



Evaluating Untraditional Agro-Industrial Wastes in *Pleurotus ostreatus* Cultivation and its Spent Countervailing Composted Soil in Lettuce Plantation as an Added Value

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Received: 18 Feb. 2023

Accepted: 25 Mar. 2023

Published: 30 Mar. 2023

ABSTRACT

The oyster mushroom *Pleurotus ostreatus* production in submerged culture (SMC) was conducted on variable agro-industrial wastes including rice straw (RS), sugarcane bagasse (SCB), orange peels (OP), olive mill pomace (OMP), moringa leaves (ML) and jojoba meal (JM), where RS, SCB, OP and OM offered *P. ostreatus* growth the highest dry weights and protein contents. Solid state culturing (SSC) for *P. ostreatus* was applied on RS, SCB, OP and OMP in combination and permutation. The best biological efficiencies (BE) and production rates (PR) for *P. ostreatus* were achieved in presence of OP mixed in combination with either RS, SCB or OM to be 86.6%, 173; 56.1%, 104 and 34.5%, 61 [BE%, PR], respectively. As the resulting spent mushroom substrates (SMS) were applied in pot experiment with soil for lettuce (*Lactuca sativa*) plantation, it was found to gradually countervail the role of compost and clay soil. The harvested *L. sativa* grown on SMS mixed in variable ratios of 25, 50 and 75% with composted clay soil increased harvested dry weight by 7, 15 and 3%, respectively, compared to composted soil alone. Correlation coefficients of chemical and physical analysis for both *P. ostreatus* and *L. sativa* against detected laccase, peroxidase and catalase activities were discussed.

Keywords: *Pleurotus ostreatus*, *Lactuca sativa*, SMS, catalase, laccase, peroxidase agroindustrial wastes

1. Introduction

Agroindustrial wastes, either as residues from agricultural field processes or as wastes resulting from food industries are considered added valuables rather than being an environmental problem. Their constituents of holocellulose, proteins, phenols and oils have been targeted in many production fields for human prosperity. Some of widely cultivated or newly introduced crops as sources for agroindustrial wastes in Egypt are shown in Table (1). Agroindustrial wastes availabilities were correlated to most recent data available for their source crop type, cultivation area and annual yield.

The structural content of wastes resulting from processing any crop and its amount could be predicted depending on their annual mass production and type of process applied during and after harvest. For instance, the amount of rice straw (RS) taken off the field depends mainly on the cutting height (Maguyon-Detras *et al.*, 2019). Also, moringa leaves (ML) analytical contents varied according to irrigation water quality and cultivation soil (Abdelaty *et al.*, 2022). The olive mill pomace (OMP)

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resulting from olive oil extraction methods including traditional pressing, 3-phase and 2-phase systems differ in their structural analysis (Dermeche *et al.*, 2013).

On the other hand, the phenolic compounds as secondary metabolites in plants and their lignocellulosic wastes have free radical scavenging and antimicrobial effects. They vary in their function and structure including phenolic acids, flavonoids, lignins and tannins, which are widely discussed in many research studies, for instance: in RS (Elzaawely *et al.*, 2017), SCB (Zheng *et al.*, 2017), OMP (Chanioti *et al.*, 2021), CC (Hernández *et al.*, 2018), OP (Omoba *et al.*, 2015), ML (Hassan *et al.*, 2021) and JM (Wagdy *et al.*, 2011).

Table 1: Agroindustrial wastes availability in Egypt presented in descending order according to source crop cultivation area

Crop				Agroindustrial waste		
Name	Area (ha)	Annual yield (MMT)	Year	Name		Estimated (MT)
Sugarcane	128298 ^(a)	12.36 ^(a)	2021 ^(a)	Sugarcane bagasse	SCB	4176 ^(b)
Corn	0.93x10 ⁶ ^(c)	7.44 ^(c)	2022-2023 ^(c)	Corn cobs	CC	-
Rice	0.60x10 ⁶ ^(c)	3.60 ^(c)	2022-2023 ^(c)	Rice straw	RS	2.1 ^(d)
Olive	99102 ^(a)	0.98 ^(a)	2021 ^(a)	Olive mill pomace	OMP	50% ^(e)
Orange	17200 ^(c)	3.6 ^(c)	2022-2023 ^(c)	Orange peel	OP	30-40% ^(f)
Jojoba	-	229 ton ^(g)	2017 ^(g)	Jojoba meal	JM	-
Moringa	-	-	-	Moringa leaves	ML	-

MMT: million metric ton, MT: million ton,

(a) FAO statistical data (2022), (b) United nations database (2020), (c) USDA FAS (2023), (d) Central Agency for Public Mobilization and Statistics (CAPMAS), (e) Jerman and Mozetič (2012), (f) Meselhy *et al.* (2022), (g) Gabt (2020), (h) Barakat and Ghazal (2016)

Establishing consolidated bioprocesses using those agroindustrial wastes in mushroom production and even as soil amendments after being biologically modified are considered an important target worthy to study. Choosing mushroom cultivation on agroindustrial wastes was motivated by their capabilities and benefits. Mushrooms are the sporophore type of fungi that have fleshy fungus fruiting structure, some of which are edible and have worthy nutritional values. On the other hand, mushrooms as saprophytic basidiomycetes that belong to wood-degrading white-rot fungi are well known of their capabilities to grow on lignocellulosic wastes efficiently under controllable conditions, because of their extracellular enzymatic consortium that catalyze lignocellulosic degradation (Camassola *et al.*, 2013).

The oyster mushroom *Pleurotus ostreatus* is a species of white-rot fungi belongs to a subclass of lignin-degrading microorganisms that produce extracellular enzymes including cellulases, laccases (EC 1.10.3.2) and peroxidases (EC 1.11.1.7) (Palmieri *et al.*, 1997; Giardina *et al.* 2000; Kumla *et al.*, 2020; Melanouri *et al.*, 2022). Laccases and peroxidases catalyze oxidation of several substances, particularly phenols and aromatic amines (d’Acunzo *et al.*, 2002). They both differ in their prosthetic groups, beside that laccases have lower oxidation potential than peroxidases (Kirk and Farrell, 1987; Hayashi and Yamazaki, 1979; Farhangrazi *et al.*, 1994). Mushroom growth in submerged and solid-state conditions on lignocellulosic wastes of different composition were widely studied among many researches (Elisashvili *et al.*, 2008; Téllez-Téllez *et al.*, 2008; An *et al.*, 2016). The growth of *P. ostreatus* begins with vegetative growth where its mycelia colonize the substrate followed by reproductive growth where its fruit body called basidiome is formed representing the mushroom itself (Díaz-Godínez *et al.*, 2017). In the submerged growth conditions or so-called submerged fermentation (SMF) the substrate is immersed in liquid state both sterilized and the application is progressing under more controlled aseptic conditions for studying and producing bioactive organic substances (Vamanu, 2012; Dudekulav *et al.*, 2020). On the other hand, the solid-state growth or so-called solid-state fermentation (SSF) is applied for mushroom fruiting production (Díaz-Godínez *et al.*, 2017). The enzyme production capacities vary under either growth conditions in which they favor SSF which have higher enzyme/biomass yield fraction (Viniegra-González *et al.*, 2003; Díaz-Godínez *et al.*, 2017).

The residue remaining from mushroom cultivation on agroindustrial wastes under solid state conditions and after being harvested was called spent mushroom waste (SMW), otherwise known as spent mushroom substrate (SMS). The remaining mushroom stipe and base in SMS was found to be

full of nutrients that shared in SMS quality (Cunha Zied *et al.*, 2020). SMS was directed by many research studies to compost production or to be used directly in soil mixtures as an added value. Composted SMS (SMC) was utilized as fertilizer and soil conditioner for horticulture applications (Umor *et al.*, 2021). On the other hand, the insist of using SMS as nutritional conditioner was reviewed in many studies (Zhang and Fadel, 2002; Medina *et al.*, 2012; Carmo *et al.*, 2021; Oliveira *et al.*, 2022). The use of spent mushroom substrate and its compost, specifically resulting from *P. ostreatus*, as soil conditioner and a competitive fertilizer in lettuce cultivation proved their reliability and efficiency in many previous studies (Zied *et al.*, 2021; Hernández *et al.*, 2021).

Soil enzymes are an important parameter in carbon sequestration and soil nutrient dynamics (Lemanowicz *et al.*, 2023). Soil enzymes activities are indicators for soil quality measuring soil microbial activity related to nutrient transformations (Ahamadou and Huang, 2012). Soil dehydrogenases (EC 1.1.1.) represent oxidoreductase enzymes that do not accumulate extracellularly in soil but occur inside microbial cells linked to their oxidoreduction processes and indicate overall soil microbial activity, as they do share in biological oxidation of soil organic matter (OM) upon which their activities are found proportional to microbial biomass in soil (Wolinska, and Stepniewsk, 2012). Peroxidase (EC 1.11.1.7) and catalase (EC 1.11.1.6) activities were used as indicators to quantify the stress conditions facing lettuce, specifically in their leaves (Aires *et al.*, 2021; Leitão *et al.*, 2021).

In the present research, the efficiency of using agroindustrial wastes in *P. ostreatus* growth under submerged and solid-state conditions followed by the use of resulting SMS in combination and permutation with composted soil in lettuce (*Lactuca sativa* L.) pot cultivation were studied.

2. Materials and Methods

2.1. Wastes

Sources from where the agro-industrial wastes were gathered included local farms for Rice straw (RS) and corn cobs (CC), juice shops for sugarcane bagasse (SCB) and orange peel (OP), while Olive milled pomace (OM), moringa leaves (ML) and jojoba meal (JM) were gathered from Agricultural Research Center (ARC) at Giza governorate – Egypt. All wastes were air dried, chopped or crushed to minimum size of 0.5 mm.

2.2. Mushroom and spawn preparation

Oyster mushroom (*Pleurotus ostreatus*) was generously offered by department of microbiology at SWERI - ARC – Egypt. The white-rot fungus was maintained on sterilized potato dextrose agar medium slants (PDA) containing: 200 g/l diced potatoes; 20 g/l glucose; 15 g/l agar, grown at 25°C for regular subculture. Inoculums were prepared on PDA plates from pre grown slants and used as source for mycelial agar disks (1 cm in diameter) for inoculation. Spawn was prepared as described by Atila (2016). The sorghum grains presoaked in water for 12 h were mixed with 10% wheat bran and 1% CaCO₃, on weight bases and were sterilized in glass bottles at 121°C for 30 min. Cooled bottles were inoculated with mycelial agar discs and incubated in the dark at 28 °C until completion of mycelial growth.

2.3. Submerged culturing (SMC)

Different wastes (20g) were distributed individually into 500ml jars, each containing 200 ml synthetic medium (g. L⁻¹): yeast extract (0.5), (NH₄)₂SO₄ (1.0) and tween 80 (1.0), pH was adjusted at 4.5, while the wide jars nozzles were firmly covered with thick cotton cloth sandwich pads and sterilized at 121°C for 15 min. The waste in each jar was inoculated with five mycelial agar discs and incubated at 28°C for 50±5 days until mushroom flush and harvest.

2.4. Solid State culturing (SSC)

Waste types on which *P. ostreatus* gave its highest yield were chosen as substrates for SSC. Dry wastes individually or mixed in combinations and permutations with other wastes in a weight ratio of 1:1, after which 10% (w/w) wheat bran and 1% (w/w) CaCO₃ were added to construct the substrate final form. Adequate water quantity was added to each substrate and mixed well so that they form a clump when squeezed without any excess water coming out. The moistened substrates of 1kg were filled into heat-resistant polypropylene bags and compressed well, after which a plastic neck was inserted around each bag mouth, plugged-in with cotton and sealed with a suitable rubber or aluminium

cap. After pasteurization, the bags were let to cool down to room temperature prior to inoculation with mushroom spawn under sterile conditions. Incubation was conducted in sanitized incubator at 28°C / humidity 70% in dark for 3-4 weeks, as primordia will be observed 1 week after complete colonization of the substrates. Mushroom fruiting bodies were harvested from two successive flushes (Yield) during production time (PT).

2.5. Plantation pot experiment:

Lettuce seedlings (*Lactuca sativa* L.) of 40 days old were brought from Horticulture Research Institute at ARC- Giza governorate. Lettuce transplants were selected for uniformity in size were transplanted in pots with size capacity of 10 kg soil (transplant /10 kg pot). The soil was mixed thoroughly with compost (35 g compost. Kg⁻¹soil). Pots were filled with soil compost mixture in 4 categories by volume, as shown in Table (2). The soil mixture introduced with SMS were mixed thoroughly as three combinations at instructed ratios before filling pots and SMS with/without compost as the 4th and 5th combinations. The pot experiment was conducted in pentaplicates (5 pots).

The NPK fertilization was followed after the recommendation of Agricultural Extension Office – Ministry of Agriculture – Egypt, at rates of 150: 45: 65 kg/ha as N: P₂O₅: K₂O, respectively, as mentioned by El-Mogy *et al.* (2020). NPK was applied as ammonium nitrate (33%N), calcium super phosphate (15.5% P₂O₅) and C (48% K₂O) equivalent to 63, 8 and 23 Kg/fed of NPK, respectively. Calcium super phosphate was added once to soil combinations in each pot before transplantation, while either of ammonium nitrate and potassium sulfate was added as half dose at a time after 15 and 30 days of plantation. After harvest, both lettuce and soil samples were collected for determination of yield parameters, chemical and biochemical properties. The other agricultural practices were done according to the recommended methods for lettuce crop (El-Ghinbihi, and Mahmoud, 2007).

Table 2: Pot design for planting *L. sativa* in combinations and permutations of soil, compost and spent mushroom substrate (SMS) mixtures.

Pot content	Combination	Soil % (V/V pot)	Compost (g/pot)	SMS % (V/V pot)
Soil mix (soil + compost)	C1	100	35	0
Soil mix + 25% SMS	C2	75	26.3	25
Soil mix + 50% SMS	C3	50	17.5	50
Soil mix + 75% SMS	C4	25	8.8	75
100% SMS (no soil mix)	C5	0	0	100
100% SMS + compost	C6	0	35	100

2.6. Physical and chemical analysis

Soil texture was identified by the amount of sand, silt, and clay, confirmed by soil saturation percentage (SP) as mentioned by Jones (2001). The soil pH values were measured according to Thomas (2018), soil electric conductivity (EC, dSm⁻¹) was determined according to Rhoades (2018), while the total dissolved solids TDS were estimated based on measured EC value, as TDS (ppm) = EC x 640 (Rusydi, 2018). Agro-industrial wastes, mushroom, lettuce harvests and soil were all dried in forced air oven at 70°C until stable weight to calculate their dry weights.

The concentrations of available elements in soil including calcium (Ca), magnesium (Mg), phosphorus (P) and sodium (Na) as major elements, copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) as micronutrients in soil sample digest (using a nitric acid/hydrogen peroxide (HNO₃/H₂O₂) digestion mixture) were determined by inductively coupled plasma spectrometry (ICP) (Ultima 2 JY Plasma) done at Research and Studies Unit (component of analyzes and studies) at SWERI-ARC, as described by Kalra (1997). On the other hand, potassium was determined in the same digest by flame photometry according to the method of Chapman and Pratt (1962).

Organic matter (OM) and organic carbon (OC) were determined according to Nelson and Sommers (1996). Total nitrogen (TN) was determined using the micro-kejedahl method and protein content was calculated based on determined nitrogen value (protein = 6.25 x N) as described by Miller and Horneck (1997). Holocellulose contents were determined using modified method described by Wise and Ratliff (1947). The samples were vacuum-dried at 40°C overnight and extracted with ethanol/toluene mixture (1:2 v/v) for 6h in Soxhlet, dried and weighed to calculate extractives.

Delignification of extracted dried sample (0.5 gram) was done by treating it with 0.2g NaClO in 30ml of 7.5% acetic acid solution at 80°C for 1h (4 times). The product was filtered, washed with distilled water several times, vacuum dried at 40°C for 24h and weighed as holocellulose. Fats were determined according to the methods described in AOAC (1990).

The total soluble solids (TSS) in lettuce leaves were measured by refractometer according to Magwaza and Opara (2015), as the results in “degrees Brix” (°Brix) were equivalent to percentages (%) (Dongare *et al.*, 2014).

Chlorophylls and carotenoids were extracted with acetone (100%) and determined according to Lichtenthaler and Buschmann (2001). Spectrophotometric absorption measurements (A) for chlorophyll a (C_a) and b (C_b) in acetone were done at 661.6nm and 644.8nm, respectively, while carotenoids (Carot) including carotene and xanthophyll were measured at 470nm. The following equations were applied (ug/ml):

$$\begin{aligned}C_a &= 11.24 A_{661.6} - 2.04 A_{664.8} \\C_b &= 20.13 A_{644.8} - 4.19 A_{661.6} \\Carot. &= (1000 A_{470} - 1.90 C_a - 63.14 C_b) \div 214\end{aligned}$$

1.7. Enzyme activities

Fresh sample material of 0.5g was homogenized in 10 ml cold phosphate buffer (50 mM, pH 7). The homogenates were centrifuged at 4000 rpm at 20°C for 20 min. The supernatant was used as a raw extract for the enzymatic assay. Reagents used in the following assays were prepared in the same previously indicated buffer.

Laccase activity was assayed by determining the rate of ABTS oxidation (Childs, and Bardsley, 1975) following the update described by Palmieri *et al.* (1997). Oxidation of ABTS (1 ml, 0.03%) by the sample (1 ml) was monitored by increase in absorbance at 420 nm ($\epsilon_{420 \text{ nm}} 36,000 \text{ M}^{-1} \text{ cm}^{-1}$) at 30°C. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1.0 μmol of ABTS per min.

Peroxidase activity was determined using methylene blue assay according to Magalhaes *et al.* (1996). The assay mixture contained 2.2 ml of the diluted supernatant, 0.1 ml of methylene blue (1.2 mM). The reaction was started by the addition of 0.1 ml of 2.7 mM H₂O₂. The conversion of the dye to Azure C was monitored by the measurement of the decrease in absorbance at 664 nm. One unit of peroxidase was defined as the amount of enzyme required to oxidize 1.0 μmol of methylene blue per min.

Catalase activity was assayed based on the breakdown of H₂O₂ detected in UV at 240nm, as mentioned by Beers and Sizer (1952). The method steps were done according to Pine *et al.* (1984), as 0.1 ml of sample was added to freshly prepared 1.4ml H₂O₂ (13.2M). The mixed solution was detected for loss in absorbance at 240nm. Units of catalase were calculated by using a molar absorbance index for H₂O₂ of 43.6. One unit of catalase was defined as the amount of enzyme required to degrade 1.0 μmol of H₂O₂ per min.

Dehydrogenase activity (DeH-ase) was assayed using tri-phenyl tetra-zolium chloride (TTC) method according to Casida *et al.* (1964). Fresh soil sample of 2g added to 20ml capacity test tube followed by 2 ml TTC 1% in TRIS-HCl buffer (0.1M, pH 7) and incubated at 30°C/24 h. Extract with 10 ml methanol, shake and filter, after which absorbance was detected at 485nm. One unit of dehydrogenase was defined as the amount of enzyme required to hydrolyze of TTC to form 1.0 μmol of tri-phenyl formazan (TPF) per hour ($\mu\text{mol g}^{-1}\text{h}^{-1}$).

Phosphatase activity (P-ase) was measured using para nitro phenyl phosphate (PNPP) according to Tabatabai and Bremner (1969). As fresh soil of 1g was placed in 250ml Erlenmeyer flask, it was followed by adding 4ml of modified universal buffer (MUB) pH 6.5, 0.25ml toluene and 1ml PNPP. Incubation was processed at 30°C for 1h, after which 1ml CaCl₂ (0.5M) and 4ml NaOH (0.5M) were added and the resulting filtrate was measured for absorbance at 420nm. Phosphatase activity of one unit was defined as the amount of enzyme required to release 1 μg of p-nitro phenol hydrolyze per hour.

1.8. Mushroom yield parameters

Mushroom was harvested from the first and second flushes in each trial, where its fresh weight (g) from the two flushes were gathered and represented the total yield (Y) from each trial. Biological

efficiency % (BE) and production rate (PR) were calculated afterwards as described by Moonmoon *et al.* (2011) according to the following equations:

$$\text{Biological efficiency \% BE} = \frac{\text{Yield (g)}}{\text{waste wt. (g)}} \times 100 ,$$

Waste wt.: 1000 g on dry basis

Yield: fresh weight in (g) of total mushroom fruiting bodies harvested from two flushes

$$\text{Production rate PR} = \frac{\text{BE}}{\text{PT}} ,$$

PT: the total time in days required for production

1.9. Statistical analysis

All experimental trials were done in triplicates (n=3), except for the pot experiment which was done in pentaplicates (n=5). Data means, standard errors and correlation coefficients were analyzed and their graphs were constructed using Excel statistical program software (Microsoft office, 2007). Standard curve equations on which most of enzymatic assays were depending were constructed using Prism program version 5.03 for windows (GraphPad software, Inc.). Data analysis of variance (ANOVA) was done to determine their least significant differences (LSD) through one way completely randomized Duncan's Multiple Range test at significance level of 5% for one factor at a time (one way analysis) using CoStat 6.45 program (CoHort software 6.311).

3. Results and Discussion

3.1. Wastes analysis

Agroindustrial waste analysis presented in Figure (1) showed that the highest organic matter (90%) and fat (15%) contents were in OMP, holocellulose (61%) in RS and CC, protein in JM (23%) and most wide C/N ratio (82:1) was found in both SCB and RS.

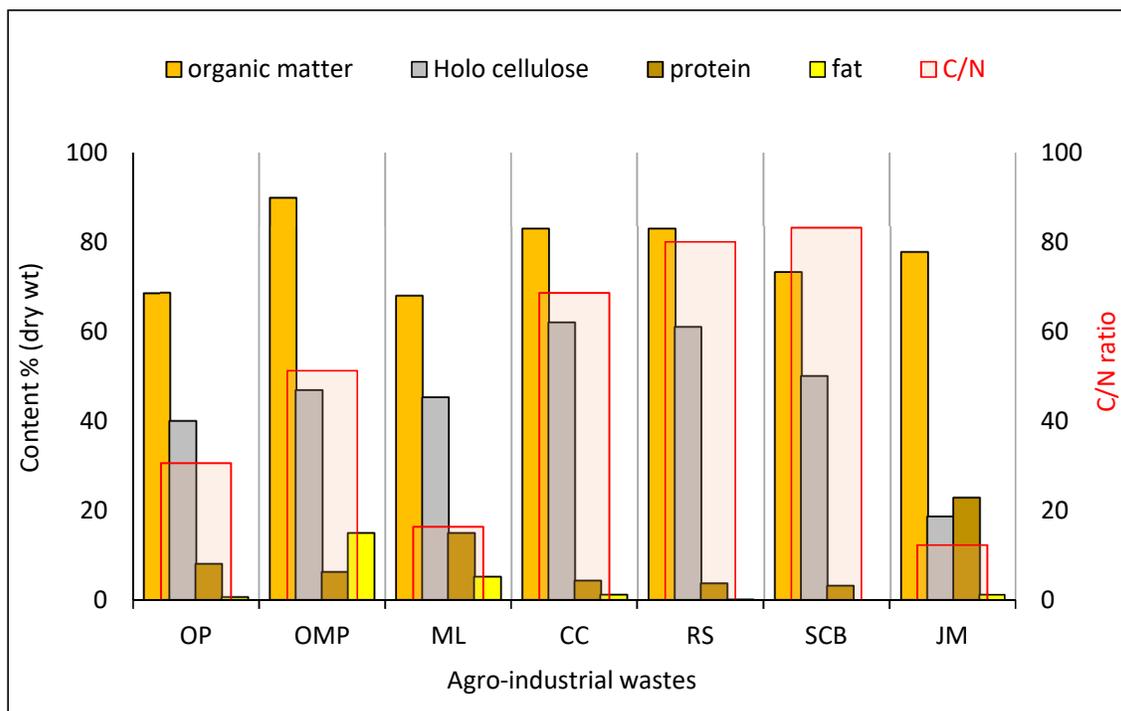


Fig. 1: Agroindustrial wastes major contents (%) analyzed on dry weight bases.

Compared to the presented waste analysis in our study, previous analytical studies reviewed on those wastes were close in their results. RS consisted of cellulose (32-47%) and hemicellulose (20-30%) (Karimi *et al.*, 2006). Also, SCB had high content in cellulose and hemicellulose to be 25-50 and 20-35, respectively (Mahmud and Anannya, 2021). CC structure of cellulose and hemicellulose were in range of 28-52 and 32-39%, respectively, while protein contents vary 3-11% (Medic *et al.*, 2012; Liu *et al.*, 2014; Shariff *et al.*, 2016). OMP contents of Protein 8-9%, while fat content varied widely from

5-30% (Ribeiro *et al.*, 2020; Nunes *et al.*, 2021; Podgornik *et al.*, 2022). OP comprised of holocellulose and oils (Pathak *et al.*, 2017; Czech *et al.*, 2021). JM was found to contain 10% oil (Mahmoud *et al.*, 2022) and 25-30% protein, (Abbassy *et al.*, 2007; Abdou, 2018). ML were found to be rich in proteins 23-30%, carbohydrates 53% and fat 4-9.5% (Abouel-Yazeed, 2019; Sultana, 2020).

3.2. Submerged culture SMC

Using SCB, OMP, OP and RS wastes in mushroom SMC achieved highest harvested dry weight results ranked a and ab based on statistical analysis (LSD 0.05 = 1.19) enforced with highest protein contents arranged by the same dwt descending order (LSD = 2.10), as presented in Figure (2a). The influence of C/N ratio was positive on both protein content (%) and mushroom yield (dwt) with mean correlation coefficient of +0.8, while on the contrary the fat content had negative impact on both by -0.8, as shown in Figure (3a).

Agro-industrial wastes used as substrates in mushroom cultivation are mostly low in nitrogen content materials, as the carbon/nitrogen (C/N) ratio was found to have influence on mushrooms growth, yield and protein content in their fruiting bodies (Hoa *et al.*, 2018; Grimm and Wosten, 2018). As for *P. ostreatus*, the optimum C/N of substrate used for highest yield was 45-66:1 (Kumla *et al.*, 2020).

On the other hand, either peroxidase and laccase activities presented in Figure (2b) had positive effect on shortening cultivation periods among the used wastes as shown in Figure (2a), clarified by negative correlation values of -0.6 and -0.4, respectively (Figure 3b). Compared to the present study, it was found that in many mushroom strains with higher level of peroxidases have shorter cultivation periods (Xu *et al.*, 2012).

Never the less, those agroindustrial wastes contained other constituents rather than holocellulose, proteins and fats that were found to affect microbial metabolism. For instance, lignins in RS, SCB and CC comprised about 5-24% (Karimi *et al.*, 2006), 14-23% (Mahmud and Anannya, 2021) and 10-19% (Medic *et al.*, 2012; Liu *et al.*, 2014; Shariff *et al.*, 2016). OMP contents of phenolic compounds included simple phenolic compounds, benzoic acids, cinnamic acid and secoiridoids (Miklavčič *et al.*, 2020), which were recorded to have many antibacterial activities (Nunes *et al.*, 2021). OP comprised lignin, pectin, chlorophyll pigments, β -carotene and other low-molecular weight compounds that some of them had been reported to have antibacterial effect (Pathak *et al.*, 2017; Czech *et al.*, 2021). Jojoba meal wastes (JM) was found to contain polyphenols, phytic and simmondsin glucoside toxin possessing antifungal activities (Abbassy *et al.*, 2007; Abdou, 2018).

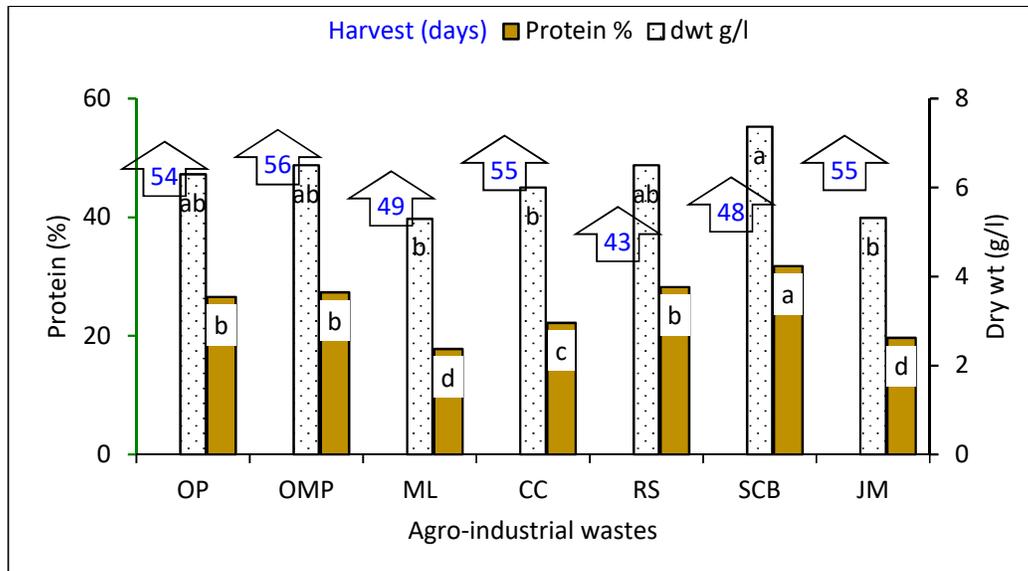


Fig. (2a): Harvest period (days), dry weight (dwt) and protein content of *P. ostreatus* grown on different agro-industrial wastes at harvest time, ranked statistically (LSD_{0.05} = 2.10 for protein% and 1.19 for dwt).

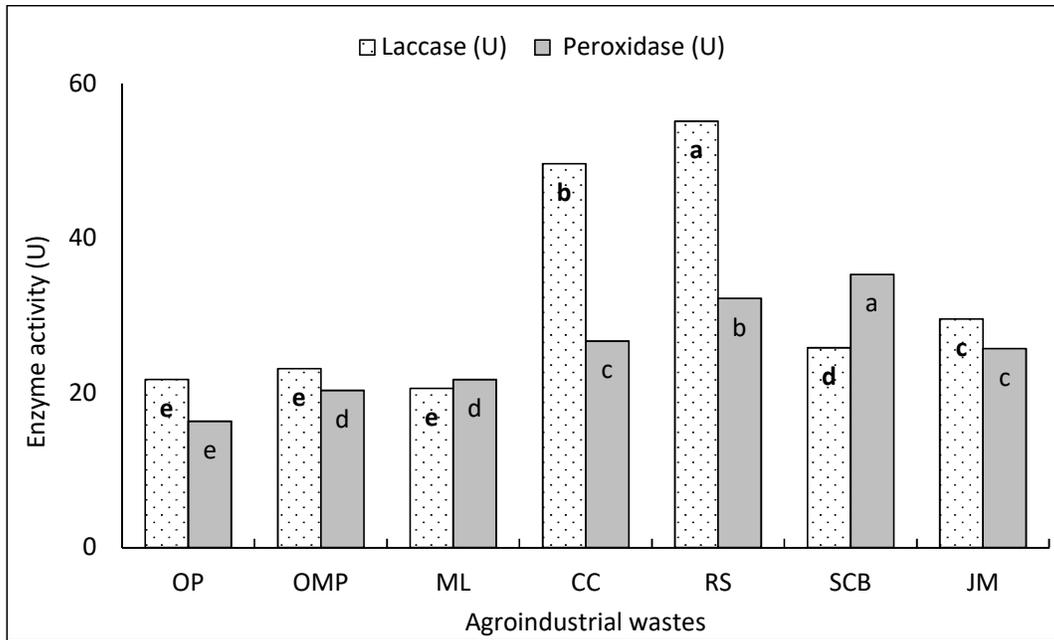


Fig. (2b): Laccase and peroxidase activities detected in culture medium broth of *P. ostreatus* grown on agroindustrial wastes at harvest time, ranked statistically (LSD_{0.05} = 2.436 for laccase and 2.349 for peroxidase).

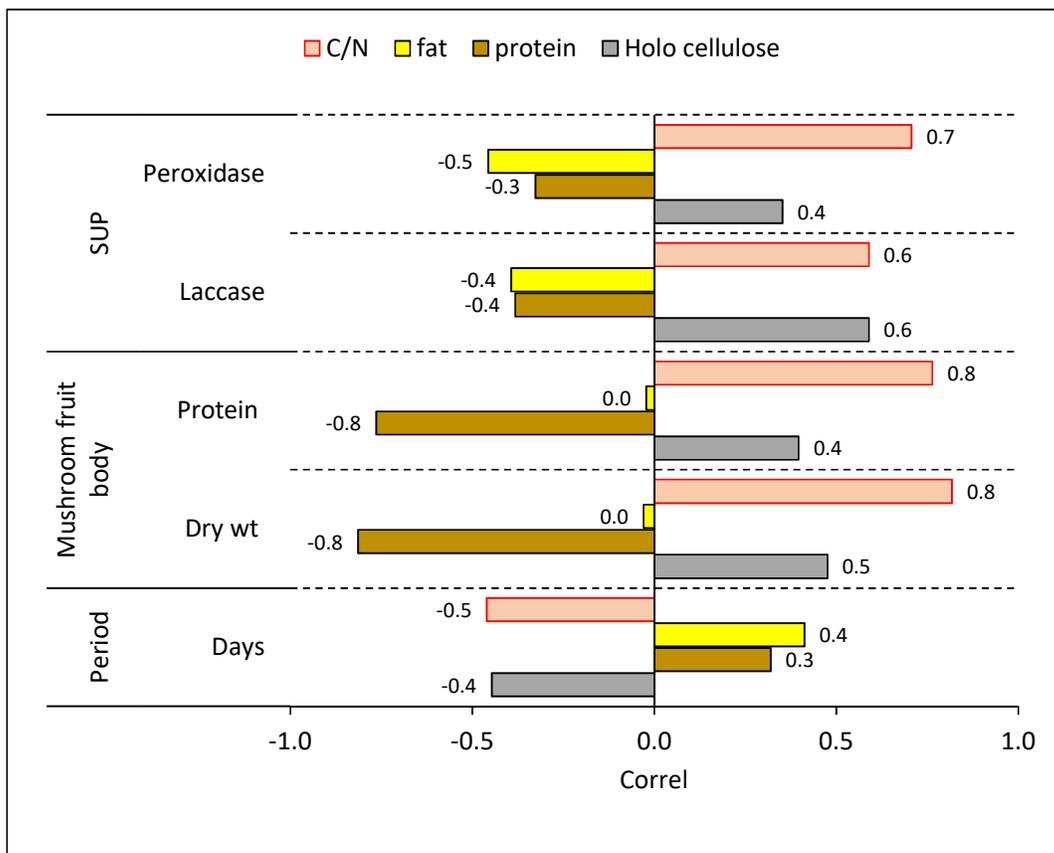


Fig. (3a): Correlation coefficient for agroindustrial wastes main contents used as substrate collectively against *P. ostreatus* laccase and peroxidase activities, protein content and dry weight.

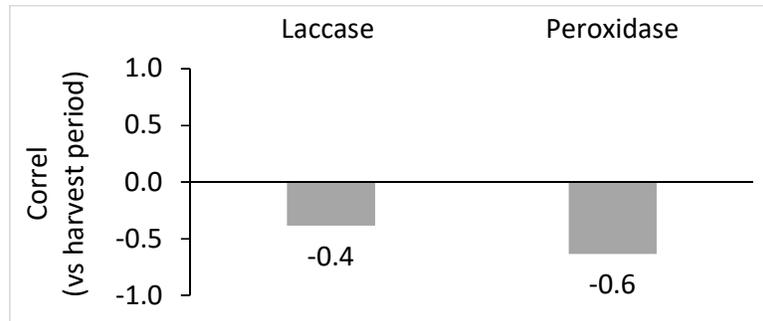


Fig. 3b: Correlation coefficient for *P. ostreatus* laccase and peroxidase activities vs harvest period (days).

2.1. Solid state culture

As presented in Table (3), the SCB, OMP, OP and RS wastes were used in combinations and permutations, of which *P. ostreatus* growth on RS+OP and SCB+OP achieved highest BE of 86.6% and 56.1%, respectively. Added to that, *P. ostreatus* growth on both waste mixtures achieved highest PR of 173 and 104, respectively, compared to other waste forms. The impacts of OP mixtures with RS or SCB were clarified by increasing BE and PR by >5 and >3 folds, compared to RS and SCB alone, respectively, calculated and presented in Table (3).

Not only with the presence of OP in waste mixtures achieved the best BE and PR, but the wider C/N ratios within those OP mixtures was a crucial factor positively correlated (+0.74 to +0.76) with better results as deduced in Figure (4). On the contrary, the fat contents had negative impact on BE and PR (-0.8).

Table 3: Biological efficiencies (BE%) and production rates (PR) for the yields of two flushes gathered from harvested *P. ostreatus* grown on agroindustrial wastes.

Waste mixtures	Content		Mushroom harvested						
	C/N	Fat%	BE% ± SE	Rank (LSD 8.6)	BE increase fold vs.		PR	PR increase fold vs.	
					RS+OP	SCB+OP		RS+OP	SCB+OP
RS	80.1	0.1	16.7 ± 2	e	5.2	3.4	33	5.3	3.2
RS+OMP	65.7	8	26.2 ± 1.5	cd	3.3	2.1	49	3.5	2.1
RS+OP	55.4	0.4	86.6 ± 6.2	a	1.0	0.6	173	1	0.6
SCB	83.2	0	10.8 ± 0.9	e	8.0	5.2	20	8.8	5.3
SCB+OMP	67.3	7.9	19.3 ± 0.9	de	4.5	2.9	34	5	3
SCB+OP	56.9	0.4	56.1 ± 2.4	b	1.5	1.0	104	1.7	1
OMP+OP	41	8.3	34.5 ± 2.1	c	2.5	1.6	61	2.9	1.7

BE from RS+OP and SCB+OP was considered profitable as they both exceeded 50%, according to Kumla *et al.* (2020). RS+OP achieved better BE compared to previous studies on *P. ostreatus* cultivation on agroindustrial wastes. For instance, combining straw from either rice or wheat with soya stalk recorded *P. ostreatus* BE of 82% and 78%, respectively (Liang *et al.*, 2019), while in other study, the cultivation using RS+SCB recorded lower BE of 50% (Ahmad *et al.*, 2020). Nevertheless, *P. ostreatus* grown on several wastes recorded biomass of 115 up to 454 mg/g dwt equivalent to BE of 12 to 50 % (Melanouri *et al.*, 2022). Worthy to notice that gathering either OP or OMP with wide C/N ratio waste such as RS or SCB enhanced BE values than that for either RS or SCB alone.

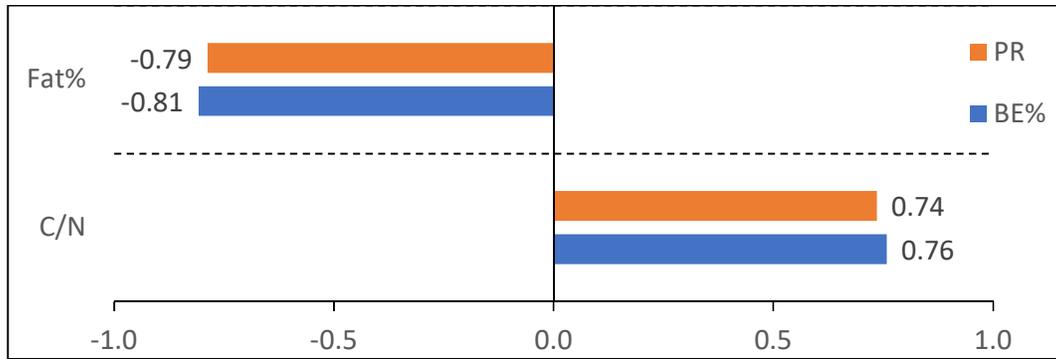


Fig. 4: Effect of C/N ratio and Fat content in (RS+OP, SCB+OP and OMP+OP) on their harvested mushroom BE% and PR submitted as correlation coefficient analysis.

2.1. Soil mixture analysis

2.1.1. Before harvest

Both SMS and compost were richer in OM with 136 and 70.8 fold than in soil, respectively, whereas the C/N ratio of SMS was found to be much bigger compared to that of soil and compost, as shown in table (4a). Added to that, SMS and compost constituent of P₂O₅ reformed about 17.6 and 76 folds, while K₂O reformed about 1.8 and 3.8 folds than those in soil, respectively.

According to data analysis in Table (4c), soil texture was found to be clay. Worthy to mention that saturation percentage (SP) that was found to be 47% ensured that soil sample had clay texture, as the SP lying in the range of 35-50% was found to characterize clay soils (Jones, 2001).

Table (4a): SMS, compost and soil analysis before plantation

Analyte	SMS	Comp	Soil
O.M	81.82 %	42.50 %	0.60 %
C	47.46 %	24.65 %	0.40 %
N	1.04 %	1.80 %	0.2 %
C/N	46	14	16
pH	5.7	7.3	8.48
EC	1.93 dS.m ⁻¹	2.3 dS.m ⁻¹	1.66 dS.m ⁻¹
P ₂ O ₅	0.22 %	0.95 %	12.4 ppm
K ₂ O	0.42 %	0.87 %	230.0 ppm

Table (4b): Soil soluble ions and available nutrients before plantation

Soluble anions	meq.l ⁻¹	Soluble Cations	meq.l ⁻¹
(CO ₃) ⁻¹	ND	(K) ⁺¹	0.32
(HCO ₃) ⁻¹	1.00	(Ca) ⁺²	5.50
(Cl) ⁻¹	9.50	(Mg) ⁺²	3.50
(SO ₄) ⁻²	6.06	(Na) ⁺¹	7.25
Available Micronutrients	mg.kg ⁻¹	Available Macronutrients	mg.kg ⁻¹
Cu	0.040	N	215.0
Fe	1.040	P	12.4
Mn	0.386	K	230.0
Zn	0.152		

ND: not detected

Table (4c): Measurements identifying soil texture

Sand (%)	Silt (%)	Clay (%)	SP (%)
24.23	33.6	42.17	47

3.2.1. After harvest

Collectively among the six soil combinations used, both DeH-ase and P-ase residual activities measured in soil were found to be parallelly correlated to SMS share in soil recording +0.5 and +0.4, respectively, as presented in Table (5). Added to that, the TDS was much correlated to the SMS share in soil combinations (+0.6).

Consequently, enzymatic residual activities showed their strong parallel correlations with TDS in soil of (+0.76/0.78) collectively among the six soil combinations, that might be due to the important role of TDS in activities of the enzymes and/or their inducing effect on M.O colonizing soil combinations.

Table 5: Pot content combinations before transplantation and their analysis after harvest

Pot combinations	Pot content			Measured parameters in soil					
	Compost (g/pot)	SMS % (v/v)	Clay soil % (v/v)	pH	EC	TDS	DeH-ase	P-ase	
C1	35	0	100	7.4	1.33	851	3.15	0.55	
C2	26.3	25	75	7.4	1.47	941	2.83	3.12	
C3	17.5	50	50	7.3	1.67	1069	1.81	2.09	
C4	8.8	75	25	7.2	2.07	1325	2.92	2.05	
C5	0	100	0	7.2	2.55	1632	7.19	4.64	
C6	35	100	0	7.3	1.42	909	3.07	1.36	
Correlation coefficients						TDS	0.76	0.78	
						Compost	-0.96	-0.59	-0.77
						SMS	0.62	0.48	0.45
						Clay soil	-0.62	-0.48	-0.45

Correlation strength variations from strong colored as green (+) and red (-) to weak and no correlations towards yellow.

2.1. Lettuce yield and analysis at harvest

Lettuce harvested as presented in Figure (5) was labeled by its dimensions, while its analysis shown in Table (6), followed by further analysis revealing cross correlation coefficients between analyzed parameters as shown in Table (7).

Replacing composted clay soil with SMS by 25 and 50% (V/V) increased shoot length, leave no., shoot dry weight and protein content by 6.4, 2.1; 10.6, 19.8; 7, 15 and 1.8, 0%, respectively, calculated from results shown in Table (6). Within lettuce leaves harvested from 25 and 50% SMS combinations, both total chlorophyll (TC) and carotenoid (Carot) contents increased by 217, 105 and 225, 117% than 100% composted clay soil, respectively. On the other hand, catalase activities decreased by 81.7 and 33.4%, while peroxidase activity increased by 95.3 and 4.7%, respectively.

High positive correlations (+0.8 to +0.9) gathered leaves dry weight with shoot height, shoot to root length ratio, head diameter, leaves protein content and considerable positive correlation (+0.4 to +0.6) with leaves no., total chlorophylls and total carotenoids, which are considered good parameters for economic evaluation. On the contrary, the leaves dry weight was negatively correlated (-0.8 to -0.9)

Table 6: Lettuce *L. sativa* analysis after harvest

POT combinations	S	R	S/R	L	H	L/H	DW	TSS	P	C _a	C _b	C _a /C _b	TC	Carot	TC /Carot	Cat	POD
C1	23.50	7.00	3.36	21.70	1.60	13.56	176	4.87	20.7	130	25.2	5.2	155	15.5	10.01	7.0	0.086
C2	25.00	7.00	3.57	24.00	2.00	12.00	188	4.63	21.1	369	122.0	3.0	491	50.4	9.73	1.3	0.168
C3	24.00	7.50	3.20	26.00	1.70	15.29	202	5.21	20.7	245	72.5	3.4	317	43.0	7.37	4.7	0.090
C4	20.50	9.00	2.28	15.00	1.44	10.42	181	4.36	22.4	97	15.0	6.5	112	22.7	4.94	11.1	0.059
C5	17.50	10.50	1.67	15.00	0.80	18.75	106	3.98	18.9	176	50.6	3.5	227	23.9	9.47	4.2	0.289
C6	17.50	14.00	1.25	19.70	1.00	19.70	104	2.95	18.9	103	10.1	10.2	113	19.0	5.94	10.0	0.072

Shoot height (S), Root depth (R), Leaves no (L), Head diameter (H), Dry Weight (DW), total soluble solids (TSS), Protein (P), Chlorophyll Ca (C_a), Chlorophyll (C_b), Total chlorophyll (TC), Carotenoids (Carot), Catalase (Cat)

Table 7: Correlation coefficients characterizing interactions between analyzed parameters of harvested lettuce *L. sativa* collectively among the six soil combinations used in pots.

	R	S/R	L	H	L/H	DW	TSS	Prot	C _a	C _b	C _a /C _b	TC	Carot	TC /Carot	Cat	POD
S	-0.9	1.0	0.8	1.0	-0.7	0.9	0.8	0.6	0.7	0.6	-0.6	0.6	0.6	0.4	-0.5	-0.3
R		-0.9	-0.5	-0.8	0.7	-0.9	-1.0	-0.7	-0.5	-0.6	0.8	-0.5	-0.5	-0.5	0.5	0.0
S/R			0.7	0.9	-0.7	0.9	0.9	0.6	0.6	0.6	-0.7	0.6	0.6	0.5	-0.5	-0.2
L				0.7	-0.1	0.6	0.5	0.1	0.6	0.6	-0.3	0.6	0.6	0.3	-0.5	-0.3
H					-0.8	0.9	0.7	0.7	0.6	0.6	-0.5	0.6	0.7	0.2	-0.4	-0.4
L/H						-0.8	-0.6	-1.0	-0.2	-0.3	0.3	-0.2	-0.3	0.0	0.0	0.4
DW							0.9	0.8	0.4	0.4	-0.5	0.4	0.6	0.1	-0.2	-0.4
TSS								0.6	0.5	0.5	-0.8	0.5	0.5	0.4	-0.5	-0.1
Prot									0.1	0.1	-0.2	0.1	0.3	-0.3	0.1	-0.5
C _a										1.0	-0.7	1.0	0.9	0.5	-0.9	0.4
C _b											-0.8	1.0	0.9	0.5	-0.9	0.4
C _a /C _b												-0.7	-0.6	0.5	0.8	-0.5
TC													0.9	0.5	-0.9	0.4
Carot														0.2	-0.7	0.2
TC /Carot															-0.8	0.6
Cat																-0.6

Shoot height (S), Root depth (R), Leaves no (L), Head diameter (H), Dry Weight (DW), total soluble solids (TSS), Protein (P), Chlorophyll Ca (C_a), Chlorophyll (C_b), Total chlorophyll (TC), Carotenoids (Carot), Catalase (Cat). Correlation strength variations from strong colored as green (+) and red (-) to weak and no correlations towards yellow.

to root length and leaves no to head diameter ratio, with considerable negative correlation (-0.4 to -0.5) with chlorophyll C_a to C_b ratio and peroxidase activity in leaves.

Catalase activity was negatively correlated to total chlorophyll and carotenoids. On the other hand, leaf number and shoot height were negatively correlated to catalase but were positively correlated to total chlorophyll and carotenoids, as same results were deduced by Zhao *et al.* (2022). Catalase activity in leaves of lettuce *L. sativa* was found to share in scavenging the reactive oxygen species resulting from oxidative stress. In plants, catalase scavenges H₂O₂ generated mainly during mitochondrial photorespiratory oxidation and electron transport, beside β-oxidation of the fatty acids. (Leitão *et al.*, 2021)

Most obvious that SMS pushed lettuce to increase its carotenoid contents which was considered as nonenzymatic antioxidants (Sharma *et al.*, 2012), that was more correlated to shoot length, leave no, dry weight and total chlorophyll, while on the contrary catalase activity decreased. On the same trend, chlorophyll C_a to C_b ratio (C_a/C_b) decreased with increasing carotenoid level while C_a/C_b increased with catalase activity. These results indicated that the plant leaves tended to convert more chlorophyll C_a to C_b (ie: decrease in C_a/C_b) parallel to catalytic activity facing any possible stress, while in reversible correlation with carotenoid level that seemed to be dependable on chlorophyll C_a form than C_b. The chlorophyll C_a to C_b ratio was found to decrease, as chlorophyll C_a tended to be replaced by chlorophyll C_b (Sarijeva *et al.*, 2007), with many forms of stress factors including decreasing N availability, high light conditions or increasing EC above 2 dSm⁻¹ among most lettuce cultivars (Kitajima and Hogan, 2003; Adhikari *et al.*, 2019).

Worthy to mention that increasing SMS to 75% in pot increased EC above 2 dSm⁻¹ at which stress began to affect lettuce growth and final yield, as mentioned before by Adhikari *et al.* (2019), which was reflected as drop in dry weight, total chlorophyll, carotenoids and peroxidase activity, while catalase activity increased.

microbial origin, reflecting the whole residual microbial activities at harvest time. Microbial DeH-ase and P-ase had their impact on lettuce growth extended during cultivation period that were translated into lettuce yield and analysis at harvest time. Their correlations revealed their positive impact on L/D, TC/Carot and POD in lettuce as shown in Table (8).

Table 8: Correlations between Lettuce analytical data vs soil analysis after harvest.

Lettuce analysis at harvest	Soil analysis at harvest			
	pH	TDS	DeH-ase	P-ase
S/R	0.69	-0.46	-0.51	-0.22
L/D	-0.22	0.16	0.48	0.21
Dry Weight	0.40	-0.36	-0.70	-0.30
TSS	0.33	-0.10	-0.35	-0.07
protein	0.07	-0.12	-0.56	-0.27
C _a /C _b	-0.15	-0.28	-0.18	-0.53
TC	0.47	-0.14	-0.14	0.43
TC/Carot	0.55	-0.08	0.36	0.27
Cat	-0.42	-0.01	-0.18	-0.55
POD	-0.24	0.65	0.86	0.89

In lettuce: Shoot height/Root height (S/R), Leaves no/Head diameter (L/D), Total soluble solids (TSS), Chlorophyll Ca/Chlorophyll Cb (C_a/C_b), Total chlorophyll (TC), Total chlorophyll/Carotenoids (TC/Carot), Catalase (Cat), Peroxidase (POD).

In soil: pH value, Total dissolved solids (TDS), Dehydrogenase (DeH-ase), Phosphatase (P-ase).

Correlation strength variations from strong colored as green (+) and red (-) to weak and no correlations towards yellow.



Figure (5): *L. sativa* grown on soil, compost and SMS mixtures at harvest time. Yellow ruler bars (cm) showing lettuce part dimensions.

3.3. Conclusion

The orange peel increased *P. ostreatus* production only when it participated with other agro-industrial wastes with wide C/N ratio, in spite of containing antimicrobial contents, as both production rate and transformation of RS to mushroom yield (BE) increased by >5 folds compared to usual rice straw alone. On the same trend, orange peel leveled up production rate and transformation of SCB by >3 folds. Those achievements of bigger yield in shorter time improvement are considered of high economic outcome.

Never the less, the harvested *L. sativa* grown on SMS mixed with composted clay soil in variable ratios of 25 and 50% (V/V) increased harvested dry weight by 7 and 15%, respectively, with higher protein, total chlorophyll and carotenoid contents almost doubled compared to composted soil alone, adding undeniable economic value for using SMS soil mixture in lettuce cultivation.

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