

Full Length Research Paper

Evaluation of the effects of the combination of NPK fertilizer, cow dung, humus soil and poultry droppings with sawdust on the number of days to primordia formation, maturity and harvest of *Pleurotus ostreatus*

Onuoha C. I., Nwachukwu E. C*, Ezeibekwe I. O. and Nwagbara E. C.

Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State, Nigeria.

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This study on the effects of combination of NPK fertilizer, cowdung, humus soil and poultry droppings with sawdust on the number of days to primordia formation, maturity and harvest of *P. ostreatus* was carried out in the Department of Plant Science and Biotechnology, Imo State University, Owerri. The objectives of the study were to identify the most suitable substrate combination in the determination of the number of days to the formation of primordia, maturity and harvest of *P. ostreatus*. Seventeen (17) treatments were laid out in completely randomized design (CRD). The data collected were subjected to analysis of variance (ANOVA) and means were separated by Fisher's least significant difference (LSD). From the experiment, treatments T6, T0, T8, T1, T3, T4, T7 and T2 formed primordia in 49, 37.67, 35.67, 34.67, 34, 28, 26.33 and 17 days, respectively. The result further revealed the mean days for maturity and harvesting of *P. ostreatus* which were 52 days in T6 (400g sawdust + 100 g humus soil) followed by 37.67 days in T0 (sawdust alone), 35.67 days in T8 (300g sawdust + 200 g humus soil), 34.67 days in T1 (450 g sawdust + 50 g cow dung), 34 days in T3 (300g sawdust + 200 g cow dung), 28 days in T4 (300 g sawdust + 200 g cow dung), 26.33 days in T7 (350 g sawdust + 150 g humus soil) and 17 days in treatment two (T2) (400g sawdust + 100 g cow dung). The study further reveals a significant difference between the treatments and the control at 5% probability ($P = 0.05$).

Key words: *Pleurotus ostreatus*, primordia formation, maturity, harvest, substrates.

INTRODUCTION

Mushroom biology is the branch of mycology that deals with mushrooms. Etymologically, we say that mycology is the study of mushrooms, since mushrooms are fungi. Fungi are a large group of simple thallus-like achlorophyllous and heterotrophic thallophytes, made up of hyphae, which together constitute mycelium. They are

majorly cosmopolitan in distribution and found in all available habitats on earth where organic materials are present. Some species occur in fresh or marine water, others are terrestrial while some are airborne, with majority preferring to grow in dark, dim lighted moist habitat.

*Corresponding author. E-mail: ebygod87@yahoo.com

Mushroom is defined as a macrofungus with a distinctive fruiting body which can either be epigeous (growing on or close to the ground) or hypogeous (growing under the ground) (Chang and Miles, 1991). The fruiting bodies are large enough to be seen with naked eyes, and to be picked up by hand, and appear in different shapes and sizes.

Mushrooms are heterotrophs as they lack chlorophyll and cannot photosynthesize, but instead they take nutrients from outer sources. They are often found growing on dead wood and other decaying organic matter, and are rich in protein, vitamins and mineral salts (Singh et al., 2009; Sinha and Vashishta, 2005; Oei, 2003). Some mushrooms are edible while some are inedible. Edible mushrooms are a low calorie food usually eaten cooked or raw and as garnish to a meal. They are a good source of B vitamins, such as riboflavin, niacin and pantothenic acid, and the essential minerals (selenium, copper, potassium etc). Fat, carbohydrate and calorie contents of mushroom are low, with absence of vitamin C and sodium.

Pleurotus ostreatus is of great importance in Nigeria and many parts of the world including America, India, Asia, Australia, Japan, Cameroon, etc, because of its high nutritional value and high level of vitamins and proteins (Onuoha, 2007; Shah et al., 2004). It is cultivated worldwide for food, as they are often used in preparing continental dishes, stews, soups, sauces, sandwiches, burgers and salads (Chang and Chiu, 1992). The medicinal values of mushrooms have long been recognized in China, Korea, Japan, India and partly in Nigeria. The mushroom, *P. ostreatus* not only serve as vegetables (food), but also produces several bioactive compounds that are usually associated with the cell wall and are of medicinal value including being used as complementary medicine/dietary supplements for anticancer, antiviral, antitumor, anti-hypertensive, antimicrobial, immunopotentiating, hypocholesterolaemic and hepatoprotective agents (Chang and Buswell, 1996; Gregori et al., 2007). *P. ostreatus* contains lovastatin that helps in reduction of cholesterol in man, thus making it suitable for patients with high blood pressure, heart diseases and diabetes (Gunde and Cimerman, 1995; Eger et al., 1976). It also contains lectins which are glycoproteins and have been shown to have anti-tumor and immunomodulatory activities (Wang et al., 1996).

Mushrooms in recent years are used as bioconversion of agricultural and industrial wastes into food has attracted the world attention. Mushrooms like *P. ostreatus* are either harvested wildly or cultivated by individuals in the laboratory, homes or farmland, as such mushroom cultivation is environment friendly, having beneficial impacts such as reduction of environmental pollution, biogas and biofertilizer production, bioremediation/mycoremediation, mycofiltration, mycopesticides and mycoforestry. This study is therefore designed to assess the efforts of nutrient combination on the numbers of days to

primordia formation, maturity and harvest of *P. ostreatus*.

MATERIALS AND METHODS

Source of materials

The spawn used in this work was obtained from the Department of Plant Science and Biotechnology of the University of Port Harcourt in Rivers State. Fresh hard wood sawdust was gotten from the Njoku and Sons Wood Mill in Owerri, Imo State, two months old cow dung was gotten from the relief veterinary centre along Egbu road in Owerri, two months old poultry droppings was collected from the Onyekwere's poultry farm, Egbu in Owerri North Local government Area of Imo State. NPK fertilizer was bought at the Eke Ukwu market, Owerri while the humus soil was gotten from the university farm.

Preparation and sterilization of substrates

To a heap of sawdust on a cement platform, water was added in the ratio of 1:2 (v/v) and mixed thoroughly, the substrate was then piled up into a heap of 11:3 m high by 1.2 m diameter, covered with a black plastic polyethylene sheet to undergo fermentation for four (4) weeks, with regular turning. After four weeks, the fermented sawdust was measured, mixed with 1% lime dust and the other substrates were added indigenously, due to the presence of the animal droppings, the mixed substrates were given another two (2) weeks to reach a uniform temperature of 28°C. The treatments include: T0: 500 g fermented sawdust (Control); T1: Mixture of 450 g fermented sawdust and 50 g of cow dung; T2: Mixture of 400 g fermented sawdust and 100 g of cow dung; T3: Mixture of 350 g fermented sawdust and 150 g of cow dung; T4: Mixture of 300 g fermented sawdust and 200 g of cow dung; T5: Mixture of 450 g fermented sawdust and 50 g of humus soil; T6: Mixture of 400 g fermented sawdust and 100 g of humus soil; T7: Mixture of 350 g fermented sawdust and 150 g of humus soil; T8: Mixture of 300 g fermented sawdust and 200 g of humus soil; T9: Mixture of 450 g fermented sawdust and 50 g of NPK; T10: Mixture of 400 g fermented sawdust and 100 g of NPK; T11: Mixture of 350 g fermented sawdust and 150 g of NPK; T12: Mixture of 300 g fermented sawdust and 200 g of NPK; T13: Mixture of 450 g fermented sawdust and 50 g poultry droppings; T14: Mixture of 400 g fermented sawdust and 100 g poultry droppings; T15: Mixture of 350 g fermented sawdust and 150 g poultry droppings; T16: Mixture of 300 g fermented sawdust and 200 g poultry droppings.

Bagging and pasteurisation

Five hundred grams (500 g) of the treatments were measured into polypropylene plastic bags (8 cm high x 18 cm width). The concentrations of the treatments used are 50, 100, 150, 200 g, produced in three replicates. Each bag was watered thoroughly, properly sealed, and sterilized at 100°C at 121psi for five (5) hours, using a pressure pot. After the sterilization, the substrates were left to cool for 48 h to enable them cool to an ambient temperature (30°C), so that the substrates containing the animal droppings could stabilize and be uniform with the other substrates.

Inoculation and incubation

Having reached a uniform temperature of 28°C, the bags were inoculated with the spawns of the *P. ostreatus* mushroom, at the rate of twenty grams (20 g) per bag, (plugged with cotton wool and



Plate 1. a and b showing primordia of *P. ostreatus* on cowdung substrate.

banded to keep it sealed and air proof), all processes were done under aseptic condition, then the bags were kept in a dark room at a temperature of $(25 \pm 2^\circ\text{C})$ and watered very properly and regularly, to create a very humid environment for mycelium colonization. After about 30 days, the bags were transferred to the main laboratory (the cropping area) and opened to monitor the rate at which the mycelia had colonized the bags, and then they were for fructification (sporulation). The growing area and the substrate bags were slightly watered daily to keep them always relatively humid (RH) in the morning and evening during cropping.

Experimental layout

All the treatments for the experiment were laid out, and arranged in a completely randomized block (CRD) design, and each treatment was set up in three replicates. For each treatment, there were 3 replicate bags, making up a total of sixty (60) bags in the experimental block. The total number of days to primordia appearance was calculated and noted. Also, calculated is the number of days for maturity and harvesting of the *P. ostreatus*.

RESULTS

The outcome of the experiment showed that all the treatments had mycelial colonization after 30 days in varied proportions, except those of NPK and poultry droppings lost with mycelia before maturity. Furthermore, it showed that the highest mean number of days to primordia formation was recorded in 400 g of sawdust and 100 g of humus soil (49 days) and it is significantly different from all other treatments while the least mean number of days recorded in 400 g of sawdust and 100 g of cowdung (16.33 days) is significantly different from the

highest and all other treatments. 32.67 days was gotten for 450 g of sawdust and 50 g of cowdung, 32 days for 350 g of sawdust and 150 g of cowdung, while 36 days were gotten for sawdust alone which acted as the control. Furthermore, 26.33 days were gotten for 300 g of sawdust plus 200 g of cowdung, 24 days for 350 g of sawdust and 150 g of humus soil and 27 days for 300 g of sawdust and 200 g of humus soil. Other treatments did not form any primordia as shown in Plate 1, Table 1 and Figure 1.

The result further shows the highest mean number of days reached for maturity and harvest is 52 days recorded in 400 g of sawdust plus 100 g of humus soil is significantly different from the mean number of days in 400 g of sawdust plus 100 g cow dung which is 17 days, and from the other treatment with mean number of days as 500 g of sawdust (37.67 days) of equal significance, which in turn are significantly different from that of 300 g of sawdust + 200 g of cow dung (28 days) at $P < 0.05$. Also, 35.67 days were gotten for 300 g sawdust + 200 g of humus soil while 34 days was recorded for treatment three (T3) which is 350 g of sawdust + 150 g of cow dung as shown in Table 2, Plate 2 and Figure 2.

According to the results, treatment T5 did not produce fruiting bodies, this development could be attributed to environmental factor, dryness, imbalance of the mixtures used (Ayodele and Okhunya, 2007).

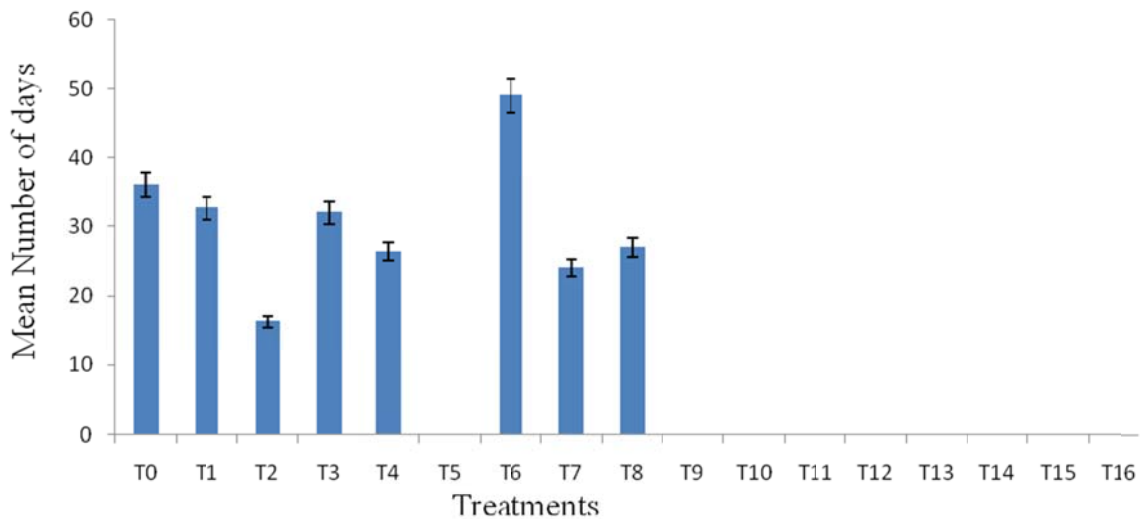
DISCUSSION

The result of this research work has revealed that growth

Table 1. Effect of combination of NPK, cow dung, humus soil and poultry droppings on the number of days to primordia formation.

Treatment (T)	Mean number of days to primordia formation
500 g sawdust (control) (T0)	36.00 ^{ab}
450 g Sawdust + 50 g Cow dung (T1)	32.67 ^{ab}
400 g Sawdust + 100 g Cow dung (T2)	16.33 ^{bc}
350 g Sawdust + 150 g Cow dung (T3)	32.00 ^{ab}
300 g Sawdust + 200 g Cow dung (T4)	26.33 ^{abc}
450 g Sawdust + 50 g Humus soil (T5)	0.00 ^c
400 g Sawdust + 100 g Humus soil (T6)	49.00 ^a
350 g Sawdust + 150 g Humus soil (T7)	24.00 ^{abc}
300 g Sawdust + 200 g Humus soil (T8)	27.00 ^{abc}
450 g Sawdust + 50 g N.P.K (T9)	0.00 ^c
400 g Sawdust + 100 g N.P.K (T10)	0.00 ^c
350 g Sawdust + 150 g N.P.K (T11)	0.00 ^c
300 g Sawdust + 200 g N.P.K (T12)	0.00 ^c
450 g Sawdust + 50 g Poultry dropping (T13)	0.00 ^c
400 g Sawdust + 100 g Poultry dropping (T14)	0.00 ^c
350 g Sawdust + 150 g Poultry dropping (T15)	0.00 ^c
300 g Sawdust + 200 g Poultry dropping (T16)	0.00 ^c
LSD Value	28.96

Each value is a mean of 3 replicates. Means in the same column, with the same letter (s) are not significantly different at $P < 0.05$.

**Figure 1.** Effect of combination of NPK, cow dung, humus soil and poultry droppings on the number of days to Primordia formation.

of *P. ostreatus* was supported by 500 g of sawdust, 450 g sawdust + 50 g cowdung, 400 g sawdust + 100 g cowdung, 350 g sawdust + 150 g cowdung, 300 g sawdust + 200 g cowdung, 400 g sawdust + 100 g humus soil, 350 g sawdust + 150 g humus soil and 300 g sawdust + 200 g humus soil. This can be attributed to the fact that

cowdung contained the essential nutrients needed by the mushroom to grow properly. This is in line with the works of Zadrazil (1980) who reported that the growth of *Pleurotus* species is favoured on substrates low in nitrogen content and this also is the reason why all the combinations containing NPK fertilizer did not support the

Table 2. Effect of combination OF NPK, cow dung, humus soil and poultry droppings on the number of days to maturity and harvest.

Treatment (T)	Mean Number of days to Maturity & Harvest
500 g sawdust (control) (T0)	37.00 ^{ab}
450 g Sawdust + 50 g cow dung (T1)	34.67 ^{ab}
400 g Sawdust + 100 g cow dung (T2)	17.00 ^{bc}
350 g Sawdust + 150 g cow dung (T3)	34.00 ^{ab}
300 g Sawdust + 200 g cow dung (T4)	28.00 ^{abc}
450 g Sawdust + 50 g humus soil (T5)	0.00 ^c
400 g Sawdust + 100 g humus soil (T6)	52.00 ^a
350 g Sawdust + 150 g humus soil (T7)	26.33 ^{abc}
300 g Sawdust + 200 g humus soil (T8)	35.67 ^{ab}
450 g Sawdust + 50 g N.P.K (T9)	0.00 ^c
400 g Sawdust + 100 g N.P.K (T10)	0.00 ^c
350 g Sawdust + 150 g N.P.K (T11)	0.00 ^c
300 g Sawdust + 200 g N.P.K (T12)	0.00 ^c
450 g Sawdust + 50 g poultry dropping (T13)	0.00 ^c
400 g Sawdust + 100 g poultry dropping (T14)	0.00 ^c
350 g Sawdust + 150 g poultry dropping (T15)	0.00 ^c
300 g Sawdust + 200 g poultry dropping (T16)	0.00 ^c
LSD VALUE	31.56

Each value is a mean of 3 replicates. Means in the same column, with the same letter (s) are not significantly different at $P < 0.05$.



Plate 2. Matured *P. ostreatus* ready for harvest.

growth of *P. ostreatus*. Shah et al. (2004) reported that *P. ostreatus* gave maximum biological efficiency on saw-

dust. This also supported the result of this study as sawdust equally supported the growth of the mushroom

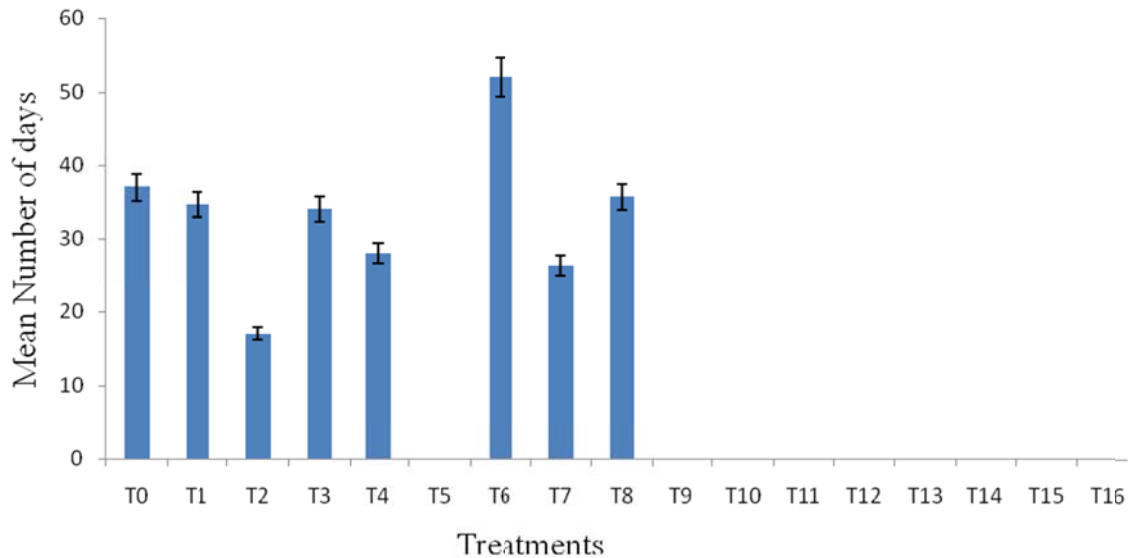


Figure 2. Effect of combination of NPK, cowdung, humus soil and poultry droppings on the number of days to maturity and harvest.

when used alone. This confirmed the works of Candy (1990) who grew mushrooms on different pure sawdust types and obtained the best result from Eucalyptus sawdust.

The results further showed that all possible combinations with poultry droppings and NPK fertilizer did not show any growth signs. This could be attributed to the fact that poultry droppings produced heat and there is every likelihood that the heat produced by the treatment might have destroyed the nutrients needed by the *P. ostreatus*. Also, NPK fertilizer combinations yielded no growth as the NPK on its own may have burnt up the spawn due to its corrosive nature or probably that the concentrations of the treatments were too much or too little for the normal growth of the mushroom. Their combinations might change the sequence of decomposition of the substrate components as reported by (Ayodele and Okhunya, 2007).

Shah et al. (2004) and Ponmurugan et al. (2007) reported that full colonization in *P. ostreatus* takes 17-20 days on different substrates thus supporting the result gotten from 400 g sawdust + 100 g cow dung which took 16.33 days to attain full colonization, while other results gotten from 500 g sawdust, 450 g sawdust + 50 g cow dung, 350 g sawdust = 150 g cow dung, 300 g sawdust + 200 g cow dung etc took longer days for full colonization. This is in line with the works of Royse et al. (2004), Oseni et al. (2012), Khare et al. (2010) and Mane et al. (2007). The production of enzymes such as cellulases, hemicellulases and lignases by fungal mycelium, is a crucial part of the colonization process and thus this is important in determining mushroom growth and maturity (Buswell et al., 1996). This might be the reason for the longer days for maturity of *P. ostreatus* as shown by the

results. From the results, it was revealed that NPK fertilizer and poultry droppings are not good substrate combination for the growth and maturity of *P. ostreatus* as they showed no growth of the mushroom.

Conclusion

The study reveals that the best substrate combination which have least number of days for the formation of primordia, maturity and harvest is 400 g sawdust + 100 g cow dung followed by 350 g sawdust + 150 g humus soil as they produced in the least number of days, thereby reducing the longer days it takes for the maturity of the mushroom. Furthermore, mushroom growers should utilize more of the cow dung and humus soil in combination with sawdust, than cultivation with only sawdust since they are economically feasible and available year round.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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