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Optimization of the Yield of Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Higher Basidiomycetes), Cultivated on a Sunflower Seed Hull Substrate Produced in Argentina: Effect of Olive Oil and Copper

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ABSTRACT: Sunflower seed hulls were used as the main component of a solid substrate for the cultivation of the lingzhi or reishi medicinal mushroom *Ganoderma lucidum*. This study evaluated the effects of supplementing the substrate with olive oil and copper (II) on the mushroom production (MP) parameters and fruiting body total triterpenoid content. The addition of 1.5% olive oil increased total MP by 21.7% (dry basis) in 3 flushes. Copper (60 ppm) increased the daily productivity of the first flush (MP per day) by both reducing the time needed to harvest the crop and increasing the MP. However, the MP at the second and third flushes was reduced. When both supplements were combined, the MP at the first flush was 43% higher than with control treatment. No significant change in mushroom total triterpenoid content was observed by the addition of supplements to the substrate. An increase of 145–155% in the mushroom copper content was obtained by the addition of 60 ppm copper to the substrate. It is thus recommended to use substrate formulations containing both olive oil and copper (II) and harvest just the first flush.

KEY WORDS: medicinal mushrooms, *Ganoderma lucidum*, solid-state fermentation, mushroom yield, olive oil, copper, triterpenoids

ABBREVIATIONS: BE, biological efficiency; **Cu**, copper; **MP**, mushroom production; **MYSA**, malt yeast sucrose agar; **SSF**, solid-state fermentation; **SSH**, sunflower seed hull.

I. INTRODUCTION

The lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (Curtis: Fr.) P. Karst. (Ganodermataceae, higher Basidiomycetes), is a white rot mushroom used in Asia for its medicinal properties for over 2,000 years. The bioactive components found in this mushroom have numerous health properties to treat different pathologies such as hepatopathy, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcers, atherosclerosis, leukopenia, diabetes, anorexia, and cancer.[1] Triterpenoids are the major group of secondary metabolites in *G. lucidum*. Triterpenoids have been found in the fruiting bodies, spores, and mycelia of this mushroom and are responsible for many of its therapeutic properties.[2]

G. lucidum is very rare in nature, and the amount of wild mushroom is not sufficient for commercial exploitation. So, its cultivation in solid synthetic substrates is essential to meet the growing demand of international markets.[3] *G. lucidum* can be grown on synthetic substrates made of a variety of agro-industrial residues. In fact, several lignocellulose-rich residues have already been reported in successful substrate formulations, such as rice straw,[4] wheat straw,[5]

coffee industry residues,[6] tea wastes,[7] and ginseng extraction residues.[8] Sunflower seed hulls (SSHs) constitute an abundant and cheap by-product of the sunflower oil industry. The disposition of huge amounts of this agro-industrial waste poses a problem to the environment because it degrades very slowly in soil and it is usually burned in open fields. Previous studies have shown that SSHs can be used as the main component in the formulation of a substrate for cultivating *G. lucidum* on synthetic logs, with good crop yields.[9]

G. lucidum usually produces relatively low crop yields (i.e., its biological efficiency [BE; (kilograms of fresh mushrooms/kilograms of dry substrate) × 100]) compared with other edible mushrooms, for example, 10% BE of *G. lucidum* vs. 108% for *Lentinus edodes* or 60–112% for *Pleurotus ostreatus*.[9–11] This, taken together with the high international market value of this mushroom and the possibility of using the fruiting bodies as a source of valuable bioactive triterpenoids, makes the proposal of any improvement in crop yield an interesting approach with an economic impact.

An increase in mushroom production (MP) in terms of crop yield can be achieved by adding supplements to improve the nutritional quality of a given substrate. Vegetable oils, particularly fractions of their fatty acids, have been shown to possess growth-stimulating properties in submerged cultures of *Grifola frondosa*, *Cordyceps militaris*, and *G. lucidum*.[12–14] Also, vegetable oils and fatty acids have be proven to stimulate crop yields of *Agaricus bisporus* and *Tricholoma matsutake* in solid-state fermentation (SSF) systems.[15,16] However, available information on the effects of these substances on the growth of *G. lucidum* cultivated in SSF systems and the total triterpenoid content of the fruiting body is limited.

In our laboratory, previous studies of the ability of *G. lucidum* to accumulate copper (Cu) or zinc in fruiting bodies showed a stimulatory effect on crop yield when Cu(II) was included in a SSH solid substrate.[17] Also, Tang and Zhu[18] observed an increase in biomass with the addition of Cu(II) in submerged liquid cultivation.

The main purpose of this work was to evaluate the effects of olive oil and Cu(II) on *G. lucidum* growth in terms of productivity when cultivated on a substrate SSF system based on SSHs. Also, we studied the effect of these supplements on the total triterpenoid content of dried fruiting bodies.

II. MATERIALS AND METHODS

A. Mushroom Strain

G. lucidum strain E47 (University of Guelph, Guelph, Ontario, Canada) was cultivated on malt yeast sucrose agar (MYSA) medium (20 g malt extract, 2 g yeast extract, 10 g sucrose, and 20 g agar per liter; pH 6) at 25°C in darkness.

B. Biomass and Radial Growth on Agar Nutrient Medium

To study the effect of olive oil on *G. lucidum* biomass and mycelium radial growth, 6.5-mm-diameter portions of mycelium from stock culture were inoculated in the center of Petri dishes containing MYSA medium with edible olive oil from the first extraction by pressure (Natura; Aceitera General Deheza, Argentina) at 0.0%, 0.1%, 0.25%, 0.5%, 0.75%, 1.0%, and 1.5%. After incubating the cultures for 5 days, the diameter of both the colony (n = 8) and the biomass (n = 6) was determined. Mycelial biomass was determined according to the method described by Sánchez and Viniegra-Gonzaléz.[19] The solid agar medium containing the mycelium was melted for 1 min in a microwave oven (BGH model 16600; 1250 W). The mycelium was then gently removed using tweezers and rinsed with distilled water. Tissue paper was used to remove excess liquid from the mycelium, which was then placed in an oven at 60°C until a constant weight was reached.

C. Solid-State Fermentation

1. Inoculum Production

Inoculum (spawn) was prepared according to the method described by Curvetto et al.[20] Briefly, 1-L bottles were filled with 59.1% wheat grain (commercially available *Triticum durum*), 0.1% $CaCO_3$,

0.8% $CaSO_4$, and 40% water by weight. Bottles were autoclaved at 1 atm for 90 min and inoculated with mycelia from the MYSA medium. Incubation was performed at 25°C in darkness for 10–15 days; bottles were periodically shaken to minimize grain clumping and to allow better colonization.

2. Substrate Preparation, Inoculation, and Bag Systems

The following substrates were formulated to study the effect of olive oil and Cu(II) on the production of *G. lucidum*: (1) a basal substrate formulation containing 32.5% SSH (kindly provided by a local sunflower oil producer), 5.0% barley (commercially available *Hordeum vulgare*), 2.0% $CaSO_4$, 0.5% $CaCO_3$, and 60% water by weight; (2) a basal substrate supplemented with 1.5% olive oil; (3) a basal substrate supplemented with 60 ppm Cu(II) as copper sulfate; and (4) a basal substrate supplemented with both 1.5% olive oil and 60 ppm Cu(II). All supplements were added before substrate decontamination.

Decontamination of substrates proceeded on a concrete mixer machine coupled with an oven gas burner according to a technique described by Curvetto et al.[21] Substrate (37 kg) was continuously heated for 3 hours; the drum was alternately rotated for 15 min and still for 15 minutes. After the substrate cooled below 38°C, inoculum at an 8% ratio (w/w) was added and mixed by drum rotation for 15 min.

Polyethylene bags (100 μm, 39.5 × 13.5 cm) were filled with 0.8–0.9 kg of substrate and packed to a density of 0.5 g/mL under aseptic conditions. The open end of each bag was tied with a plastic ribbon seal. Both ends of the bags were punctured with needles (~4 punctures/cm^2) using an ad hoc device to facilitate gas exchange. Each treatment included 40 experimental units (synthetic logs).

3. Spawn Running, Mushroom Harvesting, and Crop Yields

Synthetic logs prepared as previously described were arranged horizontally on shelves and incubated at 22–26°C in darkness until the substrate was fully colonized and the first signs of secondary metabolites appeared (yellow, orange, and brownish exudates). At this time, synthetic logs were moved to the fruiting room and the plastic ribbon seals were removed to expose the mature mycelia to the following environmental conditions: 80–100% relative humidity, 25–30°C, a 12-hour photoperiod (100- to 500-lux irradiation from white fluorescent lights), and adequate air ventilation.

Mushrooms were harvested when the clearest and yellowish edge in the active growth zone was reduced to less than 1 cm, just before complete fruiting body maturation and maximum release of spores. The fruiting bodies were harvested using a scalpel. After harvesting of the first and second flushes, the synthetic logs were moved to another section within the fruiting room to separate them from synthetic logs that were not yet harvested.

The duration of spawn running in days and time to each flush in days from inoculation were obtained for each experimental unit (synthetic log). Crop yield was expressed as the BE of each flush and accumulated BE. Because *G. lucidum* fruiting bodies are sold dry, yield parameters on a dry basis also were calculated. MP for each flush and total MP were calculated as BE values but considered the dry weight of mushrooms instead of their fresh weight. Percentages of experimental units exhibiting fruiting bodies also were calculated.

D. Laccase Activity

1. Laccase Extraction

Samples were obtained from the middle portions of synthetic logs (n = 16) where laccase activity is highest,[4] just after third flush harvesting was completed. Samples were kept at −18°C until laccase extraction was performed. Distilled water (30 mL) was added into a 50-mL flat-bottomed glass tube containing 3.0 g fresh residual mushroom substrate. The mixture was chopped using a pestle and mortar for 0.5 min and kept at 4°C for 24 hours. Another portion (10 g) of residual mushroom substrate was used to obtain the dry weight. After 24 hours of extraction, the mixture was compressed (5 kg/cm^2)

to obtain an aliquot of the crude enzyme extract, and its volume was measured. Extracts were filtered using paper filters (J. Prolab type JP-3003-4) and stored at −18°C until laccase activity was analyzed.

2. Laccase Activity Analysis

Laccase activity was analyzed by spectrophotometry using syringaldazine (S7896; Sigma) as the substrate. The increase in absorbance at 525 nm ($\varepsilon 525 = 65000\ M^{-1}\ cm^{-1}$) as a result of substrate oxidation was determined.[22] An enzyme activity reaction was initiated by adding 25 µL of 20 mmol/L syringaldazine in ethanol to 1 mL of extract (0.5 mmol/L final concentration). Extract with no syringaldazine was used as a blank. Absorbance readings were obtained at 0.5, 1, and 2 min after adding syringaldazine. Sample aliquots heated at 100°C for 10 min to denaturize proteins also were included as controls. The laccase activity unit was defined as the amount of enzyme that oxidizes 1 µmol syringaldazine per minute. Laccase activity was expressed on the basis of dry residual mushroom substrate (units per kilogram dry weight).

E. Mushroom Total Triterpenoid Content and Extraction from the Fruiting Body

Triterpenoids were quantified by a colorimetric method against an ursolic acid standard curve. A standard solution was prepared by dissolving 10 mg ursolic acid (U6753; Sigma) in 100 mL absolute ethanol (Dorwil, Argentina). Triplicate aliquots (0, 0.4, 0.8, 1.0, and 1.2 mL) of the standard solution were transferred to 10-mL test tubes. After the solvent was evaporated to dryness in an oven at 60°C, 0.15 mL of 5% vanillin (W310700; Sigma)–acetic acid (Anedra, Argentina) solution and 0.5 mL of 70% perchloric acid (Cicarelli, Argentina) were added. The mixture was heated in a 60°C water bath for 45 min, cooled at room temperature, and diluted with 2.5 mL acetic acid. Absorbance was determined at 548 nm in a spectrophotometer (Genesys 20; Thermo Scientific). Standard weight showed a good linear relationship with absorbance in the range of 0.04–0.12 mg ($y = 11.47x - 0.3606$; $r^2 = 0.96$).

Dried and powdered fruiting bodies (8 g) were accurately weighted and extracted in a Soxhlet apparatus with 120 mL absolute ethanol for 4 hours to create a triterpenoid-rich extract. Three replicates were obtained for each treatment. Triplicate 0.1-mL aliquots were taken from each extract. The total triterpenoid content of the samples was determined by the colorimetric method described above.

F. Mushroom Copper Content

Powdered dried fruiting bodies (1 g) were digested with 1.5 mL of HNO_3 and $HClO_4$ (2:1 v/v) for 2 hours at 280°C and diluted with bi-distilled water. Cu content was measured using an inductively coupled plasma optimal emission spectrometer (model 1000:III; Shimadzu).

G. Statistical Analysis

Data from the production assay were analyzed by 1-way analysis of variance. The mean value separation was done using the Tukey test. These analyses were performed using Infostat software.

III. RESULTS

A. Mycelium Growth

The effect of olive oil on *G. lucidum* mycelial growth was studied using an agar nutrient medium test. Figure 1 shows the effect of olive oil on the diameter of the colony, biomass, and mycelial surface density. The addition of olive oil from 0.75% to 1.5% reduced the diameter of the colony ($P < 0.05$) on the agar nutrient medium up to ~11% at a 1.5% concentration. However, at a 1.5% olive oil concentration, the biomass obtained was 150% higher than the control. Mycelial surface density (grams per centimeters squared) increased linearly with olive oil concentration up to the highest concentration tested ($y = 0.3096x + 0.002$; $r^2 = 0.9678$). Based on this information, we decided to use 1.5% olive oil in the substrate formulation for SSF culture.

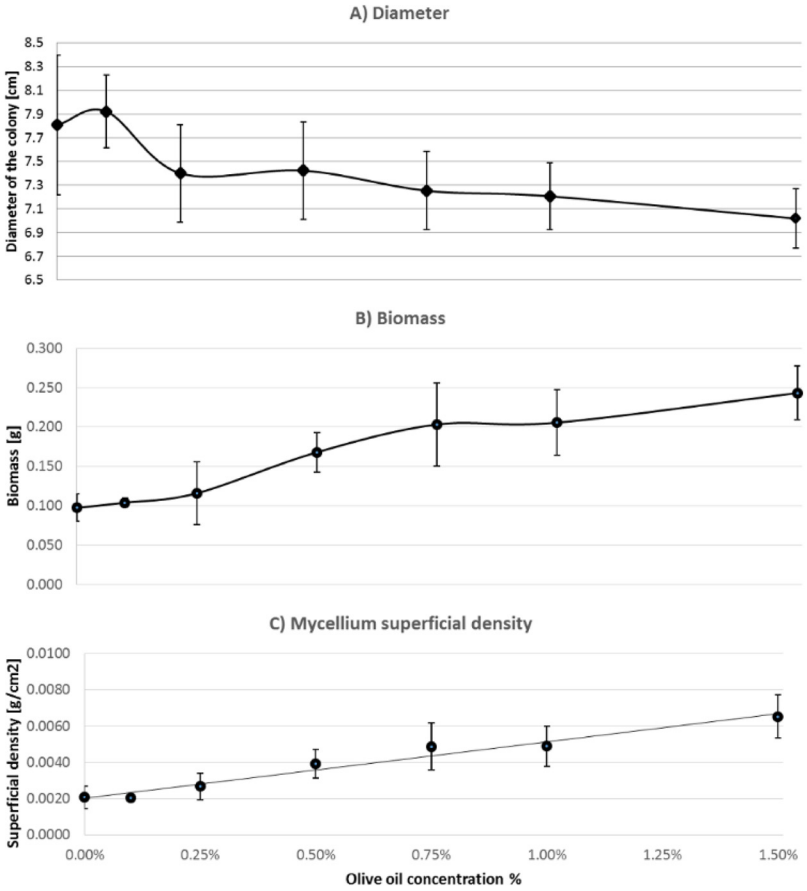

FIG. 1: Effect of olive oil on *Ganoderma lucidum* mycelium growth in agar nutrient medium after 5 days of incubation at 25°C: diameter of the colony (centimeters) **(A)**; biomass (grams) **(B)**, and mycelium surface density (grams/centimeter squared) **(C)**.

B. Solid-State Fermentation

1. Spawn Running

Addition of 60 ppm Cu(II) to the substrate significantly increased the speed at which the mycelia colonized the substrate and started to exude secondary metabolites, reducing the spawn-running time from 11 days to 7 days ($P < 0.01$) (Table 1). The addition of 1.5% olive oil delayed the spawn-running time (14 days). However, olive oil had no effect on spawn-running time when Cu(II) was also present on the substrate (Table 1).

2. Mushroom Yield Parameters

Tables 1 and 2 show the yield parameters for 3 mushroom flushes. The addition of 60 ppm Cu(II) and 1.5% olive oil increased the yield of *G. lucidum* by 27% and 30%, respectively, compared with the control. When Cu and olive oil were added together, MP was significantly increased by 43% ($P < 0.01$). Also, the addition of Cu(II) reduced the time to first flush harvest by 7 days because of a reduction in the time for both spawn running (Table 1) and fruiting body growth. Olive oil (1.5%) had no effect on the time needed for growth, development, and maturation of fruiting bodies; the increase in time taken to reach the first flush harvest was due to a slower spawn-running period (Table 1). The increase in crop yield observed following the addition of both Cu(II) and olive oil, taken together with the reduction of time needed to reach the first flush harvest, greatly improved the daily productivity of this flush by 78% ($P < 0.01$).

TABLE 1: Mushroom Productivity (First Flush): Effect of 60 ppm Copper (II) and 1.5% Olive Oil on *Ganoderma lucidum* Crop Yield

Substrate Formulation	D_{SR} (Days)	$\%_{1°F}$	$D_{1°F}$ (Days)	$BE_{1°F}$ (%)	$MP_{1°F}$ (%)	Daily $MP_{1°F}$ (%/Day)
Control	11 ± 2[b]	100	35 ± 3[b]	12.6 ± 1.6[a]	3.0 ± 0.4[a]	0.087 ± 0.011[a]
Copper (II) (60 ppm)	7 ± 1[a]	100	28 ± 1[a]	16.0 ± 2.3[b]	3.8 ± 0.4[b]	0.137 ± 0.015[c]
Olive oil (1.5%)	14 ± 1[c]	100	37 ± 3[c]	15.5 ± 2.5[b]	3.9 ± 0.7[b]	0.108 ± 0.017[b]
Copper (II) (60 ppm) + Olive oil (1.5%)	7 ± 1[a]	100	28 ± 1[a]	18.1 ± 1.9[c]	4.3 ± 0.5[c]	0.155 ± 0.018[d]

Data are means ± standard deviations (n = 40). Values within a column with the same letter are not significantly different ($P < 0.01$) according to the Tukey test.
$\%_{1°F}$, Percentage of synthetic logs exhibiting mushrooms at the first flush; $BE_{1°F}$, biological efficiency at the first mushroom flush [(kilograms fresh mushroom/kilograms dry substrate) × 100]; $D_{1°F}$, time to first flush harvest from substrate inoculation; Daily $MP_{1°F}$, daily first flush mushroom production; D_{SR}, time running spawn; $MP_{1°F}$, first flush mushroom production [(kilograms dry mushroom/kilograms dry substrate) × 100].

TABLE 2: Mushroom Productivity (Second and Third Flushes): Effect of 60 ppm Copper (II) and 1.5% Olive Oil on *Ganoderma lucidum* Crop Yield

Substrate Formulation	Second Mushroom Flush				Third Mushroom Flush			
	$\%_{2°F}$	$BE_{2°F}$ (%)*	$MP_{2°F}$ (%)*	Daily MP (%/day)*†	$\%_{3°F}$	$BE_{3°F}$ (%)*	$MP_{3°F}$ (%)*	Daily MP (%/day)*‡
Control	97.7	4.4 ± 1.2[a]	1.0 ± 0.3[b,c]	0.053 ± 0.022[a]	81.4	2.3 ± 0.9[a]	0.7 ± 0.2[b]	0.05 ± 0.015[a]
Copper (II) (60 ppm)	77	3.1 ± 1.3[b]	0.7 ± 0.3[a]	0.027 ± 0.016[b]	45.8	1.6 ± 0.7[a]	0.5 ± 0.1[a]	0.035 ± 0.012[a]
Olive oil (1.5%)	97.4	4.9 ± 1.5[a]	1.1 ± 0.3[b,c]	0.058 ± 0.017[a]	79.4	2.1 ± 1.1[a]	0.7 ± 0.2[b]	0.053 ± 0.025[a]
Copper (II) (60 ppm) + Olive oil (1.5%)	93.6	3.4 ± 1.3[b]	0.9 ± 0.4[a,b]	0.031 ± 0.018[b]	51	2.2 ± 1.3[a]	0.6 ± 0.2[a,b]	0.041 ± 0.016[a]

Data are means ± standard deviations (n = 40). Values within a column with the same letter are not significantly different (P < 0.01) according to the Tukey test.
*Only synthetic logs that showed fruit body development were considered for the calculation of this value.
†Average time to the second mushroom flush harvest from inoculation was 57 days.
‡Average time to the third mushroom flush harvest from inoculation was 72 days.
$\%_{2°F}$, Percentage of synthetic logs exhibiting mushrooms at the second flush; $\%_{3°F}$, percentage of synthetic logs exhibiting mushrooms at the third flush; $BE_{2°F}$, biological efficiency at the second flush; $BE_{3°F}$, biological efficiency at the third flush; daily $MP_{2°F}$, daily mushroom production at the second flush; daily $MP_{3°F}$, daily mushroom production at the third flush; $MP_{2°F}$, mushroom production at the second flush; $MP_{3°F}$, mushroom production at the third flush.

Figure 2 shows a comparison of fruiting bodies from representative synthetic logs from each treatment just before the harvest of the first flush. Substrates containing olive oil or Cu produced a larger number of fruiting bodies compared with the control. The second and third mushroom flush crop yields were lower than that of the first mushroom flush (Table 2). Olive oil had no effect on the second or third mushroom flush crop yields. However, the presence of 60 ppm Cu(II) decreased crop yields at this stage. At the second mushroom flush, both the BE and percentage of synthetic logs producing mushrooms were markedly lower with the Cu-containing substrate. Furthermore, time to the

FIG. 2: : Photographs showing a comparison of the first flush of *Ganoderma lucidum* from 5 representative synthetic logs 27 days after inoculation. When substrate formulations included, copper (second row), olive oil (third row), or both (bottom row), a larger number of fruit bodies formed in relation to the ones formed on the control substrate (top).

second flush was longer for the substrate containing Cu (29 days vs. 20 days in the control). However, the presence of 1.5% olive oil improved the number of Cu-containing synthetic logs that produced fruiting bodies for the second mushroom flush (93.6% vs. 77%) (Table 2).

Just half or less of Cu-supplemented synthetic logs showed fruiting body development by the third mushroom flush, and production yield was significantly lower than the yields obtained with other substrate formulations (Table 2). Synthetic logs with no Cu showed mushroom development in ~80% of synthetic logs. Total mushroom yield is presented in Table 3 both as accumulated BE and as total MP. Olive oil significantly improved the total yield by 22%. On the other hand, Cu(II) had no effect on the total mushroom yield after 3 flushes.

Considering the first mushroom flush, Cu induced a significant increase in daily productivity by both increasing the yield and reducing the time to harvest. However, the first flush was followed by an important decrease—up to 80%—in daily MP. Synthetic logs without fruiting body development at the second and third mushroom flushes were not considered when calculating that value. In the case of the control substrate and those containing 1.5% olive oil, mushroom productivity gradually decreased across mushroom flushes, and 30–35% of the total mushroom yield came from the second and third flushes.

3. Substrate and Mushroom Water Content

Moisture content was determined in residual substrate logs after the third flush. Results are shown in Table 3. A marked decrease in moisture content occurred in all synthetic logs (initial water content, 60%). However, the reduction was even more abrupt in those substrates containing 60 ppm Cu(II). Water content in mushrooms from the first and second

TABLE 3: Effect of 60 ppm Copper (II) and 1.5% Olive Oil on *Ganoderma lucidum* Production Yield

Substrate Formulation	n*	Accumulated Biological Efficiency* (%)	Total Mushroom Production† (%)	Substrate Water Content (%)§
Control	43	18.8 ± 2.7[a]	4.6 ± 0.6[a]	42.6 ± 2.3[a]
Copper (II) (60 ppm)	48	19.2 ± 3.5[a]	4.6 ± 0.7[a]	32.6 ± 4.2[b]
Olive oil (1.5%)	39	22.0 ± 4.0[b]	5.6 ± 0.9[b]	39.3 ± 5.3[a]
Cu (II) (60 ppm) + Olive oil (1.5%)	47	22.4 ± 3.0[b]	5.4 ± 0.6[b]	32.2 ± 4.9[b]

Data are means ± standard deviations. Values within a column bearing the same letter are not significantly different ($P < 0.01$) according to the Tukey test.
*number of synthetic logs per treatment
†In three flushes, calculated as [(kilograms fresh mushroom/kilograms dry substrate) × 100].
§Substrate water content was measured after 3 mushroom flushes (82 days).

flushes was 74–78%, with no significant differences between treatments ($P < 0.05$). Water content was 61–67% in mushrooms obtained at the third flush. When compared with previous flushes, the reduction in water content was significant ($P < 0.05$), but again, no significant differences between treatments was observed ($P < 0.05$).

C. Laccase Activity

Laccase activity of aqueous extracts obtained from residual substrates after 3 mushroom flushes is shown in Fig. 3. After 3 mushroom flushes, laccase activity significantly decreased in Cu-containing substrates.

D. Mushroom Total Triterpenoid Content

Total triterpenoid content in the fruiting bodies was in the range of 23.4–26.4 mg ursolic acid equivalent/g dry fruiting body for all treatments studied. Supplementation of the substrate with olive oil, Cu, or both did not significantly affect the total triterpenoid content in the fruiting body.

E. Mushroom Copper Content

An increase of 145–155% in mushroom Cu content—from 53 to 130–135 mg/kg—was observed in Cu-enriched substrates.

IV. DISCUSSION

In recent years there has been an increasing trend toward more efficient disposal of agro-industrial wastes, especially lignocellulosic residues. Lately there has been much effort in cultivating *G. lucidum* in solid media for fruiting body production on lignocellulosic wastes. The *G. lucidum* fruiting body is a popular and reputed dietary supplement and can also be used as a source of bioactive triterpenoids. However, because SSF requires more time than other methods to cultivate *G. lucidum*, several studies have focused on optimizing submerged cultures. Supplementation of substrate becomes one of the major aspects of *G. lucidum* cultivation for the purpose of increasing the biological yield while reducing the time required for mushroom production in SSF.

The presence of olive oil in substrate formulations produced a dose-dependent increase in both *G. lucidum* biomass and mycelium surface density when cultured on MYSA medium. However, a slight reduction in the colony surface area was observed. Mycelium surface density—that is, the biomass–to–surface area ratio—showed a better linear relationship in relation to olive oil concentration and thus resulted in a useful parameter with which to evaluate *G. lucidum* mycelial growth.

The addition of 1.5% olive oil to an SSH-based substrate in an SSF system significantly increased

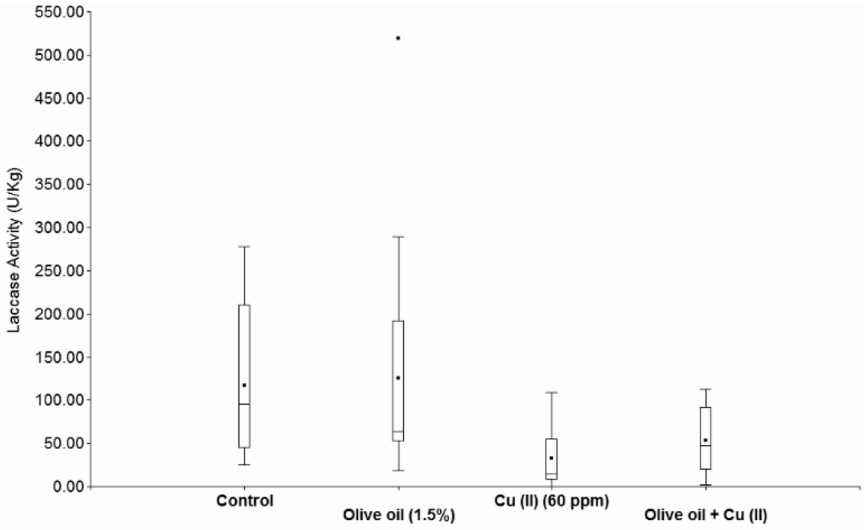

FIG. 3: Laccase activity of crude aqueous extracts obtained from residual synthetic logs of *Ganoderma lucidum* after 3 mushroom flushes. The graphs shows the influence of substrate supplementation with 60 ppm copper (II), 1.5% olive oil, and a combination of both. Laccase activities (units per kilogram on a dry-weight basis) are presented using Tukey box-and-whisker plots (n = 16).

productivity yields after 3 mushroom flushes. Working with *A. bisporus*, Schisler[15] found that substrate supplementation with olive oil and other edible oils increased crop yield mainly at the first mushroom flush, and in this case it was due to more fruiting bodies being formed.

Increased crop yields could be the result of the growth of more mycelial biomass caused by olive oil. Fatty acids, in particular oleic acid, have been linked to the growth-stimulation effect of olive oil.[13,14,23] This stimulation could be a consequence of an increment of the permeability of the hyphae, which would then facilitate better nutrient uptake.[14,16,24] This phenomenon could also explain the slight reduction in colony diameter observed on the MYSA medium and the longer time for spawn running produced by olive oil; the nutrients could be more readily available without the need for the hyphae to explore new fresh substrate.

The addition of 60 ppm Cu(II) in the substrate formulations significantly reduced the time needed for both spawn running and fruiting body development and maturation, and also increased mushroom yield at the first crop harvest. The shorter time to the first mushroom flush and a larger crop yield contributed to the observed increase in daily MP. Cu(II) has been found to stimulate both the production and the activity of fungal laccases.[25,26] It would be expected that enhanced lytic enzyme excretion and activity would increase nutrient availability and facilitate substrate colonization. Postemsky et al.[4] reported that Cu stimulated laccase activity at first flush in a *G. lucidum* rice straw SSF culture. The higher mycelial growth observed on the first flush could be the result of Cu laccase stimulation. However, it could also induce the depletion of nutrients and faster mycelium exhaustion. This becomes evident by an abrupt reduction in crop yield at the second and third flush harvests, lower substrate moisture content, and lower laccase activity after the third flush. In fact, laccase activity in the control was similar to that observed after the first flush obtained on a rice straw–based substrate.[4]

In general, the reduction of yield from the second to third mushroom flush was less marked than that from the first to the second flush. However, the water content of third-flush mushrooms was lower than those from the first and second flushes.

Triterpenoids are responsible for many of the medicinal properties reported for *G. lucidum*.

Substrate supplementation did not affect total triterpenoid content on the fruiting body. Total triterpenoid content was similar to that reported for different samples cultivated in China.[27] As previously reported by Gonzalez Matute et al.,[17] there was an increase in mushroom Cu content when it was added to the substrate. Cu is an essential micronutrient for human health.[28]

V. CONCLUSION

From an economic point of view, the use of a substrate formulation based on SSHs containing both olive oil and Cu would be cost-efficient for the production of *G. lucidum* fruiting bodies. Substrate supplementation with 60 ppm Cu(II) together with 1.5% olive oil increased the average daily productivity until the first mushroom flush up to 78% by greatly increasing the yield of the first mushroom flush and by reducing the time needed to achieve a harvest. It should be noted that 80% of the total production for this substrate formulation was obtained for the first flush in just 28 days. Furthermore, because Cu and olive oil did not change the total triterpenoid concentration in the fruiting bodies, an increase in crop yield implies an increase in total triterpenoid yield relative to substrate weight—from 0.7–0.8 to 1.0–1.1 g ursolic acid equivalent/kg dry substrate. Also, the fruiting bodies had the added value of increased Cu content, which is interesting from a medicinal and nutritional point of view.

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