Journal of Agriculture and Ecology Research International

12(4): 1-10, 2017; Article no.JAERI.36331
ISSN: 2394-1073

Cultivation of *Pleurotus* spp., as an Alternative Solution to Dispose Olive Waste

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAERI/2017/36331
Editor(s):
(1) Normala Halimoon, Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, Malaysia.
Reviewers:
(1) Jayath P. Kirthisinghe, University of Peradeniya, Sri Lanka.
(2) Mary Obodai, CSIR-Food Research Institute, Ghana.
Complete Peer review History: http://www.sciencedomain.org/review-history/20867

Original Research Article

Received 23rd August 2017
Accepted 5th September 2017
Published 8th September 2017

ABSTRACT

Disposal of olive press cake (OPC) produced during olive oil production is an important problem in Mediterranean Basin. The objective of this paper is to investigate effectively removing OPC by production of *Pleurotus* spp. In the study, four substrate formulations containing variable OPC concentration were tested for cultivation of *Pleurotus* spp and determined effect of these substrates on productivity of *P. djamor, P. eryngii and P. citrinopileatus* species. Zero (0%), 25%, 50% and 75% portions of OPC were added to beech sawdust to prepare the growing substrates. It was observed that different portions of OPC affect spawn run time (day), time to first primordia initiation (day), time to first harvest (day), yield (g/kg) and biological efficiency (BE) of *Pleurotus* spp. No negative effect on mycelial growth of the three *Pleurotus* spp. was observed in any substrates during the spawn running period, but high concentration of OPC had a negative effect on earliness. On the other hand, 25 OPC and 50 OPC substrates showed higher biological efficiencies than the control substrate in all *Pleurotus* spp. Morever, the highest yield and BE were obtained in P. *djamor* grown on 75 OPC substrate. This study demostrated that high portions of OPC are promising as a substrate for *P. djamor, P. citrinopileatus* and *P. eryngii* cultivation and using OPC as a substrate in mushroom cultivation provides an eco-friendly method for disposing of OPC.

Keywords: Olive press cake; Pleurotus djamor; Pleurotus citrinopileatus; Pleurotus eryngii.

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1. INTRODUCTION

The cultivation of olive and the production and use of olive oil has been known in the Mediterranean region for more than 7000 years [1]. The vast majority of olive oil production 98% occurs in the Mediterranean region [2]. The three major olive oil producers worldwide are Spain, Italy, and Greece, followed by Turkey [3]. In parallel with the large scale production, disposal of large amounts of wastes produced during olive oil production is a important problem in this area.

The olive oil production is characterized by relevant amounts of by-products, namely solid residues mainly containing the olive skin and stone (olive press cake) and aqueous effluent (vegetation water), usually named olive mill wastewater [4]. Olive press cake (OPC) is produced about 376.704 tonnes annually in Turkey as by-product from olive oil production process [5]. This waste has little or no use. Caputo et al. [6] reported that limited storage life and the high transportation costs of olive press cake are raising the problem of its disposal. These by-products are left to rot in the field or are disposed off through burning. Although several studies, such as utilising it as feedstock for ruminants [5], biodiesel production [6] and energy source [7] as well as other ways have been proposed, these are not sufficiently feasible and cost-effective to meet the need of olive oil industries on a massive scale.

Fungal treatments have been considered as a promising method for improving the physical and chemical structure of agrowaste [8,9]. Utilizing olive press cake for mushroom cultivation may be one of the solutions.

OPC has a slightly acidic pH values, very high content of organic matter such as lignin, hemicellulose and cellulose and has a considerable proportion of fats, proteins, water-soluble carbohydrates and a small but active fraction of hydrosoluble phenolic substances [10]. So it can serve as carbon, nitrogen and energy sources for growth of mushroom [11].

Pleurotus spp. can be grown on various agro-industrial wastes, such as sawdust, straw, cotton seed hulls, corn cobs, etc. due to its extensive enzyme systems. Therefore, they have been widely used in the bioremediation of pollutants and the degradation of lignocellulosic residues by the action of different enzymes [12]. The substrates used in each region depend on the locally available agricultural wastes for *Pleurotus* spp. cultivation. In the study, the possibility of using OPC as a potential substrate for cultivation of *Pleurotus* spp. was evaluated. Although some papers have been published on the use of OPC in the cultivation of some edible mushrooms [13,14,15] a detailed research and optamized substrate formulation are a need for practical large-scale production of commercial *Pleurotus* spp.

In present study, different substrate formulations containing variable OPC concentration were tested for cultivation of *Pleurotus* spp and determined effect of these substrates on productivity of *P. djamor, P. eryngii and P. citrinopileatus* species.

2. MATERIALS AND METHODS

2.1 Materials

Mushroom strains used in this study were the commercial strains *P. djamor, P. citrinopileatus* obtained from Agroma Co. Ltd (Denizli, Turkey). *P. eryngii* was supplied by the Ataturk Agricultural Research Institute (Yalova, Turkey).

Olive press cake (OPC) was obtained from a two-phase continuous olive oil mill plant extraction (Helvacıköy, İzmir, Turkey). Sawdust used for mushroom cultivation was purchased at Kırşehir, Turkey.

The cultures were maintained in a malt extract agar (MEA) medium (Merck). This study was carried out at the Mushroom Production Unit of Agriculture Faculty of Ahi Evran University in Kırşehir, Turkey.

2.2 Experimental Design

Four different substrate formulations were tested in the study. Zero (0%), 25%, 50% and 75% portions of OPC were added to beech sawdust to prepare the growing substrates (Table 1).

Table 1. Content of substrates used in the study

	Ratios (%)		
Substrates	**Olive press cake**	**Sawdust**	**Gypsum**
Control	0	99	1
25 OPC	25	74	1
50 OPC	50	49	1
75 OPC	75	24	1

Pure sawdust substrate was used as a control medium. The experiment was conducted in a completely randomized plot design, with ten replications.

2.3 Culture Media and Spawn Preparation

Subcultures of the mycelium were recovered on petri plates containing MEA and stored in a refrigerator at 4°C. Spawn substrate was prepared by boiled wheat grains in glass bottles and sterilized in an autoclave for 90 min at 121°C, cooled and inoculated bottles were incubated in the dark, at 25°C until the completion of mycelial growth.

2.4 Mushroom Cultivation

Substrate was mixed with increasing amounts of OPC from zero to 75% (v/w) and tap water was added until it was moistened to about 70%. Then, 1 kg (wet weight) of each substrate was packed into a polypropylene autoclavable bags. The plastic bags containing substrate were sterilized in an autoclave at 121°C for 90 min. After cooling, bags were inoculated in a laminar flow chamber using 3% *Pleurotus* spp. spawn (on a w/w wet weight basis). Bags were incubated at 25±2°C with 80% humidity in the dark for several days. When substrates were fully colonized, the cotton plugs were removed and the tops of the bags were folded down. The temperature and humidity of production chamber were changed to 16-18°C and 85%, respectively. Cool white fluorescent bulbs provided 8 h of light daily. Sufficient air changes were maintained. Substrate bags were prepared in ten replicates for each *Pleurotus* species and for each type of substrate.

Mushrooms were harvested as soon as the fruiting bodies developed and attained their full size above the substrate with a sharp knife from each treatment bag.

2.5 Evaluation of the Cultivation Parameters

Several cultivation parameters were evaluated during *Pleurotus* spp. cultivation on control substrates (containing only sawdust) and on substrates including OPC supplementation. The following data were recorded; spawn running time (day), time to first primordia initiation (day), time to first harvest (day) yield (g/kg), biological efficiency (BE%). The incubation time required before pinning (pinhead appearance) and for harvesting of the first, second and third flushes were recorded. Morever, moisture content, ash, C and pH of the substrates were measured using standard methods. The Kjeldhal method was used to determine the total nitrogen content of each substrate. Then the carbon/nitrogen ratio of each substrate was calculated. Substrate samples were taken using the randomized sampling technique, with three replication.

Yield expressed as grams of fresh mushrooms harvested at maturity per gram of wet substrate (w/w), biological efficiency (BE%) defined as the percentage ratio of the fresh weight of harvested mushroom per gram of dry substrate [16]

Results were obtained from ten replicates for each *Pleurotus* spp grown on each type of tested substrate.

2.6 Statistical Analysis

The data obtained from the experiment were subjected to variance analysis and the statistical significance was compared employing Tukey's test, using the SPSS 16.0 for Windows statistical computer program at a significance level of 5%.

3. RESULTS

3.1 Growing Media

Significant differences were found among the growing substrates regarding ash, N content, and C:N ratio *(P<0.01),* while there was not difference between C content of substrates (*P>0.05*) (Table 2).

Table 2. Chemical composition of substrates used in the study

Properties	Control	25 OPC	50 OPC	75 OPC
Moisture (%)	70.0** [a]	67.1 [b]	65.4 [c]	64.2 [c]
Ash (%)	5.40** [b]	5.79 [a]	6.04 [a]	6.18 [a]
C (%)	47.1 [ns]	47.3	47.0	46.9
N (%)	0.41 ** [d]	0.63 [c]	0.76 [b]	1.02 [a]
C:N	115.4 ** [a]	74.8 [b]	61.8 [c]	46.0 [d]

*Asterisks indicate significance at *P <0.05, **P <0.01, [ns] Nonsignificant, values within the same row followed by the same letter are not significantly different. Mean values in the same row followed by the same letters are not significantly different by Tukey multiple range test*

Moisture content of substrates varied between 64.2% (75 OPC) and 70.0% (control) The ash and N contents were determinated between 5.40%-6.18 and 0.41%-1.02%, respectively. Also, the C:N ratio of substrates varied between 46.0 (75 OPC) and 115.4 (control).

3.2 Spawn Running Time and Cultivation Cycle

Significant differences were observed among treatments in terms of days taken for spawn running time, time to first primordia initiation and time to first harvest of *P. djamor, P. citrinopileatus and P. eryngii*) (*P<0.01*).

As seen in the Table 3, *Pleurotus* species grown on substrates supplemented with different portion of OPC ranged between 17.8-25.2 days for *P. djamor*, 17.4-23.6 days for *P. citrinopileatus* and 18.6-21.2 days for *P. eryngii*. *P. djamor* strain cultivated on control, 25 OPC and 50 OPC started forming pinheads after the 22.0, 21.8 and 22.4 days, respectively, whereas pinheads grown on 75 OPC substrate appeared on 29.2th day. Significant differences were also observed among substrates in terms of time to first primordia initiation of *P. eryngii* and *P. citrinopileatus*. The strain of *P. eryngii* formed pinheads between 46.6 days (control) and 49.2 days (25 OPC). There was no statistical difference between the 25 OPC and the control medium, but the pinheads formation was longer (7 days) in the 50 OPC medium than control in *P. citrinopileatus*. Morever pinhead forming was not obtained on substrate 75 OPC in *P. citrinopileatus* and *P. eryngii.*

The time to first harvest was determinated between 25.6 and 34.8 days for *P. djamor*, 52.4 and 57.0 days for *P. eryngii* and 32.4 and 40.6 days for *P. citrinopileatus*. There was no difference between control substrate and 25 OPC and 50 OPC substrates, while the time to first harvest substrate increased significantly *for P. djamor* grown on 75 OPC. Although statistical significant difference was not observed between the control and 25 OPC substrate, time to first harvest get longer for *P. citrinopilatus* grown on 50 OPC substrate. Morever, production cycle was also elongated with increased olive press cake content on substrate in *P. eryngii*.

3.3 Yield and Biological Efficiency

Significant differences were observed among treatments in terms of yield and BE (P<0.01) (Table 4). The yield and BE showed a gradually increasing trend as the ratio of OPC increased in *P. djamor*. Substrate 75 OPC gave the highest yield (252.7 g/kg), which was 30.4% higher than the control substrate. The BEs were determined as 58.6%, 59.5%, 71.1% and 70.2% for control, 25 OPC, 50 OPC and 75 OPC, respectively. Yield and BE of *P. eryngii*, cultivated on growing media added OPC was found to be increased significantly over control, but no fruiting body was obtained on substrate 75 OPC. The total mushroom yield of *P. eryngii* ranged from 135.5 g/kg to 227.4 g/kg, while the BEs were 44.9%, 64.2%, 65.0% for control, 25 OPC and 50 OPC, respectively. Yield of 25 OPC and 50 OPC were 40.8% and 36.6% higher than the control, respectively. It was also observed that significant difference between the substrates (*P* <0.01) in terms of yield and BE (%) in *P. citrinopileatus*. Obtained yields from substrates 50 OPC and 25 OPC were significantly higher than from the control. The yield and BE was increased with increasing OPC content in the substrates. Total yield and BEs ranged from 169.7 g/kg and 56.6% (control) to 234.6 g/kg and 67.0% (50 OPC). When OPC were mixed with equal amount (50%) of sawdust, biological efficiency was found to increase to 27.7% compared with control.

The yields of three flushes were recorded in *P. djamor*, as shown in Table 4. Substrates showed significant difference in terms of yield in *P. djamor* in the first flush. 75 OPC showed highest results by producing 166.91 g, but there are not significant differences between control, 25 OPC and 50 OPC in first flush. Among the substrates, 50 OPC gave maximum yield (89.6 g) whereas control substrate showed lowest values by yielding 59.70 g in second flush. Similarly, the highest and lowest yields were obtained on 50 OPC and control in third flush, respectively.

Two flushes were recorded in *P. citrinopileatus*. In the first flush, although yields from 50 OPC (*P* <0.01) was significantly higher than from the control and 25 OPC, in second flush, the highest yield was recorded on 25 OPC in *P. citrinopileatus*. *P. eryngii* grown on 50 OPC were recorded best in terms of yield in first flush by producing 172.4 g whereas the highest yield was recorded in second flush by producing 82.8 g on 25 OPC substrate. On the other hand, only 25 OPC and 50 OPC produced fruiting body but nothing was obtained on control substrate in second flush in *P. eryngii.*

Table 3. Effect of different substrates on spawn running time, first primordia initiation and harvest start date of *Pleurotus* spp.

	***Pleurotus* spp.**											
	P. djamor				***P. citrinopileatus***				***P. eryngii***			
	Control	**25 ZP**	**50 ZP**	**75 ZP**	**Control**	**25 ZP**	**50 ZP**	**75 ZP**	**Control**	**25 ZP**	**50 ZP**	**75 ZP**
SRT (day)	17.8** [b]	18.4 [b]	18.6 [b]	25.2 [a]	18.8 **[b]	17.4 [b]	19.8 [b]	23.6 [b]	19.6** [b]	18.6 [b]	19.4 [b]	21.2 [a]
TFPI(day)	22.0** [b]	21.8 [b]	22.4 [b]	29.2 [a]	28.4** [b]	29.0 [b]	35.4 [a]	0.00[c]	46.6 **[c]	49.2 [a]	48.8 [b]	0.00[d]
TFH(day)	25.6** [b]	25.8 [b]	26.6 [b]	34.8 [a]	32.4** [b]	33.8 [b]	40.6 [a]	0.00[c]	52.4** [c]	54.8 [b]	57.0 [a]	0.00[d]

SRT: Spawn run time (day); TFPI: Time to first primordia initiation (day); TFH: Time to first harvest (day)

*Asterisks indicate significance at *P <0.05, **P <0.01; values within the same row followed by the same letter are not significantly different. Mean values in the same row followed by the same letters are not significantly different by Tukey multiple range test*

Table 4. Effect of different substrates on yield of each flush, totally yiels and biological efficiency (%) of *Pleurotus* spp.

	***Pleurotus* spp.**														
	P. djamor					***P. citrinopileatus***					***P. eryngii***				
	First flush (g)	**Second flush (g)**	**Third flush g)**	**Total Yield (g)**	**BE (%)**	**First flush (g)**	**Second flush (g)**	**Third flush (g)**	**Total Yield (g)**	**BE (%)**	**First flush (g)**	**Second flush (g)**	**Third flush (g)**	**Total Yield (g)**	**BE (%)**
Control	101.3 [b]	59.7 [b]	14.7 [c]	175.7[c]	58.6	122.8 [c]	46.9 [b]	0.00	169.7[c]	56.6[c]	134.5 [b]	0.00 [c]	0.00	134.5 [b]	44.9 [b]
25OPC	100.1 [b]	64.5 [b]	31.6 [b]	196.2 [b]	59.5[b]	146.4 [b]	61.9 [a]	0.00	208.2[b]	63.1 [b]	144.6 [a]	82.8 [b]	0.00	227.4 [a]	64.2 [a]
50OPC	108.1 [b]	89.6 [a]	51.3 [a]	249.0 [a]	71.1[a]	173.1 [a]	61.5 [a]	0.00	234.6[a]	67.0 [a]	172.4 [b]	39.6 [a]	0.00	212.0 [a]	65.0 [a]
75 OPC	126.9 [a]	84.1 [a]	41.7 [a]	252.7 [a]	70.2[a]	0.00 [d]	0.00 [c]	0.00	0.00 [d]	0.00 [d]	0.00 [c]	0.00 [c]	0.00	0.00 [c]	0.00 [c]

*Asterisks indicate significance at *P <0.05, **P <0.01; values within the same column followed by the same letter are not significantly different. Mean values in the same column followed by the same letters are not significantly different by Tukey multiple range test*

4. DISCUSSION

The study aimed to eliminate olive press cake which constitute a problem in terms of environment especially in Mediterranean basin by converting into a valuable produce such as mushroom. For this purpose, four different substrate supplemented with different portion of OPC were examined in terms of spawn run time, time to first primordia initiation and time to first harvest, yield and BE (%). In the direction of the results obtained, it was determined substrate formulations for practical large-scale production of some commercial *Pleurotus* spp such as *P. djamor*, *P. citrinopileatus* and *P. eryngii.*

Moisture content was increased when the amount of OPC in the substrate increased. This could be explained by low water retaining capacity of OPC [14]. The concentration of C and N content of substrate affect C:N ratio [17]. The lowest C:N ratio was determinated in 75 OPC because of low C and high N content of this substrate. Although Philippoussis et al. [8] mushroom yield was positively correlated to the C:N ratio of the growing substrate in *P. eryngii* and *Agrocybe aegerita*. a negative correlation was found between mushroom yield and C:N ratio of the growing substrate in the present study.

There was no statistical significant difference between control and 25 OPC and 50 OPC substrates in terms of spawn run time in *P. djamor, P. eryngii* and *P. citrinopileatus*, but their mycelial growth is slower on 75 OPC substrate. This show that the spawn run time was increased in high olive press cake content in *Pleurotus spp*. Conversely, in our study, addition of 75% OPC did not reduce mycelial growth in all *Pleurotus* spp. This can be caused by the low porosity structure, lower substrate aeration and low water retaining capacity as well as the phenol compounds of the OPC [14]. They reported that the substrate containing 80% OPC has a negative impact on the mycelial growth of *Lentinula edodes*. But there was a degree of elongation in time with using of high OPC on growing substrate, no negative effects on mycelial growth were observed amongst the three strains during the spawning period for all substrates tested, indicating that the addition of OPC did not inhibit mycelial growth of *Pleurotus* spp.

Spawn running time period of *P. djamor* was a little further short in present study than the results from the study of Chaubey et al. [18] who reported the completion of spawn running on different season to be between 21.33 and 31.0 days for *P. djamor.* Kibar [19] reported that the spawn run time of *P. eryngii* was 13.6- 36.6 days, while Obotake et al. [20] confirmed that this varied, in different growing media, between 20 and 25 days. However, Liang et al. [21] reported spawn running of *P. citrinopileatus* to be completed within 22.2- 24.2 days on different substrates. These results are consistent with our findings.

On *P. djamor*, addition of OPC up to 50% did not influence the time to first primordia innitiation and time to first harvest compared to control. However, the time required for formation of pinhead and maturity of fruiting body was significantly more on 75 OPC compared to control. There was no statistical difference between the 25 OPC and the control medium, but the pinheads formation was longer in the 50 OPC medium than control in *P. citrinopileatus*. Similarly, the longest time to first primordia initiation was recorded in *P. eryngii* grown on 50 OPC substrate. Morever pinhead formation was not obtained on substrate 75 OPC in *P. citrinopileatus* and *P. eryngii.* In parallel with, the first flush of *P. citrinopileatus* and *P. eryngii* cultivated on substrate including 50% OPC concentrations was harvested later, because they needed a longer time for primordia initiation. Zervakis et al. [22] also reported high concentration of OPC had a negative effect on earliness. This could be about high phenolic content of OPC [23].

P. djamor demonstrated an exceptionally good adaptation to high OPC content since all of the substrates contained OPC provided significantly higher yield in comparison to the control. Garg [24] reported the biological efficiency of *P. djamor* on cotton seed hulls (106%), followed by sunflowerstalks (67.49%). Shukla and Jaitly [25] reported that the yield of *P. djamor* were 656.09 gr/ kg dry substrate. Although our findings are lower than those of these studies, yield was expressed as grams of fresh mushrooms harvested at per gram of wet substrate. When the dry substrate weight was considered, the values are similar to those of previous studies. Previous cultivation studies on *P. eryngii,* growing on various agroindustrial wastes BE was reported to be 46.67%-81.33% [19] and 31%-120% [22]. These results are consistent with our findings. Morever, our results are confirmed by

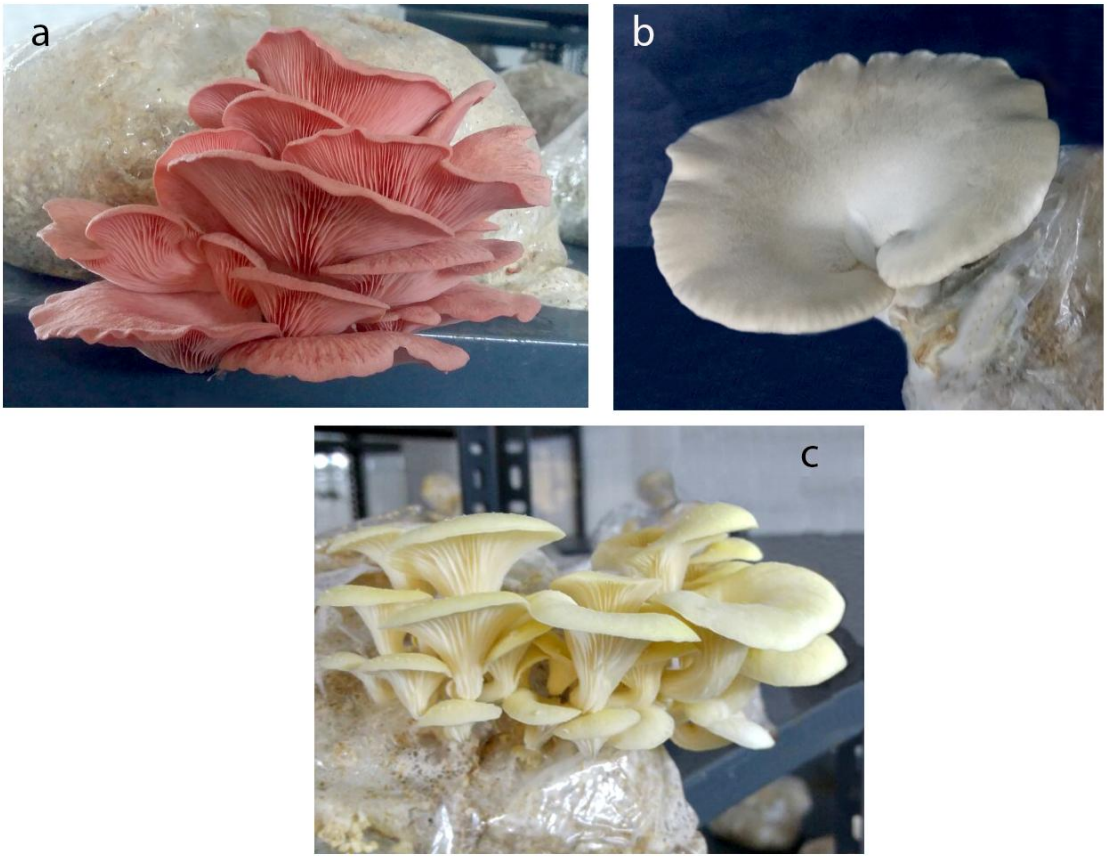

Fig. 1. (a) *Pleurotus djamor* grown on 75 OPC (b) *Pleurotus eryngii* grown on 50 OPC (c) *Pleurotus citrinopileatus* grown on 50 OPC

Liang et al. [21] who reported that the BE results of *P. citrinopileatus* grown on different substrates were between 53.6%-65.4%.

As a whole, in the first flush, the yields for all three species showed a gradually increasing trend as the ratio of OPC increased. Yields in the second and third flushes were lower than those from lower portion of OPC. Accordingly Yang et al. [26] the low yields in the second and third flushes on some substrates may be due to high yields in the first flush, which might have consumed most of the nutritional components and energy the substrate could provide. Yield was higher than those from the control substrate, particularly for 50 OPC substrate in the second flushes for all *Pleurotus* species tested. For control substrate, the highest yields for all species were in the first flush. On the other hand, yield obtained on control substrate in *P. djamor* was 8.4% of total yield, in third flush and in *P. citrinopileatus* was 27.6% of total yield in second flush. Morever, no fruiting body was obtained in second flush in *P. eryngii* grown on control substrate. The stuation was same in terms of total yield of *Pleurotus* spp grown on 100% sawdust (control) substrate. The lignocellulosic materials such as sawdust are insufficient for cultivation of mushrooms because of their low in protein content [27]. Additional materials such as bran, cotton seed hulls are important factor for the mycelial growth and yield of mushrooms [28,29,30]. Substrates supplemented with OPC may provided organic matter and some nutrients for mushroom yield. Lower yield values on control substrate could be about low nutrition content of sawdust. Nitrogen content of sawdust was determinated as 0.41% in present study. So, there was inadequate N for the second and third flushes due to a large part of the nitrogen was used for mycelial growth. On the other hand, high N content of 50 OPC could lead to high

mushroom yield in second and third flush. Adenipekun and Gbologade [31] reported that the yield and quality of the fungus depend on the C: N ratio, vitamin, phytohormones, and micro-macro element content of the growing media. Our results was corroborated Balakrishnan and Nair [32] *who reported that* the highest yield was obtained from the substrate with 0.7–0.9% nitrogen content in dried weight or the C/N ratio of the substrate was 50 or higher than 50. These values are similar with N content and C:N ratio of 50 OPC in present study.

Although, 25 OPC and 50 OPC showed higher biological efficiencies than the control substrate, fruitbody was not obtained on substrate 75 OPC in *P. citrinopileatus* and *P. eryngii.* Gregori and Pohleven [14] also reported that BE (%) of *Grifolia frondosa* and *Ganoderma lucidum* decrease with increasing share of OPC (60-80%). Ruiz-Rodriguez et al. [33] was determinated significant reduction of yield, biological efficiency and productivity of *Pleurotus ostreatus* strains on high OPC concentrations. On the other hand, *Pleurotus* species ability to utilize OPC and its tolerance to higher content of polyphenolic compounds in the substrate is different. Although no fruiting body was obtained on substrate 75 OPC in *P. eryngii* and *P. citrinopileatus*, same substrate provoked a significant increase on productivity for *P. djamor*. This result was corroborated by Visscher [34] who reported that different strains of king oyster mushroom response differently to different substrates and cultivation conditions in mycelial growing, average yield and mushroom quality.

Gregory and Pohleven [14] reported that 40-60% of OPC contained in cultivation substrates caused deformation of *Lentinula edodes, Ganoderma lucidum* and *Grifola frondosa* fruiting bodies. Conversely, in present study, fruitbody deformation was not determinated on high portions of OPC in any *Pleurotus* spp (Fig. 1). On the other hand, Altieri et al. [35] and Parati et al. [36] reported that composted olive mill solid waste can be used safely for industrial-scale cultivation of *Agaricus bisporus*. Zervakis et al. [37] found that composting of olive mill waste greatly increases the BE of produced fruiting bodies.

5. CONCLUSION

This study demostrated that OPC has a promising substrate for use as an substrate for *P. djamor, P. citrinopileatus* and *P. eryngii*. This suggested that 50% of OPC improved biological efficiency. In addition, 25 OPC substrate also showed higher biological efficiencies than the control substrate. Negative effects were observed on 75 OPC substrate for *P. citrinopileatus* and *P. eryngii*, but same substrate was preferable for *P. djamor*.

When considering previous studies, using composting method should be investigated on next work for reducing its negative effect on pinhead and fruitbody formation on 75 OPC substrate for *P. citrinopileatus* and *P. eryngii*. So more waste could used effectively.

In conclusion, the utilization of OPC for the production of *Pleurotus* spp could be a solution to solve one of the most important problems of olive oil industry. Using of OPC as a substrate not only provides an environmental method of disposing of OPC but also converts this waste into protein-rich food.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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