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Full Length Research Paper

Utilization of Brown Seaweeds *Sargassum species* Organic Supplements in Grass Basal Substrate to Enhance Yield of Edible *Coprinus cinereus* (Schaeff) S. Gray S. Lato. Mushroom in Tanzania

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Abstract
The effects of supplementing grass as substrate for Coprinus cinereus mushroom with brown seaweeds Sargassum species on yield was investigated. Sargassum species fronds were sorted into five categories; basal part (older), middle part (mid age), tip part (young), whole and unsorted. The categorized samples were sundried and washed twice with fresh water before were milled. Grass 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). All substrates and supplement formulations investigated were pasteurized at 70°C for 3 hours, when cooled were inoculated with spawn at 5% on wet wt basis and transferred into solid-state fermentation bioreactors (SSFBs) made up of rectangular plastic for incubation. Highest biological efficiency (BE) average of 51.39 %, biological yield (BY) of 531.98 g fresh mushroom/kg dry substrate and mushroom yield (MY) 166.86 g fresh mushrooms/kg fresh were recorded from supplement 4 comprised of Sargassum poligocytum, 8 cm tip-part which, represented young parts of the frond at concentration supplement rate of 15%. The lowest BE 3.35%, BY 33.47 g fresh mushroom/kg dry substrate and MY 10.87 g fresh mushroom/kg fresh substrate, were obtained from grass supplemented with supplement 8 comprised of unsorted Sargassum species at supplement concentration rate of 15%. Highest BE, BY and MY increased by 72% compared to control grass alone and were also 3.6 times higher than those recorded for grass alone. In conclusion incorporation of 15% brown seaweed Sargassum poligocytum young parts in grass substrates for cultivation Coprinus cinereus is promising and viable formulation to increase mushroom yield.

Key words: *Brown, Seaweeds, Sargassum, Coprinus, Yield, Supplement*

Introduction

Coprinus cinereus (Schaeff.) S. Gray s. lato in Tanzania is one of mushroom wild genetic resources that found growing naturally normally in piles of decomposed sisal wastes (Härkönen et al., 2003). It has been domesticated on composted and non-composted substrates without supplement on sisal wastes substrates or with supplements such as chicken manure, rice husks, cow dung manure and human urine under local climatic conditions and low technology environments (Mshandete, 2011). The Tanzanian *Coprinus cinereus* has been reported to be a good source of protein, ascorbic acid, fibre and minerals coupled with medicinal bioactive agents that could be used for development of new drugs for the treatment and prevention of disease and high antioxidants activities and phytochemical contents (Mshandete and Cuff, 2007;Ndyetabura et al., 2010; Tibuhwa et al., 2012). Furthermore, *Coprinus cinereus* (Schaeff.) S. Gray s. lato is a warm tropical, robust and fast growing saprophytic edible mushroom, which completes its life cycle within two to three weeks. Therefore, is edible, nutritious protein-rich food and medicinal fungus, which matches the request of natural, nourishment, nutraceuticals and health care.

In Tanzania another natural resource worthy for intensified research is seaweeds particularly brown seaweeds (McHugh, 2003;Oliveira et al., 2005). The brown seaweeds of the genus *Sargassum are* found in tropical and sub-tropical waters amongst many brown seaweeds (Lobban and Harrison, 1994). They are known to contain wide range of essential minerals as well contain unique polysaccharides in their tissues, growth enhancing natural products (auxins and cytokinins) and a number of biomaterials for diversity of applications (Gade et al., 2013; Kannan, 2014). *Sargassum* species brown seaweeds are found along the coast of Tanzania as a natural beach-cast, discarded as underutilized biomass often leading to environmental pollution of coastal sea water due to rapid break down resulting in offensive smell and accumulation of large amounts of organic sediments in mud.

Grass is yet another available and abundant virtually untapped bioresource in Tanzania constituting waste. The grass lignin content tends to increase as it matures making it poor animal folder. The high lignin content is very attribute, which make it excellent substrate material for cultivation of white rot, lignin-degrading mushroom such as *Coprinus cinereus* (Schaeff.) S. Gray s. lato. On the other hand *Sargassum* species brown sea weeds lack lignin and have low cellulose content, rich in bio-stimulants and inorganic minerals thus, could be an easier material for biological biodegradation to release nutrients (Gade et al., 2013; Kannan, 2014). Therefore mixing grass *and Sargassum* species brown sea weeds during cultivation of *Coprinus cinereus* (Schaeff.) S. Gray s. lato. is practically and technically sound. However, such seeming biological innovation complementary blending of grass and *Sargassum* species brown sea

weeds remains to be investigated to establish the effects on mushroom yield, agronomy characteristics, bioactive constituents, nutritional composition, antioxidants activities, phytochemical content etc.

Nevertheless, preliminary investigation on growing the oyster mushroom, *Pleurotus sajor-caju* on Kelp brown sea weed, red sea weeds *Gracilaria gracilis* and brewing industries as well on various combinations of the sea weeds and scrub grass have been conducted (Molloy et al.,1999; 2003). It was found that fruiting (mushroom production) was best on those substrates containing kelp rather than *Gracilaria gracilis*, probably due to the high salt content of the *G. gracilis*. No mushrooms were produced on 100% seaweed substrates probably due to nutrients being too readily available. In contrast, Resmi (2007) reported successful *Ganoderma lucidum and Pleurotus ostreatus* mushrooms production on 100% *Gracilaria* red seaweed-containing solid wastes as substrate obtained after jelly powder production. Recently Kaaya et al. (2012) reported utilization of brown seaweed *Laminaria schinzii* as a supplement in grass as substrate for cultivation of the oyster mushroom *Pleurotus sajor caju*. They found the optimum concentration of seaweed to be 10%, which resulted into highest numbers and biomass of mushrooms. It was also revealed that concentrations of seaweed above 10% suppressed mushroom growth while no growth occurred above 25%.

These results suggest that brown seaweeds and red sea weeds can be utilized as supplements in lignocellulosic substrates such as grass or can be used as whole in *Ganoderma* and *Pleurotus* mushroom species cultivation. However, this kind of study has to be extended to other species of mushroom and brown seaweeds locally occurring in Tanzania. On the other hand, scientific reports on incorporation of *Sargassum* brown seaweeds biomass as a supplement of lignocellulosic grass biomass in cultivation of *Coprinus cinereus* (Schaeff.) S. Gray s. lato. are totally non-existent. Therefore the present study endeavoured to investigate the possibility of using Tanzanian grass materials as substrate and *Sargassum species* material as organic supplement for producing high value mushroom crop of *Coprinus cinereus* (Schaeff.) S. Gray s. lato.

Materials and Methods

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

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 sub-culturing, short and long-term culture storage was carried out as reported previous by Mshandete (2011).

Spawn preparation (mushroom seed preparation)

Spawn was prepared with intact sorghum grains and was inoculated with 4 day-old cultures *Coprinus cinereus* (Schaeff.) S. Gray s. lato as per Ndyetabura et al. (2010).

Sargassum plant specimens' 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. Biomass (fresh weight) was estimated according to Chirapart and Ohno (1993). Preliminary identification of *Sargassum* species brown sea weeds found on both sites was done in the field by Dr.Amelia S. Buriyo using available keys (Jaasund, 1976; Oliveira et al., 2005). Gross morphology, anatomy and reproductive morphology criteria were used for further identification and description of *Sargassum* species brown seaweeds. Voucher specimens were prepared, pressed on herbarium sheets and deposited in the Herbarium of Botany Department, University of Dar es Salaam (UDSM). Preparation of samples of *Sargassum* species brown sea weeds were carried in the laboratory at Botany Department, University of Dar es Salaam. Each sample was washed initially with copious tap water (fresh water) immediately from the field. The each sample was sun dried in a green house. Then it was washed again with copious tap water to remove the surface deposited salts. Some sample were sorted and cut to obtain three different age groups from different parts of *Sargassum* frond referred to basal (older parts), middle (mid-age) and tip parts (young parts) (Table 1). Finally the treated samples were milled using a laboratory mill sieve (Model 4, Thomas Wiley; Arthur K Thomas, Philadephia, PA, USA). Milling was necessary in order to increase the surface area to optimise the action of mushroom fungal enzymes. Then each prepared milled *Sargassum* species brown seaweeds fronds specimens hereby referred as supplement 1-9 was packed in transparent heat-tolerant polyethylene bags.

Substrate, supplements preparation and experimental design

Fresh grasses were collected from Mwalimu J.K. Nyerere Mlimani main Campus, University of Dar es Salaam (UDSM), Tanzania and sun dried for 7 days in a green house. The dried grass was be chopped into 2-3 cm long using a locally made manual chopper. The dried biomass was soaked in water for three hours and excess water was drained off and packed in transparent heat-tolerant polyethylene bags. Fresh cow dung manure was obtained from Vingunguti abattoir, Ilala Municipal, Dar es Salaam, Tanzania and was sun dried for 7 days in a green house and ground to pass through a 2 mm mesh and packed in transparent heat-tolerant polyethylene bags. The dried grass, cow dung manure and *Sargassum* species brown seaweeds were all in separate packages pasteurized by steaming at 70°C for 3 hours in a 200 L food grade drum (not used for chemicals) in order to deactivate any residual microorganisms prior to mixing in various combinations.

Table 1. Glossary of *Sargassum* species brown seaweeds fronds sample treatments

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

Thereafter, aseptically grass main mushroom substrate was supplemented with different amounts (in grams equivalent to 0.5-20 % based on wet weight of the substrate) of *Sargassum powder* from different parts of frond (Table 2). Grass was supplemented with cow dung manure equal amount (10 g) equivalent to 2 % of wet weight of 500 g of the substrate. The blended lot comprised of grass basal substrate, supplemented with *Sargassum* species brown seaweeds frond and cow dung manure were aseptically thorough mixed. Finally for each 500 g lot of the substrate with or without supplements an equal amount of spawn (mushroom seed) of 25 g equivalent to 5 % of wet weight of 500 g of the substrate was aseptically inoculated and mixed thorough. Grass basal substrate without *Sargassum* species brown seaweeds supplement was used as a control i.e. grass alone. The different mixture combinations were then transferred into rectangular plastic containers as cropping containers here referred to solid state fermentation bioreactors (SSFBs) each measuring 3 litres, 23 cm x 14 cm x 9 cm (length, width and height, respectively) (Cello® Domestoware (Mkate), Dar es Salaam, Tanzania. A total of 136 aeration holes of 0.7 cm in diameter and 3 cm apart were made in all the sides of the container (Mwita et al., 2011). A total of 195 SSFBs were set up including 189 experimental with nine (9) supplements of *Sargassum* species brown seaweeds fronds at seven different concentration (%) rates (0.5, 1, 2, 5, 10, 15, 20), 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 combinations.

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**

Spawn-running and fructification

The inoculated substrates combinations in rectangular plastic containers cropping containers here referred as SSFBs were incubated as per Mshandete and Cuff (2008). The time taken to complete spawn-running for various experimental set up and the mycelia vegetative growth as well as surface mycelial density of *Coprinus cinereus* (Schaeff.) S. Gray s. lato were recorded. The mycelial density was rated as described by Kadiri (1998) as follows:

+ = Very Scanty
2+ = Scanty
3+ = Moderate
4+ = Abundant
5+ = Very abundant

Fructification (fruit body-mushroom) development. Spawn-running (mycelia vegetative growth) i.e. the colonization of mycelia on the grass basal substrate without or with various supplement *Sargassum* species brown seaweeds concentrations ended when it was fully ramified by mycelia. Afterwards various strategies were applied in order to induce fruiting as described by Mshandete and Cuff (2008).

Mushroom harvesting and determination of mushroom crop yield and productivity

Fresh *Coprinus cinereus* (Schaeff.) S. Gray s. lato fruiting bodies were harvested when young, firm and flesh (immature) when suitable for food (edible) before the mushroom cap disintegrates, turning into an inky mass according to Härkönen et al. (2003). Crop period (sum of incubation and fruiting periods) was calculated. The following aspects of mushroom crop yield and productivity were evaluated:

(i). Biological efficiency (BE) expressed as percentage BE values were calculated as [Weight of fresh mushrooms harvested (g) /dry substrate weight (g)] x100 (Royse et al., 2004).
(ii). Mushroom yield (MY) was determined as weight of fresh mushrooms harvested (g) per fresh weight (moist) substrate weight including the supplement weight (and was expressed as g fresh mushrooms/kg fresh substrate weight according to Morais et al. (2000).
(iii). Biological yield (BY) was determined as [Weight of fresh mushrooms harvested (g) per dry substrate weight] and was expressed as g fresh mushrooms/kg dry substrate weight according to Amin et al. (2008).
Nb.- Wet weight of grass was 500 g and dry weight 162.32 g all including supplements.

Statistical analysis

All experiments were carried out in triplicates to ensure reproducibility and all data were expressed as mean ± S.D. The experiments were completely randomized design (CRD) with the grass basal substrate, nine (9) *Sargassum* species brown seaweeds fronds supplements, seven (7) supplement concentration 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 biological efficiency, mushroom yield and biological yield 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

Vegetative mycelia growth of Coprinus cinereus (Schaeff.) S. Gray s. lato mushroom

The grass basal substrate supplemented with nine (9) samples of *Sargassum* species brown seaweeds at seven (7) concentrations supplement (%) rates of 0.5-20% based on wet substrate all supported the vegetative mycelial growth of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom (Figures 1 and 2). Furthermore all also sustained growth and development into mushroom fruiting bodies. This study is the first attempt in obtaining the vegetative mycelial growth and fruiting bodies of *Coprinus cinereus* (Schaeff.) S. Gray s. lato on grass as a substrate supplemented with *Sargassum* species brown seaweeds frond at supplement different concentrations up to 20%. Molloy et al. (2003) also reported for the first time preliminary investigation on cultivation of oyster mushroom, *Pleurotus sajor-caju* on various combinations scrub grass, seaweeds and brewing industries wastes. It was observed that *Pleurotus sajor-caju* white rot fungus mycelia vegetatively colonized well on all blends of substrate with exception of pure spent barley, but was slower on substrates with higher salt concentrations. Though the 100% kelp substrates were completely colonized, the mycelium was very thin and did not produce fruiting bodies. On the other hand, best mushroom production was recorded on those substrates combinations containing Kelp rather than *Gracilaria gracilis***,** which was implicated probably due to the high salt content of the *G. gracilis***.** Finally Molloy et al. (2003) noted that 100% seaweed substrates produced no mushrooms possibly due to nutrients being too readily available**.**

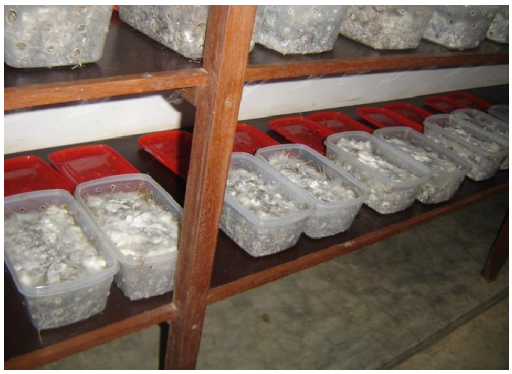

Figure 1. Differential *Coprinus cinereus* (Schaeff.) S. Gray s. lato vegetative mycelial colonization of grass basal substrate supplemented with *Sargassum* species brown seaweeds frond regardless of supplementation rates in SSFBs.

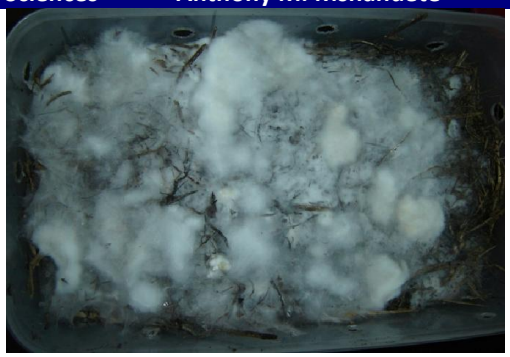

Figure 2. *Coprinus cinereus* (Schaeff.) S. Gray s. lato surface mycelial density colonization of grass basal substrate supplemented with *Sargassum* species brown seaweeds.

After five (5) days of incubation although at different extent, generally there was a progressive surface mycelia density increase with increasing supplement concentration (%) rates in particular from 1 to 20% for all nine *Sargassum* species brown seaweeds frond supplements (Table 3). However, surface mycelial density for 10% supplement rate was abundant for all 1-9 supplements, which could be considered optimum among all rates investigated. Scanty surface mycelial density (i.e. very thin mycelium) recorded from control without supplement (0 % i.e. 100 % grass and 0.5% *Sargassum* species brown seaweeds supplement). Similar scanty surface mycelial density was also recoded from 15 and 20% supplement rates for 8 and 9 supplements. The extent of vegetative mycelia growth on various substrates possibly depended on their civil structure nature, porosity, nutrients proximate composition as well as the type of mushroom and species/strain cultivated. Oei (2003), Assan and Mpofu (2014) observed that water contents, aeration, availability of nutrients, pH, microbial activity, substrate formulations and species and/or strain variations, physical-chemical nature of the medium, additives/supplements, physical make up of substrate are amongst factors which determine the quality of mushroom substrates which influences mycelia growth and ultimate development into mushroom fruiting bodies.

Table 3. *Coprinus cinereus* (Schaeff.) S. Gray s. lato surface mycelial density on grass basal substrate supplemented with *Sargassum* species brown seaweeds frond at different supplement concentration (%) rates after 5 days of colonization.

Supplement seaweeds concentration in substrate (%)	Suppl. 1	Suppl. 2	Suppl. 3	Suppl. 4	Suppl. 5	Suppl. 6	Suppl. 7	Suppl. 8	Suppl. 9
0	++	++	++	++	++	++	++	++	++
0.5	++	++	++	++	++	++	+++	++	++
1	+++	+++	+++	++	++	++	+++	++++	++
2	+++	+++	+++	++	+++	++	+++	++++	+++
5	+++	+++	+++	+++	+++	++	++++	++++	+++
10	++++	++++	++++	++++	++++	+++	++++	++++	++++
15	++++	++++	++++	++++	++++	+++	++++	++	++
20	++++	++++	++++	++++	++++	++++	++++	++	++

Key: + = Very Scanty growth; 2+ = Scanty growth; 3+ = Moderate growth; 4+ = Abundant growth and 5+ = Very abundant growth Suppl. = Supplement

Productivity and yield of Coprinus cinereus (Schaeff.) S. Gray s. lato cultivated on grass basal substrate supplemented with Sargassum species brown seaweeds fronds at different supplement rates.

This is the first scientific report on cultivation of *Coprinus cinereus* (Schaeff.) S. Gray s. lato on grass basal substrate supplemented with *Sargassum* species brown seaweeds frond at different supplement concentrations (rates). In all grass basal substrate formulation incorporating seaweeds supplement at different concentrations (rates) there was complete vegetative mycelia colonization and fruiting bodies production even though the extent differed amongst formulations. Results showed that primordial were observed in all nine (9) *Sargassum* species brown seaweeds fronds supplements and all seven (7) supplement rates (0.5-20%) on day 6 after colonization. First mushroom fruiting bodies harvest was on day 7 and day 9, except 15 and 20 % supplement concentration (%) rates for supplements 8 and 9 first harvest was delayed until day 14. Crop period (sum of incubation and fruiting periods) lasted for 23 days with mushrooms harvested from 500 g of fresh substrate, which was equivalent to162.32 g dry weight including the supplements. Productivity and yield attributes include biological efficiency (used to express the productivity of fungus), biological yield and mushroom yield for *Coprinus cinereus (Schaeff.)* S. Gray s. lato grown on grass basal substrate supplemented with nine *Sargassum* species brown seaweeds fronds at seven different supplement concentrations (%) rates are presented in (Table 4, 5 and 6). The data on biological efficiency, biological yield and mushroom yield for nine *Sargassum* species brown seaweeds fronds at seven different supplement concentrations (%) rates were statistically analyzed by repeated measures of ANOVA. A post-test was performed using Tukey-Kramer Multiple comparisons test. It was found that biological efficiency; mushroom yield and biological yield were statistically considered extremely significant ($p<0.0001$) on different supplement concentrations (%) rates tested. The biological efficiency was calculated to determine

how the *Coprinus cinereus (Schaeff.)* S. Gray s. lato mushrooms utilized efficiently nutrients present grass basal substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentrations (%) rates in the substrates combinations.

Biological efficiency and biological yield

The biological yield refers to the measure of total fresh weight of mushrooms harvested to the dry weight of substrate utilized. There were significant differences in biological yield with respect to the various grass basal substrate formulation incorporating seaweeds supplement at different concentrations % (rates). Therefore biological efficiency was expressed as a percentage of the proportion. Results in (Table 4 and 5) for biological efficiency and biological yield revealed that different supplement *Sargassum* species brown seaweeds fronds at different supplement concentrations (%) rates had an effect on how the *Coprinus cinereus (Schaeff.)* S. Gray s. lato utilized nutrients in grass basal substrate. The mean and standard deviation biological efficiency and biological yield were variable and extremely significant ($p<0.0001$) different among different supplement *Sargassum* species brown seaweeds fronds and different supplement concentration (%) rates. Results showed that there was no any particular remarkable trend of direct proportionality on increasing supplement concentration (%) rate with increasing biological efficiency and biological yield across all nine *Sargassum* species brown seaweeds fronds supplements. Beside *Sargassum* species brown seaweeds variations, treatment of samples by sorting of *Sargassum* species brown seaweeds fronds into five categories namely; basal part (older), middle part (mid age), tip part (young), whole and unsorted had a profound effect on biological efficiency and biological yield. Sorting of *Sargassum* species brown seaweeds by age represented one remarkable finding, which could be considered novel in this study. To that effect supplement 4 comprised of (*Sargassum poligocytum* brown sea weed, 8 cm tip-part which represented the young parts of the frond recorded the three relative maximum biological efficiency average of 51.39 % and biological yield of 531.98 g fresh mushroom/kg dry substrate at supplement concentration rate of 15%, followed by BE of 49.48% and biological yield of 494.80 g fresh mushroom/kg dry substrate obtained from 20% supplement concentration rate and BE of 47.97% and biological yield of 479.75 g fresh mushroom/kg dry substrate recorded from 10% supplement concentration rate. The minimum BE of 3.35% and biological yield of 33.47 g fresh mushroom/kg dry substrate was obtained from unsorted *Sargassum* species brown seaweeds supplement 8 at supplement concentration rate of 15%. This was followed closely by minimum BE of 3.57% and biological yield of 35.71 g fresh mushroom/kg dry substrate at supplement concentration rate of 20%. Direct comparison of biological efficiency and biological yield data obtained from *Coprinus cinereus (Schaeff.)* S. Gray s. lato grown on grass supplemented by *Sargassum* species brown seaweeds fronds at different concentration rates was not possible due to dearth of information in the literature which address the integrated marine and terrestrial bioresources in mushroom cultivation. The meager available literature on utilization of seaweeds (organic biomass marine bioresource) supplements and grass substrate (terrestrial organic biomass bioresource) in mushroom cultivation will be employed to provide general perspectives, trends, guidance and scenarios. Molloy et al. (2003) reported for the first time technical feasibility for the cultivation oyster mushroom, *Pleurotus sajor-caju* on various combinations of brewing and seaweeds industries and scrub grass. Mushroom production was best on those substrates combinations containing kelp (40% spent barley, 40% grass and 20% kelp hold fast or kelp sweepings) rather than *Gracilaria gracilis* combinations viz (40% spent barley, 40% grass and 20% *Gracilaria*, spent barley 50%, 40% grass and10% *Gracilaria*, spent barley 60%, 20% grass and 20% *Gracilaria*). The reason behind the scenario was speculated to be high salt content of the *G. gracilis* (Molloy et al., 2003). Recently Kaaya et al. (2012) reported growth of highest numbers and biomass of *Pleurotus sajor caju* mushrooms at 10% concentration seaweed (*Laminaria schinzii*) in grass as substrate. However, concentrations of seaweed above 10% were observed to suppress *Pleurotus sajor caju* mushroom growth while concentration of seaweeds above 25% supported no growth at all of *Pleurotus sajor caju* mushroom. Concentrations of 25% and above, inhibited *Pleurotus sajor caju* mushroom growth, probably due to high concentration of salt which was difficult to eliminate by soaking overnight in tap water and washing once (Kaaya et al., 2012). In this study the maximum biological efficiency of was recorded at 15% *Sargassum* species brown seaweed supplement concentration rate of 15% in grass substrate, followed closely by 49.48% observed at 20% supplement concentration rate. The main reason could be that in this study *Sargassum* species brown seaweed supplements were sundried at least twice in green house, whereby salts deposited on the surface was washed twice in fresh water, which probably lowered salt concentrations.

Mushroom yield

The results of mushroom yields are important both on scientific and mushroom growers point of views. Mushroom yields results represented the quantity of fresh *Coprinus cinereus* (Schaeff.) S. Gray s. lato, which can be obtained from wet or dry weight grass substrate. Therefore in this study the mushroom yield represented the proportion of fresh weight of *Coprinus cinereus* (Schaeff.) S. Gray s. lato harvested mushrooms to wet weight of grass basal substrate supplemented with *Sargassum* species brown seaweeds, recorded from 23 days cropping period. There were mean and standard deviation variations and statistically extremely significant ($p<0.0001$) differences mushroom yield (total fresh weights) with respect to the various grass basal substrate formulation incorporating nine *Sargassum* species brown seaweeds fronds supplements at seven different concentrations % (rates). The mushroom yields results (Table 6) demonstrated that each various grass basal substrate formulation incorporating nine *Sargassum* species brown seaweeds fronds supplements at seven different concentrations % (rates) supported the growth of mushrooms *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom differently. In this study the three relative maximum mushroom yields were all obtained on supplement 4 comprised of (*Sargassum poligocytum* brown seaweed, 8 cm tip-part, which represented the young parts of *Sargassum* frond.

Table 4: Biological efficiency (BE%) for *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom grown grass basal substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentrations (%) rates. (Values Mean±SD, n=3).

Supplement seaweeds concentration insubstrate (%)	Suppl. 1	Suppl. 2	Suppl. 3	Suppl. 4	Suppl. 5	Suppl. 6	Suppl. 7	Suppl. 8	Suppl. 9
0	14.25±7.07	14.25±7.07	14.25±7.07	14.25±7.07	14.25±7.07	14.25±7.07	14.25±7.07	14.25±7.07	**14.25±7.07**
0.5	23.81±1.49	25.40±2.23	17.14±4.00	38.72±1.54	22.66±0.69	35.91±4.80	31.85±3.03	31.09±4.33	**26.09±1.60**
1	23.99±3.03	36.77±1.86	13.30±0.72	31.34±1.37	34.54±5.85	23.06±5.83	36.99±1.07	18.24±10.29	**35.87±4.98**
2	32.79±1.91	30.48±0.08	17.98±0.35	44.52±8.92	37.21±10.36	29.24±2.01	32.69±2.93	28.96±3.54	**33.36±5.63**
5	23.53±6.13	44.34±5.66	28.99±1.00	36.42±1.02	39.48±6.13	35.86±3.35	31.57±1.76	14.99±4.29	**27.32±4.12**
10	15.19±2.10	20.47±0.14	42.19±0.21	47.97±0.72	45.02±6.57	26.10±3.46	28.41±5.03	13.84±8.95	**20.69±7.62**
15	26.87±0.54	26.93±4.41	37.03±6.77	51.39±3.98	38.63±1.08	38.63±1.12	34.29±7.49	3.35±0.75	**13.13±1.86**
20	**27.95±0.47**	**17.64±5.35**	**24.16±4.01**	**49.48±0.57**	**39.92±1.28**	**33.42±11.03**	**18.34±0.25**	**3.57±0.13**	**17.52±4.74**

Table 5. Biological yield (BY) g fresh mushroom/kg dry substrate for *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom grown grass basal substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentrations (%) rates. (Values Mean±SD, n=3).

Supplement seaweeds Concentration in substrate (%)	Suppl. 1	Suppl. 2	Suppl. 3	Suppl. 4	Suppl. 5	Suppl. 6	Suppl. 7	Suppl. 8	Suppl. 9
0	142.54±70.71	142.54±70.71	142.54±70.71	142.54±70.71	142.54±70.71	142.54±70.71	142.54±70.71	142.54±70.71	**142.54±70.71**
0.5	236.13±14.88	254.11±22.30	171.43±39.96	387.19±15.40	226.69±6.90	359.12±48.03	318.54±30.34	310.96±43.31	**260.88±16.05**
1	239.93±30.25	367.73±18.56	133.06±7.16	313.49±13.75	345.54±5.85	230.65±58.20	369.90±10.74	182.37±102.94	**358.77±49.78**
2	327.90±19.10	304.89±0.74	179.80±3.56	445.25±89.22	372.16±103.36	292.40±20.05	326.78±29.14	289.59±35.36	**333.64±56.29**
5	235.30±61.30	443.44±56.62	290.02±9.97	364.25±10.18	394.82±5.73	358.61±33.52	315.77±17.60	149.91±42.85	**273.26±41.20**
10	151.87±20.97	204.77±1.37	412.95±2.08	479.75±7.17	450.28±65.68	261.02±34.60	284.10±50.26	138.43±89.50	**206.93±76.23**
15	268.67±5.41	269.38±44.08	370.34±67.74	513.98±39.82	399.21±12.76	386.33±11.19	342.96±74.90	33.47±7.50	**131.32±18.65**
20	**279.53±4.69**	**176.48±53.45**	**242.03±38.72**	**494.80±19.90**	**129.60±4.14**	**334.21±110.26**	**183.42±2.55**	**35.71±1.30**	**175.20±47.38**

The mushroom yield mean of 166.86 g fresh mushrooms/kg fresh substrate was recorded at supplement concentration rate of 15%, followed by mushroom yield of 160.64 g fresh mushrooms/kg fresh substrate obtained from 20% supplement concentration rate and mushroom yield 155.75 g fresh mushrooms/kg fresh substrate recorded from 10% supplement concentration rate. The minimum mushroom yield of 10.87 g fresh mushroom/kg fresh substrate was obtained from supplement 8 with unsorted *Sargassum* species brown seaweeds at supplement concentration rates of 15%. This was followed closely by minimum mushroom yield of 11.59 g fresh mushroom/kg fresh substrate at supplement concentration rates of 20%. It follows therefore that the results in (Table 6) definitely implied furthermore that the mycelia *Coprinus cinereus* (Schaeff.) S. Gray s. lato had different colonizing potentials on grass basal substrate formulations in which they were grown in SSFBs, which ultimately, corresponded to the mushroom yield obtained. To support that speculation different researchers had reported different mushroom yields for different mushroom species or strains, for different growing substrates with or without organic or inorganic additives/supplement as well as for different cultivation techniques (Assan and Mpofu, 2014). In fact the luxury and fast growth of a particular mushroom partly depend on the appropriate substrate nutrient composition used in its cultivation, strain used, duration of cropping period, which accordingly affect mushroom yield (Assan and Mpofu, 2014). To that effect the maximum mushroom yield range of 156-167 g/kg fresh substrate weight obtained in this study was fair representative for the specific experiments undertaken and was within a mushroom yield range between 102-331 g fresh mushrooms /kg fresh substrate reported previous for *Coprinus comatus* (O.F.Mull.) Gray cultivated on combinations of pararubber sawdust, kapokwaste and boiled sorghum (Chaiyama et al., 2007).

Enhanced Coprinus cinereus (Schaeff.) S. Gray s. lato mushroom yields by Sargassum species brown seaweeds organic supplementation

In order to demonstrate the effect of *Sargassum* species brown seaweeds organic supplement on mushroom productivity and yield comparisons were made between experiments supplemented with different supplements at different concentrations (%) rates and grass alone control (un-supplemented grass basal substrate). However, in order to be more focused and concise comparisons for biological efficiency, biological yield and mushroom yield were performed on maximum results obtained from supplement 4 comprised of (*Sargassum poligocytum* brown seaweed, 8 cm tip-part, which represented the young parts of *Sargassum* frond) and minimum results obtained from supplement 8 with unsorted *Sargassum* species brown seaweed (Tables 4, 5 and 6) compared to grass alone (un-supplemented grass basal substrate).

Table 6. Mushroom yield (MY) g fresh mushroom/kg fresh substrate for *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom grown grass basal substrate supplemented with *Sargassum* species brown seaweeds fronds at different supplement concentrations (%) rates. (Values Mean ± SD, n=3).

Supplement seaweeds concentration in substrate (%)	Suppl. 1	Suppl. 2	Suppl. 3	Suppl. 4	Suppl. 5	Suppl. 6	Suppl. 7	Suppl. 8	Suppl. 9
0	46.27±22.96	46.27±22.96	46.27±22.96	46.27±22.96	46.27±22.96	46.27±22.96	46.27±22.96	46.27±22.96	**46.27±22.96**
0.5	77.65±5.25	82.51±7.25	55.65±12.97	125.71±4.99	73.59±2.24	116.59±15.59	103.41±9.85	100.95±14.06	**84.89±5.21**
1	77.90±9.82	119.38±6.02	43.20±2.33	101.77±4.46	112.14±18.98	74.88±18.90	120.09±3.49	59.21±33.42	**116.81±16.54**
2	106.47±6.21	98.98±0.24	58.37±1.16	144.55±28.96	120.82±33.62	94.93±6.51	103.90±10.04	94.01±11.48	**108.31±18.27**
5	75.33±18.52	143.96±18.38	94.15±3.24	118.25±3.31	128.17±19.90	116.42±10.88	102.51±5.71	48.67±13.91	**88.71±13.38**
10	49.31±6.82	66.47±0.45	136.99±0.68	155.75±2.33	146.18±21.32	84.74±11.23	92.23±16.32	45.01±29.14	**67.18±24.75**
15	87.24±1.74	87.45±14.31	120.23±21.99	166.86±12.93	125.43±3.51	125.42±3.63	111.34±24.32	10.87±2.43	**42.63±6.06**
20	**90.77±1.52**	**57.29±17.35**	**78.66±12.69**	**160.64±1.86**	**129.60±4.14**	**108.50±35.80**	**59.55±0.83**	**11.59±0.42**	**56.88±15.38**

The three relative maximum viz; BE (51.39% at 15% supplement rate, followed by 49.48% at 20% supplement rate and 47.97% at 10% supplement rate) and the three relative maximum biological yield namely;(513.98 g fresh mushroom/kg dry substrate at 15% supplement rate, followed by 494.80 g fresh mushroom/kg dry substrate at 20% supplement rate and 479.75 g fresh mushroom/kg dry substrate at 10% supplement rate) increased by the range of 70-72% when compared to BE 14.25% and biological yield 142.54 g fresh mushroom/kg dry substrate obtained from control grass basal substrate alone (grass un-supplemented with *Sargassum* species brown seaweeds). It also implied that the three relative maximum BE % and biological yield were higher 3.1-3.6 times more than BE % and biological yield obtained from control grass alone (grass un-supplemented with *Sargassum* species brown seaweeds). The minimum BE (3.35% and 3.57%) and biological yield g fresh mushroom/kg dry substrate of 33.47 and 35.71 obtained from supplement 8 comprised of unsorted *Sargassum* species brown seaweeds at supplement rates of 15% and 20%, decreased by -299% and -325% respectively, compared to BE of 14.25% and biological yield of 142.54 g fresh mushrooms/kg dry substrate recorded from control grass basal substrate alone. That also meant that BE and BY obtained from supplement 8 at 15 and 20% supplement concentration rates were lower by a factor of 4 compared those recorded from control grass basal substrate alone. On the other hand, a similar trend and phenomenon was observed for mushroom yield. Best results were obtained from supplement 4 at 15% followed by 20% and 10% supplement concentration rates and worst results from supplement 8 at supplement concentration % rate of 15 and 20. Compared to mushroom yield 46.27 g fresh mushrooms/kg fresh substrate recorded from control, the three relative maximum yield 166.86 g fresh mushrooms/kg fresh at supplement concentration rate 15%, increased by (72.27%) and by a factor increase of 3.60, followed by mushroom yield of 160.64 g fresh mushroom/kg fresh at supplement concentration rate 20%, which increased by (71.19%) and by a factor increase of 3.47, finally mushroom yield 155.75 g fresh mushrooms/kg fresh at supplement concentration rate 10%, increased by (70.29%) and by a factor increase of 3.36. The minimum mushroom yields were recorded from supplement 8 with unsorted *Sargassum* species brown seaweeds. Mushroom yield of 10.87g fresh mushrooms/kg fresh substrate at 15% supplement concentration rate, decreased by (-325.6%) and was lower by a 4.25 factor. It was followed closely by the minimum mushroom yield of 11.59 g fresh mushrooms/kg fresh substrate at 20 % supplement concentration rate, which decreased by (-299.2%) and was lower by a factor of 3.99.

From the above interpretation scenarios for the best results it is apparent clear that the reason for such increase in biological efficiency, biological yield and mushrooms yield is linked to addition of *Sargassum* species brown seaweeds in particular (*Sargassum poligocytum* brown seaweed, 8 cm tip-part, which represented the young parts of *Sargassum* frond, at 15, 20 and 10% supplement concentration rates) to grass basal substrate employed for growing *Coprinus cinereus* (Schaeff.) S. Gray s. lato, which lacked in the control formulation with grass alone. It demonstrated good productivity of the grass substrate due addition of *Sargassum* species brown seaweeds, which resulted into quantity of materials readily available and absorbed by fungus during the mycelial development process and fruit body production of *Coprinus cinereus* (Schaeff.) S. Gray s. lato. That can be implicated to positive synergism established in the grass basal substrate medium and possibly there was supply of additional nutrients by *Sargassum* species brown seaweeds supplementation. It well documented in the literature by other researchers in mushroom biotechnology that supplements in basal mushroom substrates contain a mixture of protein, carbohydrate and fat, where the protein is the main source of nitrogen, they also contain minerals and vitamins that also exert profound influence on the growth of the fungus. The addition of these supplements aims mainly to increase the levels of nitrogen and carbohydrates available (Assan and Mpofu, 2014). However, limited information is available in the literature on use of seaweeds in particular brown seaweeds as supplements in mushroom substrate aimed at enhanced mushroom productivity and yield. Nevertheless, Molloy et al. (2003) and Kaaya et al. (2012) reported that incorporation of seaweeds into the mushroom substrates (terrestrial organic waste such as scrub grass, spent barley, brewery wastes etc) for oyster mushroom *Pleurotus sajor caju* reduced the rate of desiccation and increased water retention, which were found to be important aspects in production of the mushrooms. Additionally the incorporation of seaweed 10% (*L. schinzii*) to grass substrate significantly enhanced *Pleurotus sajor caju* mushroom yield than that of the grass alone suggesting that the seaweed might have provided some additional micronutrients to the substrate mixture. This yield enhancement might also be due to enhanced moisture retention capacity of the seaweeds and richness in polysaccharides (Kaaya et al., 2012).

Conclusion

This first scientific study was initiated to establish the possibility of using commonly available grass terrestrial bioresource as substrate and marine bioresource *Sargassum* species brown seaweeds beach cast waste as supplement to enhance yield of valuable *Coprinus cinereus* (schaeff) S. gray s.lat. mushroom crop. The maximum biological efficiency, biological yield and mushroom yield in grass substrate supplemented with *Sargassum* species brown seaweeds, makes its use feasible for growing edible and medicinal *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom from Tanzania. In particular supplement 4 comprised of (*Sargassum poligocytum* brown seaweed, 8 cm tip-part, which represented the young parts of *Sargassum* frond at 15% supplement concentration rates.

It can therefore be concluded that amongst nine supplements investigated, supplement 4 which was comprised of (*Sargassum poligocytum* brown seaweed, 8 cm tip-part, which represents the young parts of *Sargassum* frond at 15% followed by 20% and 10% supplement concentration rates would be a viable and promising formulation for cultivation *Coprinus cinereus* (Schaeff.) S. Gray s. lato on grass basal substrate that can be adopted to produce maximum biological efficiency, biological yield and mushroom yield. The positively demonstrated enhanced *Coprinus cinereus* mushroom production on grass supplemented with *Sargassum poligocytum* brown seaweed young frond could be replicated in all coastal regions in Tanzania, coastal countries in southern Africa and indeed elsewhere in the world. Most members of the brown seaweed have been reported to contain high iodine levels in their tissues (Molloy et al., 2003). *Coprinus cinereus* mushroom grown on grass supplemented with *Sargassum* species brown seaweed would contain iodine absorbed from the substrate combinations, but the claim needs validation through analysis of the mushrooms.

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