Review Article

Synergistic Impact of Biofertilizers in Enhancing Yield and Quality of Button Mushroom

Abstract

Biofertilizers in button mushroom (*Agaricus bisporus*) cultivation has emerged as an innovative and eco-friendly approach to enhance yield and quality. Biofertilizers, such as nitrogen-fixing bacteria, phosphorus-solubilizing microorganisms, and mycorrhizal fungi, have demonstrated significant potential in improving mycelial growth, fruiting body formation, and nutrient uptake. These microbial inoculants not only augment nutrient availability but also stimulate enzyme activity, leading to faster substrate colonization and healthier fruiting bodies. In comparison to chemical fertilizers, biofertilizers promote a more sustainable cultivation practice by enhancing soil health, minimizing chemical input, and reducing environmental impact. Studies have shown that applying biofertilizers can result in higher mushroom yields, improved nutritional profiles, and better resilience to environmental stress. Specifically, nitrogen-fixing bacteria help in assimilating atmospheric nitrogen, making it available to the growing mushrooms, while phosphorus-solubilizing bacteria increase the availability of phosphorus, which is essential for energy transfer and mycelial growth. Mycorrhizal fungi form symbiotic relationships with mushroom mycelium, facilitating better nutrient absorption from the substrate. The combined effects of these biofertilizers ensure optimal conditions for both growth and quality enhancement. Moreover, biofertilizers contribute to the reduction of heavy metal accumulation in mushrooms, thereby ensuring safer and healthier produce for consumers. In addition to their nutritional benefits, biofertilizers also play a role in improving the physical structure of the growing medium, making it more conducive for mushroom cultivation. However, challenges remain in optimizing the use of biofertilizers, as their effectiveness can be influenced by various factors such as the type of substrate, environmental conditions, and the specific microbial strains used. Variability in performance and limited shelf life of some biofertilizers also pose challenges in their widespread adoption. Nonetheless, with ongoing research and technological advancements, biofertilizers are increasingly becoming a sustainable and cost-effective solution for mushroom farmers. By reducing reliance on chemical fertilizers, they not only support better yields and quality but also contribute to a more environmentally sustainable approach to agriculture.

Keywords: biofertilizers, button mushroom, mycelial growth, nutrient uptake, sustainable agriculture, fruiting body formation.

Introduction

The use of biofertilizers in agriculture, particularly in mushroom cultivation, has garnered significant attention in recent years due to growing concerns over the sustainability of chemical fertilizers. Button mushrooms (*Agaricus bisporus*), one of the most widely cultivated mushrooms globally, have traditionally relied on chemical fertilizers to enhance yield and growth. However, the negative environmental impacts associated with chemical fertilizers, such as soil degradation, pollution, and the disruption of microbial communities, have necessitated a shift towards more eco-friendly alternatives. Biofertilizers, which are composed of living microorganisms such as nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, and mycorrhizal fungi, offer a natural and sustainable approach to mushroom cultivation by promoting nutrient availability, improving substrate quality, and enhancing crop resilience (Deepak et al., 2015).

Biofertilizers are advantageous due to their role in nutrient cycling and soil health improvement. Nitrogen-fixing bacteria, such as *Azotobacter* and *Azospirillum*, are among the most common biofertilizers used in mushroom cultivation. These bacteria fix atmospheric nitrogen and convert it into ammonia, making it available to the mushroom mycelium. This process is essential because nitrogen is a critical element for protein synthesis, cell development, and enzymatic activities within the mushroom. Phosphorus-solubilizing bacteria, such as *Bacillus* and *Pseudomonas*, also play a vital role by converting insoluble phosphorus compounds into soluble forms that are easily absorbed by the mycelium (Abo Nouh 2019). Phosphorus is necessary for energy transfer and cellular processes, especially during the fruiting body formation stage of mushroom development. Additionally, mycorrhizal fungi, though not directly interacting with mushrooms, enhance the growing medium's microbial diversity, promoting better nutrient retention and cycling (*Zhang et al 2016*).

The role of biofertilizers in promoting mycelial growth and fruiting body formation is closely tied to their ability to improve nutrient availability and induce enzymatic activity. Nitrogen-fixing bacteria, for example, increase the nitrogen supply in the substrate, leading to enhanced mycelial colonization and faster fruiting body development (Kumar et al., 2018). The improved nitrogen content supports the synthesis of proteins, which are critical for the growth and development of the mushroom's vegetative structure. Similarly, phosphorus-solubilizing bacteria ensure that the mycelium receives an adequate supply of phosphorus, which is vital for energy transfer and cellular division. These microorganisms also induce the production of growth-promoting enzymes, such as cellulases and ligninases, which break down complex organic materials in the substrate, making them more accessible to the growing mycelium (Janusz et al 2017). This enzymatic activity is crucial for efficient nutrient utilization, leading to healthier mycelial growth and robust fruiting body production.

Beyond enhancing growth, biofertilizers have a direct impact on the nutritional quality of button mushrooms. Nitrogen-fixing bacteria, by providing a steady supply of nitrogen, increase the protein content of mushrooms, making them a more nutritious food source (Gray & Smith 2005). Similarly, phosphorus-solubilizing bacteria enhance the availability of essential micronutrients such as zinc and magnesium, which are important for human health. Mushrooms grown with biofertilizers also exhibit higher levels of bioactive compounds, including antioxidants, which contribute to their health-promoting properties (Khalid et al., 2017). This is particularly significant in today's health-conscious consumer market, where nutrient-dense foods are highly valued.

Another key advantage of biofertilizers is their role as a sustainable alternative to chemical fertilizers. Chemical fertilizers, while effective in the short term, can cause long-term damage to soil health and biodiversity. They often lead to nutrient imbalances, soil acidification, and contamination of water bodies through runoff (fig1). In contrast, biofertilizers are environmentally friendly, promoting natural nutrient cycling without causing soil or water pollution (Chatterjee et al., 2017). By enhancing soil structure, increasing microbial diversity, and reducing the need for synthetic inputs, biofertilizers contribute to the sustainability of agricultural systems, particularly in mushroom farming. Their ability to provide nutrients in a slow-release form ensures that mushrooms receive a consistent supply of essential nutrients, reducing the risk of over-fertilization and nutrient leaching.

Fig 1. Mixing of Biofertilizers in compost **(Source- Koderma District)**

Despite the many benefits, the use of biofertilizers in mushroom cultivation is not without challenges. One major limitation is the specificity of microbial strains. Not all biofertilizers are effective across different substrates or environmental conditions, necessitating careful selection and testing. Additionally, environmental factors such as temperature, humidity, and pH can affect the efficacy of biofertilizers, leading to inconsistent results across different farming environments (Sinha et al., 2020). The limited shelf life of biofertilizers, which consist of living microorganisms, also presents a challenge, as they require proper storage conditions to maintain their viability. This can be a logistical issue for small-scale farmers who may not have access to the necessary storage infrastructure. Moreover, the lack of regulatory oversight in the production and distribution of biofertilizers can lead to the sale of substandard or ineffective products, further complicating their adoption in mushroom farming (Saha et al., 2023).

1.Types of Biofertilizers and Their Application in Mushroom Cultivation

Biofertilizers are living microorganisms that promote plant growth by enhancing the availability of nutrients to the plants. In mushroom cultivation, particularly for button mushrooms (*Agaricus bisporus*), biofertilizers have gained attention due to their capacity to improve yield, quality, and sustainability of production. The main types of biofertilizers applied in mushroom cultivation include nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, and mycorrhizal fungi. These biofertilizers play distinct roles in improving substrate quality, nutrient availability, and overall mushroom growth.

Nitrogen-Fixing Bacteria

Nitrogen is one of the most crucial nutrients for plant growth, and its role in mushroom cultivation cannot be understated. Nitrogen-fixing bacteria, such as *Rhizobium*, *Azotobacter*, and *Azospirillum*, are widely used biofertilizers in agriculture for their ability to convert atmospheric nitrogen into ammonia, a form readily absorbable by plants. In mushroom cultivation, nitrogen is essential for mycelial growth, which is the vegetative part of the fungus. By introducing nitrogen-fixing bacteria into the substrate, these microorganisms work symbiotically to make nitrogen available to the mycelium, thus accelerating the colonization of the substrate. This ultimately results in improved mushroom yields and faster fruiting body development (Yeom et al., 2021).

Phosphorus-Solubilizing Microorganisms

Phosphorus is a vital component for energy transfer and metabolic processes in mushrooms. However, in many soils and substrates, phosphorus is present in an insoluble form, making it unavailable to mushrooms. Phosphorus-solubilizing microorganisms, such as *Bacillus* and *Pseudomonas*, secrete organic acids that convert insoluble phosphorus compounds into forms that the mushroom mycelium can absorb. The application of these biofertilizers enhances the availability of phosphorus, leading to increased mycelial growth and higher mushroom yields (Cheng et al., 2023). The integration of phosphorus-solubilizing bacteria into compost or other substrates used for mushroom cultivation optimizes nutrient cycling, leading to more efficient substrate utilization and faster mushroom production cycles (fig 2).

Fig 2. Utilization of Phosphorus by Button Mushroom from Phosphorus-Solubilizing Microorganisms. **(Source- Koderma District)**

Mycorrhizal Fungi

Mycorrhizal fungi form symbiotic relationships with plant roots, aiding in nutrient and water absorption. Although mushrooms like *Agaricus bisporus* are not mycorrhizal themselves, incorporating mycorrhizal fungi into mushroom cultivation systems can indirectly benefit mushroom growth. Mycorrhizal fungi improve the nutrient status of plants growing alongside mushrooms or can enhance the compost substrate's microbial diversity, creating a more conducive environment for mushroom growth. These fungi improve water retention, nutrient mobilization, and overall soil health, which are important factors in creating optimal conditions for mushroom cultivation (Bagyaraj, 2014).

Application Methods

Biofertilizers can be applied to mushroom cultivation through several methods. They can be mixed directly into the substrate before spawning, applied during the composting process, or integrated as part of casing material used for fruiting body formation. Each method has its advantages, with direct substrate incorporation often resulting in more immediate benefits for nutrient availability. For example, nitrogen-fixing bacteria are typically introduced during substrate preparation to ensure that the mycelium has access to a steady supply of nitrogen from the beginning of colonization (Thakur et al., 2012). Similarly, phosphorus-solubilizing microorganisms are usually introduced at the composting stage to help break down organic material and release phosphorus for better mycelial development. The selection of appropriate biofertilizer types and application methods can significantly enhance the success of mushroom cultivation by creating a nutrient-rich environment that promotes vigorous growth and high yields (fig 3).

Fig 3. Application Methods of Biofertilizers application at farmers' field of Koderma district.

2.Mechanisms of Biofertilizers in Promoting Mycelial Growth and Fruiting Body Formation

Biofertilizers play an essential role in promoting the growth and development of button mushrooms (*Agaricus bisporus*) by facilitating mycelial expansion and fruiting body formation through various mechanisms. These mechanisms include the enhancement of nutrient availability, the induction of enzymatic activity, and the improvement of substrate conditions. The key types of biofertilizers used in this process include nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, and mycorrhizal fungi, which work together to create an environment conducive to mushroom growth.

Nitrogen Fixation and Mycelial Growth

Nitrogen is critical for mushroom mycelium as it is involved in protein synthesis and cellular processes. Nitrogen-fixing bacteria, such as *Azospirillum* and *Rhizobium*, fix atmospheric nitrogen into ammonia, which is easily taken up by the mycelium. In the context of mushroom cultivation, nitrogen-fixing bacteria significantly enhance mycelial growth by increasing nitrogen availability in the substrate. The increased nitrogen boosts protein synthesis and accelerates substrate colonization, leading to faster mycelial development and the early initiation of fruiting bodies (Kaur & Purewal 2019). These bacteria create a more balanced nutrient profile, providing a continuous nitrogen supply that fosters robust mycelial growth and enhances mushroom productivity.

Phosphorus Solubilization and Fruiting Body Formation

Phosphorus is another essential nutrient for mushrooms, playing a role in energy metabolism and cellular development. However, phosphorus often exists in an insoluble form in soils and substrates, making it unavailable for mushroom growth. Phosphorus-solubilizing bacteria, such as *Pseudomonas*and*Bacillus megaterium*, secrete organic acids that convert insoluble phosphorus compounds into soluble forms (Bhattacharyya & Jha, 2012). By increasing phosphorus solubility, these bacteria ensure that the mycelium has adequate access to this nutrient during its growth stages. Phosphorus is especially important during the transition from vegetative mycelium to fruiting bodies, as it provides the energy required for this process. Therefore, biofertilizers that enhance phosphorus availability play a critical role in improving fruiting body formation and ensuring higher mushroom yields.

Mycorrhizal Associations for Enhanced Nutrient Uptake

Although button mushrooms do not form direct mycorrhizal relationships, the presence of mycorrhizal fungi in the cultivation environment can indirectly benefit their growth. Mycorrhizal fungi, such as *Glomus* species, improve nutrient and water absorption in the growing medium. These fungi form symbiotic relationships with plants or nearby organisms in the substrate, improving nutrient cycling and microbial activity (Kalamullaet al., 2022). This creates a more balanced and nutrient-rich environment that supports mycelial growth and enhances fruiting body development.

Mycorrhizal fungi help improve phosphorus and nitrogen uptake, indirectly benefiting mushrooms by optimizing substrate health and nutrient availability.

Enzymatic Activity and Substrate Utilization

Biofertilizers also enhance the production of growth-promoting enzymes that facilitate the breakdown of complex organic materials in the substrate. Nitrogen-fixing bacteria stimulate the production of cellulases and ligninases, which help decompose organic matter and make nutrients more accessible to the growing mycelium (Abioye et al., 2024). These enzymes are vital for efficient substrate utilization, enabling the mushroom mycelium to absorb nutrients more effectively and grow more rapidly (Fig 4). The enhanced enzymatic activity resulting from biofertilizer application ensures that the substrate is used optimally, leading to more vigorous mycelial growth and the faster formation of fruiting bodies.

Fig 4. Enzymatic Activity and Substrate Utilization (Source- Koderma District)

Microbial Competition and Disease Suppression

Biofertilizers contribute to creating a competitive microbial environment in the substrate, which helps suppress the growth of harmful pathogens. Beneficial microorganisms introduced through biofertilizers can outcompete harmful fungi and bacteria, reducing the incidence of diseases and contamination in mushroom cultivation. For example, *Bacillus subtilis* has been shown to inhibit pathogenic fungi, such as *Trichoderma,* which can otherwise interfere with mushroom growth (Akond et al., 2016). By promoting a healthy microbial balance in the substrate, biofertilizers help ensure that the mushroom mycelium can grow without competition or interference from harmful organisms. This contributes to healthier and more productive fruiting body development.

3. Impact of Biofertilizers on the Nutritional Quality of Button Mushrooms

The use of biofertilizers in mushroom cultivation is increasingly recognized not only for its role in enhancing yield and growth but also for improving the nutritional quality of button mushrooms (*Agaricus bisporus*). Biofertilizers such as nitrogen-fixing bacteria, phosphorus-solubilizing microorganisms, and mycorrhizal fungi influence the nutrient content of mushrooms, increasing levels of essential nutrients, including proteins, vitamins, minerals, and bioactive compounds (fig 5). The application of biofertilizers can enrich the nutritional profile of mushrooms, making them a more valuable dietary source of nutrients, thus providing health benefits to consumers.

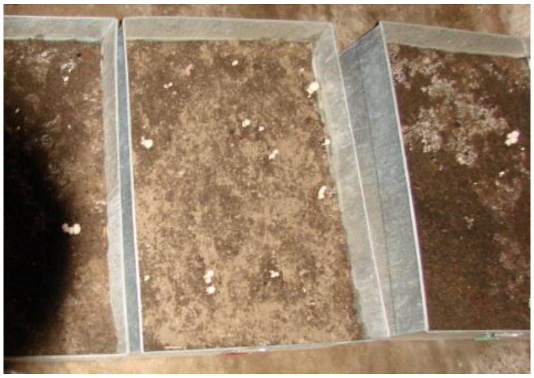

Fig 5. Biofertilizers on the Nutritional Quality of Button Mushrooms **(Source- Koderma District)**

Enhancement of Protein Content

One of the primary contributions of biofertilizers to mushroom cultivation is the enhancement of protein content. Mushrooms, especially button mushrooms, are valued for their high protein content, which is a critical nutritional component. Nitrogen-fixing bacteria, such as *Azospirillum* and *Azotobacter*, play a significant role in boosting nitrogen availability in the substrate, which is essential for protein synthesis. Nitrogen is a key element in amino acids, the building blocks of proteins. Studies have shown that mushrooms grown with nitrogen-fixing biofertilizers have a higher protein content compared to those grown without such microbial inputs (Agarwal et al., 2020). By increasing nitrogen availability, biofertilizers promote the synthesis of proteins, thus enhancing the nutritional value of button mushrooms.

Increased Vitamin and Mineral Content

Biofertilizers also have a profound effect on the vitamin and mineral content of mushrooms. Phosphorus-solubilizing bacteria, such as *Bacillus megaterium* and *Pseudomonas fluorescens*, improve the bioavailability of phosphorus in the substrate, which is crucial for the energy metabolism and overall health of mushrooms. Phosphorus is not only vital for growth but also plays a role in the synthesis of B-vitamins such as niacin and riboflavin, which are abundant in button mushrooms (Cheng et al., 2023). In addition, the solubilization of other micronutrients like zinc and magnesium, facilitated by biofertilizers, further enhances the mineral content of mushrooms. Zinc, for instance, is essential for enzyme function and immune system support, while magnesium plays a critical role in muscle and nerve function. Biofertilizers contribute to a more nutrient-rich substrate, leading to an increase in the concentration of these vital nutrients in the fruiting bodies.

Enhancement of Antioxidant Activity

Mushrooms are known for their antioxidant properties, which are linked to bioactive compounds such as polyphenols, flavonoids, and ergothioneine. Biofertilizers can influence the synthesis of these compounds, thus enhancing the antioxidant activity of button mushrooms. Mycorrhizal fungi, for instance, are known to promote the production of bioactive compounds by facilitating better nutrient uptake and improving stress tolerance in mushrooms (Reis et al., 2011). The increased availability of phosphorus and micronutrients through biofertilization helps in the synthesis of antioxidants, making the mushrooms not only nutritionally superior but also beneficial for health by reducing oxidative stress. Studies have indicated that mushrooms grown with biofertilizers exhibit higher antioxidant activity than those grown in non-fertilized substrates, further emphasizing the role of biofertilizers in boosting the health-promoting properties of mushrooms (Chakraborty & Akhtar 2021).

Bio fortification with Essential Micronutrients

Biofertilizers also contribute to biofortification, a process through which the nutrient density of crops is enhanced. For example, phosphorus-solubilizing bacteria can release minerals such as iron and calcium from organic matter in the substrate, making them more available to the growing mushrooms. Iron is a vital component of hemoglobin, and its presence in higher concentrations improves the nutritional quality of mushrooms as a source of this essential micronutrient. Calcium, on the other hand, is important for bone health and nerve function. Biofertilization strategies that focus on increasing the availability of these minerals can lead to mushrooms that are richer in micronutrients, providing enhanced health benefits to consumers (Słyszyket al., 2024). This biofortification effect of biofertilizers ensures that button mushrooms not only serve as a good source of macronutrients but also provide essential micronutrients necessary for human health.

Reduction of Heavy Metal Accumulation

Another significant impact of biofertilizers on mushroom nutrition is their ability to reduce the accumulation of harmful heavy metals, such as cadmium and lead, in the fruiting bodies. Biofertilizers such as mycorrhizal fungi form protective barriers around the root zones and mycelium, preventing the uptake of toxic metals from the substrate. This results in cleaner, safer mushrooms with lower levels of heavy metal contamination, thereby improving the nutritional safety of the produce (Malik et al., 2021). Reduced heavy metal content is particularly important for mushrooms, as they are known to accumulate metals from the substrate, which can pose health risks to consumers. By minimizing heavy metal uptake, biofertilizers help ensure that the nutritional quality of mushrooms is not compromised by environmental contaminants.

4.Biofertilizers as a Sustainable Alternative to Chemical Fertilizers in Mushroom Cultivation

In modern agriculture, including mushroom cultivation, the overuse of chemical fertilizers has raised significant environmental and health concerns. Chemical fertilizers, though effective in enhancing yields, have detrimental effects on soil health, biodiversity, and ecosystems due to nutrient leaching, contamination, and heavy metal accumulation. Biofertilizers, on the other hand, offer a sustainable alternative to chemical fertilizers in mushroom cultivation by providing natural, eco-friendly solutions to enhance growth, yield, and nutrient availability. Biofertilizers, including nitrogen-fixing bacteria, phosphorus-solubilizing microorganisms, and mycorrhizal fungi, not only improve the nutritional status of the substrate but also support the broader goals of sustainable agriculture by reducing environmental impact.

Environmental Benefits of Biofertilizers in Mushroom Cultivation

One of the most important advantages of biofertilizers over chemical fertilizers is their minimal environmental footprint. Unlike chemical fertilizers, which contribute to soil degradation and water pollution through nutrient runoff, biofertilizers are living microorganisms that enhance nutrient availability naturally. This process is more aligned with the ecological cycles and ensures that nutrients are slowly released into the substrate, thus minimizing the risk of leaching and contamination of water bodies (Kumar et al., 2022). Moreover, biofertilizers improve the structure and microbial diversity of the substrate, creating a more conducive environment for sustainable mushroom production. They help restore soil fertility and maintain ecological balance, which is crucial in long-term mushroom farming.

Reduction of Chemical Inputs and Soil Health

The reliance on chemical fertilizers can lead to the depletion of essential soil nutrients, disruption of microbial communities, and accumulation of salts, which negatively impact soil fertility. Over time, the excessive use of chemical fertilizers can cause nutrient imbalances and reduce the ability of soils to support healthy crops, including mushrooms. Biofertilizers help mitigate these issues by providing a balanced nutrient supply, which is released slowly into the substrate (Giri et al., 2019). Nitrogen-fixing bacteria like *Azotobacter* and *Azospirillum* improve nitrogen availability, while phosphorus-solubilizing bacteria such as *Bacillus* species enhance phosphorus availability, leading to better nutrient cycling in the substrate. This balanced nutrient input promotes soil health, ensuring that mushroom substrates maintain their fertility and productivity over longer cultivation cycles.

Promotion of Biodiversity and Soil Microbial Activity

Chemical fertilizers often disrupt the natural balance of microbial communities in soils and substrates by creating nutrient imbalances. Biofertilizers, in contrast, promote microbial diversity by encouraging the growth of beneficial bacteria and fungi in the substrate (Kawalekar, 2013). For example, the introduction of mycorrhizal fungi into the substrate not only improves nutrient uptake for mushrooms but also stimulates microbial activity that enhances soil health. These beneficial microbes help decompose organic matter, solubilize minerals, and support the establishment of a resilient microbial ecosystem that favors mushroom growth. By promoting biodiversity, biofertilizers contribute to a healthier growing environment, where beneficial microorganisms outcompete pathogens, reducing the need for chemical interventions such as fungicides or pesticides.

Economic Viability and Cost Efficiency

Biofertilizers provide a cost-effective alternative to chemical fertilizers in mushroom cultivation, particularly for small-scale and organic mushroom farmers. Chemical fertilizers can be expensive, and their overuse often requires supplementary inputs to counteract the negative effects on soil health. In contrast, biofertilizers are generally more affordable and can be produced locally, reducing dependency on external inputs (Amoo et al., 2021). Furthermore, biofertilizers are renewable, as the microbial populations can multiply and sustain themselves in the substrate, reducing the need for continuous application. This economic advantage makes biofertilizers a particularly attractive option for resource-limited farmers looking to improve yield without compromising soil health or incurring high input costs.

Enhanced Nutritional Quality of Mushrooms

Another major benefit of biofertilizers is their positive impact on the nutritional quality of mushrooms. Mushrooms grown with biofertilizers often have higher protein content, better mineral profiles, and enhanced antioxidant activity compared to those grown with chemical fertilizers (Kadam et al., 2017). Nitrogen-fixing bacteria improve protein synthesis in mushrooms by providing a steady nitrogen supply, while phosphorus-solubilizing bacteria increase the availability of essential micronutrients such as zinc, magnesium, and iron. The result is a more nutritionally enriched product that offers greater health benefits to consumers. Moreover, biofertilizers reduce the risk of heavy metal accumulation in mushrooms, ensuring a safer and cleaner product for consumption.

Contribution to Sustainable Agriculture Practices

Biofertilizers are at the core of sustainable agriculture practices, which seek to reduce the environmental impact of farming while maintaining productivity and profitability. In mushroom cultivation, biofertilizers contribute to sustainability by reducing the reliance on synthetic chemical inputs and promoting natural nutrient cycling. They help create a closed-loop system where waste products from one cycle (such as spent mushroom substrate) can be reused as inputs for future cycles, further minimizing environmental impact (Cetin & Atila 2024). This sustainable approach not only protects natural resources but also ensures the long-term viability of mushroom farming.

5. Challenges and Limitations in the Use of Biofertilizers for Button Mushroom Cultivation

While biofertilizers present numerous advantages in button mushroom (*Agaricus bisporus*) cultivation, including enhanced yield, improved nutritional quality, and environmental sustainability, their use is not without challenges. Several factors, such as microbial strain specificity, substrate compatibility, variability in efficacy, and storage issues, can hinder the widespread adoption of biofertilizers in mushroom farming.

Microbial Strain Specificity and Compatibility

One of the significant challenges in using biofertilizers in mushroom cultivation is the specificity of microbial strains. Biofertilizers are composed of living microorganisms that interact with the substrate and the mushroom mycelium. However, not all microbial strains are equally effective across different substrates or environmental conditions. Some strains of nitrogen-fixing bacteria or phosphorus-solubilizing bacteria may thrive in certain substrates but may not perform well in others. For instance, a strain of *Azospirillum* may be effective in one type of compost but may not show similar results in another (Kumari, &Naraian, 2021). This strain specificity necessitates careful selection and testing of biofertilizer products for specific mushroom cultivation systems. Additionally, the interaction between biofertilizers and the mushroom mycelium can vary, requiring precise matching of the microbial strains to the substrate to achieve optimal results.

Substrate Compatibility and Nutrient Availability

Another limitation is the compatibility of biofertilizers with different substrates used in mushroom cultivation. Button mushrooms are typically grown on composted agricultural residues, and the effectiveness of biofertilizers can be influenced by the type of substrate used. Some biofertilizers may not be compatible with certain substrates, leading to reduced efficacy. For example, phosphorus-solubilizing bacteria may be less effective in substrates with low organic matter content or high pH levels, as these conditions can limit the microbial activity needed to release phosphorus (Muswati et al., 2021). The variability in substrate composition across different mushroom farms poses a challenge in achieving consistent results with biofertilizer application. This variability requires tailored solutions, where biofertilizer formulations are adjusted to match the specific substrate conditions.

Variability in Efficacy Due to Environmental Factors

The efficacy of biofertilizers in mushroom cultivation is highly dependent on environmental factors such as temperature, humidity, and pH. Mushrooms require specific environmental conditions to grow, and any deviations from these optimal conditions can affect the performance of biofertilizers. For instance, nitrogen-fixing bacteria like *Azotobacter* and *Rhizobium* may not function effectively at extreme temperatures or under low moisture conditions, reducing their ability to supply nitrogen to the mycelium (Igiehon, & Babalola, 2017). Similarly, phosphorus-solubilizing microorganisms may require specific pH levels to perform optimally, and fluctuations in pH can hinder their ability to make phosphorus available to the mushrooms. This variability in efficacy due to environmental factors makes it challenging to standardize biofertilizer use across different mushroom farms, especially those with varying climatic conditions.

Limited Shelf Life and Storage Issues

Biofertilizers consist of living organisms, which makes their storage and shelf life a significant challenge. The viability of the microbial populations in biofertilizers can decline over time, particularly if they are not stored under ideal conditions. Exposure to high temperatures, moisture, or light can reduce the effectiveness of biofertilizers, leading to poor results when applied to mushroom substrates. This limited shelf life requires proper storage infrastructure, which may not always be available to small-scale mushroom farmers (Dawadi et al., 2022). Furthermore, the transportation and handling of biofertilizers require care to maintain their viability, adding to the logistical challenges faced by growers in adopting biofertilizer-based systems.

Inconsistent Results and Lack of Standardization

Inconsistent results are one of the primary limitations faced by mushroom farmers when using biofertilizers. The performance of biofertilizers can vary significantly from one farm to another due to differences in substrate composition, environmental conditions, and application methods. For example, some farmers may observe substantial improvements in yield and mushroom quality, while others may see little to no effect from the same biofertilizer product (Hamid et al., 2021). This inconsistency makes it difficult for growers to rely on biofertilizers as a guaranteed solution for improving mushroom production. The lack of standardization in biofertilizer formulations and application protocols further complicates their adoption, as farmers may struggle to find reliable products and methods that consistently deliver the desired outcomes.

Regulatory and Quality Control Issues

The regulatory framework for biofertilizers is still evolving in many countries, leading to challenges in ensuring the quality and efficacy of commercially available products. Some biofertilizers may not meet the required standards for microbial count or strain purity, resulting in reduced effectiveness when applied to mushroom substrates (Bharti, & Suryavanshi, 2021). The lack of quality control in the production and distribution of biofertilizers can lead to the sale of substandard or ineffective products, undermining the confidence of farmers in using these inputs. Additionally, the absence of clear guidelines on the appropriate application rates and methods for biofertilizers in mushroom cultivation can lead to incorrect usage, further reducing their potential benefits.

Conclusion

The application of biofertilizers in button mushroom cultivation offers significant benefits in terms of yield enhancement, improved nutritional quality, and sustainability. Biofertilizers, including nitrogen-fixing bacteria, phosphorus-solubilizing microorganisms, and mycorrhizal fungi, improve nutrient availability, allowing the mushroom mycelium to grow more efficiently and produce higher yields. Nitrogen-fixing bacteria such as *Azotobacter* and *Azospirillum* ensure a steady supply of nitrogen, which is essential for protein synthesis and mycelial growth. Phosphorus-solubilizing bacteria, on the other hand, enhance phosphorus availability, which is crucial for energy transfer and the development of fruiting bodies. Mycorrhizal fungi contribute to improving substrate quality by facilitating better nutrient retention and cycling, creating a healthier growing environment for mushrooms.

Biofertilizers not only boost growth but also significantly enhance the nutritional quality of mushrooms. Mushrooms grown with biofertilizers have higher protein content, improved mineral profiles, and increased levels of bioactive compounds such as antioxidants. These nutritional improvements make biofertilizer-grown mushrooms a more valuable food source for consumers, offering greater health benefits compared to mushrooms grown with chemical fertilizers. Furthermore, biofertilizers help reduce the accumulation of harmful heavy metals in mushrooms, ensuring that the final product is safer for consumption.

From an environmental perspective, biofertilizers offer a sustainable alternative to chemical fertilizers, reducing the need for synthetic inputs that can degrade soil health and pollute ecosystems. By promoting natural nutrient cycling and enhancing microbial diversity, biofertilizers support the sustainability of mushroom farming systems. They also reduce the risk of nutrient leaching and water contamination, making them an environmentally friendly option for farmers seeking to minimize their ecological footprint. In addition, biofertilizers are cost-effective, particularly for small-scale and organic farmers, as they are renewable and provide long-term benefits without the need for continuous application.

However, despite these advantages, the widespread adoption of biofertilizers in mushroom cultivation is limited by several challenges. One of the primary obstacles is the specificity of microbial strains, as not all biofertilizers perform equally well across different substrates or environmental conditions. This requires careful selection of the appropriate microbial strains tailored to the specific needs of the cultivation system, which can be time-consuming and costly. Additionally, environmental factors such as temperature, humidity, and pH significantly influence the efficacy of biofertilizers. Variability in these conditions can lead to inconsistent results, making it difficult for farmers to achieve uniform yields across different growing seasons or locations.Another challenge is the limited shelf life and storage requirements of biofertilizers, as they consist of living organisms that need to be stored under optimal conditions to maintain their viability. Improper storage can result in reduced microbial activity, leading to poor performance when applied to mushroom substrates. This poses logistical challenges, particularly for small-scale farmers who may lack access to the necessary storage infrastructure.

Inconsistent results are another limitation that hinders the full adoption of biofertilizers. Some farmers may experience significant improvements in yield and quality, while others may see minimal benefits due to differences in application methods, substrate composition, and environmental conditions. The lack of standardization in biofertilizer products and protocols further complicates their effective use in mushroom farming. Moreover, the absence of stringent regulatory frameworks and quality control measures can lead to the availability of substandard or ineffective biofertilizer products in the market, undermining farmer confidence.

Despite these challenges, biofertilizers remain a promising and sustainable alternative to chemical fertilizers. Addressing these limitations through ongoing research, development of standardized protocols, and improved storage solutions will be crucial in maximizing the potential of biofertilizers in mushroom cultivation. By optimizing biofertilizer use, farmers can enhance yields, improve nutritional quality, and contribute to more sustainable agricultural practices. As the demand for eco-friendly farming methods continues to grow, biofertilizers are poised to play an increasingly important role in the future of mushroom cultivation, offering both environmental and economic benefits for farmers and consumers alike.

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