



SPENT MUSHROOM SUBSTRATE AS A MEDIA FOR LEAFY VEGETABLE CULTIVATION IN URBAN AGRICULTURE

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Abstract

The main objective of this study was to investigate the yield performance and disease suppression ability of SMS on leafy vegetable cultivation, i.e. Mukunwenna (*Alternanthera sessilis*). A field experiment was carried out from July to December 2011 at the research field of Agricultural Research Station, Telijjawila in Matara district located in Low country intermediate zone (IL1) of Sri Lanka. Simultaneously an in vitro experiment carried out at the Department of Agricultural Biology, Faculty of Agriculture, and University of Ruhuna, Sri Lanka to study the antagonistic effect of SMS on red leaf spot disease of Mukunwenna. The field experiment was arranged in Randomized Complete Block Design (RCBD) with four treatments and four replicates. The data obtained were tabulated and analyzed subjected to the Analysis of Variance (ANOVA) procedure of Statistical Analysis System (SAS). Results clearly showed that the total fresh weight of Mukunwenna was not significant different ($p < 0.05$) among different treatments tested. The in vitro study conducted using 72 bacterial isolates obtained from the SMS samples revealed that the ability of 35 of the 72 unidentified bacterial isolates significantly inhibit the *Cercospora*, i.e. causal organism of the red leaf spot disease in *Alternanthera sessilis*. The study enumerated the vast potential of SMS as an alternative growth substrate for a subsequent cultivation of leafy vegetables particularly for urban agriculture where solid waste disposal is problematic due to limited land availability.

Keywords

Spent mushroom substrate; leafy vegetable cultivation; yield performance; urban agriculture; *Alternanthera*; *Cercospora*

Introduction

After completion of the mushroom cropping cycle, proper disposal of the spent substrate is important as it can cause environmental pollution and ground water contamination. General way of removing Spent Mushroom Substrate (SMS) is piling in a corner of home garden, in the farm yard or in the road side. This allows pathogens to grow in the material and putrefying organic matter creates organic acids that often have a strong odor which is toxic to plant roots in concentrations

as low as 1 ppm [1]. Laboratory studies have shown that water extracts of SMS contain high concentrations of organic carbon, K, Ca, Mg, Cl, and SO₄ [2]. SMS is leached of soluble constituents by rainwater and percolates into ground water and pollute it. These considerations require an efficient and more meaningful way of handling these solid wastes. However, SMS has been found to be a good nutrient source for agriculture mainly because of its rich nutrient status, high cation exchange capacity (CEC) and slow mineralization rate which retain its quality as an organic matter. Further, SMS contains 45% water through bulky, is light in weight [3]. Spent mushroom substrate normally contains 1.9:4:2.4. %N-P-K before weathering. [4]. SMS contains comparatively less heavy metals than sewerage sludge, which precludes its classification as hazardous substance [5]. Also the ability of SMS as a plant diseases suppressant has been reported. The actinomycetes, bacteria and fungi inhibiting the mushroom substrate, not only expedite play role in its further decomposition but also exert antagonism to the normal pathogens surviving and multiplying the soil ecosystem [6]. Due to the unique chemical constitution and the microflora present in SMS, its application can be more diversified than what is normally predicted. According to above characteristics there is a potential to use SMS as leafy vegetable manure. Most of the Sri Lankans gain their vitamin and minerals requirement by having leafy vegetables. It has been reported that farmers apply excessive amounts of chemical fertilizer for the Mukunwenna cultivation and fertilizer application practices of all the respondents are not in agreement with the DOA recommendations. They have their own type, rate and the interval of fertilizer application. The negative impact of this practice is that it not only increases the cost of cultivation but also causes health risks. In the light of these situations it is important to find out the possibility of using SMS as an alternative remedy to minimize the use of chemical fertilizers and pesticides of Mukunwenna cultivation due to its rich nutrient value and disease suppression ability. This would not only cut down the cost for the cultivation but also enable to maintain a pest and disease free cultivation. There has been an upsurge of interest in home gardening even among the urban society despite many constraints faced by urban poor. Due to the limited land availability in urban agriculture for crop production it is very important to devise a practice to increase the productivity per unit land area. Mushroom cultivation which utilizes both horizontal and vertical space is regarded as the highest protein producer per unit area and time almost 100 times more than the conventional agriculture and animal husbandry [7]. Leafy vegetables particularly Mukunwenna is popular and mostly grown by many farmers for domestic consumption as well as for commercial purposes. Therefore, integrated cultivation of mushroom and leafy vegetables could be introduced to empower woman headed households, which are on the rise after the tsunami disaster and the civil war.

Materials and methods

Experimental site

The experiment was conducted in two steps, i.e. as a Field experiment and a Laboratory experiment (in vitro). Field experiment was carried out from July to December 2011 at the research field of Agricultural Research Station at Telijjawila in Matara district located in Low country intermediate zone (IL1) of Sri Lanka. The climate of

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this region is characterized by a mean monthly temperature of 27-30°C and mean annual rainfall of 1400mm-1500mm. The Soil group belongs to the Red Yellow Podzolk with pH range of 5.4-5.7. At the same time an in vitro experiment carried out at the Faculty of Agriculture, University of Ruhuna, Sri Lanka to study the antagonistic effect of SMS on red leaf spot disease of Mukunuwenna.

Field Experiment - SMS use as a leafy vegetable manure

Experimental treatments and design

The field experiment was arranged in a Randomized Complete Block Design (RCBD) with 4 treatments and four replicates. The treatments were: (T1) - Spent mushroom substrate (SMS) 1kg/sqm; (T2) - Cattle manure 1kg/sqm; (T3) - Poultry manure 1kg/sqm and (T4-control) - Compost 1kg + 8.5g Urea + 13g TSP+10g MOP/sqm (current fertilizer recommendation). Most widely grown Mukunuwenna variety in Southern Wet Zone of Sri Lanka, i.e. Piliyandala selection was selected to compare the influence of four fertilizer treatments on growth, yield and its diseases suppression ability. Fertilizers (treatments) were thoroughly incorporated to the soil and stem cuttings of Mukunuwenna were planted and watering was done according to the requirement. The crop was cultivated in commercial scale with recommended production practices.

Measurements

First harvest of Mukunuwenna was measured (Kg) one month after the crop establishment. Subsequent harvests were measured in monthly intervals. Furthermore, the disease incidences were evaluated by using a 25cm x 25cm quadrat. Quadrat was thrown to the beds randomly to take a count on healthy and red leaf spot infected plants in order to calculate the infected percentage and it was repeated thrice. Severity of disease incidence was recorded using 0-100% scale, i.e. where (0- represents no diseases incidence and 100-severe infection). The severity of disease incidences recorded as follows; 0% - no disease incidence; 0-25% - low disease incidence; 26-50% - moderate disease incidence; 51-75% - high disease incidence; 76-100% -very high disease incidence.

Laboratory Experiment

A sample of newly disposed SMS was obtained and single SMS bacterial colonies were isolated from the SMS. Each of the unidentified bacterial isolate was tested in vitro for its ability to inhibit *Cercospora* leaf pathogen isolates obtained from Mukunuwenna leaves. The Antagonistic activity of SMS bacteria against *Cercospora* was studied via Dual Plate Culture method and Antagonistic was determined by measuring the radius of the fungal colonies.

Statistical analysis

Data of the field experiment were tabulated and analyzed by using Analysis of Variance (ANOVA) Procedure of Statistical Analysis System (SAS). Duncan's New Multiple Range Test (DNMRT) was used to compare the differences among the treatment means at $p = 0.05$. The Laboratory experiment was verified based on one way ANOVA and mean comparison of different treatment was performed using Dunnett's Multiple Range Comparison Test at $p=0.05$ in SAS 9.1.

Results and discussions

Effect of SMS on yield

The first yield was harvested at 30 days after planting and the highest yield was obtained in the treatment four (control), i.e.

recommended level of fertilizer. Lowest yield was shown in the treatment two (T2), i.e. Cattle manure. Furthermore T2 and T4 are significantly different while T1 and T3 are not significantly different at $p<0.05$ level. Second yield was harvested at 60 days after planting, and the highest yield was again recorded from treatment four (T4) and the lowest was from treatment two (T2). Also T2 and T4 are significantly different while T1 and T3 are not significantly different ($p<0.05$). The third harvest was collected at 90 days after field establishment and the highest yield was recorded treatment four (T4) and the lowest was from treatment two (T2). Also T2 and T4 are significantly different while T1 and T3 are not significantly different at $p<0.05$ level [Table 1]. Results clearly revealed that the total yield of Mukunuwenna has no significant difference ($p>0.05$) among different treatments tested. That was a good indication of SMS as a nutrient amendment and showed a vast potential to use as leafy vegetable manure. This is in agreement with [8] who has stated spent mushroom substrate makes the soil suitable for raising vegetables. Ability of SMS to maintain high organic matter content in the soil has also been reported by [9] stated that SMS contains higher percentage of three primary nutrients such as nitrogen, phosphorus, and potassium as a fertilizer. Further [10] suggested that *Pleurotus* releases humic acid like fractions when added to soil which increase its fertility. [11] Has also stated humic substances may affect the plant biochemical process. This is may be a reason for the good yield performance of Mukunuwenna due to the application of SMS as manure. Furthermore the results of the current study on SMS is in agreement with the findings of [12] who reported that application of spent mushroom substrate to a potato cultivation improved the water holding capacity of the soil and resulted in increased plant uptake of nutrients. The yield obtained from the SMS treated beds showed a steady increase of yield over the cropping period as a result of the improvement of physical and chemical structure of soil due to weathering of SMS. The study conducted by [13] on spent mushroom substrate also recorded the positive impact on physical and chemical structure of soil.

Table 1: Effect of different treatments on subsequent yield (kg) of Mukunuwenna

Treatments	Mean yield (kg) of Mukunuwenna			Mean yield
	30 days after planting	60 days after planting	90 days after planting	
T1	1.37 ^{ab}	1.75 ^{ab}	2.02 ^{bc}	1.71 ^a
T2	1.18 ^b	1.7 ^b	1.85 ^c	1.57 ^a
T3	1.78 ^{ab}	2.02 ^{ab}	2.13 ^b	1.94 ^a
T4	1.95 ^a	2.03 ^a	2.38 ^a	1.99 ^a
<i>CV</i>	25.73	11.64	7.60	10.82
<i>LSD</i>	0.62	0.33	0.26	0.43

Note: Means with same letters along the columns are not significantly different at $p<0.05$ Measurements are the means of four replicates.

Effect of SMS on Red leaf spot disease

Percentage disease incidence of red leaf spot disease on subsequent harvest of Mukunuwenna was measured in monthly intervals. The disease incidence percentage under four treatments were significantly different at $p<0.05$ level [Table 2]. The results clearly revealed that the SMS has a significant impact on the quality of the yield as it recorded the lowest disease incidence in comparison to the rest of the treatments. [Table 2] Furthermore, the percentage disease incidences have decreased during the advanced stages of the growth of Mukunuwenna. This could have attributed as a result of applying

spent mushroom substrate as topdressing to fulfill the subsequent nutrient requirement after each harvest. After topdressing application plenty of water was applied to the plant beds and this might influenced to act SMS as water based slurry against foliage pathogen because, similar result has obtained by [14] who reported that application of spent mushroom substrate to control apple scab disease caused by *Venturia inaequalis*. Furthermore, the results of this experiment are in agreement with [15] who demonstrated the application of spent mushroom substrate as a soil amendment reduces early dying of potato caused by *Verticillium dahlia*. Also [16] has mentioned that organic substrates such as mushroom compost encourage a population of antagonistic microorganisms that interfere with the activity of plant pathogens. Due to unique chemical constituents and the micro flora present in SMS, its application can be more diversified than what in normally predicted.

Table 2: Effect of different treatments on red leaf spot disease (%) of Mukunuwenna

Treatments	Disease incidence (%) of Mukunuwenna			overall mean
	30 days after planting	60 days after planting	90 days after planting	
T1	8 ^b	4 ^c	2 ^c	4.7 ^c
T2	27 ^a	13 ^b	12 ^b	17.58 ^b
T3	22 ^a	14 ^b	13 ^b	16.25 ^b
T4	25 ^a	25 ^a	25 ^a	25.37 ^a
<i>C V</i>	20.86	7.80	11.26	22.90
<i>LSD</i>	6.59	1.69	2.31	7.30

Note: Means with same letters along the columns are not significantly different at $p < 0.05$. Measurements are the means of four replicates.

In vitro assay for bacterial antagonists in SMS

Second objective of this study is to determine the antagonistic effect of SMS on red leaf spot disease. In this experiment, antagonisms of SMS bacteria were assays under in vitro condition. A total of 72 bacterial isolates were obtained from the spent mushroom substrate (SMS) sample and tested for antagonism against the red leaf spot pathogen (*Cercospora*). Thirty five of the 72 unidentified bacterial isolates significantly inhibited ($p=0.05$) the mycelial growth of (*Cercospora*) in vitro. In this study different bacteria of SMS were isolate in the screening process to test their antagonistic effect individually. The bacterial isolates varied in their inhibitory capacity. The average length of the inhibition zones between the fungus and the antagonistic bacterial colonies ranged from 0.17-2.13 cm.

The findings of this investigation are in agreement with below findings. [17] have reported a total of 849 bacterial isolates from the two spent mushroom substrate (SMS) samples and tested for antagonism against the gray leaf spot pathogen of perennial ryegrass (*Lolium perenne*). Thirty-two of the 849 unidentified bacterial isolates (3.8%) significantly inhibited ($p= 0.0001$) the mycelial growth of *Pleurotus grisea* in vitro. The results of this preliminary study clearly indicated the presence of antagonistic bacteria in spent mushroom substrate which are highly effective against red leaf spot pathogen. Therefore, this study confirms that the disease control potential of SMS lies with the available bacterial population in the medium. Hence, there is a potential to utilize this important biological control agent in a commercial scale. However, it is important to identify the individual bacteria using a precise technique such as fatty acid evaluation or molecular identification method. Furthermore, efficacy of the bacterial isolates under in vivo conditions must also be tested to evaluate their stability in the outside environment. Results clearly indicated that the total fresh weight of Mukunuwenna has no significant difference

among four treatments tested on the crop yield. Nevertheless SMS treatment has a significant impact on the quality of the yield as it recorded the lowest disease incidence ($p < 0.05$) in comparison to the rest of the treatments. The disease incidence in the cattle manure (CM), poultry manure (PM) were moderate but were significantly lower than of the DOA Fertilizer recommendation (DR) treatment. The results indicate the suitability of SMS as alternative manure for leafy vegetable cultivation in urban agriculture. Furthermore the results of the in vitro experiment validate the disease suppression ability of 35 bacterial isolates extracted from the SMS.

Dispute these evidences; farmers are still using chemical fertilizers for leafy vegetable cultivation thus incurs unnecessary expenditure on inputs. Excessive use of chemical fertilizers are adherence to sole crop for a longer duration with no crop rotation were observed as some draw backs which would further aggravate the crop vulnerability to fungal disease. This situation has created a scenario in which haphazard use of chemical fungicides and chemical fertilizer has posed environmental and health risks. The findings of this investigation are in agreement with [18] who have reported the influence of *pleurotus ostreatus* in association with plant growth promoting rhizobacteria (PGPR), to promote plant growth and disease management.

In urban agriculture, land availability for crop production is become a limiting factor.

As there is no cost involved in applying SMS, it provides simple cost effective and safe strategy for the cultivation of leafy vegetables. Also it is reported that woman headed households are on the risk in Sri Lanka and provision of food and livelihood security has become a burning issue. Female headed households have in existence in Sri Lanka for many decades, the advent of war in the north east and the political unrest in the South in the late 80s and early 90s has resulted in emergence of female headed households as a critical challenge to policy makers. Therefore in door cultivation of mushroom and leafy vegetable cultivation would provide them self-employment as a remedy to gain their livelihood.

CONCLUSIONS

The study findings clearly revealed that the feasibility of utilization of SMS to promote plant health and productivity in agricultural systems. Therefore, results of this study enumerated the vast potential of SMS as an alternative growth substrate for a subsequent cultivation of leafy vegetable particularly for urban agriculture where solid waste disposal is problematic due to limited land availability.

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