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Pathological Effects and Transmission Patterns of White Muscardine Disease in Silkworms and control measures

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ABSTRACT

White muscardine is a fungal disease primarily affecting silkworms, particularly during rainy and winter seasons. Silkworms (Bombyx mori L.) face several major diseases throughout the year, including grasserie, flacherie, muscardine, and microsporidian infections. Among these, white muscardine is especially severe, caused by the entomopathogenic fungus Beauveria bassiana, also known as mycosis. This highly infectious disease mainly impacts silkworms in their larval and pupal stages, leading to significant cocoon crop losses annually. Conditions of high humidity (90–95%) and temperatures below 25°C favour the spread and growth of the fungus.

Research indicates that infected larvae typically die within four to five days post-infection, with their bodies becoming mummified and covered with white fungal conidia within 24 hours. Infected larvae may attempt to spin cocoons but often die inside due to secondary infection, preventing moth emergence. The disease spreads when spores from dead larvae are transmitted, with high humidity and low temperatures accelerating infection within the rearing environment. This paper explores the relationship between environmental factors and the spread of white muscardine disease in silkworms, focusing on the morphological symptoms and transmission patterns and control measures by using bed disinfectants which is very effective to prevent the spreading of fungal diseases especially in rainy and winter seasons.

KEY WORDS

Silkworm Bombyx mori L., Fungal Beauveria bassiana and symptoms of white muscardine disease and control measures.

INTRODUCTION

White muscardine disease, caused by the fungal pathogen Beauveria bassiana, is a serious infectious disease affecting silkworms (Bombyx mori) and poses a significant threat to sericulture. This disease is recognized by the white powdery fungal growth that envelops the infected silkworms, leading to high mortality rates and consequently causing substantial losses in silk production. The fungus, a natural entomopathogen, infects silkworms by penetrating their cuticle, ultimately spreading throughout the host's body and resulting in death within a short period. Once the host dies, the fungus sporulates, releasing new spores into the environment and increasing the risk of transmission to other silkworms.

White muscardine disease is influenced by several environmental factors, such as humidity and temperature, which can affect the spread and severity of outbreaks in silkworm-rearing facilities.

This research aims to investigate the pathogenicity of B. bassiana in silkworms, assess the impact of environmental conditions on disease incidence, and explore potential biological or chemical control measures to limit the spread of white muscardine.

White muscardine, a disease primarily caused by the entomopathogenic fungus Beauveria bassiana, is among several muscardine diseases that affect silkworms. The term "muscardine" comes from the Italian word "moscardino," meaning musk or a confection resembling grapes, while "Calcino" refers to the white powdery appearance characteristic of the white muscardine fungus. Agostino Bassi, an Italian entomologist, first identified the disease in 1763, later naming it muscardine in 1835.

In Karnataka, white muscardine is locally known as "Sunnakaddi" or "Sunnakattu roga" (Janakiraman, 1961), whereas it is called "Chuna-Kete" in West Bengal (Mukerji, 1912).

There are different types of muscardine, identified by the color of the conidia on the infected silkworms, such as white, green, yellow, brown, and black. Over a thousand fungal species are recognized as muscardine agents (Yokohama, 1954). Silkworms face multiple microbial threats, but muscardine, a fungal disease, is particularly damaging. This highly contagious infection causes severe losses in cocoon production worldwide each year (Steinhaus, 1949).

In India, seasonal conditions affect the prevalence of white muscardine. For instance, it peaks during winter in Karnataka (Anonymous, 1975) and the rainy season in West Bengal (Mukherji, 1912). The disease's incidence also varies based on climatic factors, as Pringle (1984) observed over the past four decades. Favorable conditions for its spread include low temperatures and high humidity, particularly in winter and rainy seasons (Jayaramaiah et al., 1986). Losses from white muscardine can range from 5-50% in different countries (Jayaramaiah & Kuberappa, 1987). This fungus primarily infects silkworms in their third and fourth instars, with symptoms appearing in later stages and affecting all life stages. Reports from farmers in various sericultural regions of India suggest that silkworm diseases are a major cause of cocoon crop losses (Samson et al., 1990)

MATERIAL AND METHODS

Experimental Materials:

Materials used included Bombyx mori (silkworm) larvae, mulberry leaves, fungal pathogen spores (conidia), chemicals, glassware, and equipment for rearing.

Media Preparation, Culture Method, Stock Dilution, and Rearing Techniques:

To prepare the Potato Dextrose Agar (PDA) medium, an appropriate amount of PDA powder was dissolved in double-distilled water, then sterilized in a steam pressure cooker at 121ºC for 45 minutes. The sterilized medium was poured into Petri dishes and allowed to solidify for about an hour. Conidia were collected from diseased, mummified silkworm larvae using a sterilized inoculation loop and were cultured on the PDA plates. These plates were then incubated at 25ºC to promote fungal growth. To ensure purity, the fungus was re-cultured and purified using the monohyphal tip method, and all procedures were conducted under aseptic conditions within a Laminar Air Flow Chamber.

For inoculum preparation, fresh conidia of Beauveria bassiana were harvested from pure cultures and diluted with sterilized distilled water to the desired concentration. The concentration of the stock inoculum was determined using a Neubauer hemocytometer, following the procedure outlined by Cantwell (1973). The experiment was conducted using newly molted 4th instar larvae, with a dose of 1x10^6 conidial suspension per milliliter applied to 100 larvae through spraying.

After inoculation, larvae were kept in plastic trays lined with blue polyethylene sheets. Temperature was maintained at 25ºC ± 1ºC, with a high humidity level of 90-95% achieved using wet paper towels or foam pads, following the guidelines of Chandrashekaran and Nataraju (2008).

Disease Diagnosis:

A drop of conidial spore suspension was placed on a glass slide, stained with lacto phenol cotton blue, covered with a cover-slip, and observed under an electron microscope to examine germinated spores. Fungal cultures on PDA and microscopic images of Beauveria bassiana conidia were also observed.

Result and Discussion:

Diseases in silkworms represent abnormal conditions caused by various physical or physiological disruptions, which often result from insect interactions. Adverse climatic conditions, like increased humidity and low temperatures, can weaken the silkworms, making them more vulnerable to infections. These conditions also enhance pathogen multiplication, facilitating disease spread. Pathogens typically originate from already infected silkworms, and secondary infections can exacerbate disease severity. Progressive symptoms were observed daily after treatment, with noticeable signs appearing by the fourth day post-inoculation. The fungus Beauveria bassiana infects silkworm larvae through direct contact of its conidia with the larval body integument.

Visual Symptoms:

Infected larvae exhibit loss of appetite, sluggishness, reduced mobility, and decreased elasticity as the infection progresses. Moist, oily specks may appear on the body surface, and the larvae often vomit before dying within five days. Initially soft, the bodies of deceased larvae gradually stiffen, becoming rubbery before hardening and eventually mummifying as white fungal conidia cover the body surface. Mummified larvae, resembling a piece of chalk, are highly contagious, with the entire body, except the head region, covered in white mycelium, producing millions of conidia. Unlike other diseases such as grasserie, flacherie, and pebrine, mummified larvae do not decay, spoil, or emit odour. Larvae that survive infection may spin cocoons, though many infected pupae do not emerge as moths due to secondary infections (Ishikawa & Miyajima, 1964). Infected pupae exhibit slowed responses, die within the cocoon, and develop shriveled thoraxes and wrinkled abdomens covered with hyphae.

Microscopic Diagnosis:

Haemolymph samples from diseased and mummified larvae were collected, stained with lactophenol cotton blue, and examined under an electron microscope. Microscopic analysis revealed mycelial hyphae and cylindrical blastospores on conidia branches.

Muscardine infection results from fungal contamination and direct penetration by germ tubes, with the disease progressing acutely in younger worms and chronically in older ones. Transmission occurs through spore germination on the larvae's surface following death.

Control measures:

Early detection of fungal diseases in silkworms is essential for effective prevention. Maintaining appropriate humidity and temperature levels on rearing beds is crucial, as these factors significantly influence the spread of fungal infections.

To control humidity, applying slaked lime on the rearing bed can be beneficial. Additionally, certain chemicals like calcium hypochlorite (bleaching powder), chlorine dioxide, calcium chloride, sodium hypochlorite, and other chlorinated compounds can help kill fungal spores that infect silkworms.

Bed Disinfectants: Disinfectants play a key role of managing and controlling fungal diseases:

1. For Muscardine Control: Using sulfur, formalin chaff, and lime can help manage this disease.

2. Fungicides: Products like Diethane M-45, captol, bavistin are mixed with slaked lime powder can be dusted on the bed (@ 5gm-ft²) in measured amounts after each moult.

3. Bed Disinfectants: Products like RKO, Vetcare Vijetha Supplement, Ankush, Sanjeevani, Shakthi, Resham Jyothi, Labex, and Sericillin are effective in disease management.

4. Preventing Fungal Entry: Ensuring that fungal pathogens do not enter the rearing room is critical.

5. Controlling Alternate Hosts: Managing alternative hosts of fungal pathogens is vital in reducing the incidence of muscardine.

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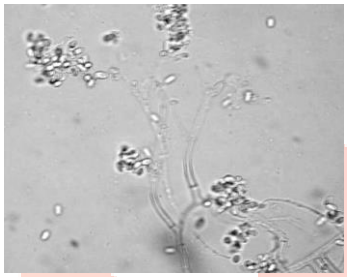
Healthy larvae

White muscardine infected larvae

Mummified larvae



Conidiagenous cell producing conidia in the form of rachis