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The Effect of Auxin and Oat in Combination with Casing Soil on Growth and Biochemical Components of *Agaricus blazei*

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ABSTRACT

Mushroom production using lignocellulosic waste is one of the most important recycling processes of agricultural and industrial residues, which, in addition to producing protein foods, will prevent environmental pollution. The present study was conducted in 2021 at the mushroom production center of Islamic Azad University, Isfahan (Khorasgan) Branch in a completely randomized design with 18 casing soil treatments (casing soil, SMC, vermi SMC, oat, vermi oat, and IAA with different compositions) in 3 replications. The qualitative and quantitative properties of *Agaricus blazei* mushroom were evaluated. The results indicated that the highest number of fruiting bodies was observed in casing soil treatment + SMC (50:50%) and the highest stipe was in casing soil treatments + oat (70:30%) and casing soil 100. The maximum diameter of the mushroom cap was obtained in casing soil treatments100 and SMC 100 + IAA, and the largest diameter of the mushroom stipe was obtained in casing soil treatment 100. In addition, casing soil treatment + vermi SMC (50:50%) showed the highest amount of nitrogen, protein and the minimum time to first pin-head formation. The highest amount of iron was observed in SMC treatment 100% and the highest amount of zinc was in casing soil treatment + oat (70:30%). Among the investigated treatments, an acceptable yield can be obtained from the substrate containing casing soil + SMC (50:50%), casing soil + oat vermicompost (70:30%), and casing soil + oat (70:30%) indicated promising results in most of the studied traits. SMC has been very effective in the growth of mushroom spawn and stimulation of the mycelium phase due to its rich mineral elements and nutrients required by mushroom spawn. On the other hand, since SMC is one of the important wastes of the mushroom production industry, which is produced in a large volume annually, can be used as a casing soil composition again, which is important in this sense.

***Keywords*:** Protein, Spent mushroom compost, Vermicompost, Casing soil, Time to first pin-head formation

INTRODUCTION

Optimal cultivation of edible-medicinal mushrooms requires special conditions and the use of appropriate inputs (Bellettini *et al*., 2019). One of the mushroom's cultivation industry problems, which accounts for about 30% of its raw material costs, is the preparation of suitable casing soil, which is extracted from non-renewable and valuable resources (Ashrafi *et al*., 2017). Most species of button mushrooms require a layer of soil before the production of fruiting bodies, called casing soil. Casing soil is used to stop vegetative growth and induce mushroom mycelium to produce fruiting bodies (Pardo-Giménez *et al*., 2017). Casing soil provides a good support for the establishment of fruiting bodies. Also, the necessary moisture for the formation and growth of the mushroom fruiting body is provided by the casing soil (Tokuda Martos *et al*., 2017). *Agaricus blazei* mushroom belongs to the Agaricaceae family. This mushroom has been cultivated in Japan and has been studied pharmacologically. It is also native to mountainous areas near Sao Paulo in Brazil (Mehwish *et al*., 2021). *Agaricus blazei* mushroom is one of the most expensive medicinal mushrooms and its other names are A. brasiliensis and A. subrufescens, which is also known as Himematsutake in Japan and Sun mushroom in Brazil. The key constituents in the fruiting body of *Agaricus blazei* include beta (1 and 3) diglucan, beta (1 and 4) d-glucan, beta (1 and 6) d-glucan (anti-tumor and immune system enhancer), and proteoglycan (anti-tumor). *A. blazei* mushroom contains the highest amount of beta-glucan and its derivatives compared to other mushrooms (Mehwish *et al*., 2021). The fruiting body of the mushroom has 5-15% dry matter, 19-35% protein, very low-fat content, vitamins B1, B2, D2, and important mineral elements (Ahmad *et al*., 2012; Mehwish *et al*., 2021). Biochemical compositions including proteins in the fruiting body of edible-medicinal mushrooms are under the influence of different species and strains, studied mushroom organ, age, and stage of fruiting organ development, substrate composition, growth techniques, mushroom harvest time, culture media preparation methods and genetic characteristics of the mushroom (Xu *et al*., 2011).

Reducing agricultural waste in the direction of production is considered a vital issue that has become a modern science in the world. Among the production industries, using agricultural wastes is the production and cultivation of edible-medicinal mushrooms, are used to produce the food needed by humans (Colauto *et al*., 2012). Oats (*Avena sativa* L.) is an herbaceous plant belonging to the family Poaceae which is one of the agricultural wastes studied in this research. Oat is a potential source of antioxidant compounds such as vitamin E (tocopherols), phenolic acids, flavonoids, sterols, and phytic acid (Ihsan *et al*., 2021). It is also rich in various fibers such as beta-glucan, arabinoxylan, and cellulose (Kadam *et al*., 2019).

Vermicompost is also used extensively in various agricultural sectors (Lee *et al*., 2018). Much research has been done on the use of vermicompost to replace casing soil. Vermicompost is an organic fertilizer that can improve the physical and chemical properties of the medium (Yadav and Garg, 2019). Due to the presence of worm-shaped droplets in

vermicompost, they have lower specific gravity than conventional compost, which increases the porosity, ventilation, and water permeability of the substrate (Zhang *et al*., 2020). Due to its high moisture-holding capacity, this organic matter always provides the appropriate amount of usable water to the product, which prevents the occurrence of severe moisture stress (Huang *et al*., 2018). Another important agricultural waste is edible mushroom compost, which after mushroom harvesting is called spent mushroom compost (SMC) (Paula *et al*., 2017). Mushroom waste is cheaper than other soil conditioners, which can reduce production costs (Roy *et al*., 2021). Mushroom compost provides plant nutrients for crops and thus replaces mineral fertilizers (Hernández *et al*., 2021). The addition of SMC to casing soil improves the physical environment of fruiting organ growth, increases the rate of water penetration into the compost, and improves its moisture capacity (González-Marcos *et al*., 2015). The most important property of SMC is the amount of organic matter that increases compost fertility and improves nutrition conditions for mushroom (Hernández *et al*., 2021).

Plant growth regulators are widely used in advanced agriculture and large inputs. Auxin affects the distribution of substances in the plant, cell division, cell growth, etc., and thus causes the development of plant organs (Bhutani *et al*., 2018). Auxins may also affect processes other than cell elongation, but cell elongation is their most important function (Mikaeili, 2016). Other auxin activities include fruit development. The increase in fruit size is mainly due to cell enlargement (Khan *et al*., 2014) According to these descriptions, it can be acknowledged that different organic materials have the potential to be used as casing soil for edible mushroom media, therefore, studying this field to determine the ability and optimal conditions for mushroom cultivation can be of great importance. According to the mentioned cases, different organic compounds were investigated such as casing soil, SMC, vermi SMC, oat, oat vermicompost, and indole-3-acetic acid to evaluate the usability in the production of the edible-medicinal mushroom *Agaricus blazei*.

MATERIALS AND METHODS

The present study was conducted in 2021 at the mushroom production center of Islamic Azad University, Isfahan (Khorasgan) Branch in a completely randomized design with 18 treatments (different compositions of casing soil) and 3 replications. Treatments included casing soil (100%), spent mushroom compost (SMC) (100%), vermi SMC (100%), oat (100%), oat vermicompost (100%), casing soil + SMC (50:50%), casing soil + vermi SMC (50:50%), casing soil + oat (70:30%) , casing soil + oat vermicompost (70:30%) , vermi SMC+ oat vermicompost (70:30%) , casing soil + SMC (70:30%) + IAA (0.5 mg l^{-1}), casing soil + oat (70:30%) + IAA (5.0 mg l^{-1}), vermi SMC + oat vermicompost (70:30%) + IAA (0.5 mg l^{-1}), casing soil (100%) + IAA (0.5 mg l^{-1}), SMC (100%) + IAA (0.5 mg l^{-1}), vermi SMC (100%) + IAA (0.5 mg 1^{-1}), oat (100 %) + IAA (0.5 mg l^{-1}) and oat vermicompost (100%) + IAA (0.5 mg l^{-1}).

Spawn, compost, and casing soil preparation

For this purpose, 5 kg of *A. blazei* spawn (TMU herbarium, TMU175) was purchased under herbarium conditions. 500 kg of compost was purchased from A mushroom production unit and transferred to the mushroom production hall. The dimensions of each compost bag were 40×60×60 cm and its height was 12 cm (equivalent to 17 kg). Each experimental plot was considered 33×200×200 cm. The amount of compost in each plot was 14.16 kg. The casing soil used by mushroom production unit located in Isfahan was prepared in 25 kg bags with specifications of pH = 7-7.5 and EC =< 3000. The results of the biochemical analysis of compost used are reported in Table (1).

Table 1. Analysis of chemical properties of compost

	EC (dS/m)	RH (%)	C/N	ASH (%)	NH_3	C (%)	N (%)	pH
Example 1	10	67.95	16.96	29.78	0.02	35.11	2.07	7.98
Example 2	9.37	67.75	16.45	29.6	0.035	35.2	2.14	7.91

Spawning

On each block, 1 meter by 2 meters, 882 grams of the spawn was used, which required 147 grams for each plot. After spawning with a sterilized flat board, the leveling and compacting were performed in a completely uniform manner. On the beds, sterilized plastics with dimensions of 1.5 by 2.5 meters were stretched. The moisture content of the substrates was 75-80%. It took 26 days for the mycelium run to become complete. At this stage, casing soil treatments were added to the composts simultaneously and evenly to a diameter of 3.5 cm. The constituents of the casing soil included black peat, brown peat, and calcium sulfate. Also, the casing soil used was free of any insect eggs and larvae, fungi, bacterial, and nematode contamination. Several substrates were treated with indole acetic acid. For this purpose, 0.5 mg/l of indole acetic acid was dissolved in 20 liters of distilled water and sprayed on the substrates. The results of biochemical analysis of casing soil compositions are reported in Table (2).

Table 2. Results of biochemical analysis of different treatments

Compounds	Ca (mmol/l)	(mg/kg) K	C/N (%)	N (%)	C (%)	P (mg/kg)	EC (dS/m)	pH
Casing soil (100 %)	10.66	1799	0.02	9.16	0.26	1085.84	0.51	5.62
Avena sativa (100%)	7.66	1889	0.06	8.26	0.55	837.13	1.52	5.84
(100%) Vermi *Avena sativa*	9.33	3736.66	0.04	8.53	0.42	317.74	4.38	5.57
Spent mushroom compost	10.66	1799	0.02	9.16	0.26	1085.84	2.24	6.48
Vermi Spent mushroom compost	13.66	2266	0.02	8.46	0.24	1230.50	2.53	5.62

After the casing soil stage, the substrates were covered again with sterile plastics to perform mycelialization operations in the casing soil well and to maintain soil moisture. During the mushroom growing period, the indoor temperature was set between 25-27 °C and the indoor humidity was adjusted between 80-90%. To evaluate the biochemical and morphological traits, the harvested mushroom samples were collected in separate containers and transferred to the Laboratory. Then some of the fruiting bodies were dried in an oven at 70 °C for 48 hours and some of them transferred to the freezer at -80 °C.

Variables

The number of fruiting body

The number of fruiting bodies during the harvest period was counted for all mushrooms and at the end was calculated for each of their treatments.

Stipe height

Stipe height of *Agaricus blazei* mushroom was also measured using a caliper.

The diameter of the mushroom stipe and cap

For this purpose, the diameter of the cap and stipe was measured using calipers and the results were recorded.

Time to first pin-head formation

The yield rate was obtained based on the number of days between the casing soil and the first fruiting body production (Lotfi *et al*., 2018).

Nitrogen, iron and zinc elements in fruiting body

Kjeldahl digester was used to measure the amount of nitrogen. The concentration of iron and zinc elements in the extract of the fruiting body of *Agaricus blazei* mushroom was observed by atomic absorption device (Ebadi *et al*., 2012; Lotfi *et al*., 2018).

Crude protein

To evaluate the crude protein percentage of the mushroom, the amount of total nitrogen was first measured by the Kjeldahl method and the protein percentage was calculated through Equation (1) (Masamba and Kazombo-Mwale, 2010).
Equation (1): Percentage of crude protein = percentage of total nitrogen × 6.25

Statistical analysis of data

Finally, the data were analyzed using SAS 9.4 statistical software and the means were compared by Duncan's multiple range test at a 5% probability level. Excel. 2016 software was used to draw the graphs.

RESULTS

The number of mushrooms

According to the analysis of variance results, the effect of different casing soil treatments on the number of mushrooms was significant at the 1% probability level (Table 3). The highest number of mushrooms was observed in the casing soil treatment + SMC (50:50%) with 118/33. The lowest amount was also obtained in vermi SMC treatments 100 + IAA, casing soil + SMC (70: 30%) + IAA, SMC 100% and casing soil + oat (70:30%) + IAA respectively with 10, 14, 16/33 and 22/66. No statistically significant difference was observed between some treatments at the 5% level of Duncan's test (Table 4).

Stipe height

The analysis of variance results conducted that the effect of different casing soil treatments on the stipe height of the fruiting body of *Agaricus blazei* mushroom was significant at the 1% probability level (Table 3). The maximum stipe height of the fruiting body of the mushroom was observed in the treatments of casing soil + oat (70:30%) and casing soil 100 with 88.50 and 85.4 mm, respectively. The lowest amount was obtained in the 100% SMC treatment with 46.87 mm. No statistically significant difference was observed between some treatments at the 5% level of Duncan's test (Table 4).

Table 3. Analysis of variance results of the treatments effect of on some morphological properties of *Agaricus blazei*

Source of variations	Degree of freedom	mean square								
		Number of mushrooms	Stipe height	Cap diameter	Stipe diameter	Time to first pin-head formation	N	Fe	Zn	Crude protein
Treatment	10	5271.73**	1502.89**	87.549**	809.07**	71.87**	21.30**	601.11**	40055.5**	834.62**
Error	22	83.29	429.32	5.448	54.25	1.12	0.16	58	72.3	6.44
CV (%)		5.03	8.03	4.11	6.90	2.97	3.29	1.20	2.62	3.38

**: Significantly difference at the 1% probability level.

Table 4. Average comparison results of the effect of the studied treatments on some morphological and biological properties of *Agaricus blazei*

Casing soil compositions (in percentage)	Number of mushrooms	Stipe height (mm)	Cap diameter (mm)	Stipe diameter (mm)	Time to first pin-head formation (Day)	N (%)	Fe (mg/kgFW)	Zn (mg/kgFW)
Casing soil (100)	81.66^{c}	85.4^{a}	66.36^{ab}	70.18^{a}	32^{d}	15.06^{b}	749.66^{e}	230.33^{g}
Casing soil + Vermi *Avena sativa* (70:30)	26.66^{e}	64.73^{abc}	59.3^{ab}	26.46^{de}	40^{b}	13.65^{c}	133^{i}	483.66^{b}
Casing soil + *Avena sativa* (70:30)	101.33^{ab}	88.50^{a}	61.43^{ab}	28.71^{cde}	41^{b}	11.29^{e}	615^{g}	503.33^{a}
Casing soil + *Avena sativa* (70:30) + IAA	22.66^{ef}	78.33^{ab}	59.64^{ab}	40.42^{bc}	40.33^{b}	14.98^{b}	116^{j}	371.33^{d}
Casing soil (100) +IAA	64.33^{d}	63.51^{abc}	54.89^{ab}	27.92^{cde}	32^{d}	10.26^{f}	213.66^{h}	196.33^{h}
Spent mushroom compost (100)	16.33^{ef}	46.87^{c}	49.04^{b}	45.58^{ab}	32^{d}	12.22^{d}	1401.33^{a}	343.33^{e}
Spent mushroom compost (100) + IAA	85^{bc}	54.19^{bc}	61.50^{a}	34.24^{bcd}	32^{d}	11.06^{e}	135.33^{i}	389^{c}
Vermi spent mushroom compost (100) + IAA	10^{f}	64.41^{abc}	58.02^{ab}	27.58^{cde}	32^{d}	7.46^{h}	830^{d}	191^{hi}
Casing soil + Spent mushroom compost (50:50)	118.33^{a}	53.09^{bc}	51.31^{ab}	21.47^{de}	32^{d}	11.57^{de}	1228^{b}	180.66^{i}
Casing soil + Vermi spent mushroom compost (50:50)	86^{bc}	79.50^{ab}	51.87^{ab}	26.48^{de}	45^{a}	15.77^{a}	885.66^{c}	390.33^{c}
Casing soil + Spent mushroom compost (70:30) + IAA	14^{ef}	53.96^{bc}	52.03^{ab}	20.44^{e}	34^{c}	8.75^{g}	673.66^{f}	287.66^{f}

In each column, the means that have at least one common letter, there is no significant difference in the level of 5% probability of Duncan test.

Cap diameter

The results of variance analysis of the data showed that the effect of different casing soil treatments on the diameter of the cap of the fruiting body of *Agaricus blazei* mushroom was significant at the 1% probability level (Table 3). The results of comparing the average data showed that the maximum diameter of the cap of the mushroom fruiting body was observed in the treatments of casing soil 100% and SMC 100% + IAA with 66.36 and 61.50 mm, respectively. The lowest amount was obtained in the 100% SMC treatment with 49.04 mm. No statistically significant difference was observed between other treatments at the 5% level of Duncan's test (Table 4).

The diameter of the stipe of the fruiting body

The analysis of variance results conducted that the effect of different casing soil treatments on the basal diameter of the fruiting body of *Agaricus blazei* mushroom was significant at the 1% probability level (Table 3). The maximum diameter of the base of the fruiting body of the mushroom was observed in the 100% casing soil treatment with 70.18 mm. The lowest amount was obtained in the casing soil treatment + SMC (70:30%) + IAA with 20.44 mm. No statistically significant difference was observed between some treatments at the 5% level of Duncan's test (Table 4).

Time to first pin-head formation

The results of variance analysis showed that the effect of different casing soil treatments on the yield rate of *Agaricus blazai* mushroom was significant at the probability level of 1% (Table 3). The highest production rate of *Agaricus blazai* mushroom was observed in the treatment of casing soil + vermi SMC (50:50%) with 45 mushrooms per day. The lowest amount was also obtained in the treatments of casing soil 100%, casing soil 100 + IAA, SMC 100, SMC 100+ IAA, vermi SMC 100 + IAA, casing soil + SMC (50:50%) with the amount of 32 in a day (Table 4).

Nitrogen, iron and zinc elements

The analysis of variance results indicated that the effect of different casing soil treatments on the amount of nitrogen, iron and zinc elements in the fruiting body of *Agaricus blazei* mushroom was significant at the probability level of 1% (Table 3). According to the results of the average data comparison, the highest amount of nitrogen was obtained in the casing soil treatment + vermi SMC (50:50%) with 15.77%. The lowest amount was also observed in the vermicompost treatment with 100 + IAA mushroom with

7.46% (Table 4). The highest amount of iron was observed in the 100% SMC treatment with 1401.33 mg/kg fresh weight. The lowest amount of iron was also obtained in the treatment of casing soil + oat (70:30%) + IAA with 116 mg/kg of fresh weight (Table 4). The highest amount of zinc was observed in the treatment of casing soil + oat (70:30%) with 503.33 mg/kg fresh weight. The lowest amount of zinc was observed in the treatments of casing soil + SMC (50: 50%) and vermi SMC 100 + IAA with 180.66 and 191 mg/kg fresh weight, respectively. There was no significant statistical difference between some treatments at the 5% level of Duncan's test (Table 4).

Crude protein

The results of variance analysis showed that the effect of different casing soil treatments on the amount of crude protein in *Agaricus blazei* was significant at the level of 1% probability (Table 3). The highest amount of crude mushroom protein was observed in the casing soil + vermi SMC treatment (50:50%) with 98.58%. Among the studied treatments, vermi SMC treatment of 100 + IAA with 46.66% showed the lowest amount of crude protein (Figure 1).

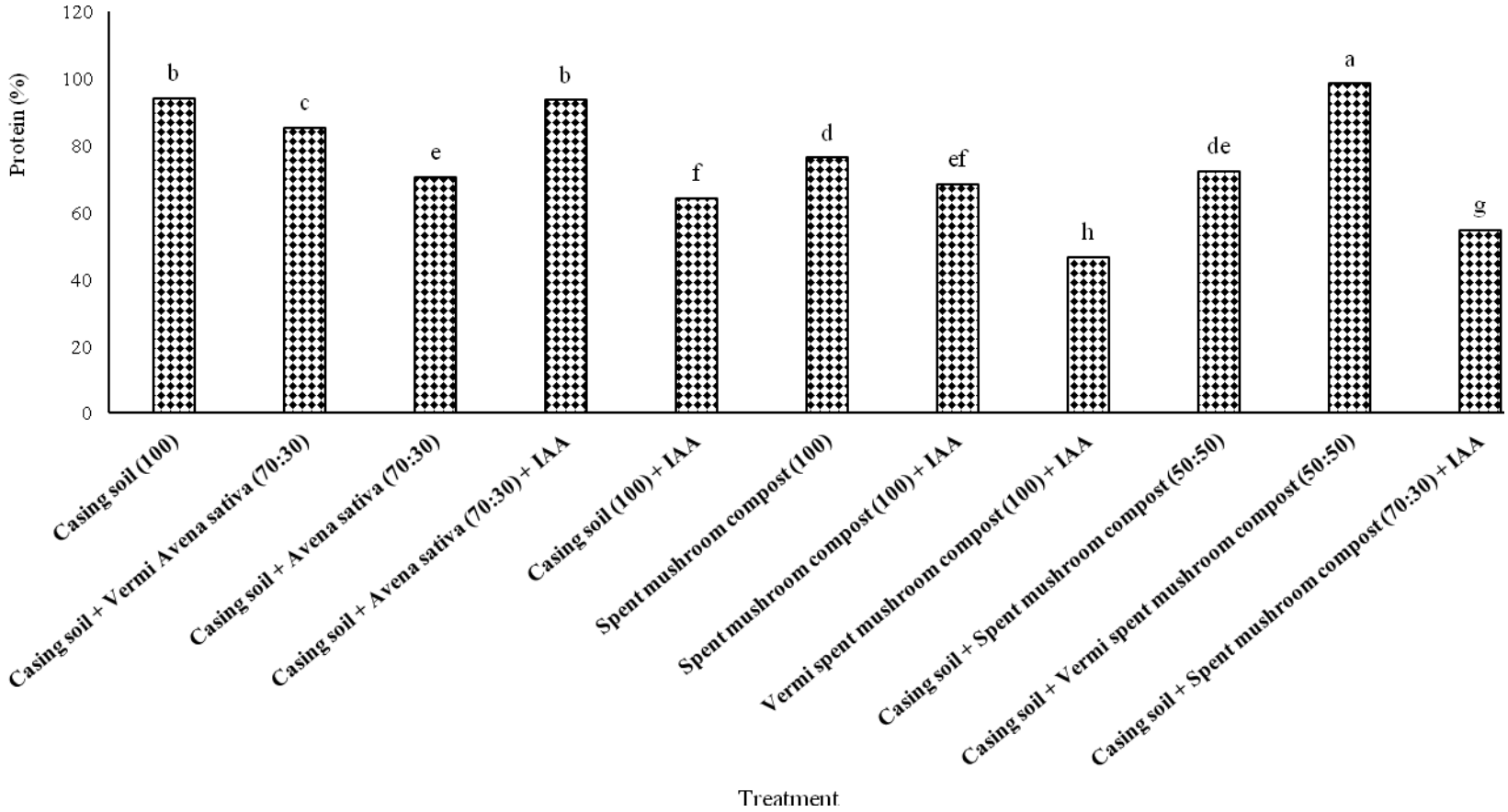

Figure 1. Results of comparison of the effect of the studied treatments on the mushroom protein, the means that have at least one common letter, there is no significant difference in the level of 5% probability of Duncan test.

DISCUSSION

The difference in the performance of *Agaricus blazai* mushroom in substrates with different casing soils is largely due to the physical structure of the substrate, the ratio of carbon to nitrogen, the ability of the mushrooms to secrete enzymes and chemical properties, including salinity (Ashrafi *et al*., 2017). Different casing soil treatments showed different effects on the number of mushroom and its function. So that the casing soil in combination with SMC (50:50%) caused a significant increase in the number of mushrooms, which is probably due to the physical structure and ratio of carbon to nitrogen suitable for this substrate. In addition, the difference in the performance of *Agaricus blazai* mushroom in substrates with different casing soils is probably due to the inability of mushrooms to secrete enzymes that can convert waste materials into amino acids and compounds that can be used by mushrooms (Antunes *et al*., 2020). The formation of a low number of mushroom fruiting bodies in the substrate containing casing soil vermi SMC + IAA can be caused by the inappropriate physical structure of this substrate and the inability of *blazei* mushroom to use this substrate (Dias *et al*., 2013). The presence of more mineral elements in the vermi SMC, such as phosphorus, potassium and calcium, has reduced the number of *blazei* mushroom in this treatment (Huang *et al*., 2018). In the vermi SMC treatment 100 + IAA, the mycelium growth was very weak and many primordia of the mushroom died in the layer of casing soil. Similar results were also reported in the research of Hosseini *et al*. (2017). Despite the fact that vermicompost has a high-water absorption capacity, the high adhesion of vermicompost particles after irrigation and the reduction of ventilation between the compost and the environment caused a decrease in the number of mushrooms in the substrates where vermicompost was applied alone or in combination with casing soil and other treatments (Rajkhowa *et al*., 2017). Vermi SMC 100%, due to its higher amount of calcium compared to other treatments, causes the formation of calcium oxalate crystals on the mycelium and reduces the number of mushrooms. According to the results of the SMC 100% treatment, it also caused a decrease in mushroom yield, which strengthens the possibility of the presence of ionic compounds in this composition (Lee *et al*., 2018). The increase in yield in the treatment of casing soil + SMC (50:50%) may be due to the increase in the amount of food available in the spawn, which provides large amounts of energy needed for the growth and development of mycelium. Investigations have shown that the higher the amount of nitrogen in the substrate, the activity of substrate-decomposing enzymes is increased. As a result, the growth rate of mycelium will increase and ultimately increase the yield of the product (Lou *et al*., 2017).

The results of this research indicated a significant effect of different casing soil compositions on the growth characteristics of the fruiting body of *blazei* mushroom. The increase in the size of the fruiting organ is mainly caused by cell division and cell enlargement. The application of auxin increases the internal levels of gibberellin, cytokinin

and indole-3-acetic acid and decreases abscisic acid, which is an important and effective balance between hormonal levels in the size of the fruiting organ. The diameter of the mushroom cap can be affected by the type of cultivation substrate and the type of casing soil (Bunsangiam *et al*., 2021). The type of casing soil played a decisive role in the number of mushroom pin-heads, and the lower this number is in the cultivation substrate, the higher the amount of nutrients and water absorbed from the cultivation substrate and less competition, so the diameter of the cap becomes larger. (Ratnoo and Doshi, 2012). In the treatments that had poor porosity and ventilation, due to the creation of less pin-heads in the casing soil and the growing climate of each mushroom, which had less competition, the mushroom was larger and its cap diameter was larger. In the casing soil treatments 100 and SMC 100 + IAA, the number of fruiting bodies appeared less, so there was less competition in receiving nutrients in these substrates, and as a result, the cap diameter showed a significant increase compared to other casing soil treatments.

It can be said that the use of indole-3-acetic acid is one of the effective mechanisms in increasing mushroom growth. The effect of growth stimulating hormones, especially indole-3-acetic acid, on the stimulation of mycelium growth, germination speed, hyphae growth, cap diameter and mushroom protein has been reported. Indole-3-acetic acid hormone increases the growth of mushroom by stimulating cell elongation and differentiation (Velazhahan *et al*., 2020). Another reason for the increase in the diameter of the cap of the fruiting body due to the use of auxin is because auxin increases the permeability and permeability of the cell wall in the fruiting body of the mushroom. As a result, it allowed more water penetration and dissolution of substances into the cell and will bring better growth of the fruiting body (Khan *et al*., 2014). Ebadi *et al*. (2012) investigated the effect of IAA producing bacteria on edible mushrooms and conducted that these bacteria increase fresh weight, dry weight, protein content, and increase the number and diameter of the mushroom cap. Eventually, they concluded that the production of IAA can be one of the effective mechanisms in increasing mushroom growth. The results of this research on increasing the diameter of the cap of the fruiting body of *Agaricus blazai* under the influence of IAA hormone are consistent with the results of Ebadi *et al*. (2012), Lotfi *et al*. (2018), Guo *et al*. (2009) and Alam *et al*. (2007) on the *blazei* mushroom.

Among the investigated treatments, the casing soil treatment + vermi SMC (50:50%) increased the yield rate of *Agaricus blazai* mushroom. Vermicompost contains enzymes that play an effective role in breaking down the organic matter of the growing medium and thus making available the nutrients required by plants (Rajkhowa *et al*., 2019). The presence of high amounts of nitrogen, potassium, calcium, magnesium and some fat has made the nutrients dissolve in the compost and facilitate the access of the fruiting organ to these materials and the material is directly absorbed by the fruiting organ. As a result, the yield rate is affected and its rate increases (Rajkhowa *et al*., 2017). In addition, the vermi SMC contains substances that stimulate plant growth such as auxin, cytokinin, iron, copper

and humic acid, and it stimulates crop growth and increases the yield rate (Tokuda Martos *et al*., 2017). In terms of minerals, vermicompost leads to the improvement of mushroom cultivation conditions, such as a significant increase in mycelium growth, high water absorption properties, and a reduction in the time first pin-head formation (Neyazi *et al*., 2017).

Also, the type of casing soil used in the cultivation bed showed a major effect on the biochemical properties of the grown *blazei* mushroom (Atila *et al*., 2018). The nutritional composition of mushroom fruiting body is affected by the type of culture medium. The investigations have shown that investigating the relationship between the properties of the fruiting bodies of mushrooms and the physicochemical properties of various agricultural wastes that are used as cultivation substrate or casing soil will improve the quality of the produced product. The studies indicated the existence of a direct relationship between the amounts of high-use and low-use elements in the culture medium with the content of nutrients in the fruiting body of *Agaricus blazei*. The results showed the effect of using mushroom vermicompost on the nitrogen percentage of the plant, the ratio of 50% of this organic fertilizer in combination with casing soil increased the nitrogen of the mushroom fruiting body. The reason for this can be attributed to the significant increase in the concentration of total nitrogen and organic matter in the substrate due to the consumption of mushroom compost (Alidadi *et al*., 2014). SMC as a suitable amendment improves the physicochemical condition of the casing soil. This organic fertilizer is rich in nutrients needed by the plant, and its consumption increases the concentration of essential elements in the culture medium and fruiting body of the mushroom (Roy *et al*., 2021). With the increase in the use of SMC, the amount of macro and micro nutrients in the product increased dramatically. The reason for this can be seen as the increase in the product's access to nutrients and water (Ratnoo and Doshi, 2012). In treated substrates with casing soil containing SMC, the release of nitrogen, phosphorus, sodium and especially iron ions have taken place, which can provide the required elements to the fruiting body and provide better growth in this substrate (Gumus and Seker, 2017). Low consumption elements are especially important for the activation of various enzymes and are needed for making vitamins and other metabolites as well as for normal growth. These elements also play a very important role in the activation of beneficial compost microorganisms. These microorganisms use elements and reproduce faster and after death are used as food by mycelium (Rzymski *et al*., 2017). In addition, the mushrooms themselves can absorb and use these elements directly. In this research, the highest amount of iron and zinc absorption was obtained from the substrates containing SMC 100% and casing soil + oat (70:30%) (Gumus and Seker, 2017).

Mushrooms have high amounts of high-quality protein, the amount of which is influenced by the composition of the culture medium and casing soil. Among the examined casing soil compositions, the highest amount of mushroom protein was obtained in the

substrate containing casing soil + vermi SMC (50:50%). The amount of protein in the fruiting body of edible-medicinal mushrooms has a direct relationship with the physical and chemical properties and the ratio of carbon to nitrogen of the culture medium and is influenced by the amount of nutrients in the culture medium, the mushroom isolate, the developmental stage and the life after harvest (Kothari *et al*., 2018). According to the nitrogen measured in the substrates, the reason for this is probably the difference in the amount of nitrogen that can be used, which can affect the quantity and quality of edible mushrooms. Therefore, it seems that the use of vermi SMC in the composition of casing soil due to its high nitrogen content and protein compounds obtained from earthworm corpses and remains has increased the amount of protein in the fruiting organ of *blazei* mushroom (Chu *et al*., 2012). Therefore, the increase in the amount of protein in the treatment of SMC in combination with casing soil may be due to the provision of a significant number of prerequisite elements for protein production from the organic matter of vermi SMC. In another study, it was observed that oyster SMC in combination with protein-rich products, including commercial supplements Calprozime, Champfood and Promycel 600, increased the total nitrogen content in the substrate, and subsequently, the crude protein content also increased (Picornell -Buendia *et al*., 2016). The results of this research are consistent with the results of the research of Picornell-Buendia *et al*. (2016) and the research of Chu *et al*. (2012).

CONCLUSION

Efforts to improve yield depend on several factors such as casing soil, culture medium formulation, particle size, type of spawn, type of supplement, amount of inoculation, and selection of strains. In general, different casing soil treatments conducted a significant effect on the growth and quality properties of *blazai* mushroom. According to the intended purpose of growing this mushroom, it is possible to select different combinations of casing soil that brought the best results. Among the investigated treatments, an acceptable yield can be obtained from the substrate containing casing soil + SMC (50:50%), casing soil + oat vermicompost (70:30%), and casing soil + oat (70:30%), which indicated promising results in most of the studied traits. Therefore, the indole-3-acetic acid hormone in combination with casing soil showed a significant effect on the growth properties of the plant, including the diameter of the cap. Moreover, these compounds can be considered scientifically and economically feasible because their continuous use is available in large quantities throughout the year at a low cost. SMC is produced annually in a large volume and can be used as a casing soil composition, and it is important in this sense.

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