

Research Progress on Pathogens of Main Diseases of *Dictyophora rubrovalvata* and Their Occurrence

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Abstract *Dictyophora rubrovalvata* is an edible fungus with rich nutritional value. It contains various nutrients and bioactive components, and has immunomodulation, anti-tumor, anti-oxidation, anti-fatigue, anti-aging, anti-inflammation and alcoholic hepatitis-protection effects. With the continuous expansion of planting area of *Dictyophora*, the disease problem has become a major problem affecting the development of *Dictyophora* industry. In this paper, the pathogens, harmful symptoms and causes of main diseases in *Dictyophora* were summarized, so as to provide reference for comprehensive control of *Dictyophora* diseases and promote the high-quality development of *Dictyophora* industry.

Key words *Dictyophora rubrovalvata*; Disease; Pathogen; Research progress
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Dictyophora rubrovalvata has the titles of "Flowers of Fungi" and "Queen of Fungi". It contains crude protein, vitamins, minerals and rich bioactive substances, among which active polysaccharides are a type of good non-specific immunostimulants, which can enhance the functions of human macrophages, lymphocytes and other immune cells, and also have immunomodulation, anti-tumor, anti-oxidation, anti-fatigue, anti-aging, anti-inflammation and alcoholic hepatitis-protection effects^[1-4]. However, the growth cycle of *D. rubrovalvata* is relatively long, and the traditional cultivation mode takes one and a half years. It takes half a year to complete a production cycle even if the process of removing bags of mushroom rods and covering them with soil is adopted^[5], while such process is extremely vulnerable to pests and diseases. With the increasing planting area of *D. rubrovalvata*, the yield and quality of *D. rubrovalvata* are seriously damaged by diseases. At present, the reported diseases of *D. rubrovalvata* include yellow rot disease, green mold, slime mold, brown rot disease, soft rot disease, brown plaster mold and sooty mold^[6-8], among which green mold, brown rot disease and yellow rot disease are the main diseases, which are common and harmful. In this study, the pathogens, harmful symptoms and causes of the main diseases in *D. rubrovalvata* were summarized, in order to provide reference for the comprehensive control of *D. rubrovalvata* diseases and promote the high-quality development of *D. rubrovalvata* industry.

Pathogenic Mechanisms and Transmission Routes of Green Mold

Trichoderma fungi cause green mold of *Dictyophora*.

Biological symptoms of *Trichoderma* fungi *Trichoderma* spp. belongs to Hypocreaceae of Hypocreales in Sordariomycetes of Pezizomycotina in Ascomycota. At present, more than 400 taxonomic units have been reported, and *Trichoderma* is widely distributed and exists in air, soil, plant residues and oceans. Meanwhile, it is also a common disease in the cultivation of edible fungi^[9-10]. In 2021, Li *et al.*^[11] isolated and purified six strains, belonging to *Trichoderma*, from the part of *D. rubrovalvata* affected with the rot disease at the late stage, namely, *Trichoderma tomentosum*, *Trichoderma velutinum*, *Trichoderma dorotheopsis*, *Trichoderma guizhouense*, *Trichoderma virens* and *Trichoderma harzianum*. It is proved that many fungi of *Trichoderma* can cause the rot disease in *D. rubrovalvata* for the second time. In the same year, Chen *et al.*^[12] collected 12 symptomatic samples of green mold in the production base of *D. rubrovalvata* in Ceheng County, Guizhou Province. After separation and purification, it was observed that these colonies were light green and regularly round in the early stage, and turned dark green after 10 d, and the hyphae were fused, and the bottom of colonies was light yellow. Conidia were produced at the top of mycelia, in an oval shape, with stipe branches distributed at right angles, and primary branches appear in pairs or single. The morphological characteristics of these strains are consistent with *Trichoderma*. The pathogen was identified as *Trichoderma koningiopsis* by molecular biological identification. It is the first time in the world that *T. koningiopsis* is reported as the pathogen of *D. rubrovalvata*.

Harmful symptoms and causes *Trichoderma* has a wide range of survival, and the cultivation materials are not completely sterilized, which easily causes the germination of *Trichoderma* spores to hyphae in the cultivation materials. Once infected with *Trichoderma* conidia, they will germinate rapidly to form hyphae.

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Trichoderma grows very rapidly at the initial stage, and often shows fine and white flocculent aerial hyphae, which are abundant, and green conidia clusters will be produced about 2 d later^[13]. When the ventilation condition in the greenhouse is poor, *Trichoderma* conidia will be produced in large quantities and attached to the wall of the greenhouse and the surrounding environment, and at the same time, toxins will be secreted to inhibit the growth of *D. rubrovalvata* hyphae, so that the strains will be scrapped. Therefore, once *Trichoderma* is found, measures must be taken to prevent and control it immediately^[14–15].

Penicillium* fungi cause green mold of *Dictyophora

Biological symptoms of *Penicillium* fungi *Penicillium* belongs to Aspergillaceae of Eurotiales in Eurotiomycetes of Ascomycota^[16]. *Penicillium* mycelia are highly branched and network-like, belonging to multicellular. They are colorless, or light or bright in color, and conidia are generally green, in an oval, spherical or short-column shape^[17]. *Penicillium* is a common fungal species in the process of seed production and cultivation of *D. rubrovalvata*, which mainly harms the mycelia of *D. rubrovalvata*^[7]. In 2022, according to the report of Qin *et al.*^[18], green mold was found in the production base of Baiyun District, Guiyang City, Guizhou Province, and 15 symptom samples were collected and separated and purified. The colonies were white at first, and turned green with the development of conidia. The mycelia are flask-shaped, transparent, and punctate. Conidia are smooth and light green, nearly spherical, and the peduncle is tuberculate, in the shape of three wheels. These morphological features are similar to the description of the genus *Penicillium*, and after molecular identification, it was determined that *Penicillium citrinum* is one of the pathogens causing the green mold disease of *D. rubrovalvata*.

Symptoms and causes of harm *Penicillium* has a wide distribution range and is most prone to occurrence under high temperature, high humidity, poor ventilation, and acidic conditions. Incomplete sterilization of the culture medium and lax inoculation process are important reasons for contamination in strain production. On culture media contaminated with *Penicillium*, white powdery hyphae appear in the early stage, and after 1–2 d, the colonies gradually turn into a green powdery mold layer^[19]. During the growth period, a white edge with a width of 1–2 mm is often visible. After a period of growth, a thin film is often formed on the surface of the colonies, covering the surface of the culture media. It reacts chemically with culture media by isolating the air, toxins are secreted to inhibit and kill the growth of *Dictyophora* hyphae^[20].

Pathogenic Mechanisms and Transmission Routes of Yellow Rot Disease

Yellow rot disease, also known as bamboo egg rot, is the most serious disease in the process of cultivating *D. rubrovalvata*, which can be called "cancer" of *Dictyophora*. This kind of disease spreads rapidly, has strong pathogenicity, and is difficult to prevent and control. It has become the main limiting factor for the

development of *D. rubrovalvata* industry and caused huge economic losses^[21]. In 2015, Pan *et al.*^[22] put forward the rotten skin disease of *Dictyophora* for the first time. Through the investigation and analysis of the environment and cultivation materials in mushroom sheds, preventive measures were put forward from the perspective of agricultural control. In 2018, Lu *et al.*^[23–24] discussed the symptoms and occurrence regularity of *D. rubrovalvata* rot, but their pathogens were not clear. In 2021, Li *et al.*^[25] found that *Trichoderma* spp. and *Penicillium* spp. were the pathogens that caused the secondary disease in the late stage of *D. rubrovalvata* rot. According to the research results of Yuan *et al.*^[26] in 2021, it was proved that a fungus named *Saccharomycopsis phalli* was the pathogen of yellow rot disease. The colonies of the pathogen are white with regular edges, and the spores are oval and elongated, appearing singly or in pairs, and no sexual structure is observed. Studies have proved that the mycelia of *D. rubrovalvata* shrink and brown after inoculation with the pathogen, and the pathogen spread and infect inward through the mycelium gap, and yellow droplet exudate becomes more and more obvious until the bamboo eggs fester^[21].

The initial diseased parts of the bamboo eggs of *D. rubrovalvata* are all in the outer skin, and the top, side and bottom of the bamboo eggs of *D. rubrovalvata* will all be affected. Needle-sized spots appear in the early stage of the disease, and after one day, light yellow juice drops flow out from the diseased spots, which become round or oval, rapidly expand and turn brown, showing darkest center and slightly shallow edge. With the expansion of the disease spots, the skin of the diseased part is corroded and broken, and colorless, milky white or brown liquid flow out. When the humidity is high, white or green mold grows on the disease spots, and gradually erodes the internal tissues. After being infected, the skin of immature bamboo eggs of *Dictyophora* gradually becomes wet and rotten, and necrotic lesions occur. After 3–6 d, the whole bamboo eggs of *Dictyophora* shrink and mildew, which can involve whole planting bases. According to the research results, bamboo eggs of *Dictyophora* are exposed to high temperature and high humidity for a long time in the growth stage, and high temperature is the inducement of rot in the cultivation process of *D. rubrovalvata*. With the increase of temperature, the invasion of pathogen on mycelia of *D. rubrovalvata* is strengthened, and the immunity of the surface layer of bamboo eggs is reduced. Coupled with the harm of pests such as slugs, earwigs, ants, nematodes, springtails, mites, mosquitoes and flies, the skin wound of bamboo eggs of *Dictyophora* will be caused, and the pathogen will quickly infect from the wound^[27], and fungal pellets will rot in a large area in a short time.

Research Progress of Other Diseases

Brown rot

Brown rot, also known as *Mycogone perniciosus* magn., is a fungal disease. It is the most common and serious disease in the growth stage of *Dictyophora*^[8,28–29]. In the early stage, the lesions

are brown, and when the humidity is high, white fluffy hyphae will grow around the lesions. As the condition progresses, the lesions will turn dark brown and exude brown juice, and *Dictyophora* will begin to rot and emit a foul odor. In severe cases, bamboo eggs may fail to grow *Dictyophora* fungus due to rot, resulting in a significant reduction in yield or even a complete crop failure. Harmful *Mycogone* can cause brown rot disease in various edible mushrooms, including *Pleurotus ostreatus*, *Agaricus bisporus*, *Flammulina velutipes*, *Pleurotus citrinopileatus*, *Pleurotus erythrii*, and *Ganoderma lucidum*^[30–31]. However, there have been no reports on the pathogens of brown rot disease in *D. rubrovalvata*.

Slime mold

In 2003, according to a research report by Liu *et al.*, the pathogen of slime mold disease is *Comatricha pulchella* (C. Bab) Rost., belonging to *Stemonitis* of *Stemonitaceae* in *Stemonitales* of *Myxomycetes* in *Myxomycota*^[32]. It is one of the common diseases in *Dictyophora* cultivation, especially the most harmful to bed cultivation, and it rarely occurs in other edible mushroom varieties. The pathogen grows rapidly under high temperature and high humidity, and at the initial stage of the disease, there are sticky and sparse reticular hyphae on the soil cover, which are pink, yellowish brown and grayish brown. Different from other diseases, the pathogen can move around, in the direction of high humidity and weak light in the initial stage, and in the direction of relative dryness and strong light in the middle stage, and it stops movement in the later stage, and begins to form spores to expand the harm. The fruiting body decays into mucus after being infected^[33–34].

Sooty mold

The mold is a flocculent miscellaneous fungus, also known as *Doratomyces*^[35]. In the initial stage, white fluffy hyphae appear and grow symmetrically. Subsequently, a large number of black spores are produced, and the colonies are smoky gray, mainly causing the breakage of *Dictyophora* hyphae. Sooty mold is prone to occurrence in environments with high temperature and humidity. Rotting wood, soil used for planting and film are common places where sooty mold spread quickly and produce a moldy odor in the later stage^[36–37].

Aspergillois

Aspergillus is a common miscellaneous fungus in the production and cultivation of *Dictyophora*. High temperatures often cause *Aspergillus flavus* and *Aspergillus niger*, and the disease names are yellow mold disease and black mold disease. In the production of strains, these diseases are mainly affected when cotton stoppers become damp, so they are often seen on cotton stoppers and bottle caps^[38–39].

There are many types of *Aspergillus*, and contaminated strains, bags and beds quickly grow out granular mold layers of black, yellow green, blue green and other colors on the surface of culture media. *Aspergillus* is widely distributed and can survive in organic residues, soil, water, and other environments. When spreading through the air, *Aspergillus* has a wide range of temperature adaptability and prefers high-temperature environments. It is

more likely to occur in environments with pH values close to neutral and when there are too much carbohydrates^[38].

Conclusions

In the *Dictyophora* industry, disease prevention and control is a crucial link. However, currently there are relatively few papers on the diseases in *D. rubrovalvata* compared with other edible fungi. Taking yellow rot disease as an example, although the pathogen has been found, the problem of yellow rot disease prevention and control has not been properly solved. The problem of yellow rot disease in *D. rubrovalvata* production is still serious. In order to better study the prevention and control of *D. rubrovalvata* diseases, it is necessary to start from multiple aspects. First of all, it is necessary to deeply study the interaction between hosts, pathogens and the environment^[40–41], understand the biological characteristics of pathogens and explore the relationship between disease occurrence and host resistance, soil conditions and environmental conditions. The ecological system of the planting base can be improved and optimized by strengthening cultivation management and rational fertilization, so as to reduce the occurrence of diseases. Secondly, it is necessary to study the pathogenic mechanisms of pathogens and the genetic mechanisms of host disease resistance^[42], and establish a comprehensive prevention and control technology system with the exploration of disease resistant varieties as the core combined with various prevention and control techniques. In this way, we can better protect the high-quality development of *D. rubrovalvata* industry and provide technical support for it.

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