

**Full Length Research Paper**

Performance Characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s. lato grown on Grass Substrate Incorporating Brown Seaweed *Sargassum* Species Supplements at Various Rates in Tanzania

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Abstract

Coprinus cinereus (Schaeff.) S. Gray s. lato, is a warm tropical, robust and fast growing saprophytic edible and medicinal indigenous Tanzanian mushroom. Grass basal substrate was supplemented with nine different *Sargassum* species supplements referred as (supplement 1, 2, 3, 4, 5, 6, 7, 8 and 9) at seven different concentration rates % (0.5, 1, 2, 5, 10, 15, 20). The combinations were tested for their influence on performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom namely; basidiocarps numbers, weights, pileus and stipe lengths, mushroom size, production rate and productivity using solid-state fermentation bioreactors (SSFs). The substrate and supplements pasteurization, spawn preparation and spawn running were carried out following standard methods. The combinations mixtures were inoculated with 5% spawn wet/wet basis. The inoculated mixtures were arranged in a completely randomized design on shelves in the mushroom growing room and incubated at ambient temperature (25±2°C). Manually spraying water on the walls and placing open containers filled with water in the corners of the room controlled the relative humidity of the mushroom growing room. Data were analyzed using SPSS version 15.0. Results showed that regardless of grass substrate, the interaction of nine brown seaweeds *Sargassum* species supplements with seven varying concentrations of supplements produced extreme significant effect ($p < 0.0001$) on mushroom performance characteristics variable analyzed. Mean durations from pinning to harvestable young immature fruiting bodies ranged from 1 to 3 days. Among nine supplements comparatively overall best mushroom performance characteristics variables analyzed were recorded from supplement 4 (comprised of *Sargassum poligocytum* brown seaweed, 8 cm tip-part, which, represented the young parts of *Sargassum* frond) at 15% concentration rate. It gave highest mean number of fruiting bodies of 85.00, highest average mushroom dry weight of 4.81g, highest mean production rate of 2.23 and highest mean productivity P, of 16.69%. On the other hand, supplement 4 recorded highest mean total fresh mushroom weight of 83.43 g at 10% concentration rate while relatively longest mean pileus (cap) of 20.70 mm was obtained at 0.5% concentration rate. The present findings indicate the potential of brown seaweeds supplement 4, which could be recommended for cultivation *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom on grass substrate for culinary, nutraceutical and therapeutic purposes.

Key words: *Sargassum*, *Coprinus cinereus*, Supplement, Basidiocarps, Productivity

Introduction

The conventional mushroom cultivation method, which utilizes wood logs, sawdust and wood chips means cutting down trees. Resulting into conflict between mushroom production, forest protection, environment safety and sustainability. Successful substitution of wood in mushroom growing by grasses would set the mushroom cultivation industry on an eco-friendly and sustainable path (Lin and Lin, 2001). Although 70% Tanzania total area is covered by natural grassland area of 658,563 km², grasses are regarded of little use hence underutilized and viewed as a waste. Nevertheless, worldwide there has been a change of the idea that grass is of little use but a potential strategic bioresource for biovalorization into valuable bio-products such as feed, biofuels, biofertilizer and food. To that effect JUNCAO technology developed by Chinese researchers in 1980's utilizing grasses in growing edible and medicinal mushrooms (Rolim et al., 2014). It is considered a significant technology amongst various cultivation methods that have been developed so far. It also represents a revolution in the mushroom production system by increasing the amount of mushrooms produced, increasing nutritional value of mushrooms and enhancing bioactive compounds concentration of the active principles in mushrooms compared to classical methods (Zhanxi and Zhanhua, 2001; Liang et al., 2011; Rolim et al., 2014). JUNCAO technology current utilizes 45 types of wild grasses to grow 55 types of edible and medicinal mushrooms. Mushrooms, which have been, tested with varying degrees of success thus far, include *Agaricus*, *Ganordema*, *Lentinula*, *Pleurotus*, *Coprinus comatus* (O.F.M&unml;II) Gray etc. For each mushroom type, specific local ground-up grasses substrate formulations with ingredient supplements, temperature and other requirements have been developed and some process have been patented (Zhanxi and Zhanhua, 2001).

Coprinus cinereus (Schaeff.) S. Gray s. lato (sisal compost mushroom) indigenous Tanzanian mushroom, is a multicellular basidiomycete with a typical mushroom form that undergoes a complete sexual cycle, it usually complete its entire crop period within four weeks (Mwita et al., 2011a, Mshandete, 2014). This confers it a unique potential for developing year round production technologies and manipulation in research activities considering its fastest growing nature under artificial condition. To that effect a number of researches have been conducted on *Coprinus cinereus* (Schaeff.) S. Gray s. lato such as domestication and optimization of growth conditions, analysis of nutritive value, determination of antimicrobial activities, antioxidants as well as studied on various effects of supplements such as cow dung manure, rice bran, chicken manure and human urine on yield and performance characteristics (Mshandete and Cuff, 2007; 2008, Ndyetabura et al., 2010; Mwita et al., 2011a; Mshandete, 2011; Tibuhwa et al., 2012; Raymond et al., 2012).

Most of the previous research endeavours on cultivation of *Coprinus cinereus* (Schaeff.) S. Gray s. lato has limitedly utilized sisal wastes namely; sisal fibres, sisal leaves, sisal dust, composted sisal waste and sisal bole (single or mixed with other sisal wastes) as substrate mainly because it is a the natural substrate where it is found growing in the wild in sisal processing factories in Tanzania. As such could also limit small-scale, medium-scale and commercial-scale mushroom production in those areas only considering that the substrates will be obtained within the vicinity sisal processing factories. Since *Coprinus cinereus* (Schaeff.) S. Gray s. lato is defined as mushroom having edible and medicinal value, it has a potential also be utilized as biological reactors in industry for the synthesis of proteins and biological active compounds. The proteins manufactured in mushrooms are believed to have higher specific biological activities in humans than those produced from other sources such as plants and animals. With such envisaged future anticipated potential of *Coprinus cinereus* (Schaeff.) S. Gray s. lato it is imperative to look for alternative substrate, which is more readily available in Tanzania for higher biomass mushrooms production in mushroom growing facility with the option of manual and automation in medium-scale and commercial scale.

The most alternative potential substrate could be grasses, which is abundant, readily available as well as the whole plants could utilized. Grass could not only provide as alternatives substrate for tropical *Coprinus cinereus* growing they can also significantly increase protein content and reduce production time. Despite wide diversity, varieties, ecological distribution, abundance and high productivity of native tropical grass species such as *Echinochloa hypoclada* (stapf), *Heteropogon contortus*, *Panicum maximum* (jacq), *Chloris roxburghiana* (schult), *Chloris gayana* (kunth) and *Hypparhenia rufa* (Nees) stapf etc found growing in many places in Tanzania. The utilization grass as basal substrate for cultivation of *Coprinus cinereus* (Schaeff.) S. Gray s. lato is limited only to recent work by Ndyetabura (2010) and Mshandete (2014). Furthermore, regardless of high diversity of seaweeds (marine bioresources) in Tanzania, in particular genus *Sargassum*, are generally untapped, not realized and underutilized particularly in the tropical Western Indian oceans region (Lobban and Harrison, 1994, McHugh, 2003; Oliveira et al., 2005). Their usage as supplements (seaweeds marine resources) in grasses (terrestrial bioresource) as main substrates for cultivation of *Pleurotus* and *Coprinus* mushroom is quite uncommon in Africa and elsewhere in the world (Molloy et al., 1999, 2003, Kaaya et al., 2012 and Mshandete, 2014). There are no research findings that have been conducted regarding effect of brown seaweeds *Sargassum* species as supplement on agronomic performance of *Coprinus cinereus* (Schaeff.) S. Gray s. lato grown on grass. Moreover, incorporation of locally grounded *Sargassum* brown seaweeds marine bioresource as grass terrestrial bioresource substrate supplement considered in this study was the first of its kind in Tanzania and elsewhere in the world (Mshandete, 2014). Most reports showed that substrates for *Coprinus cinereus* (Schaeff.) S. Gray s. lato could be supplemented with rice bran, chicken manure, cow dung manure or human urine (Ndyetabura et al., 2010; Mshandete, 2011; Mwita et al., 2011a and Raymond et al., 2012). Therefore the present study investigated the influence of different brown seaweeds *Sargassum* species supplement at various rates on performance characteristics mushroom size, basidiocarp numbers, weight, pileus and stipe length, productivity and production rate of *Coprinus cinereus* (Schaeff.) S. Gray s. lato grown on grass as main substrate.

Materials and Methods

Source and maintenance of Coprinus cinereus (Schaeff.) S. Gray s. lato culture

The culture was obtained from author's collection kept in culture bank of Department of Molecular Biology and Biotechnology, University of Dar es Salaam in Tanzania. The stock culture subculturing, short and long-term culture storage was carried out as reported previous by Mshandete (2011).

Spawn preparation

Spawn (mushroom seed) was prepared with sterilized sorghum grains and was inoculated with 4 day-old agar blocks cultures of *Coprinus cinereus* (Schaeff.) S. Gray s. lato and allowed for mycelial ramification per Ndyetabura et al. (2010). After full colonization, grains were aseptically inoculated into pasteurized grass substrate supplemented with *Sargassum* brown seaweeds based formulations and transferred in solid-state fermentation bioreactors (SSFs).

Sargassum plant specimen's collection and preliminary identification

Sargassum brown seaweeds specimens were collected by hand from Mji Mwema and Oyster Bay beaches, along the Dar es Salaam coast during spring low tide in Tanzania. They were identified, sorted into five categories namely; basal part (older), middle part (mid age), tip part (young), whole frond and unsorted and were pre-treated by sun drying and washing with fresh water twice (Table 1) before milled into fine powder as described in details by Mshandete (2014).

Table 1: Glossary of *Sargassum* species brown seaweeds fronds sample treatments (Adapted from Mshandete, 2014).

| Supplement No | Name of the Species | Treatment | Fresh water washing regime |
|---------------|------------------------------|----------------------------------|----------------------------|
| Supplement 1 | <i>Sargassum oligocytum</i> | Whole plant | Washed twice |
| Supplement 2 | <i>Sargassum polycystum</i> | Basal part with variable lengths | Washed twice |
| Supplement 3 | <i>Sargassum polycystum</i> | 10 cm, middle-part | Washed twice |
| Supplement 4 | <i>Sargassum poligocytum</i> | 8 cm tip- part | Washed twice |
| Supplement 5 | <i>Sargassum equifolium</i> | Basal part with variable lengths | Washed twice |
| Supplement 6 | <i>Sargassum equifolium</i> | 10 cm, middle-part | Washed twice |
| Supplement 7 | <i>Sargassum equifolium</i> | 8 cm tip- part | Washed twice |
| Supplement 8 | <i>Sargassum spp</i> | Unsorted | Washed twice |
| Supplement 9 | <i>Sargassum spp</i> | Unsorted | Washed twice |

Substrate, supplements preparation and experimental design

Fresh grasses were collected from Mwalimu J.K. Nyerere Mlimani Campus, University of Dar es Salaam (UDSM), Tanzania while fresh cow dung manure was obtained from Vingunguti abattoir, Ilala Municipal, Dar es Salaam, Tanzania. Grasses and cowdung manure were prepared and treated as described in details by Mshandete (2014). Grass basal substrate formulation incorporating seaweeds supplement at different concentrations % (rates) and cow dung manure during inoculation of the substrate mixtures (Table2) were as per Mshandete (2014). Grass alone without *Sargassum* species brown seaweeds was used as a control to assess the effects of supplemented *Sargassum* species brown seaweeds.

The different mixture formulations were then transferred into rectangular plastic containers as cropping containers here referred to solid-state fermentation bioreactors (SSFs) described previously by Mwita et al. (2011a). A total of 195 SSFs were set up including 189 experimental with nine (9) supplements of *Sargassum* species brown seaweeds fronds at seven different concentration (%) rates (Table 2) in triplicates and 6 control (0% supplement or 100% grass) i.e. grass basal substrate alone without *Sargassum* species brown seaweeds fronds.

Table 2: Grass basal substrate formulation incorporating seaweeds supplement at different concentrations % (rates) and cow dung manure during inoculation of the substrate mixtures. (Adapted from Mshandete, 2014).

| Seaweeds concentrations (%) in substrate | Equivalent supplement seaweed in substrate (grams) | Substrate grass (grams) | Cow dung manure in substrate (grams) | Spawn (grams) |
|--|--|-------------------------|--------------------------------------|---------------|
| (Control) 0 | 0 | 500 | 10 | 25 |
| 0.5 | 2.5 | 497.5 | 10 | 25 |
| 1 | 5 | 495 | 10 | 25 |
| 2 | 10 | 490 | 10 | 25 |
| 5 | 25 | 475 | 10 | 25 |
| 10 | 50 | 450 | 10 | 25 |
| 15 | 75 | 425 | 10 | 25 |
| 20 | 100 | 400 | 10 | 25 |

Developmental stages of basidiocarp formation

The full colonized of sorghum grains were aseptically inoculated into solid-state fermentation bioreactors (SSFs) containing grass basal substrate formulation incorporating seaweeds supplement at different concentrations % (rates) and cow dung manure during inoculation of the substrate mixtures. SSFs were incubated at room temperature range of 25±2 °C and humidity of 78±2 % and until the substrates was completely ramified by mycelia. The fully colonized substrates were removed from the SSFs and placed on the container lid but were covered with the main SSFs on top to avoid moisture loss. They were sprayed with tap water twice a day using a hand sprayer to keep them moisturized (Figure 1). Manually spraying water on the walls and placing open containers filled with water in the corners of the room controlled the relative humidity and maintained moisture in the mushroom growing room. To allow fruiting initials to develop into basidiocarps, the fully ramified SSFs were opened occasionally as fruiting progressed. The developmental stages of fruit body formation from mycelial extension to the production of mushroom primordia “pinning”, the successive development of primordia into mushrooms “fruiting” were monitored and photo-documented. However, in all substrates, the number of pinhead abortions, which did not grow into marketable products were observed but not quantified (counted) per each SSFs.



Figure 1: A section of fully colonized substrates placed on red top lid and covered with main SSFBs container on top to avoid moisture loss and also sprayed with fresh water to maintain moisture.

Performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushrooms variables analyzed

Coprinus cinereus (Schaeff.) S. Gray s. lato fruiting body easily matures and once the cap has expanded and opens it easily deteriorates and ultimately becomes inky mass due to its autolytic character (deliquescence) resulting to the discharge of its basidiospores (Reyes et al., 2009). Since *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushrooms are highly perishable, fruiting bodies were harvested when whitish young, firm and flesh (immature/juvenile) when suitable for food (edible) (Reyes et al., 2009). Crop period (sum of incubation and fruiting periods) was calculated.

(i) Mushroom numbers (MN)

The numbers of harvested young, firm and flesh (immature) fruiting bodies were counted and recorded daily until the end of harvest period. The accumulated data were kept separate for each SSFB in each supplement of *Sargassum* species brown seaweeds fronds at each concentration rate and expressed as mean numbers. The number of pinhead abortions was observed but were not counted in all treatments investigated.

(ii) Fresh weight of mushrooms (FWM)

Fresh weight of mushrooms (FWM) was determined daily for each SSFB during the harvesting phase using a weighing scale Adventurer TM balance (Ohaus Corp, Pine Brook, NJ, USA). The accumulated data were kept separate for each SSFB in each supplement of *Sargassum* species brown seaweeds fronds at each concentration rate and expressed as mean grams (g) fresh weight/SSFB. The accumulated weight of mushrooms determined the proportion of the total fresh weight mushrooms harvested in each supplement of *Sargassum* species brown seaweeds fronds at each concentration rate and was expressed in grams.

iii) Dry weight of mushrooms (DWM)

Fresh mushroom were dried in drying oven at 45°C to a constant weight as per Mshandete and Cuff (2007), Sanyo OMT oven (GallenKamp, UK) daily for each SSFB until the end of harvest period and the dry weight was determined using a weighing scale, Adventurer TM balance (Ohaus Corp, Pine Brook, NJ, USA). The accumulated data were kept separate for each SSFB in each supplement of *Sargassum* species brown seaweeds fronds at each concentration rate and expressed as mean grams (g) dry weight/SSFB.

(iv) Mushroom size (MS)

Mushroom size was determined as the ratio of total weight of fresh mushrooms harvested divided by total effective number of mushrooms harvested (Royse et al., 2004). The accumulated data were kept separate for each SSFB in each supplement of *Sargassum* species brown seaweeds fronds at each concentration.

(v) Pileus length (PL) and Stipe length (SL)

Measurements of pileus (cap) length and the stipe length were done for whitish young, firm and flesh (immature/juvenile) fruiting bodies using a string and transparent ruler and were expressed in mm.

(vi) Production rate (PR)

Production rate was determined as the ratio of biological efficiency (BE) % divided by crop period (Chang and Hayes, 1978).

(vii) Productivity (P, %)

Productivity was determined as percentage as the ratio of mushroom fresh weight and substrate fresh weight multiplied by 100 according to Andrade et al. (2007).

Statistical analysis

All experiments were carried out in triplicates to ensure reproducibility and all data were expressed as mean \pm S.D. The experiments were completely randomized design (CRD) with grass basal substrate; nine (9) *Sargassum* species brown seaweeds fronds supplements, seven (7) different supplement concentrations rates and *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom. Data analysis was done primarily using descriptive statistics such as the comparison of treatment means and percentages. The collected data for mushroom numbers, mushroom weights, cap and stipe lengths, mushroom size, production rate and productivity % were subjected to analyses of variance (one-way ANOVA) at the 5% level (significant different at $p < 0.05$) using the Statistical Package for Social Sciences (SPSS) Program 15.0. Version SPSS, (SPSS, 2006).

Results and discussion***Progressive development of Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom.**

The progressive development of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom in most treatment solid-state fermentation bioreactors (SSFs) containing grass incorporating nine (9) *Sargassum* species brown seaweeds fronds supplements, at seven (7) different concentrations % (rates) showed that most treatment were fully colonized after five days of incubation. However, the extent of colonization differed depending on the amount of supplement and the type of supplement added. Primordial (pinning) were observed for most supplements types and concentrations on day 6 that is one day after fully colonization. Most treatment solid-state fermentation bioreactors (SSFs) took 1 day i.e. day 7 to 2 days i.e. day 9 from pinning to attain immature mushroom fruiting bodies harvesting stage (Figure 2f). Harvesting of mushrooms went up to two weeks when very few or no mushroom fruiting bodies were flashing from the substrates, which implied that the entire crop period took 23 days. However with exception of 15 and 20 % concentrations rates for supplement 8 and 9 immature mushroom fruiting bodies harvesting stage was delayed until two weeks. Generally the duration to attain immature mushroom fruiting bodies harvesting stage after pinhead formation showed variations among different nine (9) *Sargassum* species brown seaweeds fronds supplements and seven (7) different concentrations % (rates) for each particular replicates. Figure 2 shows the progressive development of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom. However, the successive development from mycelia extension on the substrate (vegetative or colonization phase) into mushroom primordia "pinning" and final fruiting body formation "fruiting" (reproductive phases) seems were influenced by *Sargassum* species brown seaweeds fronds supplements and their different rates applied in grass basal substrate. That implied that it is most probably that incorporation of *Sargassum* species brown seaweeds in grass in return influenced the mycelium, which grows through the substrate, biodegrades its components and supports the formation of fruiting bodies. Similarly Wood and Smith (1987) had previously observed that both vegetative and reproductive phases are very much influenced by the physiological condition and nutritional state of the mycelium. Cropping period is of the imperative factors applicable for any commercial mushroom growing venture anywhere in the world (Gume et al., 2013). The completion of crop period within three weeks demonstrated by *Coprinus cinereus* (Schaeff.) S. Gray s. lato implied it is a fast growing genetic mushroom resource in Tanzania, potential for further development of production technologies for commercial attributes. The present findings collaborates previous findings for *Coprinus cinereus* (Schaeff.) S. Gray s. lato grown either, on grass alone, non-composted sisal wastes (sisal bole, sisal leaf wastes, sisal dust waste, and sisal fibre wastes) composted sisal wastes alone or mixed supplemented with chicken manures, cow dung manure, human urine at various rates (Mshandete and Cuff, 20008; Ndyetabura et al., 2010; Mwita et al 2011a; Mshandete, 2011 and Raymond et al., 2012).. The current findings also furthermore collaborates previous studies on completion of mycelium colonization on the substrates after spawning of 4-8 days; pin head formation (appearance minute fruiting bodies, primordia) of 8-10 days, first harvestable mushrooms picking of 6-10 days and 21-28 days for completion of cropping period (Mshandete and Cuff, 20008; Ndyetabura et al., 2010; Mwita et al 2011a; Mshandete, 2011 and Raymond et al., 2012). Cropping period of three weeks for *Coprinus cinereus* (Schaeff.) S. Gray s. lato is relatively short which, implies that that more mushroom biomass can be realized in a short period of time, which is beneficial for mass production in industrial mushroom production.





Figure 2: Progressive development of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom: (a), Pure plate culture of *Coprinus cinereus* (Schaeff.) S. Gray s. lato; (b), Spawns (mushroom seeds) of *Coprinus cinereus* (Schaeff.) S. Gray s. lato ready for use; (c), Full *Coprinus cinereus* (Schaeff.) S. Gray s. lato mycelia extension on grass substrate incorporating *Sargassum* species brown seaweeds in SSFBs; (d), Miniature *Coprinus cinereus* (Schaeff.) S. Gray s. lato fruiting bodies (pinheads) emerging; (e), Elongating *Coprinus cinereus* (Schaeff.) S. Gray s. lato pinheads; (f), harvestable immature (young/juvenile) *Coprinus cinereus* (Schaeff.) S. Gray s. lato fruiting bodies.

Performance characteristics of Coprinus cinereus (Schaeff.) S. Gray s. lato grown on grass incorporating some brown seaweed Sargassum species supplements at varying concentrations

Brown seaweeds *Sargassum* species is among the organic additives available in large quantities in Tanzania along Indian Ocean which current constitute waste. In this study, an attempt was made in which *Coprinus cinereus* (Schaeff.) S. Gray s. lato an indigenous Tanzanian mushroom species was grown on grass substrate supplemented with brown seaweeds *Sargassum* species at varying concentrations. Data was collected and observations were made on performance characteristics including; morphological parameters namely; basidiocarps numbers, weight (total fresh weight and dry mushroom weight), pileus and stipe lengths, mushroom size, and production rate as well as on productivity % of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom. In general, results revealed that there was interaction of grass substrate with nine (9) brown seaweeds *Sargassum* species organic supplements at seven (7) varying concentrations on morphological parameters as presented in (Tables 3-8), production rate (Table 9) as well as on productivity % (Table 10). Regardless of grass substrate, the nine (9) different brown seaweeds *Sargassum* species organic supplements at seven (7) varying concentrations had effects on *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom performance characteristics variable parameters analyzed which was extreme significant ($p < 0.0001$). This further meant that highly significant results were observed among treatments in terms of mushroom performance characteristics basidiocarps numbers, weight (total fresh weight and dry mushroom weight), pileus and stipe lengths, mushroom size, and production rate and productivity %. Those nine brown seaweeds *Sargassum* species organic supplements types behaved drastically different on seven, 0.5, 1, 2, 5, 10, 15 and 20% varying concentrations resulting into mushroom performance characteristics which were highly significant (Tables 3-10).

Among nine (9) brown seaweeds *Sargassum* species organic supplements, supplement 4 comprised of (*Sargassum poligocytum* brown seaweed, 8 cm tip-part, which, represented the young parts of *Sargassum* frond) gave comparatively overall best mushroom performance characteristics analyzed variables compared to other eight supplements which was significant at ($p < 0.0001$) with exception of mushroom size and stipe lengths (Tables 3-10). On the other hand, relatively most worst mushroom performance characteristics results were obtained from supplement 8 with unsorted *Sargassum* species brown seaweed compared to other eight supplements and even with grass alone, un-supplemented grass substrate (Tables 3-10).

Morphological performance characteristics of Coprinus cinereus (Schaeff.) S. Gray s. lato

Morphological parameters namely; basidiocarps numbers, weight (total fresh weight and dry mushroom weight), pileus and stipe lengths, mushroom size, constituted part of performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s. lato. The total mushroom weight, were significantly affected by brown seaweeds *Sargassum* species types and varying concentrations mixture as shown in (Table 3). Supplement 4 recorded the highest (maximum) mean number of effective fruiting bodies (mushrooms) of 85.00 at 10% supplement concentration rate, which was three times more than that of control, grass alone. Supplement 8 had least average number of effective fruiting bodies (mushrooms) of 8 for both 15 and 20% supplement concentration rates, which were lower by a factor of three compared to 23.14 recorded for control grass substrate alone.

Table 3: *Coprinus cinereus* (Schaeff.) S. Gray s. effective numbers harvested from grass substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean \pm SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|
| 0 | 26.67 \pm 8.50 | 26.67 \pm 8.50 | 26.67 \pm 8.50 | 26.67 \pm 8.50 | 26.67 \pm 8.50 | 26.67 \pm 8.50 | 26.67 \pm 8.50 | 26.67 \pm 8.50 | 26.67 \pm 8.50 |
| 0.5 | 51.67 \pm 11.50 | 58.00 \pm 6.00 | 42.00 \pm 7.00 | 60.67 \pm 7.51 | 26.67 \pm 2.52 | 51.67 \pm 11.50 | 51.00 \pm 1.00 | 39.00 \pm 4.58 | 28.67 \pm 17.10 |
| 1 | 35.33 \pm 5.51 | 65.00 \pm 2.00 | 31.00 \pm 5.00 | 46.00 \pm 5.00 | 51.67 \pm 7.09 | 35.33 \pm 5.51 | 58.67 \pm 4.51 | 22.00 \pm 15.52 | 59.00 \pm 3.00 |
| 2 | 38.00 \pm 1.00 | 50.50 \pm 2.50 | 42.67 \pm 1.53 | 74.00 \pm 19.00 | 51.00 \pm 8.00 | 38.00 \pm 1.00 | 59.00 \pm 4.00 | 38.00 \pm 1.00 | 46.00 \pm 8.00 |
| 5 | 50.67 \pm 2.52 | 65.00 \pm 2.00 | 41.67 \pm 1.53 | 59.67 \pm 0.58 | 59.00 \pm 1.00 | 50.67 \pm 2.52 | 52.00 \pm 2.00 | 19.00 \pm 2.00 | 31.00 \pm 2.65 |
| 10 | 51.00 \pm 10.00 | 27.67 \pm 1.53 | 64.67 \pm 0.58 | 85.00 \pm 4.00 | 85.00 \pm 6.00 | 51.00 \pm 10.00 | 57.00 \pm 13.00 | 25.33 \pm 14.50 | 24.67 \pm 8.50 |
| 15 | 73.00 \pm 10.00 | 39.67 \pm 15.50 | 53.67 \pm 10.50 | 80.67 \pm 11.50 | 51.67 \pm 2.52 | 73.00 \pm 10.00 | 55.00 \pm 10.00 | 8.00 \pm 1.00 | 13.00 \pm 1.00 |
| 20 | 52.00 \pm 15.00 | 39.00 \pm 11.00 | 42.00 \pm 19.00 | 76.67 \pm 34.50 | 66.00 \pm 3.61 | 52.00 \pm 15.00 | 33.67 \pm 1.53 | 8.00 \pm 1.00 | 28.67 \pm 11.50 |

Coprinus cinereus (Schaeff.) S. Gray s. lato mushroom grown on grass supplemented with supplement 4 at 15% concentration rate recorded highest mean total fresh mushroom weight of 83.43 g which was 3.6 times higher than that was obtained from grass alone control while lowest total fresh mushroom weight of 5.34 g was obtained from supplement 8 at 15% concentration rate, which was four times lower than that of 23.14 g recorded from grass alone control (Table 4).

Table 4: *Coprinus cinereus* (Schaeff.) S. Gray s.lato total fresh weight (g) mushrooms harvested from grass substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean \pm SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 0 | 23.14 \pm 11.48 | 23.14 \pm 11.48 | 23.14 \pm 11.48 | 23.14 \pm 11.48 | 23.14 \pm 11.48 | 23.14 \pm 11.48 | 23.14 \pm 11.48 | 23.14 \pm 11.48 | 23.14 \pm 11.48 |
| 0.5 | 38.66 \pm 2.41 | 41.25 \pm 3.63 | 27.83 \pm 6.49 | 62.85 \pm 2.49 | 36.80 \pm 1.12 | 58.29 \pm 7.80 | 51.71 \pm 4.92 | 50.48 \pm 7.03 | 42.35 \pm 2.61 |
| 1 | 38.95 \pm 4.91 | 59.69 \pm 3.01 | 21.60 \pm 1.16 | 50.89 \pm 2.23 | 56.07 \pm 9.49 | 37.44 \pm 9.45 | 60.04 \pm 1.74 | 29.80 \pm 16.71 | 58.24 \pm 8.08 |
| 2 | 53.24 \pm 3.11 | 49.49 \pm 0.12 | 29.19 \pm 0.58 | 72.27 \pm 14.48 | 60.41 \pm 16.81 | 47.46 \pm 3.25 | 53.04 \pm 4.73 | 47.01 \pm 5.74 | 54.16 \pm 9.14 |
| 5 | 38.20 \pm 9.95 | 71.98 \pm 9.19 | 47.08 \pm 1.62 | 59.13 \pm 1.65 | 64.08 \pm 9.95 | 58.21 \pm 5.44 | 51.26 \pm 2.86 | 24.33 \pm 6.95 | 44.36 \pm 6.69 |
| 10 | 24.66 \pm 3.41 | 33.24 \pm 0.23 | 68.49 \pm 0.34 | 73.09 \pm 10.66 | 80.32 \pm 0.93 | 42.37 \pm 5.62 | 46.12 \pm 8.16 | 22.50 \pm 14.57 | 33.59 \pm 12.37 |
| 15 | 43.62 \pm 0.87 | 43.73 \pm 7.16 | 60.11 \pm 11.00 | 83.43 \pm 6.46 | 62.72 \pm 1.76 | 62.71 \pm 1.82 | 55.67 \pm 12.86 | 5.34 \pm 1.22 | 21.32 \pm 3.03 |
| 20 | 45.39 \pm 0.76 | 28.65 \pm 8.68 | 39.33 \pm 6.35 | 77.87 \pm 1.16 | 64.80 \pm 2.07 | 54.25 \pm 17.90 | 29.77 \pm 0.41 | 5.80 \pm 0.21 | 28.44 \pm 7.69 |

Coprinus cinereus (Schaeff.) S. Gray s.lato mushroom dry matter varied among different treatments investigated (Table 5). There was least mushroom dry weight of 1.16 g at 1% concentration rate recorded from supplement 3. The highest average mushroom dry weight 4.81g was obtained in supplement 4 at 15% concentration rate, which was three times higher than dry matter obtained from grass control alone of 1.39 g under experimental conditions.

Variations of mushroom size, pileus (cap) length and stipe length in *Coprinus cinereus* (Schaeff.) S. Gray s.lato were observed in the nine *Sargassum* species brown seaweeds fronds at seven different concentrations rates used in this study (Tables 6, 7 and 8). The size of fresh fruiting bodies is desirable characteristics for marketable quality and plays a significant role during grading, packing and distribution of mushroom. For *Coprinus cinereus* (Schaeff.) S. Gray s.lato mushroom pileus length is very important since is picked at button stage. Supplement 1 comprised of *Sargassum oligocytum* whole plant at 20% concentration rate gave the relatively largest mean mushroom size (2.64), followed by (2.50) at 15% on the same supplement, which were three times higher than mushroom size (0.83) recorded from control grass alone while supplement 8 at 15% concentration rate gave the smallest mean mushroom size (0.67) (Table 6).

Table 5: *Coprinus cinereus* (Schaeff.) S. Gray s.lato dry weight (g) mushrooms harvested from grass substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean±SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 0 | 1.39 ± 0.32 | 1.39 ± 0.32 | 1.39 ± 0.32 | 1.39 ± 0.32 | 1.39 ± 0.32 | 1.39 ± 0.32 | 1.39 ± 0.32 | 1.39 ± 0.32 | 1.39 ± 0.32 |
| 0.5 | 2.77±0.36 | 3.00±0.38 | 1.92±0.27 | 4.47±0.08 | 2.68±0.04 | 3.73±0.58 | 3.91±0.08 | 4.56±1.04 | 2.91±0.08 |
| 1 | 2.68±0.04 | 4.27±0.16 | 1.16±0.10 | 3.84±0.07 | 4.28±0.40 | 2.91±0.21 | 4.31±0.22 | 2.86±0.21 | 4.27±0.16 |
| 2 | 3.81±0.17 | 2.84±0.24 | 1.66±0.29 | 4.74±0.17 | 4.57±0.37 | 3.35±0.50 | 4.17±0.16 | 3.86±0.94 | 4.28±0.40 |
| 5 | 2.87±0.26 | 4.74±0.17 | 2.81±0.05 | 4.27±0.16 | 4.57±0.37 | 4.20±0.21 | 4.27±0.16 | 1.95±0.59 | 4.16±1.49 |
| 10 | 1.82±0.08 | 2.53 ±0.16 | 4.57±0.37 | 4.54±0.30 | 4.30±0.71 | 2.99±0.08 | 4.30±0.40 | 2.79±0.76 | 2.81±0.05 |
| 15 | 4.07±0.08 | 4.16±1.49 | 4.30±0.71 | 4.81±0.16 | 4.27±0.16 | 4.50±0.39 | 4.34±0.29 | 1.31±0.05 | 1.91±0.64 |
| 20 | 4.28±0.40 | 2.91±0.98 | 3.40±0.90 | 4.21±0.04 | 4.82±0.75 | 4.21±0.81 | 2.63±0.23 | 1.26±0.10 | 2.84±0.24 |

Table 6: *Coprinus cinereus* (Schaeff.) S. Gray s.lato mushroom size of mushrooms harvested from grass substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean±SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|--------------------|
| 0 | 0.83 ± 0.17 | 0.83 ± 0.17 | 0.83±0.17 | 0.83 ± 0.17 | 0.83 ± 0.17 | 0.83 ± 0.17 | 0.83 ± 0.17 | 0.83 ± 0.17 | 0.83 ± 0.17 |
| 0.5 | 1.70±0.23 | 0.71±0.02 | 0.69±0.05 | 1.05±0.18 | 1.39±0.09 | 1.14±0.11 | 1.01±0.11 | 1.29±0.03 | 1.09±0.03 |
| 1 | 1.58±0.09 | 0.91±0.08 | 0.70±0.08 | 1.11±0.07 | 1.08±0.05 | 1.05±0.13 | 1.03±0.05 | 1.49±0.37 | 0.98±0.09 |
| 2 | 2.35±0.11 | 0.98±0.05 | 0.68±0.01 | 0.99±0.06 | 1.17±0.15 | 1.25±0.06 | 0.90±0.02 | 1.24±0.16 | 1.18±0.01 |
| 5 | 1.76±0.16 | 1.10±0.11 | 1.13±0.01 | 0.99±0.04 | 1.08±0.15 | 1.15±0.05 | 0.99±0.09 | 1.32±0.50 | 1.45±0.33 |
| 10 | 1.12±0.05 | 1.20±0.07 | 1.06±0.01 | 0.86±0.17 | 0.95±0.06 | 0.84±0.05 | 0.81±0.04 | 0.83±0.26 | 1.35±0.04 |
| 15 | 2.50±0.05 | 1.18±0.31 | 1.12±0.02 | 1.06±0.23 | 1.22±0.09 | 0.87±0.09 | 1.01±0.06 | 0.67±0.07 | 1.63±0.11 |
| 20 | 2.64±0.24 | 0.90±0.30 | 1.02±0.36 | 1.18±0.58 | 0.98±0.02 | 1.03±0.05 | 0.90±0.05 | 0.73±0.12 | 1.03±0.16 |

The relatively large sized and button unopened (immature/juvenile) *Coprinus cinereus* (Schaeff.) S. Gray s.lato mushrooms could possibly be more attractive to customers and attract highest return in the market place. Data in (Table 7) showed that the relatively three longest mean pileus (cap) of 25.90 mm at concentration rate of 15%, 22.20 mm at concentration rate of 20% and 20.90 mm at concentration rate of 0.5% were recorded from supplement 9 comprised unsorted *Sargassum* species. There were also comparable relatively longest mean pileus worthy mentioning, supplement 8 comprised of unsorted *Sargassum* species gave 21.10 mm at 5% concentration rate while supplement 4 recorded 20.70 mm mean pileus at 0.5% concentration rate.

Table 7: *Coprinus cinereus* (Schaeff.) S. Gray s.lato pileus (cap) length (mm) of mushrooms harvested from grass substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean±SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|------------------|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 0 | 11.6± 3.5 | 11.6± 3.5 | 11.6± 3.5 | 11.6± 3.5 | 11.6± 3.5 | 11.6± 3.5 | 11.6± 3.5 | 11.6± 3.5 | 11.6± 3.5 |
| 0.5 | 16.90±0.70 | 12.80±1.60 | 10.50±0.80 | 20.70±1.70 | 14.60±2.80 | 14.90±0.80 | 14.50±1.00 | 13.90±0.30 | 20.90±4.10 |
| 1 | 17.90±0.30 | 17.50±0.90 | 15.40±0.03 | 19.10±2.20 | 14.60±0.60 | 11.90±3.40 | 14.00±1.00 | 18.10±2.50 | 12.00±0.80 |
| 2 | 16.90±0.90 | 16.90±1.20 | 12.10±1.10 | 17.10±2.00 | 16.30±0.30 | 12.70±1.50 | 14.30±1.20 | 13.40±0.90 | 14.30±2.10 |
| 5 | 15.40±2.60 | 19.10±1.30 | 13.10±3.40 | 14.80±0.60 | 15.80±0.50 | 12.90±3.50 | 14.10±2.70 | 21.10±5.70 | 16.80±0.40 |
| 10 | 15.30±2.50 | 19.00±0.90 | 18.90±1.50 | 14.30±0.30 | 16.50±1.20 | 18.80±0.40 | 14.70±0.90 | 16.30±1.90 | 17.30±7.60 |
| 15 | 13.50±0.50 | 14.90±4.10 | 19.30±0.10 | 15.20±0.20 | 14.00±0.90 | 13.10±1.40 | 14.50±0.50 | 17.30±1.20 | 25.90±0.40 |
| 20 | 14.70±4.2 | 10.90±0.70 | 13.2±3.20 | 11.80±3.40 | 13.00±0.70 | 16.30±3.30 | 15.00±1.00 | 15.30±5.50 | 22.20±2.90 |

Stipe length of mushrooms is desirable performance characteristics for marketable quality of *Coprinus cinereus* (Schaeff.) S. Gray s.lato in particular relatively long mushroom stipe length coupled with longest stipe length and/or widest mushroom cap diameter. The relative average shortest pileus length of 10.50 mm at 5% concentration rate was recorded from supplement 3 comprised of *Sargassum polycystum* with 10 cm, middle-part followed closely by 10.90 mm obtained from *Sargassum polycystum* with basal part with variable lengths at 20% concentration rate. Nevertheless, both were shorter than mean pileus cap of 11.60 mm recorded from grass alone control. The stipe length results presented in (Table 8) revealed that the longest mean stipe length (18.80 mm) was recorded from supplement 1 comprised of *Sargassum oligocytum* whole plant at 1% concentration rate, which was 1.7 times higher than 10.80 mm mean stipe length obtained from control grass alone. On the other hand, the shortest mean stipe length (6.20 mm) was recorded from supplement 8 at supplement concentration rate of 20%, followed by 6.30 mm mean stipe length obtained from supplement 6 comprised of *Sargassum equifolium* with 10 cm, middle-part at supplement concentration rate of 10%. Also equally shortest 6.70 mm mean stipe length was obtained from supplement 7 comprised of *Sargassum equifolium* with 8 cm tip-part at supplement concentration rate of 15%.

Table 8: *Coprinus cinereus* (Schaeff.) S. Gray s.lato stipe length (mm) of mushrooms harvested from grass substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean±SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|------------------|------------------|--------------------|
| 0 | 10.80± 1.20 | 10.80± 1.20 | 10.80± 1.20 | 10.80± 1.20 | 10.80± 1.20 | 10.80± 1.20 | 10.80± 1.20 | 10.80± 1.20 | 10.80± 1.20 |
| 0.5 | 15.00±1.40 | 11.00±2.60 | 7.80±0.60 | 13.60±2.60 | 13.20±1.90 | 9.70±2.00 | 14.80±3.50 | 11.20±4.00 | 12.00±1.20 |
| 1 | 18.80±2.80 | 15.50±0.70 | 12.70±0.30 | 11.30±0.60 | 14.30±0.30 | 12.40±3.00 | 11.40±2.00 | 14.40±2.40 | 9.00±2.40 |
| 2 | 13.60±1.00 | 13.50±1.30 | 10.30±0.80 | 10.10±0.90 | 16.10±1.80 | 9.90±1.10 | 12.50±0.80 | 9.80±1.70 | 8.20±1.80 |
| 5 | 17.10±5.90 | 17.90±0.70 | 10.30±2.10 | 7.60±1.50 | 15.50±5.50 | 12.30±5.00 | 9.80±0.20 | 11.50±0.60 | 10.20±5.30 |
| 10 | 12.50±1.00 | 13.40±0.70 | 17.70±2.30 | 9.70±2.40 | 14.70±0.70 | 6.30±4.60 | 8.20±1.10 | 6.70±1.10 | 10.40±1.70 |
| 15 | 10.70±1.40 | 15.40±3.80 | 12.40±0.20 | 9.40±0.60 | 9.20±1.40 | 14.70±0.90 | 6.70±0.60 | 8.00±1.40 | 10.70±1.70 |
| 20 | 17.30±0.60 | 13.10±0.10 | 11.10±0.30 | 10.30±3.50 | 13.50±1.50 | 9.40±2.30 | 9.80±0.20 | 6.20±0.80 | 13.30±4.80 |

Production rate and productivity, P, % performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s. lato

The nature, type and composition of the mushroom substrate and mushroom strain/species used and climatic conditions are interrelated factors for successful mushroom production. However, the quantity and quality of harvested mushrooms depends upon duration of the cropping period and growing practice applied e.g. additives/supplement incorporated in the basal substrate, which influence response of yield attributes such as production rate and productivity %. In this study the duration of the cropping period was 23 days while fresh grass main substrate was (500 g), which incorporated brown seaweed *Sargassum* species at different supplement concentration rates. The highest mean production rate (2.23) which, reflects bioconversion of organic biomass into mushroom at particular cropping period was obtained from supplement 4 at concentration rate of 15%, which was followed by 2.15 mean production rate at 10% concentration rate, recorded from supplement 5 comprised of *Sargassum equifolium* with basal part of variable lengths, both were three times higher than the mean production rate of 0.62 observed from grass alone control (Table 9). The lowest mean production rate of 0.15 at 15 % concentration rate and 0.16 mean production rate at 20 % concentration rate both were recorded from supplement 8 which were below by a factor of four compared to 0.62 obtained from grass alone control.

Table 9: *Coprinus cinereus* (Schaeff.) S. Gray s.lato production rate (the ratio of biological efficiency (BE) % divided by cropping period) of mushrooms harvested from grass substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean±SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 0 | 0.62±0.31 | 0.62±0.31 | 0.62±0.31 | 0.62±0.31 | 0.62±0.31 | 0.62±0.31 | 0.62±0.31 | 0.62±0.31 | 0.62±0.31 |
| 0.5 | 1.22±0.02 | 1.10±0.10 | 0.74±0.17 | 1.68±0.07 | 0.99±0.03 | 1.56±0.21 | 1.38±0.13 | 1.35±0.19 | 1.13±0.07 |
| 1 | 0.96±0.06 | 1.59±0.08 | 0.57±0.03 | 1.36±0.06 | 1.50±0.25 | 1.00±0.25 | 1.61±0.05 | 0.79±0.45 | 1.56±0.22 |
| 2 | 1.12±0.03 | 1.33±0.01 | 0.78±0.02 | 1.94±0.39 | 1.62±0.45 | 1.27±0.09 | 1.42±0.13 | 1.26±0.15 | 1.45±0.24 |
| 5 | 1.16±0.30 | 1.93±0.25 | 1.26±0.04 | 1.58±0.04 | 1.72±0.27 | 1.56±0.15 | 1.37±0.08 | 0.65±0.19 | 1.19±0.18 |
| 10 | 1.01±0.07 | 0.89±0.01 | 1.83±0.01 | 1.96±0.29 | 2.15±0.02 | 1.13±0.15 | 1.23±0.22 | 0.60±0.39 | 0.90±0.33 |
| 15 | 0.88±0.03 | 1.18±0.18 | 1.61±0.29 | 2.23±0.17 | 1.68±0.05 | 1.68±0.05 | 1.49±0.33 | 0.15±0.03 | 0.57±0.08 |
| 20 | 0.89±0.03 | 0.77±0.23 | 1.05±0.17 | 2.09±0.03 | 1.74±0.06 | 1.45±0.48 | 0.80±0.01 | 0.16±0.01 | 0.76±0.21 |

Productivity P, % that reflects the quantity of mushroom per substrate fresh weight implies mushroom, which can be harvested by grower, the higher the P % means more mushrooms harvested. The highest mean P % (16.69) was obtained from supplement 4 at concentration rate of 15%, which was 3.6 times higher than P% of 4.63 recorded from grass alone control (Table 10). The lowest mean P% of 1.07 recorded at concentration rate of 15% and mean P (1.16%) at concentration rate 20 % both were recorded from supplement 8 which were below by a factor of 15 compared to highest mean P % obtained from supplement 4.

Table 10: *Coprinus cinereus* (Schaeff.) S. Gray s.lato productivity (P, %) percentage ratio of mushroom fresh weight and substrate fresh weight for grass supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentration rates. (Values Mean±SD, n=3).

| Concentration rates % | Suppl. 1 | Suppl. 2 | Suppl. 3 | Suppl. 4 | Suppl. 5 | Suppl. 6 | Suppl. 7 | Suppl. 8 | Suppl. 9 |
|-----------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 0 | 4.63 ± 2.29 | 4.63 ± 2.29 | 4.63 ± 2.29 | 4.63 ± 2.29 | 4.63 ± 2.29 | 4.63 ± 2.29 | 4.63 ± 2.29 | 4.63 ± 2.29 | 4.63 ± 2.29 |
| 0.5 | 7.73±0.48 | 8.25±0.72 | 5.56±1.29 | 12.57±0.49 | 7.36±0.05 | 7.36±1.56 | 10.34±0.98 | 10.09±1.40 | 8.47±0.52 |
| 1 | 7.79±0.98 | 11.94±0.60 | 4.32±0.23 | 10.18±0.44 | 11.21±1.89 | 7.49±1.89 | 12.00±0.35 | 5.96±3.34 | 11.65±1.62 |
| 2 | 10.65±0.62 | 9.89±0.02 | 5.83±0.11 | 14.45±2.89 | 12.08±3.36 | 9.49±0.65 | 10.61±0.94 | 9.40±1.15 | 10.83±1.82 |
| 5 | 7.64±1.99 | 14.39±1.83 | 9.41±0.32 | 11.82±0.33 | 16.06±1.99 | 11.64±1.08 | 10.25±0.57 | 4.87±1.39 | 8.87±1.34 |
| 10 | 4.93±0.68 | 6.64±0.02 | 13.69±0.07 | 14.62±2.13 | 16.06±0.18 | 8.47±1.12 | 9.22±1.63 | 4.50±2.91 | 6.72±2.47 |
| 15 | 8.72±0.17 | 8.74±1.43 | 12.02±2.20 | 16.69±1.29 | 12.54±0.35 | 12.54±0.36 | 11.13±2.57 | 1.07±0.24 | 4.26±0.60 |
| 20 | 9.08±0.15 | 5.73±1.73 | 7.87±1.27 | 15.57±0.23 | 12.96±0.41 | 10.85±3.58 | 5.95±0.08 | 1.16±0.04 | 5.69±1.54 |

This is a first scientific report on performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s.lato grown on grass incorporating some brown seaweed *Sargassum* species supplements at varying concentrations. Therefore in the literature there was limited information to offer credible rational comparisons on findings presented in (Tables 3-10) described on performance characteristics including; basidiocarps numbers, weight (total fresh weight and dry mushroom weight), pileus and stipe lengths, mushroom size, and production rate and productivity % of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom. But it suffices to speculate on the trends and phenomenon observed and revealed in the present experiment findings accrued in this study.

Variations in performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s.lato variables analyzed among brown seaweed *Sargassum* species supplements at varying concentrations clearly indicated the possible influence of the quality and quantity of nutritious components contained supplements (additives) incorporated in grass basal substrate. However, it was eminent that the interaction of nine brown seaweed *Sargassum* species supplements at various concentrations in grass substrate on performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s.lato variables observed, collected, measured and results presented in (Tables 3-10) in this study was very complex phenomenon. In some situations the expected logical and/or theoretical trend was not followed. For instance, some interactions of nine brown seaweed *Sargassum* species supplements at lowest or highest concentrations induced negative effects on the performance characteristics variables analyzed. Additionally in some cases, brown seaweed *Sargassum* species supplemented grass substrate had smaller recorded numerical data than that recorded from non-supplemented grass (grass alone control). That was contrarily to what was mostly possibly expected for incorporation of additives or supplements in grass basal mushroom substrate. It was possibly expected that the increased level of nutrient available at higher brown seaweed *Sargassum* species concentration rates would have provided more energy and nutrients for vegetative mycelial growth, primordia (minute fruiting

bodies) and fruiting bodies formation. Also it was expected that brown seaweed *Sargassum* species supplement would have increased the water-holding capacity, and decreased number of pinheads aborted due to water shortage given the physical nature and high porosity of the grass, and also the fact that it dries up very rapidly. However, in this study neither pinhead abortion nor young fruiting bodies mortality was established. Also the detailed compositions analysis of grass substrate and brown seaweed *Sargassum* species supplement mixtures used in this study (Table 2) were beyond the scope of this study and were not analyzed. A similar comparable phenomenon have been reported recently on the effect of the interaction of varying chicken manure supplement levels with three different solid sisal wastes substrates on sporocarp cap lengths and diameters, stipe lengths and diameters and dry weights of *Coprinus cinereus* (Schaeff) S. Gray s.lat (Mwita et al., 2011b). It was found that the complex phenomenon which induced varied effects on *Coprinus cinereus* (Schaeff) S. Gray s.lat mushroom size/dimensions and dry weight were possibly attributed to incorporating chicken manure supplement in sisal wastes substrates which was implicated to have affected the composition and qualities such as water holding capacity, degree of aeration, intrinsic microclimate, nutrients resource allocations of the combinations (Mwita et al., 2011b). The preceding speculations collaborates the previous observation by Zadrazil (1993) that organic or inorganic supplements or additives usually change the decomposition rate and also the sequence of decomposition of mushroom substrates components, which consequently influence performance characteristics such morphological features and yield of mushrooms. Additionally Chang et al. (1993) had also previously reported that successful mushroom production involves a variety of interrelated factors depending upon genetic characterization of the mushroom and the formulation of growing substrates that contains those nutritional substances necessary to optimize mushroom productivity.

The relationship between numbers of effective harvestable fruiting bodies, total fresh weight and mushroom caps/pileus lengths and productivity, P% observed in this study was peculiar and worthy noting (Table 3, 4,7 and 10). Individual observations in this study revealed that highest mean number of fruiting bodies of 85.00 was recorded for supplement 4 while the least mean number of 8.00 was observed from supplement 8 (Table 3). Similarly, supplement 4, recorded highest mean total fresh mushroom weight of 83.43 g while lowest total fresh mushroom weight of 5.34 g was obtained from supplement 8 (Table 4). The highest mean P % (16.69) was obtained from supplement 4 while the lowest mean P% of 1.07 was recorded from supplement 8 (Table 10). Similarly Gume et al. (2013) reported that rice straw (or wheat straw) substrate supplemented with cotton seed hull for growing *Pleurotus* (oyster mushrooms) gave higher yield also contained higher number of mushrooms. On the other hand, the relatively longest mean pileus (cap) of 25.90 mm followed by 22.20 mm was recorded from supplement 9. There was also comparable relatively longest mean pileus of 20.70 mm recorded from supplement 4 (Table 7). Supplement 4 had also highest number of mushrooms as well as highest total fresh weight as opposed to supplement 9 which had amongst least mushroom numbers and lowest total fresh weight. Such observations contradicted other previous observations. Gume et al. (2013) recently reported that big *Pleurotus* (oyster mushrooms) mushroom caps with wider pileus diameter occurred when fewer numbers of fruit bodies were harvested on rice straw (or wheat straw) substrate supplemented with cotton seed hull. The possible reasons behind the contradictory findings of the current study with the past could be type of mushroom species used i.e. *Coprinus cinereus* (Schaeff) S. Gray s.lat, grass substrate and type and treatment of the brown seaweed *Sargassum* species supplements used. It is very possible that quality and quantity of nutritious components contained in the grass substrate after brown seaweed *Sargassum* species supplementation changed the physical-chemical properties of grass substrate resulting into variations observed in the present study.

Conclusion

The findings presented herein in for the first time on the influence of different brown seaweeds *Sargassum* species supplement at various rates on performance characteristics of *Coprinus cinereus* (Schaeff.) S. Gray s. lato grown on grass. Considering the performance characteristics basidiocarp numbers, total fresh weight, dry mushroom weight, production rate and productivity P, % overall best results were obtained from supplement 4 comprised of (*Sargassum poligocytum* brown seaweed, 8 cm tip-part, which represented the young parts of *Sargassum* fronds) at 15% concentration rate. Therefore grass mushroom substrate incorporating supplement 4 is a feasible, viable and promising formulation that can be adopted to produce a good productivity of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom for year-round availability for culinary and nutraceutical purposes.

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