

INFECTIOUS MYCOTIC DISEASES OF *BOMBYX MORI* L. SILKWORMS – REVIEW

BOLILE INFECȚIOASE MICOTICE ALE VIERMILOR DE MĂTASE *BOMBYX MORI* L. – REVIZUIRE

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Abstract

The silkworm can be affected in all stages of development (larva, chrysalis, butterfly) by the evolution of some diseases (mycotic, bacterial, viral, parasitic). Mycotic diseases of silkworms (muscardina, aspergillosis) are caused by fungi that parasitize the body of larvae and chrysalises and can seriously affect silkworm populations, causing significant production losses. To investigate the influence and evolution of fungal diseases on silkworms, we consulted the publications available in scientific databases, covering the period 2000-2024. Information from more than 52 publications was collected, and finally 23 relevant studies were analyzed. Statistical data indicate that temperature and humidity influence the development of these diseases. A relative humidity between 90 and 100% favors the appearance of diseases, while a level of 70% does not allow them to develop. Also, a temperature between 15 and 18°C is extremely favorable for the development of fungi, while spore germination is not recorded at 33°C. Proactive monitoring of silkworm health to prevent fungal diseases and prompt intervention when signs of disease appear are essential to maintaining healthy and efficient silk production.

Keywords: *silkworms, contagious mycotic diseases.*

Rezumat

Viermele de mătase poate fi afectat în toate stadiile de dezvoltare (larvă, crisalidă, fluture) de evoluția unor boli (micotice, bacteriene, virale, parazitare). Bolile micotice ale viermilor de mătase (muscardina, aspergiloza) sunt produse de către fungi care parazitează corpul larvelor și crisalidelor putând afecta serios populațiile de viermi de mătase, determinând pierderi semnificative ale producției. Pentru a investiga influența și evoluția bolilor micotice asupra viermilor de mătase, am consultat publicațiile disponibile în bazele de date științifice, acoperind perioada 2000-2024. S-au adunat informații din mai mult de 52 de publicații, iar în cele din urmă au fost analizate 23 de studii relevante. Datele statistice indică faptul că temperatura și umiditatea influențează dezvoltarea acestor boli. O umiditate relativă între 90 și 100% favorizează apariția bolilor, în timp ce un nivel de 70% nu permite dezvoltarea acestora. De asemenea, o temperatură cuprinsă între 15 și 18°C este extrem de propice pentru dezvoltarea fungilor, în timp germinarea sporilor nu se înregistrează la 33°C. Monitorizarea proactivă a stării de sănătate a viermilor de mătase pentru prevenirea bolilor micotice și intervenția promptă în cazul apariției semnelor de boală sunt esențiale pentru menținerea unei producții sănătoase și eficiente de mătase.

Cuvinte cheie: *viermi de mătase, boli contagioase micotice.*

INTRODUCTION

The silkworm can be affected in all stages of development (larva, chrysalis, moth) by the evolution of some diseases (fungal, bacterial, viral, parasitic). Fungal diseases of silkworms (muscardina, aspergillosis) are caused by fungi that parasitize the body of larvae and chrysalises and can seriously affect silkworm populations, causing significant production losses (5). Fungi that infect the body of silkworm larvae and pupae can lead to the development of fungal diseases.

The term "*muscardine*" generally attributed to mycoses affecting silkworms comes from the fact that the larvae, when they die of this disease, become rigid and are covered by a mycelium, which, depending on the pathogenic fungus responsible, can be of various colors (16). There are four types of muscardine, named after the color the larva's body acquires as a result of the disease, each caused by a specific fungus: white muscardine (*Beauveria bassiana*), green muscardine (*Spicaria prasina*), yellow muscardine (*Isaria farinosa*) and brown or black muscardine (*Aspergillus spp*). These are fungal pathogens that can affect various insect pests, and some of them are used in the biological control of these pests. Among these mycoses, white muscardina or "lime disease" is the most widespread, caused by *Beauveria bassiana*, a fungus that goes through three stages in evolution: conidia, vegetative mycelium and aerial mycelium. The conidia, which have an oval or globular shape and are visible as a white powder, penetrate the integument of the larva after germination. *Beauveria bassiana* spores can survive in the environment for several months to a year. *Beauveria bassiana* is a facultative parasite, being able to develop in different sources of infection (19, 22, 23). The main source of infection is diseased corpses and droppings that contaminate various other insects with which they come into contact. Contamination occurs through the growth of vegetative hyphae and small hyphae, which consume nutrients and water from the silkworm's body. This is the main cause of the formation of crystals in the hemolymph, disrupting its functions and circulation. The vegetative mycelium produces toxins which are the main cause of poisoning and death (28). Other sources of contamination include: transmission of spores through all surfaces that come into contact with diseased larvae, direct contact with persons carrying the disease, use of non-disinfected premises for rearing silkworms (29).

The route of infection in primary infection is based on contact with the infected environment. The disease is transmitted orally through food, the main routes of contamination being: digestive, respiratory, skin pores (32).

Predisposing factors are primarily related to the age of the larvae. In older ages (III-V), worms are much more sensitive than in younger ages (I, II). They are also sensitive during larval sleep, especially during the sleep exit phase. Statistics show that this disease is influenced by temperature and humidity. The conditions favoring the appearance of the disease are low temperature (15-18°C) and a humidity of 90-100%, when the fungus develops rapidly (8). The main source of spreading the disease is the corpse of the sick larva on which the spores of the fungus are produced. Also, the temperature of 24-28°C seems quite favorable for the development of the fungus, but the spores do not germinate at 33°C, it being impetuously necessary to control the microclimate factors, as a first measure to prevent and combat mycoses.. The disease frequently occurs in spaces where ventilation is not adequate or when these spaces are located near dirty water. The presence of elastic corpses to the touch of silkworms can be a first element in establishing the diagnosis. If these corpses, in humid conditions, develop conidia and become hard, with a chalky appearance, the diagnosis is certain. In the case of an asymptomatic evolution, the laboratory examination will highlight the presence of vegetative hyphae, useful for specifying the diagnosis.

The clinical signs of evolution of muscardine that affect silkworm larvae are: inappetence of the larvae (lack of appetite or refusal of food), the color of the larvae takes on a slightly red hue, the abdominal legs turn black, a black line appears on the line separating the head from the thorax - collar, after 10 days the larva's body becomes rigid and a white powder appears on its surface, giving the larvae a chalky appearance (chalky or matte surface), dark or black spots appear on the larvae's body (common sign of muscardina), loss of mobility and elasticity and the appearance of immobility (they lose mobility and elasticity, becoming immobile), and death occurs in 6-12 hours from the first signs of infection. Blackening of the abdominal legs in the terminal part occurs, suggesting a burn, corpses are stiff red-white, then white dust-like (red-white stiffness), which later turns into a white dust-like texture, with the death of the embryo in the inside of the egg. These clinical signs may vary depending on the severity of the infection and the fungal species involved. After a few days of infection, spots similar to small drops

of oil appear on the larva's body, initially with reddish hues, and before they die, the larvae present soft excrement and vomit. After death, the body which is soft and elastic becomes rigid, its color changing to pink-red. After 1-2 days, the appearance of the corpse changes and aerial hyphae appear, which develop on the intersegmental membranes and become covered with a white powder consisting of conidia. The number of conidia that appear on the body of the fifth-stage larva can be several million (35).

Infection in the chrysalis stage produces mummification of the chrysalis. The reaction to external stimuli is slow, the thorax shrinks, the abdomen is wrinkled, and hyphae and conidia grow between the intersegmental limbs.

In the adult stage, the body of the moth hardens and the wings become very fragile, with frequent breaks. The time from infection to death is generally 2-3 days for first-instar larvae, 3-4 days for second-instar larvae, 4-5 days for fourth-instar larvae, and 5-6 days for those in the fifth stage. These periods can vary depending on temperature, humidity and the general health of the silkworms.

Aspergillosis is a mycotic disease of silkworms that is produced by the etiological agent represented by fungi of the genus *Aspergillus sp.*, being a strongly saprophytic fungus, able to live in a saprophytic state on wood, tools, in bird feed, where there are humid conditions. Because of these varied sources of fungal presence, the spread is high.

Conidia are large, spherical in shape, 3-7 μ in diameter, with one surface smooth and the other rough, bright yellow in color that gradually changes to brown. Germination occurs at a temperature of 30-35°C, and this disease generally occurs in the first and second instars, with morbidity decreasing as the larvae age (13). After infection, in the 1st larval instar there is an interruption of mulberry leaf consumption, the larvae retreating under the leaf in a lethargic state. The corpses are covered by hyphae at the site of invasion, after a day the aerial hyphae also appear, and later, yellow and brown conidia cover the entire body of the larva. During the disease, by chance, large spots may appear on the body of the larva, and before death, the head and thorax relax and vomiting occurs. Characteristic in this disease is the fact that, in the place where the fungus has penetrated, the corpses are hard, and the other parts begin to blacken and rot. The larvae affected by the disease in the first age die during sleep, the disease appears in the adult stages, the presence of spots is sometimes observed, which may disappear during sleep and the silkworm recovers.

The infection of the mature larvae leads to the appearance of the disease in the chrysalis, manifested by its hardening, the color becoming dark brown. Increased humidity causes an abundant proliferation of aerial hyphae, the chrysalis becoming blackish. Silkworm eggs kept in a humid environment tend to be parasitized by *Aspergillus sp.*, the symptom of this parasitism being their darker than normal color and under these conditions, the embryo suffocates and dies (15).

Germination of conidia and invasion of the body is achieved slowly and only at the site of infection, when the fungus begins to secrete protease and aflatoxin, and symptoms of intoxication and mass mortality appear rapidly. Suffocation of the embryo in the egg is caused by the obstruction of the pores on the surface of the egg by the growth of aerial hyphae on its exterior.

MATERIAL AND METHOD

To explore new data on fungal infectious diseases of the silkworm *Bombyx mori* L., we considered publications accessible in the published scientific databases (2000-2024) Elsevier, MDPI, PubMed, Web of Science, Research Gate, Springer and Wiley Online Library. To search for articles on this topic, we used the following keywords: silkworms, muscardine, aspergillosis and infectious mycotic diseases. The criteria for selecting the publications consisted of the topic of the papers, years of study, articles in English that met the eligibility criteria. Data were collected from over 52 publications, considering 39 major studies, from which 23 relevant studies were finally selected. In the future, it is possible to witness the development of new investigative methods and methodological approaches in our

country, which could significantly contribute to the understanding and management of fungal infectious diseases in silkworms.

RESULTS AND DISCUSSION

Epidemiological investigation based on laboratory diagnosis is an essential component of efforts to prevent and control mycotic diseases in silkworms and can significantly contribute to maintaining the health and productivity of silkworm populations (Figure 1, 2, 3). By using modern techniques and appropriate equipment, laboratories can provide important information for managing the health and productivity of silkworm populations, thereby helping to maintain the sustainability of the sericulture industry (18).



Figure 1. Larvae with clinical signs of muscardine (Pau, 2000) / Larve cu semne clinice de muscardină (Pau, 2000)



Figure 2. Biological cycle I of *Beauveria bassiana*. Outside the body (vegetative mycelium, aerial mycelium, conidia, germinal spore, small mycelium). Inside the body (integument, middle intestinal wall, adipose layer, musculature) (Pau, 2000) / Ciclul biologic I al *Beauveria bassiana*. În afara corpului (miceliu vegetativ, miceliu aerian, conidii, spor germinal, miceliu mic). În interiorul corpului (tegument, peretele intestinal mediu, stratul adipos, musculatură) (Pau, 2000)

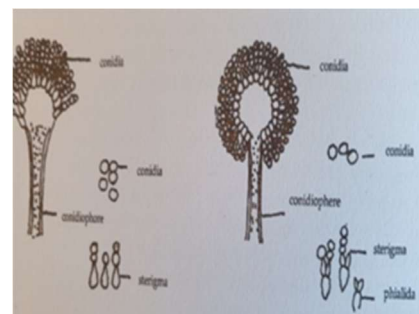


Figure 3. The species that produce aspergilloz; a) *Aspergillus flavus*; b) *Aspergillus oryzae* (Pau, 2000) / Specie care produc aspergiloza; a) *Aspergillus flavus*; b) *Aspergillus oryzae* (Pau, 2000)

Identification of mycotic diseases of silkworms by modern diagnostic technologies

Advanced molecular technologies, namely polymerase chain reaction (PCR) and DNA sequencing, are an essential component in the diagnosis of silkworm diseases (2, 5), presenting significant advantages in terms of sensitivity, specificity and diagnostic accuracy (37). PCR is a molecular technique that allows the selective amplification of specific DNA sequences, thus facilitating the detection and identification of the pathogen even in very small amounts of biological samples (6). This method is highly sensitive and can identify even incipient or subclinical infections (12).

PCR can be adapted for the detection of different pathogens (bacteria, viruses, fungi and parasites), which makes it a highly effective method in the diagnosis of silkworm diseases. DNA sequencing can provide essential information about the structure and evolution of pathogens, as well as the mechanisms of resistance to the treatments used. By using these advanced molecular technologies, laboratories can achieve accurate and rapid disease diagnosis in silkworms (40), thus contributing to the management of the health and productivity of silkworm populations.

Bioinformatics in the diagnosis of fungal diseases in silkworms represents a modern and efficient approach for the analysis of genetic data and for the rapid identification of specific genetic sequences associated with various conditions. The method consists of analyzing genetic data, identifying specific genetic sequences associated with different diseases, rapidity and efficiency of identification, personalization of treatments and disease management strategies (24). Bioinformatics involves the use of computers and specialized algorithms to analyze genetic data (DNA sequences, transcriptomes and proteomes) obtained from silkworm samples. This analysis can reveal important information about the

silkworm genome and its interactions with pathogens. By applying advanced bioinformatics techniques, such as genomic and transcriptomic sequence analysis, specific genetic sequences that are associated with various diseases in silkworms can be identified. By identifying the fungal pathogen and analyzing the molecular mechanisms involved in disease detection in silkworms we can gain significant insights into how diseases evolve and affect the health and productivity of these insects. This deep understanding of diseases can serve as the basis for developing effective prevention and control strategies, helping to maintain the health and stability of silkworm populations and protect the sericulture industry. Bioinformatics enables rapid and efficient identification of specific genera associated with silkworm diseases compared to traditional genetic analysis methods and can facilitate rapid implementation of control and treatment measures in silkworm farms (3). By applying advanced genetic data analysis techniques, bioinformatics can help identify relevant genetic markers for specific diseases (38), understand the molecular mechanisms involved in their pathogenesis, and develop personalized diagnostic and treatment strategies.

Immunodiagnosis of fungal diseases in silkworms involves the development and use of immunoassays and immunodetection technologies for the identification of disease-specific antigens and antibodies. This approach relies on the host's immunological reactions to the presence of a pathogen and can provide accurate and sensitive information about infection or exposure to a particular pathogen (35). Immunodiagnosics includes the development of immunological tests and their optimization such as ELISA (*Enzyme-Linked Immunosorbent Assay*) or agglutination tests for the detection of specific antigens or antibodies of certain diseases in silkworms and the evaluation of the effectiveness of treatments. These tests are designed to provide fast and accurate results and can be adapted to detect various pathogens (bacteria, viruses, fungi and parasites) (37). By detecting and quantifying these antigens in tissue or excrement samples, the presence of the pathogen can be confirmed and the degree of infestation in the silkworm population can be assessed. Immunological tests can also be used to detect specific antibodies produced by the host organism in response to the presence of a pathogen. This detection can provide information about the previous exposure of silkworms to certain diseases (carriers of pathogens) and can indicate the immunity status of the respective population. By monitoring the levels of antigens or antibodies before and after treatment, one can assess the host's response and determine whether the treatment has been effective in reducing the incidence of the disease (34).

The use of nanotechnology in the diagnosis of fungal diseases in silkworms represents an innovative and promising approach that may provide more sensitive and accurate methods of pathogen detection (9). Nanotechnology in this context can be integrated by using nanomaterials with unique properties for sensitive and specific detection of pathogens at the molecular level, developing nanosensor-based diagnostic systems, amplifying detection signals, and implementing automation in diagnostic and analysis processes (10, 11, 20). For example, functionalized nanoparticles can be designed to selectively interact with certain specific pathogen molecules, facilitating their identification in tissue or excreta samples (26). Nanotechnology allows the construction of advanced diagnostic systems (nanosensors), which can detect and quantify the specific molecules of pathogens with extremely high sensitivity. These nanosensors can be integrated into handheld or automated devices, enabling rapid (39) and on-site diagnosis of silkworm diseases (20). Nanotechnology can also be used to amplify detection signals, thus improving the sensitivity and specificity of diagnostic tests (27). For example, functionalized nanoparticles can amplify fluorescence or electrochemical signals generated during interaction with pathogens, facilitating the detection of even very small amounts of pathogens in biological samples, thereby facilitating real-time disease diagnosis and management (19). By integrating nanotechnology into silkworm disease diagnosis, more efficient, faster and more affordable diagnostic methods can be achieved, which can contribute to improving the health and productivity of silkworm populations and supporting the sericulture industry (26).

Early and preventive diagnosis of fungal diseases in silkworms requires the use of sensitive and specific technologies that can detect even incipient or subclinical infections at an early stage. Key aspects of early diagnosis in this context include the use of sensitive and specific technologies (regular and systematic monitoring of silkworm health), using sensitive and specific technologies that can detect even small amounts of pathogens or early signs of disease. Early diagnosis involves establishing a program of regular and systematic monitoring of the health of silkworms to detect any early signs of disease or changes in their health status by periodic examination of tissue or excrement samples using appropriate diagnostic techniques (36). Automatic and digital health monitoring of silkworms is a modern and effective technique for monitoring their populations and quickly detecting any abnormalities or changes in their behavior or health, which can be achieved by: using specialized digital devices and sensors to constantly monitor various aspects of worm health, collect real-time data and transmit it to a centralized monitoring system, quickly detect anomalies through automatic analysis of collected data, automatically generate alerts and notifications for farmers when a problem is identified, and analyze data to make more informed and effective management decisions (33). Early and preventive diagnosis of diseases in silkworms can ensure effective and sustainable management of their health, helping to maintain the sustainability and success of the sericulture industry. Digital sensors can transmit the data collected in real time to a centralized monitoring system (sudden changes in temperature or humidity, or unusual behavior of the worms), allowing farmers to obtain up-to-date and detailed information about the health of the silkworm population and environmental conditions on their farms, which require rapid intervention. It can also help prevent serious complications or mortality associated with untreated or undiagnosed diseases (14). Early diagnosis can help reduce the economic impact of diseases on the sericulture industry by minimizing production losses and costs associated with disease treatments and management.

The integration of artificial intelligence technologies in the diagnosis of mycotic diseases in silkworms is an advanced and promising approach that facilitates efficient data analysis and provides accurate and rapid disease diagnosis using modalities such as data analysis, rapid and accurate diagnosis, identification of subtle signs of diseases, personalizing treatments and optimizing health management (33). Artificial intelligence can be used to analyze and interpret data collected from various sources (laboratory test results, silkworm health monitoring data or epidemiological information), and artificial intelligence algorithms can identify patterns, correlations and trends in the raw data, thereby facilitating the identification of risk factors and early signs of diseases (25). With the help of predictive models and machine learning algorithms, artificial intelligence can generate accurate and rapid diagnoses of silkworm diseases based on available data and previously accumulated knowledge. These diagnoses can be obtained in real time and can serve as a guide for the immediate establishment of treatment and control measures. Some silkworm diseases can develop without clinical signs or be difficult to diagnose in the early stages, and artificial intelligence can be trained to recognize these subclinical signs and associate them with certain conditions, thus enabling early disease identification and rapid intervention. Based on the individual data and medical history of each silkworm lots, artificial intelligence can recommend personalized treatments tailored to the specific needs of each case, helping to improve the effectiveness of treatments and minimize the risk of treatment resistance. By continuously analyzing health and performance data, artificial intelligence can help optimize silkworm health management and identify best practices for disease prevention and control, significantly improving the ability to diagnose and manage their health.

Personalized medicine in the treatment of mycotic diseases of silkworms adapts to individual characteristics (including genome sequencing, assessment of the growth environment, diagnostic tests and personalized treatments), as well as continuous monitoring and adjustment of therapy, tailored treatments, continuous monitoring and adjustment (1). DNA sequencing can identify genetic variations and genetic markers relevant to the health and disease susceptibility of each silkworm lots, information

that can then be used to identify potential disease risks and develop diagnostic and treatment strategies adapted (7). By evaluating the specific growth environment of each silkworm lots, environmental factors that could contribute to disease risk can be identified and measures can be implemented to minimize these risks.

Based on genetic and environmental information, personalized diagnostic tests can be developed to specifically detect pathogens or risk factors for each individual silkworm batch, after which personalized treatment strategies tailored to the individual characteristics of the silkworm can be developed. to each batch of worms. These treatments may include the use of specific drugs, changes in diet or management of the rearing environment, and other tailored interventions (25). Personalized medicine involves continuously monitoring the health status of silkworms and adjusting diagnostic and treatment strategies according to their evolution (31).

Prophylactic and necessary measures in mycotic diseases of silkworms

Prophylactic measures

Cleaning and disinfection of silkworm rearing areas, inventory and litter at each rearing series. Solutions recommended according to the legislation in force can be used for the premises and the inventory. During the rearing of silkworms, the following measures are necessary: collection of diseased or suspected diseased larvae and carcasses followed by their destruction by burning; carrying out daily disinfection in the growth spaces with recommended antimycotic solutions; disinfection of the larval body with an antifungal solution, finely sprayed from a distance of 30-40 cm above the rearing beds, at the beginning of each evening and once at each larval stage, after emerging from sleep. If necessary, the application can be done therapeutically every day. Maintaining a suitable microclimate for the larvae, is recommended for the prevention on the development of fungi (temperature to be between 22-26°C, humidity below 75%, ensuring adequate ventilation, hygiene of premises and equipment). Protecting doors and windows against biological vectors or drafts have to be used. Periodic application of phytosanitary treatments to mulberry plantations, using antifungal substances with low remanence are also necessary.

If a pathogen is detected at an early stage or if there is an increased risk of infection, preventive treatments (antiparasitic or antimicrobial drugs, according to the recommendations of veterinary specialists) can be given to prevent the occurrence or spread of the disease in the silkworm population (30). If the presence of a pathogen is identified in a silkworm lot it is important to take immediate measures to isolate the affected lot in order to prevent the spread of the disease to other lots and limit the negative impact on the entire silkworm population. Early identification of diseases can lead to the implementation of changes in farm management (improving hygiene, adjusting rearing conditions or applying more rigorous biosecurity measures), which can help reduce the risk of infection and maintain the health of silkworms. After implementing preventive measures, it is important to continuously monitor the health status of the silkworm population and evaluate the effectiveness of the measures taken by performing periodic diagnostic tests and carefully observing signs of improvement or worsening of the disease over time. By implementing preventive measures following an early identification of diseases, their negative impact on the health and productivity of silkworms can be significantly reduced.

Control and prevention measures in fungal diseases

The measures to combat and prevent mycotic diseases are carried out by: isolating the lots and shelters where the disease has appeared and the lots where the disease has appeared massively will be burned; collecting diseased larvae, corpses and litter in containers with antiseptic solutions or plastic bags and destroying them by burning; decontamination with disinfectants recommended according to the legislation in force of the woody parts of the shoots, together with the change of the litter and its evacuation to the platform; daily disinfection in the growing spaces with antifungal solutions; disinfection of the body of the larvae with antimycotic solution sprayed on their body and on the litter

by applying it once a day before feeding; ensuring microclimate conditions, food and premises hygiene; reducing excess moisture in growing spaces by regulating ventilation and using chlorinated lime as a powder spread under bedding, under racks and on indoor walkways and using protecting doors and windows from growing spaces with gauze curtains to prevent pathogenic fungi from entering silkworms.

CONCLUSIONS

1. After exploring and comparing classical and modern diagnostic methods for mycotic diseases in silkworms, it can be concluded that technological progress has brought a series of innovations that improve the efficiency, accuracy and speed of diagnosis.

2. From traditional methods, such as microscopic examination, to advanced technologies (genome sequencing and the use of artificial intelligence), there are a variety of tools available for the detection and management of fungal diseases in silkworms.

3. The integration of these classical and modern techniques can help to improve the health and productivity of these valuable insects, having a positive impact on the sericulture industry.

4. In the future, a diagnostic laboratory may move from using simpler diagnostic techniques to more advanced and sophisticated technologies due to the rapid advancement of technology and the increasing availability of equipment and resources. This may involve purchasing specialized equipment, such as PCR or other molecular diagnostic technologies, and training specialized personnel to use it effectively.

5. Thus, in a relatively short period of time, the laboratory can adopt more advanced techniques for the diagnosis of mycotic diseases in silkworms.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Agriculture and Rural Development through the ADER Program within the projects ADER 24.1.1./2023, with the title "*Family sericulture farm model for the innovative capitalization of secondary sericulture productions obtained from sericulture and sericulture in the Farma-Farming system*".

BIBLIOGRAPHICAL REFERENCES

1. Atoe B., Ifijen I.H., Okiemute I.P., Emmanuel O.I. and Maliki M.; Silk Biomaterials in Wound Healing: Navigating Challenges and Charting the Future of Regenerative Medicine; 2024; In TMS Annual Meeting & Exhibition (pp. 904-920). Cham: Springer Nature Switzerland.
2. Bebitha B., Mohanra J.P., Manimegalai S., Mahalingam C.A.; Silkworm disease diagnosis through molecular approach and their management. *International Journal of Plant Protection*; 2016; 9(1), 343-352.
3. Brahma D., Bora N.R., Narzary P.R., Chakraborty R., Sarma J. and Rajkhowa A.; Tools and Applications of Bioinformatics in Sericulture: A Review; 2024; *Asian Journal of Current Research*, 9(1), pp.86-94.
4. Bura M., Acatincăi S., Pădeanu I.; *Viermii de mătase, biologie și creștere*; 1995; Editura Helicon, Timisoara, România.
5. Chen K. & Lu Z.; Immune responses to bacterial and fungal infections in the silkworm, *Bombyx mori*; 2018; *Developmental & Comparative Immunology*, 83, 3–11.
6. Dai W., Qi J., Chen H., Zhang Z., Shang R., Zhang Y., Tang S. and Shen Z.; Rapid and sensitive detection of *Nosema bombycis* using loop-mediated isothermal amplification and colorimetric nanogold; 2019; *Science Asia*, 45(3).
7. Deepika I., Ramesh K.V., Kumar I., Singh A., Debnath R., Dubey H., Shukla P., Ponnuvel K.M., Moorthy S.M. and Subrahmanyam G.; Molecular diagnostics in sericulture: A paradigm shift towards disease diagnosis in silkworms; 2024; *Entomologia Experimentalis et Applicata*.
8. Doliș M.; *Sericultura*; 2008; Editura ALFA, Iași, România.
9. Hassan S., Karaila G.K., Singh P., Meenatchi R., Venkateswaran A.S., Ahmed T., Bansal S., Kamalraj R., Kiran G.S. and Selvin J.; Implications of fungal nanotechnology for sustainable agriculture-applications and future perspectives; 2024; *Biocatalysis and Agricultural Biotechnology*, p.103110.
10. Huang W., Ling S., Li C., Omenetto F.G. and Kaplan D.L.; Silkworm silk-based materials and devices generated using bio-nanotechnology; 2018; *Chemical Society Reviews*, 47(17), pp.6486-6504.

11. Kaman P.K., Das A., Verma R., Narzary P.R., Saikia B. and Kaman N.; The Importance of Nanotechnology on Sericulture as a Promising Field; 2023; Agriculture Association of Textile Chemical and Critical Reviews Journal (2023) 40-46.
12. Kang G.P., Guo X.J.; Overview of silkworm pathology in China; 2011; African Journal of Biotechnology. 10(79): 18046-18056.
13. Kumari K.M., Rajan R.K., Himantharaj M.T., Nataraj B., Rekha M.; Influence of temperature and relative humidity on the rearing performance and disease incidence in CSR Hybrid Silkworms, *Bombyx mori L.*; 2001; International Journal of Industrial Entomology, 3(2): 113-116.
14. Liu Y., Deng S., Li Y., Zhang Y., Zhang G. and Yan H.; Fast identification of the BmNPV infected silkworms by portable NIR spectroscopy and chemometrics; 2024; Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, p.124158.
15. Majima H., Arai T., Kamei K. and Watanabe A.; *In vivo* efficacy of pitavastatin combined with itraconazole against *Aspergillus fumigatus* in silkworm models; 2023; Microbiology Spectrum, 11(5), pp. e02666-23.
16. Manjunatha S.R., Narayanaswamy K.C., Gowda M., Chandrashekar S., Kumar M.P. and Benherlal P.S.; Studies on the Effect of White Muscardine Infection on Growth and Yield Performance of Bivoltine Silkworm, *Bombyx mori* Breeds; 2023; Mysore Journal of Agricultural Sciences, 57(2).
17. Matei A.; Creșterea viermilor de mătase; 2002; Editura ALEX and ALEX.
18. Nagashetti S.M., Biradar S., Dambal S.D., Raghavendra C.G. and Parameshchhari B.D.; Detection of disease in *Bombyx mori* silkworm by using image analysis approach; 2021; IEEE Mysore Sub Section International Conference (MysuruCon) (pp. 440-444). IEEE.
19. Pandey K. and Kachhwaha N.; Effects of green nanoparticles on the pathogenic fungi of silkworm: a review; 2021; Uttar Pradesh J Zool, 42(21), pp.28-38.
20. Park S.K., Kwon H.G., Kang S.K. and Kim S.R.; Isolation and identification of insect pathogenic fungus from silkworms with suspected white muscardine disease; 2024; International Journal of Industrial Entomology, 48(1), pp.42-47.
21. Pau E.; Bolile și dăunătorii viermilor de mătase; 2000; S.C. Sericarom, Filiala de cercetare, București, România.
22. Praveena M.S., Rani A.S. and Savithri G.; Day-to-day analysis of nitrogenous products in Different Mulberry Silkworm Breeds during the Progressive infection of fungal pathogen *Beauveria bassiana*; 2024; Korean Journal of Physiology and Pharmacology, 28(1), pp.107-112.
23. Puneet C., Raghavendra Cg., Mohana Ks., Bhaskar R.N.; Assessment of diseases in *Bombyx mori* silkworm – A survey, Global Transitions Proceedings; 2021; 2 (1): 133-136, ISSN 2666-285X.
24. Punyavathi H.B.M.; Seri-bioinformatics: emerging trends and challenges in silkworm research; 2013; Advances in Biochemistry, 1(2), pp.33-42.
25. Raju C.G., Sarkar S., Canamedi V., Parameshwaranaiik J. and Sarkar S.; A Review of Silk Farming Automation Using Artificial Intelligence, Machine Learning, and Cloud-Based Solutions; 2022; In International Conference on Data Analytics & Learning (pp. 101-116). Singapore: Springer Nature Singapore.
26. Ramos N., Miranda M.S., Franco A.R., Silva S.S., Azevedo J., Dias I.R., Reis R.L., Viegas C. and Gomes M.E.; Toward spinning greener advanced silk fibers by feeding silkworms with nanomaterials; 2020; ACS Sustainable Chemistry & Engineering, 8(32), pp.11872-11887.
27. Rohela G.K., Srinivasulu Y. and Rathore M.S.; A review paper on recent trends in bio-nanotechnology: implications and potentials; 2019; Nanoscience & Nanotechnology-Asia, 9(1), pp.12-20.
28. Sahana K.P., Gowda M., Narayanaswamy K.C. and Prasanna Kumar M.K.; Characterization of *Beauveria bassiana* isolate SHDL: a white muscardine-causing fungus in silkworm, *Bombyx mori L.* from Karnataka, India; 2023; Journal of Experimental Zoology India, 26(1).
29. Saharia S., Nath M., Kalita S., Kalita D. and Bardoloi S.; Identification and Toxicity Evaluation of *Beauveria* sp. Associated with White Muscardine Disease in Muga Silkworm *Antheraea assamensis* Helfer; 2024; Indian Journal of Entomology, pp.1-5.
30. Șapcaliu A., Savu V., Mihalcea T., Gheorghe A., Hăbeanu M., Măicănescu M.; Rolul diagnosticului de laborator în controlul bolilor contagioase la viermii de mătase, starea actuală, perspective și impactul asupra sericiculturii; 2023; Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.
31. Schaly S., Ghebretatios M. and Prakash S.; Baculoviruses in gene therapy and personalized medicine; 2021; Biologics: Targets and Therapy, pp.115-132.
32. Shanmugam V, Seethapathy P.; Isolation and characterization of white muscardine fungi *Beauveria bassiana* (Bals.) Vuill. A causative of mulberry silkworm; 2017; J. Entomol. Zool. Stud; 5: 512-515.
33. Shi H., Xiao W., Zhu S., Li L. and Zhang J.; CA-YOLOv5: Detection model for healthy and diseased silkworms in mixed conditions based on improved YOLOv5; 2024; International Journal of Agricultural and Biological Engineering, 16(6), pp.236-245.
34. Sivaprasad V., Rahul K. and Makwana P.; Immunodiagnostic of silkworm diseases; 2021; In Methods in Microbiology (Vol. 49, pp. 27-46). Academic Press.

35. Subrahmanyam G., Kalita M., Krondashree D., Chutia M. & Das R.; Isolation and morphological characterization of a fungal isolate obtained from muscardine diseased Muga silkworm *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae); 2018; International Journal of Microbiology Research, 10, 1435–1440.
36. Subrahmanyam G., Ponnuvel K.M., Arunkumar K.P., Rahul K., Moorthy S.M. and Sivaprasad V.; Molecular methods for diagnosis of microbial pathogens in *Muga silkworm*, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae); 2023; International Journal of Industrial Entomology, 47(1).
37. Vankadara S., Kamidi R., Pooja M.; Chapter 2-Immunodiagnosis of silkworm diseases, Editor(s): Volker Gurtler, Gangavarapu Subrahmanyam, Methods in Microbiology; 2021; Academic Press, 49: 27-46, ISSN 0580-9517, ISBN 9780128211458.
38. Yashaswini B., Madhusudhan N., Suresh D.; Automated smart sericulture based on IoT and image processing technique; 2020; 10 (6), ISSN 2321(3361).
39. Zhao P., Hong S., Li Y., Chen H., Gao H. and Wang C.; From phyllo sphere to insect cuticles: silkworms gather antifungal bacteria from mulberry leaves to battle fungal parasite attacks; 2024; Microbiome, 12(1), p.40.