



Study on antagonistic effect of spent *Pleurotus* mushroom substrate

N P Vidanapathirana

Department of Agro-Technology, University of Colombo Institute for Agro-Technology and Rural Sciences, Hambantota, Sri Lanka

Abstract

The compost left after the completion of the mushroom cropping cycle better known as “spent mushroom substrate” (SMS). Disposal of SMS is a matter of great concern particularly in urban agriculture where land area is limited. The use of SMS as a bio fertilizer and bio pesticide is an alternative for this disposal SMS. Therefore, this research has been conducted to find out the reutilization potential of SMS of *Pleurotus ostreatus* mushroom for subsequent mushroom cultivation and its’ dual utility as a bio-fertilizer and bio-pesticide in leafy vegetable cultivation. Further to find out the most effective micro-organisms in the SMS having antagonistic effect. An *in vitro* experiment was carried out to study the antagonistic effect of microbes residing in SMS to evaluate its efficacy in managing red leaf spot disease in Mukunuwenna (*Alternanthera sessilis*). A sample of newly disposed SMS was obtained to isolate SMS associated single bacterial colonies. Each of the unidentified bacterial isolate was tested *in vitro* to determine its growth suppressive ability against *Cercospora* leaf pathogen isolated from infected Mukunuwenna leaves. Different bacteria of SMS were isolated in the screening process to test their antagonistic effect individually. The antagonistic activity of SMS associated bacteria against *Cercospora* was studied via co cultivation method and degree of suppression was determined as percent inhibition of radial growth relative to a control. Ten treatments (10 bacteria samples from SMS), 03 replicates used in CRD design. Inhibition zone of each treatment has measured. Sequence of the two bacterial samples with high inhibition/suppression rate – (Bacteria 07-/T7) were tested by sending samples to Genetech, Sri Lanka. According to the blast results given, bacteria in those two samples were *Klebsiella pneumoniae* strain OKF08 and *Klebsiella pneumoniae* strain 211. It can be concluded that SMS is having an antagonistic effect and it can be used to control red leaf spot disease in Mukunuwenna. Therefore, this can be used as biological control method for controlling diseases in leafy vegetable cultivation. The bacteria in SMS which is having this bio control effect is *Klebsiella pneumoniae*.

Keywords: antagonistic effect, bio fertilizer, bio pesticide, *Pleurotus ostreatus*, spent mushroom substrate

Introduction

Edible mushroom cultivation is a unique exploitation of microbial biotechnology, wherein nutritionally valueless agricultural waste is converted into protein rich food. *Pleurotus* mushrooms are the second most cultivated edible mushroom worldwide next to *Agaricus* species. Unlike most other mushrooms, Oyster Mushrooms (*Pleurotus* species) has the unique adaptation to a wide range of ligno-cellulosic substrates to complete its’ life cycle. In Sri Lanka soft wood sawdust is the main ingredient sought by farmers for oyster mushroom cultivation but its prevailing scarcity necessitate the search for alternative substrates.

The compost left after the completion of the mushroom cropping cycle better known as “spent mushroom substrate” (SMS) is often discarded as waste contrary to the acclaimed benefits of SMS as a biofertilizer and biopesticide (Ahlawat and Sagar, 2007) [4]. Disposal of SMS is a matter of great concern particularly in urban agriculture where land area is limited. Utilization potential of spent mushroom substrates of some species of the genus *Pleurotus* (e.g. *P. eous*) as a supplementary substrate component to wheat straw-based substrate for the cultivation of other species of the same genus (*P. sajor-caju*) has been reported (Singh, 2009; Sharma and Jandaik,1995). The ability of SMS to control foliar diseases have also been documented (Oei, 2007) [15]. Despite this empirical evidence on SMS elsewhere, no effort has been taken to explore the beneficial aspects of SMS in Sri Lanka to convert this “waste into wealth”.

Red leaf spot disease is particularly common in Mukunuwanna cultivation. Red spot disease causes red spots on the top of the leaf. Red leaf spot disease caused by a fungi decreases the value of the quality of the cultivation and the demand of consumers will decrease. To prevent this fungus, the farmers are using fungicides with high amounts. Adding chemicals to leafy vegetables is a risk for non-communicable diseases. This is especially due to the fact that leafy vegetables are only cooked for at least one steam. In a low boiling point chemicals are not destroyed and they are consumed.

Farmers are using heavy doses of pesticides to leafy vegetable cultivations especially for Mukunuwenna and Gotukola. The ability of SMS to control foliar diseases have also been documented (Oei, 2007) [15]. Therefore, it is important to find out bio control method of controlling foliar diseases of leafy vegetables. Hence considering this, an experiment was designed with the objective of to find out the reutilization potential of SMS of *Pleurotus ostreatus* mushroom for subsequent mushroom cultivation and its’ dual utility as a bio-fertilizer and bio-pesticide in leafy vegetable cultivation.

Materials and Methods

An *in vitro* experiment was carried out to study the antagonistic effect of microbes residing in SMS to evaluate its efficacy in managing red leaf spot disease in Mukunuwenna. A sample of newly disposed SMS was obtained to isolate SMS associated single bacterial colonies

during July to October 2018. Each of the unidentified bacterial isolate was tested in vitro to determine its growth suppressive ability against *Cercospora* leaf pathogen isolated from infected mukunuwenna (*Alternanthera sessilis*) leaves. In this study, different bacteria of SMS were isolated in the screening process to test their antagonistic effect individually. The antagonistic activity of SMS associated bacteria against *Cercospora* was studied via co cultivation method and degree of suppression was determined as percent inhibition of radial growth relative to a control.

Treatment structure

There were ten treatments in the experiment and each treatment was replicated three times in Complete Randomized Design (CRD). Treatments were defined according to the different bacteria in SMS. These Bacteria were Unidentified. The treatments were as follows: T1= Bacteria 1; T2= Bacteria 2; T3= Bacteria 3; T4= Bacteria 4; T5= Bacteria 5; T6= Bacteria 6; T7= Bacteria 7; T8= Bacteria 8; T9= Bacteria 9 and T10= Bacteria10

Preparation of the PDA Media

Peeled potato pieces were boiled and strained. Distilled water was added to make the solution into 1l. About 30g of sugar was added into the stock after it had cooled. pH of the solution was adjusted into 7. Then 10g of Agar was added into the stock solution and heated that to a condensed solution until bubbling occurs. After cooling of solution, it was poured into test tubes and to flasks. Then flasks were kept in the autoclave for the sterilization for 30 minutes.

Bacteria Isolation from SMS

It has taken 20g of SMS and dissolve that with distil water. Kept the solution in a shaker for about one hour. The solution was filtered by using a filter paper. Two samples were prepared to use in the centrifuge. The two samples were centrifuged for 10 minutes 1000rpm (the recommended time is 3 minutes in 8000rpm)

Bacteria Dilution

The dilution has done in a range. The 1ml from the supernant has taken out by using a micro pipet and mixed that with 9ml of distil water in a conical flask. Repeated this in a range of distil water for four times. Diluted the microbes properly.

Bacteria Culturing

Bacteria samples were grown in PDA media which had prepared separately. It has used 0.1ml from the bacteria samples for each petri dish with PDA. Then allowed them to grow in the lab.

Re-culture the separately grown bacterium

The diluted bacteria were allowed to grow in 4 petri dishes per 1 ml of 4 samples. 10 petri dishes were re-cultivated in a numbered petri dishes, clearly differentiated from the bacteria grown in the petri dishes.

Isolation of Red leaf Spot Disease Pathogen

A leaf fungus found in Mukunuwenna, infected with red spot, was carefully cleaned and cultivated in a medium. Washed the Infected *Alternanthera* leaves properly the by

using distilled water and dried them and cultured in PDA media.

Co-cultivation

The simultaneous cultivation of two or more species of microorganism, or types of tissue, in the same medium is called as Co-cultivation. Co-cultivation was performed by cultivating Bacteria and fungi in the same medium.

After the numbered bacteria were allowed to grow separately, 10 well-developed bacteria were selected and allowed to grow in a single culture medium with the fungus grown. One bacterium was allowed to grow in three petri dishes as three samples.

Red spot disease with a fructose stem obtained from the fungus, and the fungus were cultivated in the same medium with 10 undetected bacteria. Each bacteria was grown in each petri dish with the fungus. It was later developed to allow growth to increase the growth of the fungus through the bacteria.

Data collection process

Bacteria and fungi were grown in the same media and growth was measured. It was observed that the bacteria and the fungus grew in the petri dish and bacteria controlled the growth of the fungus. It was used to measure the gap between the fungus and the bacterium. Inhibition zone between the bacteria and the fungus was measured.

Data analysis

It has selected the 2 most effective bacteria having the highest antagonism against pathogenic fungi and identified the bacteria (by Gene-Tech) which can suppress pathogenic fungi. The collected data were analyzed in analysis of variance with MINITAB statistical data analysis software.

Results and Discussion

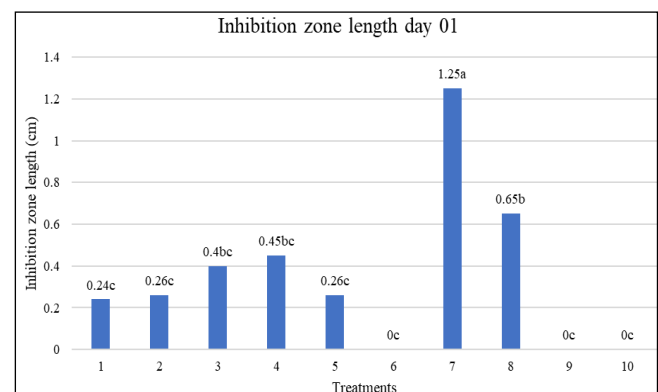
Bacterial Identification

Sequence of the two bacterial samples with high inhibition/suppression rate – (Bacteria 07-/T7) were tested by sending samples to Gene tech.

According to the blast results given Bacteria in those two samples are

- Klebsiella pneumoniae* strain OKF08
- Klebsiella pneumoniae* strain 211

Inhibition zone length (Day 01)



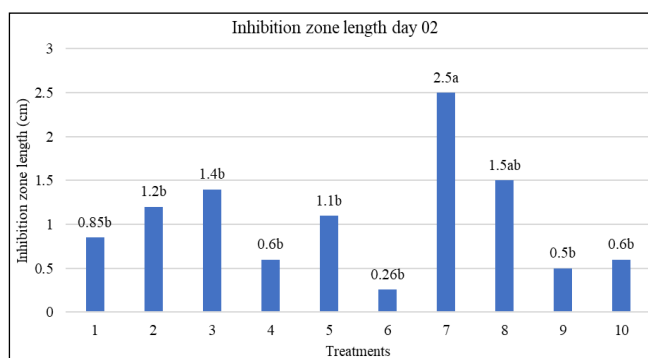
Means that do not share a letter are significantly different

Fig 1: Inhibition Zone length Day 01

It was revealed that treatment no 07 and treatment no 08 was observed the significantly highest inhibition zone length at day 01 ($p > 0.05$) with compared to other treatments. Treatment no 6, 9 and 10 has observed zero inhibition zone. Treatment 07 has given the highest inhibition. According to the bacterial identification it has revealed that the bacteria in treatment 07 is bacterium *Klebsiella pneumoniae* and it has been reported to protect rice from sheath blight and seedling blight, and also to control Nematodes in Soybean (Liu, *et al* 2018) [13]. The World Health Organization considered infections brought on by pathogens resistant to drugs to be a public health problem because the unchecked use of antibiotics has caused serious problems in human and animal health. As a result, it is necessary to find new pharmacological strategies, among which we can find natural products such as plants and fungi (Shanmugavelu and Sevugaperumal, 2021) [20].

The mycelium of *P. ostreatus* has been utilized in investigations on the antibacterial activity against a variety of microorganisms using water and alcoholic extracts. Water extract had the maximum effectiveness, particularly against the germs *Staphylococcus aureus* and *Escherichia coli* as well as the fungus *Candida albicans*, *Cryptococcus humicola*, *Trichosporon cutaneum*, and (Piska *et al.*, 2017; Verma, 1986) [17]. The antibacterial characteristics of mushroom extracts were emphasized, along with some of the found active components, such as low- and high-molecular weight compounds that exhibited antagonistic action against gram positive bacteria. Sesquiterpenes and other terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolines represent the majority of LMW chemicals, although there are also some primary metabolites like oxalic acid. The majority of HMW substances are peptides and proteins.

Inhibition zone length (Day 02)



Means that do not share a letter are significantly different

Fig 2: Inhibition Zone length Day 02

It was revealed that treatment no 07 and treatment no 08 were observed the significantly highest inhibition zone length at day 02 ($p > 0.05$) with compared to other treatments. From those two treatments, treatment 07 has given the highest inhibition. According to the bacterial identification it has revealed that the bacteria in treatment 07 is bacterium *Klebsiella pneumoniae* and it has been reported to protect rice from sheath blight and seedling blight, and also to control Nematodes in Soybean (Liu, *et al* 2018) [13]. Growing mushrooms is an environmentally benign hobby since it makes use of leftover materials from horticulture, agricultural, poultry, brewing, and other industries.

However, the accumulation of "spent mushroom substrate," which is discharged after the harvesting of mushroom crops, may result in a number of environmental issues, such as ground water pollution and annoyance (Beyer 1996; Ahlawat *et al.*, 2011) [3]. For every kilogram of mushrooms produced, 5 kilograms of discarded leftover material, also known as spent mushroom substrate, are produced (SMS). 24 t of SMS are typically discarded by a farm each month (Singh *et al.*, 2011) [21]. According to Barry *et al.* (2012) [6], Ireland produces over 254,000 t of SMS annually, and The Netherlands produces more than 800,000 t. (Oei and Albert, 2012) [14].

Farmers in various nations struggle greatly with SMS trash management. Increasing demand for SMS by looking into new applications for use seems to be the logical approach. If SMS were to be recycled and utilized again, that would be more advantageous and cost-effective. Due to the high organic content of SMS, recent years have seen tremendous advancements and a surge in scientific study. The *Pleurotus* sp. spent mushroom compost utilized in this investigation contained *Bacillus*, *Clostridium*, *Pseudomonas*, and *E. coli* bacteria. This confirms the research on the microbial makeup of *Pleurotus* sp. wasted mushroom compost (Ahlawat *et al.*, 2007) [4]. For suppressing soil-borne diseases like *Meloidogyne* sp. in tomatoes and *Venturia inaequalis* in apples, commercial fungicides and nematicides have been shown to be less effective than SMS applied as a soil supplement (Ahlawat *et al.*, 2007) [4]. This study's findings from the dual culture experiment using the SMS microbial isolates and plant pathogenic fungi verified previous reports that some of these microorganisms exhibit antagonistic properties (Yohalem *et al.*, 1994) [25].

Severe plant diseases caused by fungi like *Phytophthora* and *Fusarium oxysporum* affect plant yields and the quality of the products. Additionally, they have a diverse range of hosts and infect economically significant agricultural crops globally with illnesses (Agrios, 2005) [1]. These fungal phytopathogens are challenging to manage because to their vast range of hosts as well as the fact that they are soil-borne (Summeral *et al.*, 2003) [23]. Due to its capacity to create highly toxic and carcinogenic aflatoxins, *Aspergillus flavus* is the most harmful fungus that may grow on stored rice grains (Scheidegger and Payn, 2003) [19].

The culture filtrates of the bacterial strains *Bacillus megaterium* KU143, *Microbacterium testaceum* KU313, and *Pseudomonas protegens* strongly suppressed the growth of *A. flavus*, according to research on the antifungal activity of microorganisms isolated from SMS. The *Pseudomonas* sp. isolate from SMS was shown to have antifungal activity against *Phytophthora* sp. The growth of *Rhizoctonia* sp., *Phytophthora parasitica*, *P. palmivora*, and *Fusarium solani* was shown to be inhibited by the antifungal chemicals isolated from this bacterium, which was also found to be effective in preventing mycelial growth (Shanmugavelu and Sevugaperumal, 2021) [20]. According to the findings of the study by (Shanmugavelu and Sevugaperumal, 2021) [20], discarded mushroom substrate has a significant number of native beneficial microorganisms that are capable of inhibiting soil-borne diseases. This SMS feature may be used as a substitute substrate for mass-producing biocontrol agents for field application, which might result in the eradication of diseases and higher agricultural output.

Conclusion

SMS is having an antagonistic effect and it can be used to control red leaf spot disease in Mukunuwenna. Therefore, this can be used as biological control method for controlling diseases in leafy vegetable cultivation. The bacteria in SMS which is having this bio control effect is *Klebsiella pneumonia* according to the blast result.

References

1. Agrios GN. Plant Pathology. 5th Edn., Elsevier Academic Press, New York, USA, 2005, 922.
2. Ahlawat OP, Ahlawat Kusum, Indurani C, Vijay B, Dhar BL. Chemical composition and enzymatic activity of spent paddy straw and oyster mushroom substrates. *Mushroom Science*,2004a:16:553-559.
3. Ahlawat OP, Manikandan K, Sagar MP, Dev R, Vijai B. Effect of composted button mushroom spent substrate on yield, quality and disease incidence of Pea (*Pisum sativum*). *Mushroom Res.*,2011:20:87-94.
4. Ahlawat OP, Sagar MP, Raj D, Indu Rani C, Gupta P, Vijay B. Effect of spent mushroom substrate on yield and quality of capsicum (*Capsicum annuum*). *Indian J Hort.*,2007:64:430-434.
5. Alhawawt OP, MP Sagar. Management of Spent mushroom substrate. Tech. bulletin; National Mushroom Research Centre, India, 2007.
6. Barry J, Doyle O, Grant J, Grogan H. Supplementing of spent mushroom substrate (SMS) as a casing material to improve the structure and productivity. In: 18th Congress of the International Society for Mushroom Science. Beijing, China, 2012, 735-742.
7. Beyer M. The impact of the mushroom Industry on the environment. *Mushroom News*,1996:44:6-13.
8. Chong C, Rinker DL, Cline RA. A comparison of five spent mushroom composts for container culture of ornamental shrubs. *Mush Sci*,1991:13:637-644.
9. Herrero-Hernandez E, Andrades MS, Marin-Benito JM, Sanchez-Martin MJ, Rodriguez-Cruz MS. Field-scale dissipation of tebuconazole in a vineyard soil amended with spent mushroom substrate and its potential environmental impact. *Ecotoxicology and Environmental Safety*,2011:74:1480-1488.
10. Jarecki MK, Chong C, Voroney RP. Evaluation of compost leachates for plant growth in hydroponic culture. *Journal of Plant Nutrition*,2005:28(4):651-667.
11. Jarecki MK, Chong C, Voroney RP. Evaluation of compost leachate for growing nursery trees on a waste-rehabilitated field site. *Compost Science & Utilization*,2012:20(3):171-180.
12. Litterick AM, Harrier L, Wallace P, Watson CA, Wood M. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production – a review. *Critical Reviews in Plant Sciences*,2004:23(6):453-479.
13. Liu D, Chen L, Zhu X, Wang Y, Xuan Y, Liu X, *et al.* *Klebsiella pneumoniae* SnebYK Mediates Resistance against *Heterodera glycines* and Promotes Soybean Growth. *Frontiers in microbiology*,2018:9:1134.
14. Oei P, Albert G. Recycling casing soil. In: 18th Congress of the International Society for Mushroom Science. Beijing, China, 2012, 757-765.
15. Oei P, Zeng H, Liao J, Dai J, Chen M, Cheng Y. The alternative uses of spent mushroom compost., 2007. Retrived on www.tuibouw.nl.
16. Phan CW, Sabaratnam V. Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. *Applied microbiology and biotechnology*,2012:96(4):863-873.
17. Piska K, Sulowska-Ziaja K, Muszyńska B. Edible mushroom *Pleurotus ostreatus* (Oyster mushroom) – its dietary significance and biological activity. *Acta Sci. Pol., Hortorum Cultus.*,2017:16:151-161.
18. Rinker DL. Spent mushroom substrate uses. *Edible and medicinal mushrooms: technology and applications.* Wiley, Hoboken, 2017, 427-454.
19. Scheidegger KA, Payne GA. Unlocking the secrets behind secondary metabolism: a review of *Aspergillus flavus* from pathogenicity to functional genomics. *J. Toxicol.*,2003:22:423-459.
20. Shanmugavelu M, Sevugaperumal G. Screening and Potential Uses of Contaminated Spent Mushroom (*Pleurotus spp.*). In *Emerging Contaminants.* IntechOpen., 2021.
21. Singh AD, Vikineswary S, Abdullah N, Sekaran M. Enzymes from spent mushroom substrate of *Pleurotus sajor-caju* for the decolourisation and detoxification of textile dyes. *World J. Microbiol. Biotechnol.*,2011:27:535-545.
22. Stoner KA, Ferrandino FJ, Gent MPN, Elmer WH, LaMondia JA. Effects of straw mulch, spent mushroom compost, and fumigation on the density of Colorado potato beetles (*Coleoptera: Chrysomelidae*) in potatoes. *Journal of Economic Entomology*,1996:89(5):1267-1280.
23. Summeral BA, Salleh B, Leslie JF. A utilitarian approach to *Fusarium* identification. *Plant Dis.*,2003:87:117-128.
24. Verma RR. Efficacy of organic amendments against *Meloidogyne incognita* infesting tomato. *Ind. J. Nematol.*,1986:16:105-106.
25. Yohalem DS, Harris RF, Andrews JH. Aqueous extracts of spent mushroom substrate for foliar disease control. *Compost Sci. Util.*,1994:2:67-74.