Promoting Yield and Oyster Mushroom Cycle Production by Using Date Kernel and Wheat Straw Mixture as a Cultivation Substrate

Ghada Lamraoui,[a] Choukri Tefiani,[a] Asma Maouedj,[a] Abdelmalek Chaalel,[b] Nerdjes Spiga,[a] Diego Cunha Zied,[c] Salim Rouar,[d] Kab-Yeul Jang,[e] Fehmi Boufahja,[f] Walid Elfalleh,[f] and Hamdi Bendif [f,]*

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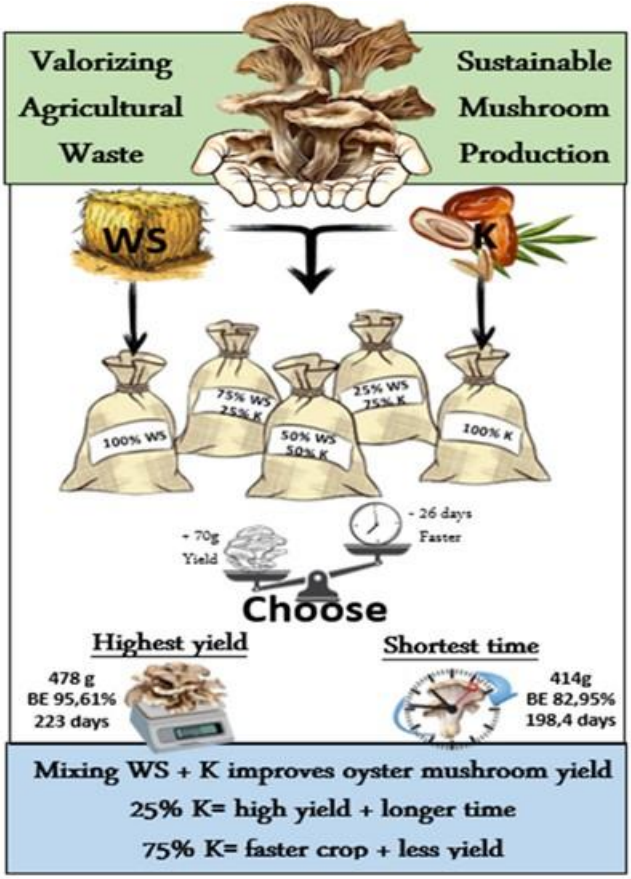
GRAPHICAL ABSTRACT



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Mushroom production can contribute to food security through producing food of high nutritional value and medicinal interest. This study examined the effectiveness of using date kernel (K) in a mixture with wheat straw (WS) as substrate on yield and different crop stages duration for *Pleurotus ostreatus* (P.O) cultivation. Five substrate formulas were investigated using K and WS, alone and in combination. The results indicated that there was a significant difference between formulas. Using wastes separately showed the lowest yield, whereas the substrate with 25% K gave the highest total yield (478 g) and biological efficiency (BE) of 95.61%, in 223 d and 78.52% as BE of three first flushes in 110.8 d. substrate with 75% K was more effective in term of time; it gave in 198.4 d 414 g of mushroom (BE: 82.95%) and 310.6 g (BE: 62.12%) for the three first flushes in 83.2 d. It is more effective to use K and WS in mixture than separately to cultivate P.O., The proportion depends on which we can give up; around 70 g of yield or 26 d as time difference; for more yield in longer time, using 25% K is more suitable, and 75% K is used for shorter time and less yield.

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Keywords: Biological efficiency; Cropping; Lignocellulosic waste; Mushroom; Yield

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INTRODUCTION

Most cultivated edible mushrooms are saprophytic fungi (decomposers), meaning that they are able to decompose lignocellulosic materials by producing extensive enzymes (lignocellulolytic enzymes). An example is the *Pleurotus ostreatus* (oyster mushroom), which is the second most important cultivated edible mushroom worldwide after *Agaricus bisporus* (Chang 1991). It is considered as a dietary food with an interesting medicinal value (Carrasco-Gonzalez *et al.* 2017; Farhan and Chechan 2020; Otali *et al.* 2024).

Pleurotus ostreatus uses lignocellulolytic enzymes to decompose materials and use it as nutrients for their growth, and the cultivation is often associated with the recycling of vast amounts of agricultural residues (Kumla *et al*. 2020). These residues include straw, leaves, saw dust of different trees, wastes of coffee, corn cobs, cotton, cherry, coconut, soybean, sunflower seed, sugarcane bagasse, and palm residue (Bonatti *et al.* 2004; Iqbal *et al.* 2005; Salmones *et al.* 2005; Tabi *et al.* 2008; Abdulhadi and Hassan 2013; Al-Qarawi *et al* 2013; Hoa *et al.* 2015; Mardiana *et al.* 2021; Dissasa 2022; Petković *et al.* 2025).

Date fruit seeds are considered as by-products generated from the agro-industrial sector that transfer date fruit into syrups, jam, date powder, and vinegar. In fact, these agro-residues are thrown away or sometimes used for feeding animals (Nurhayati 2019; Mrabet *et al.* 2020). or for seed oil extraction (Ali *et al.* 2015; Fakhfakh et al. 2019; Hasan *et al.* 2024). Despite its high content of cellulose and hemicelluloses, few studies have investigated the use of date seeds as a substrate for mushroom cultivation (Abdulhadi and Hassan 2013; Al-Qarawi *et al.* 2013). This study aims to confirm the hypothesis that date kernel seeds, when mixed with wheat straw for the first time, can be used as a substrate for *Pleurotus ostreatus* production, by examining its effects on yield and production time.

EXPERIMENTAL

Fungal Culture

The species used was *Pleurotus ostreatus*, which grew on wheat seeds and was obtained from a local producer of mushrooms in Batna, Algeria.

Substrate Formulation

The mushroom cultivation and harvesting were done according to Abdulrazzaq *et al.* (2017) with some modifications.

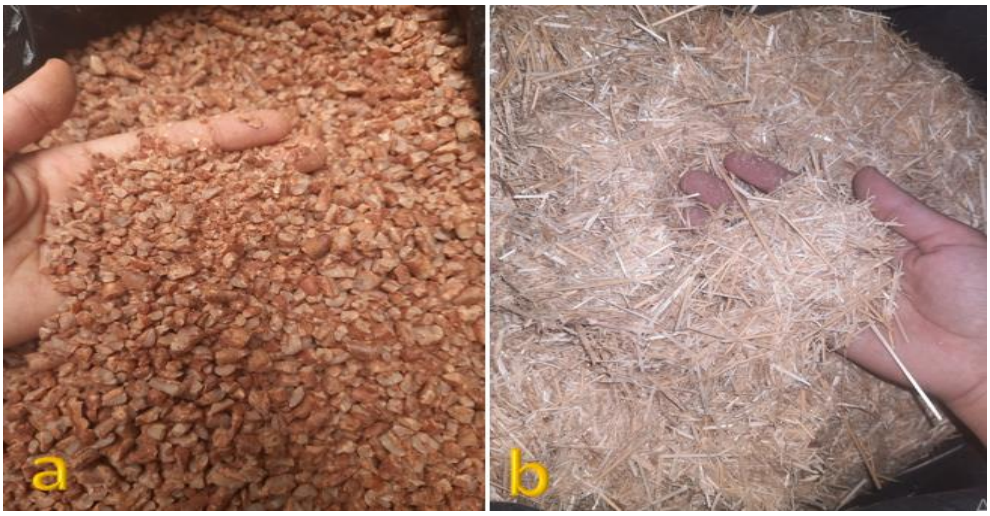

Fig. 1. Substrate preparation for *Pleurotus ostreatus* cultivation: a: grounded date kernel; and b: chopped wheat straw

Date kernels of *Phoenix dactylifera* L. (mish degla variety) were collected from local producers of date flour in Biskra, a region located approximately 200 km from the cultivation site and recognized for its total production of around 459,000 tons of date per

year which accounts for 41% to 42% of Algerian total production (Rekis 2020). Given that kernel represent from 10% to 15% of fruit wight (Kiesler *et al*. 2024), the region is estimated to produce more than 45000 tons of date kernel per year. The date kernels were ground into small particles (0.2 to 0.5 cm) using a stainless-steel coffee grinder. Wheat straw was collected from a local farm in the Batna region and cut into small pieces ranging from 2 to 4 cm (Fig. 1). Five different substrates were investigated in this study and were prepared using wheat straw and date kernel agro-wastes. One substrate (WS) contained only wheat straw (100%) and served as the control, another (K100) contained 100% grounded date kernel, and the remaining three substrates were prepared by mixing the two agro-wastes, date kernel and wheat straw mixing respectively in the following proportions: 25%–75% (K25), 50%–50% (K50), and 75%–25% (K75).

Preparation of Substrate and Crop Cycle

A total of 500 g of each substrate formula was placed in containers and hydrated with water overnight. The excess water was drained the next day, and then the hydrated substrate was transferred into polypropylene bags with 2% $CaCO_3$. The polypropylene bags containing the substrate and $CaCO_3$ were sealed and autoclaved for 20 min at 121 °C. After sterilization, the bags were left to cool down and then inoculated with 50 g of *Pleurotus ostreatus* spawn (10% of the substrate weight). The bags were then incubated in the dark at 23 °C to 26 °C until the substrate was fully colonized by the mycelium. After full colonization, the colonized bags were transferred to another room with specific conditions (temperature: 13 °C to 17 °C; relative humidity: 90% to 95%). This room was equipped with an air conditioner, a humidifier, a sensor to automatically regulate temperature and humidity, ventilators to renew the air, and a lamp to provide light. At this stage, after fructification, mature mushroom flushes were harvested one by one until the substrate produced no more mushrooms.

Registered Parameters

Throughout the cultivation experiment, several parameters were recorded and analyzed, including spawning running time (the number of days required for the mycelium to fully colonize the substrate), time for the first pinhead formation (the number of days necessary until the appearance of the first primordia), earliness (the number of days between spawning and the first flush harvest), the interval in days between successive mushroom harvests, total crop duration (the total duration in days from inoculation to the last harvest), total number of flushes, yield by calculating fresh weight of mushrooms (single flush, 3 first flushes and total flushes) harvested per bag (g), and biological efficiency (BE) which is as the ratio of fresh mushroom weight (single flush, 3 first flushes and total flushes) to dry substrate weight, expressed as a percentage (%)

Carbon and Nitrogen Analysis

After grinding the different substrates into powder, the samples were homogenized, weighed, and placed in CHN-628 elementary analyzer (LECO corporation, Michigan state, USA) to determine and calculate the total carbon and nitrogen contents. The analysis was done in the National Institute of Horticultural and Herbal Science in Eumseong, South Korea.

Statistical Analysis

The experiment was carried out in a randomized complete block design with five treatments and five replications per treatment. The statistical analysis was conducted using the Kruskal-Wallis test to assess the significant effect of treatment type, followed by Mann-Whitney tests to identify the significant differences among the five treatments with 95% confidence level. Data processing and analysis were performed using SPSS statistical software (IBM Corporation, version 27, NY, USA), and Excel (Microsoft Corporation, Excel 2016, WA, USA).

RESULTS AND DISCUSSION

Carbon and Nitrogen Analysis

The composition of carbon (C) and nitrogen (N) contents in the substrate was analyzed, and results showed that the highest carbon content was in K100 (44.52%), while the lowest was in WS (42.42%). Carbon content increased gradually with the addition of date kernel, indicating a proportional relationship. Similarly, nitrogen content ranged from 0.42% in WS to 0.52% in K100. However, the C/N ratio displayed an inverse trend, decreasing with the increasing percentage of date kernel in the substrate. The C/N ratio ranged from 81.9 in K100 to 100.5 in WS. These results are summarized in Table 1.

Table 1. Deferent Substrates Content in Carbon and Nitrogen

Substrate Formulation	Carbon Content (%)	Nitrogen Content (%)	C/N Ratio
100% WS	42.424 ± 0.132^{a}	0.424 ± 0.034^{a}	100.446 ± 7.998^{c}
75% WS + 25% K	43.723 ± 0.237^{b}	0.461 ± 0.026^{b}	95.061 ± 4.959^{c}
50% WS + 50% K	43.775 ± 0.051^{b}	0.475 ± 0.026^{b}	92.287 ± 5.240^{c}
25% WS + 75% K	44.049 ± 0.076^{c}	0.524 ± 0.016^{c}	84.176 ± 2.429^{b}
100% K	44.529 ± 0.087^{d}	0.545 ± 0.023^{c}	81.874 ± 3.503^{a}

The numbers in the table represent the means ± standard error
Statistical analysis was done by using non-parametric Kruskal-Wallis test followed by Mann-Whitney test to compare between groups (five repetitions of five treatments). The same letters in a row indicate no significant difference (Kruscal-Wallis test and Mann-Whiney test with $P < 0.05$)

The total nitrogen, total carbon, and C/N ratio in the substrate is crucial for mushroom cultivation, as they directly affect mycelial colonization and mushroom development (Nieuwenhuijzen and Oei 2005; Figueiró and Graciolli 2011). The carbon content observed in this study aligns with the values reported by Hoa *et al.* (2015), though the nitrogen content was significantly lower, resulting in a higher C/N ratio. The findings also correspond with the results of Atila and Cetin (2024) and Costa *et al*. (2023). The behavior of increase and decrease depicted in the probe can be attributed to the chemical composition of date kernels, which contain approximately 4% protein and over 20% cellulose, hemicellulose, and lignin (Kocheki 2015; Nabili *et al.* 2017; Abu-Thabit *et al.* 2020).

Spawning or Mycelium Full Colonization

The overall colonization times are presented in Table 2. The formulation K100 required the shortest colonization time, taking only 12 d. The other substrates, ranging from 19.8 d (K75) to 22.8 d (K25), showed no significant differences in colonization times.

The results of spawning indicated faster times compared to the findings of Hoa *et al*. (2015). K100 is consistent with previous studies using materials such as paddy straw, waste paper, and industrial cardboard (Afify *et al*. 2012; Subedi *et al.* 2023). Similar results were observed with cottonseed husk and maize cob (Emiru *et al.* 2016). The results for the other substrates are in agreement with Atila and Cetin (2024), who reported around 20 d for full colonization when using poplar sawdust, lavender straw, and lavender flower waste. According to Patel *et al.* (2009), mycelial growth is facilitated by enzymes that degrade the substrate, with growth usually detectable 2 to 3 d after inoculation. Samuel and Eugene (2012) emphasized the critical role of nitrogen in promoting mycelial growth. Some studies suggest that nitrogen absence inhibits growth, while low nitrogen levels promote ligninolytic enzyme production, with higher levels (greater than 1.5%) suppressing it (Hoa and Wang 2015; Neelam *et al.* 2013). Carbon, an energy source for mycelial growth, is also essential for mycelial cell structure (Walker and White 2017). Date kernels contain higher carbon and nitrogen contents compared to WS, contributing to faster spaw run in substrates with a higher percentage of date kernel seed. The rapid colonization in K100 can also be attributed to the particle size of the substrate, as larger particles allow better air circulation and less compression, which accelerates mycelial growth (Zhang *et al*. 2002; Chang and Miles 2004).

Primordia Formation

After inoculation, the bags were moved to the second room for monitoring other parameters, starting with the time required for pinhead formation. WS exhibited the longest time (15.8 d) for the appearance of the first primordia, compared to the other treatments: K25, K50, and K100, which took 13, 11, and 11.4 d, respectively. K75 had the shortest time at 9 d (Table 2).

The time of first pinhead appearance align with previous studies, where pinning duration ranged from 17 to 58 d depending on substrate composition (Girmay *et al.* 2016; Otieno *et al.* 2022). Oei (2003) stated that materials with high lignin and cellulose content, like WS, take longer to primordia formation, which is why the addition of date kernel, with its lower cellulose content, accelerated the process

First Harvesting Flush

The harvesting of different flushes is conducted after maturation of fruiting bodies (Fig. 2). The time required for the first harvesting flush, after primordia formation, ranged from 7.8 to 9.4 d across all treatments, as indicated in Table 2, but there was a significant difference when measured from the inoculation day. K100 produced the first flush in the shortest time (31.4 d), followed by K75 (36.6 d). The remaining substrates, K50, K25, and WS, required longer times (42.2, 45.2, and 44.6 d, respectively).

In the literature, the time to complete formation of the fruiting bodies (ready to harvest) generally ranged from 22 to 35 d (Emiru *et al.* 2016), with some studies reporting longer durations up to 56 d (Afify *et al.* 2012; Atila and Cetin 2024). Since there was no significant difference in the maturation time of primordia, this indicates that the main time difference occurred during spawning period, as delayed spawn run and primordia formation extended the harvesting period (Hoa *et al.* 2015).

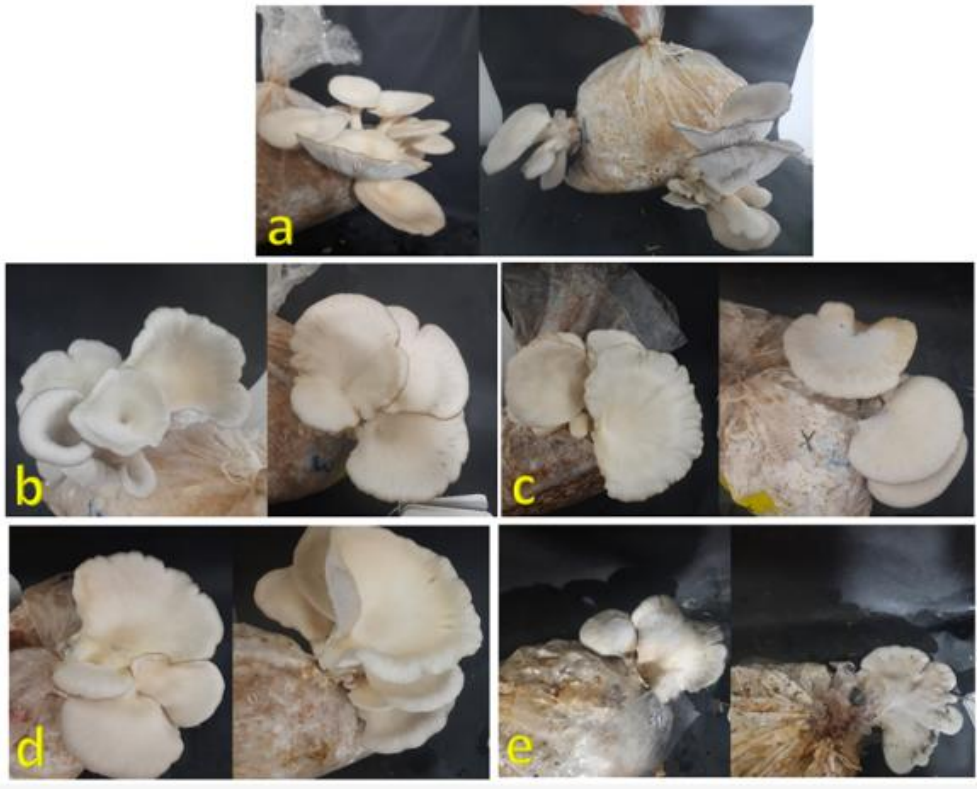

Fig. 2. Mushroom fructification on different substrates: (a: 100% wheat straw; b: 25% date kernel + 75% wheat straw; c: 50% date kernel + 50% wheat straw; d: 75% date kernel + 25% wheat straw; e: 100% date kernel)

Crop Cycle

After the first flush, subsequent flushes were harvested depending on the substrate composition. The shortest total harvest phase (47.6 d) was recorded for K100, followed by K50 (51.8 d), and K75 (54.4 d). The longest durations were observed in WS (69.6 d) and K25 (75 d). K100 showed the shortest total crop cycle of 71 d, with only three flushes, while WS, K25, and K75 had four, five, and six flushes, respectively. K50 exhibited the highest number of flushes (6.2), which did not differ significantly from K25 and K75. The second shortest cropping time after K100 was observed with WS, which took 140 d from inoculation to complete the cropping of 4.2 flushes. For the other formulas, no significant differences were found in either harvesting time (ranging from 169.6 to 187.2 d from the first to the last flush) or total harvest phase. However, K50 and K75 produced more flushes in a shorter period (207.6 and 198.4 d, respectively) compared to K25, which took 223 d to complete the harvest of the last flush.

The variation in times between flushes, especially between the three first flushes, were shorter than those reported by Paudel and Dhakal (2020), who found a longer separation time, with 5 to 6 weeks between the first and second flush and 7 to 10 weeks between the second and third flush. The total harvesting period, from the first to the last harvest, was longer than what was recorded by Hoa *et al.* (2015). This method of mushroom cultivation would be called subsistence fungiculture with low production costs, unlike controlled commercial cultivation which could not have such a long crop cycle.

Table 2. The Overall Time Measurements of *Pleurotus ostreatus* Mushroom Cultivated on Different Substrate Mixture between Wheat Straw and Date Kernel

	Necessary Time from Previous Stage (d)								
	Spawning	**Pinning**	**1st Flush**	**2nd Flush**	**3rd Flush**	**4th Flush**	**5th Flush**	**6th Flush**	**7th Flush**
100% WS	20.6±0.98^{a}	15.8±1.02^{a}	8.2±0.58^{a}	21.8±1.59^{a}	39.6±4.48^{a}	24±10.01^{b}	10±6.12^{b}	-	-
75% WS + 25% K	22.8±1.80^{a}	13±1.55^{ab}	9.4±0.93^{a}	27±5.84^{a}	38.6±7.98^{ab}	48.8±3.17^{a}	34.2±3.02^{a}	29.2±12.23^{a}	-
50% WS +50% K	22.2±1.20^{a}	11±1.26^{b}	9±0.32^{a}	18.6±1.75^{a}	24.2±1.77^{b}	39±1.76^{b}	35.2±1.56^{a}	42.4±8.84^{a}	6±6.00^{a}
25% WS + 75% K	19.8±1.80^{a}	9±0.84^{b}	7.8±0.49^{a}	21.2±1.28^{a}	25.4±2.14^{b}	50.6±10.51^{ab}	33.4±7.23^{ab}	28±9.70^{a}	3.2±3.20^{a}
100% K	12±0.00^{b}	11.4±1.29^{b}	8±0.32^{a}	16.4±1.86^{a}	23.2±1.39^{b}	-	-	-	-

	Necessary Time of Different Stages (d)					
	From Inoculation to Pinning	**From Inoculation to 1st Flush**	**From Inoculation to 3rd Flush**	**From 1st Flush to 3rd Flush**	**From 1st Flush to last Flush**	**From Inoculation to Last Flush**
100% WS	36.4±1.99^{a}	44.6±1.54^{a}	106±3.97^{a}	69.6±5.23^{a}	103.6±8.99^{b}	140±10.46^{b}
75% WS + 25% K	35.8±1.96^{a}	45.2±2.75^{a}	110.8±11.03^{ab}	75±11.19^{ab}	187.2±9.20^{a}	223±8.31^{a}
50% WS +50% K	33.2±1.56^{a}	42.2±1.59^{a}	85±2.59^{b}	51.8±3.34^{b}	174.4±10.22^{a}	207.6±9.32^{a}
25% WS + 75% K	28.8±1.11^{b}	36.6±1.47^{b}	83.2±1.66^{b}	54.4±1.17^{b}	169.6±11.87^{a}	198.4±11.13^{a}
100% K	23.4±1.29^{c}	31.4±1.50^{b}	71±2.70^{c}	47.6±1.50^{b}	47.6±1.50^{c}	71±2.70^{c}

The numbers in the table represent the means ± standard error
Statistical analysis was done by using nonparametric Kruskal-Wallis test followed by Mann-Whitney test to compare between groups (five repetitions of five treatments). The same letters in the same row indicate groups with no significant difference (Kruscal-Wallis test and Mann-Whiney test with $P < 0.05$)

A shorter production duration is an important factor when selecting substrates for mushroom cultivation. The total crop duration, from the first day of inoculation to the last flush harvested contrasts with the results of Paudel and Dhakal (2020), who found a duration of 69 to 96 d with only 3 flushes. However, the duration observed in this study is consistent with Khanna and Garcha's (1982) finding that mushroom harvesting can take up to 104 d.

The variation in cropping periods among different substrates may stem from differences in spawn running times, primordia formation, fruiting body maturation, intervals between flushes, and the number of flushes

Yield and Biological Efficiency

After collecting and weighing each flush separately, the first flush recorded the highest weight, and there was a decrease in yield from one flush to the next, with a considerable yield registered in the first three flushes. The highest first flush yield was observed in K25 (211.6 g), followed by WS (169.45 g), and the lowest yield was in K100 (93 g), followed by K75 (126 g) and K50 (127.8 g), with no significant differences (Table 3). K100 produced only 3 flushes with a total yield of 220.2 g/bag (44.04% as BE) which was almost the same as the first flush yield of K25 (211.6 g), comparing the results of the three first flushes of the other substrate, it was the last effective substrate followed by K50 (59.04%); K75 (62.12%); ws (67.3%) and the highest BE of three 1st flushes was noted in K25 with 78.52% equivalent to 392.6 g as yield (Table 3 and Fig. 3).

Yield also is considered as an important parameter for substrate selection. The decrease in yield per flush confirms the findings of Hoa *et al*. (2015) and Dedousi *et al*. (2023). Such yield distribution is common in *Pleurotus* spp. (Rizki and Tamai 2011; Koutrotsios *et al*. 2018; Melanouri *et al*. 2022).

This decrease is due to the reduced nutrient content of the substrate, as the mushrooms consume the available nutrients during growth, accompanied by the appearance of pests and diseases. *Pleurotus* spp. are lignocellulolytic, breaking down and feeding on the cell wall contents of the substrate (Adebayo and Martinez-Carrera 2015). Additionally, *Pleurotus* spp. prefer higher cellulose content for growth and development (Shah *et al.* 2004).

Considering only the first flush, the yield observed with K25 was similar to that of cotton seed (Girmay *et al*. 2016). The highest yield recorded in K25 was the same as the lowest yield reported by Subedi *et al*. (2023). Conversely, even the lowest yield in K100 was higher than most of the results reported by Emiru *et al.* (2016).

When considering all flushes, the use of date kernel as a substrate with WS resulted in yield and BE comparable to those of Sales-Campos *et al.* (2011). The same ratio of coconut to sawdust was used, although coconut 75 was significantly higher than K25. Another study using date kernel confirmed the same BE for K100 as found in the present work (Abdulhadi and Hassan 2013). This is likely due to the fact that low in protein in lignocellulosic materials limits their suitability for mushroom cultivation (Obodai *et al*. 2002) and adding substrates with high nitrogen content can improve the yield and quality of *Pleurotus* spp. (Mahari *et al*. 2020).

Table 3. Number of Flushes and Yield of *Pleurotus ostreatus* Mushroom Cultivated of Different Substrates Mixture between Wheat Straw and Date Kernel

Substrate Formulation	Total Flushes	Yield (g bag^{-1})								
		1st Flush	2nd Flush	3rd Flush	4th Flush	5th Flush	6th Flush	7th Flush	1st +2nd +3rd Flush	Total Yield
100% WS	4.2±0.58^{bc}	167±26.86^{ab}	115.2±7.96^{a}	54.284±9.93^{a}	21.576±11.95b^{c}	13.79±8.82^{b}	0^{c}	0^{a}	336.48±29.98^{ab}	374.3±41.21^{a}
75% WS + 25% K	5.6±0.24^{ab}	211.6±5.59^{a}	109.2±10.47^{ab}	71.8±15.73^{a}	43.068±10.48^{b}	36.122±1.16^{a}	6.27±3.24^{bc}	0^{a}	392.6±21.02^{a}	478.06±28.87^{a}
50% WS + 50% K	6.2±0.20^{a}	127.8±9.02^{b}	74.4±16.40^{b}	93±14.99^{a}	80.2±7.02^{a}	24.892±5.08^{ab}	22.73±4.63^{a}	8.69±8.69^{a}	295.2±14.65^{b}	431.72±12.70^{a}
25% WS +75% K	5.8±0.37^{ab}	126±9.51^{b}	93.2±15.19^{ab}	91.4±13.27^{a}	58.26±16.46^{ab}	18.37±2.67^{b}	19.72±6.39^{ab}	7.8±7.80^{a}	310.6±23.19^{bc}	414.75±20.48^{a}
100% K	3±0.00^{c}	93±20.91^{b}	78±8.81^{b}	49.2±6.78^{a}	0^{c}	0^{c}	0^{c}	0^{a}	220.2±15.97^{c}	220.2±15.97^{b}

The numbers in the table represent the means ± standard error

Statistical analysis was done by using non parametric Kruskal-Wallis test followed by Mann-Whitney test to compare between groups (five repetitions of five treatments). The same letters in the same column indicate no significant difference (Kruscal-Wallis test and Mann-Whiney test $P < 0.05$)

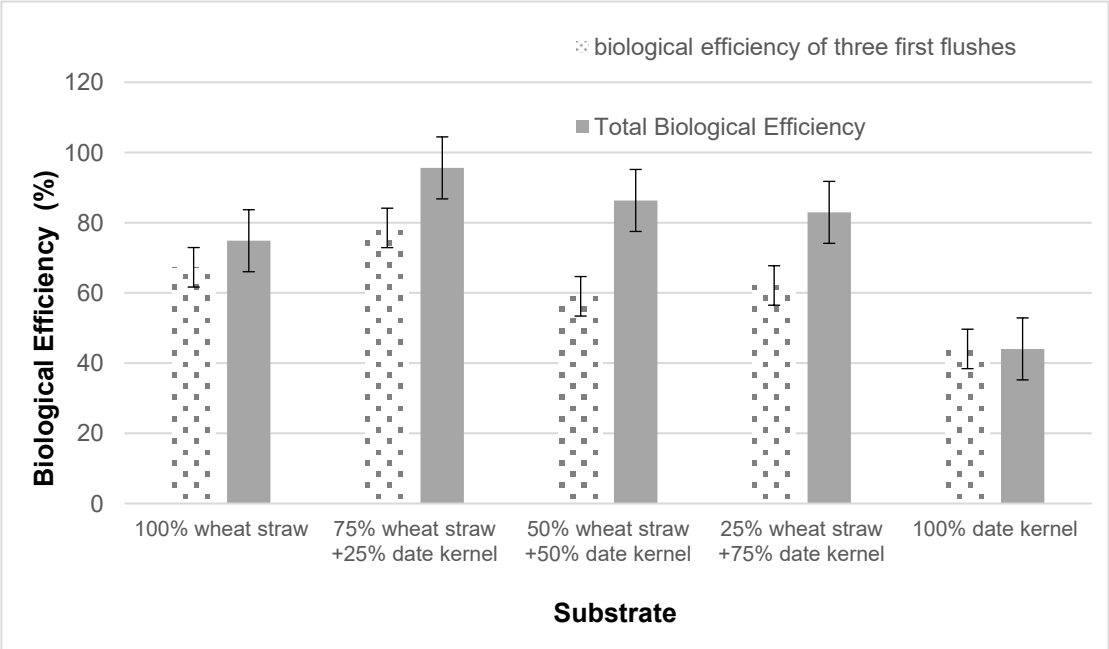

Fig. 3. Mean BE of three first flushes and total BE of *Pleurotus ostreatus* mushroom cultivated on different substrate mixture between wheat straw and date kernel. Bar in columns shows the standard error of means (Kruskal-Wallis test and Mann-Whiney test $P < 0.05$).

CONCLUSIONS

1. The effectiveness of the substrates was evaluated based on both total cultivation time and biological efficiency (BE). It was found that substrates composed of 100% date kernel or 100% wheat straw were less effective.
2. A substrate with 25% date kernel and 75% wheat straw registered the highest biological efficiency and yield over a longer cropping period, making it suitable when quantity of production is the priority. However, a substrate with 75% date kernel and 25% wheat straw demonstrated more rapid production cycle while maintaining relatively high BE, making it perfect when time efficiency is also critical.
3. The choice between the two optimal substrates depended on the production goal, whether it was the maximum yield and BE or shorter production time, and in both cases, a mixture of wheat straw with date kernel was shown to be effective for *Pleurotus ostreatus* cultivation.

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