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Review Article

Muscardine disease in silkworm: Pathogen biology, host-pathogen interactions, epidemiology and integrated management strategies

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Abstract

Muscardine is a fungal disease of silkworm which appears as powder-like efflorescence on the body of infected silkworm. The temperature around 25 °C and humidity ≥90 percent favors growth of muscardine causing fungi. The young age silkworms are more prone to fungal disease infections due to increased humidity and temperature in the rearing environment. The white muscardine caused by *Beauveria* (Bals) vuill is the most predominant fungal disease influencing Indian sericulture industry. It causes a 10-40% of total cocoon crop loss annually. Different physical and chemical methods are in vogue to manage muscardine disease. More emphasis is laid on the development of eco-friendly approaches of disease control; however the early detection can help in combating disease and prevention of cocoon crop loss. In this review, we have discussed different aspects of muscardine disease of silkworm including disease dissemination, diagnostic strategies and innovative management methods to manage the disease.

Keywords: Botanicals, Diagnostics, Fungal, Humidity, Mycelium, Oily specks

Introduction

Sericulture refers to rearing of silkworms for the production of cocoons. It involves many on-farm and off-farm activities *viz.,* growing of mulberry plant and rearing of silkworms industrial activity and activities like reeling of fiber from silk cocoon, twisting, dyeing, printing, finishing, knitting of silk fiber to produce raw silk (Astudillo *et al.,* 2014). The different processes and activities involved to obtain finished silk products from silkworm refer to sericulture (Altman & Farrell, 2022). Silkworm is susceptible to different pathogens like bacteria, viruses, fungi and microsporodia causing flacherie, grasserie, muscardine and pebrine diseases respectively and leads to significant cocoon crop losses (15-47 percent) annually (Tayal & Chauhan, 2017).The muscardine disease cause significant crop losses of 10-40% (Chopade *et al.,* 2021). The word muscardine originated from 'Italian word "muscardino" meaning 'musk', 'confit' or 'grape'. It is also known as "Calcino" or "Hardening disease" which refers to the white powder-like efflorescence of the white muscardine on insect bodies (Imoulan *et al.,* 2017). Since temperature around 25 °C and humidity above 90 percent favor growth of pathogenic fungi, muscardine is more prevalent in summer and autumn crops in temperate regions and crops reared during the rainy season in tropical regions (Tayal & Chauhan, 2017; Pandey & Kachhwaha, 2021). Muscardine poses a significant threat for the sustainability of sericulture industry in total loss due to diseases (Banerjee *et al.,* 2016). The Agostino Basi Italian entomologist was the first who reported of the white muscardine in insects in 1763 (Ansari *et al.,* 2012). In insects, *Beauveria bassiana* (Bals) vuill (1912) was the first entomopathogenic fungus observed to cause white muscardine in silkworm in 1835 (Shanmugam & Seethapathy, 2017). However, the widespread outbreak of white muscardine resulted in greater cocoon crop losses in France and Italy in 1925 (Seema *et al.,* 2019). White muscardine is named as Sunnakaddi or Sunnakattu roga in Karnataka and Chuna-Kete in West Bengal (Nirupama, 2014). Throughout this article, we intended to throw light on the pathogen, disease development, diagnosis and management strategies of muscardine disease in silkworm.

Causal organism

The most common species causing white muscardine in silkworms is *B. bassiana* infects over 100 different insect species coming from different insect orders. The colony of *B. bassiana* is velvety measures 6-23 nm in diameter and grows to a height of 5 mm. The condiospores are characterised by bearing hyaline conidia singly or sterigmata. Condia measure 2×2.5 µm in diameter and are smooth and globose to ellipsoidal. They are colorless but appear light green under microscope. These conidia are infective stages of the fungal life cycle and are resistant to environmental extremes. The conidia infect directly through the outside.

Muscardine disease is contagious caused by a wide range of fungal species. More than one thousand species of fungi exist are known to be causative agents of muscardine (Nirupama, 2014). They form differently colored conidia *viz.,* white, black, yellow, red, brown, black and named according to the color of conidia formed on the body of the insect (Pandey & Kachhwaha, 2021). In general, the mode of infection, life cycle, disease development and symptomatology of most of the muscardine infections is similar (Nirupama, 2014). The White muscardine

(*Beauveria bassiana*) and Green (*Metarhizium anisopliae*) muscardine are very common (Ribeiro *et al.,* 2017). The different types of muscardine diseases and their causal organisms are mentioned in Table 1.

Host range of B. bassiana

Host specificity or host range of an entomopathogenic fungus is defined as the number and taxonomic diversity of hosts it can infect (Brodeur, 2012). *B. bassiana* is the most common cause of insect diseases affecting a wide host range of over 700 insect species (Rohrlich *et al.,* 2018). The range of insect hosts includes economically important agricultural and forest pests, i.e., tephritides, arthropods, and termites; insect disease vectors; and ticks (Imoulan *et al.,* 2017). A host of agricultural and forest significance includes Colorado potato beetle, codling moth, several genera of termites, and bollworm, *Helicoverpa armigera* (Sinha *et al.,* 2016). Thirty-eight strains of the entomopathogenic *B. bassiana*, isolated from diverse species of Lepidoptera (Pyralidae) or Coleoptera (Curculionidae, Chrysomelidae, and Scolytidae) from various geographical sites, were reported (Maurer *et al.,* 1997). Twenty-nine genetically diverse *B. bassiana* strains were pathogenic to nine insect species across five orders, with significantly different virulence levels (Devi *et al.,* 2008). Chandrasekharan and Nataraju (2006) reported the incidence of white muscardine disease caused by *B. bassiana* in the Uzi fly (*Exorista bombycis* Louis) for the first time.

Entomopathogenic fungi in the Hypocreales are ubiquitous members of the soil microbiota. The entomopathogenic fungal species most frequently isolated from soils in temperate regions belong to the genera Beauveria, Isaria (Cordycipitaceae) and Metarhizium (Clavicipitaceae) (Sinha *et al.,* 2016; Peng *et al.,* 2019). Soil is a habitat for many potential insect hosts, some of which occur at high densities; continuity in proximity to potential hosts is a factor in the evolution of fungal entomopathogenicity. However, soil is also infused with antimicrobial metabolites secreted by microbes that can impair the ability of entomopathogenic fungi to infect their hosts (Vega *et al.,* 2009). For instance, Groden and Lockwood (1991) identified a significant trend of lower mortality of Colorado potato beetle by *B. bassiana* with increased soil fungistasis levels. A dead or dying insect infected by an entomopathogenic fungus represents a potential source of energy for other, opportunistic soil microorganisms. Some species of hypocrealean entomopathogens produce secondary metabolites within their insect hosts that are postulated to help the fungus outcompete opportunists during the saprotrophic phase of insect utilisation (Vega *et al.,* 2009).

A recombinant dsRNA-expressing strain of the entomopathogenic fungus Isaria fumosorosea has been developed to infect the whitefly, *Bemisia tabaci*, via cuticular penetration and then knock down an immunity gene, *TLR7*. The synergistic approach of using a fungal pathogen to enhance its own virulence via RNAi chimes with the wider concept that RNAi is probably most effectively deployed in concert with other pest control strategies. Intriguingly, the authors speculated that it may be the initial immune attack itself that damages the invading blastospores, thus liberating the dsRNA (Suhag *et al.,* 2021). While *I. fumosorosea* lacks host-specificity, other species and strains of entomopathogenic fungi are available that exhibit greater specificity (Sarkar *et al.,* 2024), and these could in future be exploited to deliver RNAi to disease vectors such as mosquitoes.

Developmental stages of fungal spores (B. bassiana)

Conidium or resistant spore

The conidium is oval or globular in shape and appears colourless, porcelain white in a mass. It germinates within 8-10 hours upon onset of favourable temperature and humidity conditions (Kumar *et al.,* 1999). These spores being infective stage of the disease remain viable at 45 °C for 48 hours and are killed if exposed for longer period and temperature higher than 48 °C. The conidia of *B. bassiana* survive for two and half years at 4 °C but less than 12 weeks at 23 °C (Lingg & Donaldson, 1981; Zimmermann, 2007). The conidial concentration (1×10^7 conidia/mL) can be estimated using Neubar hemocytometer under a Leitz DialuX20 EB microscope (400x magnifications) (Yeo *et al.,* 2003). The spore concentration may be adjusted to 2×10^6 conidia/mL (Mallikarjuna *et al.,* 2024).

Germ tube

Conidium germinates into a thin filamentous structure known as germ tube. The germ tube uses mechanical and enzymatic forces to secrete chitinase enzyme for proper penetration into the cuticle and facilitates further multiplication (Anwar *et al.,* 2017; Sharma & Sharma, 2021). It develops into vegetative hyphae in the haemocoel (Kumar *et al.,* 1999) (Figure 1).

Table 1: Muscardine diseases of silkworm and their causative agents

Muscardine type	Causative organism	Reference
White muscardine	*Beauveria bassiana*	Chen *et al.* (2015)
Black muscardine	*Metarrhizium anisopliae*	Rustiguel *et al*. (2012)
Green muscardine	*Nomuraea rileyi (Spicaria prasina)*	Zhang *et al*. (2016)
Yellow muscardine	*Paecilomyces farinosa (Isaria farinosa)*	Madelin (1963)
Red muscardine	*Sporosporella uvella*	Kumari *et al.* (2011)
Yellow-red muscardine	*Paecilomyces fumosorosea*	Zhu *et al.* (2022)
Orange muscardine	*Sterigmatocystis japonica*	Nesa *et al.* (2020)
Aspergillosis (brown muscardine)	*Aspergillus flavus*, *A. tamarii*, *A. sp.*	Kumar *et al.* (2004)

Vegetative mycelium

The multiplication of germ tube after invading larval blood forms the vegetative hyphae in the larval body (Yup-Lian, 1991). It appears as round or oval shaped short hyphal filaments which elongate and get detached from the main hyphal structure and produce vegetative spores (Samanta, 2015). The vegetative spores get dispersed throughout the haemocoel. The vegetative mycelium derives all the nourishment from insects and produces toxins, secretes calcium and magnesium oxalate crystals (Benz, 2012). These products make changes in the pH of insect gut and haemocoel influencing all the metabolic functions of the insect body which ultimately results in insect death (Nirupama, 2014).

Conidiophores

The hyphal filaments come out of insect skin and form many simple branched hyphae outside the insect body. The fungus grows saprophytically even after death of insects and penetrates all the body parts including silk gland, malpighian tubules, fat bodies, ganglia, and muscles of silkworm (Kumar *et al.,* 1999). The hyphal filaments develop further and produce aerial hyphae known as conidiophores which bear one or two conidia with characteristic color of species. The larval body turns hard, stiff and mummified due to growth of fungus on the insect body (Babulal *et al.,* 2023).

Isolation and purification of fungus

The larvae showing typical symptoms of muscardine are sterilized with 70% ethanol rinsed thrice with water and cut into small pieces and transferred to sterile petri plates containing Saboured Dextrose Agar (SDA) under aseptic conditions (Shanmugam & Seethapathy, 2017). B. *bassiana* is also cultured on Potato Dextrose Agar slants and sub-cultured in Petri dishes according to Linday (1962). PDA is prepared by mixing 200 g of peeled and diced potatoes, 20 g of dextrose, 15 g of agar in 1 L of distilled water (Mallikarjuna *et al.,* 2024). The inoculated petri plates are incubated at 28±1 °C under sterilized bell jars and observed periodically (Shanmugam & Seethapathy, 2017). The cultured fungus develops on the bits on SDA plates. A loop full of fungal culture is taken on a glass slide for the microscopic examination of presence/absence of conidia and conidiophores. The plates are examined for presence of fungal mycelium mostly after 4-5 days (Mallikarjuna *et al.,* 2024). The fungus is sub-cultured on Saboured Dextrose Agar (SDA) and allowed to grow for seven days at 28±1 °C and preserved at 4 °C, sub-cultured under aseptic conditions. The pathogens are visually examined for the morphological and spore characters in the culture media and also examined under microscope. The *B. bassiana* isolates are characterized based on the colony colour and spore structure. It produces white velvety to powdery type colonies which may turn yellowish in colour (Figure 2). The spores are mostly spherical or oval and non-septate (Shanmugam & Seethapathy, 2017). In the pathogenicity test, the re-inoculation of isolated fungus in the healthy silkworm population shows similar symptoms of disease. It is reported that inoculation of fungus after 12 hours of moulting shows highest mortality (100%) after inoculation (Taha *et al.,* 2016; Shanmugam & Seethapathy, 2017).

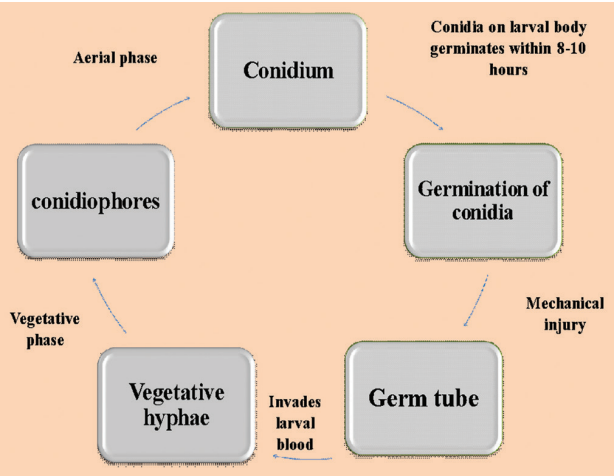


Figure 1: Disease cycle of fungi

Fungal spores can be isolated by adding 25 mL of sterile distilled water from week's old PDA plates by scraping each plate. A sterile funnel containing autoclaved glass wool is used to separate the spores from mycelia structures. The collected spore suspension is centrifuged at 4000 × g and washed three times with distilled water and finally re-suspended in 0.5 mL water. Spores are counted using a haemocytometer and diluted to the desired concentration (Shanmugam & Seethapathy, 2017; Nguyen *et al.,* 2024).

Disease diagnosis of white muscardine

The conventional technique to diagnose fungal disease infection in silkworm is based on the post-infection symptoms. The presence of oily specks on the integument of silkworm body or hardened powdery patches after death is some peculiar symptom to identify fungal disease (Deepika *et al.,* 2024). This technique is not reliable as



Figure 2: *B. bassiana* culture (Courtesy: Division of Silkworm Crop Improvement, College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and technology, Kashmir)

disease is diagnosed only after definite cocoon crop losses. The traditional methods to diagnose fungal disease infection include laboratory culturing and microscopy which largely depend on microscopic studies and staining protocols for the identification of pathogens (Gadwala *et al.,* 2021). These methods are found to lack sensitivity to pathogen specificity which poses inaccuracy and inefficiency in diagnosis of pathogen.

The serological techniques *viz.,* Enzyme-linked immunosorbent assay (ELISA), Lateral flow assay (LFA) kits are widely used in sericulture (Gani *et al.,* 2021). These are based on antigen-antibody complex visualization and rapidly detect and quantify various analytes, including viruses, bacteria and proteins (Koczula & Gallotta, 2016; Deepika *et al.,* 2024). However, these immunoassays are most sensitive and specific for the detection of viral and microsporodian pathogens of silkworm, not reliable for fungal pathogens

The molecular diagnostic techniques being rapid provide accurate and efficient diagnosis of pathogen can help in timely detection of disease causing pathogen. It involves use of DNA/RNA or protein-based molecular biology techniques to identify and diagnose disease causing pathogens (Deepika *et al.,* 2024). These molecular techniques for disease diagnosis are beneficial for timely prevention of crop losses incurred due to diseases (Chen & Lu, 2018). For the study of fungal disease pathogens in silkworm, following techniques were found to have potential of detection as well as differentiation of different fungal strains causing muscardine disease in silkworm.

The serological techniques *viz.,* ELISA, LFA is used to detect the pathogen causing muscardine disease in silkworm. However the differentiation among fungal pathogens is needed to ascertain the exact cause of disease. Polymerase chain reaction (PCR) is an efficient technique which employs use of DNA of causative agent to detect presence of particular disease. It has been used to differentiate strains of *B. bassiana* (Gençer *et al.,* 2023). The genetic relatedness among different strains of fungal pathogens can be studied by Random amplified polymorphic DNA-PCR (RAPD-PCR) profiles (Hassan & Nath, 2015). Another technique is loop-mediated isothermal amplification (LAMP) used for rapid amplification of DNA or RNA at a constant temperature range of 65-68 °C even in the presence of high levels of contaminants (Deepika *et al.,* 2024). LAMP has some special attributes *viz.,* its products can be visualized by the naked eye and requires no prior technical expertise to diagnose pathogens. It can be used in sericulture for the detection of various pathogens, including bacteria, viruses and fungi (which reduces the need for specialised equipment and makes it easier to perform in resource-limited settings (Deepika *et al.,* 2024). LAMP assay was exploited as an efficient technique to detect apple tree fungus *Valsa mali* which causes valsa canker disease in apples (Xu *et al.,* 2021).

Prevalence of muscardine disease

The influence of the environment on fungal disease spread is a significant and complex phenomenon driven by many factors. The growth, germination and sporulation of fungal spores are governed by climatic factors. Temperature and humidity are the main two factors regulating the fungal disease incidence (Tayal & Chauhan, 2017). The fungal growth and development is found to be optimum at temperature range of 20-30 °C and humidity range of 50-95% (Chandrasekharan & Nataraju, 2013; Tayal & Chauhan, 2017). The ultraviolet radiations (UV) were also found to influence the fungus that attacked silkworms. It is investigated that exposure of UV light to fungus infected silkworms can elicit toxin production (Zimmermann, 2007). However it requires acidic pH and diurnal light conditions in addition to optimum temperature and humidity for more fungal toxin production (Sundar *et al.,* 2021). The exposure of UV light to affected silkworms for four hours showed significant increase in toxin production which may be due to cell mutations (Qian *et al.,* 2020). The rainy season or winter season favours the growth, sporulation and development of fungal spores (Goettel *et al.,* 2010). Hence the disease is more prevalent in the rainy and winter season.

Mode of action of fungal toxins in the insect immune system

The fungal toxins of *B. bassiana* are primarily the secondary metabolites and small molecular compounds, such as beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, oxalic acid, calcium oxalate crystal activate immune system of silkworm (Wang *et al.,* 2021). The mycelia-secreted beauvericin is one of the most important toxins (Molnár *et al.,* 2010; Rohlfs & Churchill, 2011; Safavi, 2013) and activates innate immune system through different pathways by activation of reactive oxygen species (ROS), humoral melanization and phagocytosis to defend fungal pathogens (Zibaee & Malagoli, 2014). Beauvericin possesses nematicidal activity and both cytotoxicity and insecticidal activity is reported as well (Xu *et al.,* 2007). The mode of action of toxins is host-specific in insects, but the same toxin may have similar mechanisms of pathogenicity and toxicity in different insect hosts. However, the fungal insecticidal effect is a cumulative approach of *B. bassiana* toxins. There are many stages of insecticidal mechanism towards fungal toxin (Wang *et al.,* 2021) which involves:

a) Proliferation of virulence factors for continuous virulence
b) Hindrance to activation of the host immune system
c) Disruption of the nerve conduction pathways
d) Damage to the epidermis of the insect host to facilitate hyphae penetration
e) Clogging of the insect host spiracles.
f) Absorption of water and nutrients from the host body

The physical, chemical environment and host immune barrier are always adverse to fungal spores for successful invasion into the insect body. The immune responses are entry points which influence the accessibility and

adaptability of fungi inside the silkworm body. The pathogenic fungus enters the epidermis and invades the haemolymph of silkworm (Wang *et al.,* 2021). There are some direct and in-direct factors that regulate the pathogenicity of fungus inside the body (Figure 3).

Direct factors

Melanin

Melanin is a phenolic or indole compound with a lipid or protein component in its structure. It can provide cyto-toxicity against metal, Ultra Violet light and lysozymes in insect epidermis (Wilson *et al.,* 2001). However, melanin plays a greater role in formation of anti-fungal compounds in the forms of defensins to counter the host response (Langfelder *et al.,* 2003).

Upregulation of oxidative stress response genes

Fungi enhance the expression of oxidative stress response genes to prevent host immune response. For instance, superoxide dismutase enhances the conversion of superoxide ions into molecular oxygen and hydrogen peroxide. It results in enhancing the *B. bassiana* tolerance to oxidative stress by eliminating the insect-produced Reactive Oxygen Species (ROS) (Xie *et al.,* 2010). In addition, fungi upregulate the expression and the translation of oxidative stress response genes to counter the host immune response— for example, upregulation of superoxide dismutase accelerates the conversion of superoxide ions into molecular oxygen and hydrogen peroxide, thereby enhancing the *B. bassiana* tolerance to oxidative stress by eliminating the insect-produced ROS (Xie *et al.,* 2010). The overexpression of heat shock factor 25 likewise facilitates fungus penetration and resistance against host immune attack even at 35 °C in insect epidermis (Liao *et al.,* 2014). *B. bassiana* also exerts other factors that can directly inhibit the host immune system. These are primarily the active proteins or secondary metabolites with insecticidal properties, such as cyclooligomer non-ribosomal peptides (beauvericin), cyclic peptides (beauverolides), and 2-pyridone tenellin (Molnár *et al.,* 2010; Wang *et al.,* 2021). These factors may also function as immunosuppressive compounds. Notably, a study in Triatoma infestans showed that, compared with the

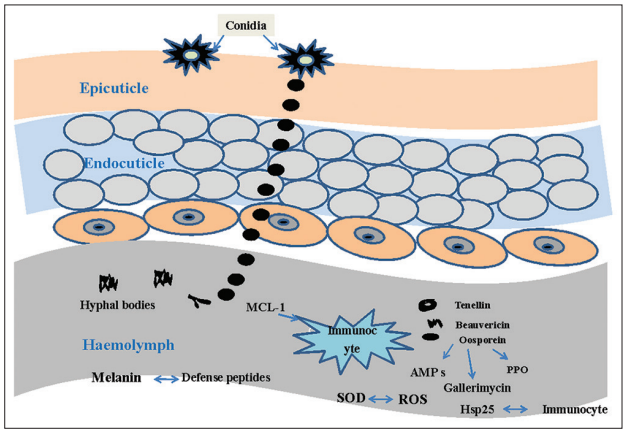


Figure 3: Fungal invasion inside silkworm body

third day of infection, many of the antimicrobial peptide and defensin genes were significantly inhibited on the ninth day of infection (Mannino *et al.,* 2019). Although the regulatory mechanism was not confirmed, it was largely due to fungal toxins (Baldiviezo *et al.,* 2020).

Indirect factors

Oosporein

Apart from bactericidal and fungicidal activities, oosporein also inhibits tumour cell proliferation (Feng *et al.,* 2015). It has been extensively studied for its wide spectrum of insecticidal properties and strong economic and environmental benefits. It causes 20% mortality in sap-sucking insects *viz.*, white-flies. In addition to fungal conidia, it is more lethal and causes 92% mortality (Namara *et al.,* 2019). It promotes infection by inhibiting the immune or defense mechanism (Amin *et al.,* 2011). It involves three mechanistic pathways which suppress the insect immune system.

1) It inhibits the splitting of prophenoloxidase into polyphenol oxidase (PPO), which then hinders the activation of prophenoloxidase (PO)
2) It directly inhibits the expression of antifungal peptide gallerimycin (from Gal gene) at the post-transcriptional level
3) It blocks the antimicrobial peptide cascade response (Feng *et al.,* 2015)

Symptoms of muscardine disease

The fungus infects all the developmental stages of silkworm. However, the disease symptoms are vibrant at the late stage of infection (Yup-Lian, 1991; Yu *et al.,* 2021). The diagnostic symptoms are:

a) The infected larvae lose appetite and become inactive.
b) The body of the larvae becomes limp, loses its skin elasticity, stops movement and finally they die.
c) Before death, symptoms of diarrhoea and vomiting appear.
d) After death, the body is initially soft, but within 6-8 hours it becomes stiff and hard.
e) The wooly aerial hyphae grow out between intersegment membranes and the whole body gets covered with white powdery conidia except the chitinous parts of the head.
f) The larvae unlike other diseases do not rot or decay but remain hard as the fungus secretes double oxalate - crystals of ammonium and magnesium (Figure 4).

Susceptibility of silkworms to B. bassiana

Most of the diseases are chronic in nature and curative measures are not practical (Sivaprasad *et al.*, 2021). The different larval stages and type of leaves provided to silkworm were also found to show co-related response towards fungal disease induction in silkworm (Ram *et al.,* 2016). The larval mortality due to fungal disease was reported to be higher when 4th and 5th instar (late age) larvae were reared on tender mulberry leaves when compared to



Figure 4: Muscardine infected silkworms (Courtesy: Division of Silkworm Crop Improvement, College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and technology, Kashmir)

1st - 3rd instar (young age) larvae fed with same type of leaves (Krishnakumar, 1998; Chauhan & Tayal, 2017; Saad *et al.,* 2019). The young age silkworms (Chawki worms) are more prone to fungal disease infections due to increased humidity and temperature in the rearing environment (Chauhan & Tayal, 2017; Gautam *et al.,* 2022). Silkworms are more susceptible to *B. bassiana* infection during the moulting period. Shanmugam & Seethapathay (2017) reported 100 percent larval mortality in silkworms inoculated with *B. bassiana* spores before 6 and 12 hrs of commencement of moulting period. Early instars/young age silkworms are more susceptible to infection by microbial pathogens and it decreased with larval ageing from first to fourth instars (Aruga & Watanabe, 1964). The integument in insects forms a strong barrier to microbes due to the presence of several chemicals present in it which inhibit the growth and penetration of disease causing microbes. The waxy epicuticular layer of silkworm integument contains free medium chain saturated fatty acids like caprylic acid, capric acid and linoleic acids that inhibit the invasion of fungi.

However, the thermo-tolerant bivoltine hybrids *viz.*, B1×B4 and B1×B8 exhibited better performance against muscardine infection than their parental breeds (Chandrakala *et al.,* 2022). The B4×B6, B6×B4 and B1×B6 proved better heterotic performance under muscardine infection (Jayashree *et al.,* 2024). It indicates the possibility of developing multiple tolerant breeds *viz.,* thermo-tolerant as well as muscardine resistant breeds (Thrilekha *et al.,* 2024). Nirupama (2014) screened the multivoltine and bivoltine silkworm breeds of Bombyx mori L with higher dose of conidial *B. bassiana* concentration ($1x10^7$) suspension to identify the resistant and susceptible against white muscardine disease. It was reported that out of 27 silkworm breeds, the multivoltine breed 2000H and bivoltine CSR6 exhibited relatively more resistant and susceptible breeds.

Development of disease resistance/tolerance in silkworm

Tolerance to diseases particularly to white muscardine is one of the important objectives in silkworm disease management and crop production because, if the breed is highly productive both in terms of quantity and quality and not tolerant to diseases, it is regarded as inferior. The development of disease tolerance is an important objective to manage the silkworm diseases. So, the development of muscardine resistant or tolerant breeds through conventional breeding methods is to tackle muscardine disease outbreak during silkworm rearing. Keerthana (2018) identified four muscardine resistant breeds *viz.*, B1, B4, B6 and B8 showing relatively higher tolerance to muscardine disease infection. It is reported that the disease resistance for fungi *B. bassiana* is polygenic. The muscardine disease resistance is determined by major genes, one is the 'mus' gene and the other is 'cal' gene located in the 11th chromosome and 7th chromosome respectively (Shimada, 1999). Similarly, muscardine gene susceptibility may also be related to several polygenes. The genetic materials having strong disease resistance can form the substantial basis for breeding disease resistance (Li *et al.,* 2023). The screening and identification of resistant breeds is the foremost step to evaluate the genetic base for development of high yielding genotypes (Makkar & Bentur, 2017).

Physiological changes caused by muscardine infection

The fungi are reported to cause several biochemical and physiological changes in insect tissues and accounts for 30-40% loss in cocoon yield (Babu *et al.,* 2009). Muscardine or mycosis is the most fatal disease which inflicts heavy loss to cocoon crops every year in the world (Nirupama, 2014). The fungal spores invade the gut of silkworm and influences pH, glycogen, total sugar content, and trehalose level of silkworm haemolymph (Boucias & Pendland, 1998). Significant dip in pH range of silkworm *B.mori* is observed when infected by *B. bassiana* spores (Helaly *et al.,* 2020). The pH range reached an extreme acidic state by the fifth day of 5th instar due to B. *bassiana* infection indicating the acidemia disease (Begum & Savithri, 2023). Due to acidemia, sudden biochemical changes in the organic components of certain silkworm breeds were reported. The significant increase in fat body glycogen in Pure Mysore (PM) and PM×KA (hybrid) with significant decrease in total sugar and fat body trehalose was reported. In the silkworms, infested with *B. bassiana*, significant increase in uric acid with significant decrease in ammonia content and osmotic pressure was reported (Chandrasekharan & Nataraju, 2008).

Management of muscardine

No silkworm disease can be controlled but can be managed by adopting precautionary measures like proper disinfection of the rearing room and appliances before commencement and after completion of rearing. The maintenance of proper spacing between silkworms in the rearing bed helps optimum hygrothermic conditions with regard to different instars (Chandrasekharan & Nataraju, 2008). The routine dusting of lime powder before moulting of silkworms and recommended bed disinfectant before half an hour of resumption and separation of muscardine infected larvae from rearing bed and their proper disposal by burying and burning are some prophylactic measures

to prevent disease spread. However, the different methods followed for muscardine disease prevention are broadly classified into physical and chemical methods.

The physical methods involve use of Ultra violet (UV) light, solar heat and flame in the rearing environment (Surapwar *et al.,* 2019). UV light exposure beyond 40 minutes to *B. bassiana* inoculated silkworms inactivates fungal spores (Chandrasekharan & Nataraju, 2008). A significant improvement in rearing parameters, larval mortality, spinning percent and pupation in *B. bassiana* inoculated silkworm trays treated beyond 30 minutes of UV exposure was reported (Chandrasekharan & Nataraju, 2008; Trivedy *et al.,* 2011). Solar heat treatment of fungal inoculated trays for more than two days effectively inactivates white muscardine. A significant improvement in rearing parameters of silkworm trays which received three to five days of solar treatment when compared to control (Chandrasekharan & Nataraju, 2008).Flame is used to destroy silkworm pathogens on wooden rearing equipments and cement surfaces, mud surfaces of rearing house. A minimum exposure period of three seconds is recorded to destroy fungal pathogens (Chandrasekharan & Nataraju, 2008).

The chemical methods refer to use of specific chemicals possessing anti-microbial activity against microbes for efficient disinfection (Trivedy *et al.,* 2011).The foremost approach of chemical method relies on the broad spectrum activity to destroy all the pathogenic microbes in the same specific feature Chemical disinfectants should be safe and non-toxic to untargeted ones (Pandey & Kachhwaha, 2021). Different chemicals are used in routine disinfection and showed efficacy against fungal pathogens. Captan was found more effective for control of white muscardine followed by formalin chaff and Dithane M-45. Captan & foltaf at percent concentration was found effective against muscardine and revealed no adverse effects on economic characters of cocoon due to fungicides (Chandrashekharan & Nataraju, 2008). Lime and bleaching powder mixture increased the survival by 20.75% over control in muscardine infected silkworm population (Usha, 1996; Chandrasekharan & Nataraju, 2008).

The traditional methods of disease management rely on the use of chemical agents which may influence silkworm rearing and cocoon crop production either directly or indirectly. These chemicals provide quick results but may be lethal at some stage due to underlying residual toxicities (Saad *et al.,* 2019; Bhat, 2021). More emphasis is laid on development of eco-friendly approaches of disease control for sustainable sericulture. Many plant-based products possess anti-microbial potential and show efficacy in prevention of silkworm diseases in sericulture. Most of the fungal growth inhibition was recorded when tested with garlic extract followed by *Vinca rosea*, ginger and turmeric extract (Chandrasekharan & Nataraju, 2008). Bhat (2021) reported the efficacy of many botanicals against *B. bassiana* and found *Juglans regia* with highest area of inhibition zone. The aloevera gel extract inhibited the growth of cultured fungus, *B. bassiana* on the 7th day of inoculation in culture media (Chandrasekharan & Nataraju, 2008). Aqueous and alcoholic extracts of herbal extracts of *Acalpha indica, Ocimum sanctum* and *Tridax procumbens* revealed efficacy in prevention of pathogens causing flacherie and muscardine disease in silkworm *B. mori* L. (Isaiarasu *et al.*, 2011). Ethanolic extract (3%) of *O. sanctum* has been found most effective for managing of white muscardine disease in silkworm, *B. mori* (Devi & Bai, 2014). *O. basilicum* leaves extracts and Nigella *sativa* seeds extract suppressed the bacterial and fungal disease spread when used three times during 4th and 5th larval instars (Saad *et al.,* 2019). Bhat (2020) reported the anti-fungal activity of *Ficus carica*, *Vitis vinifera* and *Datura stramonium* and are effective against white muscardine disease of silkworm.

Conclusion

Muscardine disease poses an obstacle in achieving successful cocoon crop production and leads to significant economic losses in sericulture. The effective disease diagnostics strategies should be adopted for early detection particularly in prevailing seasons for fungal growth. The pest infested or attacked mulberry leaves should be avoided for feeding silkworms as the insect pests may serve as alternate hosts of fungi attacking silkworm. Since muscardine prevails more in summer and autumn season in temperate regions and in rainy seasons in tropical regions. The development of muscardine resistant silkworm breeds should be a prime concern while developing season specific breeds for temperate regions and tropical regions respectively. The modern technologies *viz.,* image processing and Internet of Things (IoT) play a significant role in early disease detection, monitoring ecological factors influencing silkworm growth and managing disease outbreaks. The model of IoT is apparent for control of muscardine disease to achieve successful cocoon crop and promote smart sericulture.

Author contributions

Jasmeena Qadir: Writing – original draft preparation. Shabir Ahmad Bhat: Conceptualization and supervision. Ifat Bashir: Review and editing. All authors read and approved the final manuscript.

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