

Research Article

Cultivation of *Schizophyllum commune* Fries (CP-433) on corn stubble (*Zea mays* L.) and peanut shell (*Arachis hypogaea* L.) in Mexico

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Abstract

Schizophyllum commune Fr. is a mushroom with a worldwide distribution; it is a food source in several countries and produces medicinal substances. It has been cultivated on sawdust and agricultural byproducts. In the present study, corn stubble (*Zea mays* L., stem with dry leaves) and peanut shell (*Arachis hypogaea* L., dry pericarp) were used as substrates for the cultivation of a strain (CP-433) of *S. commune*. The parameters evaluated were days in the formation of fruiting primordia, the number of harvests, days of the total cycle, biological efficiency (BE), production rate (PR) and yield (Y). Primordia formed between 12 and 13 days and later at 16 days. Two harvests with a total cycle of 22 days were made. The BE was 16.8±7.0% and 17.9±3.2%, the PR was 0.39±0% and 0.29±0.05%, and the Y was 1.68±0.7% and 1.79±0.3% for corn stubble and peanut shell, respectively, without significant differences between the two. The cultivation cycle in this study was shorter than the cultivation of *S. commune* on cocoa shell and coconut fiber (47 days), on sunflower seed shell (30-31 days), on oil palm fruit waste (29 days), and on palo mulato, cocoa shell and banana leaf (26-27 days). The values reached in BE are similar to other studies in which *S. commune* was cultivated on corn stalks and cobs (5.5%) and on palo mulato (6.4%). Corn stubble and peanut shells are promising substrates for the cultivation of *S. commune* on a small or large scale, mainly in the regions where they are discarded.

Keywords: Agricultural byproducts, Medicinal mushroom, Mushroom cultivation, *Schizophyllum commune*, Solid fermentation.

INTRODUCTION

Schizophyllum commune Fr. is a Basidiomycete that grows on fallen tree trunks or on freshly cut branches, generally forming groups. The basidiocarpus measures 7-25 mm wide x 10-30 mm from base to edge, fan-shaped, sessile, or understipited, with wavy and irregular margins. The pileus is grayish-white, plush, of leathery consistency. The hymenium has longitudinal lamellae and a bifid light brownish color (Carreño-Ruiz *et al.*, 2014).

S. commune is one of the most common saprophytic species found and is widely distributed throughout the world (Rosnan *et al.*, 2019). It is considered a food resource because it contains significant amounts of proteins, vitamins, lipids and minerals (Adejoye *et al.*, 2007; Herawati *et al.*, 2016; Pathania and Chander,

2018). Its consumption has been reported in Benin, China, Ethiopia, Ghana, Hong Kong, Madagascar, Malawi, Peru, the Central African Republic, Laos and Zambia (Boa, 2005), Guatemala (Bran-González *et al.*, 2009), Argentina (Figlas *et al.*, 2014), and Sri Lanka (Ediriweera *et al.*, 2015; Dasanayaka and Wijeyaratne 2017).

In Mexico, *S. commune* has been recorded in almost the entire country, and it is consumed as food in the southeast region in Veracruz, Puebla, Oaxaca, Chiapas, Tabasco and Quintana Roo (Carreño-Ruiz *et al.*, 2014; Ruan-Soto, 2018; Ruan-Soto *et al.*, 2020-2021) and commercialized in traditional markets in Oaxaca and Tabasco during the fruiting season (Ruan-Soto *et al.*, 2006).

S. commune contains antioxidant compounds such as polyphenols, hydroxybenzoic acid, protocatechial acid

and tocopherol (Basso *et al.*, 2020); it also has polyphenols and terpenoids that have shown antidiabetic activity (Sharma *et al.*, 2021), in addition to extracellular dark pigments with antioxidant, antibacterial, antifungal and anticancer properties (Arun *et al.*, 2015). *S. commune* produces the polysaccharide schizophyllan, which has antitumor and immunomodulatory properties (Sutivisedsak *et al.*, 2013; Zhong *et al.*, 2013) and contains iminolactones in the fruiting body that are capable of inhibiting the growth of cancer cell lines (Liu *et al.*, 2015).

Studies have been carried out on the cultivation of *S. commune* on sawdust and various agricultural byproducts used as substrates, such as oak sawdust, corn bran, coir, shells of cacao, banana leaves, sawdust from cedar, sunflower seed hull, cocoa shell, and palo mulato (Montoya *et al.*, 2013; Carreño-Ruiz *et al.*, 2014; Figlas *et al.*, 2014; Capello *et al.*, 2018; Carreño-Ruiz *et al.*, 2020).

In Mexico, various agricultural crops are grown, such as corn (*Zea mays* L.) and peanuts (*Arachis hypogaea* L.) that generate large amounts of byproducts. In the 2020 agricultural cycle, 27,424,527 tons of corn and 101,251 tons of peanuts were produced (SIAP, 2020).

The present study evaluated the potential of corn stubble and peanut hull in the cultivation of a strain of *S. commune* to determine the possible biotechnological use and promote the consumption of basidiocarps food.

MATERIALS AND METHODS

In the cultivation of *S. commune*, the techniques indicated by Carreño-Ruiz *et al.* (2020) were followed in the preparation of the inoculum, with modifications in the disinfection of the substrates and the type of containers that were bags with a filter on the upper part.

Strain and inoculum preparation

A strain of *S. commune* with code CP-433 belonging to the Unit of Genetic Resources of Edible, Functional and Medicinal Mushrooms of the Postgraduates College, Campus Puebla, Mexico, was used. The strain was kept in Petri dishes with malt extract agar (MEA, Difco) between 27°C and 28°C in the dark.

Wheat grain (*Triticum aestivum* L.) previously hydrated (45% humidity) was used to prepare the inoculum. It was placed in polypropylene bags (ca. 250 g) and sterilized at 121°C for 1 hour. Immediately, the grain was cold, and 1 cm² of MEA medium with previously developed mycelium was added to each bag. The inoculated bags were incubated at 28°C in the dark for three weeks.

Substrate preparation and inoculation

Corn stubble and peanut shells were broken into segments between 1 and 3 cm in length and immersed in

water for 14 hours. Once drained, 1 kg in wet weight for each substrate was placed in polypropylene bags of 14 cm x 10 cm x 47 cm, with a filter on the upper part of 3.8 cm x 3.8 cm and 5 microns of pore opening sterilized at 121°C for 1 hour. Once cold, they were then inoculated in an approximate proportion of 10% by the wet weight of the substrate. The dry weight was 19.5 g and 28 g in 100 g wet corn stubble and peanut shell, respectively. Five replicates were prepared for each substrate.

Incubation and harvests

Inoculated bags were incubated between 25°C and 27°C in the dark, with a relative humidity of 50% to 55%. On the second day of incubation, several incisions were made on the sides of each bag, approximately 1.5 cm long, to allow gas exchange. The fruiting primordia were formed first in some incisions and later in the upper part of the substrate; therefore, these incisions were enlarged, and the upper part of the bag was removed to allow the full development of the basidiocarps. The substrates were maintained with 12 hours of natural light and 12 hours in darkness, and fine watering was carried out with a manual sprinkler. The temperature in the production stage ranged from 21°C to 26°C with relative humidity between 75% and 85%.

Productivity was based on the number of days in the formation of primordia, fresh weight of basidiocarps, total days of culture counted from inoculation, biological efficiency (BE = fresh weight of harvested basidiocarps/dry weight of substrate); production rate (PR = BE/total days of production) and yield (Y = fresh weight of the basidiocarps/fresh weight of the substrate). BE, PR and Y are expressed as a percentage.

Experimental design and statistical analysis

A completely randomized design was used. An analysis of variance was carried out on the data obtained, and the mean values were compared with the multiple range test according to Tukey's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

During incubation, both substrates were colonized by the mycelium at 100%, and between 12 and 13 days, primordia were formed on the lateral parts of the bags, while on the upper surface, they were formed at 16 days, counted from inoculation. The primordia required six days to reach maturity; therefore, the production of two harvests was evaluated with a total crop cycle of 22 days (Fig. 1).

Thus, the days of the cultivation cycle were less than those reported by Capello *et al.* (2018) (47 days) on cocoa shell (*Theobroma cacao* L.) and coconut fiber (*Cocos nucifera* L.); Figlas *et al.* (2014) (36 to 31 days) on sunflower seed shell (*Helianthus annuus* L.) with and

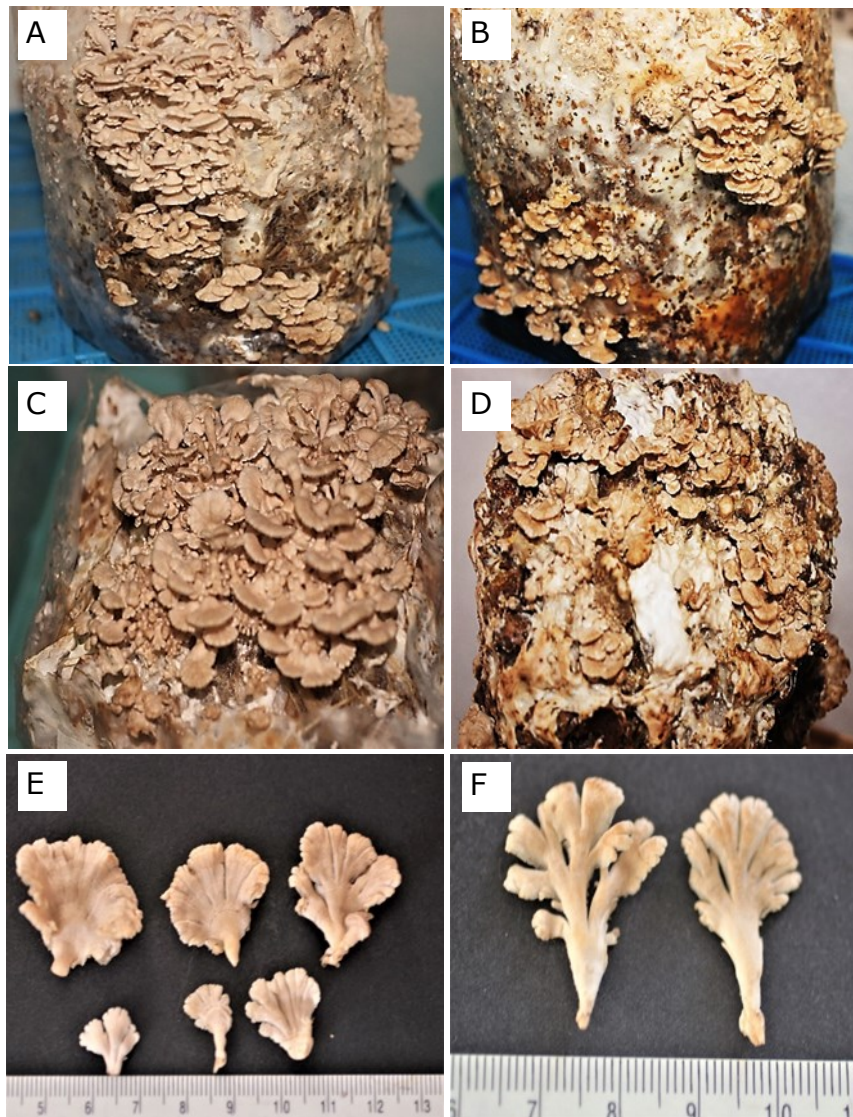


Fig. 1. A and B, Basidiocarps of *S. commune* growing on the lateral parts of the substrates corn stubble and peanut shell, respectively. C and D, Basidiocarps of *S. commune* growing on the upper parts of the substrates corn stubble and peanut shell, respectively. E and F, Morphology and size of the basidiocarps harvested on both substrates.

without supplements; Ediriweera *et al.* (2015) (20 to 25 days) on coconut leaves and coir dust; Herawati *et al.* (2016) (29 days) on oil palm fruit waste with supplements and Carreño-Ruiz *et al.* (2020) (26 to 27 days) on palo mulato [*Bursera simaruba* (L.) Sarg.], cocoa shell and banana leaf (*Musa paradisiaca* L) with two strains and two types of containers (bag and tray).

The basidiocarps developed into connate groups, with the pileus labelliform or marked lobed and substipitate. They measured 7-30 mm wide x 11-35 mm from base to edge (Fig. 1). These measurements were larger in width but smaller from base to edge than those recorded by Carreño-Ruiz *et al.* (2020), who obtained basidiocarps with sizes from 3-8 mm wide x 10-50 mm from base to edge on the cocoa shell at a temperature above 30°C.

Regarding BE, the corn stubble reached an average of $8.62 \pm 3.6\%$, while the peanut shell presented

$6.39 \pm 1.2\%$, without significant differences (Table 1). These percentages were similar to the BE (5.5%) achieved by Bran-González *et al.* (2009) on corn stalks and cobs (1:1) and with the BE (6.4%) obtained by Carreño-Ruiz *et al.* (2020) on palo mulato (strain CCG013). However, they exceeded the value (3.73%) reached by Herawati *et al.* (2016) on oil palm fruit waste but were lower than those reached (40.7% to 48.3%) by Figlas *et al.* (2014) on sunflower seed shell and by Carreño-Ruiz *et al.* (2020) (12.8%) on cocoa shell contained in bags and on palo mulato (10.7%) contained in trays used for cultivation (strain CCG009).

The PR values were $0.39 \pm 0.16\%$ for corn stubble and $0.29 \pm 0.05\%$ for peanut shell, without significant differences (Table 1) and are lower than the percentages (1.1% to 1.6%) reported by Figlas *et al.* (2014) but are similar or slightly lower than the values (0.1% to 0.7%) registered by Carreño-Ruiz *et al.* (2020) with two

Table 1. Averages in productivity evaluation achieved in the cultivation of the CP-433 strain of *S. commune* on corn stubble (*Z. mays*) and peanut shell (*A. hypogaea*)

Substrates	1st harvest (days)	2nd harvest (days)	1st harvest (g±σ)	2nd harvest (g±σ)	Total weight of mushroom (g±σ)	Biological efficiency (%±σ)	Production rate (%±σ)	Yield (% ±σ)
Corn stubble	18	22	9.0±7.4 a *	7.8±4.0 a	16.8±7.0 a	8.62±3.6 a	0.39±0.16 a	1.68±0.7 a
Peanut shell	18	22	13.9±5.6 a	4.9±2.8 a	17.9±3.2 a	6.39±1.2 a	0.29±0.05 a	1.79±0.3 a

*Equal letters in the columns indicate nonsignificant differences between the mean values according to Tukey's multiple range test ($\alpha = 0.05$).

strains and two types of containers.

Regarding the percentages reached in Y, they were 1.68±0.7% for corn stubble and 1.79±0.3% for peanut shell, without significant differences (Table 1) and were similar or lower than those registered by Carreño-Ruiz et al. (2020) (2.0%) with a strain (CCG013) on palo mulato and the substrate contained in bags, while with another strain (CCG009), the percentage was 4.1% on cocoa shell contained in bags and 3.3% on palo mulato contained in trays.

In general, the production rates achieved in this research were within those recorded in other works. However, the variation in temperature (21°C to 26°C) during the production stage probably influenced the delay in maturation, the decrease in the size and weight of the basidiocarps, and the number of harvests. On the basis of the results obtained in this work, it is important to continue studies to increase production, cultivating several strains of *S. commune* on the same or other agricultural byproducts under different environmental conditions.

Conclusion

Corn stubble and peanut shell proved to be potentially suitable substrates for the cultivation of *S. commune* (strain CP-433) because mycelial colonization and the formation of basidiocarps in both substrates were fast, with a culture cycle of 22 days from inoculation, obtaining two harvests. Therefore, there is the possibility of continuing with the studies to carry out the cultivation of *S. commune* on a small or larger scale and, at the same time, promote its consumption as a functional food.

Conflict of interest

The authors declare that they have no conflict of interest.

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