International Scholars Journals

African Journal of Tropical Agriculture ISSN 2375-091X Vol. 7 (10), pp. 001-006, October, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of agrowastes, pH and temperature variation on the growth of *Volvariella volvacea*

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Accepted 11 March, 2019

The effect of pH and temperature variations on the growth of *Volvariella volvacea* cultivated on various agricultural wastes singly and in various combinations was studied. A pH range of 5.5 to 8.5 recorded the maximum mycelia yield and the highest mycelia weight was recorded at pH 6.5. The mycelia yield decreased at pH above 6.5 while poor mycelia growth of the mushroom and the least mycelia weight was recorded at pH 2.0. High mycelia growth of the mushroom was also observed between 25°C and 30°C with the highest mycelia dry weight of 80.0 mg obtained at 30°C, and the least mycelia dry weight of 0.5 mg obtained at 10°C . The use of these wastes in mushroom cultivation enhances the biological recycling of nutrients.

Key words: Agricultural wastes, temperature, pH, *Volvariella volvacea*.

INTRODUCTION

Mushrooms are good sources of sugars, fibre, proteins and minerals (Senatore, 1990; Adewusi et al., 1993), with comparable amino acid with animal protein (Aletor, 1995). Inadequate supply and high cost of animal protein necessitate the search for and cultivation of locally available and cheap protein sources. The ability of fungus to degrade agricultural materials and the ready availability of many agricultural by- products like rice husk, cotton waste, groundnut shell, cassava peel, corn cob and oil palm pericarp, may provide a sustainable way of addressing protein deficiency via cultivation of mushrooms like *Volvariella volvacea*.

The objective of this work was to study the effect of pH and temperature variation on the growth of *V. volvacea* using agricultural wastes.

MATERIALS AND METHODS

Collection and identification of *Volvariella volvacea*

Fruiting bodies of *V. volvacea* were harvested from the heap of discarded oil palm pericarp waste at the oil palm mill, Federal College of Agriculture, Akure, Ondo State, Nigeria. Identification of mushroom was done using the methods of Alofe et al. (1996), Oso (1975) and Zoberi (1972). The mycelium of *V. volvacea* was obtained by culture. These pieces of tissue were placed aseptically on sterilized potato dextrose agar (PDA) and the mycelium was allowed to develop from the spores. The developed mycelium was maintained on PDA by regular subculturing during the period of investigation.

Preparation of growth medium

The agricultural wastes, namely: rice husk (RH), rice straw (RS), cotton waste (CW), groundnut shell (GS), cassava peel (CP), corn cob (CC), white afra dust (WAD), red afra dust (RAD),oil palm pericarp (OPP) and red sorghum shaft (RSS) and their blends (ratio 1:1), RH-RAD, OPP-GS, CC-GS, CP-SS, CW-RH, and OPP- CP were chopped (where necessary), soaked in hot water (80°C, 30 min), and then pressed to expel excess water till the moisture content was about 60%. 10 g of rice bran and 4.0 g of $CaSO_4$ were added to 56.0 g of each waste and mixed together properly in a container. 10 g of each mixture was then transferred into a clean petri dish, sterilized at 121°C for 30 min, cooled, inoculated with a 72-h old culture of mycelia using a 5 mm disc and incubated at 30 ± 2ºC for 8 days. The radial growth and mycelia density of mushroom were measured and recorded for each treatment. Uninoculated sterile plates were used as control.

Effect of agrowaste extract on growth of *V. volvacea*

This was done by the method of Ogundero (1982) as modified by Akinyosoye et al. (1995). 20 g of each powdered agrowaste was

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Table 1. Radial growth and density of *Volvariella volvacea* on various agricultural wastes.

Agricultural wastes	Mycelia extension (mm)	Mycelia density
Rice husk	80.67f ± 0.2	9+
Oil plam pericarp	69.33e ± 0.5	4+
Cassava peel	41.67a ± 1.3	3+
Red sorghum shaft	42.33a ± 1.0	7+
Cotton wastes	98.23g ± 0.1	8+
Rice straw	59.67d ± 0.3	5+
Red afra dust	50.46c ± 0.2	3+
White afra dust	39.67a ± 0.1	3+
Ground nut shell	65.62e ± 0.4	4+
Corn cob	45.38b ± 0.1	3+

+: Scanty mycelia growth.
3+: Moderate mycelia growth.
5+: Very abundant mycelia growth.
Values followed by different letters are statistically significant different from each other.

transferred into 100 ml of distilled water in a container and soaked overnight. The mixture was filtered and 30 ml of filtrate was dispensed into 100 ml conical flasks to obtain the cold water extract (CWE). The hot water extract (HWE) was obtained by boiling 2.0 g of each powdered agrowaste in 100 ml of distilled water for 1 h, cooled, filtered and dispensed into 100 ml conical flasks. CWE and HWE were sterilized at 121°C for 15 min, cooled, inoculated with a 72 h-old mycelia culture of *V. volvacea*, and incubated at 30 ± 2°C for 7 days on a rotary shaker at 100 rpm. Mycelia dry weight and pH of culture filtrate were determined from three replicates and the average recorded.

Effect of pH on growth of *V. volvacea*

The basal medium used in the experiment was prepared as described by Kadiri (1998). The basal medium contained $FeSO_4.7H_2O$ (0.01 g), $MgSO_4.7H_2O$ (0.05 g), KH_2PO_4 (0.05 g), KCl (0.05 g), yeast extract (2.50 g), KNO_3 (1.55 g) and D-glucose (10.0 g) in 1000 ml of deionized water. 30 ml of liquid medium was poured into each of the 250 ml flasks and the medium adjusted to different pH of 2.0, 3.0, 4.0, 5.0 with a Pye Unicam pH meter using 1 M NaOH or 1 M HCl that was added in drops. Each pH medium was dispensed into 100 ml conical flask and all flasks autoclaved at 121°C for 15 min and 50 mg/100 ml of streptomycin added to suppress bacteria growth. The flasks were then inoculated with 5 mm disc mycelia plug of 72 h old culture of the mushroom. The flasks were incubated at 30 ± 2°C for 7 days on a rotary shaker at 100 rpm. Mycelia dry weight and pH of culture filtrate were determined from three replicates and the average recorded.

Effect of temperature on growth of *V. volvacea*

To determine optimum temperature for mycelia growth, the basal medium employed in the experiment was prepared according to Kadiri (1998) . The basal medium contained all the components stated above and in the same proportions. The pH of the medium was adjusted to 6.0. Thirty millilitres of the liquid medium was dispensed into 100 ml flasks, and 50 mg/100ml of streptomycin added to suppress bacteria growth. The flasks were covered with aluminium foil, autoclaved at 121°C for 15 min, inoculated with a 72-h old mycelia culture of *V. volvacea* using a 5 mm diameter disc and incubated for 7 days at 10, 15, 20, 25, 30, 35, 40, 45 and 50°C. Each treatment was replicated three times. The mycelia produced were harvested by filtration through pre-weighed filter paper with the aid of suction pump. Mycelia dry weight and pH of culture filtrate were determined from three replicates and the average recorded.

Statistical analyses

Means of readings from three replicates were determined and subjected to analysis of variance. Means were separated using Duncan's Multiple Range Test with the aid of statistical package for social scientists (SPSS version 10.0).

RESULTS

Growth of *V. volvacea* on various agrowastes

Tables 1 and 2 show the radial growth and density of *V. volvacea* on the agrowastes and their blends. Mycelia extension ranged from 39.67 ± 0.1 to 98.23 ± 0.1 mm. CW gave the yield with a mycelial extension of 98.23 mm, followed by RH (80.67 mm), OPP (69.33 mm), while RSS had the least (42.33 mm). The highest mycelia density was observed in RH (9+), RSS (7+), CW (8+) and RS (5+) . CP, RAD, WAD and CC had the least (3+). CW-RH and OPP-GS blends gave the highest mycelia extension of 101.87 and 100.67 mm, respectively. The least mycelia extension was recorded in CC-GS blend (26.67mm). Mycelia density is very high in RH-RAD and CW-RH blends. Moderate growth was observed in the other combinations.

The effect of cold water extract (CWE) and hot water extract (HWE) of agrowastes on the growth of *V.*

Table 2. Radial growth and mycelia density of *V. volvacea* on combination of various agricultural wastes.

Combination of wastes	Mycelia extension (mm)	Mycelia density
Rice husk + red afra dust	80.10c ± 0.2	5 +
Oil palm pericarp + groundnut shell	100.67e ± 0.3	4+
Corn cob + groundnut shell	26.67a ± 0.1	4+
Cassava peel + sorghum husk	90.52d ± 0.2	4+
Cotton waste + rice husk	101.87e ± 0.4	8+
Oil palm pericarp + cassava peel	58.67b ± 0.3	3+

+: Scanty mycelia growth.
3+: Moderate mycelia growth.
5+: Very abundant mycelia growth.
Values followed by different letters are statistically significant different from each other.

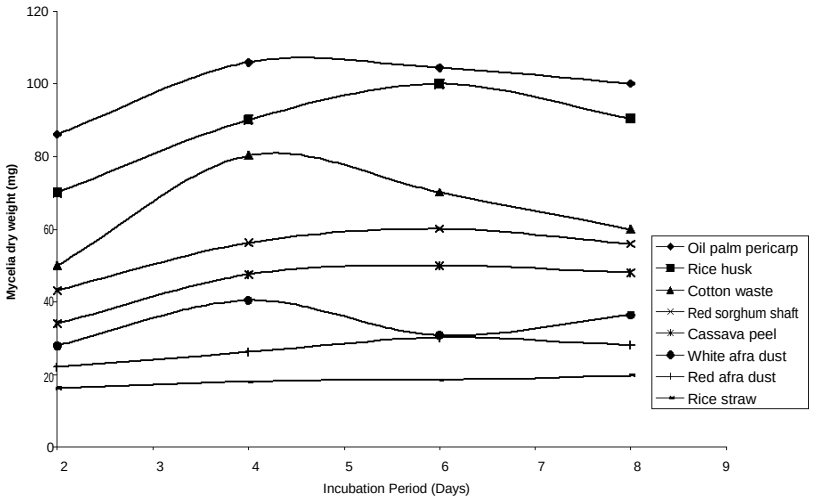

Figure 1. Growth of *V. volvacea* in cold water extract of various agricultural wastes.

volvacea mycelia are shown in Figures 1 and 2. CWE and HWE of OPP gave the highest mycelia dry weight (86.0 – 106.0 mg and 105.6 – 131.0 mg, respectively), followed by RH (70.0 – 90.0 mg and 71.4 – 120.1 mg, respectively) and CW (50.0 – 80.0 mg and 60.0 – 98 mg, respectively). RS gave the least mycelia dry weight (20.0 mg and 30.8 – 40.0 mg, respectively). The highest mycelia dry weight was obtained on day 6 for all the agrowastes.

Effect of pH and temperature on growth of *V. volvacea*

The effects of pH and temperature variation on *V. volvacea* mycelia growth are shown in Figures 3 and 4. Maximum mycelia yield was observed between pH 6 and 8 while the highest mycelia dry weight was recorded at pH 7.0. Poor growth was recorded in the acidic pH region with the least mycelia weight observed at pH 2.0.

Appreciable mycelia growth was recorded between 25 and 40°C with the highest mycelia dry weight (80.0 mg) obtained at 30ºC. The least mycelia weight (0.5 mg) was obtained at 10°C.

DISCUSSION

The cultivation of edible mushroom using agricultural residues such as rice and wheat straw is a value added process to convert these materials, which are otherwise considered to be wastes, into human foods. *V. volvacea* mycelia grows very well on a wide range of cellulosic wastes. Rice husk, cotton waste, oil palm pericarp, groundnut shell as well as red afra dust all supported good growth and fast mycelia extension of the mushroom. This is not surprising as rice straw has been used for the indoor cultivation of *V. volvacea* since the beginning of the 19th century, a practice from which the

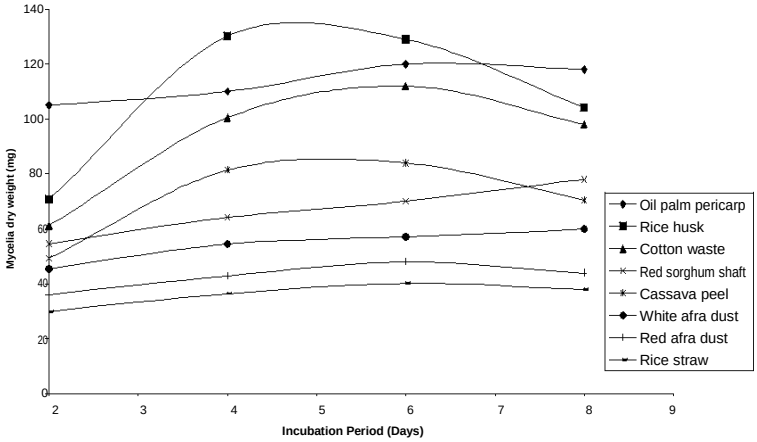

Figure 2. Growth of *V. volvacea* in hot water extract of various agricultural wastes.

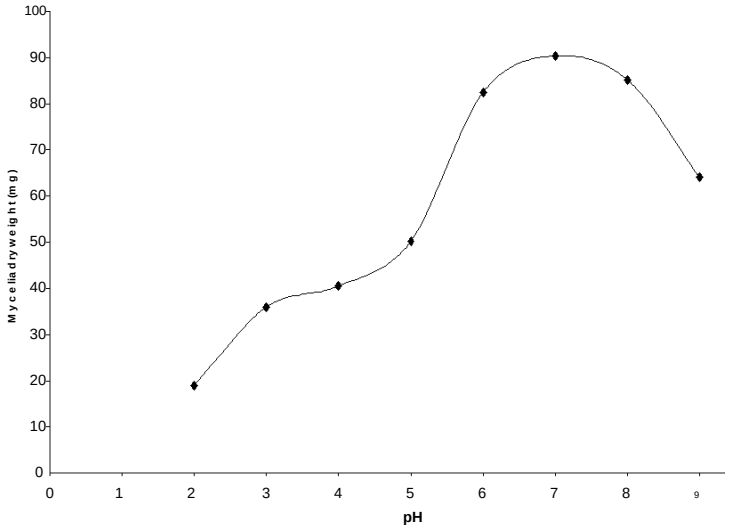

Figure 3. Effect of pH on growth of *V. volvacea*.

mushroom has been given the common name straw mushroom, and has been cultivated under natural conditions in many countries (Quimio, 1990). Fasidi (1996) reported rice straw as the natural substrate on which *V. esculenta* grew and led to naming the mushroom delicious straw mushroom. Rice husk supported the highest mycelia density of *V. volvacea.*

Mixtures of the various agricultural wastes also gave appreciable yield of the mushroom mycelia. For instance a combination of oil palm pericarp and groundnut shell as well as mixture of cassava peel and sorghum shaft gave high mycelia dry weight of the mushroom. Bolton and Blair (1982) and Fasidi (1996) reported that rice husk is good for the production of *V. esculenta* because of its richness in oils and vitamins which are good stimulants for high mushroom yield. In the past, *V. volvacea* do not fruit on corn cob and sawdust. This may be due to the looseness, high proportion of cellulose and compactness on wetting of these wastes (Chang, 1983). *V. volvacea* have also been known to grow on several materials such as banana leaves, water hyacinth, cotton wastes, oil palm pericarp wastes, oil palm bunch wastes and sawdust but their mean mycelia yields are comparably low in some of these wastes (Chen and Graham, 1973; Chua and Ho, 1973).

The presence of agricultural wastes poses disposal problems as well as causing environmental pollution. The wastes are either burnt or left to rot openly thereby creating a health hazard to human life. The use of these wastes in the cultivation of edible mushrooms will enhance the biological recycling of nutrients (Madan et al., 1987).

Mushrooms have also been reported to be potential contributors to world's food supply since they have the

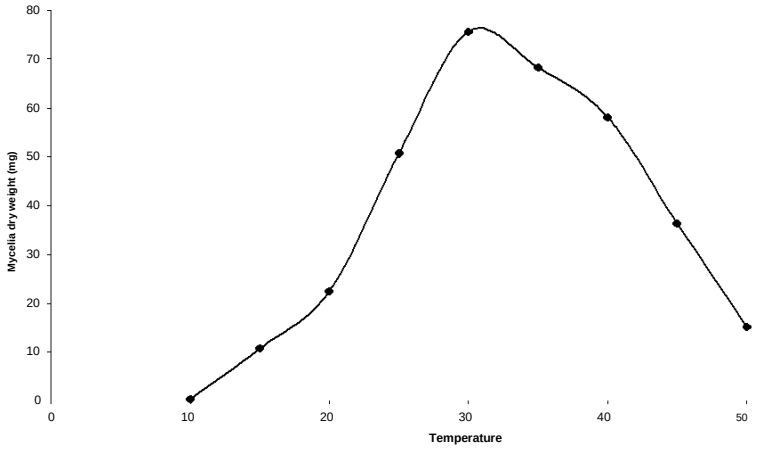

Figure 4. Effect of temperature (ºC) on growth of *V. volvacea*.

ability to transform nutritionally worthless wastes into protein rich food and have been confirmed to be sources of single cell protein (Alofe, 1985; Alofe et al., 1998). Cotton wastes, maize stalks, sorghum shaft, sugar cane bagasse, sawdust and water hyacinth have been used for cultivation of mushrooms (Chang and Quimio, 1982; Chang, 1993).

The results of the growth performance of *V. volvacea* in cold and hot water extracts of various agricultural wastes showed a broad nutritional pattern. The mushroom was able to utilise all the substrates for mycelia growth. It was observed that oil palm pericarp supported good mycelia yield of mushrooms. Also, rice husk and cotton wastes supported significant growth of *V. volvacea* mycelia. Akpata (1986) observed that the nutrient enrichment from the deposition of sawdust in Lagos metropolis favoured growth of the cellulolytic fungi that are able to breakdown components of wood wastes. Rice husks, oil palm pericarp as well as cotton wastes were observed to support maximum mycelia growth of the mushroom *V. volvacea.* Stone (1954) suggested that extracts of cellulosic materials may contain active ingredients that act as nutrient sources to support the growth of microorganisms. Therefore, there is a possibility that the constituents of the agricultural wastes employed were easily released more in hot water than in cold water resulting in the higher mycelia weight over the period of growth (Belewu and Banjo, 2000). Rice straw and sawdust have been reported to contain lignin, lignocellulose, starch and simple sugars which are probably difficult to break into soluble forms in cold water (Jonathan and Fasidi, 2001).

Temperature and pH are found to be important environmental factors that control the growth of most microorganisms. Fasidi (1996) reported that *Volvariella esculenta* was able to tolerate temperature range of 20 – 40ºC and pH range of 3 – 10. According to the author, optimum temperature for the growth of the mushroom was found to be 35ºC while the optimum pH was 6.0. This probably explains the ability of the mushroom to flourish very well on various agricultural wastes in the tropics. The pH range for the growth of *V. volvacea* was found to be 5.5 – 8.5, while the optimum pH was found to be 6.5 and the mushroom was able to tolerate a temperature range of 27 - 40ºC with the optimum temperature of 30ºC.

The optimum pH of 6.5 and optimum temperature of 30º C reported for *V. volvacea* in Figures 3 and 4 agree with the report of Chang and Yau (1977) of temperature range 30 – 35ºC for the same mushroom. Kuforiji and Fasidi (1998) obtained an optimal temperature of 35ºC for *Pleurotus tuberregium* and pH range of 5 – 7 for same mushroom. Chang et al. (1981) reported a temperature range of 10 – 20ºC for *Pleurotus Sajor-caju* while Jonathan and Fasidi (2000) reported appreciable growth of *Psathyrella atroumbonata* at 30ºC and pH 6.5. Similar observations were made by Anyakuorah et al. (1998) on the cultivation of *Lentinus squarrosulus.* This work has shown that significant improvement in the mycelia growth of *V. volvacea* can be attained through cultivation with agrowastes. Cotton wastes, rice husk and oil palm pericap and its blends gave the best mycelia growth while pH 7 and temperature of 30ºC were the most suitable for its cultivation. The cultivation of *V. volvacea* on these agrowastes may provide a sustainable means of adding value to them and also result in increasing human protein intake through cultivation of *V. volvacea*.

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