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DEVELOPMENT OF A HIGH-YIELDING UV-MUTANT STRAIN OF *VOLVARIELLA VOLVACEA* FROM THE NIGERIAN STRAIN VNW

Adewoyin A. G.[a,*], Barooah M.[b] and Oloke J. K.[c]

[a]Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, P. M. B. 4000, Nigeria.
[b]Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat Assam 785013, India.
[c]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, P. M. B. 4000, Nigeria.

Corresponding Author: Adewoyin A. G.
Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, P. M. B. 4000, Nigeria.

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ABSTRACT

Volvariella volvacea is a fast-growing edible mushroom with a low yield that has greatly limited its commercial cultivation compared to other commercially cultivated mushrooms. Mutagenesis was induced by UV irradiation in the C region at varying distances and time intervals. Phylogenetic analysis suggests a close relatedness of surviving and mutants to the wild strain than to other strains considered. Comparative fructification of fast-growing mutants and other strains was carried out using rice straw as substrate. Mutant M5 (UV irradiation for 75min from a distance of 60cm) exhibited exceptional growth qualities at 35ºC and pH 6.0. The mutant also showed a significantly ($\rho \leq 0.05$) higher growth rate and yield (Biological Efficiency of 23.98%) and a significant ($\rho \leq 0.05$) improvement in the nutrient composition. This study highlights the development of a high-yielding UV mutant strain from the Nigerian wild stain that is suitable for commercial cultivation.

KEYWORDS: *Volvariella volvacea*, UV-mutant, wild type, rice straw, Biological Efficiency.

1.0 INTRODUCTION

Volvariella volvacea known as the straw mushroom or Chinese mushroom occurs in both tropical and subtropical regions of the world (Ukoima *et al*., 2009) and is a preferred type of mushroom by most consumers because of its texture, aroma, and taste (Tharun, 1993, Ahlawat *et al*., 2008). Reports have indicated that it grows well on cellulosic agricultural waste materials like rice straw (Thiribuvanamala *et al*., 2012), cotton waste (Imram *et al*., 2011), banana leaves, and cassava peels (Obodai and Odamtten, 2012; Apertogbor *et al*., 2015), oil palm empty fruit bunch (Tryono, 2019). This mushroom is adapted to areas with high temperatures (30-36ºC) but is not as popular amongst mushroom consumers as button, oyster, or shiitake mushrooms, probably due to its low yield (Buswell and Chen, 2005; Ding *et al*., 2006). Earlier efforts to improve the yield of *V. volvacea* have proved abortive as a result of the non-availability of basic genetic information (Royse *et al*., 1987). However, it has been shown recently that factors like the multinucleate nature, lack of clamp connection, and incompletely identified sexual reproductive nature have made breeding of *V. volvacea* very difficult (Chen *et al*., 2016).

Various methods have been utilized for the yield improvement of *V. volvacea* such as cultivation using circular compact bed method (Thiribuvanamala *et al*., 2012), likewise supplementation of mushroom growth substrate with proteinaceous materials and micronutrients (Thiribuvanamala *et al*., 2012; Corrasco *et al*., 2018). Studies carried out by Payapanon *et al*. (2011) indicated that supplementation with *Paenibacillus polymyxa* N10 and *Bacillus subtilis* B2 to straw mushroom compost resulted in increased mushroom yield. Composting of oil palm bunch used as substrate for cultivation of *V. volvacea* within 8 (eight) days has also been reported to improve its yield (Tryono, 2019). However, Thuc *et al*. (2020) attributing the unstable fruiting nature and yield of *V. volvacea* to unstable weather reported an improved yield performance from indoor cultivation. The use of electrical stimulation to improve yield of mushrooms is another method that has been reported (Takaki *et al*., 2009; Takaki *et al*., 2014). Chemical mutagens have been used to breed cold-tolerant strains of *V. volvacea* (Liu *et al*., 2011). Report have shown that the use of UV irradiation have led to yield improvement in some mushrooms (Teichmann *et*

al., 2007; Elfallal *et al*., 2013; Ngamnit and Saovapong 2014).

In Nigeria, *V. volvacea* is commonly found growing on empty oil palm bunch and there is still no commercial cultivation despite the availability of suitable weather conditions and substrate for its cultivation. This work is aimed at developing UV-mutants strains from the Nigerian strain *V. volvacea* VNW (KC894923) with improved yield performance suitable for commercial cultivation.

2.0 MATERIALS AND METHODS

2.1 Volvariella volvacea strain

The Nigerian strain *V. volvacea*, VNW (KC894923) was isolated from discarded oil palm waste in the southwestern part of Nigeria (Adewoyin *et al*., 2017), while 3 Indian commercial strains (V11, V245, and V247) were procured as cultures on PDA slants from the Directorate of Mushroom Research, Indian Council of Agricultural Research (ICAR), Chambaghat, Solan, India (courtesy of DBT-AAU, Jorhat). All stock fungi cultures were maintained on PDA slants and kept at room temperature.

2.2 UV irradiation of Nigerian strain VNW (KC894923)

Mutagenesis was induced by UV irradiation (15Watt, λ=250nm) of plate cultures following the method of Elfallal *et al*. (2013) with little modification. Briefly, in the first group, five-day-old actively growing plate cultures of *V. volvacea* on PDA medium were uncovered and subjected to UV-irradiation given at 60 cm distance from the source and at time intervals (15, 30, 45, 60, 75, and 90) min. In the second group, uncovered plate cultures of *V. volvacea* were subjected to UV-irradiation given at a 30 cm distance from the source and at time intervals of 30 min (30, 60, and 90). Irradiated plate cultures were incubated briefly for 30 min in the dark. Plate cultures that remained viable after repeated subcultures were regarded as viable mutants.

2.3 Determination of Mycelia morphology of mutant strains on PDA plate

Morphological examination of mycelia was carried out macroscopically by visualizing the growth pattern of 5-day-old cultures on PDA plates. Patterns and characteristics of mycelia were described as texture (cottony or floccose), mycelia density (high, regular, or low), color (white, off-white, white, or pale pink), and growth (scanty, moderate, or abundant) mycelia nature (compact or aerial) and recorded accordingly (Sobal *et al*., 2007).

2.4 Physiological study of mutant strains

The effect of temperature on mycelial growth was determined by the cultivation of the mycelia at 20, 25, 30, 35, and 40ºC (Jonathan and Fasidi, 2004). Mycelia plugs (5.0 mm in diameter) obtained from an actively growing margin of a 5-day-old culture were used as inocula. The diameter of each fungal colony was the average of 5 replicates.

The optimal growth pH for mycelia extension was evaluated by the cultivation of the mycelia of the UV mutants on PDA at 35ºC. The inoculums' size and diameter of the growing colony were determined as described above.

2.5 DNA Isolation, PCR Amplification, Sequencing, and Phylogenetic Analysis

The DNA extraction, Polymerase Chain Reaction (PCR), and purification of each amplicon were carried out as earlier described (Adewoyin *et al*., 2017). Amplicons were sequenced using fluorescent dye terminator chemistry and were run on ABI 3130 (4-capillary) or 3730XI (96-capillary) Automated Sequencer (Perkin Elmer Applied Biosystems, Foster City, CA), according to the manufacturer's instruction. The final sequences obtained with each primer set were blasted against the GenBank database (www.ncbi.nlm.nih.gov/BLAST). The Phylogenetic analysis of the partial sequence data of 5.8S ribosomal RNA gene of the mutant strains, wild strains, and corresponding GenBank data of related species and strains was conducted using MEGA X (Kumar *et al*., 2018). Phylogenetic trees were constructed using the Neighbours-Joining (NJ) method with 1000 bootstrap resampling.

2.6 Sporophore (fruiting body) production using rice straw as substrate

The in-door method of Quimo (1993) was adopted for mushroom cultivation with little modification using woven bamboo as a supporting bed. Whole rice grains were used as the substrate for the mother spawn after cleaning, brief par-boiling, and addition of 4 % (w/w) $CaCO_3$ and 2 % (w/w) $CaSO_4.2H_2O$ (gypsum). Mycelia plugs cut from an actively growing margin of 5- day old plate cultures were used as inocula after autoclaving for 1 hour. Inoculated substrates were incubated at 35ºC in the dark for 10-15 days. The daily observation was made for full ramification and appearance of conspicuous and well-formed chlamydospores. Using the mature mother spawns as inocula, and chopped rice straw as substrate, the planting spawn was prepared after the addition of 5 % w/w coarsely ground *C. cajan* powder, and the planting spawn was incubated. The maturity of planting spawn was indicated by the presence of abundant chlamydospores.

The straws for mushroom cultivation were bundled to sizes of about 8 - 10 cm in diameter, about 50 cm in length, and soaked in boiled water for 3 hours to soften and afterward drained. The straw bundles were arranged by laying 4 side by side (parallel) on the bamboo frame and these four bundles constituted the first layer. Small pieces (thumb-sized) of the planting spawn were placed 4 - 6 cm deep and about 8 cm apart along the edges of the bundles. The spawn was then dusted with coarsely ground *C. cajan* powder. The second layer of 4 bundles

was placed across the first layer and spawned as that of the first layer. This was followed by the third layer across the second in the same manner and finally, the fourth layer was used to cover the others, spawned and pressed lightly. After the arrangement of the bundles and spawning, the beds were completely but loosely covered with a transparent polythene sheet.

2.7 Fructification and Harvest

Each day the beds were sprayed with clean water and after a full spawn run, the polythene cover was removed and water was sprayed over the beds 2 - 3 times daily to maintain the moisture level of the bed. Fully matured mushrooms were harvested after about 15 days of spawning. Fruiting bodies were harvested weighed and the values obtained were used to calculate biological efficiency –BE (Girmay *et al*., 2016). Harvested mushrooms were dried in the laboratory oven at 60ºC until constant weight. The oven-dried sample was cooled in desiccators, pulverized in an electric blender and kept until needed.

2.8 Determination of the proximate composition of fruiting bodies

Moisture content was determined by the direct oven drying method. The weight loss after oven drying of each sample (1 g) at 103±2ºC to constant weight was expressed as % moisture content (Sivrikaya, 2002). The nitrogen content was determined using the micro-Kjeldahl method and crude protein was calculated by multiplying the nitrogen content with a factor of 6.25 (Thimmaiah, 2004). The crude fiber was determined according to the standard method of AOAC (2005) where the sample was digested successively with acid and base. The difference in the weight of the residue obtained after digestion expressed in percentage gave the crude fiber content. Crude fat was determined by using the Soxhlet extraction method using petroleum ether as the solvent (AOAC 2005). Ash content of 1 g powdered sample was determined as the residue of incineration at 550°C in a muffle furnace (AOAC, 2005). Mineral constituents (calcium, phosphorous, sodium, potassium, magnesium, iron, copper, manganese, zinc, and cobalt) were determined by atomic absorption spectrophotometry (AOAC, 2005). All proximate analyses of the mushroom powder were carried out in triplicate and reported in percent.

2.9 Sample extraction

Extraction was performed using 10g of the powdered sample according to the method of Gasecka *et al*. (2015) with 80% methanol. The residual solvent of methanol extracts was removed using a rotary evaporator to the dry form. The obtained concentrated extracts were stored in dark at 4ºC until further analysis.

2.10 Estimation of total phenol content (TPC) of methanol extract

Folin-Ciocalteu reagent was used for the estimation of total phenol content of methanol extract using gallic acid as a standard and following the methods described by Ainsworth and Gillespie (2007). Absorbance at λ=765 nm was measured. A standard curve was prepared from 0 - 42 mg/L gallic acid. Total phenolic content was expressed in mg gallic acid equivalents/g dry extract. All samples were analyzed in triplicates

2.11 Estimation of total flavonoid content (TFC) of methanol extract

Aluminum chloride ($AlCl_3$) was used to determine the total flavonoid content (TFC) of the methanolic extracts according to the methods of Singh *et al*. (2012). Absorbance was measured at λ=510 nm and Quercetin was used for the calibration curve from 10 - 1000 µg/mL. Total flavonoid content was expressed as mg quercetin equivalent (mg QE)/g of dried extract. All experiments were carried out in triplicates.

Data availability

The data of molecular identification of sequences in this study were deposited with NCBI GenBank with accession numbers KC894927, KC894928, KC894929, and KC894930.

Statistical analysis of data

All data obtained were subjected to analysis of variance (One-way ANOVA) and significance was accepted at a 5% probability level according to Duncan's multiple range test using the Statistical Package for Social Sciences (SPSS) 23.0 version software. Analyzed data were reported as the mean ± standard error.

3.0 RESULTS

After UV irradiation of plate cultures of the Nigerian wild strain at different time intervals and different distances from the source of irradiation, surviving strains showed different growth characteristics and patterns. The pattern and rate of growth of mycelia (Table 1) observed on plate cultures of the surviving mutant strains were different for all the UV mutants. Mutants M5 and M7 appeared cottony aerial mycelia which made the mycelia appear abundant on the plate cultures. However, mutants M3 and H6 showed compact floccose mycelia and moderate mycelia on the plate cultures. With the increase in incubation temperature (20-40°C), results obtained (Table 2) show a gradual increase in the mycelia radial extension of the mutants. Optimum mycelial growth was recorded at 35°C for all the mutant strains. Mutant M5 and M7 have a significantly higher ($\rho \leq 0.05$) mycelia radial extension. On the other hand, the effect of pH on mycelial radial extension (Table 3) did not follow any regular pattern. The optimum mycelia radial extension was observed at pH 6.0, and mutants M5 and M7 showed a significantly higher ($\rho \leq 0.05$) radial extension.

Table 1: Pattern of growth and mycelial density of surviving mutants on plate cultures.

Mutant	Texture	Density	Colour	Growth	Mycelia nature
M3	Fluccose	Regular	Off-white	Moderate	Compact
M5	Cottony	High	White	Abundant	Aerial
M7	Cottony	Regular	White	Moderate	Aerial
H6	Fluccose	Cottony	White	Moderate	Compact

M3= Mutant at 45minutes UV exposure from a distance of 60cm; M5= Mutant at 75minutes UV exposure from a distance of 60cm; M7= Mutant at 30minutes UV exposure from a distance of 30cm; H6= Mutant at 60minutes UV exposure from a distance of 30cm.

Table 2: Effect of incubation temperature on mycelia radial extension of mutant strains.

Temperature °C	M3	M5	M7	H6
20	2.90 ± 0.19^{ab}	3.50 ± 0.63^{b}	2.80 ± 0.12^{a}	2.85 ± 0.31^{a}
25	10.80 ± 0.12^{ab}	11.40 ± 0.19^{b}	10.70 ± 0.25^{ab}	9.65 ± 0.36^{a}
30	18.20 ± 0.46^{b}	15.60 ± 0.29^{a}	15.60 ± 0.19^{a}	15.45 ± 0.83^{a}
35	28.20 ± 1.16^{b}	29.00 ± 0.61^{c}	29.50 ± 0.22^{c}	26.00 ± 0.76^{a}
40	25.40 ± 0.19^{b}	$25.50 \pm 0.27b^{b}$	25.90 ± 0.29^{b}	24.20 ± 0.42^{a}

Each value is a mean of 5 replicates ± standard error, values accompanied by identical superscript letters are not significantly different ($\rho \leq 0.05$) in rows.

M3= Mutant at 45minutes UV exposure from a distance of 60cm; M5= Mutant at 75minutes UV exposure from a distance of 60cm; M6= Mutant at 90 minutes UV exposure from a distance of 60cm; M7= Mutant at 30minutes UV exposure from a distance of 30cm; H6= Mutant at 60minutes UV exposure from a distance of 30cm.

Table 3: Effect of pH on mutant mycelia radial extension.

pH	M3	M5	M7	H6
5.0	35.10 ± 0.22^{b}	33.40 ± 0.37^{a}	33.20 ± 0.60^{a}	34.50 ± 0.69^{ab}
5.5	22.60 ± 0.92^{b}	22.10 ± 0.19^{b}	21.30 ± 0.44^{a}	22.75 ± 0.65^{b}
6.0	37.50 ± 0.67^{a}	40.10 ± 0.51^{b}	40.10 ± 0.33^{b}	37.15 ± 0.34^{a}
6.5	25.00 ± 0.22^{b}	23.90 ± 0.48^{ab}	22.60 ± 0.19^{a}	24.30 ± 0.50^{ab}
7.0	36.30 ± 0.34^{a}	35.50 ± 0.22^{a}	35.80 ± 0.34^{a}	35.37 ± 0.47^{a}
7.5	36.50 ± 0.22^{b}	33.40 ± 0.76^{a}	34.50 ± 0.50^{a}	34.20 ± 0.61^{b}

Each value is a mean of 5 replicates ± standard error, values accompanied by identical superscript letters are not significantly different ($\rho \leq 0.05$) in rows.

M3= Mutant at 45minutes UV exposure from a distance of 60cm; M5= Mutant at 75minutes UV exposure from a distance of 60cm; M6= Mutant at 90minutes UV exposure from a distance of 60 cm; M7= Mutant at 30minutes UV exposure from a distance of 30cm; H6= Mutant at 60minutes UV exposure from a distance of 30cm.

The evolutionary history involving 14 nucleotides sequences (comprising of the Nigerian wild, 3 Indian wild, 4 mutant strains, and 6 other strains from GenBank database NCBI) inferred using the Neighbor-Joining method is as represented in Figure 1. The unrooted phylogenic tree shows three (3) clades. The first clade comprise the six (6) strains obtained from the GenBank database (Strains V5, V23, OSM-2, ZJ0001GBJO2, H3 and JAC12235). The second clade comprises the Indian wild strain V247, Nigerian wild stain (VNW), and the 4 surviving UV mutant strains (H6, M7, M3 and M5) while the third clade comprise only the Indian strain V11. The derived strains shower closer relatedness to the Nigerian wild strain than to the Indian strains and other strains from GenBank. The Indian strain V247 is closer to the Nigerian wild strain since they share a common ancestor and belong to the same clade because of their relatedness.

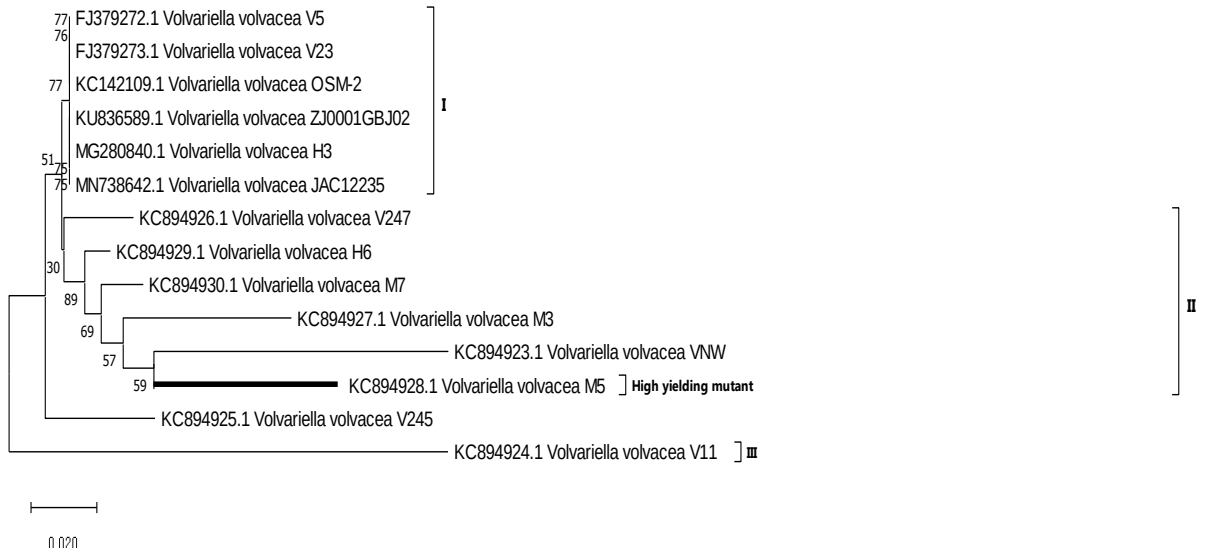

Figure 1: Phylogenetic tree constructed using ITS sequences from the Nigerian wild strains of *V. volvacea* VNW (KC894923), three Indian strains - V11 (KC894924), V245 (KC894925) and V247 (KC894926), 4 UV mutants – M3 (KC894927), M5 (KC894928), H6 (KC894929) and 7 other sequences in GenBank (accession numbers KU836589, FJ379272, MN738642, FJ379273, MG280840, HM367073 and JN086672) using the neighbor-joining method and Bootstrap analysis with 1,000 replications.

The full ramification of both the mother (grain) and planting spawns was fastest for mutant M5 (5 days), followed by other mutants. The Nigerian wild strain ramified faster than the Indian wild strains (7 days), while the Indian strains V11 and V245 ramified completely in 9 days (Table 4). The Indian strain V247 however took 11 days to fully ramify. The chlamydospores appeared on the planting spawn of the mutants in less than 10 days and more than 10 days in the three wild strains, indicating a longer time for maturation than in the mutant strains. The appearance of chlamydospores in all the planting spawns within two weeks was in varying degrees ranging from scanty in the Indian strain V247 to abundant in the mutant strain M5. The sporophore yield (Table 5) as indicated by the Biological Efficiency (BE) ranged from 15.85% to 23.98%. The mutant strain M5 had the highest percentage of 23.98 %, which was higher than that of its wild parent, VNW (20.33 %). The Indian wild strain V247 showed the lowest yield with BE 15.85%.

Table 4: Growth details and maturity of spawn (Planting).

Strain	Full ramification (in days)	First appearance of Chlamydospore (in days)	Aboundace of chlamydospore in 2 weeks
VNW	7.33 ± 0.33^{d}	10.00 ± 0.58^{b}	++
V11	9.00 ± 0.58^{bc}	13.00 ± 1.00^{d}	++
V245	9.00 ± 0.58^{bc}	11.33 ± 0.33^{c}	++
V247	11.67 ± 0.33^{a}	13.68 ± 0.88^{d}	+
M3	6.33 ± 0.33^{d}	8.33 ± 0.33^{a}	++
M5	5.33 ± 0.33^{e}	8.33 ± 0.33^{a}	+++
M7	6.67 ± 0.33^{d}	9.33 ± 0.33^{b}	++
H6	8.67 ± 0.33^{c}	13.33 ± 0.33^{d}	++

VNW=Nigerian wild strain of *V. volvacea*; V11=Indian wild strain 11 of *V. volvacea*; V245=Indian wild strain 245 of *V. volvacea;* V247=Indian wild strain 247 of *V. volvacea.*; M3= Mutant at 45minutes UV exposure from a distance of 60cm; M5= Mutant at 75minutes UV exposure from a distance of 60cm; M7= Mutant at 30minutes UV exposure from a distance of 30cm.

Key: Scanty chlamidosphore = +, Moderate Chlamidosphore = ++, Abundant chlamidosphore = +++

Table 5: Details of sporophore yield of strains cultivated on rice straw.

Strain	First flush (g)	Second Flush (g)	Biological Efficiency (%)
VNW	357.30 ± 7.71^{c}	235.73 ± 8.58^{bc}	20.33
V11	320.53 ± 10.31^{ab}	212.60 ± 6.59^{b}	17.78
V245	344.53 ± 11.98^{b}	227.43 ± 8.47^{bc}	19.07
V247	293.63 ± 12.71^{a}	181.80 ± 4.89^{a}	15.85
M5	431.13 ± 9.85^{d}	288.17 ± 10.09^{d}	23.98
M7	393.63 ± 4.47^{c}	245.63 ± 12.53^{c}	21.30

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different (ρ≤0.05) in columns.

VNW=Nigerian wild strain of *V. volvacea*; V11=Indian wild strain 11 of *V. volvacea*; V245=Indian wild strain 245 of *V. volvacea;* V247=Indian wild strain 247 of *V. volvacea.*; M5= Mutant at 75minutes UV exposure from a distance of 60 cm; M6= Mutant at 90 minutes UV exposure from a distance of 60 cm; M7= Mutant at 30 minutes UV exposure from a distance of 30 cm; H6= Mutant at 60 minutes UV exposure from a distance of 30 cm

The proximate composition of all the strains determined from the fruiting bodies harvested from the rice straw is as represented in Table 6. The crude protein and ash content of the two mutant strains M5 and M7 was higher than that of the wild strains (both Nigerian and Indian). The crude fibre content was however lower in the mutant strains and the Indian wild strain V247 compared to the other strains. There was no significant difference in the fat content of all the strains.

The potassium content was higher than all the minerals investigated for all the mushrooms samples. Values ranged from 1024.67±12.7 mg/kg in the Indian strain V11 to 1204.67±10.9 mg/kg in the Indian strain V247 (Table 7). All the mushrooms showed lower magnesium content in comparison with the four mineral content determined. There was no significant difference in the sodium content of all the mushroom samples.

The Total Phenol Content of all the strains ranged from 0.159±0.01 to 0.221±0.012mg GAE/g dried weight extract. The Total Phenol Content (TPC) of Mutant M5 was significantly higher than that of the Indian wild strains but not significantly different from that of the Nigerian wild strain. Mutant M5 had a significantly higher Total Flavonoid Content (TFC) followed by Mutant M7. The TFC of Mutant M7 is not significantly different from that of the Nigerian wild strain which is also not significantly different from that of the 3 Indian wild strains. The Indian wild strain V247 has the least TFC of 18.24 mg/g (Table 8).

Table 6: Proximate composition (%) of dried fruiting body.

Strains	Moisture	Ash	Crude Fibre	Crude Protein	Fat
VNW	10.33±0.71^{b}	12.30±0.21^{ab}	11.20±0.30^{b}	29.18±0.62^{c}	1.60±0.01^{a}
V11	9.78±0.33^{ab}	11.67±0.26^{a}	10.50±0.41^{ab}	27.73±0.64^{b}	1.37±0.03^{a}
V245	8.74±0.65^{a}	11.70±0.18^{a}	11.82±0.11^{b}	26.70±0.53^{ab}	1.39±0.06^{a}
V247	11.13±0.22^{b}	12.27±0.34^{ab}	7.57±0.12^{a}	22.23±0.33^{a}	1.35±0.03^{a}
M5	10.65±0.31^{b}	17.35±0.17^{c}	8.14±0.10^{a}	30.52±0.19^{c}	1.30±0.02^{a}
M7	11.20±0.15^{b}	14.68±0.33^{b}	8.39±0.21^{a}	30.14±0.56^{c}	1.52±0.01^{a}

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different (ρ≤0.05) in columns.

Table 7: Mineral composition (mg/kg) of dried fruiting body.

Strains	Calcium	Magnesium	Potassium	Sodium
VNW	496.00±8.10^{b}	47.67±1.01^{ab}	1069.33±13.31^{ab}	245.67±2.13^{b}
V11	475.67±7.41^{ab}	48.67±2.11^{ab}	1024.67±14.00^{a}	216.67±1.95^{a}
V245	490.67±6.90^{b}	45.67±0.98^{a}	1040.33±11.98^{a}	238.00±2.33^{ab}
V247	430.67±5.79^{a}	48.40±1.57^{ab}	1104.67±15.10^{b}	233.00±3.10^{ab}
M5	596.33±9.20^{d}	45.67±2.05^{a}	1204.67±10.89^{c}	238.33±3.75^{ab}
M7	525.00±9.57^{c}	49.67±1.00^{b}	1102.67±13.45^{b}	244.67±2.93^{b}

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different (ρ≤0.05) in columns.

Table 8: Total phenol content (TPC) and total flavonoid content of mushroom extracts.

Mushroom sample	TPC (mg GAE/g dried extract)	TFC (mg QE/g dried extract)
VNW	0.182 ± 0.025^{ab}	0.068 ± 0.001^{ab}
V11	0.165 ± 0.023^{a}	0.057 ± 0.002^{a}
V245	0.175 ± 0.021^{a}	0.060 ± 0.001^{a}
V247	0.159 ± 0.019^{a}	0.057 ± 0.003^{a}
M5	0.221 ± 0.012^{b}	0.101 ± 0.005^{c}
M7	0.189 ± 0.013^{ab}	0.073 ± 0.003^{b}

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different ($\rho \leq 0.05$) in columns.

4.0 DISCUSSION

The exposure of the Nigerian wild strain of *V. volvacea* to UV radiation successfully produced viable mutants that survived several subcultures. This supports earlier findings regarding the use of UV mutagenesis in strain improvement (Teichmann *et al*., 2007; Beejan and Nowbuth, 2009; Elfallal *et al*., 2013; Ngamnit and Saovapong, 2014) and has been employed for improving enzyme production in *Aspergillus niger* (Kang *et al*., 1999), mycelia cell and sporophore production in *P. florida* and *P. sajor-caju* (Ravishanker *et al*., 2006).

The evolutional analyses conducted in MEGA X (Tamura *et al*., 2004; Kumar *et al*., 2018) indicate that all surviving mutants are closer to the Nigerian wild strain than to other strains considered in the analysis. However, the highest yielding mutant strain shows the closest relatedness since they both share a recent common ancestor.

The developed mutant strain was able to grow within a wide range of temperatures (20–40 °C) with good growth characteristics at the optimum temperature of 35 °C, agreeing with previous studies that have established the optimum temperature range for growth of *V. volvacea* at 30-37 ^{o}C (Zervakis *et al*., 2001; Akinyele and Adetuyi, 2005; Ahlawat *et al*., 2008; Ahlawat and Harleen, 2018). *Volvariella esculenta*, which has been reported to possess similar growth requirements to *V. volvacea*, has also been reported to tolerate a temperature range of 20–40 °C (Jonathan and Fasidi 2004). The optimum pH (6.0) for the mycelia radial extension of the mutant strain falls within the range that has been reported earlier (Akinyele and Adetuyi, 2005; Kumar *et al*., 2016). This result also coincides with what has been reported earlier for the Nigerian wild strain (Adewoyin *et al*., 2017), and this proves the similarity in growth requirements of the mutant strain and the wild strain.

Mycelia growth properties of the derived mutants have also been enhanced, which has affected mycelia formation. Hyphal growth and mycelial development in the substrates have been shown to be one of the most important steps of mushroom production (Da Silva *et al*., 2013). This finding corroborates the report of Ahlwalat and Harleen (2018). The duration of maturation of the planting spawn for the mutant strain was shorter than that of the wild strains, as indicated by the presence of abundant chlamydospores, a faster spawn run, and a higher yield as represented by the BE. This depicts the improvement in the yield of the mutant strain compared to the wild strain, indicating that the yield of mushrooms is also affected by the strain used for cultivation. This finding also corroborates the submission of earlier authors (Beejan and Nowbuth, 2009; Ngamnit and Saovapong, 2014) of the use of UV irradiation to develop high-yielding strains of mushrooms. This study also clearly reveals the direct correlation between the abundance of chlamydospore and the maximum yield of the fruiting body, supporting earlier findings (Thiribhuvanamala *et al*., 2012). This is further in line with the submission of Quimo (1994) that suggested that chlamydospores act as storage units of balanced cell contents, additional inocula during artificial cultivation of the mushroom, and a survival mechanism during storage.

The yield reported by Tripathy *et al*. (2011) for the study of the effect of various lignocellulose wastes on mycelia growth and the yield of *Volvariella* spp. is lower than what is reported in this study. However, the yield obtained for the best-growing mutant falls within the range recorded by some authors (Thirbhuvanamala *et al*., 2012; Apetorgbor *et al*., 2015). This suggests that apart from the strain of mushroom used for cultivation, additives like growth boosters can affect yield (Thirbhuvanamala *et al*., 2012). Likewise, substrates used in mushroom cultivation also affect the yield of the mushroom (Ahlawat *et al*., 2011; Apetorgbor *et al*., 2015).

The increased protein content in the mutant strain suggests that it improved the nutritional composition of the mushroom. The crude protein content of all the strains cultivated was higher than the range reported by some authors (Jonathan *et al*., 2006; Mshandete, 2007; Hung and Nhi, 2012). Some earlier reports, however, indicated a higher protein content of *V. volvacea* than what is reported in this study (Thirbhuvanamala *et al*., 2012; Ahlwalat and Harleen, 2018; Tryono, 2019). While in some other studies, the results obtained fall within the range reported (Imran *et al*., 2011; Abena *et al*., 2018). Protein has been reported to be one of the important nutritional components in most edible mushrooms (Hung and Nhi, 2012). The calcium, potassium, magnesium, and sodium content recorded in

this study for the mutant exceeds that of the wild strain, showing the improvement in the mineral content of the mutant strains. However, the calcium, potassium, and magnesium content for all the strains were lower than what was reported by Alhawat and Harleen (2018) except for the sodium content, which was higher. All the strains also show lower fat content, establishing earlier reports of the preference for mushrooms because of the low cholesterol content. The difference in proximate composition observed in different reports of this study could be attributed to factors like substrate, substrate treatment, growth condition, and strain used. The higher protein content of *Volvariella* spp. compared to most edible mushrooms explains the possible reason for its distinct flavour and tastiness and why it is mostly preferred among mushroom consumers. This is an indication that, in addition to the substrate used for cultivation, the strain used for cultivation also has a significant effect on the proximate composition of mushrooms.

The TPC and TFC content of the mutant strains show an improvement, which is depicted in the higher value recorded for mutant strains compared to the wild strains. The values recorded in this study differ from the values reported by Dulay *et al*. (2016) from mycelia of *V. volvacea* cultivated in different broths for both the TPC and TFC. On the other hand, values obtained were higher than the values reported for *V. volvacea* by Payapanon *et al*. (2016) but lower than those reported by Batkhup *et al*. (2018). The values, however, fall within the range reported by Boonsong *et al.* (2016). The different values for both the TPC and TFC in *V. volvacea* by different authors could be due to the method of extraction of the extract (Puttaraju *et al.*, 2006), solvent used in the extraction process (Cheung *et al*., 2003; Boonsong *et al.*, 2016) and the strain used for extraction. Reports have shown the correlation of TPC and TFC of mushrooms with antioxidant properties (Boonsong *et al.*, 2016) and have also been associated with their free radical scavenging activity (Barros *et al*., 2007; Carmel and Rajasekaran, 2014).

5.0 CONCLUSION

This study suggests that UV irradiation of actively growing mycelia of the Nigerian strain *V. volvacea* VNW produced two (2) viable mutants. The yield performance of these mutants, along with the Nigerian wild strain and the Indian wild strains, shows that mutant M5 (irradiated from a distance of 60cm for 75 minutes) proved to be significantly better than the other surviving mutants, the Nigerian wild strain and the Indian wild strains. The mutant strain's fast growth rate and higher nutritional composition make it suitable for commercial cultivation in the Tropics. Developing such high-yielding strains of *V. volvacea* mushroom will enhance commercial mushroom production and improve the supply to meet the increasing demand of consumers.

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6.0 REFERENCES

1. A. O. A. C., Official methods of analysis of the Association of Official Analytical Chemist- AOAC. (18th Edn.). Washington DC. (2005).
2. Abena A. A., Essel E. A., Agbenorhevi, J. K., Oduro, I. N. (2018). Effect of Processing Methods on the Proximate Composition, Total Phenols and Antioxidant Properties of Two Mushroom Varieties *Ame. J Food Nutri*, 6(2): 55-59.
3. Adewoyin, A. G., Barooah, M., Oloke, Bora, S. S. (2017). Identification and physiological properties of a Nigerian strain of *Volvariella* sp. isolated from oil palm waste. *World J Microbiol Biotechnol*, 33: 135. doi 10.1007/s11274-017-2293-7.
4. Ahlawat, O. P. and Harleen, K. (2018). Characterization and optimization of fruit body yield in *Volvariella volvacea* white strain. *Indian J. Exp. Biol.*, 56: 112-120.
5. Ahlawat, O. P., Pardeep, G., Shwet, K. and Dhar, B. L. (2008). Development of molecular and biochemical markers for selecting a potential high yielding strain of paddy straw mushroom (*Volvariella volvacea*). *J. Plant Biochem. Biotechnol*, 17(1): 57–63.
6. Ahlawat, O. P., Singh R., Kumar, S. (2011) Evaluation of *Volvariella volvacea* strains for yield and diseases/insect-pests resistance using composted substrate of paddy straw and cotton mill wastes. *Indian J Microbiol*, 51(2): 200–205.
7. Ainsworth, E. A. and Gillenspie, K. M. (2007). Estimation of total phenolic content and other oxidation substances in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, 2: 875-877.
8. Akinyele, B. J. and Adetuyi, F. C. (2005). Effect of agrowastes, pH and temperature variation on the growth of *Volvariella volvacea*. *Afr. J. Biotechnol*, 4(12): 1390-1395.
9. Apetorgbor, A. K., Apetorgbor, M. M. and Derkyi, N. S. A. (2015). Comparative Studies on Growth and Yield of Oil Palm Mushroom, *Volvariella Volvacea* (Bull. Ex. Fr.) Sing. on Different Substrates. *Greener J. Agric. Sci.*, 5(5): 177-189.
10. Barros L., Calhelha R. C., Vaz, J. A., Ferreira, I. R., Baptista, P., Estevinho, L. M. (2007). Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *Eur. Food Res. Technol*, 225: 151–156.
11. Beejan, P. H. F. and Nowbuth, R. (2009). Use of radiation for the induction of mutation in oystermushroom for improvement of strains. *In*: Shu, Q. Y. (Ed), Induced plant mutation in the

genomic era (pp. 289-292). Rome, Italy, Food and Agricultural. Organization of the United Nation.
12. Boonsong, S., Wanwimol K., Pongtep W. (2016). Antioxidant activities of extracts from five edible mushrooms using different extractants *Agric. Nat. Resour*, 50: 89-97.
13. Buswell, J. A. and Chen, M. (2005). Cultivation, biochemical, molecular biological and medical aspect of the culinary medicinal straw mushroom, *Volvariella volvacea* (Bull. Fr.) Singer (Agarcomycetideae). *Intl. J. Med. Mushr.*, 1(1 & 2): 157-166.
14. Butkhup, L., Wannee S., Sujitar J. (2018). Evaluation of bioactivities and phenolic contents of wild edible mushrooms from north-eastern Thailand. *Food Sci Biotechnol*, 27(1): 193–202.
15. Carmel, S. P. and Rajasekaran, M. (2014). Free radical scavenging activity of fruiting body extracts of an edible mushroom, *Volvariella volvacea* (Bull. ex Fr.) Singer: an *in vitro* study. *Asian J. Biomed. Pharm. Sci*., 4(30): 6-11.
16. Chen, B., Arend, F. van Peer, J. Y., Xiao, L., Bin, X., Juan, M., Qianhui, H. *et al*., (2016). Fruiting Body Formation in *Volvariella volvacea* can Occur Independently of Its MAT-A-Controlled Bipolar Mating System, Enabling Homothallic and Heterothallic Life Cycles. *Genes, Genomes, Genetics*, 6: 2135-2146.
17. Cheung, L. M., Cheung, P. C., Ooi, V. E. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem*., 8(2): 249-255.
18. Corrasco, J., Diego, C. Z., Jose, E. P., Gail, M. P., Arturo, P. G. (2018). Supplementation in mushroom crops and its impact on yield and quality. *AMB Expr*, 8: 146.
19. Da Silva, M. C. S., Mateus, D. N., Jose, M. R., Da Luz, K. M. C. (2013). Mycelia growth of *Pleurotus* spp. in Se-enriched culture media. J. *Adv. Microbiol*, 3: 11-18. http://dx.doi.org/10.4236/aim.2013.38A003.
20. Ding, S., Ge, W. A., Buswell, J. A. (2006). Cloning of multiple cellulose cDNAs from *Volvariella volvacea* and their differential expression during substrate colonization and fruiting. *FEMS Microbiol. Lett*, 263(2): 207-213.
21. Dulay, R. M. R., John, J. A. Vicente, A. G., Dela, C., Jude, M. G., Whilma, F., Sofronio, P. K., Renato, G. R. (2016). Antioxidant Activity and Total Phenolic Content of *Volvariella volvacea* and *Schizophyllum commune* Mycelia Cultured in Indigenous Liquid Media. *Mycosphere*, 7(2): 131–138.
22. El-Fallal, A. A., A., El-Sayed, K. A., Hoda. A., El-Gharabawy, M. (2013) Induction of low sporulating-UV mutant of oyster mushroom with high content of vitamin D2. 3rd International conference on Biotechnology and its Application in Botany and Microbiology, *Aprile*, 17-18.
23. Gasecka, M., Mleczek, M. Siwulski, M. Niedzielski, P., Kozak, L. (2015). The effect of selenium on phelonics and flavonoids in selected edible white rot fungi. LWT *Food Sci Technol*, 63: 726-731, doi:10.1016/j./wt.2015.03.046.
24. Girmay, Z., Gorems, W., Birhanu, G., Zepdie, S. (2016) Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express*, 6: 87. https://doi.org/10.1186/s13568-016-0265-1.
25. Hung, P. G. and Nhi, N. N. Y. (2012). Nutritional composition and antioxidant capacity of several edible mushrooms grown in southern Vietnam. *Int. Food J.*, 19(2): 611-615.
26. Imram. U. H., Muhammad, A. K., Sajid, A. K. and Maqshoof, A. (2011). Biochemical analysis of fruiting bodies of strain Vv pk, grown on six different substrates *Soil Environ*, 30(2): 146-150. https://doi.org/10.5897/AJB11 .4066.
27. Jonathan, G., Adetolu, A., Ikpebivie, O., Donbebe, W. (2006). Nutritive Value of Common Wild Edible Mushrooms from Southern Nigeria. *Global J. Biotechnol. Biochem*, (1): 16-21.
28. Jonathan, S. G. and Fasidi, I. O. (2004). Physico-chemical studies on *Volvariella esculenta* mass (Singer), a Nigerian edible fungus. *Food Chem*., 85: 339- 342.
29. Kang, S. W., Ko, E. H., Lee, J. S., Kim, S. W. (1999). Overproduction of beta-glucosidase by *Aspergillus niger* mutant from lignoocellluosic biomass. *Biotechnol. Lett*., 21: 647-650.
30. Kumar, N., K., Krishnamoorthy, A. S., Kamalakannan, A., Amirtham, D. (2016). Influence of temperature and pH on mycelial growth and chlamydospore production of paddy straw mushroom *Volvariella volvacea* (Bull. Ex Fr.) Sing. *J. Res. ANGRAU*, 44(1/2): 1-7.
31. Kumar, S., Stecher, G., Li, M., Knyaz C., Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547-1549.
32. Liu, Z., Kai Z. I., Jun-Fang L., C. and Li-Qiong G. (2011) Breeding cold tolerance strain by chemical mutagenesis in *Volvariella volvacea*. *Scientia Horticulturae*, 130: 18–24.
33. Mshandete, A. M. and Cuff, J. (2008). Cultivation of three types of indigenous wild edible mushrooms: *Coprinus cinereus*, *Pleurotus flabellatus* and *Volvariella volvocea* on composted sisal decortications residue in Tanzania. *Afri. J. Biotechnol*, 7(24): 4551-4562.
34. Ngamnit S. and Saovapong C. (2014) Development of straw mushroom strain for high yield by gamma radiation, *J Agric. Tech.*, 10(5): 1151-1164.
35. Obodai, M. and Odamtten, G. T. (2012). Mycobiota and some physical and organic composition of agricultural wastes used in the cultivation of the mushroom *Volvariella volvacea. J. Basic Appl. Mycol.*, 3: 21-26.
36. Payapanon, A., Suthirawut, S., Shompoosang, S., Tsuchiya, K., Furuya, N., Roongrawee, P., K., *et al*., (2011). Increase in Yield of the Straw Mushroom

(*Vovariella volvacea*) by Supplement with *Paenibacillus* and *Bacillus* to the Compost. *J. Fac. Agr., Kyushu Univ*., 56(2): 249–254.
37. Puttaraju, G. N., Venkateshaiah, S. U., Dharmesh, S. M., Nanjarajurs, S. M., Somasundaram, R. J. (2006). Antioxidant activity of indigenous edible mushrooms. *Agric. Food Chem.,* 54: 9764−9772.
38. Quimio, T. H. (1993). Indoor cultivation of the straw mushroom, *Volvariella volvacea*. 43. *Mush. Res*., 2: 87-90.
39. Quimio, T. H. (1994). Role of chlamydospores on the fruiting and survival of *Volvariella volvacea*. *Philippines J. Biotechnol.* agris.fao.org 5(12): 160.
40. Ravishanker, S. Pandey, M., Tewari, R. P. Krishna, V. (2006). Development of sporeless/low sporing strains of *Pleurotus* through mutation. *World J Microbiol Biotechnol*, 22: 1021–1025. DOI 10.1007/s11274-0052891-7.
41. Royse, D. J., Jordon, M. H., Antoun, G. G., May, B. P. (1987).Confirmation of intraspecific crossing and single joint segregation of biochemical loci of *Volvariella volvacea*. *Exp*. *Mycol.,* 11: 11-18.
42. Singh, V., Guizani, N., Essa, M. M., Hakkim, F. L. and Rahman, M. S. (2012). Comparative Analysis of Total Phenolics, Flavonoid Content and Antioxidant Profile of Different Date Varieties (*Phoenix dactylifera* L.) from Sultanate of Oman. *Intl. Food Res. J*., 19: 1063-1070.
43. Sivrikaya, H., Bacak, L., Saracbasi, A., Toroglu, L. and Eroglu, H. (2002) Trace Elements in *Pleurotus sajorcaju* cultivated on Chemithermomechanical Pulp for Bioleaching. *Food Chem*., 79: 173-176. https://doi.org/10.1016/S0308-8146(02)00128-0.
44. Sobal, M., Martínez-Carrera, D., Morales, P., Roussos, S. (2007). Classical characterization of mushroom genetic resources from temperate and tropical regions of Mexico. *Mycol Apl Int.,* 19: 15-23.
45. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*), 101: 11030- 11035.
46. Takaki, K., Yamazaki, N., Mukaigawa, S., Fujiwara, T., Kofujita, H., Takahasi, K., Narimatsu, M., Nagane, K. (2009). Improvement of Edible Mushroom Yield by Electric Stimulations *J. Plasma Fusion Res.,* 8: 556-559.
47. Takaki, K., Yoshida, K., Saito, T., Kusaka, T., Yamaguchi, R., Takahashi, K., *et al*., (2014).
48. Effect of Electrical Stimulation on Fruit Body Formation in Cultivating Mushrooms *Microorganisms, 2:* 58-72; doi: 10.3390/microorganisms2010058.
49. Teichmann, A., Paresh, C., Dutta, A. S., Margaretha, J. (2007). Sterol and vitamin D2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. *LWT Food Sci. Technol*, 40: 815–822.
50. Tharun, G. (1993). Promotion of mushroom production and Bioconversion of wastes for income generation in rural areas. CDG 3EAPO's Biotechnology Training Project. *In* Chang, S. T., Bussivell, J. A. and Chril, S. (eds) Mushroom Biology and Mushroom products. Hong Kong Chinese University Press, 307 – 318.
51. Thimmaiah, S. R. (2004). *Standard Methods of Biochemical Analysis*. Kalyani Publishers, New Dehli, India.
52. Thiribhuvanamala, G., Krishnamoorthy, S., Manoranjitham, K., Praksasm, V., Krishnan, S. (2012). Improved techniques to enhance the yield of paddy straw mushroom (*Volvariella volvacea*) for commercial cultivation. *Afr. J. Biotechnol*, 11(64): 12740–12748.
53. Thuc, L. V., Rizal G. C., Julius T. S., Ngo Thi T. T., Phan H. H., Remelyn E. R., Elmer B., (2020). Rice straw mushroom production http://ricestraw.irri.org/resources-1
54. Tripathy, A., Patel, A. K., Sahoo, T. K. (2011). Yield evaluation of paddy straw mushrooms various lignocellulosic wastes. *Asian J. Plant Sci*., 4: 566-569.
55. Triyono, S. Agus, H., Mareli, T., Dermiyati, J. L., Filip, T. (2019). Cultivation of straw mushroom (*Volvariella volvacea*) on oil palm empty fruit bunch growth medium. *Intl. J. Recycl. Org. Waste Agric*., 8: 381–392.
56. Ukoima, H. N., Ogbonnaya, L. O., Arikpo, G. E. and Ikpe, F. E. (2009). Cultivation of mushroom *Volvariella volvacea* on various farm wastes in Obrubra Local Government of Cross Rivers State, Nigeria. *Pak. J. Nutr.,* 8(7): 1059-1061.
57. Zervakis, G., Philippoussis, A., Ioannidou, S. and Diamantopoulou, P. (2001). Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible Mushroom species on Lignocellulosic substrates. *Pak. J. Nutr.,* 46(3): 231- 234.