PHYSICAL-CHEMICAL EVALUATION OF RESIDUES FROM THE SHIITAKE MUSHROOM PRODUCTION FOR THE SEEDLING GERMINATION IN NURSERY

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ABSTRACT

The substrates most utilized for the production of forest seedling in México are Peat and forest soil; however, the high cost of the forest production, creates the necessity and search for alternative substrates, which must be capable of promoting healthy and good quality plant. There are many byproducts of different productive processes that can be used as a substitute for peat in forest nurseries. The residues form the Shiitake mushroom production (*Lentinula edodes*) has not yet been characterized, therefore, its potential, as an alternative substrate remains unknown. It was conducted a physical and chemical characterization of the compost made from the residues of Shiitake mushroom production as well as an evaluation of the germination of *Pinus Pseudostrobus* at nursery. The compost showed a 4.37% content of N, 0.904% K, and 0.117% P, additionally it has a porosity of 30% among other physical and chemical properties. After 30 days of sowing, the plants that developed in the Shiitake compost 50%+vermiculite 25%+perlite 25%, presented a 92% of seedling, which was higher than the control treatment (Peat Moss 33%+vermiculite 33%+perlite 33%). From the results obtained, it is proposed that the substrate based on the compost from cultivation of Shiitake, allows a high germination rate contributing to the forestry sector.

Keywords: Shiitake mushroom (*Lentinula edodes*), alternative substrate, germinative capacity, *Pinus pseudostrobus*.

INTRODUCTION

Nowadays, humans have shown a tendency towards the consumption of healthy and good quality products, which furthermore, are processed in a way that generates less impact on the Agroecosystems. This has motivated producers to introduce crops under a sustainable management, where agroecological practices are incorporated, such as the use of byproducts from other productive processes, thus in this way, accomplish the recovery of residues and avoid pollution by the same [1, 2].

Within this context, the production of edible mushrooms has shown 11% of annual average increase in production during the last decades, principally due to its nutritional value and pharmaceutical constituents. Worldwide, the most cultivated and marketed species are champignon (*Agaricus* spp) with a production level of over two million metric tons annually. In second place is shiitake *(Lentinula* spp), with more than 1.5 million tons, followed by the production of seta (*Pleurotus* spp) with about 1 million tons per year. Mexico is the largest producer of edible mushrooms in Latin America, generating about 80.8% of total production in this region, with a 25.4% of growth during 1986 to 1997 [3, 4, 5].

Shiitake mushroom marketing has spread from Asian countries to Europe and America, mainly due to its excellent medicinal and nutritional properties. It is scientifically proven that its consumption provides certain benefits to human health because of their high content of protein, essential vitamins like B1, B2, B6, B12 and minerals, besides having anti-cancer, antiviral, antioxidant properties and activate the immune system [6, 7].

The production chain of edible mushrooms, including Shiitake mushroom, can be categorized of economic and social importance since their production levels are similar to conventional products [3]. Particularly, the Shiitake mushroom has had an important evolution in Mexico, reaching a significant production and therefore a considerable residue generation. The main residue generated from this crop have an organic character since the substrate for the development and growth of Shiitake mushroom entails the use of materials such as tree trunks, sawdust and other agricultural or agro-industrial by products available in the region [8].

Against this background, it is necessary to provide a suitable alternative for the use of wastes generated in the production of edible mushrooms; otherwise, it contributes greatly to environmental pollution. There have been accomplished different studies showing that residual substrates from the production of edible mushrooms, are materials that can have various applications in other productive processes such as organic fertilizer, nematicide and mainly as a substrate for bioremediation in situ of soils contaminated by aromatic hydrocarbons, pesticides and biphenyl polychlorinated compounds. This property of the residual substrate is attributed to the large amount of hydrolytic and oxidative enzymes that are release by the mushrooms for nutrient uptake. It's important to highlight that all organic amendments such as compost improve the physical, chemical and biological properties of nearly all soils; adjusting pH and improving fertility. However, despite knowing its nutritional properties either composted or not, there still have not been done studies that analyze their physicochemical characteristics of residual substrate from Shiitake production that may contribute to the cultivation of forest seedling [8, 4, 9].

Forest seedling production is an activity that has regained importance in recent decades, due to the need to restore the vegetation cover through reforestation activities. It is well known that sustainable ecosystems over time are those with greater abundance in biodiversity, as a number of synergisms that maintain their functionality are generated, that is where lies the interest in native forest seedling production in each region. In Mexico 94% of the production plant is located in forest nurseries, using a mixture of organic materials, mainly peat moss and inorganic materials such as perlite and vermiculite, increasing in a significant way the production costs in forest nurseries. Furthermore, soil forest has been used for the forest seedlings production, but it has been limited since it generates an undesirable environmental impact and plant diseases in forest production. For this reason there is a need to evaluate alternative substrates, which produce healthy seedlings, good quality and also reduce production costs in nursery [10, 11, 12, 13 and 14].

In recent years there have been studies in which the properties of different substrates using organic waste for the production of forest seedlings are evaluated. It is proven that the quality of the produced plant depends mainly on the substrate on which it grows and develops, specifically on its physical and chemical characteristics, since the operation of the roots is linked to the conditions of aeration, water content and delivery of necessary nutrients for their development and growth [10]. Studies show that the properties of certain products from production processes such as in the case of nutshell (*Juglans regia* L.) are ideal for the establishment of forest seedling, it was demonstrated that its use as an alternative substrate allows the production of healthy and good quality seedling [15]. Sawdust as a byproduct of the forest industry has also shown to have positive effects on the production of forest seedlings [16]. This research work had the aim to characterize the physical and chemical properties of the compost based on the residues of the Shiitake mushroom production and additionally, evaluate its characteristics as a substitute for commercial substrate Peat Moss in the germination of *Pinus pseudostrobus* (var Lindl. *pseudostrobu*s) in the nursery "Flor Del Bosque" located in Amozoc de Mota, Puebla-Mexico.

MATERIALS AND METHODS

Compost elaboration

The composting process was conducted at the Center for Agroecology Sciences Institute of the BUAP (ICUAP), its geographical coordinates are parallel 18º50'42 "and 19º13'48" north latitude, and meridians 98º 00'24 "and 98º 19'42" west longitude. The municipality has a humid temperate climate with summer rains (C-w1) with an average annual temperature between 12 ºC and 18 ºC [17, 18].

The residual substrates were obtained from the production plant of edible mushrooms "Nanacatlán" located in the city of Puebla, Mexico. The residues were placed in a black plastic container 3m x 8m. Later on, it was added 1 kg of biological activator dissolved in 10 L of water, which is based on yeast and actinomycetes and it was covered with black plastic to increase the temperature to 45°C. To keep the humidity at 60%, 3 irrigations per day and oscillatory movements each week were performed throughout the process. After three and a half months, a dark color, and pleasant smelling material was finally obtained, this was dried and sieved to obtain a homogeneous material used in the preparation of the treatments.

Physical-chemical analysis

The characterization of the compost based on residues of the mushroom production was carried out by the University Center for Bonding and Technology Transfer (CUV and TT) of the BUAP, using the methodology established in the Mexican Standards NOM-021-SEMARNAT-2000 and NOM-021-SEMARNAT-2002 [19, 20]. It was determined the macronutrients (N, P, K,) which are involved in plant development, as well as humidity retention, organic matter, cationic exchange capacity, apparent density, pH, electric conductivity and % of humidity.

Germination capacity

The germination test of *Pinus pseudostrobus* (var Lindl. *pseudostrobu*s) was carried out in the nursery "Flor Del Bosque" located in Amozoc Mota SEMARNAT, Puebla-Mexico. For this test, 3 treatments and a control were prepared; the proportions varied from 80% of compost until reaching the same amount used of Peat Moss which consists of 33% (Table-1).

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Table-1. Proportions of components to test using compost based on residues from the Shiitake mushroom production as a substitute for Peat Moss.

Components for the mixtures in %					
Treatments	**Shiitake compost**	**Peat Moss**	**Perlite**	**Vermiculite**	**Total**
T1*		33.3	33.3	33.3	100
T2	80		10	10	
T3	50		25	25	
T4	33.3		33.3	33.3	

* T1 = Control, mixture commonly used in "Flor del bosque" nursery seedbed for planting.

Sowing was done directly in plastic containers whose dimensions are 37.0; 34.0 and 18.4 cm in length, width and height, respectively; they were placed in trays made of polypropylene with 30% glass fiber. Each tray contains 49 cavities, where the seeds of *P. pseudostrobus* were placed, having in total 98 replicates per treatment. A maximum period of 30 days, considered sufficient for the germination process [21], was established. Irrigation was performed every third day; after this period the percentage of germination was evaluated for each treatment, taking as 100% the 98 repetitions for each treatment. It is important to highlight that during these 30 days, no fertilizers or agrochemicals to control diseases such as Damping off were added. Was used methodology [22] to determine differences between the treatments tested? The expression for the variable to be analyzed is:

Germinative capacity (GC) = (Ae x100)/M

Where

CG = Germinative capacity or final percentage of germination.
Ae = Accumulated germination until the last evaluation.
M = Evaluated sample, which corresponds to the total of sown seeds.

The statistical package SPSS Statistical 17 version (Statistical Package for the Social Sciences) was used to process the data obtained. Means comparison tests were accomplished ($p<0.05$), in order to determine significant differences between treatments. Variance analysis was obtained for each variable.

RESULTS AND DISCUSSIONS

Three important parameters for the production of compost were monitored during the composting process, in which the variability of temperature stands out. The highest temperature was recorded during the third week with a value of 42°C at noon. After four weeks the temperature began to decrease until values stabilize between 18-20°C. It´s important to mention that the temperature is closely linked to the presence of different microorganisms. It is considered that the temperatures in the composting process achieved in this investigation, remained in the mesophilic stage, which varies between 10 and 45°C [23].

Microorganisms able to grow in the compost based on residues from Shiitake mushroom production are mesophilic, which are characterized to be the most efficient for the decomposition of organic matter. On the other hand, temperatures between 35-45 °C are considered as ideal for the elimination of pathogens [24].

Physical-chemical characterization

An optimal substrate for the growth of seedling must have certain physical characteristics that facilitate their development. It´s important to know these characteristics otherwise, if they are not adequate from the beginning they can hardly be modified once the plantation is done [25, 10]. The considered parameters for the physical characterization are: humidity retention, apparent density, porosity and % of humidity. With the knowledge of these properties, we can determine the conditions of aeration, drainage, water retention and wet weight per volume present in the compost. The apparent density reflects compaction and ease of circulation of water and air. The results show a value lower than 1 g/cm^3 for the apparent density, which is within the optimum values of a good substrate [26]. According to [25], values for the porosity should not be less than 10%, because if so, the plant will not have enough ventilation especially when the sow is established in small containers, which is the case of forest nurseries using trays. It further states that the optimal value of air porosity is between 10% and 20%; compost from shiitake production has 30% of porosity which is due to the material used in the manufacture of substrates for mushroom production; 60% oak sawdust, 10% corn stover and 28.5% corn cob, which are generally very porous materials. As for humidity, a value of 37% was detected and 42.7% for water holding capacity, these values largely represent nutrient availability that is very important for the growth and development of seedlings once they have germinated [25, 27].

The chemical properties of a substrate can be modified along the plant development; the most common example is the use of fertilizers. Despite this, it is advisable to know them before the use of the substrate; otherwise if it does not meet the required characteristics we would commit the use of inputs representing an economic cost [10]. The pH of the compost is slightly alkaline, with a value of 8, this is due to the basic nature of the organic material that has undergone a process of degradation (sawdust oak), even being considered alkaline, no problems were detected during germination, as the seeds germinated before the period set as maximum (30 days). [28], reported a pH of 8.13 for different mixtures of organic materials and pine sawdust, these results are very similar to those obtained in this investigation.

The electrical conductivity (EC) is a measure of the concentration of dissolved salts in a growth substrate. EC values provide an idea of the amount of fertilizer that is available in the environment for the growth of plants or indicates whether accumulation of salts in it. [10], recommends a maximum value of 3dS / m for EC in substrates, [29] provides a suitable value between 2 to 4 dS / m for good quality compost, the value obtained for the compost in this investigation is 13.7 dS / m. Even though this value is significantly higher than those mentioned before and it is established that it may negatively interfere in the germination process or plant development, we did not detect issues during the germination, since it occurred before the established period of time. The presence of N, P, K greater than 1% on a substrate, determine the development of a healthy plant [10]. Nitrogen presence is indeed prevailing for the growth of plants, followed by phosphorus which is considered as vital nutrient to living beings for their growth and development since it can contribute in the absorption of other micronutrients and potassium [30, 31].

[32], mentions the following contents of macronutrients for different composts based on municipal waste and manure; 0.57-1.25% N, 0.03% P and 0.11-0.77% for K. For compost based on waste from the coffee production with manure, [24] shows values of 1.44% N, 0.5% P and 0.86% K. The results of this investigation are 4.37% for N, 0.117% P and 0.904% K, representing an advantage over peat with 0.84% N [10].

Organic materials have a high Cation Exchange Capacity (CEC), which relates to the availability of nutrients for plants. A substrate must contain a minimum of 30 meq/100g [33]. The compost studied has 4, 572 meq/100g, which ensures a reservoir of nutrients available for the development of seedlings [34].

The amount of organic matter in the compost is 45.8%, this value is within the average (25-70%) established for composting process [24], however this amount could have vary if the composting process had taken more time, since it is known that the sawdust (main component of the residues used for the compost) has a long term decomposition period [35]. Values ranging between 46-57% were obtained for the compost of sawdust mixed with manure in different proportions, which is similar to the results obtained in this investigation [34]. Table-2 shows the values obtained for each physicochemical parameter evaluated.

Table-2. Physico-chemical characterization of residues from the production of Shiitake mushroom (*Lentinula edodes*) at the end of the composting process.

Parameter	Analytical method	Units	Results
Humidity retention	NOM-021-SEMARNAT-2000 AS-06	%	42.7
Humidity	NOM-021-SEMARNAT-2002 AS-05	%	37
Apparent density	NOM-021-SEMARNAT-2002 AS-03	g/cm^3	<1,0
Porosity	Internal method	%	30
Total N	NOM-021-SEMARNAT-2000	%	4,37
Organic matter	NOM-021-SEMARNAT-2000 AS-07	%	45,8
Cation exchange capacity	NOM-021-SEMARNAT-2002 AS-13	meq/100g	4572
Electric conductivity	NOM-021-SEMARNAT-2002 AS-18	dS/m	13,7
Total P	EPA 6010 C- 2007	%	0,117
Total K	EPA 6010 C- 2007	%	0,904
pH	NOM-021-SEMARNAT-2002 AS-02	pH	8,0

g/cm^3= Grams per cubic centimeter, meq/100g= ppm of cation/(equivalent weight x 10), dS/m= decisiemens per meter. EPA= Determination by atomic emission spectrophotometry.

Germination test

At the end of 30 days from the date of planting on April 1 of this year the percentage of germination (CG) was evaluated for each treatment (Table-3), it was noted that treatment 3 (50% compost, 25% 25% perlite and vermiculite) had higher germination percentage followed by treatment 2 (80% compost, 10% perlite and 10% vermiculite) both exceeding the germinative capacity of the control treatment. These values are consistent with the timeframe established by Aparicio [36], which shows 80.5% germination for *Pinus pseudostrobus* at 18 days of planting. In addition to these values the final germination percentage is also consistent within the reported ranging from 80-95% [37].

Table-3. Germination percentage variable presented statistical differences between the treatments tested.

Treatments	**Germinative capacity** (GC) *	Substrate proportions %
T1	81.6 d	33-33-33
T2	89.7 c	80-10-10
T3	93.8 a	50-25-25
T4	92.8 b	33-33-33

T1= Control (Peat Moss, Vermiculite, Perlite), T2= Shiitake compost, Vermiculite, Perlite, T3= Shiitake compost, Vermiculite, Perlite, T4= Shiitake compost, Vermiculite, Perlite. * Different letters in the same column indicate significant differences with the multiple range test of Kramer Turkey (=0,05).

CONCLUSIONS

a) Compost obtained from waste Shiitake mushroom production has physical-chemical characteristics that allow it to be used as substrate in place of Peat Moss in forest seedling production.
b) Compost showed suitable content of N 4.37% and 0.904% K, Macroelements necessary for the development and growth of plants.
c) The greatest percentage of germination for *Pinus pseudostrobus* (var Lindl. *pseudostrobus*) was detected treatment 2: 50% Shiitake compost + 25% vermiculite + 25% perlite, with a 92% germination in the nursery.

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