sericulture host-pathogen interaction, silkworm immunity

Introduction

Sericulture is an ancient agro-industrial practice involving the cultivation of mulberry and the rearing of silkworms for natural silk production (Aruga, 1994; Rahmathulla, 2012). The domesticated silkworm *Bombyx mori* has been utilized for over five thousand years and remains the principal species in commercial sericulture worldwide (Aruga, 1994; Goldsmith et al., 2005; Rehman et al., 2026). Silk produced by *B. mori* is highly valued for its unique physicochemical properties, including high tensile strength, biocompatibility and lustrous appearance, making it a premium textile fiber in global markets (Datta & Nanavaty, 2007; Kundu et al., 2022). Countries such as China and India dominate global silk production, contributing a major share of raw silk output, with sericulture supporting millions of rural households in India by providing employment in both farm and allied sectors (Nagaraju, 2002; FAO, 2024). Despite its economic importance, silkworm rearing is highly susceptible to various biotic stresses, among which infectious diseases are a major constraint to sustainable production (Rahmathulla, 2012). Silkworms are vulnerable to a wide range of pathogens, including viruses, bacteria, fungi and protozoa and among the silkworm diseases that cause economic damage, the highest crop loss is attributed to viral diseases, accounting for 70% of total crop loss every year (Tanada & Kaya, 2012; Sharma & Sharma, 2020). Major diseases affecting *B. mori* include grasserie caused by *Bombyx mori* nucleopolyhedrovirus, cytoplasmic polyhedrosis caused by cytoplasmic polyhedrosis virus, flacherie associated with bacterial and viral pathogens, muscardine caused by entomopathogenic fungi and pebrine caused by the microsporidian *Nosema bombycis* (Krishnaswami, 1986; Govindan et al., 1998; Muhammad et al., 2024). Traditionally, these diseases have been studied as single-pathogen infections, where one microorganism is identified as the causative agent. However, increasing evidence from field observations and epidemiological studies indicates that multiple pathogens often coexist within the silkworm rearing environment. Contaminated mulberry leaves, rearing equipment and environmental sources such as soil and air serve as reservoirs of diverse microbial populations (Aizawa, 1964; Chen & Lu, 2018). Under such conditions, silkworm larvae may be exposed to two or more pathogens simultaneously, resulting in mixed infections involving combinations of viruses, bacteria, fungi or protozoa (Tanada & Kaya, 2012; Zhou et al., 2025). These mixed infections often produce complex disease manifestations that differ significantly from single-pathogen infections (Sharma et al., 2020; Muhammad et al., 2024). Mixed infections are

commonly associated with diseases such as flacherie, where viral agents like infectious flacherie virus and cytoplasmic polyhedrosis virus interact with bacterial pathogens including *Bacillus*, *Streptococcus* and *Serratia* species (Kawase et al., 1980; Sharma et al., 2020). Interactions among co-infecting pathogens can significantly influence disease progression, pathogen replication and host immune responses. These interactions may be synergistic, enhancing pathogenicity and accelerating disease development, or antagonistic, where one pathogen inhibits the growth of another. Such complexity makes disease diagnosis and management more challenging in sericulture systems (Deepika et al., 2024). The pathogenesis of mixed infections is often more severe than that of individual infections due to interactions that facilitate pathogen invasion and proliferation. Viral infections, for example, may damage the midgut epithelium, enabling secondary bacterial invasion into the hemocoel, while bacterial toxins can weaken host defences and promote viral replication (Doreswamy et al., 2004). These interactions result in extensive tissue damage, disruption of metabolic processes and increased larval mortality (Doreswamy et al., 2004; Muhammad et al., 2024). Consequently, infected larvae exhibit reduced feeding, impaired nutrient assimilation and abnormal development. Damage to key organs such as the midgut, fat body and silk gland adversely affects silk protein synthesis, leading to poor cocoon quality and reduced filament yield (Lv et al., 2021; Rehman et al., 2026). Recent advances in molecular biology and microbial diagnostics have improved the detection and characterization of multiple pathogens within infected silkworms, providing deeper insights into pathogen interactions and disease dynamics (Nagaraju and Goldsmith, 2002; Goldsmith et al., 2005; Mondal et al., 2024). In parallel, studies on silkworm immunity have revealed that *B. mori* possesses both cellular and humoral defense mechanisms, including haemocytes, antimicrobial peptides and enzymatic pathways such as phenol oxidase (Wan et al., 2013; Yang et al., 2018; Nesa et al., 2020). However, simultaneous exposure to multiple pathogens can overwhelm these immune responses, resulting in immune dysregulation and increased susceptibility to infections (Chen & Lu, 2018; Muhammad et al., 2024). Given the growing incidence and complexity of mixed infections, there is a pressing need to synthesize existing knowledge on pathogen interactions and their impact on silkworm health (Hu et al., 2023; Park et al., 2024). While extensive research has been conducted on individual pathogens, studies focusing specifically on mixed infections remain limited (Park et al., 2024). The present review therefore aims to provide a compre-

Table 1. Major diseases of silkworm and their causative agents

Disease	Pathogen type	Causative organism	Major symptoms
Grasserie	Virus	<i>BmNPV</i>	Swollen body, fragile larvae
Flacherie	Bacteria/virus	<i>Bacillus</i> spp., IFV	Soft body, diarrhea
Muscardine	Fungus	<i>Beauveria bassiana</i>	White fungal growth
Pebrine	Protozoa	<i>Nosema bombycis</i>	Black spots, weak larvae

Source: (Chen & Lu, 2018; Ito et al., 2021)

hensive understanding of the diversity of pathogens involved, their interaction mechanisms and their effects on silkworm physiology and cocoon production. Additionally, it highlights current disease management strategies and identifies future research directions for effective control of mixed infections in sericulture systems (Hu et al., 2023; Mondal et al., 2024).

Major pathogens affecting silkworm (*Bombyx mori*)

Silkworm diseases are primarily caused by four major groups of pathogens: viruses, bacteria, fungi and protozoa (Chen & Lu, 2018; Muhammad et al., 2024). These microorganisms vary in their pathogenicity, mode of transmission and impact on silkworm physiology. The classification of silkworm diseases based on the causative pathogens has been widely documented in sericulture literature (Krishnaswami, 1986; Goldsmith et al., 2005; Sharma & Sharma, 2020) and has been shown in Table 1 and Figure1.

Concept of mixed infection in silkworms

Mixed infection refers to the simultaneous presence of two or more pathogenic organisms infecting the same host organism at the same time. Such infections involve complex interactions between pathogens and the host immune system, which can significantly alter disease outcomes (Alizon et al., 2013).

According to Cox (2001), mixed infections may result in three possible interactions:

1. Synergistic interaction, where the presence of one pathogen enhances the virulence of another (Cox, 2001; Alizon et al., 2013).
2. Antagonistic interaction, where one pathogen suppresses the growth or pathogenicity of another (Cox, 2001; Read & Taylor, 2001).
3. Neutral interaction, where pathogens coexist without significantly influencing each other's effects (Cox, 2001).

In the context of silkworm diseases, mixed infections frequently involve combinations of viral and bacterial pathogens associated with flacherie disease (Cox, 2001; Alizon et al., 2013). These interactions can drastically modify host-pathogen dynamics and influence the epidemiology of silkworm diseases (Anusha, 2016).

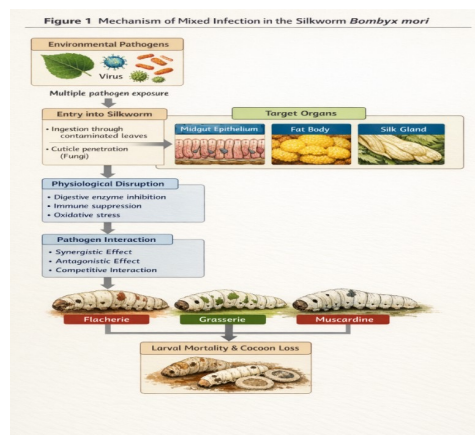


Figure 1. Mechanism of mixed infection in silkworm

The occurrence of mixed infections in silkworms is closely associated with environmental and management factors (Rahmathulla, 2012). Contaminated mulberry leaves represent one of the primary sources of pathogen transmission in sericulture. Mulberry leaves may harbor diverse microbial populations originating from soil, irrigation water and atmospheric deposition (Ponnuvel et al., 2008; Saad et al., 2019). When such contaminated leaves are fed to silkworm larvae, multiple pathogens may simultaneously enter the digestive tract and initiate infection (Hu et al., 2023). Rearing conditions also play a crucial role in determining the incidence of mixed infections. Overcrowding of larvae, inadequate disinfection of rearing equipment, poor ventilation and high humidity levels can create favorable conditions for microbial proliferation (Govindan et al., 1998; Rahmathulla, 2012; Sharma et al., 2020). Under these conditions, pathogens may rapidly spread among larvae through direct contact or through contamination of rearing trays and bed litter. Another important factor contributing to mixed infections is the weakened physiological condition of silkworm larvae. Environmental stress, nutritional deficiencies and exposure to pesticides can suppress the immune system of silkworms, making them more susceptible to opportunistic pathogens (Nagaraju & Goldsmith, 2002; Nesa et al., 2020). Once the host defenses are compromised, multiple pathogens may colonize different tissues and establish infection simultaneously (Lu et al., 2018; Zhu et al., 2023). The epidemiology of mixed infections is further complicated by the diversity of pathogens

present in the sericulture environment. Studies have shown that multiple viruses, including nucleopolyhedrovirus, cytoplasmic polyhedrosis virus and infectious flacherie virus, may coexist within the same silkworm population (Khurad et al., 2004). Similarly, bacterial communities in silkworm gut environments often include several opportunistic species capable of causing flacherie disease under favorable conditions (Doreswamy, 2002). These complex pathogen interactions make it difficult to identify the primary causative agent of disease outbreaks in silkworm farms (Tanada & Kaya, 2012; Alizon et al., 2013). Consequently, effective management of mixed infections requires a comprehensive understanding of pathogen ecology, host physiology and environmental factors influencing disease transmission (Mondal et al., 2024; Kiruthika et al., 2025; Rabha et al., 2025).

Pathogens involved in mixed infections

Mixed infections in silkworms involve diverse microbial pathogens that interact within the host to influence disease progression. Among these pathogens, viruses and bacteria play the most prominent roles, although fungal and protozoan agents may also contribute to complex disease syndromes (Goldsmith et al., 2005; Tanada & Kaya, 2012).

Viral pathogens

Viral infections are among the most destructive diseases affecting silkworm populations (Tanada & Kaya, 2012). The nucleopolyhedrovirus of *B. mori* is one of the most extensively studied pathogens in sericulture (Blissard & Rohrmann, 1990; Goldsmith et al., 2005). This virus belongs to the family Baculoviridae and infects multiple tissues, including the fat body, hemocytes and epidermal cells (Rohrmann, 2019). Viral replication results in the formation of polyhedral inclusion bodies that accumulate within host cells, ultimately causing cellular lysis and systemic infection (Rohrmann, 2019; Mei et al., 2022). Cytoplasmic polyhedrosis virus primarily infects the midgut epithelial cells of silkworm larvae (Babu et al., 2009; Tanada & Kaya, 2012). Infected cells exhibit characteristic cytoplasmic inclusion bodies that disrupt digestive processes and reduce nutrient absorption (Krishnaswami, 1986). When CPV infection occurs together with bacterial pathogens, the resulting disease complex may lead to severe digestive dysfunction and rapid larval mortality (Govindan et al., 1998; Rahmathulla, 2012). Infectious flacherie virus represents another important viral pathogen associated with mixed infections. IFV is known to damage the epithelial lining of the silkworm midgut, thereby increasing susceptibility to secondary

bacterial invasion (Sivaprasad et al., 1997; Jiang, 2021). Studies have shown that IFV frequently occurs in association with bacterial pathogens responsible for flacherie disease (Govindan et al., 1998; Ayaz et al., 2025).

Bacterial pathogens

Bacterial pathogens are commonly associated with gastrointestinal diseases in silkworms. Several bacterial species belonging to the genera *Bacillus*, *Streptococcus*, *Serratia* and *Alcaligenes* have been implicated in flacherie disease (Govindan et al., 1998; Rahmathulla, 2012; Ayaz et al., 2025). These bacteria produce toxins and enzymes that disrupt the digestive system of silkworm larvae, leading to diarrhea, body softening and eventual death (Tanada & Kaya, 2012; Ayaz et al., 2025). In mixed infections, bacterial pathogens often act as secondary invaders that exploit tissues already damaged by viral infection. For example, the destruction of midgut epithelial cells by CPV or IFV may allow opportunistic bacteria to enter the hemocoel and spread throughout the body (Sivaprasad et al., 1997; Jiang, 2021). This synergistic interaction significantly increases disease severity and mortality rates (Alizon et al., 2013; Ayaz et al., 2025).

Fungal and protozoan pathogens

Although less frequently involved in mixed infections compared with viruses and bacteria, fungal and protozoan pathogens may also contribute to complex disease syndromes (Ayaz et al., 2025). The entomopathogenic fungus *Beauveria bassiana* can infect silkworm larvae through the cuticle and produce muscardine disease (Chandrasekharan & Nataraju, 2008; Mascarin & Jaronski, 2016; Chen & Lu, 2018). Under conditions of high humidity, fungal spores may proliferate rapidly and infect multiple larvae within the rearing environment (Chandrasekharan & Nataraju, 2008; Xing et al., 2017). The protozoan parasite *Nosema bombycis* is responsible for pebrine disease and may occasionally occur in combination with other pathogens (Hu et al., 2021; Bagheri et al., 2024). Mixed infections involving microsporidia and bacteria have been reported to cause severe physiological stress and reduced cocoon productivity in silkworm populations (Suraporn & Terenius, 2021; Huang et al., 2024).

Host-pathogen interactions and immune responses during mixed infection

The interaction between pathogens and the host immune system plays a crucial role in determining the outcome of infectious diseases in the silkworm *B. mori*. When silkworm larvae encounter pathogens, a complex network of physiological and immune responses is ac-

Table 2. Reported mixed pathogen infections in silkworm

Authors	Pathogens involved	Major findings
Aizawa et al. (1964)	IFV + bacteria	Demonstrated synergistic infection leading to substantially higher larval mortality than single-pathogen infection
Iwashita et al. (2004)	CPV + IFV	Histopathological evidence of simultaneous viral infection causing severe midgut epithelial degeneration
Watanabe et al. (1968)	IFV + Bacterial secondary infection	Confirmed viral gut damage predisposes larvae to opportunistic bacterial invasion
Hukuhara (2014)	Multiple flacherie-associated pathogens	Established flacherie as a complex polymicrobial disease syndrome rather than a single-pathogen disorder
Li et al. (2015)	IFV + host midgut interaction	Showed IFV disrupts midgut cellular machinery, facilitating secondary infection processes

tivated to prevent pathogen proliferation and maintain homeostasis (Wang et al., 2020). However, in the case of mixed infections, these defense mechanisms may become overwhelmed due to the simultaneous presence of multiple pathogenic agents (Lavine & Strand, 2002; Hoffmann, 2003). The primary route of pathogen entry in silkworm larvae is through ingestion of contaminated mulberry leaves (Suraporn et al., 2024). Once pathogens enter the digestive system, the midgut epithelium acts as the first line of defense against microbial invasion (Awais et al., 2024). The midgut possesses several protective mechanisms, including digestive enzymes, antimicrobial peptides and reactive oxygen species that help neutralize invading microorganisms (Wang et al., 2020; Awais et al., 2024; Ayaz et al., 2025). However, viral pathogens such as nucleopolyhedrovirus and cytoplasmic polyhedrosis virus can infect midgut epithelial cells and disrupt their structural integrity (Sun et al., 2016; Xia et al., 2023). Damage to these cells compromises the protective barrier of the gut, allowing secondary bacterial pathogens to invade deeper tissues (Sun et al., 2016; Jiang et al., 2021). Following pathogen invasion, the silkworm immune system activates both cellular and humoral responses. Cellular immunity involves the activity of hemocytes, which are specialized immune cells present in the hemolymph. Hemocytes play an essential role in phagocytosis, encapsulation and nodulation of invading microorganisms (Lavine & Strand, 2002; Wang et al., 2021). In mixed infection scenarios, hemocytes may encounter multiple pathogens simultaneously, leading to increased immune activation and metabolic stress (Wang et al., 2020; Ayaz et al., 2025). Humoral immunity in silkworms involves the production of antimicrobial pep-

tides such as cecropins, attacins and defensins (Nesa et al., 2020). These peptides are synthesized mainly in the fat body and secreted into the hemolymph to inhibit microbial growth (Hoffmann, 2003; Muhammad et al., 2024). In addition, the phenol oxidase cascade plays a crucial role in melanization and pathogen encapsulation. Activation of this pathway leads to the production of toxic quinones that help eliminate invading pathogens (Toufeeq et al., 2019; Muhammad et al., 2024). Despite these defense mechanisms, mixed infections often result in immune suppression or dysregulation (Jiang et al., 2021). Certain viral pathogens are known to interfere with host immune signaling pathways, thereby reducing the effectiveness of antimicrobial responses. For instance, baculoviruses can suppress the production of antimicrobial peptides and inhibit apoptosis in infected cells, allowing viral replication to proceed unchecked (Jiang et al., 2021). When bacterial pathogens exploit this weakened immune environment, they can rapidly proliferate within the host, leading to systemic infection (Ayaz et al., 2025). Another important aspect of mixed infections is the interaction among pathogens themselves (Luo et al., 2025). Some pathogens may enhance the pathogenicity of others by producing toxins or enzymes that damage host tissues (Luo et al., 2025). For example, bacterial proteases can degrade epithelial barriers, facilitating viral entry into host cells (Wang et al., 2022). Conversely, certain microbial species may compete for nutrients or produce inhibitory compounds that suppress the growth of other pathogens (Sun et al., 2016; Suraporn et al., 2025). The outcome of mixed infections therefore depends on a complex interplay among pathogen virulence, host immune responses and environmental factors (Ito et al., 2021). Understanding these interac-

tions (Fig. 2) is essential for developing effective disease management strategies in sericulture (Wang et al., 2022; Ayaz et al., 2025).

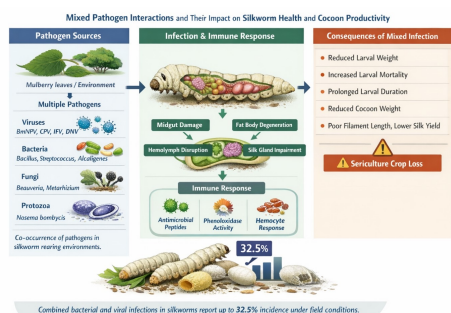


Figure 2. Host-pathogen interaction during mixed pathogen interaction

Factors contributing to mixed infection in silkworm rearing

The occurrence of mixed infections in silkworm rearing systems is influenced by several environmental, biological and management-related factors. (Nataraju et al., 2005; Rahmathulla, 2012; Zhou et al., 2025).

Environmental conditions

Environmental factors such as temperature, humidity and ventilation play a crucial role in the development and spread of silkworm diseases. Fluctuating temperature and humidity levels can create favorable conditions for the proliferation of multiple pathogens simultaneously (Rahmathulla, 2012; Selvakumar et al., 2013; Central Silk Board, 2024).

Poor rearing hygiene

Lack of proper sanitation in rearing houses can facilitate the accumulation of pathogens on rearing beds, equipment and mulberry leaves. Contaminated rearing environments can expose larvae to multiple pathogens simultaneously, thereby increasing the likelihood of mixed infections (Dandin & Giridhar, 2003; Nataraju et al., 2005; Islam & Qadir, 2024).

Nutritional stress

The nutritional quality of mulberry leaves plays a crucial role in determining the immune competence of silkworm larvae (Li et al., 2023). Poor nutrition can weaken the immune system, making larvae more susceptible to infections by multiple pathogens (Dandin & Giridhar, 2003; Rahmathulla, 2012).

High rearing density

Overcrowding in rearing trays can accelerate the spread of pathogens through direct contact between

larvae. High larval density also increases the accumulation of waste materials, which may serve as a source of microbial contamination (Nataraju et al., 2005; Wang et al., 2022; Central Silk Board, 2024).

Host susceptibility

The susceptibility of silkworms to infection can vary depending on factors such as larval stage, genetic strain and physiological condition (Ito et al., 2021). Certain developmental stages, particularly the late larval instars, are more vulnerable to infections due to increased metabolic activity and physiological stress (Rahmathulla, 2012; Xu et al., 2021; Jiang et al., 2021).

Effects of mixed infection on rearing parameters of *Bombyx mori*

Mixed infections caused by combinations of viral and bacterial pathogens have been widely reported to influence the biological performance and rearing success of silkworm larvae. These interactions can significantly modify larval growth, survival, developmental duration and physiological efficiency, ultimately affecting cocoon yield and silk productivity. The severity of disease symptoms is generally higher when silkworm larvae are simultaneously infected by multiple pathogens compared with single infections (Hukuhara, 2014; Ayaz et al., 2025).

Effects on larval growth and body weight

Larval body weight is a critical indicator of silkworm health and productivity (Suraporn et al., 2025). Several studies have demonstrated that mixed infections cause a substantial reduction in larval weight due to disruptions in metabolic processes and nutrient assimilation (Rahmathulla, 2012; Hukuhara, 2014; Feng et al., 2023). Viral pathogens such as cytoplasmic polyhedrosis virus (CPV) and nucleopolyhedrovirus (BmNPV) primarily infect midgut epithelial cells, impairing digestive functions and nutrient absorption (Sun et al., 2016; Jiang et al., 2021). When these viral infections occur in combination with bacterial pathogens, the physiological stress experienced by the larvae becomes more severe (Sun et al., 2016; Jiang et al., 2021; Ayaz et al., 2025). Experimental studies involving combined inoculation of CPV with bacterial isolates such as *Bacillus pumilus*, *Bacillus licheniformis* and *Acinetobacter baumannii* have shown drastic reductions in larval body weight compared with single pathogen treatments. The reduction in larval weight is primarily attributed to metabolic disruption caused by synergistic pathogen activity that interferes with digestive enzyme activity and nutrient assimilation (Hukuhara, 2014; Sun et al., 2016; Jiang et al., 2021). Similarly, mixed infections involving *Alcaligenes faecalis* and other bacterial

or viral pathogens have been reported to significantly reduce larval weight in *Bombyx mori*. These reductions are believed to result from the cumulative effects of pathogen-induced stress, immune activation and reduced feeding efficiency (Anusha & Bhaskar, 2018; Li et al., 2023). Viral infections such as BmNPV are known to destroy epithelial cells in the silkworm midgut, which subsequently impairs nutrient uptake and results in growth retardation (Xia et al., 2024). When bacterial pathogens colonize the digestive tract simultaneously, they further compromise digestive functions, leading to severe larval debilitation. Studies have shown that bacterial pathogens such as *Bacillus thuringiensis* and other species can cause significant larval mortality and growth inhibition in later larval instars of *B. mori* (Nataraju et al., 2005; Wang et al., 2022). In addition to physiological damage, mixed infections also increase metabolic energy expenditure because the silkworm immune system becomes highly activated in response to multiple pathogens. Consequently, the available energy resources are diverted away from growth and development towards immune defense mechanisms (Lavine & Strand, 2002; Ayaz et al., 2025).

Effects on larval duration and development

The duration of larval instars and moulting periods is another important parameter affected by mixed infections (Yang et al., 2016; Chen et al., 2019). Pathogen-induced stress often disrupts hormonal regulation involved in insect development, leading to abnormal larval duration and delayed moulting (Yang et al., 2016). Experimental investigations have demonstrated that combined infections of CPV with bacterial pathogens significantly prolong larval and moulting durations in *B. mori*. This phenomenon occurs because pathogens interfere with endocrine signaling pathways that regulate ecdysteroid and juvenile hormone levels, which are essential for normal moulting cycles (Chandana et al., 2020). Mixed infections also lead to degeneration of tissues and apoptosis in various organs, including the midgut and silk glands (Liu et al., 2019). These pathological changes impair larval metabolism and delay developmental processes. In severe cases, larvae may die before completing the final instar stage (Li et al., 2019). Studies have also reported that bacterial pathogens associated with flacherie disease rapidly multiply in the silkworm gut and subsequently invade other tissues, disrupting physiological homeostasis and leading to developmental abnormalities (Saad et al., 2019; Chen et al., 2019).

Effects on larval mortality

One of the most significant consequences of mixed infections in silkworms is the increase in larval mortal-

ity. Research has consistently demonstrated that the mortality rate is significantly higher when larvae are exposed to multiple pathogens simultaneously compared with single infections (Nesa et al., 2020; Chandana et al., 2020). Flacherie disease is often associated with mixed infections involving bacteria and viruses. Field surveys have revealed that silkworm larvae may exhibit individual infections with bacteria or viruses, but combined infections of both pathogen types can reach levels as high as 32.5% in some rearing conditions. This indicates the importance of pathogen interactions in determining disease severity (Chandana et al., 2020). Experimental evidence further indicates that sequential infections can also influence mortality outcomes. For example, when silkworm larvae are initially infected with bacterial pathogens such as *Streptococcus faecalis* followed by viral infection with BmNPV, mortality rates increase significantly compared with the reverse sequence of infection. This suggests that bacterial infections may weaken host immunity and facilitate viral replication, resulting in synergistic pathogenic effects (Xia et al., 2004; Wang et al., 2022). Similarly, combined infections of densovirus (BmDNV) with bacterial pathogens have been shown to produce higher mortality rates than single infections. The synergistic interaction between pathogens accelerates host tissue damage, leading to rapid larval death (Ito et al., 2013; Chen et al., 2019).

Effects of mixed infection on cocoon parameters

Mixed infections not only affect larval growth and survival but also significantly influence cocoon characteristics, which directly determine the economic value of sericulture production (Nesa et al., 2020; Chandana et al., 2020; Ayaz et al., 2025).

Cocoon weight and shell ratio

Cocoon weight and shell ratio are important economic parameters that determine silk yield. Several studies have demonstrated that mixed infections significantly reduce both cocoon weight and shell ratio due to physiological disturbances during larval development (Lye et al., 2024). The decline in cocoon parameters is largely due to pathogen-induced metabolic stress and impaired nutrient assimilation (Mohanta et al., 2015). When larvae are infected by multiple pathogens, the energy resources required for silk synthesis are diverted towards immune responses and survival mechanisms (Chen et al., 2019; Nesa et al., 2020). Experimental studies have shown that silkworm larvae infected with combinations of CPV and bacterial pathogens exhibit significant reductions in cocoon weight, shell weight and shell ratio compared with healthy larvae. These effects

are mainly due to disruption of silk gland development caused by pathogen activity (Chandana et al., 2020). Similarly, mixed infections involving *Alcaligenes faecalis*, *Bacillus subtilis* and viral pathogens have been reported to deteriorate cocoon characteristics in *B. mori*. Such infections impose severe physiological stress that interferes with the synthesis and secretion of fibroin and sericin proteins within the silk glands (Doreswamy et al., 2004; Anusha & Bhaskar, 2018; Chen et al., 2019).

Filament Length and silk quality

Silk filament length and denier are critical parameters that determine the quality and commercial value of silk. Mixed infections often result in shorter filament lengths and increased denier, indicating poor silk quality (Anusha & Bhaskar, 2018; Chandana et al., 2020). Pathogen-induced physiological stress can impair the functioning of silk glands, which are responsible for synthesizing silk proteins. Viral infections that damage epithelial cells of the silk gland can significantly reduce silk secretion (Chen et al., 2019). In addition, bacterial infections produce toxins that disrupt metabolic pathways and reduce protein synthesis efficiency. When these pathogens occur together, the combined effect leads to severe deterioration in silk quality parameters (Saad et al., 2019; Nesa et al., 2020). Studies have shown that the presence of viral pathogens such as Bm-NPV together with bacterial isolates causes substantial reductions in filament length due to impaired silk gland activity and reduced nutrient availability for silk synthesis (Chandana et al., 2020; Wang et al., 2022).

Mechanisms underlying pathogen interaction in mixed infection

The interaction between pathogens during mixed infection can occur through several biological mechanisms, including synergism, antagonism and immune modulation (Liu et al., 2015; Kausar et al., 2019; Wang et al., 2020).

Synergistic pathogen interactions

Synergistic interactions occur when one pathogen enhances the virulence or replication of another pathogen. In silkworm mixed infections, bacterial pathogens often compromise the host immune system, making it easier for viral pathogens to proliferate (Huang et al., 2009; Zhang et al., 2015; Wang et al., 2022). For example, bacterial toxins may damage epithelial barriers in the gut, facilitating viral entry and replication within host tissues. This results in accelerated disease progression and increased mortality (Kumar et al., 2019; Zhang et al., 2020).

Immune suppression and host response

The immune system of *B. mori* relies primarily on innate immunity mechanisms, including antimicrobial peptides, phenoloxidase activation and cellular immune responses (Tanaka et al., 2008; Kausar et al., 2019). However, during mixed infections the immune system may become overwhelmed due to simultaneous exposure to multiple pathogens (Nakahara et al., 2009; Liu et al., 2015). Recent studies have demonstrated that silkworm immune responses involve complex signaling pathways such as Toll, IMD and JAK-STAT pathways that regulate antimicrobial defense mechanisms. Viral and bacterial pathogens can interfere with these pathways, thereby weakening host resistance and facilitating infection (Huang et al., 2009; Liu et al., 2015; Zhang et al., 2015).

Pathophysiology of mixed infection in *B. mori*

The pathophysiology of mixed infections in the silkworm *B. mori* is characterized by complex interactions between pathogens and host physiological systems. When multiple pathogens infect the host simultaneously, their combined activities often produce more severe pathological effects than single infections. These effects arise from synergistic interactions that disrupt metabolic processes, impair immune responses and damage vital tissues such as the midgut and silk glands (Liu et al., 2015; Kausar et al., 2019; Wang et al., 2022). One of the primary targets of many silkworm pathogens is the midgut epithelium, which plays a crucial role in digestion and nutrient absorption. Viral pathogens such as cytoplasmic polyhedrosis virus (CPV) infect the epithelial cells of the midgut and cause extensive cellular degeneration. This infection results in impaired nutrient assimilation and reduced metabolic efficiency (Tanada & Kaya, 2012). When bacterial pathogens simultaneously colonize the digestive tract, they further aggravate tissue damage through toxin production and rapid proliferation (Huang et al., 2009). The degeneration of midgut epithelial cells disrupts the digestive process and significantly reduces the ability of larvae to convert mulberry nutrients into body biomass and silk proteins. Consequently, infected larvae exhibit reduced feeding efficiency, metabolic imbalance and growth retardation. Studies have shown that viral infections can also alter digestive enzyme activities such as proteases and amylases, further impairing nutrient utilization (Sinha & Datta, 2002; Kumar et al., 2019). Another important physiological consequence of mixed infection is systemic immune stress. The innate immune system of *B. mori* includes several defense mechanisms such as antimicrobial peptides, hemocyte-mediated

phagocytosis and activation of the phenoloxidase cascade. However, simultaneous infection by multiple pathogens can overwhelm these defense mechanisms. Viral pathogens often suppress immune responses by interfering with signaling pathways, whereas bacterial pathogens produce toxins that damage immune cells (Hoffmann, 2003; Tanaka et al., 2008; Liu et al., 2015). The combined effect of immune suppression and tissue damage accelerates disease progression and increases larval mortality. In many cases, the infection spreads from the digestive system to other tissues, including the fat body, hemolymph and silk glands, resulting in systemic infection. Detection and diagnosis of mixed infection (Chen et al., 2019). Pathogen interactions during mixed infection may also influence each other's replication dynamics. For instance, bacterial infections may compromise epithelial barriers, facilitating viral entry into host tissues. Conversely, viral infections may weaken host immunity, enabling bacterial proliferation. Such synergistic interactions significantly increase the virulence of mixed infections compared with single pathogen infections (Cox, 2001; Wang et al., 2022). In addition to physiological damage, mixed infections also induce oxidative stress in silkworm larvae. Reactive oxygen species generated during immune responses can damage cellular components, including lipids, proteins and DNA (Wang et al., 2020). This oxidative stress further contributes to tissue degeneration and metabolic dysfunction (Liu et al., 2015; Zhang et al., 2015; Kausar et al., 2019).

Detection and diagnosis of mixed infection

Accurate detection and diagnosis of mixed infections are essential for effective disease management in sericulture. Because mixed infections involve multiple pathogens, diagnostic techniques must be capable of identifying different microbial agents simultaneously (Ravikumar et al., 2011; Zhou et al., 2015).

Microscopic examination

Microscopic examination remains one of the traditional methods for diagnosing silkworm diseases. Viral pathogens such as nucleopolyhedrovirus produce characteristic polyhedral inclusion bodies that can be observed under a light microscope (Hosaka & Aizawa, 1963; Brancalhão et al., 2000). Similarly, bacterial infections can be identified through staining techniques and observation of bacterial cells in infected tissues (Huang et al., 2009). Although microscopic methods are simple and inexpensive, they may not always distinguish between closely related pathogens (Ravikumar et al., 2011; Zhou et al., 2015).

Molecular diagnostic techniques

Recent advances in molecular biology have improved the accuracy of pathogen detection in silkworm diseases. Techniques such as polymerase chain reaction (PCR) and quantitative PCR allow rapid identification of viral and bacterial pathogens at the molecular level (Singh et al., 2011; Ponnuvel et al., 2008; Ravikumar et al., 2011). Molecular diagnostics are particularly useful for detecting mixed infections because they can simultaneously identify multiple pathogens in a single sample. These techniques are increasingly being used in sericulture research to study pathogen diversity and infection dynamics (Ravikumar et al., 2011; Zhang et al., 2015).

Serological methods

Serological methods such as enzyme-linked immunosorbent assay (ELISA) are also widely used for detecting viral infections in silkworms. These techniques rely on antigen-antibody interactions to identify specific pathogens (Shamim et al., 1995; Sivaprasad et al., 2003). Serological tests are particularly useful for large-scale screening of silkworm populations and early detection of disease outbreaks (Bhattacharya et al., 1995; Sivaprasad et al., 1997; Qazi et al., 2025).

Management strategies for mixed infection

Effective management of mixed infections requires an integrated approach that combines preventive measures, improved rearing practices and disease monitoring (Central Silk Board, 2024).

Maintenance of rearing hygiene

Strict sanitation practices are essential for preventing disease outbreaks in silkworm rearing houses. Regular disinfection of rearing equipment, trays and rearing rooms helps eliminate pathogen reservoirs and reduce infection risk (Krishnaswami, 1986; Rahmathulla, 2012). Disinfectants such as bleaching powder, formalin and other approved chemicals are commonly used in sericulture to maintain hygienic conditions (Watanabe et al., 1968; Islam & Qadir, 2024).

Environmental control

Maintaining optimal temperature and humidity conditions in rearing houses is critical for preventing disease development. Proper ventilation and temperature regulation can reduce pathogen proliferation and improve silkworm health (Krishnaswami, 1986; Rahmathulla, 2012). Environmental control also helps reduce physiological stress on silkworm larvae, thereby enhancing their resistance to infections (Jolly, 1987; Rahmathulla, 2012).

Use of disease-free layings

The use of certified disease-free silkworm eggs is an important strategy for preventing the introduction of pathogens into rearing systems. Disease-free layings are produced through careful screening of parent moths for infections (Krishnaswami, 1986; Tanada & Kaya, 2012). This practice significantly reduces the risk of transmitting pathogens such as *Nosema bombycis* and other microbial agents to subsequent generations (Datta, 1992; Govindan et al., 1997).

Early disease detection

Early identification of infected larvae allows farmers to remove diseased individuals before the infection spreads to healthy populations. Prompt removal and disposal of infected larvae can significantly reduce disease transmission (Nagaraju & Goldsmith, 2002; Zhao et al., 2015). Regular monitoring of larval health and rearing conditions is therefore essential for effective disease management (Huang et al., 2009; Rahmathulla, 2012).

Future research directions

Although considerable progress has been made in understanding silkworm diseases, several research gaps remain in the study of mixed infections. Future research should focus on elucidating the molecular mechanisms underlying pathogen interactions and host immune responses. Advances in genomics, proteomics and transcriptomics provide new opportunities for studying host-pathogen interactions in silkworms. These approaches can help identify genes involved in immune defense and disease resistance. Another important research area is the development of disease-resistant silkworm breeds through genetic improvement programs. Breeding strategies aimed at enhancing resistance to multiple pathogens could significantly improve the sustainability of sericulture. Furthermore, the development of biological control agents and probiotics may offer promising alternatives for managing microbial infections in silkworm rearing systems.

Conclusion

Mixed infections represent a significant and increasingly recognized challenge in modern sericulture. The simultaneous presence of multiple pathogens within silkworm populations can lead to complex disease syndromes that are more severe than single-pathogen infections. These infections disrupt essential physiological processes in the silkworm, including digestion, metabolism and silk protein synthesis, ultimately resulting in reduced cocoon productivity and substan-

tial economic losses. Understanding the mechanisms underlying mixed infections requires an integrated approach that considers pathogen diversity, host immune responses and environmental influences. Advances in molecular biology, genomics and microbial ecology have provided valuable insights into these interactions, but further research is needed to fully elucidate the dynamics of pathogen coexistence within the silkworm host. Effective management of mixed infections will depend on the adoption of integrated disease control strategies that combine hygienic rearing practices, biological control methods and the development of disease-resistant silkworm strains. Such approaches are essential for safeguarding the sustainability of the sericulture industry and ensuring the continued production of high-quality silk.

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