GROWTH AND YIELD RESPONSES BY THE SCLEROTIA OF *PLEUROTUS TUBERREGIUM* (FR.) SING. TO DIFFERENT LEVELS OF N, P, K AND NPK FERTILIZERS IN SOIL

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RESUMEN

CRECIMIENTO Y RENDIMIENTO DE ESCLEROCIOS DE *PLEUROTUS TUBERREGIUM* (FR.) SING. AL FERTILIZAR EL SUELO CON DIFERENTES NIVELES DE N, P, K Y NPK. Rev. Mex. Mic. 15: 17-21 (1999). Se estudio el efecto de la fertilización del suelo con diferentes niveles de sulfato de amonio (21% N), superfosfato simple (20.5% P_2O_5), cloruro de potasio (60% K_2O) y NPK 15-15-15, sobre el desarrollo y producción de esporóforos a partir de esclerocios del hongo comestible tropical, *Pleurotus tuberregium*. Al añadir potasio se obtuvieron los mejores desarrollos y rendimientos. El nitrógeno también aumentó el rendimiento y favoreció mayores estípites y diámetros de píleo, mientras que el fósforo resulto en estípites mas largos. La fertilización con NPK 15-15-15 a 20 Kg/ha resultó en rendimientos significativamente mayores respecto al control. Al aplicar fósforo se obtuvo la menor relación píleus/stipite, es decir los esporóforos fueron mas vigorosos y de mayor tamaño, mientras que este parámetro fue mayor en el control, es decir cuerpos fructíferos mas pequeños.

Palabras clave: *Pleurotus tuberregium*, esclerosios, producción, fertilización

ABSTRACT

A study was carried out to determine the effect of the fertilizers, ammonium sulfate (21% N), single superphosphate (20.5% P_2O_5), potassium chloride (60% K_2O) and NPK 15-15-15, on the growth and sporophore yield from sclerotia of the tropical edible mushroom, *Pleurotus tuberregium*. In this study, potassium proved to be the most efficient mineral amendment in enhancing the growth of the fungus, producing the highest yield. Nitrogen also increased yield and encouraged stipe and pileus diameter, while phosphorus improved stipe length. NPK 15-15-15 fertilizer application at 20 Kg/ha resulted in significant difference in yield, when compared with the control. Pileus/stipe ratio was the lowest with application of phosphorus indicating more vigorous and tall sporophores, while it was the highest with the control, which showed short fruit bodies.

Key words: *Pleurotus tuberregium*, sclerotia. growth effect, fertilizers.

Introduction

Edible mushrooms abound in Africa in the wild state. However, very little effort has been made to study and cultivate them commercially. In other continents like America, Europe and Asia, the cultivation of edible mushrooms is quite common and it has developed into a multimillion-dollar industry (Chang, 1996). *Pleurotus tuberregium* (Fr.) Sing. is a basidiomycete found in the tropical and sub-tropical regions of the world (Isikhuemhen *et al.*, 2000; Zoberi, 1973). Usually, it forms a large spherical to ovoid subterranean sclerotium, sometimes up to 30 cm or more in diameter. It forms a true sclerotium and the color on the outside can vary between very light to dark brown, depending on the variety and the substrate on which it was formed, but it is usually white on the inside. *P. tuberregium* is different from all other *Pleurotus* species in its non-pleurotoid habit (Isikhuemhen *et al.*, 2000; Oso, 1977; Isikhuemhen & Nerud, 1999). It colonizes dead wood, where it produces the sclerotium either buried within the tissue of the decaying log or on the surface, bulging out and taking one of its characteristic shapes (Okhuoya *et al*, 1998; Isikhuemhen & Okhuoya, 1996). Sometimes when it grows into the underground roots of a dead tree, its sclerotium can form underground and become buried in the soil (Isikhuemhen *et al.*, 2000; Isikhuemhen & Okhuoya, 1996; Oso, 1977). If given a warm humid condition, the sclerotium continues to produce fruit bodies, one crop after another, over a period whose length depends on the size of the sclerotium (Oso, 1977). The fungus is of great importance throughout Western Africa. Both the sclerotium and the mushrooms grown from it are eaten as food. Also the sclerotium is used in combination with other herbs, in curing ailments such as headache, stomach pain, fever and cold. In some parts of Nigeria, native doctors use the sclerotium for medicinal preparation that is given to pregnant women to aid foetal development (Oso, 1977; Isikhuemhen & Okhuoya, 1995). In Ghana, it is also used as medicine for illness that relates to malnutrition and anemia in children.

In Nigeria, the sclerotium of *P. tuberregium* is usually buried in the soil in a cool, moist place for the production of sporophores which occurs one flush after another. This practice that Kurtzman and Zadrazil (1982) said could be considered the most primitive form of mushroom cultivation is as old as the Nigerian people and dates back many centuries for which records are not available (Okhuoya & Isikhuemhen, 1999). However, the knowledge and method that the local people in Nigeria always use to induce mushrooms from the sclerotia of this fungus and to maintain it in a condition that allows development of fruit bodies to maturity is more than being a primitive knowledge. We see it as basic knowledge to which further information can be added to make it usable in commercial production systems. To this end we have undertaken many studies on this fungus to enable exploitation of the potential of this fungus under commercial cultivation set up vis-à-vis what the Nigerians have been doing for many centuries past. The aim of this study was to investigate the effect of various fertilizers on the growth and development of this fungus when sporophore induction from sclerotia is done in soil.

Materials and methods

River sand was collected from Ogba River in Benin City and washed under running tap water for 30 minutes to remove plant and animal debris before use. One kilogram of the soil was put in a tin can (10.5 cm diameter and 12 cm in height); a total of 75 such tin cans were made. Three of these were to serve as controls. The fertilizers used were ammonium sulfate (21% N), single calcium superphosphate (20.5% P_2O_5), potassium chloride (60 % K_2O) and an NPK 15-15-15 (compound fertilizer). The rates used for all the fertilizers were 10, 20, 30, 40, 50 and 60 kg/ha of $NH_4(SO_4)_2$, P_2O_5 and K_2O. In determining the amount (mg) of each fertilizer equivalent to the rates used, the following formula was used: V/10000 x 8375/1. V/10000 is the rate (Kg/ha), V could be 10, 0, 30, 40 50 and 60 and 8375 is the volume of water (ml) whose equivalent in quantity of sand was used for mixing different rates of each fertilizer. Each of these rates was applied in three replicates. The fertilizers were thoroughly mixed with the sand before each can was seeded with a 25 g piece of sclerotium at a depth 3 cm below the soil surface. The controls had no fertilizers but were also seeded with sclerotia pieces as above.

The experiment was conducted outside in the shade. The cans were watered twice daily, with care to avoid leaching due to over-watering. The following data were recorded as the sclerotia started producing sporophores: time of emergence of fruit bodies, fresh weight of harvested fruit bodies, and stipe length and stipe and pileus diameter. Pileus/stipe ratio was then calculated. Values obtained for sporophore yield and stipe/pileus ratio were compared with their respective controls by using Student's t-test.

Results and discussion

In the control, where no fertilizer was applied, the average sporophore yield was 13.0 and pileus/stipe ratio was 4.0. These fruiting bodies that appeared like dwarfs when compared with those treated with the different fertilizers showed a very high pileus/stipe ratio. Ammonium sulfate application (21% N) increased yields significantly. The greatest yield was from 20 and 60 kg/ha (23.9 and 22.0 respectively, see Table 1). Also, N application was most effective in enhancing the stipe and pileus diameter of the fruit bodies. This effect was highest at 60 Kg/ha level of application giving 4.1 and 11.2 cm as stipe height and pileus diameter respectively. The average time of emergence in N treatment was second to the application of potassium, see Figure 1. However, it did not increase stipe-length growth as much as the other fertilizers, hence its pileus/stipe ratio was higher.

Although phosphate application as single calcium superphosphate (20.5 % P_2O_5), did not result in a significant yield increase, as compared with the control, it emerged as the fertilizer, which most enhanced the stipe length, indicative of vigorous growth. However, differences between rate treatment show an increase in yield. It also resulted in a low and consistent pileus/stipe ratio (Table 2).

Table 1. Average yield of *P. tuberregium* using different rates of fertilizers.

	Average yield (g fresh weight)			
Kg/ha	N	P	K	NPK
10	16.8^{a}	18.3^{a}	13.7^{a}	20.3^{b}
20	23.9^{d}*	21.0^{b}	22.8^{c}*	22.8^{c}*
30	20.4^{b}	19.9^{b}	18.3^{b}	19.1^{a}
40	17.8^{a}	19.0^{b}	25.5^{e}*	18.5^{a}
50	17.8^{a}	18.3^{a}	23.1^{d}*	17.5^{a}
60	22.0^{c}	17.2^{a}	20.5^{c}*	17.1^{a}
Control = 13.0				

+ = Average yields in the same column followed by different superscripts are statistically different at $p<0.05$

* = Statistically different from control at $p<0.05$.

Table 2. Average pileus/stipe ratio of *P. tuberregium* using different rates of fertilizers.

	Average pelius/stipe ratio			
Kg/ha	N	P	K	NPK
10	2.0a	2.0a	2.0a	2.0a
20	2.7c	2.0a	2.7b	2.7c
30	2.8c	2.0a	1.9a	2.4b
40	2.0a	2.0a	2.0a	1.8a
50	2.5b	2.6b	2.0a	2.2a
60	2.9c	2.0a	2.6b	1.7a
Control = 4.0				

Values under the same column with different superscripts are statistically different at $P<0.05$.

Potassium chloride application (60 % K_2O) at 20, 40 and 50 kg/ha resulted in the highest increase in yield compared with the control (Table 1). The time of emergence was the most rapid with this fertilizer, 16 and 13 days at 20 and 40 Kg/ha respectively (Figure 1). It also resulted in a low pileus/stipe ratio.

Application of NPK (15-15-15) at 20, kg/ha resulted in a significant increase in growth compared with the control. Also, the time of emergence was most delayed with this fertilizer (Figure 1). However, the stipe length growth was greater than the control. In the controls, the fruit bodies were dwarfs resulting in a high pileus/stipe ratio (Table 2).

Nitrogen application enhanced fruit body yields (23.9 g at 20 Kg/ha) more than other fertilizers did, except potassium. Nitrogen enhances dry matter content of plants generally and it is the most soluble nutrient element in the soil, the most readily available to the crops and the most readily leached (John *et al.*, 1971). The ease of absorption and translocation of

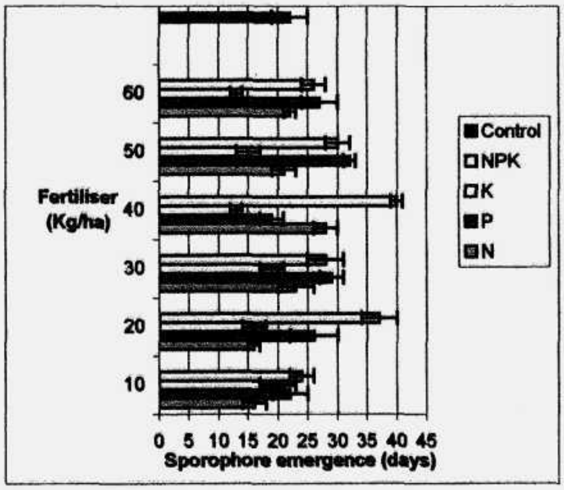

Figure 1. Effect of various fertilizers on sporophore emergence (days) in *P. tuberregium*

this nutrient by mycelia into the sclerotium used as inoculum perhaps affected the physiological and metabolic activities positively. This can then lead to earlier sporophore induction and development from the sclerotium than did the application of P or NPK 15-15-15 fertilizers; and early emergence is one of the parameters for good mushroom cultivation (Kurtzman & Zadrazil, 1982). Nitrogen application also enhanced the stipe girth growth. This observation is similar to experiments with higher plants like rubber seedling (Onuwaje & Uzu, 1982). However, it did not increase the pileus/stipe ratio, as other fertilizers did.

That the amendment with phosphate did not increase the yield significantly as compared to the control (Table 1), was perhaps due to its large granular size and insolubility, which in turn is related to its effectiveness as determined by its placement pattern in the soil. Nonetheless, phosphorus fertilizer was the most effective in enhancing growth of the stipe length, as shown by the low pileus/stipe ratio obtained (Table 2); we could only assume that some of this fertilizer probably went into solution and got absorbed, resulting in the observed effect on stipe growth. Precisely, the effect of this fertilizer cannot be unequivocally ascribed to the phosphate component of the fertilizer. This is because calcium is also present in this fertilizer. Also, when the cation and anion making up a salt go into solution, they can affect the physical environment like pH and other factors, which may determine the availability and the effect either the anion or cation may have on the fungus (Kurtzman & Zadrazil, 1982).

Potassium chloride application increased yield of the fungus significantly (Table 1) and resulted in the shortest time of emergence compared to other fertilizers. Characteristics such as its small granular size, high solubility, non-readily leached, and its "luxury consumption" in the soil, which this nutrient possesses (Stanley, *et al.*, 1971), may be in part responsible for its best performance in the growth of *P. tuberregium*. The results with NPK 15-15-15 are consistent with the results of other workers, who relate it to its insolubility due to the large granular size of the fertilizer and poor placement pattern in the soil (Grant, 1970; Philips & Webb, 1971). However, this fertilizer encouraged the stipe length growth, and hence a low pileus/stipe ratio.

The Pileus/stipe ratio as observed in the control in this experiment is slightly higher than what is usually found in the wild, where sporophores are growing out of sclerotia that are either sitting on top or buried in decaying logs. It is reported (Oso, 1975; 1977) that it can be found naturally buried in the soil in Nigeria, and when growing from sclerotia in Northern Australia and Papua New Guinea, such sclerotia are usually buried in the soil (Vilgalys, personal communication). We have never come across sporophores growing out of sclerotia buried in the soil in our field studies, so we could not compare the usual stipe/pileus ratio observed in the control in this experiment with those produced in the wild. Furthermore, we believe that sporophores growing from sclerotia buried in the soil and those growing from sclerotia in or on logs are subject to different factors, which exist in each microenvironment, soil and decaying logs. Although reports about yield and morphometric values from sporophore induction studies were conducted by burying sclerotia in soil (Oso, 1977, Okhuoya & Ajerio, 1988, Okhuoya *et al.*, 2000), information on the ability of the sclerotia of this fungus to grow and produce sporophores in an environment lacking other nutrient or carbon sources is not available. Therefore, it is not possible to unequivocally assign all observations on the control to the sclerotia only, especially so that the mycelium usually developed during sporophore induction is used for absorption of water and nutrients (Okhuoya *et al.*, 1998; Isikhuemhen *et al.*, 1999).

It has been shown in this study that fertilizers have a positive effect on the growth and yield in this fungus, in a manner similar to higher plants, and that their application could boost mushroom production. It is known that during sporophore morphogenesis in this fungus, at the point when the outline of the cap is just forming, it is soft and devoid of the leathery nature as compared to very matured sporophores and the sporophores of most other *Pleurotus spp.* with dimitic hyphal systems. It is at this point of maturity that this fungus is picked by the people and used for cooking their meals for eating. Though Kurtzman and Zadrazil (1982) hold that it is necessary to avoid even a small amount of stipe elongation in the production of mushrooms of high quality; this is not necessarily the case *in P. tuberregium*. It is the only non-pleurotoid member of the genus *Pleurotus* (Isikhuemhen *et al.*, 2000; Nerud F., 1999; Isikhuemhen & Nerud, 1999); as such; naturally, it should develop a stipe. Coincidentally, it is the fruit body with robust stipe and a just forming cap that meets the consumer's demand in Nigeria. This is understandable because this stage of sporophore morphogenesis could represent the young stage that Pegler (1983) describes to consist of monomitic hyphae system and at a later stage becomes dimitic with skeletal hyphae. Different fertilizers can affect positively different parts of sporophore development as evidenced from the various pileus/stipe ratios found. Therefore, it will be necessary to carry out further studies to determine the combinations of the different fertilizers that can give greater sporophore yield, along with shapes and sizes that will appeal most to consumers, in a commercial production set up.

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