International Journal of Recycling of Organic Waste in Agriculture (2023)12: 559- 571
Doi: 10.30486/IJROWA.2023.1964536.1513

ORIGINAL RESEARCH

Effect of substrate temperature and stages duration on recycling of agro-industrial residues through *Pleurotus ostreatus* production

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Received: 05 September 2022 / Accepted: 09 May 2023 / Published online: 10 May 2023

Abstract

Purpose This study investigated the effect of recycled Olive Pruning Residues (OLPR) and Spent Coffee Grounds (SCG) on substrate temperature (temp) and duration (dur) of *Pleurotus ostreatus* growing stages. This study also sheds light on the correlation between selected parameters, yield, and substrate composition.

Method The experiment consisted of 2 sole substrates wheat straw (WS; control) and SCG, and 6 mixtures of WS, OLPR, and SCG (v/v): WS:SCG 3:1, WS:SCG 1:3, WS:OLPR:SCG 1:1:1, WS:OLPR:SCG 3:1:1, WS:OLPR:SCG 1:3:1, and WS:OLPR:SCG 1:1:3.

Results Increased SCG proportions caused the substrate temperature to decrease, whereas, OPLR proportions caused it to rise. All stages showed earliness in productive substrates. The highest organic matter loss (OML) was reported by WS (76.7%) (76.7%). WS:SCG 3:1 OML depicted a correlation ($0.8 \leq r \geq 0.9$) with dur Stages 2 and 3. OML of WS:SCG 1:3 denoted a correlation ($0.8 \leq r \geq 0.9$) with dur Stages 1, 2, and 3. OML of WS:OLPR:SCG 3:1:1 and WS:OLPR:SCG 1:1:1 correlated ($R^2 \geq 0.7$) with dur Stages 3 and 4, respectively. WS:SCG 1:3 temp had a strong influence on economic yield (EY) at all stages. At Stage 2, combined substrate temp positively affected biological efficiency (BE) and biological yield (BY). Spawn run initiation (SRI), pinhead formation (PNF), and harvest of the first flush (HF1) strongly correlated with major minerals and fatty acids in SCG substrates. Increased Mg and Fe contents affected negatively the complete mycelial colonization (100% MC) in combined substrates.

Conclusion SCG reduces OML and causes early production.

Keywords Earliness, Olive pruning residues, Organic matter loss, Spent coffee grounds, Substrate temperature, Yield

Introduction

The oyster mushroom (*Pleurotus ostreatus*) is the second most cultivated mushroom and contributes around 20% of the world's global production (Royse 2014). It has a variety of nutritional and therapeutic benefits (Cheung 2010; Khan and Tania 2012) and is recognized for its high bioremediation feature, especially in the recycling of agro-industrial residues (Ritota and Manzi 2019; Abou Fayssal et al. 2021a). *P. ostreatus* is a selective saprophytic mushroom that can be grown on various substrates including wheat straw (Sassine et al. 2021;

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Abou Fayssal et al. 2023). Due to wheat straw scarcity in many world regions, several partial or complete substitutes were reported for oyster production, for instance, olive pruning residues (Abou Fayssal et al. 2020), spent coffee grounds (Alsanad et al. 2021) and spent mushroom substrate (SMS) (Naim et al. 2020 a, b). These residues help improve the substrate or compost quality used in mushroom production. It also enriches it with essential minerals and easily available lignocellulosic compounds needed for the growth and development of mycelium (Abou Fayssal et al. 2021c; El Hage et al. 2021; Sajyan et al. 2021). In the same vein, several reports discussed the strong interrelationship between substrate composition, and mushroom composition and its nutritional value (Kumar et al. 2021).

Olive pruning residues, largely disposed of hazardously in Lebanon (MOA/FAO 2000), have been acknowledged as being good partial substitutes in *P. ostreatus* growing (Abou Fayssal et al. 2020). Similarly, the disposal of spent coffee grounds without any pre-treatment into the environment pushed researchers to its successful incorporation into mushroom production (Alsanad et al. 2021). Because *P. ostreatus* can grow on rich lignocellulosic materials (Elbagory et al. 2022; Werghemmi et al. 2022), this incorporation reduces the danger of heavy metals in a bioremediation process (Abou Fayssal et al. 2021a).

The effect of OLPR and SMS on the substrate temperature and timing of *P. ostreatus* production was earlier investigated (Abou Fayssal et al. 2021b; Naim et al. 2021). Their studies illustrated that mushroom yields were affected by such parameters. The effect of spent coffee grounds (SCG) combined with WS and/or not with OLPR on the substrate temperature, period of growth and production stages, and yields of *P. ostreatus* was not previously studied. Therefore, the current study investigated a) the possible role of SCG combined with WS and OLPR in the regulation of substrate temperature; b) their possible role in hastening/delaying the production cycle and improving/reducing yield; c) their possible effect on the correlation between growing cycle stages, yield, and substrate composition.

Materials and methods

Experimental setup and treatments

The treatments consisted of 2 sole substrates WS(control) and SCG, and 6 mixtures of WS, OLPR, and SCG: WS:SCG 3:1, WS:SCG 1:3, WS:OLPR:SCG 1:1:1, WS:OLPR:SCG 3:1:1, WS:OLPR:SCG 1:3:1, and WS:OLPR:SCG 1:1:3. The mixtures were made on volume basis (v/v). The chemical analysis of substrates followed the methodology adopted by Alsanad et al. (2021) and Abou Fayssal et al. (2021a). All treatments were compared to the control substrate (WS) in terms of substrate temperature, stage duration, and productive and compositional characteristics. A complete randomized design (CRD) was adopted with ten replicates (bags) per treatment. The experiment was repeated thrice for better accuracy.

Experimental work and incubation

WS and one-year fermented OLPR were procured from a local private farm, while a local private company provided the needed SCG. Sun-drying and pasteurization setups followed the methodology of Abou Fayssal et al. (2020). Then, 50 g of gypsum was added to each mixture before spawning to regulate pH. *P. ostreatus* grains spawn (5% (w/w)) was used to inoculate substrates in transparent polyethylene bags. The adopted conditioning procedure during the different growth and

productive stages was based on the experiment of Abou Fayssal et al. (2021a) and Sassine et al. (2021). Briefly, the temperature in the obscure growing room was maintained at 23-25 ℃ during mycelial running stages. A relative humidity of 80-90% was stabilized and monitored using a humidity/temperature meter Lutron HT-3007SD. Ventilation (reduction of room temperature to 15 ℃), lighting (200 lx), and CO_2 reduction (to around 900 $mg.L^{-1}$) were performed to trigger pinhead formation after the full mycelial colonization of substrates. In the same vein, a high relative humidity ranging between 88 and 90% was maintained during the fruiting stage. Mature mushrooms were harvested manually when their caps were fully formed.

Growth and productive measurements

Spawn run initiation (SRI), half mycelial colonization (50% MC), complete mycelial colonization (100% MC), pinhead formation (PNF), and harvest of the first flush (HF1) were evaluated and expressed as days after spawning (DAS). Stages 1, 2, 3, and 4 represented the periods between SRI and 50% MC, 50% and 100% MC, 100% MC and PNF, and PNF and HF1, respectively.

In addition, the Biological Yield (g/bag; BY) was calculated as fruit bodies weight + clusters weight, whereas, the Economic Yield (g/bag; EY) corresponded to fruit bodies weight only. The Organic Matter Loss (OML) and Biological Efficiency (BE) were calculated based on Abou Fayssal et al. (2020) as follows:

$$OML(\%) = \frac{Initial\ dry\ mass\ of\ substrate\ (g) - Residual\ dry\ mass\ of\ substrate\ (g)}{Initial\ dry\ mass\ of\ substrate\ (g)} \times 100$$

$$BE\ (\%) = \frac{Total\ fresh\ mass\ of\ mushrooms\ (g)}{Initial\ dry\ mass\ of\ substrate\ (g)} \times 100$$

The measurement of substrate temperature was performed through different places of the growing bag using a digital thermometer (JR-1 BEL®), starting from the spawning date. Therefore, each substrate temperature is an average value of three temperature records.

Statistical analysis

One-way ANOVA and Duncan tests were performed using SPSS 25®. Simple regressions were dispatched to detect the relationship between organic matter loss (as a dependent variable), and SCG and OLPR/SCG proportions in substrates (as targeted for predictors). Predictive models based on multiple stepwise regressions were performed. The relationship between BY, BE, EY, or OML (as dependent variables), and the duration of the stages and corresponding substrate temperature (as independent variables) were evaluated. Pearson's correlations were performed between substrate components and timings of mycelial growth and production at 95% and 99% levels of confidence.

Results and discussion

Substrate temperature and timing of growth stages

SCG significantly decreased the substrate temperature during Stage 1 by a range of 0.5–1.4 °C (273.65–274.55 K) compared to WS (Table 1). The substrate fully based on SCG was not fully colonized by the mycelium and was, therefore, excluded from further temperature assessment. Over Stages 2, 3, and 4, a similar trend was clear.

During these stages, all productive substrates had significantly lower temperatures by 0.8–1.2 °C (273.95–274.35 K), 0.6–1.0 °C (273.75–274.15 K), and 0.6–1.0 °C (273.75–274.15 K) compared to WS.

Table 1 Wheat straw, spent coffee grounds, and olive pruning residues mixtures' temperature during the different growth stages (N=10 per treatment; 10 replicates per treatment)

Substrate	Stage 1	Stage 2	Stage 3	Stage 4
WS	25.8^{c}	26.8^{b}	26.4^{b}	26.3^{b}
SCG	24.4^{a}	-	-	-
WS:SCG 3:1 (v/v)	25.3^{b}	26.0^{a}	25.8^{a}	25.7^{a}
WS:SCG 1:3 (v/v)	24.8^{a}	25.6^{a}	25.4^{a}	25.3^{a}
WS:OLPR:SCG 3:1:1 (v/v)	25.3^{a}	26.2^{a}	26.1^{a}	26.0^{a}
WS:OLPR:SCG 1:1:1 (v/v)	25.5^{ab}	26.3^{a}	26.2^{a}	26.1^{a}
WS:OLPR:SCG 1:3:1 (v/v)	25.7^{b}	-	-	-
WS:OLPR:SCG 1:1:3 (v/v)	25.0^{a}	-	-	-

Means within the same column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test, WS: Wheat Straw, SCG: Spent Coffee Grounds, OLPR: Olive Pruning Residues, Stage 1: the period between SRI and 50% MC, Stage 2: the period between 50% MC and 100% MC, Stage 3: the period between 100% MC and PNF, Stage 4: the period between PNF and HF1, -: mycelium didn't colonize substrate at the corresponding stage.

During stage 1, a significant reduction in substrate temperature was ensured by the combination of WS with OLPR and SCG in 3:1:1 (v/v) and 1:1:3 (v/v) proportions in *P. ostreatus* substrates (reduction by 0.5–0.8 °C; 273.65–273.95 K) (Table 1). Whereas, the equitable distribution of agro-industrial residues in substrates and a high proportion of OLPR in the mixture (1:1:1 (v/v) and 1:3:1 (v/v), respectively) showed comparable substrate temperature with WS during the aforementioned stage. Substrates containing OLPR and SCG three-fold higher than WS were not fully colonized by the mycelium and were, therefore, excluded from further temperature investigation. Stage 2 marked a significant decrease (0.5–0.6 °C; 273.65–273.75 K) in the temperature of combined OLPR and SCG substrates compared to the control. Sympathetically, Stages 3 and 4 fetched comparable substrate temperatures between all productive mixtures.

The period between SRI and 50% MC was significantly reduced by 2.2–2.3 days in comparison with WS due to the full or partial incorporation of SCG in substrates (SCG and WS:SCG 1:3 (v/v)) (Table 2). However, the incorporation of such industrial residues in substrates resulted in an extended period between 50% and 100% MC (Stage 2) (2.5–4.5 days) compared to the control.

A comparable period between 100% MC and PNF (Stage 3) was observed between WS and WS: SCG 3:1 (v/v). The latter reached the time for the first harvest on the same day of pinhead formation (Stage 4), showing a significant hastening by 2.7 days in comparison with WS. Similarly, the whole production cycle was significantly shorter by 1.0-6.3 days with WS in comparison with substrates containing SCG. Stages 2 and 3 were primarily the main reason behind this hastening.

Table 2 Duration (days) of the different WS and SCG mixtures' growth stages (N=10 per treatment; 10 replicates per treatment)

Substrate	Stage 1	Stage 2	Stage 3	Stage 4	Complete cycle
WS	4.3^{b}	2.4^{a}	24.3^{a}	2.7^{b}	33.7^{a}
SCG	2.0^{a}	-	-	-	-
WS:SCG 3:1 (v/v)	3.8^{b}	4.9^{b}	26.0^{ab}	0.0^{a}	34.7^{b}
WS:SCG 1:3 (v/v)	2.1^{a}	6.9^{c}	29.0^{b}	2.0^{b}	40.0^{c}

Means within the same column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test, WS: Wheat Straw, SCG: Spent Coffee Grounds, OLPR: Olive Pruning Residues, Stage 1: the period between SRI and 50% MC, Stage 2: the period between 50% MC and 100% MC, Stage 3: the period between 100% MC and PNF, Stage 4: the period between PNF and HF1, -: mycelium didn't colonize substrate at the corresponding stage.

Table 3 denoted a significantly hastened Stage 1 (by 2.0 days) as a result of combined OLPR and SCG incorporation with WS in proportions of 1:1:3 (v/v) respectively.

Table 3 Duration (days) of the different wheat straw, and combined olive pruning residues and spent coffee grounds mixtures' growth stages (N=10 per treatment; 10 replicates per treatment)

Substrate	Stage 1	Stage 2	Stage 3	Stage 4	Complete cycle
WS	4.3^{b}	2.4^{ab}	24.3^{a}	2.7^{b}	33.7^{b}
WS:OLPR:SCG 3:1:1 (v/v)	4.0^{b}	2.7^{b}	25.6^{b}	2.4^{ab}	34.7^{c}
WS:OLPR:SCG 1:1:1 (v/v)	3.7^{b}	2.0^{a}	24.0^{a}	2.0^{a}	31.7^{a}
WS:OLPR:SCG 1:3:1 (v/v)	3.0^{ab}	-	-	-	-
WS:OLPR:SCG 1:1:3 (v/v)	2.3^{a}	-	-	-	-

Means within the same column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test, WS: Wheat Straw, SCG: Spent Coffee Grounds, OLPR: Olive Pruning Residues, Stage 1: the period between SRI and 50% MC, Stage 2: the period between 50% MC and 100% MC, Stage 3: the period between 100% MC and PNF, Stage 4: the period between PNF and HF1, -: mycelium didn't colonize substrate at the corresponding stage.

The equitable incorporation of raw materials in growing substrates showed a comparable period between 50% and 100% MC (Stage 2) and between 100% MC and PNF (Stage 3) with control. However, it speeded the period between PNF and HF1 (Stage 4) by 0.7 days compared to WS. The substrate with equal proportions of agro-industrial residues (WS: OLPR: SCG 1:1:1 (v/v)) showed the shortest growing cycle, being significantly shorter (by 2.0 days) than the control. The growing cycle of WS: OLPR: SCG 3:1:1 (v/v) was significantly longer (by 1.0 day) than the control. This delay has primarily occurred during Stage 3.

Managing the uprising disposal of agro-industrial residues could be a good pathway for the environmental pollution reduction program (Hamed et al. 2022; Thaisuchat et al. 2022; Wachira et al. 2022). Several initiatives were launched to find solutions for such an issue using the bioremediation concept (Kumar et al. 2022a, b, c; Širić et al. 2022). Results

indicated that the use of SCG alone or in high proportions decreased substrate temperature, conversely to their effect when combined with OLPR in mixtures. The current findings corroborate with those of Abou Fayssal et al. (2021b) who reported that OLPR substrates had higher temperatures than WS when colonized by *P. ostreatus*. On the other hand, Alsanad et al. (2021) reported that the incorporation of SCG in substrates resulted in a decreased C/N ratio. According to Fanadzo et al. (2010), lower C/N ratios are associated with an increased temperature, thus inducing faster metabolic activity. Herein, our findings corroborate partially and are partially controversial with such statements. Lower C/N ratios of SCG mixtures hastened the mycelial growth, had comparable yields with the WS substrate when found in low proportions, and reduced OML despite reducing the substrate's temperature. This may propound the following hypothesis: the type and source of SCG may affect its content in easily available holo-cellulose, wich is essential for mycelial growth and production.

The incorporation of equal proportions of OLPR and SCG decreased substrate temperature in comparison with WS. This is well supported by the fact that low OLPR proportions (Abou Fayssal et al. 2021b) and the availability of SCG in substrates are the main cause. The combination of SCG, or not, with OLPR induced an earliness in some mycelial growth stages, which may be related to their induction of high initial lignin content in substrates. Thus, such findings confirm the earlier reports of Abou Fayssal et al. (2021a) and Alsanad et al. (2021).

Mushroom production

WS: SCG 3:1 (v/v) substrate showed comparable biological yield (BY), biological efficiency (BE), and economic yield (EY) with WS substrate (Table 4). These parameter values were reduced by more or less than half in the substrate containing a high SCG proportion (WS: SCG 1:3) (v/v). On the other hand, a significant reduction in OML by 5.7–8.3% was detained by the incorporation of industrial residues (SCG) onto *P. ostreatus* substrates in comparison with WS.

Table 4 Wheat straw and spent coffee grounds mixtures' effect on vario us parameters of mushroom production

Substrate		BY (g/bag)	SE	BE (%)	SE	EY (g/bag)	SE	OML (%)	SE
WS		910.1 ±236.3^{b}	136.4	105.0 ±27.2^{b}	15.7	871.4 ±238.4^{b}	135.6	76.7 ±3.8^{b}	2.1
WS:SCG (v/v)	3:1	814.6 ±201.1^{b}	116.1	105.1 ±26.0^{b}	14.9	772.5 ±187.0^{b}	107.9	71.0 ±0.4^{a}	1.6
WS:SCG (v/v)	1:3	437.1 ±19.0^{a}	10.9	59.3 ±2.6^{a}	1.4	402.8 ±17.0^{a}	9.8	68.4 ±0.2^{a}	0.6
p-value		0.039	-	0.066	-	0.036	-	0.01	-

Means within the same column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test, WS: Wheat Straw, SCG: Spent Coffee Grounds, BY: Biological Yield, BE: Biological Efficiency, EY: Economic Yield, OML: Organic Matter Loss, SE: Standard Error (Alsanad et al. 2021).

The findings of Table 5 outlined comparable BYs, EYs, as well as BEs between all productive substrates. Sympathetically, OML was significantly reduced in WS:OLPR:SCG 3:1:1 (v/v) and WS: OLPR: SCG 1:1:1 (v/v) substrates by 12.9–14.6% compared to WS.

Table 5 Wheat straw, and combined olive pruning residues and spent coffee grounds mixtures' effect on various parameters of mushroom production

Substrate	BY (g/bag)	SE	BE (%)	SE	EY (g/bag)	SE	OML (%)	SE
WS	910.1 ±236.3[a]	136.4	105.0 ±27.2[a]	15.7	871.4 ±238.4[a]	135.6	76.7 ±2.7[b]	2.2
WS:OLPR:SCG 3:1:1 (v/v)	883.6 ±66.5[a]	38.4	101.7 ±7.9[a]	4.5	861.1 ±66.7[a]	38.5	63.8 ±0.5[a]	1.3
WS:OLPR:SCG 1:1:1 (v/v)	811.7 ±13.7[a]	7.9	95.3 ±2.7[a]	1.6	782.4 ±22.7[a]	13.1	62.1 ±0.6[a]	0.4
p-value	0.696	-	0.771	-	0.715	-	<0.001	-

Means within the same column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test, WS: Wheat Straw, OLPR: Olive Pruning Residues, SCG: Spent Coffee Grounds, BY: Biological Yield, BE: Biological Efficiency, EY: Economic Yield, OML: Organic Matter Loss, SE: Standard Error (Abou Fayssal et al. 2021a).

The comparable yields of mixtures containing low proportions of SCG based on combinations of OLPR and SCG with WS could be attributed to the high holo-cellulose contents of these substrates, and their high C/N ratios. Naim et al. (2021) reported that increasing the nitrogen content in substrates delays but increases yields and substrate temperature. Herein, controversial findings showed that the higher nitrogen content led to lower, or at best, comparable yields with WS, lower substrate temperature, and an overall comparable production period. This may simulate that the nitrogen type found in substrates (organic or inorganic) naturally affects substrate temperature, obtained yields, and growing cycle period. The decrease of OML in substrates containing OLPR and/or SCG justifies the assumption raised by Abou Fayssal et al. (2021b) regarding the big influence of increased lignin content on the OML of mushroom substrates.

Predictive models analysis

A strong quadratic relationship delineated the inversed correlation between increased proportions of SCG in substrates and decreased OML ($R^2 = 0.787$) (OML = – 3.0 x SCG^2 – 0.22 x SCG + 76.67 + 1.42 E). Likewise, increased proportions of combined agro-industrial wastes (OLPR and SCG) were concomitant with decreased OML ($R^2 = 0.81$) (OML = – 0.43 x OLPR/SCG + 74.56) (Abou Fayssal et al. 2021a). Predictive models of the performed stepwise regression (Table 6) demonstrated the close interrelationship between the duration of Stages 2 and 4, and BE and OML of WS: SCG 3:1 (v/v) substrate.

Table 6 Predictive models showing the relation of different indicators with the substrate temperature and/or duration of growth stages in substrates containing spent coffee grounds (N=10 per treatment; 10 replicates per treatment)

	Dep	Ind	Equation	Adj R^2
WS:SCG 3:1	BE	dur(Stage 2), dur(Stage 4)	BE= 18.46 × dur(Stage 2) –173.17 × dur(Stage 4) + 257.32	0.80*
	OML	dur(Stage 2), dur(Stage 4)	OML= 82.58 × dur(Stage 2) – 95.24 × dur(Stage 4) + 59.36	0.83*
WS:SCG 1:3	EY	temp(Stage 1), temp(Stage 2), temp(Stage 3), temp(Stage 4), dur(Stage 1), dur(Stage 2), dur(Stage 3)	EY= 45.11 × temp(Stage 1) + 63.29 × temp(Stage 2) – 44.53 × temp (Stage 3) – 42.62 × temp(Stage 4) + 7.02 × dur(Stage 1) – 15.20 × dur(Stage 2) – 23.09 × dur(Stage 3) + 18.17	0.95**
	OML	dur(Stage 1), dur(Stage 2), dur(Stage 3)	OML= 9.45 × dur(Stage 1) – 12.38 × dur(Stage 2) – 19.67 × dur(Stage 3)	0.94**

Dep: Dependent variable (s), Ind: Independent variable (s), Adj: Adjusted, BE: Biological Efficiency, EY: Economic Yield, OML: Organic Matter Loss, dur: duration, temp: substrate temperature, Stage 1: the period between SRI and 50% MC, Stage 2: the period between 50% MC and 100% MC, Stage 3: the period between 100% MC and PNF, Stage 4: the period between PNF and HF1. *Significant at $p < 0.05$, **Significant at $p < 0.01$

This interrelationship was described by positive correlations between BE and OML with the duration of Stage 2. It also demonstrated negative correlations between BE and the duration of Stage 4 (R^2 = 0.80 for BE and R^2 = 0.83 for OML). The substrate temperature during all production stages and all stages' durations (except the one of Stage 4) affected the EY of WS:SCG 1:3 (v/v) substrate. The substrate temperature during Stages 1 and 2 was positively correlated with EY. While, the substrate temperature during Stages 3 and 4 had a negative correlation with this productive parameter. The duration of Stage 1 was positively correlated with EY of WS: SCG 1:3 (v/v). Whereas, the duration of Stages 2 and 3 was negatively correlated with the latter (R^2 = 0.95). In addition, the OML of WS: SCG 1:3 (v/v) was positively correlated with the duration of Stage 1. While, Stages 2 and 3 negatively affected the OML of this substrate (R^2 = 0.94).

Results in Table 7 outlined a negative correlation between the duration of Stage 3 and the OML of WS: OLPR: SCG 3:1:1 (v/v) substrate (R^2 = 0.76). The BE and BY of WS: OLPR: SCG 1:1:1 (v/v) substrate were positively correlated with the latter's temperature during Stage 2 and the duration of Stage 4 (R^2 = 0.92 and 0.90, respectively). In the same vein, OML of the same substrate was positively affected by the duration of Stage 4 (R^2 = 0.79). The built predictive models showed the interesting impact of the duration of Stage 2 (the period between 50% MC and 100% MC) on the BE and OML of WS: SCG 3:1(v/v), and on EY and OML of WS: SCG 1:3 (v/v). An extended Stage 2 resulted in an increased BE in WS: SCG 3:1(v/v), and a decreased EY in WS: SCG 1:3 (v/v), pointing out the effect of SCG on the productive parameters. However, this extension was highly beneficial in terms of OML reduction in these substrates. The delay in

Stage 3 reduced the OML of WS: SCG 3:1:1 (v/v); while a hastened Stage 4 led to a reduced BE, BY, and OML of WS: SCG 1:1:1 (v/v). These findings point out the necessity of stabilizing low nitrogen contents in *P. ostreatus* substrates to optimize yields.

Table 7 Predictive models showing the relation of different indicators with the substrate temperature and/or duration of growth stages in substrates containing olive pruning residues and spent coffee grounds (N=10 per treatment; 10 replicates per treatment)

	Dep	**Ind**	**Equation**	**Adj R^2**
WS:OLPR:SCG 3:1:1	OML	dur(Stage 3)	OML= – 22.81 × dur(Stage 3) + 45.22	0.76*
WS:OLPR:SCG 1:1:1	BE	temp(Stage 2), dur(Stage 4)	BE= 2.21 × temp(Stage 2) + 12.36 × dur(Stage 4) + 6.40	0.92**
	BY	temp(Stage 2), dur(Stage 4)	BY= 4.87 × temp(Stage 2) + 11.05 × dur(Stage 4) + 8.91	0.90**
	OML	dur(Stage 4)	OML= 7.25 × dur(Stage 4) + 9.39	0.79*

Dep: Dependent variable (s), Ind: Independent variable (s), Adj: Adjusted, BE: Biological Efficiency, BY: Biological Yield, EY: Economic Yield, OML: Organic Matter Loss, dur: duration, temp: substrate temperature, Stage 1: the period between SRI and 50% MC, Stage 2: the period between 50% MC and 100% MC, Stage 3: the period between 100% MC and PNF, Stage 4: the period between PNF and HF1. *Significant at $p < 0.05$, **Significant at $p < 0.01$

Substrate composition

Pearson's correlations between substrate composition and timings of mycelial growth and production were investigated. Results delineated negative correlations between calcium (Ca), potassium (K), carbohydrates, and fructose; and SRI, PNF, and HF1 timings ($r \geq –0.88$) at a 99% level of confidence (Table 8).

C16: 0 palmitic acid, C18:0 stearic acid, C18:1 oleic acid, and C18:3 linolenic acid contents were negatively correlated with the same timings ($r \geq –0.92$) at a 99% level of confidence. On the other hand, sodium (Na), fat, and total protein contents were positively correlated with the same timings ($r \geq 0.92$) at a 99% level of confidence. Moreover, C18: 2 linoleic acid and C20:0 arachidic acid contents were positively correlated with the same timings ($r \geq 0.92$) at a 99% level of confidence. The magnesium (Mg) content of substrates was positively correlated with SRI and HF1 at a 95% level of confidence ($r \geq 0.82$) and with PNF at a 99% level of confidence ($r = 0.84$).

Negative correlations between magnesium (Mg) ($r = -0.812$) and iron (Fe) ($r = -0.828$) contents of substrates (combined OLPR and SCG mixtures), and the timing of 100% MC were detected at a 95% level of confidence. Pearson's correlations revealed negative correlations between oleic acid content in SCG substrates and all timings of mycelial growth and production.

Table 8 Pearson correlations between spent coffee grounds mixtures components and timings of mycelial growth and production (N=10 per treatment; 10 replicates per treatment)

Component	SRI (DAS)	PNF (DAS)	HF1 (DAS)
Calcium (Ca)	-0.922**	-0.923**	-0.967**
Potassium (K)	-0.912**	-0.911**	-0.958**
Sodium (Na)	0.942**	0.954**	0.981**
Carbohydrates	-0.925**	-0.927**	-0.969**
Fat	0.930**	0.933**	0.973**
Magnesium (Mg)	0.827*	0.863**	0.845*
Fructose	-0.886**	-0.880**	-0.934**
Total protein	0.928**	0.931**	0.972**
C16:0 palmitic acid	-0.924**	-0.926**	-0.968**
C18:0 stearic acid	-0.923**	-0.925**	-0.968**
C18:1 oleic acid	-0.923**	-0.925**	-0.968**
C18:2 linoleic acid	0.927**	0.929**	0.971**
C20:0 arachidic acid	0.928**	0.931**	0.971**
C18:3 linolenic acid	-0.926**	-0.928**	-0.970**

SRI: Spawn Run Initiation, MC: Mycelial Colonization, PNF: Time to Pin Head Formation, HF1: Time to Harvest of First Flush, DAS: Days After Spawning. * Correlation coefficient is significant at the 0.05 level (95%). ** Correlation coefficient is significant at the 0.01 level (99%)

Whereas, positive correlations were denoted between the linoleic acid content of SCG substrates and those timings. Such findings are in agreement with those of Yang et al. (2000) who reported the positive and negative effects of oleic and linoleic acids, respectively, on the mycelial growth of *Ganoderma lucidum*. Previously, Wardle and Schisler (1969) reported the positive impact of both fatty acids on the mycelial growth of *Agaricus bisporus*. This points out the specificity of each mushroom species and the role of each fatty acid in boosting or inhibiting mycelial growth. Kang et al. (2002) reported that oleic and palmitic acids had a major positive influence on the mycelial growth of *Hericium erinaceum*, while linoleic acid badly affected it. Herein, the palmitic acid content of SCG substrates was negatively correlated with the timings of mycelial growth and production, which agree with Kang et al. findings. The negative correlations between stearic acid and the different timings suggest its role in hastening the whole production cycle of *P. ostreatus*. The positive correlations between linolenic acid and the different timings suggest its role in extending the whole production cycle of *P. ostreatus*. On the other hand, the increased content of arachidic acid in SCG substrates suggests its role in the delay of oyster mushroom production. The decreased carbohydrates and increased total protein contents in SCG substrates were negatively and positively correlated, respectively, with the timings of mycelial growth and production. Therefore, the earliness observed in SCG substrates is a result of increased protein content; while their lower yield is caused by lower carbohydrate content found in substrates with high SCG proportions. This assumption corroborates with the

findings of Pardo-Giménez et al. (2016) who associated the high protein content of substrates with earliness. In a similar vein, the increased fat content in SCG substrates was positively correlated with all timings; thus, delaying the fruit body formation. This assumption contradicts the hypothesis of Abou Fayssal et al. (2021b) in this concern and corroborates with the findings of Picornell-Buendía et al. (2016).

Ca content in WS: SCG 3:1 (v/v) and WS: SCG 1:3 (v/v) was negatively correlated with the timings of mycelial growth and production. This outlines the shortening and extension of *P. ostreatus* production cycle. Increasing amounts of Na and Mg in SCG substrates led to a delay in mushroom production. The increased amounts of Mg and Fe in OLPR/SCG substrates were negatively correlated with 100% MC, thus resulting in an earliness in such parameters. Curvetto et al. (2002) reported that an increase in initial mineral contents in *P. ostreatus* substrate resulted in a 25% enhancement of the mycelial growth rate. Whereas, the current study findings suggest not much improvement in this concern.

Conclusion

Low proportions of spent coffee grounds in *Pleurotus ostreatus* substrate seemed to be cost-effective toward a healthier environment. Spent coffee grounds reduced organic matter loss and caused earlier production. Compared to the control, using spent coffee grounds lowered substrate temperature while olive pruning residues rose it. Organic matter loss was strongly correlated with the growth stages' duration. Substrate temperature affected biological and economic yields as well as biological efficiency. Substrates composition was strongly correlated with growth stages duration.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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