

## Biotechnological potential of plant residues to produce *Pleurotus ostreatus* in rural areas of Campeche

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### Abstract

The objective of this study was to identify the main plant residues available in smallholder farming systems and use them as substrates for the cultivation of two strains of *Pleurotus ostreatus* (CP 50 and CP 753) under laboratory conditions. 184 questionnaires were applied in 13 rural communities in Campeche, Mexico. The availability of agricultural residues (t DM ha<sup>-1</sup>) in crops of corn, chihua pumpkin, x-pelon beans and tree species was evaluated. The selection of plant residues was evaluated in the culture of *Pleurotus ostreatus* strains. The variables evaluated in the Pleurotos culture were: colonization of the substrate, appearance of primordia, biological efficiency, colonization rate and production rate. The results were analyzed with descriptive statistics and an experimental design of random blocks. The availability (t DM ha<sup>-1</sup>) of the vegetable residues was corn (10.7), chihua pumpkin (14) and x-pelon beans (17.36). The CP-753 in bean stubble presented the shortest times with 12 and 15 days in colonization and appearance of primordia, while the CP-50 inoculated in pixoi fruit was the least efficient. The bean stubble substrate inoculated with the CP-753 strain presented the best biological efficiency with values of 102.75 ± 7.48 and a production rate of 5.13 g day<sup>-1</sup>. Most of the producers surveyed have an interest in using the plant residues generated in the lots or backyards as productive alternatives using agroecological strategies that contribute to the generation of economic income for much of the year.

**Keywords:** agroecological strategy, mushroom fungus, plant residues, substrate.

Reception date: April 2020

Acceptance date: May 2020

Edible mushrooms of the genus *Pleurotus* are currently cultivated in a wide range of altitudes and have the capacity to grow in different plant residues (Skariyachan *et al.*, 2016). It is an easy-to-handle crop, which places it as an alternative with biological, economic and social viability for the rural population (Gaitán and Silva 2016). *Pleurotus* is considered a species with nutritional (Fernández *et al.*, 2015) and medicinal (Martínez-Carrera *et al.*, 2007) properties. In Latin America, Mexico is the largest producer of fresh edible mushrooms with 47 468 t year<sup>-1</sup>, it is estimated that of this figure 4.6% corresponds to mushrooms of the *Pleurotus* genus (Martínez-Carrera *et al.*, 2007).

The potential of rural areas for the production of *Pleurotus* lies in the diversity of local agricultural residues generated annually that can function as an alternative for fungus inoculation. Among the local agricultural residues are henequen bagasse and pumpkin stubble (López *et al.*, 2005), banana leaf (Romero *et al.*, 2010), bean stubble (Alejo *et al.*, 2015), corn, oat straw (Gaitán and Silva 2016) and coffee pulp (Cruz *et al.*, 2010), among others. In Campeche, 99.4% of its territory is rural and is represented by the cultivation of corn, soybeans, sugar cane, rice, sorghum, and chihua pumpkin, estimating a production of 894 764 t DM of potential residues for *Pleurotus*.

Mushrooms of the *Pleurotus* genus represent a nutritious food alternative, rich in protein, essential amino acids, fiber and low in fat, which benefit health and provide food security to people in rural areas. Therefore, the objective of the present study was to determine the potential of the main agricultural and forest residues as substrates for the cultivation of *Pleurotus ostreatus* in rural areas of the State of Campeche.

## Study area

Rural communities with less than 2 500 inhabitants (CONEVAL, 2014) were selected from the municipalities enrolled in the National Program of the Crusade Against Hunger. A total of seven municipalities and 13 communities were considered: Calakmul (Virgencita de la Candelaria, Zoh Laguna), Calkiní (Pucnachen), Campeche (Tixmucuy, Nilchi), Carmen (Chicbul, Pital Nuevo), Champoton (Revolucion, General Ortiz Ávila), Escarcega (La Victoria, Silvituc) and Hopelchen (Katab, Xmaben). A sample of 184 questionnaires was obtained using the finite population formula  $\frac{Z^2 p q N}{NE^2 + Z^2 p q}$ , (Sierra 1995). Where: n= sample size; Z= confidence level; p= positive variability; q= negative variability; N= population size (number of houses inhabited); E= precision of the error.

## Collection of information

A semi-structured questionnaire was designed with open and closed questions made up of four sections: 1) general information; 2) uses of the plant component of the lot or backyard; 3) crop management; and 4) use of plant residues (solar and crops). The questionnaire was applied during the months of August-October 2016, using the technique of free desire to participate.

## Analysis of data

The calculations of the available biomass of the residues were made using the formula proposed by Borja *et al.* (2013), considering the planting extensions reported by producers and descriptive statistics with Statistica V7.

## Laboratory phase

The second phase was carried out at the Edible, Functional and Medicinal Fungi Genetic Resources Center of the Puebla *Campus*. The selected residues were those found in the highest percentage in the first stage of the investigation: x-pelon bean stubble (*Vigna unguiculata* L. Walp), dehydrated pulp of chihua pumpkin (*Cucurbita argyrosperma* Huber), huaxin husk (*Leucaena leucocephala* Lam.), fruit of pixoi (*Guazuma ulmifolia* Lam.) and wheat straw (*Triticum aestivum* L.) as control. These agricultural residues were evaluated as substrates for *P. ostreatus* strains CP-50 and CP-753, obtaining a total of four treatments (CH, PC, FP, RF) each with four replications and a control based on wheat, for each one.

## Preparation and pasteurization of the substrates

The production units (UP) weighed 200 g of substrate on a dry weight basis. The preparation of the inoculum and the treatment of the substrates was carried out according to Sobal *et al.* (1993). The sterile substrates were measured for pH with a potentiometer (Conductronic brand pH 130) and the dry weight was calculated.

## Seeding of substrates

Each UP was seeded at a proportion of 15% of previously prepared inoculum, based on the fresh weight of the substrate under sterilization conditions. Once sown, they were transferred to the incubation area at room temperature.

## Variables evaluated

The percentage of colonization of the substrate (CS) was estimated every third day. The appearance of primordia (AP) was reported from the days elapsed from inoculation to the appearance of the first outbreaks.  $AP = \frac{DI}{DAP}$  Where: AP= appearance of primordia (days); DI= incubation time (days); DAP= time of appearance of primordia (days).

Biological efficiency (EB%) was calculated by dividing the total weight of the harvested fresh mushrooms by the dry weight of the substrate at the time of inoculation (Salmones *et al.*, 1997).  $EB(\%) = \frac{PTHF}{PSS}$ . Where: EB= biological efficiency (%); PTHF= total weight of fresh mushrooms (kg); PSS= dry weight of the substrate (kg).

The colonization rate (TC) was obtained by dividing the colonization of the substrate (%) by the time it took to colonize each strain (Reyes *et al.*, 2004).  $TC(\%) = \frac{CS}{TCC}$ . Where: TC= colonization rate; CS= colonization of the substrate (%); TCC= time to colonize the strain (days).

The production rate (TP) was calculated with the EB/TIPC formula (Reyes *et al.*, 2004).  $TP(\%) = \frac{EB}{TIPC}$ . Where: TP= production rate (%); EB= biological efficiency (%); TIPC= elapsed time from inoculation to first harvest (days).

### Statistical analysis

A two-factor randomized block experimental design was used: factor 1 the strain with two levels (CP-50 and CP-753) and factor 2 the substrates with five levels (x-pelon bean stubble, chihua pumpkin pulp, huaxin husk, pixoi fruit and wheat straw). Data analysis was performed using an Anova with Tukey's test ( $p < 0.05$ ) with Statistica V7 software.

### Diagnosis of agricultural production systems

The most planted crop was corn with 65.9%, followed by chihua pumpkin and x-pelon beans with 14.4 and 7.1%, respectively (Table 1). The total surface of the production systems in the state oscillates between  $428 \pm 5.3$ ,  $93 \pm 1.3$  and  $46.2 \pm 1$  ha for the cultivation of corn, chihua pumpkin and x-pelon beans, with an average of 2.3, 0.5 and 0.2 ha per production cycle.

**Table 1. Estimation of the production of plant residues in rural communities of Campeche, Mexico.**

Agricultural crops	Sown area (ha)	Waste generated	Waste generation (estimate)	n	Localities
Corn	428	Stubble	4 583.69	130	Katab, Xmaben, Silvituc, Chicbul and Tixmucuy
Chihua pumpkin	93.5	Pulp	1 309	49	Zoh-laguna and Xmaben
Pumpkin	50.6	Stubble	NR	25	Zoh-laguna, Pucnachen, Nilchi, Chicbul, Nuevo pital, Revolucion and Xmaben
X-pelon bean	46.2	Stubble	802.14	24	Virgencita de la Candelaria, Zoh-laguna, Xmaben and Silvituc
Peanut	0.5	Stubble	3 391	1	Nilchi
Chilli	7.8	Stubble	715.05	7	Virgencita de la Candelaria, Chicbul, Victoria and Silvituc
Sweet potato	28	Stubble	345.88	3	Pucnachen, Zoh-laguna, Revolucion and Silvituc
Watermelon	9.92	Stubble	NR	8	Chicbul, Silvituc and Virgencita de la Candelaria
Cassava	1	Stubble	NR	2	Silvituc and Revolucion
Jamaica	0.5	Stubble	171.42	1	Nilchi

Agricultural crops	Sown area (ha)	Waste generated	Waste generation (estimate)	n	Localities
Orange	0.5	Foliage and fruit	NR	1	Revolucion and Pucnachen
Lemon	2	Foliage and fruit	NR	2	Nilchi
Banana	Undefined	Stem and foliage	4.9 t DM year <sup>-1</sup> ha <sup>-1</sup>	44	General Ortiz Ávila, Chicbul, Virgencita de la Candelaria, Silvituc and Revolucion
Forest plants					
Huaxin	Undefined	Scale and foliage	13 kg DM tree <sup>-1**</sup>	66	Tixmucuy, Revolucion and Xmaben
Pixoi	Undefined	Fruit and foliage	74 kg DM tree <sup>-1***</sup>	58	Tixmucuy
Ramon	Undefined	Fruit and foliage	36 kg DM tree <sup>-1</sup> year <sup>-1****</sup>	11	Xmaben
Huaya	Undefined	Fruit and foliage	NR	54	Virgencita de la Candelaria, Xmaben, Revolucion, Victoria and Zoh-laguna

\* = García *et al.* (1993); \*\* = Anguiano *et al.* (2012); \*\*\* = Giraldo (1998); \*\*\*\* = Mendoza *et al.* (2000); NR = not registered; n = number of producers surveyed.

Likewise, it was recorded that 3.2% of the producers surveyed carry out other crops on areas of less than one hectare, among which various varieties of pumpkin (*Cucurbita* spp.), watermelon (*Citrullus lanatus*), chili (*Capsicum* spp.), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), peanuts (*Arachis hipogaea*), jamaica (*Hibiscus sabdariffa*) and banana (*Musa paradisiaca*) (Table 1).

The producers mention that huaxin, pixoi, Ramon (*Brosimum alicastrum*) and huaya (*Melicoccus bijugatus*) constitute forest resources that are available throughout the year. In this sense, Anguiano *et al.* (2012) mention that the production of foliage dry matter per huaxin tree can be 13 kg DM tree<sup>-1</sup> year<sup>-1</sup>; for pixoi Giraldo (1998) reports productions of 74 kg DM tree<sup>-1</sup> year<sup>-1</sup> and for Ramon Mendoza *et al.* (2000) report productions of 36 kg DM tree<sup>-1</sup> year<sup>-1</sup>. It was observed that, in most rural production systems, plant residues lack a previously defined process of utilization and only 13% of producers use it for livestock feed, while 18% burn it, 15% incorporates it into the ground and 46% have no use whatsoever.

These results are lower than those reported in other altitudes of the country, such as the case of the Frailesca region of the State of Chiapas where 53% of producers use agricultural residues for animal feed and 20% burn it (Guevara *et al.*, 2013). For their part, Camacho *et al.* (2013) reported 95% of producers in the central valley of the country use crop residues for animal feed and only 5% carry out sporadic burning.

Those responsible for production systems showed interest in learning how to use plant residues in the production of food for human consumption (edible mushrooms; 23%), animal feed, production of organic fertilizers (20%), handicraft production (19% ), elaboration of silos (19%) and bales (19%). This indicates their willingness to diversify their use from the simple transfer of low-cost biotechnology in order to propose alternatives for the use of this resource in the communities (Gaitan and Silva, 2016).

### Laboratory phase

The cultivation of the CP-753 strain in x-pelon bean stubble was more efficient ( $F_{9, 20} = 6.96$ ,  $p < 0.001$ ) with a colonization of the substrate of 12 days, meanwhile, the CP-50 strain in the treatment with pixoi fruit it reached 85% colonization after 33 days of incubation. Regarding the appearance of primordia, the treatment with the fastest value was CP-753 in bean stubble, showing the presence of primordia 15 days after planting, while CP-50 in pumpkin pulp and fruit of Pixoi did not obtain fructifications ( $F_{9, 20} = 695.25$ ;  $p < 0.001$ ), and the effect of the substrate was not shown ( $F_{4, 25} = 0.83$ ,  $p < 0.05$ ) (Table 2).

**Table 2. Production of *Pleurotus ostreatus* CP-753 and CP-50 strains using plant residues as substrates under laboratory conditions.**

Strain	Substrate	Substrate colonization (days)	Appearance of primordia (days)	Time to first harvest (days)	Biomass(g)	Biological efficiency (%)	Colonization rate (TC)	Production rate (TP)
CP-50	RF	18	27	32	169.25 ± 10.27 <sup>b</sup>	84.62 ± 5.13 <sup>b</sup>	5.55	2.64
	CH	24	27	32	69.25 ± 14.79 <sup>d</sup>	34.62 ± 7.39 <sup>d</sup>	4.16	1.04
	PT	18	21	25	170 ± 18.7 <sup>b</sup>	85 ± 11.46 <sup>b</sup>	5.55	2.44
	PC	21	NR	NR	NR	NR	4.76	NR
	FP	33	NR	NR	NR	NR	2.57	NR
CP-753	RF	12	15	19	205.5 ± 14.97 <sup>a</sup>	102.75 ± 7.48 <sup>a</sup>	8.33	5.13
	CH	15	19	24	118.25 ± 14.97 <sup>c</sup>	59.12 ± 7.48 <sup>c</sup>	6.66	2.46
	PT	15	21	25	186 ± 18.81 <sup>ab</sup>	93 ± 9.4 <sup>ab</sup>	6.66	3.72
	PC	21	27	32	70 ± 11.51 <sup>d</sup>	35 ± 5.75 <sup>d</sup>	4.76	1.09
	FP	27	31	36	20 ± 3.39 <sup>e</sup>	10 ± 2.19 <sup>e</sup>	3.7	0.27

RF= bean stubble; CH= huaxin husk; PT= wheat straw; PC= pumpkin pulp; FP= pixoi fruit. NR= not registered. Different literals in the same column indicate significant difference, Tukey ( $p < 0.05$ ).

Sosa (2012) reports values similar to the present investigation with 25-day averages for the appearance of primordia. On the other hand, Bernabe *et al.* (2004) indicate that at 16 days the appearance of primordia appeared in the corn stubble substrate when inoculated by *P. pulmonarius*. The biological efficiency (EB) was higher ( $F_{9, 20} = 122.39$ ,  $p < 0.0001$ ) with the CP-753 strain in x-pelon and wheat straw stubble with values of 102.75% and 93%, respectively (Table 2). The CP-753 strain presented higher EB ( $F_{1, 28} = 3.71$ ,  $p = 0.06$ ) compared to CP-50. The highest values of the present study are within the range reported by Sobal *et al.* (1993) from 98.8% to 137.6%, but lower than 111.41% with what was reported by Alejo *et al.* (2015), in cultures of *P. ostreatus* inoculated in bean stubble.

The fungus production rate (TP) was higher in the CP-753 strain in x-pelon bean stubble with 5.13 g day<sup>-1</sup> (F9, 20=181.79,  $p < 0.001$ ) (Table 2). The values obtained are higher than those reported by Romero *et al.* (2010), with the CP-50 strain inoculated in bean straws except for the values obtained with pixoi fruit in the two strains. The substrate that *P. ostreatus* efficiently colonized the x-pelon bean stubble with an average value of 15 days, while pixoi fruit with 30 days (F4, 25= 21.25,  $p = 0.0001$ ) (Table 3). The TC varied depending on the substrate, Romero *et al.* (2010) obtained a TC of 5.5 in residues of bean stubble and wheat straw.

**Table 3. Selection of the best substrate to cultivate *Pleurotus ostreatus* strains in plant residues in rural Campeche, Mexico.**

Substrate	Substrate colonization (days)	Appearance of primordia (days)	Time to first harvest (days)	Biomass (g)	Biological efficiency (%)	Colonization rate (TC)	Production rate (TP)
RF	15	21.33	25.83	187.37 ±22.73 <sup>a</sup>	93.68 ±11.36 <sup>a</sup>	6.94	3.88
CH	19.5	23.16	28.16	93.75 ±29.59 <sup>c</sup>	46.87 ±14.79 <sup>c</sup>	5.41	1.83
PT	16.5	21.33	25.66	178 ±19.36 <sup>b</sup>	89 ±9.68 <sup>b</sup>	6.11	2.38
PC	21	13.83	16.33	35 ±38.16 <sup>d</sup>	17.5 ±19.08 <sup>d</sup>	4.76	0.68
FP	30	15.66	18.16	10 ±11.07 <sup>e</sup>	5 ±5.53 <sup>e</sup>	3.13	0.19

RF= bean stubble; CH= huaxin husk; PT= wheat straw; PC= pumpkin pulp; FP= pixoi fruit. NR= not registered. Different literals in the same column, indicate significant difference, Tukey ( $p < 0.05$ ).

The x-pelon bean stubble stood out (F4, 25= 31.23,  $p = 0.0001$ ) with an average value of 93.68% EB and the lowest value was obtained in the pixoi fruit with 5% (Table 3). Similarly, the x-pelon bean stubble showed a higher production rate (F4, 25= 17.69,  $p = 0.001$ ) with 3.88 g day<sup>-1</sup>, while the pixoi fruit remained the lowest (0.19 g day<sup>-1</sup>)

## Conclusions

In rural communities in the state of Campeche there is a wide availability of plant residues, which lack systematic use by producers. The interest of the producers interviewed to learn to use plant residues in different agricultural activities was evident, for which it is necessary to establish training mechanisms. X-pelon bean stubble and huaxin husk, as agricultural substrates, were shown to be potential for the production of *P. ostreatus* strains CP-753 and CP-50 under laboratory conditions. It is necessary to carry out evaluations with the different residues and implement modules for the production of rustic mushrooms in the communities, where the use of these residues is validated and their production is disseminated as an agroecological strategy that allows improving the food security of rural families in the state of Campeche.

## Acknowledgments

This research is part of the Catedras-CONACYT 2181 project 'Agroecological strategies for food security in rural areas of the state of Campeche'. Thanks to the National Council of Science and Technology (CONACYT) for the first author's postgraduate scholarship, to the College of Postgraduates, Campeche Campus, to the Biotechnology Laboratory of Edible, Functional and Medicinal Mushrooms of the College of Postgraduates, Puebla Campus and to the participating producers from rural areas of Campeche, Mexico, for the information provided.

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