FUNGAL DISEASE OF WHITE MUSCARDINE IN SILKWORM, *BOMBYX MORI* L.

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ABSTRACT: White muscardine is a fungal disease, it is most common and more prevalence during rainy and winter seasons. Usually silkworm diseases are prevalent throughout the year in all sericultural areas/countries. Silkworm *Bombyx mori* L. are affected by major diseases, such as grasserie, flacherie, muscardine and microspordian. White muscardine disease is most dangerous, virulent in silkworm and mainly caused by entomo-pathogenic fungi of *Beauveria bassisana* also named as mycosis. This disease is highly infectious in larval and pupal stages and inflicts heavy loss to cocoon crop every year. Low temperature and high humidity plays big role for the occurrence of muscardine, fungus grows well in relatively high humidity 90 to 95% and low temperature below 25ºC. The study result revealed that, diseased larvae die within four to five days of infection. Larva mummified after 24 hrs of death, the whole body covered with white fungi conidia, and survival diseased larvae spun the cocoon and dead inside due to severe/secondary infection and fail to emerge as moth. Disease is transmitted through developed germination spores by diseased larvae after its death and consequently high humidity and low temperature plays a strong role for spread of muscardine disease inside the rearing tray/bed. In the present paper study has been made to understand in relation to the white muscardine disease in silkworm and its nature of morphological symptoms role have been discussed.

KEY WORDS: Silkworm *Bombyx mori* L., Fungual *Beauveria bassiana* and symptoms of muscardine disease.

Silk production is the ultimate goal of sericulture and mulberry silkworm, *Bombyx mori* L. is an economically important to primary producer of tradable form of silk, it is a class of fiber of excellence grace and luster (Nataraju et al., 2005). India has unique distinction of being the only country in the world bestowed by nature with all the four known species of silkworm viz., Mulberry, Eri, Muga and Tasar. Especially mulberry sericulture is practiced in Karnataka, Tamilnadu, Kerla, Andhrapradesh, Assam, Bihar, Madhya Pradesh, Uttar Pradesh, Maharastra, Punjab, Rajasthan, Gujarat, Orissa, Himachal Pradesh, Nagaland, Meghalaya, Mizoram, Arunchal Pradesh and Tripura. The bulk of world silk production is 95% of mulberry silk origin, China, India and Japan occupy top three positions in mulberry raw silk production. The diseases are one of the main constraints in cocoon production, the outbreak of diseases and crop failures in silkworm rearing are common under tropical countries. Mulberry silkworm, *Bombyx mori* L. is affected by number of disease. In 1950 Dasgupta, reported major silkworm diseases caused by Grasserie (viruse), Flacherie (bacteria), Muscardine (fungi) and Pebrine (protozoan /microspordian). Silkworm diseases are considered as a major one and its prevalence through out the year in all sericulture areas cause of high mortality at various stages due to different diseases. In India the annual cocoon crop losses estimated around 30 to 40% due to different silkworm diseases like, virus, bacteria, fungi and microsporidian, out of these 10 to 40% loss recorded for white muscardine disease (Janakiraman, 1961).

Muscardine caused by various saprophytic fungi, but white muscardine mainly caused by entomo-pathogenic fungai of *Beauveria bassiana*. The word muscardine originated from "Italian, moscardino" meaning musk, confit, grape "Calcino" refers to the white powder like efforscence of the white muscardine. The Agostino Basi Italian entomologist was the first reporter of the diseases white muscardine in 1763. later in 1835 demonstrated the name of muscardine. In Karnataka white muscardine is named as Sunnakaddi or Sunnakattu roga (Janakiraman, 1961) and Chuna-Kete in West Bengal (Mukerji, 1912). Classifiacation of white muscardine belongs to scientific name: *Beauveria bassiana*. Kingdom: Fungi, Division: Eumycota, Class: Hypomycetes, Order: Moiliales, Family: Monoiliaceae, Genus: *Beauveria*, Species: *bassiana* (Balsamo Vuillemin). Also there are different types of muscardine named according to the colors of the conidia formed on the dead body of silkworm larva namely, white, green, yellow brown and black. More than one thousands species of fungi exists known as causative agent of muscardine (Yokohama, 1954). Silkworm diseases are mainly caused by microbial pathogens, among the four major diseases, fungal disease are recognized as muscardine or mycosis, is the most fatal and disastrous one, which is highly contagious and inflict heavy loss to cocoon crop every year in the world (Steinhaus, 1949).

In Karnataka, white muscardine occurrence is extremely high in winter season (Anonymous, 1975) and rainy season in West Bengal (Mukherji, 1912). The history of diseases in India during the last four decades and it may vary from season to season and different agro climatic conditions reported by (Pringle, 1984). A condition of low temperature and high humidity is congenial for the development of the disease is more in winter season (Jayaramaiah et al., 1986). The loss due to white muscardine varies from 5-50% in different countries (Jayaramaiah & Kuberappa, 1987). The fungus infects primarily the third and fourth instars silkworm and disease symptoms appear at late stage of infection and affect all stages of life cycle of silkworm. In Several reports from farmer of different Sericultural areas in India reported that, the cocoon crop loss is mainly due to silkworm diseases (Samson et al., 1990).

MATERIALS AND METHODS

Experimental materials:

Silkworm larvae of *B.mori*, Mulberry leaves, Fungal pathogen (conidia), Chemicals, Glass wares and Rearing equipments.

Preparation of media, culture method, stock dilution and rearing techniques:

The required quantities of Potato Dextrose Agar medium was slightly dissolved in double distilled water and molten the media in a steam pressure cooker at 121°C for 45 min, media was poured into sterilized Petri plates and kept it an hour for solidification. The conidia was scrapped from dead mummified larva of silkworm with the help of sterilized inoculation wooden loop and cultured on solidified medium, and plates were kept at 25°C in room temperature for fungal growth. The fungus was again cultured and purified by monohyphal tip method and whole experiment was conducted under aseptic condition of Laminar Air Flow Chamber. The inoculum was prepared by a fresh conidium of fungus Beauveria bassiana, harvested by pure culture and diluted in sterilized distilled water to get required concentration. The stock inoculum suspension was quantified by standard procedure of Neubauer haemocytometer and counting the conidia followed by (Cant well, 1973). Experiment was done on newly ecdysed

IIIrd moult out of 1st day 4th instars larvae. The dose of 1x10^{6} conidial suspension/1ml/100 larvae were inoculated per cutaneous by spraying on the body of larvae. Treated/inoculated larvae were reared in plastic trays with polythin blue sheets, under optimum temperature at 25^{o}c±1^{o}c and wet paper folds/wet foam pad kept inside the rearing trays to maintain the high humidity of 90 to 95% RH followed by (Chandrashekaran & Nataraju, 2008).

Diagnosis of disease: A drop of conidia spore suspension was onto glass slide and stained with lacto phenol cotton blue with cover-slip and germinated conidia spores was observed under Electronic Microscope. Cultured PDA fungi and microscopic conidia of Beauveria bassiana has been given in the Figures 1 and 2.

RESULTS AND DISCUSSION

Disease is a condition of abnormality resulting from physical or physiological derangements the whole systems of the body injure with an insect. The unfavorable climatic condition provides opportunity to the pathogens to infect the silkworm by causing weak, also higher humidity and low temperature enhance the susceptibility of silkworm to infection and increases multiplication of pathogen results in the spread and development of disease. The source of pathogenic micro organisms are normally diseased silkworms and severity of the disease is due to secondary infection. A progressive infection symptom was observed every day after treatment and symptoms was found after 4th day of inoculation. The way of infection by fungus *B. bassiana* conidia contact through the integument of the body of silkworm larva.

Visual diagnosis: diseased larvae loose appetite, become sluggish, ceases to move and loses elasticity as the disease advances moist oily specks appear on the body surface, larva vomits and die within five days. Diseased dead larvae body initially soft, corpses gradually stretches become rubbery, turn to harden and finally mummify due to white powdery fungi conidia cover the body surface and mummified within 48hrs of death. Mummified larva looks like white chalky piece, different stages of diseased, healthy and dead larvae have been presented in the figures 3, 4, and 5. The mummified stage considered as highly contagious and dreaded, the whole body covered with white powdery mycelium and produces millions of conidia except the chitinous parts of the head region. Mummified larva remains hard, do not decay, spoil or smell, unlike other diseased larvae of grasserie, flacherie and pebrine. Infected survival larvae were spun the cocoons and unable to emerge as silk moth due to secondary infection was found in pupal stage. These infectious microbes cause secondary infection and spread diseases stated by (Ishikawa & Miyajima, 1964). In the pupae stage of infection, infected pupae slowdown their reaction to outside stimuli and died inside the cocoon, dead pupal thorax shrinks and abdomen is wrinkled, body covered with aerial hyphae of white conidia have been given in figure 6 and infected dead pupae fail to emerge as moth.

Microscopic diagnosis: Haemolymph of the diseased worms and mummified samples were collected, placed onto glass slide and stained with lacto phenol cotton blue with cover-slip was observed under Electronic Microscope. Microscopic examination showed the presence of mycelia hyphae and cylindrical blastopores of conidia branches have been presented in the figures 7 and 8.

Muscardine infection is caused due to body contamination by fungus and direct penetration by germ tube. This disease is acute with young worms and chronic with grownup worms. Disease is mainly transmitted by the germination of spores which are formed on the outside of diseased larvae after its death. Many

studies were carried out in India and other sericulture countries on white muscardine based on silkworm. Among different types of muscardine, white muscardine is the most common, caused by *Beauveria bassiana* (Bals.) Vuillemin. The muscardine of *B. bassiana* is a well known entomopathogen of world wide distribution (Bulmer & Formtling, 1983). In 1835, Bassi reported that, infectious nature of the Beauveria bassiana not only attack the silkworm, but also occur in other insects. Low temperature and relatively high humidity plays a great role for spread and development of muscardine disease in the rearing bed. Highest rate of infection and mortality was found during rearing, similarly there is possibility for larvae to get infected either through food or other sources of contamination. Sometimes few worms are infected, it spreads within the host and affected worms release pathogens either through excreta or by direct contact leading to the secondary infection. This may ultimately lead to the spread of diseases in the rearing bed. The incidence of muscardine disease caused by high humidity and low temperature (Samson et al., 1990; Anon, 1992b). The source of infection mainly due to mummified larva, alternate hosts, contaminated mulberry leaves, infected insects and rearing appliances.

White muscardine disease can be managed by strictly disinfections of rearing house, rearing appliance, surrounding areas and bed disinfectants. Similarly, providing optimum temperature and humidity in the silkworm rearing room, proper ventilations, spacing in the bed, periodically bed cleaning and fed good nutrient leaves ensure to avoid spread of diseases. Use of heater during rainy and winter seasons helps to reduce high humidity in the silkworm rearing room. The main reason for outbreak of muscardine is due to its wide host range and faster rate of spreading nature, improper disinfections, and non hygienic condition and irregular rearing management cause for diseases. In 1999 and 2002, Nataraju and his team worker of CSR&TI, Mysore has been made an effort to develop an integrated technology against control of silkworm diseases and used chlorine dioxide, Anukush and Vijetha as main components. Use of white muscardine bed disinfectants like Vijeth, Ankush, as well as timely application of lime powder after every moult and maintenance of hygiene condition ensure the prevent of muscardine disease.

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Figure 1. Cultured fungi *Beauveria bassiana* on PDA.

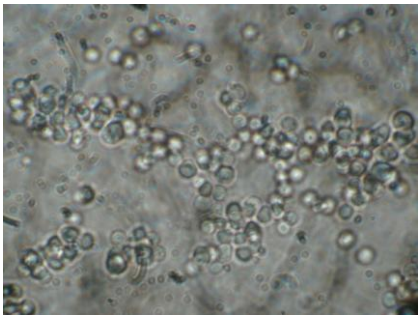

Figure 2. Conidia of fungi *B. bassiana.*

Figure 3. Diseased silkworm larvae by muscardine

Figure 4. Dead larvae Rubbery & Harden stage

Figure 5. Mummified larvae (white muscardine)

Figure 6. Mummified dead pupae (white muscardine)

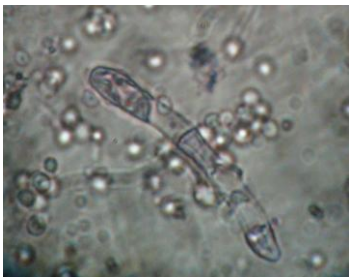

Figure 7. Mycelial hyphae with conidia in the haemolymph

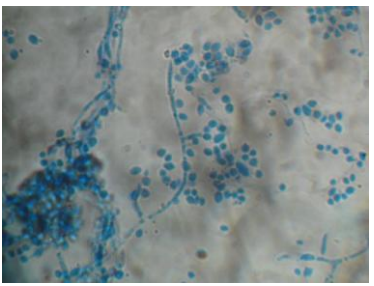

Figure 8. Conidiagenous cell producing conidia in the form of rachis.