Molecular Microbiology Research 2024, Vol.14, No.3, 141-152
http://microbescipublisher.com/index.php/mmr

Research Insight Open Access

The Role of *Aspergillus oryzae* in Bilological Control Against Rice Pests

Jun Wang, Jie Zhang
Cuixi Academy of Biotechnology, Biotechnology Center, Zhuji, 311800, Zhejiang, China
Corresponding author: jie.zhang@cuixi.org

Molecular Microbiology Research, 2024, Vol.14, No.3 doi: 10.5376/mmr.2024.14.0016
Received: 16 Apr., 2024
Accepted: 28 May, 2024
Published: 15 Jun., 2024

Preferred citation for this article:
Wang J., and Zhang J., 2024, The role of *Aspergillus oryzae* in bilological control against rice pests, Molecular Microbiology Research, 14(3): 141-152 (doi: 10.5376/mmr.2024.14.0016)

Abstract *Aspergillus oryzae* has shown great potential in biological control against various rice pests. This review provides a detailed exploration of its biological characteristics, including morphology, lifecycle, enzymatic properties, and the generation of secondary metabolites. Meanwhile, this review provides an in-depth analysis of its pest control mechanisms, particularly its ability to resist fungi, bacteria, and nematodes. Evaluate the effects of different formulations, concentrations, and application strategies of *Aspergillus oryzae* through laboratory and field application techniques. In addition, the synergistic effects of *Aspergillus oryzae* and other beneficial microorganisms were explored, demonstrating the potential of microbial consortia in enhancing pest control. At the same time, the advantages of *Aspergillus oryzae* in environmental protection, sustainability, and cost-effectiveness were also considered. This review also points out the challenges faced by the future use of *Aspergillus oryzae* for biological control, including its limitations, potential risks, and regulatory issues, and proposes future research directions. This review provides a theoretical framework for using *Aspergillus oryzae* as a pest management tool in sustainable rice cultivation.

Keywords *Aspergillus oryzae*; Biological control; Rice pests; Pest management; Sustainable agriculture

1 Introduction

Rice is a staple food for more than half of the world's population, playing a crucial role in global food security. However, rice cultivation is significantly challenged by various pests, including stem borers, leafhoppers, planthoppers, and root-knot nematodes. These pests cause extensive damage to rice crops, leading to reduced yields and compromised plant health. The economic impact is substantial, as farmers face increased costs for pest control and decreased income due to lower harvest quality and quantity. Effective pest management is essential to ensure sustainable rice production and food security (Ponce et al., 2022).

Biological control involves using natural enemies, such as predators, parasitoids, and pathogens, to manage pest populations. This approach is environmentally friendly and sustainable, providing an alternative to chemical pesticides that can have adverse effects on human health and the environment. Biological control agents target specific pests, reducing the likelihood of non-target effects and resistance development. Common methods include the use of beneficial insects, nematodes, bacteria, and fungi, which act through mechanisms like predation, parasitism, competition, and the production of toxic compounds that inhibit or kill pests (Costa et al., 2021).

Aspergillus oryzae is a filamentous fungus traditionally used in the fermentation industry for producing sake, soy sauce, and miso. Recently, its potential as a biological control agent has gained attention due to its ability to produce a wide range of secondary metabolites with antimicrobial properties. Research indicates that *A. oryzae* can inhibit the growth of various plant pathogens and pests, making it a promising candidate for integrated pest management (IPM) strategies. Its enzymatic properties and ability to thrive in diverse environmental conditions further enhance its suitability for agricultural applications (García-Conde et al., 2023).

This systematic review aims to explore the role of *Aspergillus oryzae* in the biological control of rice pests. It will provide a comprehensive overview of the biological characteristics of *A. oryzae*, its mechanisms of action against pests, application efficacy, synergistic effects with other biological agents, and the environmental and economic implications of its use. Provide a theoretical framework for using *Aspergillus oryzae* as a pest management tool in sustainable rice cultivation.

2 Biological Characteristics of *Aspergillus oryzae*

2.1 General morphology and life cycle of *Aspergillus oryzae*

Aspergillus oryzae, commonly known as the koji mold, is a filamentous fungus extensively utilized in traditional Asian fermentation processes such as sake, soy sauce, and miso production. Understanding the morphology and life cycle of *A. oryzae* is crucial for optimizing its use in various industrial applications and its potential role in biological control against rice pests.

The morphology of *A. oryzae* includes several distinct parts and structures, as illustrated in Figure 1A. The fungus forms a complex mycelial network composed of septate hyphae, which are hyphal cells divided by cross-walls known as septa. These structures provide structural support and compartmentalization. The hyphal cells typically measure between 2 to 4 micrometers in diameter, which allows for efficient nutrient absorption and growth. Asexual reproduction in *A. oryzae* involves the formation of conidiophores, which are specialized structures that arise from the mycelium and bear conidia. Conidiophores consist of a stalk, vesicle, metulae, and phialides. The phialides produce chains of conidia, which are spherical or ellipsoidal in shape and typically range from 3 to 6 micrometers in diameter. These conidia are responsible for the dispersal and propagation of the fungus (Chen et al., 2019). Figure 1B illustrates *A. oryzae* grown in a PDA medium, showing the typical colony morphology of the fungus. Figure 1C shows the growth of *A. oryzae* in steamed rice (köji), highlighting its application in traditional fermentation processes.

The life cycle of *Aspergillus oryzae* begins with the germination of conidia upon encountering favorable environmental conditions such as appropriate moisture, temperature, and nutrient availability. Germination involves the formation of germ tubes, which elongate to form hyphae and eventually develop into a mycelial network. This vegetative mycelium serves as the primary growth phase during which the fungus colonizes the substrate and absorbs nutrients.During the vegetative phase, *A. oryzae* undergoes extensive hyphal growth and differentiation, leading to the formation of conidiophores. The process of conidiation, or conidia formation, is regulated by a combination of genetic and environmental factors. Conidia are produced in large quantities, facilitating widespread dispersal and ensuring the continuation of the life cycle (Okabe et al., 2018).

2.2 Enzymatic Properties and Secondary Metabolites Production

Aspergillus oryzae is renowned for its enzymatic capabilities, which are pivotal in its widespread use in fermentation industries. The fungus secretes a diverse array of enzymes that catalyze the breakdown of complex substrates, facilitating various industrial processes.

A. oryzae produces a wide range of hydrolytic enzymes, including amylases, proteases, lipases, and cellulases. These enzymes are essential for converting complex biomolecules into simpler forms, enabling their utilization in fermentation and other industrial applications. For instance, α-amylase and glucoamylase hydrolyze starch into dextrins and glucose, crucial in producing sake, soy sauce, and other fermented foods (Chen et al., 2019).

A. oryzae also produces various proteases that break down proteins into peptides and amino acids, enhancing the flavor and nutritional value of fermented foods. These enzymes are particularly vital in soy sauce production (Frisvad et al., 2018). Additionally, *A. oryzae* lipases hydrolyze triglycerides into glycerol and free fatty acids, contributing to the breakdown of fats and oils in fermentation substrates (Jeennor et al., 2019).

In addition to its primary metabolic activities, *Aspergillus oryzae* produces a variety of secondary metabolites, which have significant industrial and biological applications. The fungus generates organic acids such as lactic acid, citric acid, and gluconic acid, which play crucial roles in food preservation, flavor enhancement, and pH regulation in fermentation processes (Son et al., 2018). Moreover, *A. oryzae* produces secondary metabolites with antimicrobial properties that inhibit the growth of pathogenic bacteria and fungi, making these compounds valuable in biological control and food safety applications (Wasil et al., 2018).

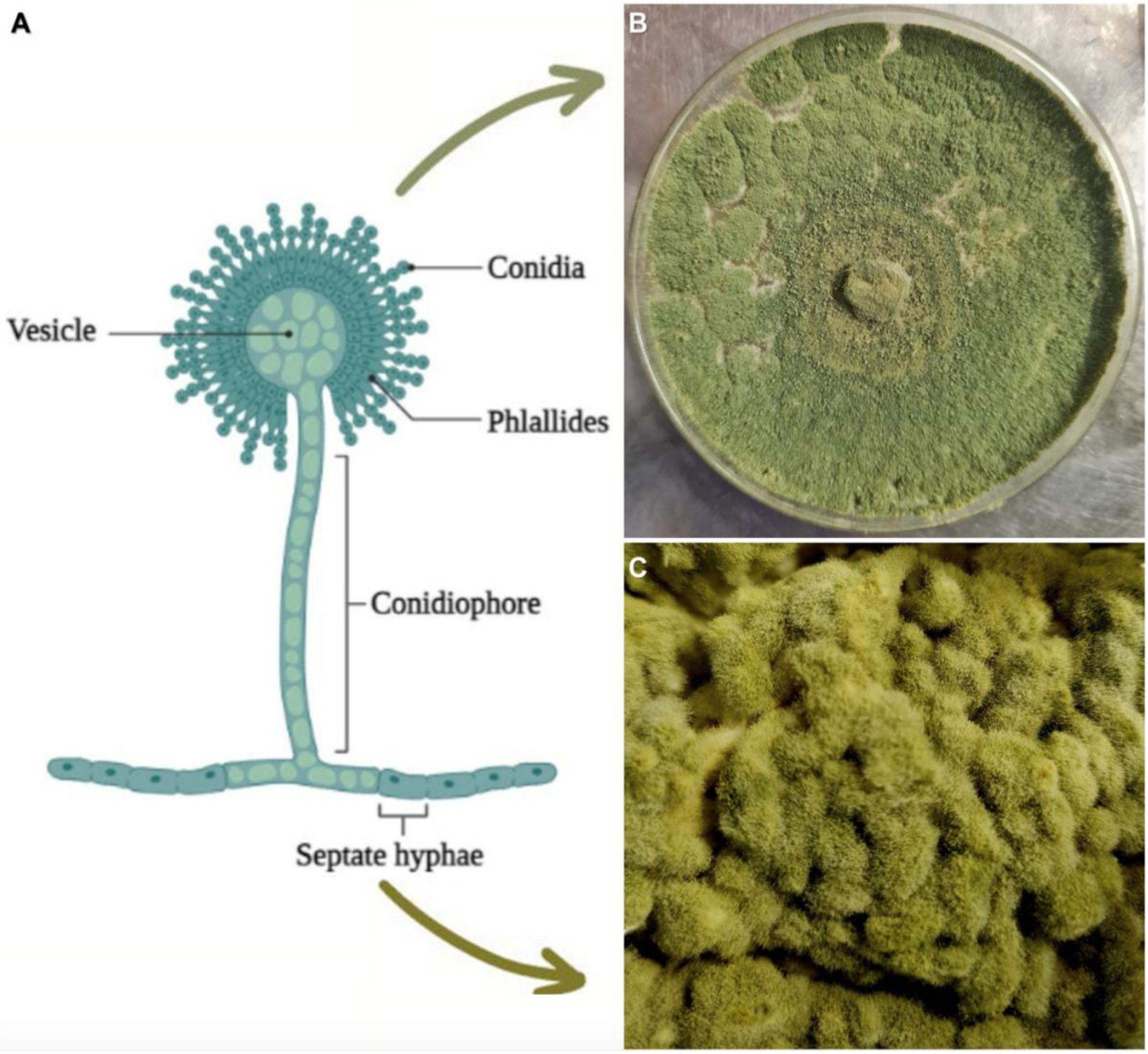

Figure 1 Morphology of *A. oryzae*; (A) Parts and structures of the fungus (Adapted from "Structure of *Aspergillus spp*.", 2023), (B) *A. oryzae* planted in a PDA medium and (C) Growth of *A. oryzae* in steamed rice (köji) (Adapt from García-Conde et al., 2023)
Image caption: Figure 1 illustrates the morphological characteristics of *Aspergillus oryzae*, including its fungal structures, growth in a culture medium, and growth in steamed rice. Figure 1 comprehensively shows the morphological characteristics of *Aspergillus oryzae*, from its structural components to its growth in various media. By analyzing these images, we gain a better understanding of *A. oryzae*'s significance in industrial fermentation and its potential applications in biological control. This information provides a solid foundation for further research and application (Adapt from García-Conde et al., 2023)

2.3 Comparison of *A. oryzae* with Other Aspergillus Species

Aspergillus oryzae belongs to a diverse genus of filamentous fungi, each species exhibiting unique characteristics and industrial applications. Comparing *A. oryzae* with other Aspergillus species, particularly *A. flavus* and *A. niger*, highlights both commonalities and distinct differences.

Both *A. oryzae* and *A. flavus* exhibit filamentous growth and produce conidia. However, *A. flavus* conidia are typically rougher and more variable in shape compared to the smooth, spherical conidia of *A. oryzae*. This morphological distinction is significant in industrial settings where the purity and consistency of fungal cultures are essential (Zhang et al., 2015). Furthermore, *A. oryzae* is non-toxigenic and safe for food production, while *A. flavus* produces aflatoxins, which are potent carcinogens posing significant health risks.

Comparing *A. oryzae* with *Aspergillus niger* reveals notable differences in enzymatic activity and industrial applications. Both species are prolific producers of hydrolytic enzymes. *A. niger* is particularly noted for its production of citric acid and pectinases, whereas *A. oryzae* is renowned for its amylases and proteases (Park et al., 2019). Additionally, *A. niger* typically forms black or dark conidia, whereas *A. oryzae* produces green to yellow conidia. These color and morphology differences are useful for distinguishing between the two species in industrial applications. The metabolic pathways of *A. oryzae* and *A. niger* differ, with *A. niger* exhibiting a broader range of organic acid production, including high levels of citric acid. This makes *A. niger* a primary organism for industrial citric acid production, whereas *A. oryzae* excels in producing enzymes critical for fermenting starch-based substrates (Daba et al., 2021).

3 Mechanisms of Action in Pest Control

3.1Antifungal properties and inhibition of fungal pathogens

Aspergillus oryzae demonstrates significant antifungal properties, making it a promising candidate for biological control against fungal pathogens affecting rice. Studies have shown that certain strains of *A. oryzae* can inhibit the growth of various pathogenic fungi. For example, the strain *Aspergillus oryzae* 18HG80, isolated from saline soil, exhibited notable antifungal activity against phytopathogens such as *Aspergillus flavus*, *Fusarium oxysporum*, and *Alternaria solani*. The strain inhibited their growth with varying degrees of effectiveness, showing the highest inhibition against *A. flavus* and *F. oxysporum* (Nacef et al., 2020).

The mechanism behind this antifungal activity involves the production of secondary metabolites such as kojic acid and butanedioic acid. These compounds interfere with the cellular processes of the target fungi, leading to inhibited growth and reduced pathogenicity. Additionally, environmental factors such as temperature and pH were found to influence the antifungal activity of *A. oryzae*, with optimal activity observed at 30 °C and pH 6 (Nacef et al., 2020).

Further research demonstrated that essential oils and other bioactive compounds derived from *A. oryzae* could be effective in controlling fungal pathogens in agricultural settings. For instance, studies have shown that essential oils with antifungal properties significantly inhibit the growth of *Aspergillus oryzae*, suggesting potential applications in the preservation of cultural relics and possibly in crop protection.

3.2 Antibacterial activity against rice pathogens

In addition to its antifungal properties, *Aspergillus oryzae* also exhibits antibacterial activity against several rice pathogens. This antibacterial potential is particularly significant in controlling diseases caused by bacterial pathogens such as *Xanthomonas oryzae pv. oryzae*, which is responsible for bacterial leaf blight in rice.

Research has identified strains of *A. oryzae* that produce metabolites with strong antibacterial effects. Jiang et al. (2019) highlighted the isolation of Aspergillus sclerotiorum strain As-75, which demonstrated significant antagonistic activity against X. oryzae pv. oryzae. The strain produced a novel compound identified as (2Z)-2-butenedioic acid-2-(1-methylethenyl)-4-methyl ester, which showed strong antibacterial activity and improved control efficiency in preventing rice bacterial blight (Jiang et al., 2019).

Moreover, Shoji et al. (2021) discovered a novel water extract from rice fermented with *Aspergillus oryzae* and *Saccharomyces cerevisiae*, suggesting broader antimicrobial potential. The extract promoted cell survival and inhibited viral infection, indicating that *A. oryzae* can produce bioactive compounds with diverse antimicrobial properties (Shoji et al., 2021).

3.3 Nematicidal effects against root-knot nematodes

Aspergillus oryzae also shows potential in controlling nematode infestations, particularly root-knot nematodes, which are a significant problem in rice cultivation. The nematicidal activity of *A. oryzae* is attributed to its ability to produce metabolites that are toxic to nematodes, thereby reducing their populations and minimizing crop damage.

Studies on the nematicidal effects of *A. oryzae* have demonstrated that certain strains can effectively inhibit the growth and reproduction of root-knot nematodes. The metabolites produced by *A. oryzae* disrupt the nematodes' life cycle, leading to decreased infection rates and improved plant health. This mode of action is particularly advantageous as it offers a biological alternative to chemical nematicides, which can have adverse environmental impacts (Zhang et al., 2022).

In conclusion, the antifungal, antibacterial, and nematicidal properties of *Aspergillus oryzae* highlight its potential as a versatile biological control agent in rice cultivation. Its ability to produce a wide range of bioactive metabolites makes it an effective tool in integrated pest management strategies aimed at reducing reliance on chemical pesticides and promoting sustainable agriculture.

4 Application Efficacy

4.1 Laboratory and field application techniques

The application techniques of *Aspergillus oryzae* in biological control have been explored both in laboratory settings and field trials to assess their efficacy against various rice pests. Laboratory experiments typically involve controlled environments where variables such as temperature, humidity, and exposure time can be precisely regulated. For instance, in vitro studies on the nematicidal effects of *A. oryzae* have demonstrated significant reductions in nematode populations when applied at specific concentrations and incubation periods (Liu et al., 2019).

Field application techniques, are more complex due to the variable environmental conditions. A common approach involves the use of *A. oryzae* spore suspensions sprayed directly onto rice plants. For example, large-scale field trials have shown that aerial spraying of *A. oryzae* spore suspensions can effectively reduce pest populations over extensive areas. In a study where *A. oryzae* was used against locusts, drone-assisted spraying resulted in significant reductions in locust populations, demonstrating the feasibility of this method for large-scale agricultural applications (You et al., 2023).

Another effective method involves incorporating *A. oryzae* into soil treatments. By mixing fungal spores into the soil, it is possible to target soil-borne pests such as nematodes more directly. This method has been shown to reduce the incidence of root galls and nematode populations in rice roots, as seen in greenhouse studies where soil applications led to significant pest suppression (Liu et al., 2019).

4.2 Efficacy of different formulations and concentrations

The application of synthetic The efficacy of *Aspergillus oryzae* in pest control can vary significantly depending on the formulation and concentration used. Different formulations, such as spore suspensions, dry spore powders, and fermented extracts, have been tested to determine the most effective method for delivering the biocontrol agent.

Spore suspensions are commonly used due to their ease of application and effectiveness. Studies have shown that higher concentrations of spore suspensions generally result in greater pest mortality. For example, a study on the use of *A. oryzae* against nematodes found that a concentration of 5×10^7 spores/ml was more effective than lower concentrations, resulting in higher mortality rates and reduced nematode reproduction (Liu et al., 2019).

Fermented extracts of *A. oryzae* have also been tested for their efficacy. These extracts contain not only fungal spores but also secondary metabolites produced during fermentation, which can enhance the biocontrol effects. For instance, the use of fermented rice extracts with *A. oryzae* demonstrated significant antifungal activity against rice pathogens, suggesting that these formulations could be particularly effective in integrated pest management programs (Shoji et al., 2021).

4.3 Comparative Analysis of Various Application Strategies

Comparing different application strategies for *Aspergillus oryzae* in pest control highlights the advantages and challenges of each method. Aerial spraying, soil treatment, and seed treatment are among the most commonly used strategies. Can be selected based on the specific pest and environmental conditions.

Aerial spraying is highly effective for large-scale applications, particularly in fields where uniform coverage is essential. This method has been successfully used to control locust populations, with drone-assisted spraying providing an efficient and scalable solution (You et al., 2023). However, this method requires precise calibration and environmental considerations to avoid drift and ensure effective coverage.

Soil treatment is advantageous for targeting soil-borne pests directly. Incorporating *A. oryzae* spores into the soil can significantly reduce pest populations, as demonstrated in greenhouse studies against nematodes (Liu et al., 2019). This method is particularly useful for pests that affect the root systems of plants.

Seed treatment is another effective strategy, especially for ensuring early protection against pests. Treating seeds with *A. oryzae* spores or extracts can enhance seedling resistance to pests and pathogens. This method has the added benefit of promoting early plant vigor and reducing the need for additional pesticide applications (Kalaivani et al., 2020).

5 Synergistic Effects with Other Biological Agents

5.1Combined Use with Other Beneficial Microbes

The combined use of *Aspergillus oryzae* with other beneficial microbes has been explored to enhance the overall efficacy of biological control methods. The synergy between *A. oryzae* and other microbes can result in improved pest management outcomes due to complementary mechanisms of action.

Jambhulkar et al. (2018) evaluated the combined use of *Aspergillus oryzae* with *Trichoderma harzianum* and *Pseudomonas fluorescens* in their study. The study demonstrated that the co-application of these biocontrol agents provided enhanced protection against rice pathogens such as *Magnaporthe oryzae* and *Xanthomonas oryzae pv. oryzae*. The combination of *T. harzianum* and *P. fluorescens* with *A. oryzae* resulted in a synergistic effect, reducing disease severity by 69.5% compared to untreated controls.

In another study, the combination of *A. oryzae* with Bacillus licheniformis was shown to be effective against multiple phytopathogens. The bacterial strain *B. licheniformis* inhibited the growth of *A. oryzae* and other pathogens through the production of antimicrobial compounds, enhancing the overall biocontrol efficacy (Albarrán-de la Luz et al., 2022).

5.2 Enhanced Pest Control Through Microbial Consortia

Microbial consortia involving *Aspergillus oryzae* can offer enhanced pest control by leveraging the strengths of multiple organisms. These consortia can create a hostile environment for pests and pathogens through various synergistic interactions.

A notable example is the use of *A. oryzae* in combination with beneficial bacteria and fungi to form a microbial consortium that targets specific pests. This approach has been tested in various agricultural systems. For instance, the consortium of *A. oryzae* with Trichoderma and Bacillus species has shown improved control of soil-borne pathogens and pests, leading to healthier crop growth and higher yields (Jambhulkar et al., 2018).

Furthermore, studies have indicated that microbial consortia can also enhance the production of secondary metabolites with pesticidal properties. The synergistic interaction among the microbes can stimulate the production of bioactive compounds that are more effective in pest control than those produced by individual strains alone (García-Conde et al., 2023).

5.3 Case Study: Successful Synergy in Pest Control

5.3.1 Case study 1: *Aspergillus oryzae* in locust control

You et al (2023) study highlighting the successful synergy of *Aspergillus oryzae* in pest control involves its application against locusts. The study evaluated the virulence of *A. oryzae* strain XJ-1 on adult locusts (*Locusta migratoria*) in both laboratory and field trials. The lethal concentration for adult locusts was determined to be 3.58×10^5 conidia/mL 15 days post-inoculation in laboratory settings. Field-cage experiments revealed that locust mortality reached approximately 92% 15 days after inoculation with 3×10^5 conidia/m². Furthermore, a large-scale field trial over 666.6 hectares, involving aerial spraying of a water suspension of *A. oryzae* at 2×10^8 conidia/mL, demonstrated a significant reduction in locust population densities by 85.4%-94.9% within 31 days (You et al., 2023).

5.3.2 Case study 2: synergistic effects with *Bacillus licheniformis*

In the study on mango disease control by Albarr á n-de la Luz et al. (2023), *A. oryzae* was tested in combination with Bacillus licheniformis M2-7 against phytopathogenic fungi affecting mango trees. The combined application of these microbes resulted in a significant reduction of fungal growth, with *A. oryzae* showing a 45% inhibition rate against itself, 40% against Colletotrichum sp., and 35% against *Aspergillus niger*. The presence of *B. licheniformis* altered the mycelial structure of the fungi, leading to fragmentation and reduced growth. This synergistic effect highlights the potential of combining *A. oryzae* with other beneficial microbes for enhanced pest control (Albarrán-de la Luz et al., 2022).

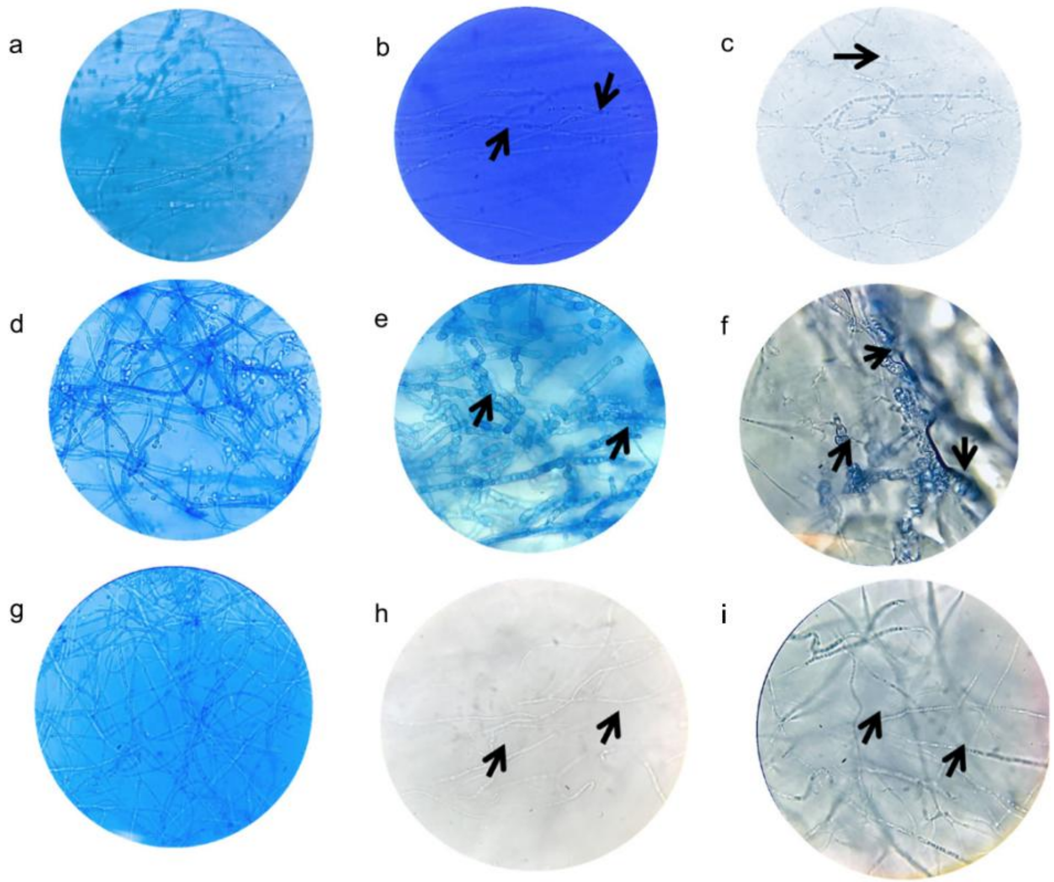

Figure 2 Damage caused to the structures of the fungi in antagonism was observed at 40X (Adapt from García-Conde et al., 2023)
Image caption: *Aspergillus oryzae* without bacterial inoculum (a); *A. oryzae* in the presence of Bacillus licheniformis M2–7 (b) and *A. oryzae* in the pre- sence of *B. licheniformis* LYA12 (c); Colletotrichum sp. without bacterial inoculum (d) in the presence of M2–7 (e), in the presence of LYA12 (f) and A. niger without bacterial inoculum (g) in the presence of M2–7 (h) and LYA12 (i), the arrows indicate hyphae malformation (Adapt from García-Conde et al., 2023)

Figure 2 effectively demonstrates the antagonistic effects of Bacillus licheniformis on various fungi, including *Aspergillus oryzae*, Colletotrichum sp., and *Aspergillus niger*. The presence of *B. licheniformis* strains M2-7 and LYA12 results in significant hyphal malformations, indicating their potential as biocontrol agents in managing fungal pathogens. These visual observations support the hypothesis that *B. licheniformis* can be employed to mitigate fungal infections in agricultural settings, promoting healthier crop growth and reducing reliance on chemical fungicides.

6 Environmental and Economic Implications

6.1 Eco-friendly Nature and Sustainability of *A. oryzae*-based Control Methods

The utilization of *Aspergillus oryzae* as a biocontrol agent is an eco-friendly alternative to chemical pesticides, offering significant benefits for sustainable agriculture. *A. oryzae* is a naturally occurring fungus that can effectively manage pest populations without introducing harmful chemicals into the environment. This biocontrol method aligns with the principles of Integrated Pest Management (IPM), which emphasizes the reduction of chemical pesticide use through biological and ecological approaches (Fahad et al., 2015).

A key environmental benefit of *A. oryzae*-based methods is their minimal impact on non-target organisms. Unlike broad-spectrum chemical pesticides that can harm beneficial insects, birds, and aquatic life, *A. oryzae* targets specific pests, reducing collateral damage and preserving biodiversity. For example, the use of *A. oryzae* to control locusts demonstrated high specificity and effectiveness, significantly reducing pest populations without affecting other species (You et al., 2023).

Moreover, *A. oryzae*-based biocontrol methods contribute to soil health by reducing the need for chemical inputs that can degrade soil quality over time. These methods support the maintenance of a balanced soil microbiome, which is crucial for plant health and productivity. The use of *A. oryzae* in soil treatments has shown to improve soil fertility and structure, further enhancing crop resilience against pests and diseases (García-Conde et al., 2023).

6.2 Cost-benefit analysis of using *A. oryzae* in rice pest management

The economic feasibility of employing *Aspergillus oryzae* in rice pest management has been increasingly supported by recent studies. The initial cost of developing and applying biocontrol agents like *A. oryzae* can be offset by the long-term benefits of reduced pest damage, lower chemical pesticide expenses, and enhanced crop yields.

In terms of cost, *A. oryzae*-based biocontrol methods may initially seem higher compared to chemical pesticides. However, the reduction in pest resurgence and resistance, which often necessitates higher doses and more frequent applications of chemicals, makes *A. oryzae* a cost-effective solution in the long run. For instance, the use of *A. oryzae* in controlling nematodes in rice fields has shown significant reductions in nematode populations, leading to healthier crops and higher yields, thereby justifying the initial investment (Liu et al., 2019).

Moreover, *A. oryzae*-based treatments often lead to better marketability of crops due to the absence of chemical residues, meeting the growing consumer demand for organic and sustainably produced food. This can lead to premium pricing and better market access, further enhancing the economic benefits for farmers (Shoji et al., 2021).

6.3 Potential Reduction in Chemical Pesticide Usage and Its Benefits

The use of *Aspergillus oryzae* in pest management significantly reduces reliance on chemical pesticides, bringing profound environmental and health benefits. Chemical pesticides are known to cause various issues, including water pollution, soil degradation, and harm to non-target species, including humans. The environmental and economic impacts of employing *Aspergillus oryzae* in biological control against rice pests are overwhelmingly positive, eco-friendly, and sustainable, highlighting the potential of *Aspergillus oryzae* as a cornerstone of sustainable agricultural practices.

Reducing chemical pesticide use through biocontrol agents like *A. oryzae* helps mitigate these negative impacts. For instance, studies have shown that biocontrol can effectively manage pest populations without the environmental persistence associated with chemical residues. This leads to cleaner water systems and healthier ecosystems. The reduction in chemical use also minimizes the risk of pesticide runoff into water bodies, thereby protecting aquatic life (Fahad et al., 2015).

Furthermore, the health benefits of reducing chemical pesticide use cannot be overstated. Pesticides have been linked to various health issues in humans, ranging from acute poisoning to long-term chronic illnesses such as cancer. By decreasing the dependency on these chemicals, *A. oryzae*-based methods contribute to safer food production and reduced health risks for both farmers and consumers (García-Conde et al., 2023).

7 Challenges and Future Directions

7.1 Limitations and potential risks associated with *A. oryzae*

While *Aspergillus oryzae* holds great promise as a biocontrol agent, there are several limitations and potential risks associated with its use. One of the primary challenges is the variability in efficacy due to environmental conditions. Factors such as temperature, humidity, and soil composition can significantly influence the performance of *A. oryzae*. For instance, studies have shown that optimal growth and metabolite production occur at specific pH levels and temperatures, which may not always be achievable in field conditions (Nacef et al., 2020).

Another significant limitation is the potential for horizontal gene transfer and unintended ecological impacts. *A. oryzae*, like other microorganisms, can potentially transfer genes to other microorganisms in the environment, which may result in unforeseen ecological consequences. This risk necessitates rigorous monitoring and regulation to prevent any adverse effects on non-target species and ecosystems (García-Conde et al., 2023).

Additionally, there is the issue of mycotoxin production. While *A. oryzae* is generally considered safe and non-toxigenic, its close relatives, such as Aspergillus flavus, produce aflatoxins, which are potent carcinogens. Ensuring the purity and strain specificity of *A. oryzae* applications is crucial to avoid contamination and potential health risks (Frisvad et al., 2018).

7.2 Regulatory and safety concerns

The use of *A. oryzae* in agricultural settings is subject to various regulatory and safety concerns. Regulatory frameworks vary by region, but generally include stringent requirements for safety assessments, environmental impact evaluations, and efficacy trials before approval for use. These regulations are designed to ensure that biocontrol agents do not pose risks to human health, non-target organisms, or the environment.

A key regulatory concern is the potential for allergenicity and pathogenicity. Although *A. oryzae* is widely used in food production and is considered safe, the large-scale application in fields could expose agricultural workers and nearby populations to fungal spores, which may cause allergic reactions or respiratory issues in sensitive individuals (Shoji et al., 2021).

Rregulatory agencies require comprehensive data on the environmental persistence and potential bioaccumulation of *A. oryzae* and its metabolites. The persistence of spores and secondary metabolites in soil and water bodies needs careful assessment to prevent long-term ecological impacts. Ensuring compliance with these regulations involves extensive field trials and monitoring, which can be resource-intensive and time-consuming (Jambhulkar et al., 2018).

7.3 Areas for Future Research and Potential Technological Advancements

Aspergillus oryzae in biological control should focus on several key areas to address current challenges and enhance its efficacy and safety. One important area is the genetic and metabolic engineering of *A. oryzae* strains to optimize their performance under various environmental conditions. Advances in omics technologies and synthetic biology can facilitate the development of tailored strains with enhanced biocontrol properties and reduced risks of mycotoxin production (He et al., 2019).

Research should explore the integration of *A. oryzae* with other biocontrol agents and agricultural practices to develop comprehensive pest management strategies. Studies have shown that microbial consortia can provide synergistic effects, enhancing pest control and reducing reliance on chemical pesticides (García-Conde et al., 2023).Technological advancements in formulation and delivery methods are also critical. Developing stable, easy-to-apply formulations such as granular products, liquid suspensions, or seed coatings can improve the practical use of *A. oryzae* in various agricultural settings. Innovations in application technologies, such as precision spraying and drone-based delivery systems, can enhance the efficiency and effectiveness of biocontrol applications (You et al., 2023).

While *Aspergillus oryzae* presents a promising biocontrol agent with significant potential, addressing its limitations and risks through focused research and technological innovation is essential. Regulatory compliance and safety assurance will be crucial in advancing its adoption in sustainable agriculture practices.

8 Conclusion Remarks

This systematic review has explored the multifaceted role of *Aspergillus oryzae* in biological control against rice pests. Key findings highlight its significant potential in pest management, supported by its antifungal, antibacterial, and nematicidal properties. Laboratory and field studies have demonstrated the effectiveness of *A. oryzae* in reducing pest populations and disease incidence in rice crops, indicating its viability as an alternative to chemical pesticides.

This review also reveals the advantages of combining *A. oryzae* with other beneficial microbes, enhancing pest control through synergistic effects. Integrating *A. oryzae* into microbial consortia has shown improved pest suppression and crop health, highlighting its role in comprehensive pest management strategies. Furthermore, the environmental and economic implications of using *A. oryzae* are discussed, emphasizing its eco-friendliness and cost-effectiveness. The reduction in chemical pesticide usage and the subsequent benefits to human health and the environment are significant points of discussion, demonstrating the broader impact of *A. oryzae*-based biocontrol methods.

Integrating *Aspergillus oryzae* into sustainable agricultural practices presents a viable pathway toward reducing dependency on chemical pesticides and enhancing crop resilience. The eco-friendly and cost-effective nature of *A. oryzae*-based biocontrol methods aligns with the goals of sustainable agriculture, which seeks to balance productivity with environmental stewardship.

To fully realize the potential of *A. oryzae*, ongoing research and development are crucial. This includes optimizing formulation and application methods, ensuring regulatory compliance, and conducting long-term field trials to validate efficacy under diverse environmental conditions. The development of genetically enhanced strains and innovative application technologies, such as precision spraying and drone delivery, can further enhance the practical use of *A. oryzae* in pest management.

Moreover, fostering collaborations between researchers, agricultural practitioners, and regulatory bodies will be essential to address the challenges and streamline the adoption of *A. oryzae*-based biocontrol strategies. Educating farmers on the benefits and application techniques of biocontrol agents will also play a crucial role in promoting widespread acceptance and implementation.

Aspergillus oryzae holds significant promise as a biological control agent against rice pests. Its integration into sustainable agricultural practices offers a pathway to reducing chemical pesticide use, protecting the environment, and ensuring food security. Continued research, technological innovation, and collaborative efforts will be key to unlocking the full potential of *A. oryzae* in sustainable pest management.

Acknowledgments

We sincerely thank to Ms. J. Zhang for her help and support during the research process. We also want to thank the peer reviewers for their valuable feedback and suggestions on my research, which greatly enriched and improved my work.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Albarrán-de la Luz L., Rodríguez-Barrera M.A., Hernández-Flores G., Lopezaraiza Mikel M., Alemán-Figueroa L., Toribio-Jiménez J., and Romero-Ramírez Y., 2022, Antagonism of *Bacillus licheniformis* M2-7 against phytopathogen fungi of *Mangifera indica* L., Revista Internacional de Contaminación Ambiental, 38: 1-10.
https://doi.org/10.20937/RICA.54217

Costa S., Summa D., Zappaterra F., Blo R., and Tamburini E., 2021, *Aspergillus oryzae* grown on rice hulls used as an additive for pretreatment of starch-containing wastewater from the pulp and paper industry, Fermentation, 7(4): 317.
https://doi.org/10.3390/fermentation7040317

Chen X., Zhou J., Ding Q., Luo Q., and Liu L., 2019, Morphology engineering of *Aspergillus oryzae* for l-malate production, Biotechnology and Bioengineering, 116: 2662-2673.
https://doi.org/10.1002/bit.27089

Daba G., Mostafa F., and Elkhateeb W., 2021, The ancient koji mold (*Aspergillus oryzae*) as a modern biotechnological tool, Bioresources and Bioprocessing, 8: 1-17.
https://doi.org/10.1186/s40643-021-00408-z

Frisvad J., Møller L.L.H., Larsen T.O., Kumar R., and Arnau J., 2018, Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*, Applied Microbiology and Biotechnology, 102: 9481-9515.
https://doi.org/10.1007/s00253-018-9354-1

Fahad S., Nie L., Hussain S., Khan F., Khan F.A., Saud S., Muhammad H., Li L., Liu X., Tabassum A., Wu C., Xiong D., Cui K., and Huang J., 2015, Rice pest management and biological control, Springer International Publishing, 16: 85-106.
https://doi.org/10.1007/978-3-319-16988-0_4

García-Conde K.B., Cerna-Chávez E., Ochoa-Fuentes Y.M., and Velázquez-Guerrero J.J., 2023, *Aspergillus oryzae*: an opportunity for agriculture, Mexican Journal of Phytopathology, 42(1): 1.
https://doi.org/10.18781/R.MEX.FIT.2302-2

He B., Tu Y., Jiang C., Zhang Z., Li Y., and Zeng B., 2019, Functional Genomics of *Aspergillus oryzae*: strategies and progress, Microorganisms, 7: 103.
https://doi.org/10.3390/microorganisms7040103

Jambhulkar P.P., Sharma P., Manokaran R., Lakshman D., Rokadia P., and Jambhulkar N., 2018, Assessing synergism of combined applications of *Trichoderma harzianum* and *Pseudomonas fluorescens* to control blast and bacterial leaf blight of rice, European Journal of Plant Pathology, 152: 747-757.
https://doi.org/10.1007/s10658-018-1519-3

Jeennor S., Anantayanon J., Panchanawaporn S., Chutrakul C., and Laoteng K., 2019, Morphologically engineered strain of *Aspergillus oryzae* as a cell chassis for production development of functional lipids, Gene, 718: 144073.
https://doi.org/10.1016/j.gene.2019.144073

Jiang B., Wang Z., Xu C., Liu W., and Jiang D., 2019, Screening and identification of *Aspergillus* activity against *Xanthomonas oryzae* pv. *oryzae* and analysis of antimicrobial components, Journal of Microbiology, 57: 597-605.
https://doi.org/10.1007/s12275-019-8330-5

Kalaivani K., Maruthi-Kalaiselvi M., and Senthil-Nathan S., 2020, Seed treatment and foliar application of methyl salicylate (MeSA) as a defense mechanism in rice plants against the pathogenic bacterium, *Xanthomonas oryzae* pv. *oryzae*, Pesticide Biochemistry and Physiology, 171: 104718.
https://doi.org/10.1016/j.pestbp.2020.104718

Liu Y., Ding Z., Peng D., Liu S., Kong L., Peng H., Xiang C., Li Z., and Huang W., 2019, Evaluation of the biocontrol potential of *Aspergillus welwitschiae* against the root-knot nematode *Meloidogyne graminicola* in rice (*Oryza sativa* L.), Journal of Integrative Agriculture, 18(11):2561-2570.
https://doi.org/10.1016/S2095-3119(19)62610-9

Nacef H.S., Belhattab R., and Larous L., 2020, Chemical composition, antimicrobial study against human and plant pathogenic microorganisms and optimization of bioactive metabolites produced by the new strain *Aspergillus oryzae* 18HG80 isolated from saline soil (El-Baida Marsh, Algeria), Journal of Microbiology Research, 10: 11-21.

Okabe T., Katayama T., Mo T., Mori N., Jin F., Fujii I., Iwashita K., Kitamoto K., and Maruyama J., 2018. BiFC-based visualisation system reveals cell fusion morphology and heterokaryon incompatibility in the filamentous fungus *Aspergillus oryzae*, Scientific Reports, 8(1): 2922.
https://doi.org/10.1038/s41598-018-21323-y

Ponce M.A., Lizarraga S., Bruce A., Kim T.N., and Morrison W., 2022, Grain inoculated with different growth stages of the fungus, *Aspergillus flavus*, affect the close-range foraging behavior by a primary stored product pest, *Sitophilus oryzae* (Coleoptera: Curculionidae), Environmental Entomology, 51: 927-939.
https://doi.org/10.1093/ee/nvac061

Park M.K., Seo J.A., and Kim Y.S., 2019, Comparative study on metabolic changes of *Aspergillus oryzae* isolated from fermented foods according to culture conditions, International Journal of Food Microbiology, 307: 108270.
https://doi.org/10.1016/j.ijfoodmicro.2019.108270

Son S., Lee S., Singh D., Lee N.R., Lee D.Y., and Lee C., 2018, Comprehensive secondary metabolite profiling toward delineating the solid and submerged-state fermentation of *Aspergillus oryzae* KCCM 12698, Frontiers in Microbiology, 9: 1076.
https://doi.org/10.3389/fmicb.2018.01076

Shoji M., Sugimoto M., Matsuno K., Fujita Y., Mii T., Ayaki S., Takeuchi M., Yamaji S., Tanaka N., Takahashi E., Noda T., Kido H., Tokuyama T., Tokuyama T., Tokuyama T., Kuzuhara T., 2021, A novel aqueous extract from rice fermented with *Aspergillus oryzae* and *Saccharomyces cerevisiae* possesses an anti-influenza A virus activity, PLoS One, 16(1): e0244885.
https://doi.org/10.1371/journal.pone.0244885

Wasil Z., Kuhnert E., Simpson T.J., and Cox R., 2018, Oryzines A & B, maleidride congeners from *Aspergillus oryzae* and their putative biosynthesis, Journal of Fungi, 4(3): 96.
https://doi.org/10.3390/jof4030096

You Y., An Z., Zhang X., Liu H., Yang W., Yang M., Wang T., Xie X., and Zhang L., 2023, Virulence of the fungal pathogen, *Aspergillus oryzae* XJ-1 in adult locusts (Orthoptera: Acrididae) in both laboratory and field trials, Pest Management Science, 79(10): 3767-3772.
https://doi.org/10.1002/ps.7561

Zhang P., You Y., Song Y., Wang Y., and Zhang L., 2015, First record of *Aspergillus oryzae* (Eurotiales: Trichocomaceae) as an entomopathogenic fungus of the locust, *Locusta migratoria* (Orthoptera: Acrididae), Biocontrol Science and Technology, 25: 1285-1298.
https://doi.org/10.1080/09583157.2015.1049977

Zhang Y.Q., Zhang S., Sun M.L., Su H.N., Li H.Y., Liu K., Zhang Y.Z., Chen X.L., Cao H.Y., Song X.Y., 2022, Antibacterial activity of peptaibols from *Trichoderma longibrachiatum* SMF2 against gram-negative *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight on rice, Frontiers in Microbiology, 13:1034779.
https://doi.org/10.3389/fmicb.2022.1034779