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Research Article

Three-plant stubble (Family: Fabaceae) as a substrate for cultivation of *Pleurotus ostreatus* (Jacq.) P. Kummer., in Mexico

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Abstract
Mushroom cultivation is an economically feasible bio-technological process for conversion of various agricultural by-products. In Mexico, a large quantity of lignocellulosic residues is generated and several of them have been used as a substrate in the cultivation of *Pleurotus* spp. Thus, high nutritional value food is produced at a relatively low cost. In this study, fermented chickpea stubble (*Cicer arietinum* L.); bean (*Phaseolus vulgaris* L.) and peanut (*Arachis hypogaea* L.) stubble sun-dried were used as a substrate for growing a strain of *Pleurotus ostreatus* (Jacq.) P. Kummer. (IE-8). On the chickpea stubble, the spawning was carried out on three, five and seven days of fermentated (FCS-3, FCS-5 and FCS-7, respectively) substrate. Highest productivity was obtained on the FCS-3 substrate with the formation of first primordia between 15 and 17 days; crop cycle between 44 to 49 days, with 156% of biological efficiency (BE), 46.8% of yield (Y) and 3.3% of production rate (PR). In the other treatments, forming first primordia was between 16 to 35 days, crop cycles between 43 and 61 days, with BE from 76.2% to 130.2%, Y between 16.8% to 39.0% and PR between 1.7% to 2.9%. Stubbles studied can be used as a substrate for the cultivation of the strain IE-8 on a small to large scale in the regions where they are generated, mainly the stubble of the chickpea plant.

Keywords: Agricultural by-products, Edible mushrooms, Mushroom cultivation, *Pleurotus ostreatus*

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INTRODUCTION

Pleurotus species are present in tropical and subtropical forests around the world and can be cultivated artificially due to their high ability to colonize and degrade a wide variety of substrates containing cellulose, hemicellulose, and lignin, which are used for development, besides having fast mycelial growth and fruiting with low production cost (Pokhrel *et al.,* 2013). On the other hand, the cultivation of *Pleurotus* species has increased because they have nutritional and medicinal values (Fernandes *et al.,* 2015). Commercial cultivation of *Pleurotus spp.,* ranks second worldwide after *Agaricus* spp. (Royse *et al.,* 2017). In Mexico, mushroom production (*Pleurotus* spp.) is 4.76% (Martinez-Carrera *et al.,* 2016) of the total that is mainly produced in Brazil, Colombia, Argentina and Guatemala (Royse and Sánchez, 2017).

In Mexico, various agricultural by-products have been used as a substrate for the cultivation of *Pleurotus* spp. using Mexican or commercial strains (Mora and Martínez-Carrera, 2007). In the agricultural cycle 2018, chickpea (*Cicer arietinum*) stood out among other crops in eight states with a production of 52,622.71 tons, also crop beans (*Phaseolus vulgaris*) and peanut (*Arachis hypogaea*) on 32 and 25 states with a production of 1'222,890.45 tons and 91,109.3 tons, respectively (SIAP, 2018).

In this study, the potential of chickpea, bean and peanut stubble was evaluated when growing a strain of *Pleurotus ostreatus* (Jacq.) P. Kummer., to establish its possible production on a small and large scale in regions where these stubble occur.

MATERIALS AND METHODS

Biological material and spawn: Strain *Pleurotus ostreatus* (IE-8) was donated by the Institute of

Ecology, Xalapa, Veracruz, Mexico. It was replanted and kept in Petri dishes in medium containing malt extract and agar (MEA) and incubated in darkness at 28°C. Wheat grains (*Triticum aestivum* L.) with 45% humidity were used to prepare the spawn and they were sterilized at 121ºC for 1 h in polypropylene bags (*ca.* 250 g/bag). After cooling the grains, 1 cm^2 of MEA with previously developed mycelium was added to each bag; then, they were incubated in a dark room between 28ºC and 29ºC for two weeks (Gaitán-Hernández *et al.,* 2002).

The experiment was conducted in a pilot plant of the biotechnology laboratory of edible and medicinal mushrooms of the Higher School of Natural Sciences dependent on the Autonomous University of Guerrero, Mexico. Stubbles of the three plants were obtained from farmland in the central region of the state of Guerrero and fragmented into segments between 4 to 6 cm long. Chickpea plants were obtained green and wet, so they were fermented aerobically for 7 days. Stubbles from the bean and peanut were obtained semi-humid and then sun-dried for 2 days. After, they were moistened and covered with plastic for 18 h.

Treatments and spawning: The following treatments were prepared: 1. Fermented chickpea stubble 3 days (FCS-3), 2. Fermented chickpea stubble 5 days (FCS-5), 3. Fermented chickpea stubble 7 days (FCS-7), 4. Bean stubble (BS) and 5. Peanut stubble (PS). All substrates were pasteurized in the water at 80ºC for 1 h. Once cold, 4 kg of the wet weight of each substrate were placed inside 50 x 70 cm polyethylene bags and the spawn (*ca.* 250 g wet) was added homogeneously. Five replicates per treatment were prepared.

Incubation and crops: Inoculated bags were incubated in the dark at room temperature between 27ºC and 29ºC and placed on metal shelves. With the formation of first fruiting primordia, the plastic cover was removed and the environmental conditions maintained were natural lighting (11±1 h light / 11±1 h dark), relative humidity between 80% and 85% and temperature between 25°C and 28°C, with ventilation provided by two electric air extractors.

Evaluation of the production of fruiting bodies: The parameters evaluated were: days in the formation of primordia; total crops days counted from spawning; fresh weight of basidiocarps in three harvests; biological efficiency (BE = fresh weight of basidiocarps harvested/dry weight substrate); yield (Y = fresh weight of basidiocarps/fresh weight substrate) and production rate (PR = BE/ total days of production). BE, Y, and PR are expressed in percentages.

Experimental design and statistical analysis: The design was completely random. The obtained values were processed by an analysis of variance and to determine the differences between treatment means, Tukey's multiple range test (α = 0.05) was applied.

RESULTS AND DISCUSSION

The formation of first primordia was earlier in FCS -3 treatment, requiring an average of 15.8 days and was statistically significant at the other treatments (16.6 to 28.8 days). In the formation of seconds and third primordia, there were no significant differences between treatments, except in BS treatment. The total crops cycle was between 44.2 to 47.4 days, except in BS treatment that took 58

Table 1. Average days in the formation of fruiting primordia and total crops cycle obtained in the cultivation of IE-8 strain of *P. ostreatus.*

Treatments	Appearance of primordia (days±σ) after spawning			TCC[1]
	First	Second	Third	
Fermented chickpea stubble 3 days (FCS-3)	15.8±1.0 c*	29.6±2.4 b	42.8±2.2 b	46.8±2.2 b
Fermented chickpea stubble 5 days (FCS-5)	16.6±0.5 bc	31.4±1.3 b	43.4±1.1 b	47.4±1.1 b
Fermented chickpea stubble 7 days (FCS-7)	18.2±1.0 bc	29.2±1.0 b	40.2±1.0 b	44.2±1.1 b
Bean stubble (BS)	28.8±3.8 a	42.8±4.2 a	54.0±5.1 a	58.0±5.1 a
Peanut stubble (PS)	20.6±3.1 b	31.8±2.4 b	41.6±4.1 b	45.4±3.8 b

[1]TCC = total crops cycle.*Different letters in the same column indicate statistical differences between mean values according to Tukey's multiple range test (α = 0.05).

Table 2. Averages in productivity evaluation achieved in the cultivation of IE-8 strain of *P. ostreatus.*

Treatments	Sustrates dry weight (g)	Total weight (g±σ)	Biological efficiency (%±σ)	Yield (%±σ)	Production rate (%±σ)
FCS-3*	1200	1872±187.5 a**	156±15.6 a	46.8±4.6 a	3.3±0.4 a
FCS-5	1198	1560±119.4 b	130.2±9.9 b	39±2.9 b	2.7±0.2 b
FCS-7	1197	1510±41.8 b	126.1±3.4 b	37.8±1.0 b	2.9±0.1 ab
BS	952	935.4±178.1 c	98.3±18.7 c	23.4±4.4 c	1.7±0.3 c
PS	881	671±97 d	76.2±11 c	16.8±2.4 d	1.7±0.3 c

*Abbreviations corresponding to the treatments in Table 1. **Different letters in the same column indicate statistical differences between mean values according to Tuke's multiple test (α = 0.05).

days (Table 1).

FCS-3 treatment reached the highest values obtaining an average of 1872 g of fresh mushrooms in three harvests; BE of 156%, Y of 46.8% and PR of 3.3% and was significantly different from the rest of treatments. The fresh weight of mushrooms in the other treatments ranged from 671g to 1560 g; BE between 76.2% and 130.2%; Y between 16.8% and 39%; PR between 1.7% and 2.9% (Table 2).

The values reached on FCS are higher than those obtained when four species of *Pleurotus, P. djamor, P. platypus, P. florida* and *P. eous* were cultivated on chickpea stubble and presented the first primordia between 15 to 19 days, total crops cycle from 34 to 41 days, BE between 48.0% and 81.2% (Deshmukh and Deshmukh, 2016) or when chickpea straw was used as main materials and cotton seed hulls (*Gossypium* sp.), olive press cake (*Olea europea* L.**)**, sunflower press cake (*Helianthus annuus* L**.)** and sugar beet pulp (*Beta vulgaris* L.) were used as additive materials in the cultivation of *P. ostreatus*. Chickpea straw without additives presented the first primordia at 15.3 days of incubation and BE of 68.3%, while the chickpea straw with additives presented the first primordia between 17.8 to 23.2 days, with BE between 55.3% and 99.8% (Atila, 2017). Moreover, the values achieved on BS are higher than those obtained when a strain of *P. ostreatus* was grown on bean stubble alone or supplemented in various proportions with dehydrated alfalfa (*Medicago sativa* L.). The bean stubble alone reached 46.8% of BE and PR of 0.39%, and when was supplemented, reached between 62.7% to 84.1% of BE and between 0.52% to 0.70% of PR (Romero-Arenas *et al.,* 2018).

Conclusion

The best treatment was FCS-3 followed by FCS-5 and FCS-7 treatments that achieved more than 100% BE, while BS and PS treatments showed BE that was close to this percentage. The results indicate the possibility of using the fermented chickpea stubble as a substrate in the cultivation of IE-8 strain. Likewise, bean and peanut stubble can be used as a substrate, since they approached 100% BE, which makes their production profitable.

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