

## GROWTH, YIELD AND ANTIOXIDANT ACTIVITY OF GREY OYSTER MUSHROOM (*PLEUROTUS PULMONARIUS*) GROWN IN SAWDUST SUBSTRATE WITH THE SUPPLEMENTATION OF ALKALINE MATERIALS

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### ABSTRACT

The study aims to evaluate the effect of different alkaline materials' supplementations on grey oyster mushroom (*Pleurotus pulmonarius*) to assess mycelial growth, initiation of primordia, yield and antioxidant activity. The sawdust substrate for mushroom cultivation was subjected to three different treatments, including lime, zeolite and gypsum, each designed in a completely randomised block with 10 replications. The prescribing of these alkaline materials significantly influences mushroom cultivation. The combinations of gypsum showed the lowest number of days taken for mycelial growth ( $30.7 \pm 4.12$  days) and the emergence of primordia ( $7.7 \pm 4.55$  days). The mushroom fruiting body collected from each flush showed that zeolite treatment produced the most acceptable yield ( $62.36 \pm 9.67$  g) compared to the other treatments. A maximum inhibitory effect against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, expressed as IC<sub>50</sub> value, was emanated by zeolite treatment from the third flush ( $63.48 \pm 28.804$  µg/ml). The total phenolic content (TPC) showed that zeolite exerted the highest phenolics in the fourth flush ( $98.96 \pm 10.07$  µg GAE/mg). The application of zeolite and gypsum as additives in mushroom breeding is highly recommended for the rapid growth, increase in yield and antioxidant properties of grey oyster mushroom.

**Keywords:** *Pleurotus pulmonarius*, Mycelial growth, Biological efficiency (%), 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, Total Phenolic Content (TPC)

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### INTRODUCTION

The mushroom industry in Malaysia is one of the trades that continuously contribute to the national economic growth; mushroom was recognised as a potential commodity that can generate higher incomes for the country. Out of 17 commercial varieties, oyster mushroom shows an upsurge in demand, receiving extensive market globally. Grey oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quél (1827), also known as *P. sajor-caju* (Fr.) Singer (Li & Yao, 2005), belonging to Pleurotaceae, Agaricomycetes, Agaricales, is among the most popular edible mushrooms that garner worldwide interest from culinary courses to scientific studies in mycology, biotechnology, nutrition, medicine, immunomodulatory and pharmacology. This highly nutritious food has been widely cultivated for commercial purposes and is an inexpensive source of protein, especially for the poor.

*Pleurotus* spp. are presently appreciated for their chemical constituents that possess many health benefits and have been considered as functional foods. The biological activities were mainly determined from their composition in a wide range of phenolic compounds, polysaccharides, amino acids, terpenes and sterols. In the

past, *P. pulmonarius* were tested into different extracts: methanol-dichloromethane extract, water fraction, hot water, aqueous extract and hexane fraction. The tests successfully revealed potent antioxidant activity against Low-Density Lipoprotein (LDL) oxidation and Human Aortic Endothelial Cell (HAEC) damage from H<sub>2</sub>O<sub>2</sub>-induced oxidative stress due to the content of ergothioneine that possesses a protective effect (Abidin *et al.*, 2016). Smiderle *et al.* (2008) also reported that the bioactive compound (1→3)(1→6)-linked β-glucan isolated from *P. pulmonarius* exhibited anti-inflammatory and analgesic properties on a rodent model. Out of the edible mushrooms, the oyster mushroom is recognised for its potentialities in antinociceptive, antitumour, antioxidant and immunological activities (Li *et al.*, 2017).

To date, *Pleurotus* spp. have been cultivated on various types of substrates through biotechnological processes that acquire selective lignocellulosic material from any accessible source of agricultural wastes. Some examples of agricultural waste are cotton waste, rice straw, and wheat straw (Yang *et al.*, 2013; Sardar *et al.*, 2017), sawdust (Obodai *et al.*, 2003), banana leaves (Reddy *et al.*, 2003), sugarcane bagasse (Hasan *et al.*, 2015), elephant grass, coat cross and coffee husks (Corrêa *et al.*, 2016). Besides, the supplementation on

mushroom planting bed is crucial for a high yield and excellent quality of mushroom. Added materials, including wheat bran, rice bran, cotton seed hulls and perilla stalks, have been reported to improve mushroom's marketable qualities (Yang *et al.*, 2013; Li *et al.*, 2017). With several research revolving around the increase in the yield of mushroom production, the selectivity of different substrates with other supplementary materials warranted a major concern.

The mushroom industry finds the solution to enhancing mushroom at the expense of yield by cultivating mushroom on a substrate prepared by composting due to cut-off cost and relatively reducing the rate of infection of other competitors (Vieira & Andrade, 2016). For the time being, the technique of composting is well established only for particular species that have been popularly applied in producing *Agaricus bisporus* (Martos *et al.*, 2017; Lishma & Das, 2017; Gea *et al.*, 2012). Similar to most mushroom substrates, compost used to have supplementation as a casing layer covering at its surface. The addition of a casing layer is useful to address the environmental conditions that support optimum mushroom growth (Colauto *et al.*, 2011). These materials should be non-toxic and have less nutritional value to avoid any negative consequences that might inhibit the initiation of mushroom growth. Amid their application, casing materials, as emphasised by several studies, exert a range of neutral to alkaline pH, calcium source, a potent cationic exchange capacity, low magnesium content and low toxic trace compound (Pardo *et al.*, 2010). Among those additives favoured in alkaline pre-treatment are lime, zeolite, bentonite, alkaline bauxite (Li *et al.*, 2012; Kim, Lee, & Kim, 2016; Awasthi *et al.*, 2016), biochar (Czekala *et al.*, 2016), gypsum (Febrisiantosa, Ravindran, & Choi, 2018), wood fly ash, lime, phosphor-gypsum, polyethylene glycol and jaggery (Gabhane *et al.*, 2012).

Alkaline materials benefit most agricultural activities, but since the study of their application in a mushroom substrate is still limited, the effect of alkaline materials on mushroom has not been resolved yet and the distinguished functional role of its uses is still obscure. One study found that the application of different calcium sources of bentonite and gypsum increases the potential of early mushroom fructification (Zied *et al.*, 2012). Therefore, the implementation of alkaline materials in a substrate medium are really important to be investigated for its outstanding effects on mushroom cultivation. The potential of using this agile method for various crops is as desirable as many had claimed, yet there is a lack of evidence of its benefits and values that apply in mushroom cultivation. Herein, the study aimed to evaluate the different alkaline materials, including agricultural lime, zeolite and gypsum, to produce a high yield and optimum antioxidant status of grey oyster mushroom.

## MATERIALS AND METHODS

**Mushroom strains and spawn preparation:** *Pleurotus pulmonarius*' pure culture was obtained from the University Agriculture Park, Universiti Putra Malaysia (UPM). The subculture of *Pleurotus pulmonarius* was prepared by inoculating (2 × 2 mm) mycelial agar on Potato Dextrose Agar (PDA) media and incubated at 25°C until a fully grown culture was obtained.

Wheat grain was used as a spawn substrate for the cultivation of *Pleurotus pulmonarius*. Wheat grains were washed and boiled in hot water for 30 min. The cooked grains were filled in a polypropylene bag and sterilised in an autoclave at 121°C for 1 hr. The sterilised grains were inoculated with one disc (7 mm diameter) of mycelial agar and incubated until full colonisation of spawn.

**Substrate preparation:** The medium substrates were analysed to compare the different treatments of alkaline materials (lime, zeolite and gypsum) as a supplement. The different substrates were prepared in the ratio of 100%: 10%: 1%, consisting of rubber sawdust, rice bran and alkaline materials, respectively. The water content of the mixed raw materials was adjusted to 75%. The mixtures were filled to 600 g in polypropylene bags and compressed densely. In each treatment, 10 replicates of polypropylene bags were used for different compositions of substrates. For all treatments, each bag was measured for pH level in order to compare the changes in the condition of the substrate (Table 1). The substrates were sterilised in a steamer at 121°C for 8 hr. The sterilised substrates were inoculated with *Pleurotus pulmonarius* at an amount of 5–8 g of grain spawn. The inoculated culture bags were then transferred to a mushroom house and incubated for 10–12 weeks before the harvesting stage. The mushroom house was maintained at 25–30°C and 60% relative humidity and kept in the dark until mycelia completely covered the bags. In the harvesting period, the environment was set to 25–30°C, 85–95% and 100–200 lux of light intensity. Mushroom growth and yield were observed in the early incubation period until mushroom was produced.

**Cultivation test of *Pleurotus pulmonarius*:** To test the growth performance and yield of *Pleurotus pulmonarius* cultivated in different treatments, selective parameters were applied, such as spawn running, initiation of primordial, yield of the fruiting body and biological efficiency. Spawn run was measured based on the number of days taken to complete the mycelial growth of the cultured bags during incubation. Through the determination of primordia's initiation, the cultured bags were observed for the time taken for primordial to form after the removal of the cap. The yield of mushroom was recorded based on the fresh weight of the fruiting body from the first flush to the fourth flush. The term 'flush'

refers to the harvesting period, with the interval between flushes normally being 7–10 days. In this experiment, four flushes of fruiting bodies were harvested and recorded over the cultivation cycle. The weight of the fruiting body was converted into biological efficiency by calculating the ratio of the weight of fresh mushroom to the dry weight of substrates. All of the aforementioned

parameters were evaluated in the experiment of 10 replicates for each different treatment in order to discriminate mushroom production performance. The grown fruiting bodies were freshly harvested from each bag and compared for their effectiveness according to the analysed parameters. The fresh mushrooms collected were subsequently screened for antioxidant activity.

**Table 1. The characteristic of pH level between different substrates.**

Substrate Formula with Alkaline Treatment	pH Level
100% SD + 10% RB + 1% Lime	7.13 <sup>ab</sup>
100% SD + 10% RB + 1% Zeolite	7.21 <sup>a</sup>
100% SD + 10% RB + 1% Gypsum	7.05 <sup>b</sup>

Note: SD, Sawdust; RB, Rice Bran.

The different connotations show a significant difference at  $P \leq 0.05$  using Tukey's test.

**Methanolic extract preparation of *Pleurotus pulmonarius* fruit body:** Mushroom samples from different treatments were prepared for methanolic extracts in accordance with Jeena *et al.* (2014). The extracts were set into screening of five different concentrations (1, 0.5, 0.25, 0.125, 0.0625 mg/ml) for further analysis.

**The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:** The free radical scavenging capacity of *Pleurotus pulmonarius* was determined according to Brand-Williams *et al.* (1995) with several modifications. The percentage of DPPH radicals scavenged by the samples was calculated using Prieto's DPPH microplate assay (Prieto, 2012). A solution of 0.06 mM of 2,2-diphenyl-1-picrylhydrazyl was prepared using methanol, sealed with aluminium foil and kept in the fridge. The tested samples (1-0.0625 mg/ml) were dissolved in 100  $\mu$ l of methanol and mixed with 100  $\mu$ l of DPPH solution in a 96-well microplate. The mixture was kept in complete darkness and incubated at room temperature for 30 min. The absorption was measured at 517 nm using a microplate reader (Multiskan FC, Thermo, Waltham, MA, USA). Each tested sample was analysed in four replicates. The standard antioxidant of ascorbic acid was used as a positive control. The radical scavenging activity was calculated as a percentage of DPPH discoloration using the following formula:

$$\text{Inhibition of DPPH radical (\%)} = [(A_0 - A_s)/A_0] \times 100$$

Where  $A_{\text{control}}$  = Absorbance of control (blank sample),

$A_{\text{sample}}$  = Absorbance of samples after 30 minutes

The half-inhibitory concentration (IC50) was calculated from the graph of radical scavenging activity percentage against the extract concentration.

**Total phenolic content determination using Folin-Ciocalteu's method:** The total phenolic content of extracts was estimated following Folin-Ciocalteu's method developed by Bobo-García *et al.* (2015) with several modifications. A solution of 20  $\mu$ l of sample

mixed with 80  $\mu$ l of diluted Folin-Ciocalteu's phenol reagent (1:4) in a 96-well microplate was shaken for 1 min and left for 2 min until dissolved. The mixture was added to 75  $\mu$ l of sodium carbonate solution (100 g/l), shaken at medium-continuous speed for 1 min and kept in the dark for 2 hr of incubation. Then, the absorption was measured at 765 nm using the microplate reader (Multiskan FC, Thermo, Waltham, MA, USA). Gallic acid was used to prepare the standard curve (1-0.0625 mg/ml). Results were expressed as mg of gallic acid equivalents (GAE) per gram of the extract.

**Statistical analysis:** The data obtained were analysed using SAS statistical software. Each of the treatment was arranged into a completely randomised design (CRD) with post-hoc Tukey's test. The data were expressed as means  $\pm$  standard deviation.

## RESULTS

**Growth performance in spawn running and primordial initiation:** The number of days for spawn running was noticeably significant among the *Pleurotus pulmonarius* cultivated with different treatments (Table 2). The treatment of gypsum showed the earliest spawn run period to complete the mycelial growth ( $30.7 \pm 4.12$  days), followed by lime ( $48.4 \pm 2.8$  days) and zeolite ( $50.5 \pm 3.5$  days). In primordial initiation, the treatment of gypsum was also the fastest ( $7.7 \pm 4.55$  days) compared to that of lime and zeolite. Statistically, the treatment of gypsum showed a high significance according to Tukey's test, which was considerably different from the treatment of lime and zeolite with a similar level of significance.

**Yield of mushroom from the first flush to the fourth flush and biological efficiency (%):** The total yield was obtained from the fresh weight of mushroom collected from each bag, 10 replicates for all treatments. The result in Table 3 shows that all of these treatments were

considerably varied in the yield of mushroom counts from every flush, as presented in Figure 1. The treatment of zeolite accumulated the biggest yield ranging from  $18.88 \pm 3.22$  g to  $62.36 \pm 9.67$  g, while the lowest was found in the treatment of gypsum ( $11.61 \pm 5.82$  g) at the fourth flush. The maximum yield of mushroom was observed in the first flush of the treatment of zeolite with  $62.36 \pm 9.67$  g. The biological efficiency of mushroom indicated that the treatment of zeolite ( $26.05 \pm 11.18$  %) was the highest compared to that of lime ( $18.77 \pm 8.34$  %) and gypsum ( $17.95 \pm 8.21$  %). There was a high significance in the treatment of zeolite according to Tukey's test as compared to the level of significance in the treatment of lime and gypsum. On average, the efficiency of yield from every flush was simplified as the highest was obtained in the treatment of zeolite ( $39.07$  g), followed by lime ( $28.15$  g) and gypsum ( $26.92$  g).

**DPPH scavenging activity:** All the tested samples had outcome into significant reactions to DPPH radicals as summarised in Table 4. The treatment of gypsum showed the greatest inhibition of radicals in the first and second flushes with  $187.38 \pm 2.97$   $\mu\text{g/ml}$  and  $207.84 \pm 11.71$   $\mu\text{g/ml}$ , respectively, while the treatment of zeolite was the most reactive in the third and fourth flushes with  $63.48 \pm 28.804$   $\mu\text{g/ml}$  and  $152.16 \pm 17.504$   $\mu\text{g/ml}$ , respectively. The minimum IC50 value, which was the highest antioxidant activity, was documented in the treatment of zeolite ( $63.48 \pm 28.804$   $\mu\text{g/ml}$ ) from the third flush of grey oyster mushroom, from as low as  $31.25$   $\mu\text{g/ml}$  to  $500$   $\mu\text{g/ml}$  of concentration. Collectively, the third flush of zeolite treatment was depicted as the most

effective among all the four flushes in scavenging the free radicals.

**Total phenolic content (TPC):** The total phenolic content was crucially comparable in tested samples among different treatments (Table 5 and Fig 2). The treatment of gypsum exerted the highest content of phenolics in the second flush with  $188.44 \pm 11.38$   $\mu\text{g/ml}$ , while the treatment of zeolite accumulated the most phenolics in the third and fourth flushes with  $321.84 \pm 21.79$   $\mu\text{g/ml}$  and  $369.44 \pm 101.31$   $\mu\text{g/ml}$ , respectively. However, all the tested samples showed no significant difference in the total phenolic content analysis in the first flush. The greatest phenolics composed in a fruiting body was found in the treatment of zeolite ( $369.44 \pm 101.31$   $\mu\text{g/ml}$ ) from the fourth flush. Therefore, the treatment of zeolite from the fourth flush was the most pronounced in phenolic content among the samples.

**Table 2. Rate of growth of *Pleurotus pulmonarius* in spawn running and primordial initiation.**

Treatment	Spawn-run (day)	Primordial initiation (day)
Lime	$48.4 \pm 2.80^a$	$10.0 \pm 2.67^{ab}$
Zeolite	$50.5 \pm 3.50^a$	$12.7 \pm 4.11^a$
Gypsum	$30.7 \pm 4.12^b$	$7.7 \pm 4.55^b$

Values are the mean  $\pm$  SD (n=10).

The different letter(s) in the column indicate considerable differences among the means within each treatment at  $P \leq 0.05$  using Tukey's test.

**Table 3. Yield of fresh samples harvested in every flush (1<sup>st</sup> – 4<sup>th</sup> flush) and biological efficiency (%).**

Treatment	Yield of mushroom (g)				BE (%)
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	4 <sup>th</sup> flush	
Lime	$43.64 \pm 9.69^b$	$28.15 \pm 7.62^b$	$26.2 \pm 6.71^b$	$14.62 \pm 2.60^{ab}$	$18.77 \pm 8.34^b$
Zeolite	$62.36 \pm 9.67^a$	$40.62 \pm 5.14^a$	$34.42 \pm 2.51^a$	$18.88 \pm 3.22^a$	$26.05 \pm 11.18^a$
Gypsum	$41.98 \pm 6.65^b$	$28.31 \pm 7.05^b$	$25.8 \pm 3.65^b$	$11.61 \pm 5.82^b$	$17.95 \pm 8.21^b$

Values are the mean  $\pm$  SD (n=10).

The different letter(s) in the column indicate considerable differences among the means within each treatment at  $P \leq 0.05$  using Tukey's test.

**Table 4. IC50 values of the methanol extracts from each treatment of *Pleurotus pulmonarius* in antioxidant activity.**

Treatment	IC50 of DPPH radical by mushroom extracts ( $\mu\text{g/ml}$ )			
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	4 <sup>th</sup> flush
Lime	$333.0 \pm 23.49^a$	$302.42 \pm 11.00^b$	$332.65 \pm 8.32^a$	$201.27 \pm 12.33^a$
Zeolite	$309.62 \pm 27.51^a$	$393.45 \pm 15.16^a$	$63.48 \pm 28.80^c$	$152.16 \pm 17.50^b$
Gypsum	$187.38 \pm 2.97^b$	$207.84 \pm 11.71^c$	$283.36 \pm 1.41^b$	$219.32 \pm 9.53^a$

Values are the mean  $\pm$  SD (n=4).

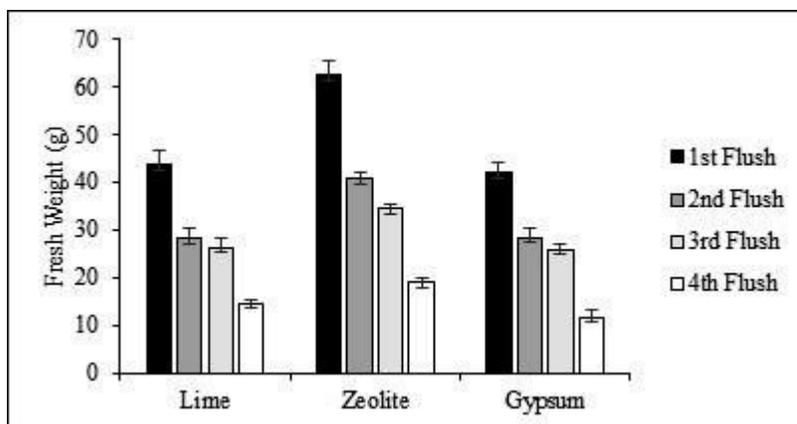
The different letter(s) in the column indicate significant differences among the means within each treatment at  $P \leq 0.05$  using Tukey's test.

**Table 5. Phenolic content in three different treatments of mushroom samples.**

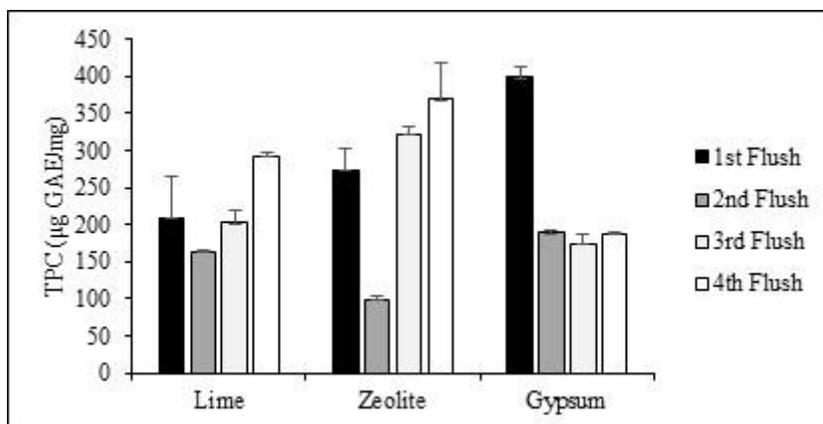
Treatment	Total phenolic content of mushroom extract ( $\mu\text{g GAE}/\text{mg}$ )			
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	4 <sup>th</sup> flush
Lime	209.51 $\pm$ 118.45 <sup>a</sup>	163.4 $\pm$ 7.20 <sup>b</sup>	202.03 $\pm$ 33.96 <sup>b</sup>	292.66 $\pm$ 8.24 <sup>ab</sup>
Zeolite	273.51 $\pm$ 59.57 <sup>a</sup>	98.96 $\pm$ 10.07 <sup>c</sup>	321.84 $\pm$ 21.79 <sup>a</sup>	369.44 $\pm$ 101.31 <sup>a</sup>
Gypsum	398.88 $\pm$ 27.42 <sup>a</sup>	188.44 $\pm$ 11.38 <sup>a</sup>	174.96 $\pm$ 23.38 <sup>b</sup>	187.25 $\pm$ 3.007 <sup>b</sup>

Total phenolic content was expressed as gallic acid equivalent (GAE) which resembled the linear equation,  $y = 0.0045x - 0.2306$ , where  $R^2 = 0.9857$ .

The values are the mean  $\pm$  SD (n=4). The mean differences with different letter notations were significant at  $P \leq 0.05$  using Tukey's test.



**Fig. 1. Total yield of mushroom of each treatment collected from the 1<sup>st</sup> flush to the 4<sup>th</sup> flush based on the weight of fresh samples (g).**



**Fig. 2. Total phenolic content of *Pleurotus pulmonarius* (methanolic extract).**

## DISCUSSION

The grey oyster mushroom provides various raw materials to be manipulated in mushroom cultivation, which often adds value to its growth, yield, flavour and nutritional properties. The treatment of alkaline materials reacts significantly with a wide spectrum of performance in terms of mycelial growth, primordial formation, yield of production and antioxidant status. In another way, our results disregarded the morphological change of the treatment on the fruiting body, as there are no appreciable

differences among distinct treatments regarding the size of pileus and stalks that have been produced.

The rate of mycelial growth is one of the measures of mushroom cultivation that considers the time taken to reach the harvesting stage. Mycelial growth is mainly influenced by the basal substrate where all those essential nutrients are available to be absorbed for the development of premature mycelium. Meanwhile, the primordial growth that occurs after the completion of spawn running is another insight into determining the productivity of mushroom cultivation. Mushroom grows

at a specific pH level that provides a suitable condition to support growth from an early stage. A previous study showed that oyster mushrooms had the best growth rate at neutral pH levels (Khan *et al.*, 2013). In the bottom line, the mushroom can only absorb the nutrients at an optimum pH level that was prescribed in the media (Arif *et al.*, 2015).

Generally, the ratio of alkaline material used plays an important role in mushroom cultivation, especially in supporting the availability and transportation of the nutrients. However, the ratio of alkaline materials used for substrate preparation is according to the ratio standard practiced by local growers. In the current study, all these alkaline materials at 1% of treatment showed notable effects on mushroom growth over the spawn running and primordial growth. The results were in line with the previous study performed by Firdaus *et al.* (2015) who reported that a substrate mixture with 1% of lime took 36 to 48 days for a complete spawn running.

The variation in the duration of spawn running might be due to the presence of different calcium concentrations from various types of alkaline materials. Specifically, the essential element that encompasses magnesium, calcium, iron, copper, manganese, zinc and molybdenum is favourable for fungal growth (Jennings, 1996). Calcium is responsible for stimulating intracellular effects through chemical, electrical and physical signals. Amidst mycelial development, calcium ions were transferred to the hyphal tips and supplied to the subapical zones through passive mechanisms whereby it initiated the gradient of H<sup>+</sup>, pH, electrical and ions (Royse & Sanchez-Vazquez, 2003). The concentration of calcium in a substrate could have an adverse effect on growth, cell proliferation and sporulation.

Yield and biological efficiency create awareness among the growers in attempting to meet the demands of both the local and global markets. In general, mushroom cultivation with the supplementation of other beneficial additives improves the yield and quality of mushroom (Yang *et al.*, 2013). For instance, many studies indicated that the variety of agricultural wastes mixed into substrates following the ratio of the mixture would be a betterment of the yield of mushroom (Alananbeh *et al.*, 2014). The full exploitation of lignocellulosic material solely without supplementation is inadequate to provide the optimum nutrient requirement to increase the performance of mushroom production (Atila *et al.*, 2017).

The considerable factors in attaining the high productivity of mushrooms presumably point out the composition of the nutrient and physical and chemical systems of the substrates (Hoa, Wang, & Wang, 2015). The recent result showed a significant difference in the yield of these treatments, which is lower than the yield recorded in *Pleurotus ostreatus* cultivation grown on rice straw (735.9–900.9 g), weed plants (154.1–195.36 g) and

maize and pineapple residues (218–675 g) (Hlerema, Eiasu, & Koch, 2017; Islam & Riaz, 2017; Cayetano *et al.*, 2018). A past study (Salmalian, Peyvast, & Olfati, 2016) reported that *Pleurotus eryngii* produced a yield between 14.71 g and 164.54 g, which was in line with the yield in the present study.

With regard to the biological efficiency percentage, a range of 17.95 to 26.05% was observed for the harvested mushroom between treatments. According to a previous study conducted by Myronycheva *et al.*, (2017), the biological efficiency obtained from *P. pulmonarius* produced from the mixture of wheat straw and sunflower husks at the ratio 2:3 ranged from 9.8 to 62% compared to the current study that recorded lower in biological efficiency. The result was less likely to contradict the study of Khan *et al.* (2017) that showed that *Pleurotus ostreatus* grown on a cotton waste substrate with wheat bran produced the maximum biological efficiency of 46.33%, whereas the lowest was only 30% in the cotton waste without supplementation. Another research also reported the contrast in result in terms of biological efficiency of *Pleurotus sajor-caju* on cotton waste with 81%, while the least was 48.25% in the sugarcane bagasse (Sardar *et al.*, 2017).

The treatment of different alkaline materials on grey oyster mushrooms resulted in crucial effects on the yield, where the treatment of zeolite outweighed the other treatments. The performance corroborates the earlier findings since the application of zeolite on crops elevated the crop production with an outstanding yield that involved *Aloe vera* (397.17 g), potato (396.35 g), sunflower (5828 kg/ha), canola (2674.1 kg/ha) and tomato (16.972 kg/m<sup>2</sup>) (Savvas *et al.*, 2004; Bybordi & Ebrahimian, 2013; Gholamhoseini *et al.*, 2013; Ghannad, Ashraf, & Alipour, 2014; Hazrati *et al.*, 2017). It has been discovered that zeolite is a subtle cationic interchanger with microporous structures, enabling it to adsorb molecules at relatively low pressure (Sozana *et al.*, 2003). The exchange capacity of zeolite depends on its nature, which only allows selective minerals and nutrients to pass through despite its ability to adsorb a wide range of molecules. Nevertheless, Ok *et al.* (2003) reported that zeolite promoted enormous potentialities as it can improve water holding capacity, solubility and nutrient retention in soil. These studies indicated that zeolite is potent for its application in mushroom breeding since it may correlate to the yield thanks to the catalysation of nutrient transportation.

The scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals measures the antioxidant capacity attributed to the plant-, fungi- or microbial-released bioactive compounds. In general, the DPPH assay is carried out in accordance with the principle that active compounds, as the free radical scavenger, donate hydrogen ions to the radicals to manifest the protective effects from oxidative damage. As the antioxidant

donates a proton to these radicals, the absorption of samples decreased. The measurement to determine the radical scavenging activity was based on the reduction of the absorbance of the samples (Srivastava *et al.*, 2006). Herein, the results of the assay were derived as an inhibitory concentration of extracts onto 50% of radicals (IC50 value).

All the different treatments demonstrated a wide spectrum of the scavenging activity in IC50 where the lowest value was recorded in the treatment of zeolite at the third flush of the harvested mushroom. This result was also supported by a study conducted by (González-Palma *et al.*, 2016) that displayed that the IC50 of *Pleurotus ostreatus* fruiting body reached the minimum value of 26.99 mg GAE/L. A few past studies show corresponding results with the current findings with *Pleurotus* spp. (43.21–52.03 µg/ml), *Pleurotus eryngii* (48.53 mg/ml) and *Pleurotus dryinus* (24.71 mg/ml) (Obodai *et al.*, 2014; Wong *et al.*, 2013; Keles, Koca, & Gencelep, 2011). Our finding supported the previous study performed by Shahbaz *et al.* (2019), where soil remediation for wheat production in nickel (Ni) polluted soil, through the employment of biochar and zeolite, and alleviated the Ni oxidative stress while increasing the activities of antioxidant in wheat. The addition of zeolite to the soil had improved the antioxidant defence machinery of sunflower, which highly scavenged the reactive oxygen species as previously reported (Shahbaz *et al.*, 2018).

The different types of alkaline materials applied to *Pleurotus pulmonarius* may have a different composition, which resulted in a wide range of IC50. The possible combination of environmental materials, such as composite additives, while prescribing the use of zeolite could be beneficial for crop management, hence promoting growth, postharvest quality and antioxidant enzyme activity in the long run of crop production (Shi *et al.*, 2016).

The phenolic compound is a secondary metabolite that possesses antioxidant properties and in which over 10,000 compound species were identified as being most abundantly extracted from plants (Veberic, 2016). The establishment of many studies on the constituents of chemicals embedded in plant samples was a result of the antioxidant activity determined by the profile of the phenolic compound (Yoon *et al.*, 2011). In the mushroom species, *Pleurotus pulmonarius* is among the edible mushrooms that implore great interest in its provision of active compounds that perform a wide range of metabolic activities. Their analysis of the phenolic compound broke it down into a large number of species such as gallic acid, homogentisic acid, procatechuic acid, catechin, chlorogenic acid, vanillin, naringin, myricetin, resveratrol and quercetin (Nguyen *et al.*, 2016).

All the treatments differed significantly in demonstrating phenolic content from 98.96 to 369.44

µg/ml. This finding corresponded with the previous study of Rashidi and Yang (2016) that included *Agaricus bisporus* (0.63 mg/g), *Hypizigus marmoreus* (0.67 mg/g), *Volvariella volvacea* (0.73 mg/g), *Flammulina velutipes* (0.75 mg/g), *P. eryngii* (0.44 mg/g), *P. ostreatus* (0.39 mg/g), *Lentinula edodes* (0.49 mg/g) and *Hericium erinaceus* (0.46 mg/g). The current research indicated that the treatment of zeolite from the fourth flush was the largest in total phenolic content (TPC) between treatments. Similarly, zeolite application improved the polyphenols content in wheat as reported from the previous research (Shahbaz *et al.*, 2019). This result also coordinated with the incorporation of zeolite in fertilisation, where it promoted the highest phytochemicals in apricot, especially phenolic compounds (Milošević, Milošević, & Glišić, 2013).

The composition of zeolite affects the chemical content in the fruiting body where zeolite was naturally a good source of K (Milosevic & Milosevic, 2009). The relationship between the amount of K and the phenolic compound was positively correlated (Radi *et al.*, 2003). Thus, this study showed that the nutrient status present in the alkaline materials may induce changes in the phenolic content of mushrooms.

**Conclusion:** The effect of alkaline materials on the cultivation of *Pleurotus pulmonarius* manifests the pivotal role in response to the yield and quality of mushroom. The treatment of zeolite considerably increased mushroom yield and biological efficiency and induced a higher level of antioxidant value in radical scavenging activity and phenolics content. In other cases, the treatment of gypsum was beneficial in terms of mycelial growth where it significantly minimised the period of spawn running and initiation of primordia. Our results depicted that the application of zeolite and gypsum as additives in substrates leads to the improvement of mushroom production. However, further research on the market price survey of alkaline materials is required to investigate whether the use of alkaline materials results in significant profit in production. The survey is useful to lead the direction of this sector towards economic manners and can be used to generate high yield prospects in the mushroom industry.

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## REFERENCES

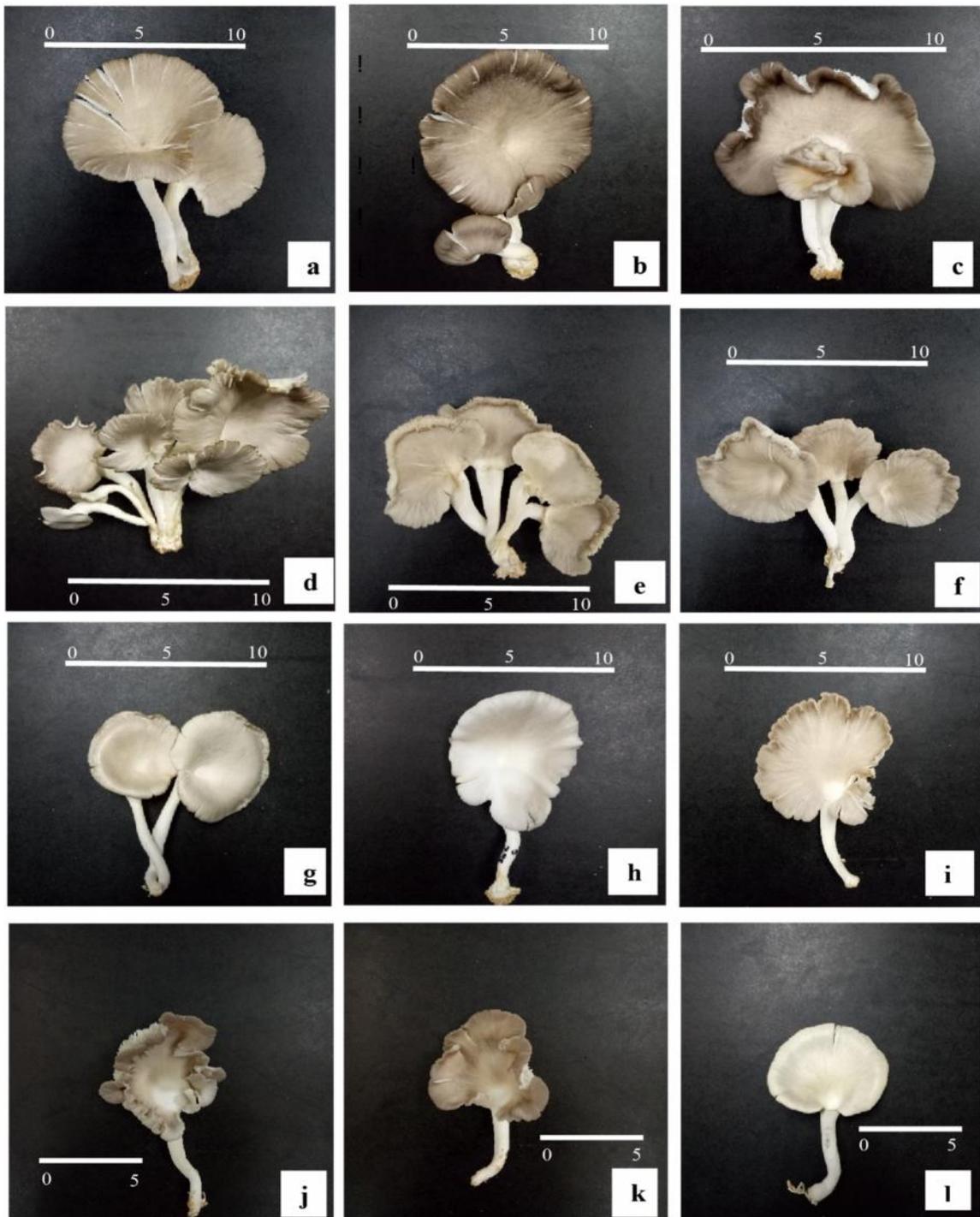
- Abidin, M. H. Z., Abdullah, N., & Abidin, N. Z. (2016). Protective Effect of Antioxidant Extracts from Grey Oyster Mushroom, *Pleurotus pulmonarius* (Agaricomycetes), Against Human Low-Density Lipoprotein Oxidation and Aortic Endothelial Cell Damage. *International J. Medicinal Mushrooms*, 18(2), 109–121. <https://doi.org/10.1615/IntJMedMushrooms.v18.i2.20>
- Alananbeh, K. M., Bouqellah, N. A., & Al Kaff, N. S. (2014). Cultivation of Oyster Mushroom *Pleurotus ostreatus* on Date-palm Leaves Mixed with Other Agro-wastes in Saudi Arabia. *Saudi J. Biological Sciences*, 21(6), 616–625. <https://doi.org/10.1016/j.sjbs.2014.08.001>
- Arif, Z., Zahid, N. Y., Abbasi, N. A., & Iqba, S. M. (2015). Effect of Different Culture Medium and pH on the Mycelial Growth of Shiitake Mushroom. *Mycopath*, 13(1), 25–28. Retrieved from <http://journals.pu.edu.pk/journals/index.php/mycopath/article/viewFile/668/349>
- Atila, F., Tüzel, Y., Faz Cano, A., & Fernandez, J. A. (2017). Effect of Different Lignocellulosic Wastes on *Hericium americanum* Yield and Nutritional Characteristics. *J. the Science of Food and Agriculture*, 97(2), 606–612. <https://doi.org/10.1002/jsfa.7772>
- Awasthi, M. K., Wang, Q., Ren, X., Zhao, J., Huang, H., Awasthi, S. K., ... Zhang, Z. (2016). Role of Biochar Amendment in Mitigation of Nitrogen Loss and Greenhouse Gas Emission During Sewage Sludge Composting. *Bioresource Technology*, 219, 270–280. <https://doi.org/10.1016/j.biortech.2016.07.128>
- Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Virseda, P., Marín-Arroyo, M. R., & Navarro, M. (2015). Intra-laboratory Validation of Microplate Methods for Total Phenolic Content and Antioxidant Activity on Polyphenolic Extracts, and Comparison with Conventional Spectrophotometric Methods. *J. the Science of Food and Agriculture*, 95(1), 204–209. <https://doi.org/10.1002/jsfa.6706>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Bybordí, A., & Ebrahimian, E. (2013). Growth, Yield and Quality Components of Canola Fertilized with Urea and Zeolite. *Communications in Soil Science and Plant Analysis*, 44(19), 2896–2915. <https://doi.org/10.1080/00103624.2013.823986>
- Cayetano, C. M., Bernabé, G. T., Bernabé, V. G., Romero, F. A., & Martínez, C. D. (2018). Experimental Study on Mushroom Yield of Commercial Strains from *Pleurotus ostreatus* Cultivated on Rice Straw in Mexico. *International J. Applied Science and Technology*, 8(4). <https://doi.org/10.30845/ijast.v8n4p5>
- Colauto, N. B., da Silveira, A. R., da Eira, A. F., & Linde, G. A. (2011). Production Flush of *Agaricus blazei* on Brazilian Casing Layers. *Brazilian J. Microbiology*, 42(2), 616–623. <https://doi.org/10.1590/S1517-83822011000200026>
- Corrêa, R. C. G., Brugnari, T., Bracht, A., Peralta, R. M., & Ferreira, I. C. F. R. (2016). Biotechnological, Nutritional and Therapeutic Uses of *Pleurotus* spp. (Oyster Mushroom) Related with Its Chemical Composition: A Review on the Past Decade Findings. *Trends in Food Science and Technology*. <https://doi.org/10.1016/j.tifs.2016.01.012>
- Czekala, W., Malińska, K., Cáceres, R., Janczak, D., Dach, J., & Lewicki, A. (2016). Co-composting of Poultry Manure Mixtures Amended with Biochar - The Effect of Biochar on Temperature and C-CO<sub>2</sub> Emission. *Bioresource Technology*, 200, 921–927. <https://doi.org/10.1016/j.biortech.2015.11.019>
- Febriantosa, A., Ravindran, B., & Choi, H. L. (2018). The Effect of Co-additives (Biochar and FGD Gypsum) on Ammonia Volatilization During the Composting of Livestock Waste. *Sustainability (Switzerland)*, 10(3). <https://doi.org/10.3390/su10030795>
- Firdaus, S. M., Bahri, A. R. S., Rahijan, M., & Rahman, A. W. A. (2015). Growth Performance and Postharvest Quality of Grey Oyster Mushroom (*Pleurotus sajor-caju*) Subjected to Different Sound Intensity Treatments Prior to Fruiting Body Formation. *Advances in Life Science and Technology*, 28, 51–60. Retrieved from [www.iiste.org](http://www.iiste.org)
- Gabhane, J., William, S. P., Bidyadhar, R., Bhilawe, P., Anand, D., Vaidya, A. N., & Wate, S. R. (2012). Additives Aided Composting of Green Waste: Effects on Organic Matter Degradation, Compost Maturity, and Quality of the Finished Compost. *Bioresource Technology*, 114, 382–388. <https://doi.org/10.1016/j.biortech.2012.02.040>
- Gea, F. J., Santos, M., Diáñez, F., Tello, J. C., & Navarro, M. J. (2012). Effect of Spent Mushroom Compost Tea on Mycelial Growth and Yield of

- Button Mushroom (*Agaricus bisporus*). World J. Microbiology and Biotechnology, 28(8), 2765–2769. <https://doi.org/10.1007/s11274-012-1081-7>
- Ghannad, M., Ashraf, S., & Alipour, Z. T. (2014). Combined Effects of Zeolite, Humic Acid and Potassium Sulphate on Yield and Qualitative Characters of Potato (*Solanum tuberosum* L.). International J. Farming and Allied Sciences, 3(6), 669–674. Retrieved from [www.ijfas.com](http://www.ijfas.com)
- Gholamhoseini, M., Ghalavand, A., Khodaei-Joghan, A., Dolatabadian, A., Zakikhani, H., & Farmanbar, E. (2013). Zeolite-amended Cattle Manure Effects on Sunflower Yield, Seed Quality, Water Use Efficiency and Nutrient Leaching. *Soil and Tillage Research*, 126, 193–202. <https://doi.org/10.1016/j.still.2012.08.002>
- González-Palma, I., Escalona-Buendía, H. B., Ponce-Alquicira, E., Téllez-Téllez, M., Gupta, V. K., Díaz-Godínez, G., & Soriano-Santos, J. (2016). Evaluation of the Antioxidant Activity of Aqueous and Methanol Extracts of *Pleurotus ostreatus* in Different Growth Stages. *Frontiers in Microbiology*, 7, 1–9. <https://doi.org/10.3389/fmicb.2016.01099>
- Hasan, M. T., Khatun, M. H. A., Sajib, M. A. M., Rahman, M. M., Rahman, M. S., Roy, M., ... Ahmed, K. U. (2015). Effect of Wheat Bran Supplement with Sugarcane Bagasse on Growth, Yield and Proximate Composition of Pink Oyster Mushroom (*Pleurotus djamor*). American J. Food Science and Technology, 3(May), 150–157. <https://doi.org/10.12691/ajfst-3-6-2>
- Hazrati, S., Tahmasebi-Sarvestani, Z., Mokhtassi-Bidgoli, A., Modarres-Sanavy, S. A. M., Mohammadi, H., & Nicola, S. (2017). Effects of Zeolite and Water Stress on Growth, Yield and Chemical Compositions of *Aloe vera* L. *Agricultural Water Management*, 181, 66–72. <https://doi.org/10.1016/j.agwat.2016.11.026>
- Hlerema, I. N., Eiasu, B. K., & Koch, S. H. (2017). Pineapple (*Ananas comusus*) Plant Material as Supplement for Maize Residue-based Oyster Mushroom Substrate and Reduction of Cadmium Soil Contamination. *HortScience*, 52(4), 667–671. <https://doi.org/10.21273/HORTSCI10880-16>
- Hoa, H. T., Wang, C. L., & Wang, C. H. (2015). The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, 43(4), 423–434. <https://doi.org/10.5941/MYCO.2015.43.4.423>
- Islam, W., & Riaz, A. (2017). Yield and Biological Efficiency of *Pleurotus ostreatus* (Jacq . Fr .) Cultivated Upon Various Weeds and Agricultural Wastes. *Pakistan J. Weed Science Research*, 23, 271–279.
- Jeena, G. S., Punetha, H., Prakash, O., Chandra, M., & Kushwaha, K. P. S. (2014). Study on In-vitro Antioxidant Potential of Some Cultivated *Pleurotus* Species (Oyster Mushroom). *Indian J. Natural Products and Resources*, 5(1), 56–61.
- Jennings, D. H. (1996). The Physiology of Fungal Nutrition. *Choice Reviews Online*, 33(07), 33-3899-33–3899. <https://doi.org/10.5860/choice.33-3899>
- Keles, A., Koca, I., & Gencelep, H. (2011). Antioxidant Properties of Wild Edible Mushrooms. *J. Food Processing & Technology*, 02(06), 1–6. <https://doi.org/10.4172/2157-7110.1000130>
- Khan, M. W., Ali, M. A., Khan, N. A., Khan, M. A., Rehman, A., & Javed, N. (2013). Effect of Different Levels of Lime and pH on Mycelial Growth and Production Efficiency of Oyster Mushroom (*Pleurotus* spp.). *Pakistan J. Botany*, 45(1), 297–302.
- Khan, N. A., Amjad, A., Binyamin, R., Rehman, A., & Hafeez, O. B. A. (2017). Role of Various Supplementary Materials with Cotton Waste Substrate for Production of *Pleurotus ostreatus* an Oyster Mushroom. *Pakistan J. Botany*, 49(5), 1911–1915.
- Kim, J. S., Lee, Y. Y., & Kim, T. H. (2016). A Review on Alkaline Pretreatment Technology for Bioconversion of Lignocellulosic Biomass. *Bioresource Technology*, 199, 42–48. <https://doi.org/10.1016/j.biortech.2015.08.085>
- Li, H., Zhang, Z., Li, M., Li, X., & Sun, Z. (2017). Yield, Size, Nutritional Value, and Antioxidant Activity of Oyster Mushrooms Grown on Perilla Stalks. *Saudi J. Biological Sciences*, 24(2), 347–354. <https://doi.org/10.1016/j.sjbs.2015.10.001>
- Li, R., Wang, J. J., Zhang, Z., Shen, F., Zhang, G., Qin, R., ... Xiao, R. (2012). Nutrient Transformations During Composting of Pig Manure with Bentonite. *Bioresource Technology*, 121, 362–368. <https://doi.org/10.1016/j.biortech.2012.06.065>
- Li, X., & Yao, Y. (2005). Revision of the Taxonomic Position of the Phoenix Mushroom. *Mycotaxon*, 91, 61–74. Retrieved from <https://agris.fao.org/agris-search/search.do?recordID=US201300984852>
- Lishma, N. P., & Das, L. (2017). Yield Performance of *Agaricus bisporus* in Different Composted Substrates. *Trends in Biosciences*, 10(27), 5890–5891.

- Martos, E. T., Zied, D. C., Junqueira, P. P. G., Rinker, D. L., Da Silva, R., Toledo, R. C. C., & Dias, E. S. (2017). Casing Layer and Effect of Primordia Induction in the Production of *Agaricus subrufescens* Mushroom. *Agriculture and Natural Resources*, 51(4), 231–234. <https://doi.org/10.1016/j.anres.2017.04.003>
- Milosevic, T., & Milosevic, N. (2009). The Effect of Zeolite, Organic and Inorganic Fertilizers on Soil Chemical Properties, Growth and Biomass Yield of Apple Trees. *Plant Soil and Environment*, 12, 528–535.
- Milošević, T., Milošević, N., & Glišić, I. (2013). Tree Growth, Yield, Fruit Quality Attributes and Leaf Nutrient Content of “Roxana” Apricot as Influenced by Natural Zeolite, Organic and Inorganic Fertilisers. *Scientia Horticulturae*, 156, 131–139. <https://doi.org/10.1016/j.scienta.2013.04.002>
- Myronycheva, O., Bandura, I., Bisko, N., Gryganskyi, A. P., & Karlsson, O. (2017). Assessment of the Growth and Fruiting of 19 Oyster Mushroom Strains for Indoor Cultivation on Lignocellulosic Wastes. *BioResources*, 12(3), 4606–4626. <https://doi.org/10.15376/biores.12.3.4606-4626>
- Nguyen, T. K., Im, K. H., Choi, J., Shin, P. G., & Lee, T. S. (2016). Evaluation of Antioxidant, Anti-cholinesterase, and Anti-inflammatory Effects of Culinary Mushroom *Pleurotus pulmonarius*. *Mycobiology*, 44(4), 291–301. <https://doi.org/10.5941/MYCO.2016.44.4.291>
- Obodai, M., Cleland-Okine, J., & Vowotor, K. A. (2003). Comparative Study on the Growth and Yield of *Pleurotus ostreatus* Mushroom on Different Lignocellulosic By-products. *J. Industrial Microbiology and Biotechnology*, 30(3), 146–149. <https://doi.org/10.1007/s10295-002-0021-1>
- Obodai, M., Owusu, E., Schiwenger, G. O., Asante, I. K., & Dzomeku, M. (2014). Phytochemical and Mineral Analysis of 12 Cultivated Oyster Mushrooms (*Pleurotus Species*). *Advances in Life Science and Technology*, 26, 35–43.
- Ok, C. H., Anderson, S. H., & Ervin, E. H. (2003). Amendments and Construction Systems for Improving the Performance of Sand-Based Putting Greens. *Agronomy J.*, 95(6), 1583–1590.
- Pardo, A., Emilio Pardo, J., De Juan, J. A., & Zied, D. C. (2010). Modelling the Effect of the Physical and Chemical Characteristics of the Materials Used As Casing Layers on the Production Parameters of *Agaricus bisporus*. *Archives of Microbiology*, 192(12), 1023–1030. <https://doi.org/10.1007/s00203-010-0631-3>
- Prieto, J. M. (2012). Procedure : Preparation of DPPH Radical , and Antioxidant Scavenging Assay. *DPPH Microplate Protocol*, 7–9.
- Radi, M., Mahrouz, M., Jaouad, A., & Amiot, M. J. (2003). Influence of Mineral Fertilization (NPK) on the Quality of Apricot Fruit (cv. *Canino*). The Effect of the Mode of Nitrogen Supply. *Agronomie*, 23(8), 737–745. <https://doi.org/10.1051/agro:2003052>
- Rashidi, A. N. M., & Yang, T. A. (2016). Nutritional and Antioxidant Values of Oyster Mushroom (*P. sajor-caju*) Cultivated on Rubber Sawdust. *International J. Advanced Science, Engineering and Information Technology*, 6(2), 161–164. <https://doi.org/10.18517/ijaseit.6.2.610>
- Reddy, G. V., Ravindra Babu, P., Komaraiah, P., Roy, K. R. R. M., & Kothari, I. L. (2003). Utilization of Banana Waste for the Production of Lignolytic and Cellulolytic Enzymes by Solid Substrate Fermentation Using Two *Pleurotus* species (*P. ostreatus* and *P. sajor-caju*). *Process Biochemistry*, 38(10), 1457–1462. [https://doi.org/10.1016/S0032-9592\(03\)00025-6](https://doi.org/10.1016/S0032-9592(03)00025-6)
- Royse, D. J., & Sanchez-Vazquez, J. E. (2003). Influence of Precipitated Calcium Carbonate (CaCO<sub>3</sub>) on Shiitake (*Lentinula edodes*) Yield and Mushroom Size. *Bioresource Technology*, 90(2), 225–228. [https://doi.org/10.1016/S0960-8524\(03\)00119-6](https://doi.org/10.1016/S0960-8524(03)00119-6)
- Salmalian, N., Peyvast, G., & Olfati, J. A. (2016). Amendments for Cultivation of Oyster Mushroom in Boxes or Plastic Bags. *International J. Vegetable Science*, 22(2), 115–120. <https://doi.org/10.1080/19315260.2014.880544>
- Sardar, H., Ali, M. A., Anjum, M. A., Nawaz, F., Hussain, S., Naz, S., & Karimi, S. M. (2017). Agro-industrial Residues Influence Mineral Elements Accumulation and Nutritional Composition of King Oyster Mushroom (*Pleurotus eryngii*). *Scientia Horticulturae*, 225, 327–334. <https://doi.org/10.1016/j.scienta.2017.07.010>
- Savvas, D., Samantouros, K., Paralemos, D., Vlachakos, G., Stamatakis, M., & Vassilatos, C. (2004). Yield and Nutrient Status in the Root Environment of Tomatoes (*Lycopersicon esculentum*) Grown on Chemically Active and Inactive Inorganic Substrates. *Acta Horticulturae*, 644, 377–383. <https://doi.org/10.17660/ActaHortic.2004.644.50>
- Shahbaz, A. K., Adnan Ramzani, P. M., Saeed, R., Turan, V., Iqbal, M., Lewińska, K., ... Mahmood-ur-Rahman (2019). Effects of Biochar and Zeolite Soil Amendments with Foliar Proline Spray on Nickel Immobilization, Nutritional Quality and Nickel Concentrations in Wheat. *Ecotoxicology and Environmental Safety*, 173(Febuary), 182–

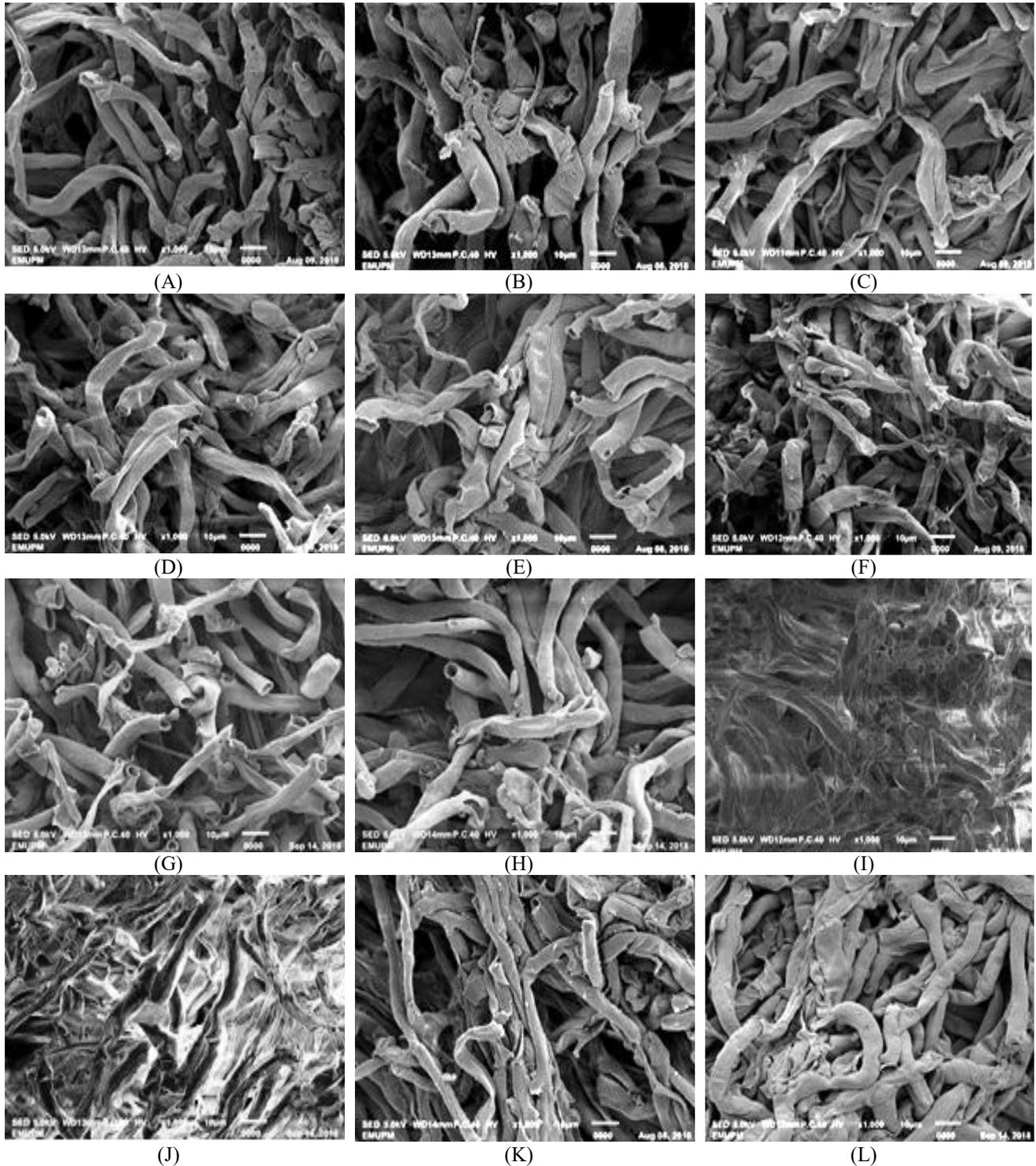
191.  
<https://doi.org/10.1016/j.ecoenv.2019.02.025>
- Shahbaz, A. K., Lewińska, K., Iqbal, J., Ali, Q., Mahmood-ur-Rahman, Iqbal, M., ... Ramzani, P. M. A. (2018). Improvement in Productivity, Nutritional Quality, and Antioxidative Defense Mechanisms of Sunflower (*Helianthus annuus* L.) and Maize (*Zea mays* L.) in Nickel Contaminated Soil Amended with Different Biochar and Zeolite Ratios. *J. Environmental Management*, 218, 256–270. <https://doi.org/10.1016/j.jenvman.2018.04.046>
- Shi, Y., Huang, Z., Liu, X., Imran, S., Peng, L., Dai, R., & Deng, Y. (2016). Environmental Materials for Remediation of Soils Contaminated with Lead and Cadmium Using Maize (*Zea mays* L.) Growth As a Bioindicator. *Environmental Science and Pollution Research*, 23(7), 6168–6178. <https://doi.org/10.1007/s11356-015-5778-7>
- Smiderle, F. R., Olsen, L. M., Carbonero, E. R., Baggio, C. H., Freitas, C. S., Marcon, R., ... Iacomini, M. (2008). Anti-inflammatory and Analgesic Properties in a Rodent Model of a (1→3),(1→6)-linked  $\beta$ -glucan Isolated from *Pleurotus pulmonarius*. *European J. Pharmacology*, 597(1–3), 86–91. <https://doi.org/10.1016/j.ejphar.2008.08.028>
- Sozana, K., Kamarudin, N., Hamdan, H., & Mat, H. (2003). Methane Adsorption Characteristic Dependency on Zeolite Structures and Properties. *Synthesis*, (December), 29–30. Retrieved from <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.599.3622&rep=rep1&type=pdf>
- Srivastava, A., Harish, R., & Shivanandappa, T. (2006). Novel Antioxidant Compounds from the Aqueous Extract of the Roots of *Decalepis hamiltonii* (Wight and Arn.) and Their Inhibitory Effect on Low-Density Lipoprotein Oxidation. *J. Agricultural and Food Chemistry*, 54(3), 790–795. <https://doi.org/10.1021/jf052433x>
- Veberic, R. (2016). The Impact of Production Technology on Plant Phenolics. *Horticulturae*, 2(3). <https://doi.org/10.3390/horticulturae2030008>
- Vieira, F. R., & de Andrade, M. C. N. (2016). Optimization of Substrate Preparation for Oyster Mushroom (*Pleurotus ostreatus*) Cultivation by Studying Different Raw Materials and Substrate Preparation Conditions (Composting: Phases I and II). *World J. Microbiology and Biotechnology*, 32(11). <https://doi.org/10.1007/s11274-016-2152-y>
- Wong, F. C., Chai, T. T., Tan, S., & Yong, A. (2013). Evaluation of Bioactivities and Phenolic Content of Selected Edible Mushrooms in Malaysia. *Tropical J. Pharmaceutical Research*, 12(6), 1011–1016.
- Yang, W. J., Guo, F. L., & Wan, Z. J. (2013). Yield and Size of Oyster Mushroom Grown on Rice/Wheat Straw Basal Substrate Supplemented with Cotton Seed Hull. *Saudi J. Biological Sciences*, 20(4), 333–338. <https://doi.org/10.1016/j.sjbs.2013.02.006>
- Yoon, K. N., Alam, N., Lee, K. R., Shin, P. G., Cheong, J. C., Yoo, Y. B., & Lee, T. S. (2011). Antioxidant and Antityrosinase Activities of Various Extracts From the Fruiting Bodies of *Lentinus lepideus*. *Molecules*, 16(3), 2334–2347. <https://doi.org/10.3390/molecules16032334>
- Zied, D. C., Pardo-Gimenez, A., de Almeida Minihoni, M.T., Boas, R. L. V., Alvarez-Orti, M., & Pardo-Gonzalez, J. E. (2012). Characterization, Feasibility and Optimization of *Agaricus subrufescens* Growth Based on Chemical Elements on Casing Layer. *Saudi J. Biological Sciences*, 19(3), 343–347. <https://doi.org/10.1016/j.sjbs.2012.04.002>

## Appendix 1

The Image of Fruiting Body between Different Treatments from Each Flush (1-4<sup>th</sup> Flush).

Diameter of pileus; a) lime (first flush) b) zeolite (first flush) c) gypsum (first flush) d) lime (second flush) e) zeolite (second flush) f) gypsum (second flush) g) lime (third flush) h) zeolite (third flush) i) gypsum (third flush) j) lime (fourth flush) k) zeolite (fourth flush) l) gypsum (fourth flush); (scale: cm).

**Appendix 2: SEM View on Mushroom Fruiting Body between Different Treatment of Alkaline Materials in Every Flushes (1-4<sup>th</sup> Flush).**



Scanning electron micrographs on cross-section of stalks of *Pleurotus pulmonarius* between different treatment of alkaline materials; a) lime (first flush) b) gypsum (first flush) c) zeolite (first flush) d) lime (second flush) e) gypsum (second flush) f) zeolite (second flush) g) lime (third flush) h) gypsum (third flush) i) zeolite (third flush) j) lime (fourth flush) k) gypsum (fourth flush) l) zeolite (fourth flush).