PASTEURIZATION OF SUBSTRATE FOR GROWING *PLEUROTUS OSTREATUS* BY SELFHEATING

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ABSTRACT

Substrate to grow *Pleurotus ostreatus* must be pasteurized in order to be used for commercial production of Oyster mushrooms. The pasteurizing treatment normally involves steam application during a variable time/temperature procedure. In this research we studied an alternative treatment by forming a substrate compost pile with sufficient size and moisture to generate the necessary heat for pasteurization. A 1 m^3 wooden box with 80 kg dry Pangola grass *Digitaria decumbens* + 2% lime ($Ca(OH)_2$) was adjusted to 55, 60, 65 and 70% moisture. The composting treatment lasted 48 h; subsequently the substrate was cooled to ambient temperature and spawned. The treatment was efficient in preventing other organisms like flies, bacteria and competitor fungi. After two flushes of mushrooms, the biological efficiency was between 70.5 and 88.1% when compared to the control (same substrate steam pasteurized 90ºC, 1 h).

Keywords: Oyster mushroom cultivation; Substrate treatment; *Digitaria decumbens*; Composting

INTRODUCTION

Several treatments are used to prepare substrate for growing Oyster Mushrooms *Pleurotus* spp, principally pasteurization (steam, hot water), alkaline soaking and composting, among others [1, 2]. As part of a strategy to support small mushroom growers, the pasteurization process has been studied in order to replace traditional steaming pasteurization with a simpler and less costly treatment by avoiding energy expenses. For this purpose, we used the self-heating (reduced compost) method already tested by Villa et al. [3] and improved by Hernández *et al.* [4] and Barrios Espinoza *et al.* [5]. These authors reported that inducing a short composting process in a substrate pile rendered possible the use of self-generated heat to inhibit the development of non-desired organisms detrimental to *P. ostreatus* cultivation. In this study, we researched the effect of substrate moisture on substrate temperature profile during the pasteurization process. Subsequently, by using the best treatment we made supplementation tests to improve Oyster Mushroom production.

MATERIALS AND METHODS

Strain. *Pleurotus ostreatus* ECS-1123 from the fungal culture collection of El Colegio de la Frontera Sur was used since it is a strain with already tested commercial quality [5].

Substrate treatment. Self-heating: dry and coarsely ground Pangola grass *Digitaria decumbens* (80 kg, 3-5 cm long particle size) plus 2% lime ($Ca(OH)_2$ was subjected to an incomplete composting process (48 h long) as recommended by Barrios Espinosa *et al.* [5] in a $1m^3$ wooden crate. Substrate moisture was adjusted to 55, 60, 65 and 70% in order to determine the effect of moisture on the temperature profile of the pile. At 24 h incubation, the pile was tumbled and mixed once to provide aeration. At 48 h, mixing and aerating the substrate to a lower temperature (25ºC) stopped the composting process. The best treatment selected was the one that provided enough heat to the substrate during the process to reach at least 50ºC during no less than 10 h in any part of the pile. According to Overtjins [6] this time/temperature treatment should be sufficient to kill most bacteria and mushroom competitor microorganisms.

Steam pasteurization was done by providing the necessary steam to reach 90ºC during an hour inside a mass of 75 kg substrate (Pangola grass, 65% moisture and 2% lime).

Supplementation treatments. Soybean flour, ground cowpeas and wheat bran were used as supplements, according to Table 1. In all cases 6% supplement was added to the self-heating pasteurized substrate (65% moisture) at the time of spawning.

Table 1. Mixtures used to supplement the substrate (Pangola grass) after self-heating pasteurization, at the time of spawning *Pleurotus ostreatus* ECS-1123. Supplementation rate 6%.

Treatment number	Supplementation		
	Soybean*	Cowpeas	Wheat bran
T1	1	0	0
T2	0	1	0
T3	0	0	1
T4	0.67	0.17	0.17
T5	0.17	0.67	0.17
T6	0.17	0.17	0.67
T7	0	0.5	0.5
T8	0.5	0	0.5
T9	0.5	0.5	0
T10	0.33	0.33	0.33

* Numbers indicate fraction of 6% supplementation rate per treatment.

Cultivation. After pasteurization, the substrate was spawned at a rate of 3% dry weight basis with sorghum spawn [7]. The supplement was added by mixing simultaneously while spawning and then 3.0 kg of substrate was placed in polyethylene bags (30X40 cm) with 6 repetitions per treatment. Incubation lasted 17 days at 25ºC initially, although incubation temperature was changed as explained later. After full colonization, in order to induce fruit body formation the polyethylene bags were discarded and fresh and moist air was introduced to maintain 85-90% relative humidity and CO_2 content in the air below 800 ppm. The mushrooms were harvested at maturity, when the caps were fully extended and, just before the pileus border rolled up. Two flushes of mushrooms were obtained. The comparison steam/self-heating pasteurization was made between substrates pasteurized by one of those methods and without supplementing. Twenty bags per treatment were used in this case.

Sampling. From the center of the compost pile, 100g of substrate were taken at the beginning and at the end of the composting process to determine pH, moisture content and main contaminants.

Parameters. For pH, ten grams of substrate taken from the center of the compost pile was added to 90 ml distilled water and then agitated. pH value was measured with a pH/ISE Orion meter model 710A. For moisture determination in substrate**,** five grams of substrate were taken from the center of the pile and placed in a thermobalance Moisture analyzer A&D M.F. 50.

For temperature measurements, the readings were taken directly from the compost pile every three hours: three holes on the wooden box front side, situated at 15 cm below the upper substrate surface, at the center of the pile and 15 cm above the bottom level were used to insert 30 cm long bimetallic lab thermometers.

The Biological Efficiency (BE) was determined according to the formula:

$$BE = (X/S)*100$$

where X is the weight of harvested fruiting bodies and S the dry weight substrate

Microbial contamination was evaluated by two methods: 1) from the compost pile used for the evaluation of supplementation mixtures, 150 g of substrate was taken from the center of the pile at the end of the pasteurization process. Approximately 15 g sample was placed aseptically in each of 10 sterile Petri dishes. The plates containing the sample were incubated at 25ºC for 15 days to search for microbial contaminants. 2) all synthetic logs spawned (60 bags) were observed at the end of the incubation period (15 days after spawning) for contamination spots. The contaminated area for each bag was estimated (%), and then an average was calculated for each treatment (6 repetitions per treatment).

Experimental design and statistical analysis. Three compost piles were made for each moisture level reported and the mean temperature was used to monitor the temperature increase in the substrate (Fig.1). For the supplementation experiments, a randomized design with 10 treatments and 6 repetitions was used. An analysis of variance (ANOVA) and a mean separation by Tukey´s test were performed ($\propto$=0.05). The statistical package JMP 4.0 from SAS (SAS Institute Inc. Cary, NC, USA, 1998) was used.

RESULTS AND DISCUSSION

Compost crate temperature profile. Figure 1 shows the temperature profile of substrate with various moisture contents. The pile with 55% moisture (1a) presented the lowest temperature profile of all four treatments tested. It was observed there that the substrate upper layer did not reach 50ºC, the medium layer stabilized between 40-45ºC after 16 hours incubation and the lower layer stabilized around 35ºC and had presented a maximum of 40ºC after 60 h incubation. The pile with 60% moisture (1b) reached 55ºC in its substrate upper layer after 24 h incubation and maintained around that temperature until the end of the 48 h process; the medium layer showed very close temperatures to the upper layer and the lowest layer in this pile reached 50ºC

after 36 h incubation and maintained this temperature for 3-4 h, then decreased slowly. In the pile with 65% moisture (1c) the upper substrate layer reached 50ºC after 26 hours incubation, same as the medium layer, and the lower layer reached 50ºC after 34 h incubation and remained there up to the end of the composting process. The three layers of pile "d" (70% moisture, 1d) reached at least 50ºC in 40 h incubation but the lower layer stabilized around 45-48ºC after 40 h incubation.

Overtjins [6] indicated that a treatment at 46ºC during 1 h was enough to kill cecids; 50ºC during 10 minutes and 4 h prevented the development of bacterial blotch and cobweb respectively. He also pointed out that a few hours at 55ºC or 60ºC were necessary to kill other bacteria and fungi frequently encountered in white button mushroom compost. It should be mentioned that *Agaricus bisporus* is a mushroom needing a very selective substrate in order to grow optimally. However, *Pleurotus ostreatus* grows well on a less selective substrate with low simple carbohydrates, low nitrogen content, high lignin content and being slightly alkaline. That is why using a grass like Pangola + lime and a temperature of 50ºC during 10 hours may be enough to produce Oyster mushrooms.

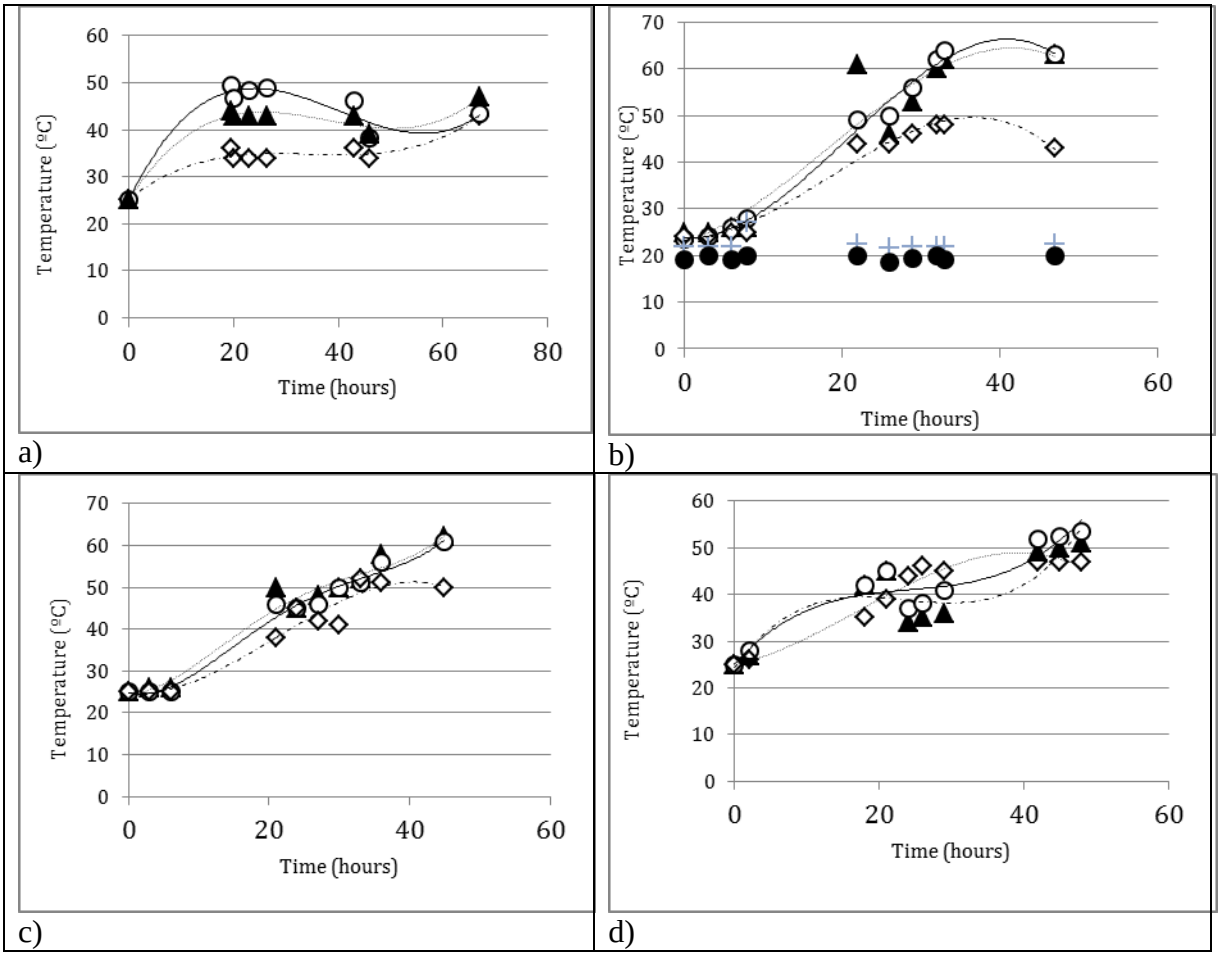

Figure 1. Temperature profile of 80 kg (d.m.) Pangola grass + 2% lime composted during 48 h in a 1m^3 wooden box, at four different moisture contents: a) 55%, b) 60%, c) 65%, d) 70%. Δ = upper layer; circle= medium layer; rhomb= lower layer; + = wet air temperature; black circle= dry air temperature.

According to the profile observed, and the need to avoid the growth of different wild organisms taken from the field in substrate (larvae and fly eggs, mites, other insects, nematodes, various fungi and bacteria), substrate piles with 60 and 65% moisture performed better since medium and upper layers reached temperatures above 50ºC in 24 h; however, only the 65% moisture pile allowed its lower layer to maintain 50ºC for 10 h. Thus the 65% moisture pile allowed a time/temperature treatment providing enough heat to kill or inhibit contaminant microorganisms. Certainly, the combined action of temperature and alkaline pH (initial pH was 9.5, data not shown) in the substrate after composting helped to inhibit most competitor organisms. The pH at the end of composting was slightly alkaline (7.5-8), allowing *P. ostreatus* spawn to develop in its selective substrate, while impeding the growth of other possible surviving fungi like *Trichoderma* spp. The absence of contaminant organisms was observed in all samples taken from the substrate and incubated in Petri plates: nor larvae nor adult flies nor pathogenic mitosporic fungi were found. Although these organisms were not added to the raw substrate, they normally come within the substrate from the field [2, 8]. On the other hand, looking at the temperature profile of each substrate layer it remains clear that substrate tumbling is important and necessary in order to aerate and provide a homogenous heat treatment to the substrate.

Self-heating against steam pasteurization. Substrates prepared by self-heating or steam pasteurization were compared by spawning strain ECS-1123 after pasteurization. The biological efficiency obtained after two harvests was 69.8±17.5% for the self-heating process and 75.6 ± 10.2% for the steam pasteurization. Statistical analysis did not find significant differences between these two treatments. These results confirm those obtained for the same strain by Barrios Espinoza et al. [5] whereby no difference between steam and self-heating pasteurization were obtained. However, these results differ from those obtained by Contreras et al. [2], as they found that steam pasteurization and alkaline disinfection were statistically better than self-heated compost, when using strain ECS-0152 of *Pleurotus ostreatus*. Although different strains were used in both cases, the contrast in findings may be due to the fact that Contreras at al., *op cit*. used a substrate with 70% moisture and a smaller crate (50x50x50 cm^3) for composting. Certainly, the temperatures attained inside the substrate were lower than those in the crate we used (1 m^3 crate, 65% moisture). Probably due to these two differences (moisture and mass), their pasteurization treatment became less effective (less self- generated heat).

Temperature profile in supplemented substrate during spawn running. A second trial was carried out to test the use of different supplementation mixtures (Table 2) in a self-heated substrate. After supplementing and spawning, all 3.0 kg substrate bags were incubated at 25ºC. The temperature at the center of each bag (Fig. 2) increased to reach after two days incubation, an average of 34ºC, and then decreased to 26ºC on the third day. This was followed by temperature increasing again and reaching an average of 32ºC. Because of this substrate temperature rise, the air temperature in the incubation room was set to 21ºC in order to avoid a further increase in substrate temperature resulting in the death of fungal mycelium. This change in air temperature allowed controlling substrate temperature, which was stabilized at approximately 25ºC by the end of the spawn run period. From these results it remains clear that temperature control is of utmost importance if substrate supplementation is required. This

conclusion confirms previous results [9, 10] indicating that supplementation increases yield but a temperature rise may occur during spawn run.

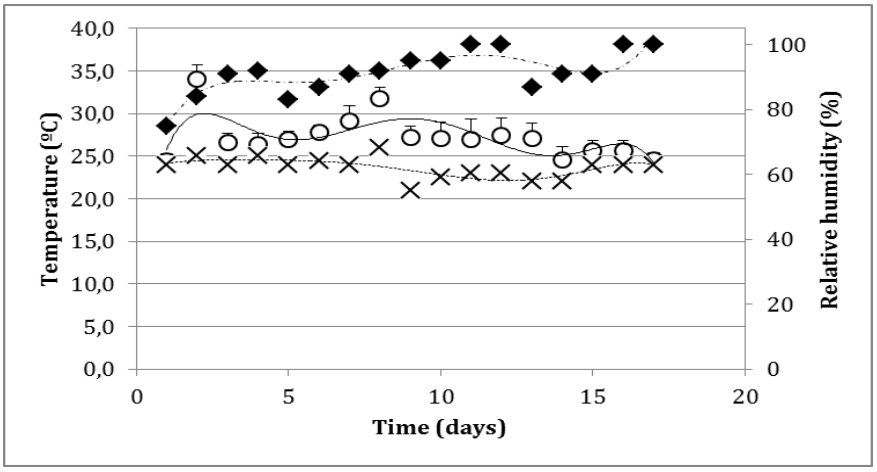

Figure 2. Average temperature profile of Pangola grass substrate supplemented with several mixtures of cowpeas, soybean meal and wheat bran (Table 1), during incubation at 25ºC (initially) then 21±1ºC. Mean of 10 treatments. Relative humidity (Rhomb), mean temperature of substrate at the center of bags (circle), air temperature (X).

Table 2. Presence of contaminated spots after colonization of substrate by *P. ostreatus* at 25ºC in each out of 6 repetitions spawned.

Treatment	Contamination (% of exposed area)						
	r_1	r_2	R_3	r_4	r_5	r_6	Mean (%)
1	0	0	0	0	25	0	4.1
2	0	5	0	0	0	0	0.8
3	0	0	0	0	0	0	0
4	0	0	0	0	0	2	0.3
5	0	0	5	0	0	0	0.8
6	1	0	0	0	0	2	0.5
7	0	1	0	0	0	0	0.2
8	2	25	0	0	0	0	4.5
9	2	0	0	0	0	0	0.3
10	0	2	0	0	0	0	0.3

Yield. Figure 3 shows the biological efficiency of *P. ostreatus* ECS-1123 grown on Pangola grass substrate pasteurized in a self-heated compost crate and supplemented with 10 different mixtures of soybean, wheat bran and ground cowpeas. Values varied between 70.5% (T_9) and 88.1% (T_4). The statistical analysis did not demonstrate any differences between treatments.

This result is lower than those reported [5] when cultivating the same strain on non-supplemented Pangola grass (110%). However, the authors of this report harvested three flushes of mushrooms while our results took only two flushes into account.

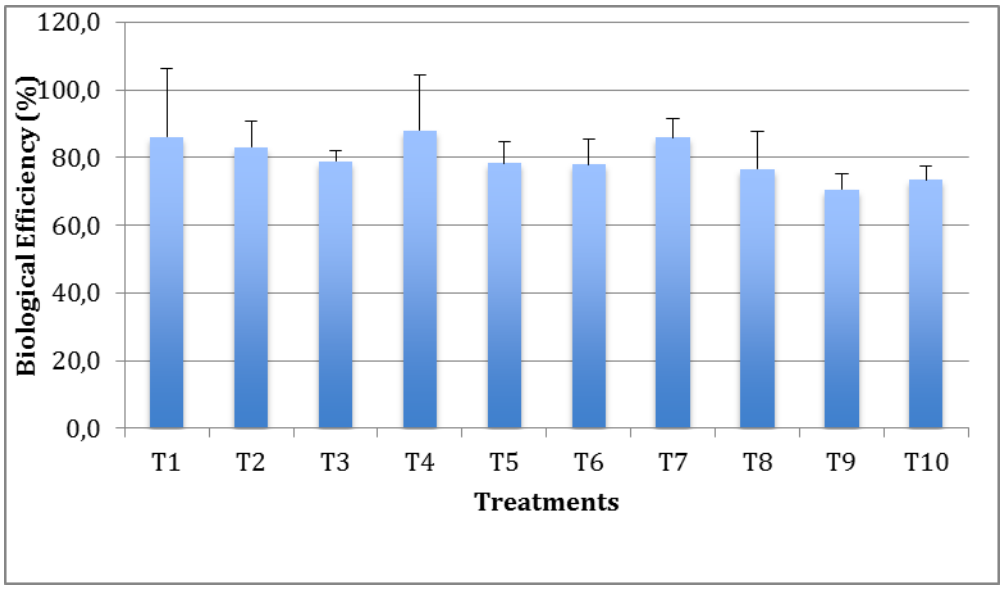

Figure 3. Biological efficiency after two flushes of *P. ostreatus* ECS-1123 on Pangola grass pasteurized by self-heating and supplemented with different mixtures of soybean, wheat bran and ground cowpeas. Incubation temperature 25ºC.

Contamination. After self-heating pasteurization, just prior to spawning, a substrate sample was aseptically taken and placed in ten Petri plates. The plates were incubated for 15 days at 25ºC to observe the appearance of microbial contaminants in the substrate. After the incubation period, it was observed that none of the plates showed presence of neither unsafe organisms nor microorganisms. The absence of contaminants or competitor fungi on the pasteurized Pangola grass suggested that at the place where the substrate sample was taken (the center of the substrate pile), the pasteurizing process was successful. It must be pointed out that temperature at the core of the compost pile (50 cm deep, in the middle of the crate) should be higher than the one reported by our thermometers (30 cm long). For future studies it would be helpful to take samples from other sectors of the compost pile, mainly near the bottom of the crate to evaluate the pasteurization treatment in the entire substrate. Table 2 shows the incidence of contaminants in substrate bags after substrate colonization by the mushroom, just prior to harvesting. Forty nine out of 60 bags spawned were totally free of contamination (82%), nine bags (15%) showed small spots with no mycelium growth in a range of 1-5% exposed surface and two bags (3.3%) were severely contaminated showing contamination spots of about 25% of exposed area. Regarding the exposed area, the total non-colonized area for all bags represented 11.8%. All but one bag showed bacterial contamination due to moisture excess in the substrate and only one bag (Treatment 1, r_5; 25% contamination) was contaminated by *Trichoderma* sp.
Based on the spreading pattern we could suggest that contamination should be decreased by a better substrate mixing to better homogenize moisture and by improving prophylactic measures after spawning.

The pasteurization method by self-heating discussed here has been successful in producing mushrooms. The use of Pangola grass as raw material with 65% moisture and 2% lime

[$Ca(OH)_2$] is suitable for self-pasteurization. The method did not decrease mushroom production and may well be considered as an ecological pasteurization treatment because it uses lime (an input allowed for organic growers) and its self-generated heat from the compost pile to provide a substrate thermo-treatment. It does not require an external energy source for pasteurization. However, the method is not yet ready to be recommended to mushroom growers: whether it is later commercially used or not remains unclear; nevertheless, it opens up new alternatives for future research. It must be further studied to confirm that after repeatedly use, certain thermophilic fungi and/or actinomycetes resistant to high temperature may affect production. On the other side, the use of more thermo-resistant materials to provide better insulation thus reducing heat losses during the process is another research alternative.

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REFERENCES

[1] Muez Ororbia MA & Pardo Núñez J. (2002). La preparación del sustrato. In: *La biología y el cultivo de Pleurotus spp.* Sánchez JE, Royse DJ. Eds. Uteha-Ecosur. Mexico D.F. 157-186.

[2] Contreras EP, et al. (2004). Soaking of substrate in alkaline water as a pretreatment for the cultivation of *Pleurotus ostreatus*. *J. Hort. Sci. Biotechnol*. 79(2): 234-240.

[3] Villa-Cruz V., et al. (1999). Solid fermentation of a corn cob-coffee pulp mixture for the cultivation of *Pleurotus ostreatus*. *Micol. Neotrop. Apl.*12:67-74

[4] Hernández D, et al. (2003). Composting a simple procedure for preparing substrate for cultivation of *Pleurotus ostreatus*. *Biores. Technol.* 90 (2):145-150.

[5] Barrios-Espinoza BM, et al. (2009). Composteo en cajones de madera como pretratamiento del sustrato para cultivar *Pleurotus ostreatus*. *Rev. Mex. Mic*.29:19-25. ISSN 0187-3180.

[6] Overtjins A. (1998). The conventional Phase II in trays and shelves. *Mush. J.* 584: 15-21.

[7] Quimio T. (2002) Preparación de semilla. In: *La Biología y el Cultivo de Pleurotus spp.* Sánchez JE, Royse DJ. Eds. Uteha-Ecosur. México, DF. 141-156.

[8] De León-Monzón JH, et al. (2004) El cultivo de *Pleurotus ostreatus* en los altos de Chiapas, México. *Rev. Mex. Mic.* 18, 31-38

[9] Lemke G. (1965). Kontrollmassnahmen beim champignonkultur verfahren nach Till. *Mush. Sci.* 6:393-402.

[10] Sinden JW, Schisler LC. (1962). Nutrient supplementation of mushroom compost at casing. *Mush. Sci.* 5:267-280.