African Scientist Vol. 17, No. 1 March 31, 2016
Printed in Nigeria

1595-6881/2016 $10.00 + 0.00
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http://www.niseb.org/afs

AFS 2015009/17103

Effect of Sclerotia Weight and Watering Regime on the Growth and Development of *Pleurotus tuber-regium* (Fr.) Singer

Faith Efosa Oviasogie[1], Emmanuel Oluwadare Akpaja[2]*, Kesiena Clementina Gbona[3] and Gloria Esohe Oriakhi[4]

[1]Department of Microbiology, Tel: +2348057829907, Email address: efosaoviasogie@yahoo.co.uk,
[2]Department of Plant Biology and Biotechnology, Tel: +2348034108935, Email address: akpajauniben@yahoo.com,
*Corresponding author
3Department of Plant Biology and Biotechnology, Tel: +2347033137729, Email address: kessydebest@yahoo.com
4Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

(Received February 20, 2015; Revised version accepted December 26, 2015)

ABSTRACT: The study was carried out to determine the effect of sclerotia weight (10, 25, 50 and 100 g), watering regime and urea solution on the growth and development of P. *tuber-regium*. The experiment comprised four watering regimes with either water or urea solution (1g/litre) and four weights of sclerotia were replicated five times in a completely randomised design. The watering regime comprised watering once daily, twice daily, thrice daily and once alternate daily. The earliest time (6.00 ±0.63 days) of primordial emergence occurred in 100 g of sclerotia watered once daily. The highest fresh weight of P. *tuber-regium* harvested was in 100 g of sclerotia watered twice daily (128.48 ±25.89g) with water and 100 g of sclerotia watered once daily with urea solution (122.91 ±23.15 g). Weight loss in sclerotia ranged from 17.60 to 44.20% for sclerotia watered with tap water and from 17.60- 70.40 % with urea solution. Fruit body yield was significantly higher (p=0.05) in 50g sclerotia pieces watered once daily with urea solution. This sclerotia weight is therefore recommended for the fruit body production of the mushroom.

Key words: P. *tuber-regium*, sclerotia size, urea solution, vegetative growth, watering regime

Introduction

Pleurotus tuber-regium (Fr.) Singer, a basidiomycetous fungus of economic importance, is an edible mushroom in tropical and subtropical regions of the world. It is found on dead decaying logs in tropical Africa, Asia and Australia (Isikhuemhen et. al., 2000). The sclerotia of P. *tuber-regium* is irregular in shape, light or dark brown in colour on the surface depending on the variety and substrate on which it was formed, but usually white inside (Okhuoya and Etugo, 1993). The sizes and weight of the sclerotia could vary and may be as large as 30 cm and weigh over 5 kg (Isikhuemhen and Okhuoya, 1995).

The sclerotia of the fungus can be stored for a very long time without loss of viability, and can later be used for sporophore induction. The induced sporophore can be consumed or used as condiment to add flavour to food. *P. tuber-regium* fruit body is highly nutritive and rich in protein, sugar, lipids, amino acids, glycogen, vitamins (B, C and D) and mineral elements (Zoberi, 1972; 1973 and Oso, 1977). The medicinal and nutritional content of the sclerotium of the fungus has been investigated by Oso (1975), Singer (1986) and Stamets (2000).
Mushrooms are cultivated on composted substrate bed, wet timber, banana pseudostem, sawdust, oil palm fibre and other agricultural wastes (Anoliefo et. al., 1990 and Quimio et. al., 1990). Commercial production of edible mushrooms is very limited in the African continent although many people still collect and eat wild edible mushrooms (Okhuoya and Ajerio, 1988).

The field trial of the production of P. *tuber-regium* is largely lacking. However, Akpaja et. al. (1999) and Akpaja and Begho (1999) used it as an intercrop with mature para rubber (Hevea brasilliensis). Akpaja et. al. (1999) showed that mulching with sawdust improves the market value.

As a follow-up to the earlier studies on the development of basic agronomic practices for the fruit body production of the mushroom from its sclerotia, this study aims to determine the effects of sclerotia weight, watering regime and supplementation on the growth and development of the test mushroom.

Materials and Methods

Planting of sclerotia and management:

The sclerotia were obtained from Ekiuwa market, Benin City, Edo state, Nigeria. Urea was bought from ADP, Benin City, Nigeria while garden soil was obtained from the Botanical Garden of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Sclerotia were sundried for 7 days and were cut into different weights namely; 10, 25, 50 and 100 g. These were soaked in water for 24 hours. The soil was filled into black polythene bags (in 15 cm x 30 cm) and was drenched with water. Each group of sclerotia were planted into the soil.

Watering regime:

Four watering regimes; once daily, twice daily, thrice daily and once on alternate day was used. In all the different watering regimes, 20 ml of water was used. In another set of the experiment, water was replaced with urea solution. Urea solution was prepared by dissolving 1g of urea in 1 litre of water (Quimio et al., 1990).

Harvest and post harvest handling:

The fruiting bodies of the mushroom were harvested four days after primordial emergence. Fresh weight of the mushroom was determined immediately after harvest. Dry weight was determined by oven-drying fresh mushroom at 1000C to constant weight.

Statistical analysis:

The experiment comprised four watering regimes with either water or urea solution (1g/litre) and four weights of sclerotia were replicated five times in a completely randomised design. Data obtained were analyzed by using analysis of variance (ANOVA) and significant means were separated using Duncan's multiple range test.

Results and Discussion

This study shows that the watering regime and sclerotia size significantly (p=0.05) affects the production of fruit bodies from the sclerotium of *Pleurotus tuber-regium*.

The time of primordial emergence varied significantly among the treatments (Table 1). The earliest time recorded (6.00 ±0.63 days) was from 100 g of sclerotia watered once daily while 10 g of sclerotia watered once alternate daily gave the longest time of primordial emergence (27.60 ±1.94 days). When water was replaced with urea solution, the time of primordial emergence also varied among treatments (Table 2). 25 g sclerotia watered thrice daily gave the earliest time of primordial emergence (6.60 ±0.93 days) while 10 g watered twice daily gave the longest (21.80 ±6.92 days) time of primordial emergence. In this study, primordial appeared much earlier than was observed by Isikhuemhen et al. (2000). They recorded time of primordial emergence of 18-62 days after inoculation. Akpaja et al. (1999) recorded 44-66 days and Okhuoya and Etugo, (1993) recorded 12-40 days of primordial emergence on different substrates. This variation in time of primordial emergence may have connection with the age of sclerotia used, method of storage before use and the inherent genetic variability common to all sets of organisms.

Table 1: Effect of watering regime and sclerotia weight on the time of primordial emergence of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	17.75 ±4.61ab	9.33 ±0.88b	7.60 ±1.03a	6.00 ±0.63b
Twice daily	8.20 ±1.02b	13.80 ±1.72ab	9.40 ±2.11a	7.80 ±0.20b
Thrice daily	24.00 ±5.87a	10.80 ±2.28b	11.00 ±3.54a	7.80 ±1.53b
Once on alternate days	27.60 ±1.94a	18.75 ±2.78a	9.75 ±2.75a	10.80 ±

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Table 2: Effect of urea watering regime on the time of primordial emergence of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	16.25 ±2.63a	12.20 ±2.63ab	10.40 ±2.77a	9.40 ±1.98a
Twice daily	21.80 ±6.92a	10.00 ±1.52ab	10.20 ±2.96a	8.40 ±1.12a
Thrice daily	13.75 ±5.51a	6.60 ±0.93b	11.00 ±2.59a	7.20 ±0.74a
Once on alternate days	14.25 ±2.51a	15.40 ±2.54a	7.80 ±0.97a	8.60 ±0.93a

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Fresh weight of fruit bodies of *P. tuber-regium* harvested increased with weight of sclerotia for all the watering regimes but varied within weights (Tables 3 and 4). The least weight recorded for water-only treated sclerotium was 5.25 ±1.55g (10 g of sclerotia watered alternate daily), while for urea, it was 7.13 ±2.04g (10 g of sclerotia watered twice daily). Oghenekaro et al. (2010a) recorded lower values of dry weight of P. *tuber-regium* watered with mushroom extract than with ordinary water. The yield obtained for 50 and 100 g of sclerotia weight was higher than that reported by Akpaja et al. (1999) but the number of flushes was generally lower.

Table 3: Effect of watering regime and sclerotia weight on the fresh weight (g) of fruit bodies of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	12.27 ±4.13a	21.23 ±3.64a	38.82 ±4.45a	112.36 ±20.44ab
Twice daily	8.70 ±2.08a	17.70 ±2.16a	48.22 ±7.00a	128.48 ±25.89a
Thrice daily	7.00 ±15.05a	21.96 ±4.82a	39.33 ±6.81a	96.22 ±18.73ab
Once on alternate days	5.25 ±1.55a	14.43 ±2.96a	45.57 ±11.40a	65.30 ±6.85b

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Table 4: Effect of urea watering regime on the fresh weight (g) of fruit bodies of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	8.70 ±1.61a	18.83 ±5.44b	80.77 ±16.74a	122.91 ±23.15a
Twice daily	7.13 ±2.04a	23.62 ±6.38ab	56.04 ±16.64a	97.49 ±21.02a
Thrice daily	11.15 ±3.13a	36.24 ±3.67a	47.61 ±9.54a	97.70 ±10.34a
Once on alternate days	8.63 ±1.92a	24.20 ±2.24ab	82.94 ±25.33a	86.74 ±12.22a

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Dry weight of P. *tuber-regium* also increased with weight of sclerotia and varied within weights (Table 5). This was also observed by Oghenekaro et al. (2010b). The highest dry weight (25.18 ± 4.49g) was obtained from 100 g of sclerotia watered twice daily. Watering with urea solution showed the same trend (Table 6). The highest dry weight obtained was 26.39 ±5.47g (from 100 g of sclerotia watered with urea solution once daily). The number of flushes varied between weights for sclerotia watered with urea solution and that watered with tap water (Tables 7 and 8).

Table 5: Effect of watering regime and sclerotia weight on dry weight (g) of fruit bodies of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	2.26 ±1.05a	4.82 ±0.21a	7.32 ±1.08a	20.44 ±4.72a
Twice daily	1.58 ±0.35a	3.01 ±0.70a	8.21 ±1.67a	25.18 ±4.49a
Thrice daily	1.71 ±0.30a	3.44 ±0.85a	6.29 ±1.16a	13.88 ±1.56a
Once on alternate days	0.82 ±0.22a	3.30 ±0.25a	9.78 ±2.44a	16.88 ±2.67a

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Table 6: Effect of urea watering regime on dry weight (g) of fruit bodies of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	1.40 ±0.27a	3.45 ±1.12a	16.02 ±3.45ab	26.39 ±5.47a
Twice daily	1.24 ±0.34a	3.90 ±0.95a	11.27 ±3.22ab	18.85 ±3.88a
Thrice daily	1.75 ±0.56a	5.85 ±0.68a	7.91 ±1.62b	21.22 ±2.39a
Once on alternate days	2.00 ±0.30a	4.13 ±0.51a	17.36 ±2.63a	24.80 ±3.97a

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Table 7: Effect of watering regime and sclerotia weight on number of flushes of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	1.67 ±0.33a	1.33 ±0.33a	1.40 ±0.25a	2.00 ±0.45a
Twice daily	1.00 ±0.00b	2.00 ±0.32a	1.60 ±0.25a	2.00 ±0.32a
Thrice daily	1.00 ±0.00b	1.40 ±0.25a	1.50 ±0.29a	2.00 ±0.45a
Once on alternate days	1.00 ±0.00b	1.25 ±0.25a	2.00 ±0.41a	1.60 ±0.25

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Table 8: Effect of urea watering regime on number of flushes of *Pleurotus tuber-regium*

	Sclerotia weight (g)			
Watering regime	10	25	50	100
Once daily	1.50 ±0.29a	1.25 ±0.25a	3.00 ±0.332a	2.20 ±0.58a
Twice daily	1.67 ±0.33a	1.60 ±0.25a	2.00 ±0.45ab	2.60 ±0.60a
Thrice daily	1.50 ±0.29a	1.60 ±0.40a	1.60 ±0.25b	3.00 ±0.55a
Once on alternate days	1.25 ±0.25a	1.50 ±0.29a	2.60 ±0.06ab	2.60 ±0.51a

Means of five replicates. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

The weight of spent sclerotia and percentage organic matter loss varied from one watering regime to the other (Tables 9 and 10). Percentage organic matter loss ranged from 17.60% for 100 g of sclerotia watered thrice daily to 44.20% for 100 g of sclerotia watered thrice daily. With urea solution, it ranged from 17.60% for 100 g sclerotia watered twice daily to 70.40% for 50 g of sclerotia watered once daily. This implies that watering regime may have a profound effect on organic matter loss of sclerotia used as planting material. Increase in fruit body yield from sclerotia will concomitantly increase its organic matter loss. For sclerotia watered with urea solution, organic matter loss was higher from 10 g to 50 g sclerotia weight. The ability of urea solution to increase the yield of mushroom was earlier mentioned by Quimio et al. (1990).

Table 9: Effect of watering regime and sclerotia weight on spent sclerotia of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	6.16 (38.40)a	15.63 (37.60)a	37.02 (25.90)a	58.19 (41.81)a
Twice daily	8.00 (22.10)a	17.80 (28.80)a	34.37 (31.20)a	55.56 (44.20)a
Thrice daily	7.54 (24.60)a	17.93 (28.40)a	32.33 (35.40)a	82.42 (17.60)a
Once on alternate days	8.02 (19.80)a	17.73 (29.20)a	37.17 (25.60)a	80.56 (1940)a

Means of three replicates in grams with organic matter loss (%) in parenthesis. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

Table 10: Effect of urea watering regime on spent sclerotia of *Pleurotus tuber-regium*

Watering regime	Sclerotia weight (g)			
	10	25	50	100
Once daily	6.67 (33.40)a	13.77 (44.80)a	14.72 (70.40)b	70.67 (29.30)ab
Twice daily	5.61 (43.90)a	15.87 (36.40)a	28.76 (40.40)a	82.38 (17.60)a
Thrice daily	4.95 (50.50)a	10.00 (60.00)b	30.40 (39.20)a	60.30 (39.70)b
Once on alternate days	6.33 (36.70)a	15.93 (36.40)a	25.70 (48.60)a	59.30 (40.70)b

Means of three replicates in grams with organic matter loss (%) in parenthesis. Means followed by the same letters within a sclerotia weight (column) are not significantly different from each other at p=0.05 by Duncan's multiple range tests.

In conclusion, the yield of P. *tuber-regium* was improved by urea. Sclerotia size also affected the yield. The development of basic agronomic practices for the production of fruit bodies via the sclerotium is on-going. The integration of the different research findings on the subject matter will therefore pave way for the large scale production of the mushroom. Till date, such an enterprise is largely lacking in Nigeria.

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