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ORIGINAL ARTICLE

Domestication of wild-growing Turkey tail mushroom (*Trametes versicolor)* from Ethiopian forests on augmented agro-industrial byproducts

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Abstract

Despite being extensively studied as a white-rot fungus, there have been no efforts to explore and cultivate the high-yielding wild *Trametes versicolor* strains in Ethiopia. Thus, this study was initiated to assess the growth performance of *T. versicolor* on various growth media. Accordingly, ten substrates (S1–S10) were formulated by a combination of agro-industrial by-products that mainly constituted sugarcane wastes and animal manures. The effect of substrates on yields, biological efficiencies, and nutritional compositions was examined. The mushroom developed a white mycelium on the growth media. *T. versicolor* cultivated on the S5 blend, comprising 80% sugarcane bagasse, 12% horse manure, and 8% poultry manure, exhibited the most substantial fruiting body yield (158.33 g/500 g bag) and the highest biological efficiency (31.5%), with an optimal C:N ratio of 31:1. It has shown good mycelial growth, short colonization, and short pinhead formation time compared to other substrates. S7, lacking nitrogen supplementation, yielded low biological efficiency and fruiting bodies at 11.50% and 57.67%, respectively. The crude protein, fiber, low fat, and carbohydrate content ranged from 7.46 to 14.65%, 12.89 to 18.38%, 0.42 to 0.53%, and 48.75 to 66.75%, respectively. Notably, the highest nutritional values, excluding carbohydrates, were obtained from S5, while the sugarcane bagasse had the highest carbohydrate content among substrates. Consequently, S5 emerged as a suitable medium for cultivating wild *T. versicolor* mushrooms, particularly in regions abundant in poultry, horse manures, and sugarcane bagasse. Therefore, S5 represents an optimal substrate for *T. versicolor* cultivation, offering improved productivity and nutritional quality at reduced costs.

Keywords Agro-industrial byproducts · Animal manures · Sugarcane bagasse · Forest resources · Yields

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Introduction

Ethiopia has natural forest ecosystems with diverse natural treasures, including wild medicinal mushrooms (Dejene et al. 2017; Sitotaw et al. 2020). This rich ecological setting offers fertile ground for the growth of various species of wild mushrooms, renowned for their medicinal properties and cultural significance. These mushrooms thrive abundantly during the rainy season, serving as vital resources for sustenance and traditional healing practices among local populations. However, despite their importance, there remains a notable gap in research and documentation surrounding these valuable fungi (Indrani Sarma 2010). Generally, wild mushrooms are the most neglected resource, receive little attention, and await extensive studies in Ethiopia. Wild mushrooms are not only a source of nutrients but can also form the basis of many of the most expensive products,

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including medicine. Mushrooms are currently being used in modern pharmacological applications for medical purposes, although their vital roles in the bioremediation of new toxins and excellent pharmaceutical uses are still underused (Platania and Santisi 2015; Fonseca et al. 2016). Therefore, there has been an increasing interest in both academia and industry due to the immense application of wild medicinal mushrooms as a source of biologically active compounds that provide health benefits for humans in preventing and treating diverse diseases (Platania and Santisi 2015). Moreover, the health-promoting effects of the mushrooms have been attributed to the presence of bioactive compounds that exhibit antioxidant, anticancer, antibacterial, antiviral, anti-inflammatory, anti-diabetic, immune-modulating, and other health-promoting effects (Wasser 2010; Platania and Santisi 2015). The nutritional and other therapeutic uses of wild medicinal mushrooms strongly prompt more intensive investigations to explore these untapped resources as alternative medicines in an attempt to combat drug-resistant microbial pathogens.

The genus *Trametes* is one of the most important medicinal mushroom species in the world (Tel-Çayan et al. 2021). Among the white-rot basidiomycete fungi that are widely distributed throughout all the continents and major climatic regions, it mainly grows on partially living or dead woody deciduous trees (Wasser 2010). Nearly 60 *Trametes* fungal species are known to inhabit the world, but a few of them are cultivated and screened for their medicinal properties (Wasser 2010). Some species of the genus *Trametes* have a long ethnomycological history as medicinal fungi in many countries as reported by Marco-Urrea et al. (2009); and Rathee et al. (2012) which encompass *T. cubensis*, *T. hirsuta*, and *T. versicolor*. The current study was mainly focused on the cultivation of *T. versicolor* (Synn. *Coriolus versicolor*), usually named Turkey tail, which is the most popular medicinal macro-fungi and a model species applied in both medicinal applications and environmental protection (Rathee et al. 2012).

T. versicolor is one of the most potent and best-studied medicinal mushrooms in the world, and it has been famous in Japan and China as a potential medicine (Elsayed et al. 2014). Many studies have reported the antiviral and antioxidant activities of polysaccharopeptides isolated from basidiocarp extracts of *T. versicolor*, but the amount of basidiocarp used for this purpose is limited in nature and in the natural habitat (Elsayed et al. 2014). In addition, this macrofungus grows seasonally depending on weather conditions during rainy seasons in the natural forest. Therefore, the cultivation of these exclusive wild medicinal mushrooms using low-cost optimized substrates constituted from diverse agro-industrial byproducts and animal manures is a very important way of solving the aforementioned problems for the availability and sustainability of mushrooms cultivation. Hence, the successful artificial cultivation of *T. versicolor* was reported in Vietnam, where the Vietnamese *T. versicolor* industry has been screening and testing potential strains that can produce high yields of bioactive molecules (Elsayed et al. 2014). For enhanced productivity and high biomass, collecting and identifying the fast-growing wild mushroom strains and optimizing the growth substrates from locally available organic wastes are useful strategies for securing sufficient inputs for commercial production. This approach can play a key role in the growth of a research wing and the maintenance of genetic resources for sustainable wild as well as commercial mushrooms cultivation in Ethiopia. Generally, in Ethiopia, information about wild mushrooms domestication and uses is dearth, and hence their status is also mysterious under rapidly degrading natural habitats including the remanent patchy forest ecosystems (Dejene et al. 2017). Hence, there is an urgent need for the conservation and domestication of wild-growing mushrooms in the country.

The selection and preparation of adequate growth substrates and culture conditions, which primarily depend on the costs and availability of lignocellulosic biomass, including agro-forest and agro-industrial wastes, are one of the primary highlights in the development of wild and commercial mushrooms (Atila 2017). In this study, the target mushroom was domesticated using locally accessible sugarcane factory wastes from Ethiopia, including sugarcane bagasse, and filter cake supplemented with cotton seed hull, cow dung, horse manure, and chicken manure. These biowastes contain cellulose, hemicellulose, lignin, nitrogen, as well as macro- and micronutrients that can be used to enhance the cultivation of wild mushrooms with adequate nutrient levels. Using the right technologies such as mushrooms farming, this (i.e., untapped enormous resource) using biowastes that can be transformed into goods with additional value in ensuring the strategy of a circular economy as suggested by Cho and Ryu (2015) and inspiring a new dimension of economic development from medicinal mushrooms in Ethiopia. Accordingly, exploring the possibility of cultivating wild-growing Turkey tail mushroom (*Trametes versicolor*) on formulated agro-industrial byproducts is deemed important.

Thus, this study was aimed at analyzing the biochemical composition of agro-industrial byproducts to be utilized as growth substrates for *T. versicolor* cultivation to better understand the nutrient requirements of the target macrofungus. The study also determined successful cultivation of *T. versicolor* and its capacity to produce fruiting bodies on optimized artificial growth substrates to commercialize this medicinal mushroom for further value-addition attempts.

Materials and methods

Study area and experimental design

The experiment was conducted at the Forest Products Innovation Center of Excellence in Addis Ababa, Ethiopian Forestry Development, between June and August 2022. The experiment was done in a completely randomized design with ten substrate treatments and three replications per treatment.

Culture source and mycelium characterization

Trametes versicolor strain (E-7) (NCBI GenBank accession number OQ621598) was isolated from Bonga Forest, Gewata Woreda, located in the Kafa Zone of the southwest regional state of Ethiopia (Desisa et al. 2023, unpublished; Fig. 1A). The mycelium of *T. versicolor* was prepared by growing on malt extract agar (MEA; Himedia) and potato dextrose agar (PDA; Eur. pharm.) medium to determine the mycelium growth (Fig. 1B; C). Typically MEA consists of malt extract (30 g), peptone (5 g), and agar (15 g). The PDA usually contains potato peptone (4 g), glucose (20 g), and agar (15 g) per liter of distilled water. The inoculated Petri dishes were incubated at 25°C in the dark for 8–10 days, and the mycelial growth was measured at regular intervals of 2 days (Fig. 2).

Spawn preparation

The *T. versicolor* strain (AAU BE-7) from stock culture was used for spawn preparation (Fig. 2A). Polypropylene plastic bottles (500 mL) were filled three-quarters full with mother spawn grain comprising 95% wheat grain, 4% gypsum, and 1% calcium carbonate on a dry weight basis (Beje et al. 2013). The spawn grain was sterilized by autoclaving at 121°C for 80 min (Atila 2017). After cooling down to room temperature, the sterilized grain was inoculated with 10 g of actively growing *T. versicolor* mycelium plugs and incubated at 25 ± 2°C for 10 days in a completely dark environment until the grains were entirely covered with mycelium (Fig. 2B).

To prepare a commercial spawn of *T. versicolor*, 7.5 × 35.0 cm polypropylene bags were filled with three-quarters full of tightly packed spawn grains and then sterilized by autoclaving at 121°C for 80 min. After cooling down to room temperature, the sterilized grains were inoculated with 15 g of mother spawn on a wet-weight basis, as described by Atila (2019). The inoculated bags were then maintained at 25 ± 2°C for 9 days until the grains were completely covered by mycelium (Fig. 2C).

Substrate preparation and *T. versicolor* cultivation

Agro-industrial waste and enhanced substrates were created in ten (10) distinct formulations (S1–S10). These were made using agro-industrial by-products that included, filter

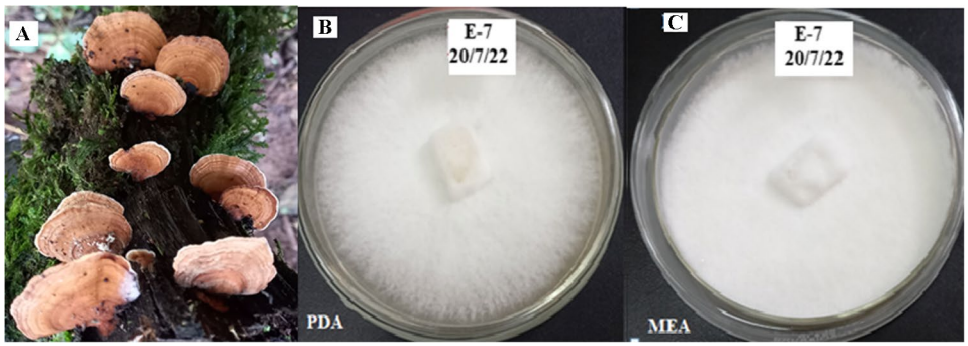

Fig. 1 Fruiting bodies of *T. versicolor* on natural logs (**A**) and mycelium characteristics on artificial growth media (PDA, (**B**), and MEA, (**C**) completed the Petri dish within 10 and 8 days, respectively

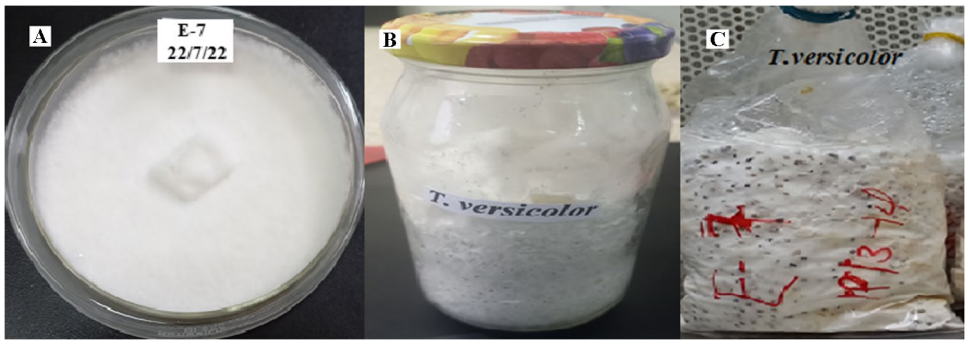

Fig. 2 Eight-day-old colony of *T. versicolor* on malt extract agar (**A**), 10-day-old mother spawn (**B**), and 9-day-old commercial spawn (**C**) for cultivation of *T. versicolor* mushroom

cake, trash, and bagasse from sugarcane collected from the Ethiopian Sugar Industry Group (Wonji Shoa Sugar Factory), Ethiopia; other materials came from Bishoftu, Oromia, Ethiopia, including cotton seed hulls, cow dung, chicken manures, and horse manures. The sun-dried sugarcane bagasse and supplements were chopped into smaller pieces of a specific length following the method of Gaitán-Hernández et al. (2011), weighed separately, and thoroughly mixed manually according to their proportions indicated in Table 1. Each substrate was soaked in water for 24 h and then drained to reduce the moisture content to 60–65%. To each substrate, 1% calcium carbonate and 1% gypsum (on a dry weight basis) were added to adjust the pH at 5.8 and prevent clumping of the substrate, respectively.

Substrates of 500 g (dry weight) were filled into polypropylene bags (20 × 35 cm) and sterilized at 121°C for 80 min. After sterilization, substrates were inoculated with 3% fresh *T. versicolor* spawn, equivalent to 15 g for 500 g of substrates, in a laminar flow chamber and sealed before being transferred to a dark incubation room for the duration of the spawn running stage with a temperature of 25°C and a relative humidity of 85 ± 5% for 15–23 days. After the surfaces of the substrates were entirely covered with mycelium, surface mycelial density and mycelial growth rate (mm/day) were evaluated according to Yang et al. (2013). The spawned mushroom bags were moved to a cropping room to facilitate pinhead formation and fructification. The substrate bags were subsequently placed at a temperature of 10–24°C, and relative humidity increased to 95–100% in the cropping room with sufficient light for 8 h daily until the pinheads began to appear. The mushroom cropping room and shelf arrangements were designed as Beje et al. (2013).

During harvesting, mature fruiting bodies were picked by a clean hand without harming the substrate. This process was repeated for three subsequent flushes. According to the method of White et al. (1990), the yield parameters were recorded with respect to the time taken for the completion of spawn running, the first appearance of pinhead formation, and the maturity of fruiting bodies. Similarly, the number of flushes and the yield of flushes from the respective treatment substrates (i.e., the total wet weight of all the fruiting bodies harvested from all three pickings) were measured and considered as the total yield of mushrooms. Pileus diameter was also measured, and the average biological efficiency (BE) of the harvests was computed (Altschul et al. 1990). The total yield (i.e., weight of fruiting body per plastic bag) of the mushrooms was weighed using a scale (g) and calculated.

Analysis of substrates chemical composition

Before inoculation, samples of each of the seven substrates were oven-dried at 60°C for 48 h and then milled to pass through a sieve with a 1-mm mesh. Moisture and total ash content were determined using the Ethiopian Standard Method ES1032-1:2005. The lignocellulosic content, that is, the alcohol-toluene solubility, the lignin content, and the cellulose and hemicellulose contents of the substrates were measured using the standard method of the American Society for Testing and Materials D1107-56, direct extraction with aqueous alkali, and the Kurchner-Hoffer methods, respectively. The total crude fiber was determined using BCTL/SOP/M017.01 in the Agricultural Food Product Analysis Manual, which is based on the International Organization for Standardization's ISO 5498:1981 agricultural food products general method for the determination of crude fiber general method.

The Soxhlet extraction technique was used to determine the crude fat content of substrates (Srigley and Mossoba 2016). The total crude protein and N contents were evaluated by the Kjeldahl method according to the method of ES (1032–1:2005) adopted from the USDA (2009) national nutrient database with appropriate nitrogen conversion factor (Drenovsky et al. 2004). The C content was calculated by determining the fixed C content, volatile matter content, and ash content of the biomass, as described by Dai et al. (2019). The C:N ratio

Table 1 Substrate formulations used for the cultivation of *T. versicolor*

Substrates	Formulation (100%)
S1	80% Sugar cane bagasse + 12% chicken manure + 8% cotton seed hull
S2	80% Sugar cane bagasse + 12% chicken manure + 8% cow dung
S3	80% Sugar cane bagasse + 12% cow dung + 8% chicken manure
S4	80% Sugarcane bagasse + 6.67% chicken manure + 6.67% cow dung + 6.67% horse manure
S5	80% Sugarcane bagasse + 12% horse manure + 8% chicken manure
S6	50% Sugarcane bagasse + 15% sugarcane trash + 15 sugarcane filter cake + 20% chicken manure
S7	100% Sugarcane bagasse alone
S8	80% Sugarcane bagasse + 20% cotton seed hull
S9	50% Sugarcane bagasse + 25% sugarcane trash + 25% sugarcane filter cake
S10	30% Sugarcane bagasse + 25% sugarcane trash + 25% sugarcane filter cake + 20% chicken manure

was calculated as C/N. Macro- (K, Ca, Mg, and Na) and micro- (Fe and Zn) concentrations were quantified by performing microwave plasma atomic emission spectroscopy.

Nutritional analysis of *T. versicolor* mushrooms

The dried fruiting bodies were milled to a fine powder by a laboratory mill (RRH-500A, high-speed multi-function comminutor (Zhejiang, China)) with a screen that was given a particle size of < 1 mm. Total ash and moisture contents by mass were analyzed following the method of Kimura (1980). Total crude protein, crude fiber, and crude fats were determined using an Agilent 4200 Series MP-AES Inductively Coupled Plasma spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) (Ethiopian Conformity Assessment Enterprise, 2022). The content of the available carbohydrate was determined using the equation of Alam et al. (2008).

Statistical analysis

Substrates had ten treatments (S1–10). They were compared based on their chemical composition and mineral content with three replications. Based on spawning time, time to pinhead formation, first harvest, yield, and biological efficiency were assessed. Data analyses were performed using Statistical Package for Social Sciences (SPSS) version 28 (Gao et al. 2020). Data were log-transformed when needed to achieve the parametric criteria of normality and homoscedasticity necessary for the analysis of variance. Differences between substrate options for the different variables were evaluated using a one-way analysis of variance. Duncan's Multiple Range Test was used to determine significant differences ($p \leq 0.05$) between substrates when needed.

Results

Mycelium characteristics of *T. versicolor* mushroom

The impact of growth media on mycelial growth, hyphae density, and complete colonization of the Petri dish showed significant differences ($p < 0.05$; see Fig. 1B, C and Fig. 3). The *T. versicolor* mycelium showed white pigmentation, smooth texture, abundant aerial hyphae, and high density in both growth media (Fig. 1), indicating that both MEA and PDA media were conducive for enhanced mycelial development. However, mycelial growth and strain performance were faster on MEA compared to PDA (Fig. 3). In both growth media, MEA and PDA, the mushroom exhibited fast growth and completely colonized the Petri dish in 8 and 10 days, respectively. Consequently, the strain's growth performance on MEA and PDA showed statistically significant differences ($p < 0.05$) at days 2, 4, 6, and 8 (Fig. 3). Notably, after 8 days of incubation, MEA and PDA displayed the highest growth diameter (mm/day) at 89.33 and 78.13, respectively. However, a mycelial growth diameter of 89.33 mm/day was observed on PDA after 10 days (Fig. 2). Comparatively weak mycelial growth was noted on PDA, indicating a more favorable growth environment on MEA with reduced medium colonization within 8 days at 25°C. Consequently, MEA medium emerged as an efficient and suitable growth medium for the rapid mycelial extension of *T. versicolor* mushrooms.

Biochemical composition of the substrates

Substrates differed significantly in terms of their C, N, and C:N, cellulose, hemicellulose, and lignin contents ($p < 0.05$, Table 2). Hence, S8 had the highest C content, followed by S7 and S2, whereas S4 and S5 had the lowest C content (Table 2). Substrates S7 (100% sugarcane

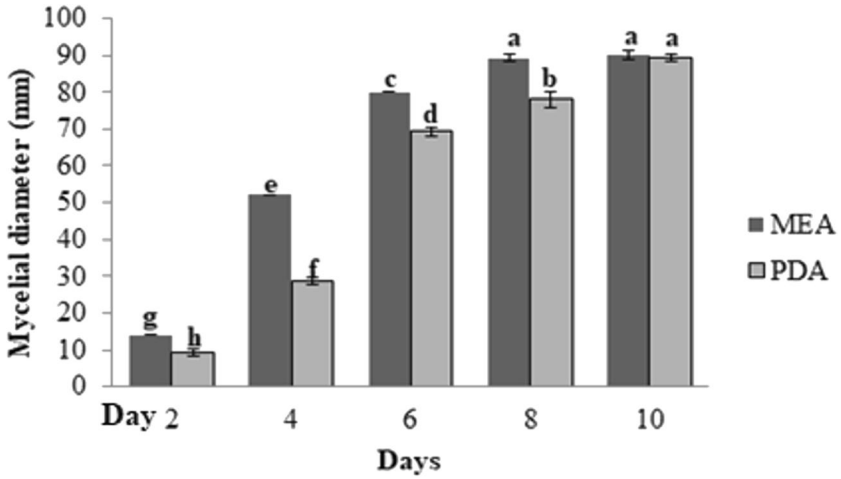

Fig. 3 The effects of growth media on the mycelial growth diameter of the *T. versicolor* strain; different letters denote statistically significant differences at $p < 0.05$

Table 2 Biochemical compositions of the substrates

Substrates	Ratio of carbon to nitrogen			Lignocellulosic contents (wt. %)		
	C	N	C:N ratio	Cellulose	Hemicellulose	Lignin
S1	40.96 ± 6.86^{e}	2.08 ± 0.36^{a}	19.69 ± 1.79^{f}	26.20 ± 0.14^{i}	19.86 ± 0.03^{c}	22.32 ± 0.29^{b}
S2	46.52 ± 3.65^{b}	1.65 ± 0.50^{d}	28.19 ± 6.64^{e}	31.29 ± 0.26^{g}	26.06 ± 0.34^{a}	19.05 ± 0.48^{cd}
S3	40.37 ± 2.36^{e}	1.89 ± 0.10^{c}	21.35 ± 0.64^{f}	32.56 ± 0.38^{f}	18.03 ± 0.50^{e}	18.00 ± 0.33^{e}
S4	38.64 ± 5.63^{g}	1.96 ± 0.26^{b}	19.71 ± 5.47^{f}	29.29 ± 0.34^{h}	15.93 ± 0.26^{f}	22.96 ± 0.11^{a}
S5	39.63 ± 5.56^{f}	1.27 ± 0.05^{f}	31.20 ± 3.76^{d}	35.59 ± 0.36^{d}	13.26 ± 0.02^{g}	21.21 ± 0.21^{c}
S6	42.12 ± 1.99^{d}	1.56 ± 0.10^{e}	26.92 ± 2.17^{e}	25.56 ± 0.45^{j}	22.57 ± 0.42^{c}	17.99 ± 0.12^{f}
S7	46.09 ± 0.10^{b}	0.53 ± 0.20^{h}	86.96 ± 3.27^{a}	40.12 ± 0.10^{b}	17.83 ± 0.15^{e}	16.43 ± 0.02^{g}
S8	49.92 ± 0.60^{a}	1.24 ± 0.10^{f}	40.25 ± 3.23^{c}	37.31 ± 0.27^{c}	22.54 ± 0.02^{b}	21.67 ± 0.01^{c}
S9	43.16 ± 0.20^{c}	0.89 ± 0.10^{g}	48.49 ± 5.35^{b}	41.55 ± 0.12^{a}	15.33 ± 0.05^{f}	18.36 ± 0.13^{d}
S10	42.95 ± 3.42^{d}	1.46 ± 4.72^{e}	29.41 ± 1.97^{e}	34.06 ± 0.55^{e}	$21.97 \pm 0.49d^{cd}$	17.99 ± 0.01^{f}

Values are expressed as means ± standard deviation. Values in the same column with different letters as superscripts are significantly different by Duncan's multiple range tests ($p < 0.05$). For substrate (S1–S10) proportions, see Table 1

bagasse) and S9 had significantly lower N content (0.53% and 0.89) than the other substrates ($p < 0.05$). Accordingly, the N content of S1 (2.04) was significantly higher than that of the other substrates ($p < 0.05$; Table 2). The C:N ratio of the different substrates ranged from 19.69 (S1) to 86.96 (S7) (Table 2). The results of this study revealed that the N content increased gradually with the addition of potential animal manures to the designed substrate formulas (Table 1).

In addition, significant ($p < 0.05$) differences were found between different substrates in terms of their cellulose, hemicellulose, and lignin contents (Table 2). S9 had the highest cellulose content (41.55%), but S6 had the lowest content (25.56%) (Table 2). Similarly, S2 had the highest hemicellulose content (26.59%), but S5 had the lowest hemicellulose content. On the other hand, S4 had the highest lignin content (22.96%), followed by S1 and S7 (Table 2).

Substrate mineral content

Mineral elements are significantly varied among substrates (Table 3; $p < 0.05$). Na and Mg contents were significantly higher ($p < 0.05$) in S3 than in the other substrates, whereas Zn and Fe contents were significantly higher in S4 and S6, respectively. Ca and K values were significantly higher in S5 and S9 ($p < 0.05$; Table 3). The contents of Zn, Mg, K, and Ca were significantly lower in S7 than in the other substrates ($p < 0.05$; Table 3).

Table 3 Mineral content (mg kg^{-1}) of substrates used for *T. versicolor* mushroom cultivation

Substrate	Elements					
	Zn	Fe	Na	Mg	K	Ca
S1	43.52 ± 0.06^{e}	2431.20 ± 0.10^{g}	1630.13 ± 0.14^{d}	1452.50 ± 0.50^{g}	$11,172.00 \pm 0.13^{c}$	5103.30 ± 0.18^{f}
S2	57.33 ± 0.05^{c}	1134.50 ± 0.20^{i}	2150.43 ± 0.26^{a}	3529.10 ± 0.10^{b}	$11,158.66 \pm 0.15^{d}$	$11,215.12 \pm 0.33^{b}$
S3	58.10 ± 0.10^{b}	2820.23 ± 0.40^{f}	2150.45 ± 0.12^{a}	3529.16 ± 0.12^{a}	$11,158.66 \pm 0.17^{d}$	$11,215.13 \pm 0.35^{b}$
S4	67.20 ± 0.05^{a}	2870.15 ± 0.23^{e}	1987.50 ± 0.23^{c}	3331.87 ± 0.17^{c}	$11,576.50 \pm 0.44^{b}$	$10,266.25 \pm 0.12^{c}$
S5	47.23 ± 0.05^{d}	3400.19 ± 0.12^{c}	1996.66 ± 0.13^{b}	2819.16 ± 0.10^{d}	$11,882.20 \pm 0.45^{a}$	4630.19 ± 0.45^{h}
S6	41.63 ± 0.30^{g}	3683.75 ± 0.34^{a}	1524.16 ± 0.16^{e}	2226.37 ± 0.04^{f}	4865.25 ± 0.14^{h}	9856.33 ± 0.14^{d}
S7	8.20 ± 0.10^{j}	2010.14 ± 0.25^{h}	1160.20 ± 0.16^{f}	627.50 ± 0.16^{j}	3591.14 ± 0.67^{i}	1040.44 ± 0.08^{i}
S8	12.80 ± 0.20^{i}	3590.21 ± 0.50^{b}	780.12 ± 0.12^{h}	1120.34 ± 0.90^{i}	$10,700.24 \pm 0.11^{e}$	4940.17 ± 0.14^{g}
S9	20.80 ± 0.02^{h}	3188.30 ± 0.12^{d}	922.66 ± 0.15^{g}	1271.80 ± 0.32^{h}	6487.00 ± 0.15^{g}	$11,331.18 \pm 0.45^{a}$
S10	41.90 ± 0.30^{f}	3683.75 ± 0.20^{a}	1524.50 ± 0.03^{e}	2226.37 ± 0.13^{e}	9671.50 ± 0.18^{f}	8498.75 ± 0.56^{e}

Values are expressed as means ± standard deviation. Values with different small letters in the same column are significantly different at $p < 0.05$. For respective substrate (S1–S10) proportions, see Table 1

Table 4 Effect of substrate formulas on morphological parameters of *T. versicolor* mushroom fruiting bodies

Substrates	Mycelial growth rate (mm/days)	Surface mycelial density	Spawn run (days)	Pinhead formation (days)	Fructification (days)	First harvest (day)	Cap diameter (cm)
S1	15.60 ± 0.85^{dc}	+ + +	19.33 ± 0.58^{b}	8.67 ± 1.00^{b}	8.21 ± 1.80^{d}	21.33 ± 0.57^{bc}	5.00 ± 0.50^{b}
S2	17.30 ± 0.30^{b}	+ + +	17.33 ± 0.52^{c}	7.67 ± 0.57^{bc}	7.33 ± 0.57^{e}	21.00 ± 1.16^{b}	5.71 ± 0.76^{a}
S3	17.40 ± 0.26^{b}	+ + +	17.33 ± 0.56^{c}	6.67 ± 1.16^{a}	6.67 ± 0.57^{f}	22.00 ± 1.15^{bc}	4.60 ± 0.36^{b}
S4	14.53 ± 0.25^{bc}	+ + +	21.33 ± 0.15^{a}	8.06 ± 1.21^{bc}	9.00 ± 1.43^{d}	24.00 ± 1.31^{b}	3.66 ± 0.28^{de}
S5	18.90 ± 0.36^{a}	+ + +	15.67 ± 0.58^{d}	5.35 ± 1.13^{f}	7.33 ± 0.58^{e}	19.00 ± 1.21^{f}	3.33 ± 0.17^{e}
S6	16.16 ± 0.38^{e}	+ + +	18.67 ± 0.53^{b}	9.12 ± 1.12^{cd}	8.67 ± 1.53^{de}	22.00 ± 1.37^{bc}	3.66 ± 0.28^{de}
S7	12.76 ± 0.25^{g}	+ +	23.33 ± 1.53^{a}	13.33 ± 1.53^{a}	12.00 ± 1.34^{a}	34.33 ± 1.52^{a}	5.76 ± 0.05^{a}
S8	14.16 ± 0.65^{f}	+ +	21.00 ± 1.20^{a}	11.21 ± 1.00^{e}	11.00 ± 1.45^{b}	23.33 ± 1.15^{c}	4.10 ± 1.44^{d}
S9	15.06 ± 0.60^{de}	+ +	19.33 ± 0.57^{c}	10.11 ± 1.00^{de}	10.00 ± 1.73^{c}	23.23 ± 1.00^{c}	4.66 ± 1.28^{b}
S10	16.36 ± 0.49^{c}	+ + +	19.13 ± 1.0^{bc}	$8.00 \pm 1.00b^{c}$	8.33 ± 1.87^{d}	21.00 ± 1.50^{b}	3.66 ± 0.05^{de}

Degree of mycelial density when the mycelia fully colonizes the substrate: + + mycelium grew throughout the whole bag but was not uniformly white, + + + mycelium grew throughout the whole bag and was uniformly white. Values are expressed as means ± standard deviation. Values with different small letters in the same column are significantly different at ($p < 0.05$). For respective substrate (S1–S10) proportions, see Table 1

Mycelial growth rate and spawn run time

The combinations of different ingredients with sugarcane bagasse had significant differences ($p < 0.05$; Table 4) in the mycelial growth rate, surface mycelial density, and spawn run time. The mycelial growth rate (mm/day) of *T. versicolor* mushroom was significantly faster on the S5 (18.90 mm/day; Table 4) than that grown on the S7 (12.76 mm/day), which is the control substrate among other substrates. Moreover, the mycelial growth rate ranged from 12.76 to 18.90 mm/day, including 100% sugarcane bagasse. Treatments S7, S8, and S9 showed poor mycelial density, while S1 to S6 showed excellent mycelial growth throughout the whole bags with a uniform and thick white mycelial appearance (Fig. 4A). Spawn run time ranged from 15.67 (S5) to 23.33 days (S7; Table 4). Hence, S5 had the shortest spawn run time (15.67 days) to fully colonize the substrate bags.

Pinhead formation, fructification, and first harvest time

The shortest pinhead formation was obtained from S5 (5.35 days), while the longest one was obtained from S7 (13.33 days), followed by S8, S9, and S6 (Table 4). However, the number of days required for pinhead formation on substrates S1, S2, S4, and S10 did not vary significantly ($p > 0.05$; Table 4). In addition to pinhead formation, uniform distributions and localization of pinheads on the growth substrate were observed (Fig. 4B). This study verifies that optimum yield and quality of fruiting bodies could be obtained when mushrooms grew on a substrate containing sugarcane bagasse with chicken manure, cow dung, and horse manure. The fruiting bodies of *T. versicolor* were produced on all substrates formulated (Table 1). Fructification differed significantly between substrates ($p < 0.05$; Table 4).

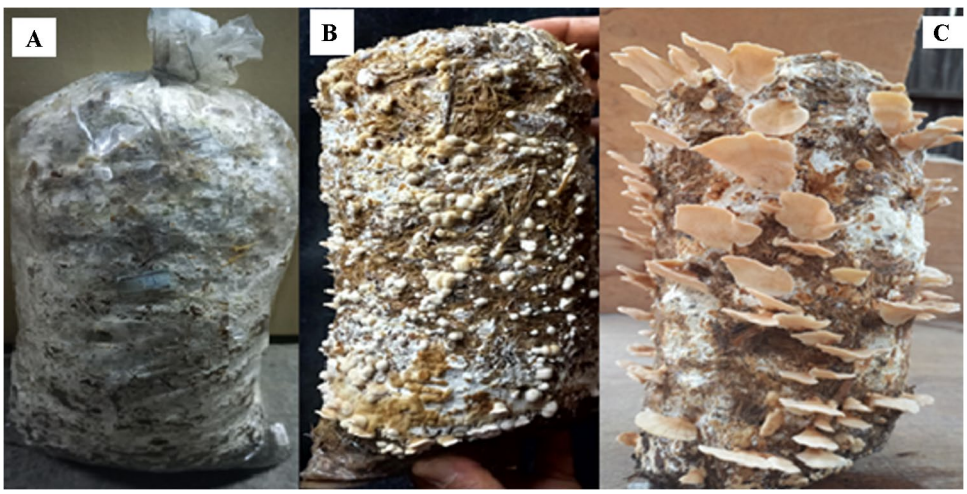

Fig. 4 Spawn running (**A**), pinhead formation and appearance of fruiting bodies (**B**), maturation of the fruiting body (**C**) of *T. versicolor* mushroom

Early fructification was observed on S3 (6.67 days), while the longest time was on S7 (12 days). However, the days to fructification on S3 did not differ significantly from those on S2 and S5 ($p > 0.05$; Table 4). On the other hand, S7 and S8 recorded the longest fructification days compared to the other substrates (Table 4). The number of days to first harvest differed significantly among the substrates ($p < 0.05$; Table 4). The shortest first harvesting time occurred after 19 days on S5, while the longest time was obtained from S7 (34.33 days) and S4 (24 days), respectively (Table 4).

Cap diameter of *T. versicolor* mushroom

The type of artificial substrate used significantly affected *T. versicolor* mushroom cap diameter ($p < 0.05$; Table 4). The cap diameters of mushrooms developed on substrates S7 (5.76 cm), S2 (5.71 cm), and S1 (5 cm). However, the cap diameters of mushrooms that developed on substrates S4, S5, S6, and S10 were smaller than those that grew on other substrates.

Total yield and biological efficiency

T. versicolor mushroom yield and biological efficiency were significantly affected by substrate type and formulations ($p < 0.05$; Table 5). The highest yield (158.33 g/500 g of substrate) and biological efficiency (31.66%) were obtained from S5, but significantly lower yield (57.67 g/500 g) and biological efficiency (11.73%) were obtained from S7 compared to other substrates ($p < 0.05$).

Nutritional composition of *T. versicolor* mushroom

The crude protein content of *T. versicolor* mushrooms from different substrates varied significantly ($p < 0.05$; Table 6). Significantly higher crude protein content (14.65%) was

Table 5 *T. versicolor* mushroom yield (wet weight) and BE grown on different substrates (S1–S10)

Substrates	Number of flushing			Total yields (g/bag)	Biological efficiency (BE %)
	1st flush (g/bag)	2nd flush (g/bag)	3rd flush g/bag)		
S1	51.23 ± 1.40^{c}	61.16 ± 1.10^{a}	31.67 ± 0.58^{b}	143.33 ± 1.53^{b}	28.70 ± 0.44^{b}
S2	66.56 ± 1.30^{a}	54.60 ± 1.20^{b}	26.33 ± 0.58^{d}	146.33 ± 1.45^{b}	29.27 ± 0.64^{b}
S3	61.17 ± 1.00^{b}	47.23 ± 1.02^{c}	28.34 ± 1.06^{c}	136.44 ± 1.53^{c}	27.20 ± 1.53^{b}
S4	38.33 ± 0.58^{e}	34.67 ± 0.58^{e}	19.22 ± 1.13^{f}	92.56 ± 4.36^{e}	18.40 ± 0.58^{d}
S5	65.67 ± 0.58^{a}	54.33 ± 1.53^{b}	38.33 ± 0.58^{a}	158.33 ± 0.58^{a}	31.66 ± 0.50^{a}
S6	45.33 ± 0.58^{d}	39.33 ± 0.58^{d}	15.67 ± 0.58^{g}	100.33 ± 1.14^{d}	20.06 ± 0.58^{c}
S7	27.34 ± 0.56^{e}	28.67 ± 1.12^{g}	22.34 ± 1.07^{e}	57.67 ± 0.58^{h}	11.73 ± 1.33^{e}
S8	38.09 ± 1.01^{e}	18.23 ± 1.67^{h}	13.67 ± 0.15^{d}	88.70 ± 1.34^{f}	17.6 ± 0.11^{d}
S9	38.67 ± 1.12^{e}	35.67 ± 1.12^{e}	15.08 ± 1.12^{g}	89.34 ± 1.65^{f}	17.86 ± 1.34^{d}
S10	46.56 ± 1.04^{d}	31.33 ± 0.58^{f}	14.67 ± 1.53^{g}	92.00 ± 3.79^{e}	18.40 ± 0.64^{b}

Values are expressed as means ± standard deviation. Values with different small letters in the same column are significantly different at $p < 0.05$. For respective substrate (S1–S10) proportions, see Table 1

Table 6 Nutritional composition of *T. versicolor* mushroom

Substrates	Nutritional composition of *T. versicolor (%)*					
	Total ash content	Moisture content	Crude protein	Crude fiber	Crude fat	Carbohydrate
S1	5.72 ± 0.01^{a}	11.64 ± 0.41^{b}	14.27 ± 0.06^{a}	17.66 ± 1.08^{a}	0.51 ± 0.01^{ab}	50.20 ± 0.57^{h}
S2	6.45 ± 0.00^{b}	11.47 ± 1.06^{c}	11.54 ± 0.13^{de}	15.93 ± 0.75^{bc}	0.50 ± 0.01^{abc}	54.11 ± 0.01^{f}
S3	5.64 ± 0.12^{a}	11.55 ± 0.58^{b}c	12.58 ± 0.02^{bc}	12.89 ± 0.49^{e}	0.49 ± 0.01^{abc}	56.85 ± 0.57^{e}
S4	5.61 ± 0.51^{a}	10.41 ± 0.12^{d}	13.22 ± 1.23^{b}	17.11 ± 0.57^{ab}	0.49 ± 0.01^{abc}	53.16 ± 0.00^{g}
S5	5.72 ± 0.31^{a}	12.34 ± 1.46^{a}	14.65 ± 1.16^{a}	18.38 ± 0.01^{a}	0.53 ± 0.01^{a}	48.38 ± 0.00^{i}
S6	4.14 ± 0.31^{d}	9.52 ± 0.01^{e}	11.67 ± 0.29^{cd}	14.11 ± 0.67^{de}	0.47 ± 0.01^{bcd}	60.09 ± 0.01^{d}
S7	5.20 ± 0.71^{b}	6.74 ± 0.53^{a}	7.54 ± 0.48^{f}	13.63 ± 0.99^{de}	0.47 ± 0.04^{bcd}	66.42 ± 0.01^{a}
S8	3.56 ± 0.49^{a}	6.84 ± 0.71^{a}	7.46 ± 0.50^{f}	14.93 ± 0.39^{cd}	0.46 ± 0.02^{cde}	66.75 ± 0.01^{b}
S9	4.12 ± 0.02^{d}	8.56 ± 0.01^{f}	$10.70 \pm 0.22b^{c}$	14.78 ± 1.36^{cd}	0.44 ± 0.03^{de}	61.40 ± 0.01^{c}
S10	4.76 ± 0.06^{c}	11.77 ± 0.07^{b}	11.11 ± 0.58^{de}	17.15 ± 1.54^{de}	0.42 ± 0.03^{e}	54.79 ± 0.01^{e}

Values are expressed as means ± standard deviation. Values with different small letters in the same column are significantly different at $p < 0.05$. For respective substrate (S1–S10) proportions, see Table 1

obtained from S5, followed by S1 (14.27%) ($p < 0.05$), but significantly lower content (7.46%) was recorded from S7 compared to other substrates ($p < 0.05$; Table 6). The crude fiber analysis revealed that there were significant ($p < 0.05$; Table 6) differences among substrates. High (18.38%) and low (12.89%) crude fiber contents were recorded from the mushrooms grown on S5 and S2, respectively. The crude fat contents of the study ranged from 0.42 to 0.52% and significantly varied ($p < 0.05$; Table 6) in different substrates. Furthermore, the carbohydrate content of *T. versicolor* mushrooms varied significantly based on the growth substrates used ($p < 0.05$; Table 6). Notably, S8 exhibited the highest carbohydrate content (66.75%), while S5 showed significantly lower carbohydrate content (48.38%) compared to other substrates ($p < 0.05$; Table 6).

Discussion

Mycelium characteristics of *T. versicolor* mushroom

The findings of this study indicate that wild mushroom *T. versicolor* strains show high adaptation and high growth performance on artificial media and laboratory culture conditions (Fig. 1). This shows substrate implications during domestication with increased mycelium growth and growth performance as compared to the natural environment (Jo et al. 2010). The study also indicated the suitable medium and optimal temperature for the best mycelium growth were MEA and 25°C; these influence successful mycelial growth and overall productivity (Bonatti et al. 2004; Kurd-Anjaraki et al. 2022). Therefore, domestication of *T. versicolor*, mycelial growth, fast colonization, and densities were high on MEA compared to PDA and showed high density with a white color during cultivation on these media (Figs. 1 and 2). This finding is similar to Veena and Pandey (2012), who also observed mycelial growth and strain performance demonstrated accelerated rates when cultivated on MEA. The influence of the media on mushrooms growth, MEA medium provides the maximum effect due to its rich complex composition and enriched with nutrients for the appropriate fungal growth (Krupodorova et al. 2021). In both media, the fungus exhibited vigorous growth and fully colonized the Petri dish in 8 days on MEA and 10 days on PDA, respectively. This colonization is by far different from Guerrero and Martínez (2011), who noted complete colonization of *T. versicolor* took 10 days on MEA. The results indicate that the MEA medium was an efficient and suitable medium for the fast mycelial growth of *T. versicolor*. Moreover, the selection of growth media could influence successful mycelial growth and the production of *T. versicolor* biomass (Borràs et al. 2008). Moreover, the observed phenomenon of slightly enhanced mycelial growth in a more natural environment (Jo et al. 2010), as the mushroom may encounter substrates and conditions more closely resembling their native habitats (Wu et al. 2019; Warnasuriya et al. 2023). This can include factors like nutrient availability, pH levels, temperature fluctuations, and the presence of other microorganisms (Wu et al. 2019). The faster colonization might be due to the growth media that we used could collectively provide a more conducive environment for the mycelium to grow and spread rapidly, leading to accelerated colonization of the substrate or growth medium (Philippoussis 2009). Comparatively weak mycelial growth was noted on PDA, indicating a more favorable growth environment on MEA with reduced colonization time within 8 days at 25°C. This factor significantly influences successful mycelial growth and the production of *T. versicolor* biomass and rapid mycelial extension (Borràs et al. 2008).

Biochemical composition of the growth substrates

Nutrient inconsistency in nature might affect the growth and productivity of mushrooms. Therefore, cellulose, hemicellulose, and lignin are the major carbohydrates used as a carbon source that is accessible for mushrooms from growth substrates. Similarly, the C:N ratio also affects the qualitative and quantitative yield of mushrooms (Harith et al. 2014). Pavlík and Pavlík (2013) have confirmed that these elements are naturally present in all raw materials but vary among the individual and formulated substrates (Dhakar and Pandey 2013). The results of this study revealed that the N content increased gradually with the addition of potential nitrogen sources such as animal manures to the designed substrate formulas (Table 1). This suggests that the higher N content of the substrates was due to the nitrogen-rich supplements incorporated into the growth medium. Hence, nitrogen is an essential nutrient for mushrooms growth and plays a crucial role in various metabolic processes (Carrasco et al. 2018). Accordingly, the N content of S1 (2.04) was significantly higher than that of the other substrates (Table 2) which might be associated with the supplements added to the substrate, such as animal manures, which are known to contain significant sources of nitrogen. These organic materials undergo decomposition and release nitrogen in the form of ammonium compounds, which are better absorbed by the mushrooms determining the growth of mycelium and stimulate fructification (Mleczek et al. 2021). This in general indicates that the presence of nitrogen-rich supplements enhances the availability of nitrogen in the growth medium, and can increase the fructification and productivity of *T. versicolor* mycelium. The optimum nitrogen content facilitates robust mycelial growth and colonization, ultimately contributing to the successful cultivation of the mushrooms (Mleczek et al. 2021).

The C:N ratio is a useful parameter that influences fungal growth and decomposition processes in substrates (Bich Thuy Thi et al. 2021). In our study, the C:N ratio of the different substrates was varied (Table 2). This wide range indicates variations in the availability of carbon and nitrogen in the substrates (Cunha et al. 2011; Ramezan et al. 2021), which could impact the growth and development of *T. versicolor* (Jo et al. 2010), as the ideal C:N ratio of 31:1 has been found to promote optimal mycelial growth yield and protein content of *T. versicolor* mushrooms (Hoa et al. 2015; Royse et al. 2017). The results suggest that the addition of potential nitrogen manures (Table 1) in the substrate formulations contributed to the gradual increase in nitrogen content with a substantial impact on mushroom yield and quality (Carrasco et al. 2018). Animal and poultry manures are known to be rich sources of nitrogen, as they contain organic compounds such as proteins that decompose into nitrogenous compounds during microbial growth, with a substantial impact on mushroom yield and quality (Noble et al. 2024). Therefore, nitrogen supply for mushroom production can be optimized by adding animal and poultry manures to the sugarcane bagasse substrates. Creating the correct balance of carbon (C) and nitrogen (N) content to produce mushroom substrate is critically important to achieving maximum mushroom yields. In other cases, the nitrogen content increased, resulting in lower C:N ratios (Bonatti et al. 2004). The occurrence of a lower C:N ratio in the cultivation substrate is more favorable for mushroom colonization and fruit body development (Royse 2002; Bellettini et al. 2019). A mixture of agro-industrial and animal byproducts can be remarkable. According to Owaid et al. (2015), productivity and biological efficiency were increased in some mixtures when compared with substrates alone, because of a variation in the capability of such substrates to aid the nutritional and environmental requirements and difference in cellulose, hemicellulose and lignin contents (Bellettini et al. 2019). Overall, the findings highlight the importance of considering the C:N ratio, nitrogen, and carbon contents in substrate formulations for optimizing substrate for *T. versicolor* cultivation.

These variations in cellulose, hemicellulose, and lignin contents among the substrates highlight the diverse compositions of the materials used in the formulation (Kumla et al. 2020). Such differences can influence substrate properties, including nutrient availability and decomposition rates, which, in turn, may impact fungal growth and biomass production during *T. versicolor* cultivation. Therefore, understanding the composition of substrates is crucial for optimizing nutrients and environmental conditions and enhancing mushroom cultivation efficiency. Hence, during the rapid growth phase of mushrooms, hemicellulose primarily serves as a substrate for fungal hyphae during early colonization, before cellulose and lignin breakdown occurs (Locci et al. 2008). This system facilitates lignin degradation into simpler compounds, serving as a carbon source absorbed by the mushrooms for their development. Moreover, substrates S1 and S2 exhibited high cellulose and hemicellulose levels, with sugarcane bagasse alone being rich in carbohydrates, thus supporting fruiting body formation as a primary carbon source and playing a crucial role in mycelial growth and fruiting body development (Sardar et al. 2017). Therefore, considering its economic feasibility, sugarcane bagasse can be used as an available carbon source for promoting radial mycelial growth and should be prioritized as a key component of substrate for *T. versicolor* cultivation. Moreover, the use of new combinations may promote increased productivity and biological efficiency of the mushroom. The ratio has a critical influence on mycelium growth, mushroom weight, yields, and protein content in the fruiting body of mushrooms (Kumla et al. 2020).

Substrate mineral content

Optimum mineral elements in mushroom substrates had a significant effect on the mycelial growth and total yields of mushrooms (Hoa et al. 2015). These mineral elements can support the growth of the mushroom since essential elements as potassium play an important role in the synthesis of amino acids and proteins (Jo et al. 2010). K is crucial for regulating water uptake by enhancing tolerance to environmental stress, and improving yield, while Mg and Ca are necessary for the uptake and transportation of other nutrients such as N (Bich Thuy Thi et al. 2021). Insufficient levels of these minerals in the substrate can delay the absorption of necessary nutrients that leads to poor growth and fruiting bodies of mushrooms (Siwulski et al. 2019). Ca can help buffer the substrate to prevent the pH from becoming too acidic, while Mg can prevent the pH from becoming too alkaline (Khalaphallah et al. 2020). Overall, this study highlights the importance of selecting a substrate with the correct balance of macro-elements, and lignocellulolytic materials to ensure the healthy growth and development of *T. versicolor* mushrooms during trials for domestication.

Mycelial growth rate and spawn run time

The combinations of different ingredients with sugarcane bagasse significantly impact on the mycelial growth rate, surface mycelial density, and spawn run time (Table 4). The inclusion of chicken and horse manure with sugarcane bagasse in S5 likely led to the fastest colonization due to factors such as both chicken and horse manure are rich sources of nutrients, including nitrogen, phosphorus, and potassium, which are essential for fungal growth and development (Sivagurunathan and Sivasankari 2015). These nutrients provide an optimal environment for mycelial expansion,

colonization of the substrate, and reducing spawn run time (Yang et al. 2013). Also, the organic matter present in the manure helps to improve the overall structure, and texture of the substrate, creating a favorable habitat for fungal hyphae to proliferate (Hyde et al. 2019).

Despite variations in substrate formulation, mycelial growth rates varied from 12.76 to 18.90 mm/day, even with 100% sugarcane bagasse. S1 to S6 treatments showed robust mycelial growth with a uniform and thick white appearance, contrasting with poorer density in S7, S8, and S9 treatments (Fig. 4a). This result could be attributed to differences in substrate composition and nutrient availability among treatments. Substrates with higher quality ingredients or better nutrient balance may have provided more favorable conditions for mycelial growth, resulting in higher density and uniformity (Bich Thuy Thi et al. 2021). Spawn run time varied in all treatments (Table 4). Hence, S5 had the shortest spawn run time for complete substrate bag colonization, and substrates S7, S8, and S4 showed longer spawn run times (Table 4). This exceeds other reports for wild *T. versicolor* strains cultivated on sawdust with additional supplements (Bich Thuy Thi et al. 2021). Moreover, the addition of poultry and horse manures significantly decreased spawn run time compared to using sugarcane bagasse as the sole substrate (Table 4). However, the exceptionally short spawn run time observed in S5 resulted in reduced fungal development and an increased risk of contamination in the substrates (Atila et al. 2017). This emphasizes the importance of striking a balance to minimize time loss, contamination risk, and delay in reaching harvest maturity (Atila et al. 2017).

Pinhead formation, fructification, and first harvest time

In this study, the shortest duration for spawn run, pinhead formation, and mycelial growth rate were observed compared to the findings of Ramezan et al. (2021), who reported 40 and 47 days for pinhead formation on sugarcane bagasse-supplemented and unsupplemented substrates, respectively. The optimal substrate, S5 (Table 4), which facilitated the shortest pinhead formation, provided optimal conditions for mushroom development, including adequate moisture levels and nutrient availability (Hoa et al. 2015). The intervals between spawn run, pinhead development, mycelial growth rate, fructification, and first harvest time varied among the formulated substrates (Table 4). This is supported by the study of Hoa et al. (2015), who found that the first fructification and harvesting time depend on the ingredients used in the substrate formulated. In addition, this time frame is particularly shorter compared to the findings of Ramezan et al. (2021), who reported 40 and 47 days for pinhead formation on sugarcane bagasse supplemented and unsupplemented substrates, respectively. This result can be attributed to the varying nutrient compositions and physical characteristics of the substrates used in the experiment (Muswati et al. 2021). Substrate S5, which facilitated the shortest pinhead formation, provided optimal conditions for mushroom development, including adequate moisture levels, and nutrient availability (Hoa et al. 2015). Conversely, substrates such as S7 may have exhibited optimal conditions, resulting in delayed pinhead formation. The lack of significant differences in pinhead formation time among substrates suggests that these substrates may have had similar nutrient profiles or physical properties conducive to pinhead initiation (Onyeka and Okehie 2018). Additionally, variations in the spawn run time, pinhead development, and mycelial growth rate across substrates highlight the intricate relationship between substrate composition and mushroom growth dynamics. Compared to the longer pinhead formation times reported in previous studies, the shorter timeframe observed in this experiment underscores the efficacy of the substrates utilized in promoting efficient mushroom development (Adedokun and George-David 2016).

Furthermore, alongside pinhead formation, we observed uniform distributions and clustering of pinheads on the growth substrate (Fig. 4B). Desisa et al. (2023) confirm that optimal yield and quality of fruiting bodies are attainable when mushrooms are cultivated on a substrate comprising sugarcane bagasse along with animal manures in our study, the fruiting bodies of *T. versicolor* were successfully produced on all ten formulated substrates (Table 1). However, fructification significantly varied among substrates (Table 4). This finding is consistent with Hoa et al. (2015), who noted that the timing of the first fruiting body of mushrooms is influenced by the substrate ingredients. The shortest duration until the first harvest was observed on S5 (19 days), whereas S7 and S4 exhibited the longest durations at 34.33 days and 24 days, respectively. These durations are notably shorter than those reported by Harith et al. (2014), which recorded a duration of 68.8 days. This discrepancy might be influenced by the carbon-to-nitrogen (C:N) ratio, cellulose, hemicellulose, and other crucial elements of the mushroom growth substrates (Kurd-Anjaraki et al. 2022). A balanced C:N ratio is vital for providing the necessary nutrients for fungal growth, metabolism, and fruiting body formation (Kumla et al. 2020). In the context of the study, variations in the C:N ratios of the substrates could explain the differences observed in the time to first harvest. Substrates with lower C:N ratios may have facilitated faster nutrient availability and mycelial growth, leading to earlier fruiting body formation and harvest (Desisa et al. 2023). Conversely, substrates with higher C:N ratios might have delayed nutrient availability and mycelial development, resulting in longer harvest times.

Total yield and biological efficiency

The yield and biological efficiency of *T. versicolor* mushrooms were significantly influenced by the type and formulation of the substrate (Table 5). Mainly, the S5 substrate is the most promising substrate for future commercial cultivation of this wild medicinal mushroom in Ethiopia. Notably, the yield and biological efficiency obtained from S5 were higher than the findings of Harith et al. (2014), who reported a mushroom yield of 27.7 g and a biological efficiency of 3.2% when *T. versicolor* was cultivated on oak sawdust. However, achieving such results not only relies on the nutritional balance of substrates but also on environmental factors like aeration, relative humidity throughout different growth stages, and substrate water retention, which highlights the potential for enhancing commercial cultivation practices (Vega et al. 2022).

The optimized substrates led to enhancements in the mycelial growth rate, early pinhead formation, and overall biological yield of the *T. versicolor* mushroom strain. In particular, S5 emerged as one of the most suitable growth substrates for cultivating the target mushroom. The incorporation of enrichment materials such as chicken manure, horse manure, and cow dung is known to elevate the nitrogen content of the growth medium, optimizing the C:N ratio (Yildiz et al. 2002). All substrates utilized for cultivating *T. versicolor* strains exhibited three flushes during the cultivation period (Table 5). However, in the second and third flushes, the harvested yields were lower compared to the first flush, with a gradual decrease observed in subsequent flushes, as previously observed by Hoa et al. (2015). This decline could be attributed to changes and reductions in available nutrients, such as hemicellulose in the medium, and the depletion of essential nutrients present in the substrates for subsequent flushes (Rizki and Tamai 2011).

Nutritional composition of *T. versicolor* mushroom

Proteins, crucial bioactive constituents found in mushrooms, are primarily influenced by the mushroom species (Łysakowska et al. 2023) and play vital roles in antioxidant, anticancer, antiviral, and antibacterial activities (Landi et al. 2022). Key bioactive fungal proteins, including lectins and enzymatic proteins like laccase, contribute to blood sugar regulation and immune modulation (Łysakowska et al. 2023). The crude protein content of *T. versicolor* mushrooms in this study was higher than the crude protein content of *T. versicolor* mushrooms reported by Kıvrak et al. (2020). This result suggests that the choice of substrate significantly impacts the crude protein content of *T. versicolor* mushrooms (Angelova et al. 2022). Moreover, substrates such as S5 and S1 demonstrate potential for enhancing the protein content of the mushrooms, highlighting the importance of substrate selection for enhanced nutritional quality and potentially influencing the therapeutic properties of the mushrooms (Benson et al. 2019). Conversely, the lower protein content observed in S7 indicates the need for careful substrate formulation to achieve desirable nutritional outcomes in mushroom cultivation. Such variations can be attributed to differences in the carbon and nitrogen richness of the original substrates used for cultivating the mushrooms (Yildiz et al. 2002; Rizki and Tamai 2011).

The analysis revealed that the highest crude fiber and carbohydrate content of *T. versicolor* mushrooms was obtained, suggesting the importance of substrate selection in optimizing the desired nutritional qualities of the mushrooms (Desisa et al. 2024). Specifically, variations in crude fiber, fat, and carbohydrate contents among different substrates highlight the importance of substrate selection in determining the nutritional quality of the mushrooms. Understanding these differences can aid in optimizing cultivation practices to produce mushrooms with desired nutritional profiles, potentially enhancing their suitability for dietary or medicinal purposes. Additionally, such insights can contribute to the development of tailored cultivation strategies aimed at maximizing the nutritional value of *T. versicolor* mushrooms for various applications. More interestingly, the parameters obtained in this study were higher than those reported in previous studies (Elkanah et al. 2022; Wang and Zhao (2023);, indicating that the chosen growth substrates or cultivation conditions have led to improved nutritional content in *T. versicolor* mushrooms. This suggests the potential for optimizing cultivation practices to further enhance the nutritional quality of the mushrooms, thereby increasing their value for various applications such as dietary supplementation or medicinal purposes (Bich Thuy Thi et al. 2021). Also, the results obtained in this study align with those of Wang and Zhao (2023), indicating that carbohydrates constitute the primary component of mushrooms, comprising approximately 60% of their dry weight. However, such finding underlines the importance of continued research and exploration in the field of mushroom cultivation to unlock their full potential as a source of valuable nutrients and bioactive compounds. The species had a high growth rate, intensely utilized a wide range of lignocellulosic substrates, and produced huge biomass. The selected low-cost locally available agro-industrial byproducts can reduce production costs, maximize utilization, and serve as solid waste management that contributes much to the development of the circular economy (Gaitán-Hernández et al. 2020). The good results obtained from the cultivation of wild-growing *T. versicolor* on a laboratory scale encourage large-scale production for commercialization and value addition.

Conclusion

The results of this study highlighted the significant potential of cultivating wild *T. versicolor* mushrooms using agro-industrial byproducts in Ethiopia. Through the successful cultivation of *T. versicolor* mushrooms on artificial media, these mushrooms demonstrate promise as genetic resources for the country, offering opportunities for sustainable commercial production. Furthermore, its high growth rate, efficient substrate utilization, and biomass production suggest domestication and producing high biomass for value addition, highlighting its role in reducing pollution. Leveraging locally available, low-cost substrates presents avenues for reducing production costs and contributing to solid waste management, while opportunities for large-scale commercialization align with Ethiopia's expanding sugar cane industry. Overall, these findings underscore the potential of *T. versicolor* cultivation for both medicinal and environmental purposes, pointing towards a promising avenue for further research and development in Ethiopia.

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Data availability Data are accessible and can be sent to the concerned entity upon request.

Declarations

Ethical approval and consent to participate By signing below, we, the undersigned, approve and agree to the release of identifying information, including the entirety of the study report to be published in the Journal. We commit to acting honestly, faithfully, and with integrity. We shall make commitments that we intend to uphold and take full ownership of our actions. No malicious injury to another person or animal will be done on purpose by us or anyone else who participates in our research paper.

Consent for publication We are aware that all Frontiers, Fungal Biotechnology journal might be accessible in print and online, as well as to a larger readership via marketing channels and other third parties. So, anyone can read everything that is published in the Journal. We are aware that readers can include not just academic scholars but also journalists and regular citizens. As a result, we accepted the conditions and indicated guidelines.

Conflict of interest The authors declare no competing interests.

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