AGROSYM

BOOK OF PROCEEDINGS

XVI International Scientific Agriculture Symposium

"Agrosym 2025"

Jahorina, October 2-5, 2025

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FOREWORD

The world is facing a range of crises that require policymakers to base their decisions on scientific data and evidence to effectively address them. Issues such as climate change, loss of biodiversity, degradation of natural resources (e.g. land and water), ongoing conflicts, persistent inequalities, and economic disruptions are progressively weakening the ability of agri-food systems to ensure food security and nutrition for all. Research plays a vital role in providing evidence and technical expertise, acting as a facilitator and policy broker in the development of agri-food strategies, investment planning, innovation policies, and other important policy processes. Additionally, research is essential for generating the knowledge, evidence, and resources required to promote responsible and inclusive innovation. It also offers support to a range of stakeholders, including farmers' organizations, businesses, and civil society groups. Integrating research with long-term policy strategies can enhance the acceptance and adoption of innovations in the agri-food sector, particularly when these innovations are promoted through inclusive platforms. To achieve a sustainable impact at scale, it is essential to improve collaboration in agri-food research and innovation. Scientific events and gatherings can be crucial in reaching this objective.

The International Agriculture Symposium AGROSYM has served as an annual forum for sixteen years, facilitating global scientific discussions on agriculture, food, rural development, the environment, and forestry. This symposium offers an excellent opportunity to share ideas, strengthen existing networks, establish new academic connections, and stimulate dialogue among academia, public institutions, the private sector, and civil society organizations. Participants engage in discussions about recent global and regional trends in the agri-food sector. The multidisciplinary findings presented at AGROSYM contribute to the dissemination of knowledge and best practices among all stakeholders in the agri-food chain, including farmers, extension agents, researchers, and policymakers. Additionally, the symposium emphasizes the importance of agriculture and food science as vital components of many national research strategies, impacting the broader community as well.

This Book of Proceedings from the 16th International Scientific Agricultural Symposium "AGROSYM 2025" encompasses articles which illustrate a wide range of research and insights across the comprehensive spectrum of agricultural disciplines. These articles address the following themes: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture and agroecology, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, and 7) Forestry and agroforestry.

We wish to express our heartfelt appreciation to all authors, reviewers, and colleagues who assisted in preparing this Book of Proceedings. Our special thanks go to co-organizers, partners, and sponsors for their unwavering support and collaboration.

We hope this multidisciplinary and international collection of research findings serves as a valuable resource for all stakeholders and encourages continuous dialogue and exchange to facilitate the transition towards sustainable, resilient, and inclusive agri-food systems.

East Sarajevo, 1 November 2025

Dr. Hamid El Bilali, CIHEAM Bari, Italy – Editor in Chief, President of the Scientific Committee
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CONTENT

THE INFLUENCE OF SUBSTRATE ACIDITY ON PRODUCTION OF MUSHROOM *LENTINULA EDODES* (BERK.) PEGLER

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Abstract

For the fungus to successfully colonize the substrate, counteract competitive species, and perform maximum fructification, certain basic environmental conditions must be met, depending on the type and strain of the fungus and the phase of wood decay. This paper examines the basic environmental conditions during the first phase of Shiitake development – the phase of substrate colonization. The aim of the research was to determine the optimal conditions for the development of the fungus *Lentinula edodes* (Berk.) Pegler. The results showed optimal acidity of the substrate stimulating the growth of this fungus, compared to the conditions under which the rival microorganisms developed. The highest weight of mycelial dry mass was formed at the substrate pH values between 3.00 and 3.63, meaning that this fungus could easily perform spontaneous infection. The changes in pH of the substrate where the examined strains of the fungus *L. edodes* grew shifted toward acidic reaction (5.15 to 3.52), suggesting that such acidity favors the development of the fungus investigated. Results show the stagnation of base pH value at the pH value of about 3.5, which indicates that these are the most favourable conditions for development of both strains of the fungus *L. edodes* in the substrate colonization phase (base pH of 3.6 being the most favourable for mycelial growth). The assumptions from the reference literature were thus confirmed by using the described methods. The fungus cannot thrive in an alkaline environment at pH 7.3.

Key words: *Substrate acidity, Mushroom Production, Shiitake.*

Introduction

The Shiitake mushroom (*Lentinula edodes* (Berk.) Pegl., Syn. *Lentinus edodes* (Berk.) Sing.) grows wild in Japan on the dead wood of *Castanopsis cuspidata*, family Fagaceae (Chang and Buswell, 1996; Wasser, 2005). This mushroom is known for its medicinal properties and has been cultivated for centuries in Japan and China, while its production in Serbia is more recent. It is important to note that no chemical substances are used in its cultivation, unlike in the case of popular button mushrooms.

Shiitake significantly increases the strength and vitality of the organism and empowers the body to ward off a large number of organic disorders (Safaie *et al.*, 2024). Shiitake contains immunostimulants, components effective against tumours and viruses which lower the cholesterol level, prevent clogging of blood vessels, regulate the blood pressure, improve the circulation, balance the blood sugar level, regulate digestion, improve the respiratory system, have antirheumatic and anti-allergenic effect, stimulate or soothe the central nervous system in an innocuous way, boost physical strength and stamina and slow down the aging process (Camilleri *et al.*, 2025). Japanese and American researchers have demonstrated that this type of fungus contains a certain number of biologically active compounds that inhibit cell degeneration. The fungus contains medicinal polysaccharides - lentinan and LEM are compounds that have shown anti-fatigue, anti-virus, anti-bacterial and hepatoprotective effects (Hobbs, 2000; Maeda *et al.*, 1998; Mizuno, 1996; Yap & Ng, 2003; Ngai &Ng, 2003), its production does not entail use of chemicals and pollution of the environment and, finally, the

production itself requires minimal investment and pays off before long (Markovic, 2003; Stamets, 1993; Tsivileva *et al*., 2008). The basic conditions that enable the fungus to perform infection are the substrate pH, the status of the nutrients within the substrate, the level of inoculum, virulence and the competition on the part of other microorganisms. For the fungus to carry out successful colonization of the substrate, to counteract the competitive species and perform maximum fructification, certain basic environmental conditions must be met, depending on the type and strain of the fungus and the phase of the wood decay. This paper examines the basic environmental conditions during the first phase of Shiitake development – the phase of substrate colonization. Concentration of hydrogen ions is one of the major factors that define the dynamics of the substrate-based fungal development (Grosser, 1985; Li, K. & Li, F., 1992, Lee *et al*., 2008, Desisa *et al*., 2023). Substrate acidity may affect the stimulation or inhibition of the growth of lignicolous fungi, while changes in pH value significantly influence the speed of nutrient consumption and substrate decomposition. Environmental acidity also affects the enzyme system of the fungi, which satisfies the vital organisms' needs for food. Decomposing wood (by oxidation and hydrolysis of the wood constituents), epixylous fungi increase wood acidity by means of oxalic acid obtained as a product of these processes. Edible fungus *L. edodes* belongs to this group of fungi. In this way, the order and succession of nutritive substrate colonization by microorganisms are arranged under natural conditions. Succession is one of the key factors of the wood colonization process, in addition to the vitality of the host plant, degree of parasitism, and the manner fungi consume food (Schmidt, 1994), which in turn regulates connexion or succession of the species on the same substrate.
In order to achieve more efficient mass production, this study examined the most important optimal conditions that stimulate its growth and development.

Material and Methods

The influence of the substrate pH value on the mycelial growth
and mass production investigation method
For the purpose of investigating the influence of the constant substrate pH values on the *L. edodes* mycelial growth and mass production, a buffered nutritive base was prepared according to the Wolpert method (Rehm & Reed, 2007). By mixing different volumes of 0.3 molar solutions of phosphates (H3PO4, KH2PO4 and K2HPO4), five series of phosphates with different pH values were obtained, yet they all had equal quantities of nutrients in certain parts of the buffer so that different quantities would not influence the results. A portion of double-concentrated malt and agar base (10 Bé sugar and 4% agar) was prepared separately and autoclaved independently of 0.3 M solutions of phosphates. Upon pH value check, the base was mixed with the phosphates under aseptic conditions. In this way, a base of standard concentration was obtained with the physiologically equal percentage of buffer (0.15 M). Thus prepared buffered base was poured into plastic Petri dishes. Five replications were used for each fungus strain and each pH value examined. The experiments were set in a poly-thermostat at the temperature of 21°C. Mycelial growth was measured every 24 hours along three axes: the dish radius and two additional lines angled at ±22.5° from the radius. To test the stability of the buffered system, a parallel culture was grown in a liquid medium. The dry mycelial mass from this culture was measured at the conclusion of the experiment.
The influence of the mycelium on the substrate pH change investigation method
For the study of the influence of the fungus on the change of nutritive substrate pH, the unbuffered (liquid) medium was prepared. A double-concentrated malt base was prepared with distilled water. According to the recipe, 1M-solutions of HCl or NaOH were added, thus providing five series of liquid base of standard sugar concentration (5 Bé). Base acidity for each series (five replications each) was measured before the sterilization. The base pH values were

also measured after the sterilization and treated as initial values. Upon inoculation of the substrate with strains of the fungus *L. edodes*, the changes in pH value of the substrate were measured every 7 days. Twenty-two days after the experiment began, the mycelium was extracted with a water vacuum pump, air-dried in a laminar flow chamber, and its dry mass was measured.

Results and discussion

The influence of the substrate pH value on the *L. edodes* mycelial growth and mass production
The mycelia of both strains of the fungus *L. edodes* developed in all the series with acidic reaction (initial pH 3.0 to 4.9) as shown in Table 1. For the most part, the A strain had a more aggressive growth than the B strain while both strains had the fastest growth and formed the greatest mycelial mass in series 2 (at the initial pH 3.6). Both strains had the smallest mycelial mass and the lowest growth in series 3 (initial pH 4.9), whereas in series 5 (at the initial pH 7.3) there was no mycelial growth in either strain. Series 5 was not lethal for the fungus which was confirmed when fragments were transferred onto the substrate at optimal pH value 3.5. Results shows the most favourable initial pH value is 3.6 as the mycelial growth is approximately 4.25 mm/day in both strains of the fungus, while for all other initial pH values (3.0, 4.6 and 4.9), the growth amounts to 3.5 to 3 mm/day. At the initial pH value of 7.3 there was no mycelial growth at all.

Table 1. Average daily increment (mm/day) and weight of dry mycelial mass of the fungus *L. edodes* (A and B strain) (g) on buffered nutritive substrates

Series no.	Fungus strain	pH of base			Average daily increment of mycelium (mm/day)	Weight of dry mycelial mass (g)
		pH of buffer	Initial pH	pH at the end of experiment		
1	A	3.0	3.0	3.1	3.48	0.195
	B			3.2	3.37	0.180
2	A	3.5	3.6	3.4	4.23	0.252
	B			3.3	4.37	0.271
3	A	4.6	4.9	4.7	3.01	0.148
	B			4.5	3.05	0.155
4	A	4.7	4.6	4.3	3.25	0.160
	B			4.2	3.07	0.161
5	A	7.5	7.3	7.0	0.0	0.0
	B			7.1	0.0	0.0

The influence of *L. edodes* mycelium on the change of pH of the nutritive substrate
Table 2 shows changes in the pH values of the malt substrate of the standard concentration under the influence of the *L. edodes* mycelium. After three weeks of the activity of the fungus, the pH of the base was reduced to the range of 3.52 to 5.15 for A strain and 3.63 to 5.05 for B strain in all the series investigated which means that those pH values were most favourable for the mycelia of both strains of the fungus *L. edodes* in this phase of growth.

Table 2. Changes of pH values of substrate provoked by fungus *L. edodes* (A and B strain) after 7, 14 and 21 days

Series no.	Initial pH	Fungus strain	Change in pH of the base				pH at the end of experiment	Weight of dry mycelial mass (g)
			After 7 days	After 14 days	After 21 days	Total change		
1	3.9	A	-0.15	-0.20	-0.03	-0.38	3.52	0.081
		B	-0.20	-0.05	-0.02	-0.27	3.63	0.085
2	5.4	A	-0.12	-0.04	-0.40	-0.54	4.86	0.062
		B	-0.07	-0.08	-0.25	-0.40	5.00	0.059
3	5.5	A	-0.10	-0.11	-0.14	-0.35	5.15	0.051
		B	0.02	-0.08	-0.45	-0.55	4.95	0.045

4	5.7	A	0.00	-0.14	-0.50	-0.64	5.06	0.040
		B	0.10	-0.15	-0.40	-0.65	5.05	0.042
5	6.1	A	-0.15	-0.20	-0.60	-0.95	5.15	0.033
		B	-0.12	-0.40	-1.05	-1.57	4.53	0.038

The greatest change of the substrate pH value through the effect of both strains of the fungi *L. edodes* was found in series 5 at the initial pH value 6.1 while the smallest one occurred in series 1 – initial pH 3.9. In three series (no. 3, 4 and 5), the B strain caused greater total change of the substrate pH than the A strain.

Results demonstrates that for the initial pH value of 3.9 (strain B) base pH slightly decreases until the seventh day when it reaches a constant value. This pH is optimal for *L. edodes*, meaning the substrate should be adjusted to this value during the colonization phase to ensure efficient production. The same occurs in the A strain at the initial substrate pH value of 3.9, the base pH decreases mildly until day 14 when it reaches its constant value. It is characteristic for the initial pH values of 5.4, 5.5 and 5.7 that both strains of the fungus (A and B strain) reach stagnation of the base pH value by the 14th day after which the base pH value suddenly drops. At the initial pH value of 6.1, the base pH value abruptly decreases since day 1 in both strains of the fungus.

Results shows that the percentage of decrease of the pH value ranges between 6 and 11% for the initial pH values of 3.9, 5.4, 5.5 and 5.7 in both strains of the fungus (A and B strain), whereas at the initial pH value of 6.1 the decrease of the acidity is far greater and amounts to approximately 16% for the A strain and 26% for the B strain.

Generally, during the experiment (22 days) it was determined that both strains of the fungus changed the initial pH values of the substrate shifting them towards the optimal values. As this shifting was within the range of tolerance, all buffered systems were considered stable. Both strains of the fungus *L. edodes* had the fastest growth and formed the greatest mycelial mass at the initial pH 3.6. According to the research results of other authors, the optimum pH for *L. edodes* mycelial growth was 3.0 to 3.5 (Hassegawa *et al*., 2005). It is well known that all fungi need different pH values depending on the phase of development – germination of spores, mycelial growth, formation of fruiting bodies, sporulation etc. Hence the fungi may prefer more acidic substrate for germination of spores, whereas this changes in the phase of fruiting body formation, and quite often less acidic or almost neutral reaction in the substrate is more suitable. This phenomenon has particular importance in the production of the fruiting bodies of the fungi for commercial purposes as is the case with fungus *L. edodes*. Practice has shown that the formation of fruiting bodies for this species is best on substrates with an acidic reaction. This should certainly be taken into consideration for the pH value of the substrate is the key factor for the infection process, substrate colonization or rot progress and dynamics.

Conclusions

The changes in pH of the substrate where the examined strains of the fungus *L. edodes* grew shifted toward acidic reaction (5.15 to 3.52), which suggests that such acidity favours the development of the fungus investigated. Results show the stagnation of base pH value at the pH value of about 3.5 which indicates that these are the most favourable conditions for development of both strains of the fungus *L. edodes* in the substrate colonization phase (base pH of 3.6 being the most favourable for mycelial growth). The assumptions from the reference literature were thus confirmed by using the described methods. On mildly acidic bases, the fungus *L. edodes* developed a colony of hyphae, which means that lower pH values resulting from the development of a rival species under natural conditions would not hinder the development of *L. edodes.* Alkaline conditions inhibit this fungus, with growth stopping at a pH of 7.3. Because development resumes when the pH is reduced, this suggests the fungus

enters a dormant state rather than losing its vitality. The competition of microorganisms on the same substrate, inhibition or growth, or the occurrence of antagonism, represents a phenomenon that may be the consequence of the metabolism of rival fungal species, excretion of mycotoxins or antibiotics from the growing front of the mycelia, and sensitivity, that is, the reaction of the rival species thereafter. This phenomenon has a direct impact on the speed, course, and consequences of the decomposition of wood as a substrate and food source.

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