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PRODUCTION OF *Pleurotus ostreatus* CULTIVATED IN SUBSTRATES MADE FROM TWO INVASIVE WEEDS

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ABSTRACT

The species *Pleurotus ostreatus* is an edible mushroom with a high commercial value, cultivated for its ease and adaptability in substrates based on agro-industrial waste. The plant species *Solanum elaeagnifolium* and *Salsola kali* are two invasive weeds that spread rapidly in crops, ruderal or urban areas, representing high-cost agricultural losses and environmental impact. The hypothesis of this research was that at least one of the weeds would increase the production and quality of *P. ostreatus.* The objective was to evaluate the biological efficiency and carpophore productivity of *P. ostreatus* with the use of substrates elaborated and combined from *S. elaeagnifolim* and *S. kali.* The fungal cultures were carried out with substrates generated from mixtures of weeds and using wheat straw as a control; from these combinations, seven treatments and four replicates were obtained in a completely randomized design. The carpophores obtained were measured for the width and length of the pileus and stipe, total production (g), biological efficiency (%), and proximate analysis of the harvested mushrooms was performed. Maximum production was 23.30 mushrooms, with minimum of 3.67 carpophores. The width and length of the pileus were 8.30-11.70 cm and 8.40-11.40 cm, respectively. The stipe length variable showed widths of 1.40-2.30 cm and lengths of 2.80-6.20 cm. The weights obtained were up to 358.33 g, with a minimum of 95.10 g. Proximate analysis showed carpophores with the following composition: 82.25-91.37 % moisture; 1.71 to 13.66 % ash; 15.70 to 20.93 % protein; 0.40 to 1.55 % lipids and 8.60-17.7 % dry matter. The variables evaluated showed differences ($p \leq 0.05$), being the substrates made with 100 % *S. elaeagnifolium* the ones with the highest biological efficiency, carpophore production, harvest weight and nutritional value. The species *P. ostreatus* proved to be efficient in providing substrate for two weeds, as well as producing carpophores with high protein content.

Keywords: edible mushrooms, invasive weeds, *Salsola kali*, *Solanum elaeagnifolium*.

INTRODUCTION

Mushroom *Pleurotus ostreatus* (Jacq. Fr.) Kumm is one of the most cultivated macromycetes worldwide, ranking second only to *Agaricus bisporus* (Romero-Arenas *et al*., 2015). This mushroom is characterized by presenting a carpophore with a fleshy texture, pleasant taste, and odour (Sekan *et al*., 2019). It also represents an alternative food source rich in protein, in all essential amino acids, carbohydrates, vitamins (thiamine and riboflavin) and minerals (K, Fe, Na, P and Ca) (Corrêa *et al*., 2016).

The cultivation technique for the *P. ostreatus* mushroom is well standardized and industrialized on a wide variety of lignocellulosic substrates, has high biological efficiency (EB) (> 40 %), is low cost, and the carpophore develops rapidly and is not usually attacked by diseases and pests (Jin *et al*., 2020). Mushrooms of the genus *Pleurotus* require substrates with sources of carbon, nitrogen and inorganic compounds, carbon being the element that this mushroom requires in greater proportion. This mushroom is efficient in degrading various cellulose, hemicellulose, lignin, and other complex carbohydrate-based substrates with ease (Koutrotsios *et al*., 2014; Pandey and Singh, 2014; Song *et al*., 2020).

Mexico has shown an increase in the production of *P. ostreatus* in the last fifteen years, being currently the country that contributes the largest production in Latin America (80 % of the total production) with a yield that exceeds 60 000 Mg per year (García-Calderón *et al*., 2021). The production of this mushroom represents a profitable industry with high biotechnological potential in the country since it allows the use of various agro-industrial wastes (Sekan *et al*., 2019), and other non-conventional materials such as seaweeds (for example, *Glacilariopsis* spp., *Laminaria* spp., *Sargassum* spp. and *Ulva* spp.) (Kaaya *et al*., 2011; Hausiku and Mupamwa, 2018).

The species *Solanum elaeagnifolium* Cav. is a sub-shrubby herbaceous perennial that inhabits scrub and grassland regions from the central United States to South America, with a preference for disturbed sites, and has become a problematic species that requires control and proper management in Mexico. The rapid spread of this weed causes significant economic losses due to reduced crop yields, derived from competition (light, water, space, and nutrients), as well as higher incidence of pests and diseases (Uludag *et al*., 2016). Nevertheless, the plant shows an interesting composition in protein, minerals, and total carbohydrates (Mellado *et al*., 2008). On the other hand, the species *Salsola kali* L. is an annual herbaceous introduced in the American continent, distributed from Canada to central Mexico, in arid and semi-arid regions; it is considered an active allelopathic species and its propagation significantly reduces agricultural production. This species also has negative impacts on the environment, given its high level of invasiveness to crops, in addition to being a host plant for the beet leafhopper (*Circullifer tenellus*), an insect that transmits the curly top virus, a disease that affects various species of agricultural importance such as sugar beets, tomatoes (*Solanum lycopersicum* L.) and beans (*Phaseolus vulgaris* L.). The latter weed has protein contents in the range of 5.40 to 22.30 %, and total carbohydrates in the range 23.10 to 58.00 % (Hanif *et al*., 2018).

The hypothesis of this study was that at least one of the weeds used as substrate would increase carpophore production and nutrient content of *P. ostreatus* compared to the use of conventional wheat (*Triticum aestivum*) straw. The objective was to evaluate the biological efficiency and productivity of *P. ostreatus* with the use of substrates based on *S. elaeagnifolium* and *S. kali* and to evaluate their effect on the composition of harvested carpophores.

MATERIALS AND METHODS

Collection and preparation of substrates

S. elaeagnifolium and *S. kali* plants were collected from disturbed areas and vacant lots in Ciudad Juárez, Chihuahua, Mexico (31° 44′ 39.7 "N 106° 26′ 25.8 "W), from July to September. Preference was given to plants that were already dry. Once collected, the plants were stored in raffia sacks and transferred to the Biodiversity Laboratory of the Universidad Autónoma de Ciudad Juárez (UACJ). In parallel, duplicates of these species of weed were collected, which were contrasted with specimens from the UACJ Herbarium, and then left in safekeeping (*S. elaeagnifolium:* 0.00778; *S. kali*: 001241). Only stems of *S. elaeagnifolium* were used, while stems and leaves were used for *S. kali.* In both cases they were cut into 2 to 4 cm fragments. The wheat straw was purchased from a commercial supplier in Ciudad Juárez; proximate composition reported for these study plants is included (Table 1).

The fragments of the weeds were placed in containers with water and kept soaking for 24 hours. Subsequently, they were subjected to a pasteurization process for 2 h in 18.92 L capacity metal pots, maintaining a constant temperature of 70 °C. Once the process had elapsed, they were spread out on metal tables to accelerate cooling; when they reached room temperature, 20 g of calcium carbonate were added to reduce the growth of yeasts and bacteria during the first days of incubation (Pandey and Singh, 2014).

Substrate inoculation and incubation

The sowing of the inoculum was carried out using sorghum grain (*Sorghum bicolor*) colonized with mycelium of *P. ostreatus* (HONCOP) and kept under refrigeration (3 − 4 °C) until use. Inoculation was carried out on surfaces disinfected with a 0.5 % sodium hypochlorite-chlorine solution.

The substrate was placed in 36 x 49 cm (2 kg) clear plastic bags and 400 g of *P. ostreatus* mycelium inoculum was applied to each replicate. For filling, a layer of substrate was placed on top of a layer of inoculum. This process was repeated until the production

Table 1. Proximate composition of dry residues of *Triticum aestivum*, *Solanum elaeagnifolium* and *Salsola kali* species.

	Wheat straw[†]	*Solanum elaeagnifolium*[¶]	*Salsola kali*[§]
Dry matter (%)	90.10	81.40	80.20
Ash (%)	11.04	11.40	12.0
Lipids (%)	0.73	2.50	2.17
Protein (%)	3.34	15.00	21.50
Total carbohydrates (%)	38.54	32.80	48.40

Source: [†]Romero-Arenas *et al*. (2018); [¶]Mellado *et al*. (2008); [§]Hanif *et al.* (2018).

units were completely filled and closed with the aid of a sterile rubber band. Finally, each bag was labelled with data on the substrate used, bag number and date of planting, according to the methodology described by Mleczek *et al*. (2021).

The experimental design was completely randomized with seven different treatments and four replicates for each. 1: substrate composed of 100 % *S. elaeagnifolium* (SE); 2: substrate composed of 100 % *S. kali* (SK); 3: control substrate with 100% wheat straw (PT); 4: combination substrate 50 % *S. elaeagnifolium* and 50 % *S. kali* (SE-SK); 5: combination substrate 50 % wheat straw and 50 % *S. elaeagnifolium* (PT-SE); 6: combination substrate 50 % wheat straw and 50 % *S. kali* (PT-SK); and 7: substrate composed of 1/3 of *S. elaeagnifolium*, *S. kali* and wheat straw (SE-SK-PT), respectively. Each bag with substrate was considered an experimental unit.

The production units were transferred to an incubation area in darkness, where they remained for 20 to 30 d at a temperature of 23-25 °C; these were monitored daily to detect possible sources of contamination (mold, bacteria, or insects) (Mleczek *et al.*, 2021). Once the mycelium colonized the substrates completely and the first primordia appeared, the cultures were transferred to a fruiting area where they received ten hours of illumination, temperature between 20 to 22 °C and humidity at 70-80 % (Lisiecka *et al*., 2021). Mature carpophores were harvested with the aid of a sterilized razor, according to the protocols described by Pandey and Singh (2014), with some modifications.

Determination of production parameters and their statistical analyses

Data collection was carried out after harvesting was completed. The variables considered were quantity of harvested carpophores, total production (g) and morphometry (length and width of the pileus and stipe) of the carpophores.

Statistical analysis consisted of a one-way analysis of variance (ANOVA) and a Tukey's test of means ($p \leq 0.05$) to identify differences between treatments. The tests were performed with the help of the statistical package IMB SPSS ver. 25.0 (IBM, 2017).

Biological efficiency (EB; capacity of mushrooms to convert a substrate into fruiting bodies) was determined for each of the treatments using the formula proposed by Díaz-Muñoz *et al.* (2019):

$$EB = \frac{\text{Total weight of fresh fruting bodies}}{\text{Weight of the dry substrate}} \times 100$$

Proximate analysis and statistical analysis

Moisture content and dry matter were determined by the stove drying method (AOAC, 2000; 926.08), ash was determined by the muffle calcination protocol (AOAC, 2000; 935.42), protein was determined by the Kjeldhal method (AOAC, 2000; 920.123-1920), using a conversion factor of 4.38, and fat was determined by the Soxhlet method (AOAC, 2000). Total carbohydrates were determined by the difference method (AOAC, 2000; FAO, 2002). The energy calculation for the different treatments was performed using Atwater's general factor system (DFI, 2015). The tests were performed in triplicate and about 80 to 100 g of powdered carpophores were used for each analysis.

A one-way analysis of variance (ANOVA) and a Tukey's multiple means test ($p \leq 0.05$) were performed to identify differences in moisture, dry matter, ash, protein, total fat, and carbohydrate composition among the seven treatments evaluated. The tests were performed in the statistical package IMB SPSS ver. 25.0. (IBM, 2017).

RESULTS AND DISCUSSION

Morphometric measurements

The quality properties of the mushroom have been related to the size of the carpophore, as well as that of the pileus (cap) and stipe (Owaid *et al.*, 2015). The results for the evaluation of morphometric measurements of the carpophores produced indicate differences in all cases ($p \leq 0.05$). For pileus width (Figure 1A) the best response was obtained when implementing a combination of *S. elaeagnifolium* and wheat straw (PT-SE) (11.74 ± 0.75 cm); the lowest averages for pileus width were found in the SE-SK-PT combination (8.3 ± 0.57) (F = 5.80; g. l. = 6; $p \leq 0.05$).

In the variable of pileus length, it can be observed that the PT-SK treatment presented a better response in length (11.38 ± 0.73 cm). The treatments SE-SK (10.46 ± 1.47 cm), PT (10.71 ± 0.82 cm), SK (10.40 ± 0.51) and SE-SK-PT (9.73 ± 0.79 cm) obtained a similar performance with values close to those presented by PT-SK (Figure 1B). In general, the treatments in combination with wheat straw (PT-SE; PT-SK) and those in combination with the proposed substrates (SE-SK) had the best response in the length and width of *P. ostreatus* carpophore pileus (F = 4.74; g. l. = 6; $p \leq 0.05$).

The results for the stipe measurements agreed with those reported by Jonathan *et al.* (2012a) who obtained average lengths of *P. ostreatus* carpophore stipes ranging from 4 to 8 cm using substrates based on rice stubble, cotton, and sawdust of *Milicia excelsa*. Atila (2016) reported diameter measurements of the pileus ranging from 4.64 cm to 7.28 cm, while Andries and Vasilica (2017) obtained carpophores ranging from 10 to 13.5 cm in length. Carpophores with large pileus dimensions represent a commercial attraction, as the consumer aims for larger mushrooms in the case of this species.

For stipe width (Figure 2A), the best response was presented in the SE-SK-PT treatment (3.30 ± 0.39 cm), followed by SK (2.3 ± 0.15 cm) and with the lowest values in SE (1.4 ± 0.15 cm) (F = 18.27; g. l. = 6; $p \leq 0.05$). In contrast to the width of the pileus, which was wider in the combination treatments, the values obtained for stipe width were more heterogeneous in all treatments.

Regarding stipe length values (Figure 2B), greater homogeneity was observed, since treatments SK (5.57 ± 0.47 cm), PT (6.23 ± 0.55 cm), SE-SK (5.40 ± 0.50 cm) and PT-SK (5.57 ± 0.43) presented similar statistical values, while the smallest length on average was recorded in SE-SK-PT (2.81 ± 0.34 cm) (F = 12.66, g. l. = 6; $p \leq 0.05$).

The length of the stipe can vary between 2.80 cm and 8.80 cm (Jonathan *et al.* (2012a), although they can also be smaller 3 cm (Andries and Vasilica, 2017). Thinning of the pileus and stipe can be caused by lack of light and faced with the presence of substances in the substrate that cannot degrade the mushroom, the carpophores tend to show abnormal growth (Aghajani *et al.*, 2018). The values reported in the present

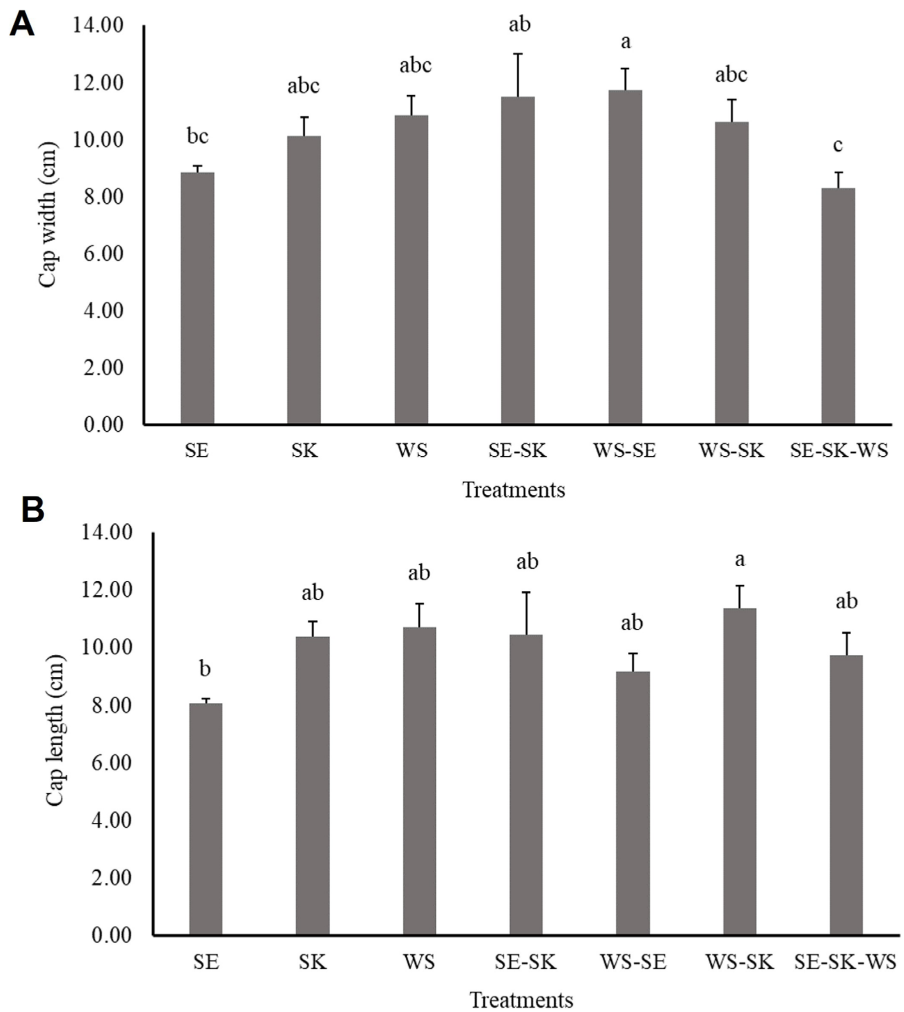

Figure 1. Means for *Pleurotus ostreatus* pileus produced in the seven treatments evaluated. A: measurements of the pileus width. B: measurements of the pileus length. Different letters in columns indicate statistical differences between treatments (Tukey; $p \leq 0.05$). SE: 100 % *Solanum elaeagnifolium*; SK: 100 % *Salsola kali;* PT: 100 % wheat straw (control); SE-SK: 50 % *S. elaeagnifolium* and 50 % *S. kali*; PT-SE: 50 % wheat straw and 50 % *S. elaeagnifolium*; PT-SK: 50 % wheat straw and 50 % *S. kali*; SE-SK-PT: 1/3 of *S. elaeagnifolium*, *S. kali* and wheat straw.

study for stipe length can be attributed to deficiencies in light conditions, but not to the presence of any substance in the substrate that has affected the development of the carpophores, because in the control group (PT) high values were also obtained for the length of the pileus (6.23 ± 2.79 cm) and this substrate is used in other investigations as a control, since very good yields have been obtained (Pandey and Singh, 2014).

The difference in the growth pattern of morphometric variables may be related to the different chemical compositions of the substrates (Jonathan *et al.*, 2012b). The development and size of the mushroom stipe and pileus can also be affected by environmental (temperature, humidity, fresh air) and spatial conditions (Jonathan

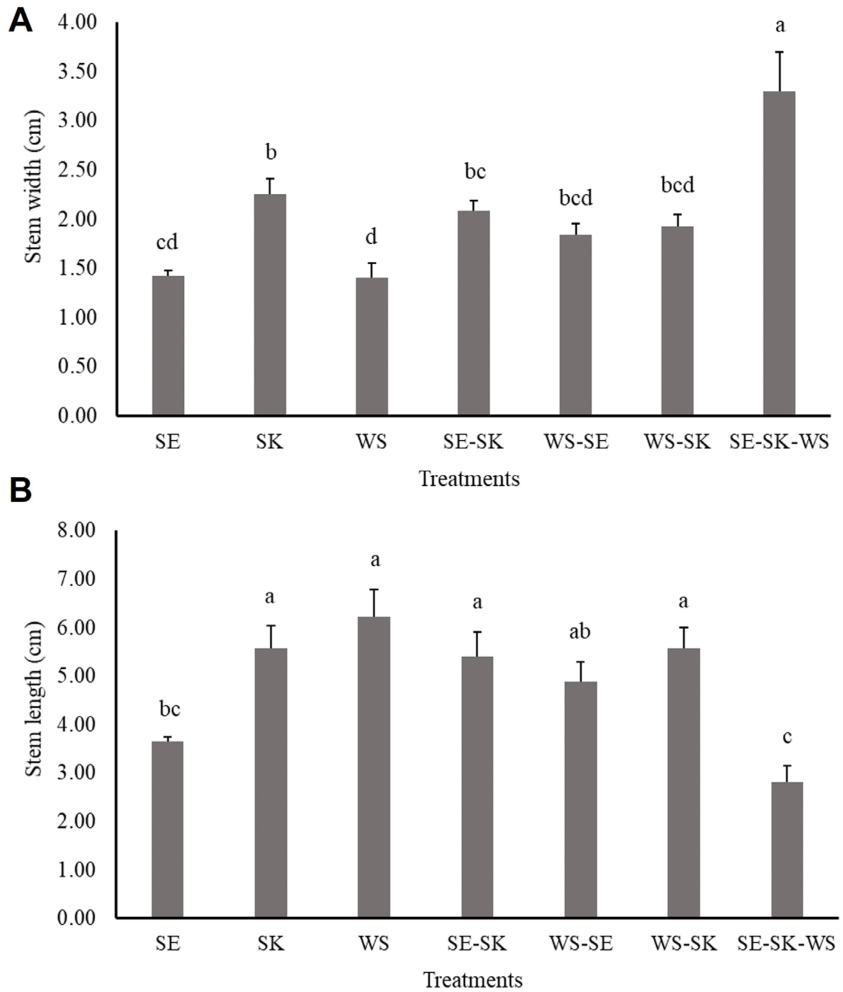

Figure 2. Means for *Pleurotus ostreatus* stipes produced in the seven treatments evaluated. A: measurements of stipe width. B: measurements of the stipe length. Different letters in columns indicate statistical differences between treatments (Tukey; $p \leq 0.05$). SE: 100% *Solanum elaeagnifolium*; SK: 100% *Salsola kali;* PT: 100% wheat straw (control); SE-SK: 50% *S. elaeagnifolium* and 50% *S. kali*; PT-SE: 50% wheat straw and 50% *S. elaeagnifolium*; PT-SK: 50% wheat straw and 50% *S. kali*; SE-SK-PT: 1/3 of *S. elaeagnifolium*, *S. kali* and wheat straw.

et al., 2012a). The latter is consistent with the treatments in which the number of carpophores produced was lower, and consequently there was more space for development, and they presented greater length and width in the stipe and pileus (Figure 3).

Carpophore production, mass, and biological efficiency

The results obtained for total production show differences (F = 8.851, g. l. = 6; $p \leq 0.05$), with the SE treatment having the highest mass production (g) with a total of 358.33 ± 8.33 g, followed by the control group PT (220.00 ± 5.00 g). Regarding the combination

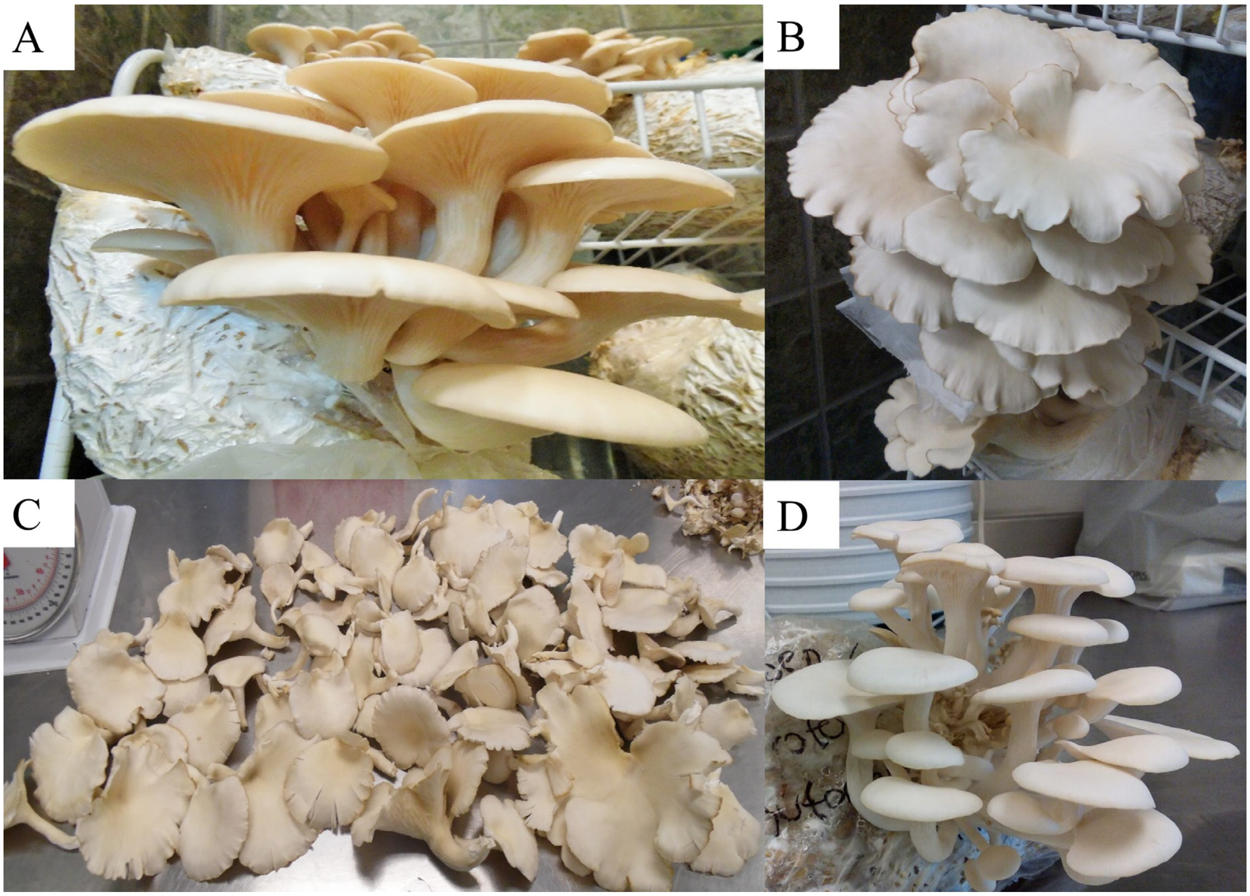

Figure 3. Carpophores of *Pleurotus ostreatus* collected from different treatments. A: carpophores from PT treatment; B: carpophores from PT-SE treatment; C: carpophores from SE treatment; D: carpophores from PT-SK treatment. SE: 100% *Solanum elaeagnifolium*; PT: 100% Wheat straw (control); PT-SE: 50% wheat straw and 50% *S. elaeagnifolium*; PT-SK: 50% wheat straw and 50% *S. kali.*

treatments, the highest values were for the PT-SE treatment (186.10 ± 61.12 g) (Table 1). This variable is related to the number of carpophores produced, being SE the treatment that also obtained the best response in this variable. Although no studies have been conducted on *P. ostreatus* production with the proposed substrates, the results match the values reported by Moran-Arellanos *et al*. (2020), who obtained a carpophore weight ranging from 93.75 g to 187. 37 g in substrates based on bean stubble, wheat straw and guaje or huaxin (*Leucaena leucocephala*) husk, although they are higher than those found with substrates based on chihua squash (*Cucurbita argyrosperma*) pulp (35 g) and guazima or pixoi (*Guazuma ulmifolia*) fruit (Moran-Arellanos *et al*., 2020).

The total yield of carpophores produced per treatment also showed differences (F = 7.66, g. l. = 6; $p \leq 0.05$), with the SE treatment producing the highest amount (23.33 ± 4.33 mushrooms), the rest of the substrates had similar statistical results, ranging from 3.67 ± 0.67-7.67 ± 0.67 ± 0.88 mushrooms produced (Table 2). These results match those

Table 2. Total production, carpophore, and biological efficiency of *Pleurotus ostreatus* in the seven treatments evaluated.

Treatment	Total production (g)	Effective carpophore production (n)	Biological efficiency (%)
SE	358.33±8.33 a	23.33±4.33 a	50.00 a
SK	200.00±14.43 bc	4.33±.33 b	42.47 b
PT	220.00±5.00 b	7.67±0.88 b	42.47 b
SE-SK	95.10±9.73 c	3.67±0.67 b	13.41 e
PT-SE	186.10±61.12 bc	5.67±1.20 b	25.85 c
PT-SK	109.03±8.24 bc	3.67±0.67 b	19.56 d
SE-SK-PT	101.70±6.38 bc	4.33±0.33 b	17.48 d

Different letters in the same column indicate statistical differences between treatments (Tukey; $p \leq 0.05$). SE: 100% *Solanum elaeagnifolium*; SK: 100% *Salsola kali*; PT: 100% wheat straw (control); SE-SK: 50% *S. elaeagnifolium* and 50% *S. kali*; PT-SE: 50% Wheat straw and 50% *S. elaeagnifolium*; PT-SK: 50% wheat straw and 50% *S. kali*; SE-SK-PT= 1/3 of *S. elaeagnifolium*, *S. kali* and wheat straw.

presented by Hoa *et al.* (2015), who reported carpophore production ranges from 2.17 to 10.32 in substrates of wood sawdust and corn stubble (*Zea mays*), obtaining the highest amount in substrates that were composed entirely of 100 % of any of these agro-industrial wastes. Andries and Vasilica (2017) harvested carpophores produced on wheat straw enriched with sunflower (*Helianthus annuus*) stubble in the range of 10.50–13.00 carpophores. In general, both authors agree that productivity depended on the type of substrate used for cultivation, as well as the strain used. The high carpophore production of the SE treatment may be due to the fact that the chemical composition of *S. elaeagnifolium* reflects that it is rich in lignocellulosic material, structural carbohydrates and crude fibre, protein, and free nitrogen extracts (Mellado *et al.*, 2008).

The EB presented ranges from 17 % in the SE-SK-PT substrate to 50 % in SE, the minimum and maximum respectively (F = 18.47; g. l. = 6; $p \leq 0.05$). In general, medium to low values are reported for the biological efficiency of *P. ostreatus* on the substrates evaluated during this study. In the composite substrates, lower EB were observed with the lowest values (25.85–12.41 %). The formation of substrates from various plant sources results in a lower biological efficiency (54.10 % in substrates of wheat straw and olive tree pruning residues (*Olea europaea*) compared to that obtained by the control group (105.00 % in wheat straw) (Abou-Fayssal *et al.*, 2020), which agrees with our results. It is important to note that the means observed in this study are higher than those observed by Díaz-Muñoz *et al.* (2019), who obtained EB less than 20 % in lignin-rich substrates based on sugarcane bagasse (*Saccharum* spp.) (16.77 %), corn stover (12.10 %), rice straw (*Oryza sativa*) (8.65 %) and grass pruning residues (9.97 %). EB can be affected by a wide variety of factors and variants present in the experiment, including the type of substrate, substrate preparation (sterilization, pasteurization), size of the container used for cultivation, presence of contaminants and pests, CO_2 concentration, light, temperature, pH, among others (Atila, 2016).

Proximate analysis

The results obtained from the proximate analysis of the carpophores harvested from the six treatments and the positive control are reported as percentage of weight on dry basis (Table 3). Significant differences were found in all treatments ($p \leq 0.05$).

The moisture content of harvested carpophores showed significant differences among treatments, ranging from 82.25 to 91.37 %. The highest moisture value was obtained in the carpophores of the PT treatment (91.37 ± 0.04 %), followed by the PT-SK combination treatment (90.84 ± 0.08 %), and the lowest moisture value was recorded in the carpophores of the SE-SK and SK treatment (86.66 ± 0.03 and 82.25 ± 0.64 %, respectively). On the other hand, in dry matter, it was found that the SK treatment presented the highest percentages (17.71 ± 1.11 %), while SE and SE-SK-PT showed the lowest amount (10.40 ± 0.10 and 10.30 ± 0.07 %, respectively) (F = 159.71, g. l. = 6; $p \leq 0.05$) (Table 3). Similar results are observed in the studies of Mintesnot *et al.* (2014), who obtained moisture values from 89.38 % to 92.42% in three different strains of *P. ostreatus* on wheat straw, similarly, they recorded the lowest moisture values for treatments made with substrate based on three invasive plant species: 86.61% with *Lantana camara;* 88.06 % with *Parthenium incanum*; and 85.92 % with *Prosopis juliflora*.

Due to the difference in moisture content of the samples, ash, protein, fat, and carbohydrate data were analysed on a dry weight basis (Table 3). Results for ash content of carpophores ranged from 1.71 to 13.66 %. The highest value was obtained in the SE-SK treatment (13.66 ± 0.16 %), followed by PT-SK (11.56 ± 0.13 %). The lowest values were presented in the treatments without substrate combination, with the control PT showing the lowest value (1.71 ± 0.02 %) (F = 1648.29; g. l. = 6; $p \leq 0.05$). The ash content is related to the substrate used and gives a general idea about the mineral content of the carpophores, which is consistent with the data obtained.

Protein content ranged from 15.70 to 20.93 %; the treatment that provided carpophores with more protein was the SE-SK combination (20.93 ± 0.06 %), followed by SK (20.87 ± 1.88 %) (F = 20.47, g. l. = 6; $p \leq 0.05$) (Table 3). These values fall within the range of 14 to

Table 3. Proximate analysis of *Pleurotus ostreatus* carpophores produced from seven evaluated treatments.

Treatment	Moisture (%)	Dry matter (%)	Ash* (%)	Proteins* (%)	Lipids* (%)	Total carbohydrates* (%)
SE	89.62±0.06 c	10.38±0.06 d	5.49±0.08 d	20.74±0.47 ab	0.40±0.00 f	62.98±0.41 b
SK	82.25±0.64 d	17.74±0.64 a	4.85±0.18 e	20.87±1.08 a	1.49±0.00 b	55.02±1.70 d
PT	91.37±0.04 a	8.62±0.04 f	1.71±0.01 f	16.64±0.33 cd	0.59±0.00 e	72.41±0.32 a
SE-SK	86.66±0.03 d	13.33±0.03 b	13.66±0.09 a	20.93±0.03 a	1.02±0.01 c	51.03±0.09 e
PT-SE	88.91±0.05 c	11.09±0.05 c	11.10±0.15 bc	19.36±0.27 ab	0.70±0.00 d	57.73±0.19 d
PT-SK	90.84±0.08 ab	9.15±0.08 e	11.56±0.08 b	17.53±0.09 c	0.99±0.00 c	60.75±0.13 c
SE-SK-PT	89.66±0.64 bc	10.33±0.04 d	10.89±0.09 c	15.70±0.19 d	1.55±0.01 a	61.50±0.17 c

Mean values ± EE. *Data on dry weight basis of the samples. Different letters indicate significant difference between the different treatments (Tukey; $p \leq 0.05$). SE: 100% *Solanum elaeagnifolium*; SK: 100% *Salsola kali;* PT: 100% wheat straw (control); SE-SK: 50% *S. elaeagnifolium* and 50% *S. kali*; PT-SE: 50% wheat straw and 50% *S. elaeagnifolium*; PT-SK: 50% wheat straw and 50% *S. kali*; SE-SK-PT: 1/3 of *S. elaeagnifolium*, *S. kali* and wheat straw.

32 % proposed by Koutrotsios *et al*. (2014). Our results are similar to those reported by Jin *et al.* (2018) who found values from 18.35 to 25.86 %, although they are lower than those presented by Li *et al.* (2017) on mushrooms grown on cottonseed (*Gossypium hirstium*) husk and perilla (*Perilla frutescens*) stems (20.50 to 26.10 %). These differences in protein content have been directly accounted for by the type of substrate used, as a result of differences in nutrient supply and ability of the mushroom to degrade the substrate (Gupt*a et al*., 2016).

Lipid content ranged from 0.40 to 1.55 %. The highest value was found in the SE-SK-PT treatment (1.55 ± 0.02 %), followed by SK (1.49 ± 0.00 %), while the lowest was for SE (0.40 ± 0.00 %) (F = 2653.19; g. l. = 6; $p \leq 0.05$). These results were lower than those reported by Jin *et al.* (2018) on *P. ostreatus* carpophores produced on corn stubble and supplemented with plant residues, who obtained a fat range from 2.34 to 2.58%. However, the lipid content did not exceed the limit of 6% proposed for mushrooms (Carrasco-González *et al*., 2017).

Total carbohydrate content was highest in the PT treatment (71.41 ± 0.56 %), followed by the SE treatment (62.98 ± 0.72 %), while the lowest was presented in the SE-SK treatment (51.03 ± 0.16 %) (F = 369.16, g. l. = 6; $p \leq 0.05$). Using wheat straw, carbohydrate contents of 62.54 to 71.26 % were also found. This matches with those reported by Jin *et al*. (2018). Carbohydrate contents of 57 % were reported in *Pleurotus ostreatus* produced on substrates made from almond husk (*Prunus dulcis*) and nutshells up to 76% in *Pleurotus* grown on substrates made from *Pinus* spp. needles (Koutrotsis *et al*., 2014).

Protein and carbohydrate content vary in mushroom carpophores when grown on different agro-industrial waste and alternative substrates, in addition to the type of soil and climate of the site (Rodriguez-Estrada and Pichica, 2017). Grown on a coffee (*Coffea arabica*) residue substrate, *P. ostreatus* showed a protein content between 28.6 and 29.7 %, higher values than those found in this study and with total carbohydrate contents (< 8 % of fresh basis weight) similar to those obtained in this research (6 to 8.5 % of fresh basis weight), except for the SK treatment which had the lowest water content (12.85 %) (Nieto-Juárez *et al*., 2019).

Regarding the calculation of energy for carpophores in each treatment, the averages of this variable ranged from 32.03 to 61.49 kcal 100 g^{-1} of sample (fresh weight basis), with significant differences (F = 72.10, g. l. = 6; $p \leq 0.05$) among treatments (Table 4). SK carpophores had the highest contribution, whereas the lowest was for PT and PT-SK. The moisture content of carpophores on a fresh basis has an important effect on their energy intake because the lower the water content (as is the case of SK), the higher the dry matter that contributes to the caloric intake, in addition to the higher protein content of SK and SE. This same behaviour was observed in raw mushrooms with variable humidity from 88.3 to 92.4 % (USDA, 2022), or very wide ranges of proteins that this mushroom can present (7.3 to 53.3 %) and variation in its total carbohydrate content (50–60%) in weight in dry basis (Torres-Martínez *et al*., 2022).

In general, in this first part of the study, the proximte composition of *P. ostreatus* carpophores indicated that substrates obtained from invasive plants such as *S. elaeagnifolium* and *S. kali* allow adequate development and growth of the fungus and

Table 4. Energy contribution of *Pleurotus ostreatus* carpophores produced in different treatments.

Treatment	Energy (kcal 100 g^{-1})
SE	37.00±0.33 bc
SK	61.49±3.05 a
PT	33.89±0.20 c
SE-SK	42.37±0.10 b
PT-SE	37.38±0.24 bc
PT-SK	32.03±0.27 c
SE-SK-PT	36.77±0.18 bc

Mean values ± EE. Data obtained on a fresh weight basis. Different letters indicate significant differences between treatments (Tukey; $p \leq 0.05$). SE: 100% *Solanum elaeagnifolium*; SK: 100% *Salsola kali;* PT: 100% wheat straw (control); SE-SK: 50% *S. elaeagnifolium* and 50% *S. kali*; PT-SE: 50% wheat straw and 50% *S. elaeagnifolium*; PT-SK: 50% wheat straw and 50% *S. kali*; SE-SK-PT: 1/3 of *S. elaeagnifolium*, *S. kali* and wheat straw.

had an important effect on protein and mineral content. However, more studies are needed to determine its safety and toxicity and to suggest potential use for animal or human consumption.

CONCLUSIONS

Substrates made from the invasive weeds *Solanum elaeagnifolium* and *Salsola kali* can be used effectively for the production of *Pleurotus ostreatus* carpophores. It is not recommended to use them in combination with each other or with wheat straw. The best substrate was 100 % *S. elaeagnifolium*, which produced the highest results in the production of carpophore number, biomass, morphometric variables, and biological efficiency.

In their proximate composition, *P. ostreatus* carpophores obtained from substrates with *S. elaeagnifolium* and *S. kali* showed higher dry matter content, particularly in protein and minerals compared to the conventional wheat straw-based substrate. The caloric contribution of carpophores is particularly related to their protein and carbohydrate content, given their low lipid content.

This study demonstrated that *P. ostreatus* has the ability to produce carpophores of good size and high protein content with the use of substrates from *S. elaeagnifolium* and *S. kali,* considered highly invasive weeds.

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