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Biological efficiency of recycling organic waste with edible fungi

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BIOLOGICAL EFFICIENCY OF RECYCLING ORGANIC WASTE WITH EDIBLE FUNGI

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Shyam S. Kurup

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Declaration of Original Work

I, Yusra Matar Al Shamsi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Biological Efficiency of Recycling Organic Waste with edible fungi*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Shyam Kurup, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Mushrooms has been known as important cultivars for their nutritional and medicinal values, the species *Pleurotus sajor caju* and *Calocybe indica* were selected in this investigation for being used in previous studies to be grown on different lignocellulosic materials. Cultivating mushrooms on plant wastes is value-added way to produce a source of human food and is an efficient way to recycle those residues. Date palm leaf waste, date palm bunch waste and mowed turfgrass waste were used as mushroom growing substrates. Date palm is the most important plant grown in the UAE and it produce tons of wastes every year similarly with the turfgrass. This study aims to use sustainable and novel technology for recycling organic waste for value addition with reference to UAE. The objectives focuses on assessing the bio efficiency of two different mushroom species in degrading the organic waste materials, the quality of spent waste to utilize it as organic matter for enriching the soil, the quality of spent waste in terms of using as ruminant feed and the comparable quality of mushrooms. Parameters that were tested are: growing period, fresh and dry weight of the yield, biological efficiency, macro-nutrients and trace minerals, proline, crude fiber and protein. It was concluded that concluded that the *Pleurotus sajor* has a higher nutritional value than *Calocybe indica* and date palm bunch waste has the higher values between three used substrates to be used in animal feed and soil enrichment.

Keywords: Mushroom, plant-waste, recycle, soil enrichment, ruminant feed.

Title and Abstract (in Arabic)

الكفاءة الحيوية لإعادة تدوير المخلفات العضوية باستخدام الفطر الصالح للأكل

الملخص

يعتبر فطر المشروم (عش الغراب) من الأنواع التي تمتاز بقيمة غذائية وعلاجية عالية. تم استخدام النوعين *Pleurotus sajor caju* و *Calocybe indica* في الدراسة لنجاحهما بالنمو على مواد ليجنينية وسيلولوزية مختلفة في دراسات سابقة.

زراعة وإنتاج المشروم على المخلفات النباتية يعتبر قيمة مضافة لإنتاج الغذاء وإعادة تدوير المخلفات بشكل فعال. تم استخدام مخلفات سعف النخيل وعذوق النخيل ومخلفات قص المسطحات الخضراء كأوساط نمو لفطر المشروم.

يعتبر نخيل التمر من أهم النباتات في دولة الإمارات العربية المتحدة بالإضافة إلى نباتات المسطحات الخضراء.

تهدف هذه الدراسة إلى إيجاد وسيلة مبتكرة ومستدامة لمعالجة المخلفات العضوية واستخدامها كإضافة نوعية لدولة الإمارات. تتلخص أهداف الدراسة في ما يلي: تقييم الكفاءة الحيوية لنوعين من الفطر من ناحية النمو على مواد المخلفات العضوية، نوعية المخلفات لاستخدامها كمادة عضوية لزيادة خصوبة التربة، نوعية المخلفات فيما يخص استخدامها في تغذية المجترّات ، والمقارنة النوعية للمشروم.

تم إجراء القياسات التالية: فترة النمو، الوزن الرطب والجاف للمحصول، الكفاءة الحيوية، العناصر الغذائية الكبرى والصغرى، البرولين، الألياف الخام، والبروتين.

خَلُصَت الدراسة إلى أن نوع *Pleurotus sajor caju* يحتوي على قيمة غذائية أكثر من النوع *Calocybe indica* ، كما أن مخلفات عذوق النخيل تمتاز بقيمة أعلى من ناحية استخدامها في تغذية المجترات وزيادة خصوبة التربة مقارنة بالأوساط الأخرى.

مفاهيم البحث الرئيسية: فطر المشروم، المخلفات النباتية، إعادة تدوير، تخصيب التربة، أعلاف الحيوانات المجترة.

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My friends and colleagues.

Dedication

To my great parents

Table of Contents

List of Tables

List of Figures

List of Plates

List of Abbreviations

MWM	*Calocybe indica*
Pl	*Pleurotus sajor caju*
DPLW	Date Palm Leaf Waste
DPBW	Date Palm Bunch Waste
MTGW	Mowed Turfgrass Waste
ADF	Acid Detergent Fiber
NDF	Neutral Detergent Fiber
NS	Not Significant

Chapter 1: Introduction

1.1 Overview

Fungi have been grown around the world for more than 400 million years, they have a wide diversity including mushrooms which have been used by people for food and medicinal purposes. They have worldwide importance as food and medicine source and one of the biggest agricultural productions (Miles et al., 2004). Mushrooms are considered as saprophytes, living on dead or decayed organic matters (Jiskani, 2001). As heterotrophs, mushrooms obtain the sufficient nutrients to grow from organic sources, using secreted enzymes that decompose dead organisms to be absorbed (Enger, 2003). They are also a good source of carbohydrates, vitamins, fats, minerals and amino acids. They are a rich protein source and they are classified among the best vegetables and animal protein source. They have double the value of protein as that in the potatoes and asparagus, four times more than carrots and tomatoes and six times more than orange (Jiskani, 2001). They contain all of the essential minerals and amino acids as well as water soluble vitamins (Adejumo T. O., 2005). According to (Ogundana, 1982) mushrooms are about 16.5% dry matter, 14.6% of the dry matter is crude protein, 7.4% crude fiber and 4.48% is the fat and oil content.

Mushrooms were collected by people in the wild until A.D 600 when the Chinese started cultivating mushrooms on logs and people kept on cultivating them in this way until 1600 when the biggest advance in cultivating mushrooms started in France where they were grown on composted substrate.

Therefore, the focus of our study is to exploit the cultivation of mushrooms on specific substrates for value addition.

1.2 Relevant Literature

1.2.1 *Pleurotus sajor caju*

Pleurotus sajor caju belongs to the fungi kingdom and is classified under the phylum Basidiomycota (Stamets, 1983). It is one of the edible mushroom species that are commercially cultivated in special methods under controlled conditions in cultivation rooms and farms (Thomas & Schumann, 1993). *Pleurotus* species are one of the most popular mushroom around the world especially in Asia and Europe, with a low cost and simple production techniques and high biological efficiency (Mane, 2007). *Pleurotus* sp. is one of the highest cultivated mushrooms worldwide as it reaches 25% of the total production of cultivated mushrooms around the world (Miles & Chang, 2004).

It can grow on different agricultural wastes due to its lignin degradation efficiency and its ability to adapt to different agro-climatic conditions (Jandaik, 1995). The cultivation of *Pleurotus* sp. in lignocellulosic wastes is a biotechnological process to recycle those wastes and it is the only way that combines producing edible mushrooms with reduced pollution in the environment (Sánchez, 2010).

Pleurotus mushrooms have nutritional and medicinal value (Agrahar-Murugkar, 2005). They are a rich source of proteins and minerals such as calcium, phosphorus, potassium, sodium and iron, similarly they are a good source of vitamin C, folic acid, thiamine, niacin and riboflavin (Çağlarırmak, 2007). They contain trace elements and they are a low caloric food (Badu & Boadi, 2011), which have all the essential amino acids to enhance the quality of the protein (Purkayastha R. P., 1981). *Pleurotus* species medicinal value is due to having significant antioxidant, anticancer (Kim et al, 2009), anti-inflammatory, antiviruses (Peres et al., 2007), antifungal

(Owaid et al., 2015), antimicrobial (Akyuz et al., 2010) and anti-parasitic activities (David et al., 2012).

1.2.2 *Calocybe indica*

Calocybe indica is a tropical edible mushroom that belongs to the family Tricholomataceae of the order Agaricales (Purkayastha R. P., 1976). It became more popular due to its attractive color, vigorous size, sustainable yield, good taste, and unusual texture (Amin et al., 2010). It is rich in protein, mineral, carbohydrate, fiber, lipid, and is rich with essential amino acids (Alam et al., 2010). Similarly it is as a premium source of thiamine, nicotinic acid, riboflavin, biotin, pyridoxine and ascorbic acid (Breene, 1990).

This mushroom variety was identified first in the eastern Indian state of West Bengal. It can be cultivated at a high temperature range (30~38 ℃) on a wide variety of substrates (Subbiah & Balan, 2015). The first occurrence of *Calocybe indica* P&C was reported in India where they call it “Dhuth chatta” which means “Milky white mushroom”. It is collected and sold in the local markets in West Bengal due to its white color and fine texture which make it attractive to consumers (Vikineswary & Chang, 2013). They are grown in nature on humus rich soil (Purkayastha R. P., 1984) between May and August every year (Subbiah & Balan, 2015).

1.2.3 Using agro-wastes to grow edible mushrooms

Edible mushrooms cultivation with agricultural wastes is a value-added way to convert those waste materials into a media to grow human food. It is an efficient biological way to recycle those residues (Madan et al., 1987). Mushrooms from nutritional point of view are rich in proteins, vitamins, moisture, minerals and fibers.

They are also low in calories due to the low fat content (Heleno et al., 2009). Some developing countries face the problem of protein shortage, they also face the problem of the rapid increase especially in agricultural wastes due to the industrial development and tremendous growth in urban landscaping, those two problems can be solved by growing edible mushrooms in recycled wastes (Erkel, 1989). Fungi have the ability to colonize wood and wood waste in order to produce edible reproductive structures, this method has been used for centuries in Asia to produce oyster mushrooms (*Pleurotus* sp.) (Leatham, 1981) (Zadrazil, 1974).

Some studies have been done on the use of lignocellulosic materials and agro-wastes to produce edible mushrooms, such as: tea waste (Gülser, 2003), rice straw, cotton waste, corn cobs waste (Owaid MN, 2015), paddy straw (Zhang et al., 2002), wood substrate (Tisdale et al., 2006), tomato tuff mixed with wheat straw (Al-Momany & Ananbeh, 2010) and date palm wastes.

1.2.4 Interaction between mushrooms and substrates

Using different substrates in mushrooms cultivation has an effect on mushroom's functional, chemical and organoleptic properties. Mushrooms get advantage from the substrate as the substrates get advantage too. A study that was done by (Michael & Pant, 2011) showed that iron, phosphorus, ash and protein content differ comparing the two substrates that were used in the study. Other studies showed that mushroom cultivation improves the substrate quality along with producing nutritious food (Patil et al., 2010). This occurs in reducing cellulose, crude fiber and lignin making the substrate a typical animal forage (Ortega et al., 1992).

1.3 The need of waste management in UAE

United Arab Emirates is one of the developing countries around the world, due to the rapid development in the country, the pollution has increased because of the high amount of wastes that are buried or burned. According to (Saifaie, 2013), the general waste influx in Dubai has increased by 1165986 tons between 1997 and 2003 where 35% of those wastes is organic.

This led to the need of a serious solution for this environmental hazard, recycling wastes has been organized by private companies in the 1990s but still it was recorded that agricultural wastes in Dubai has reached 175022 tons per year in 2011.

1.4 Focus of work

This study focuses on developing relatively simple sustainable and novel technology for recycling organic waste for value addition with reference to UAE. There are no previous report on the use of date palm bunch waste, leaves and mowed turf waste to serve as substrate in edible mushroom production for value addition. The organic agriculture waste generated in the form of date palm bunch waste, pruned date palm leaves and mowed turf grass waste from extensive landscape gardens will be biodegraded using edible fungi (Pleurotus and Calocybe) for their biological efficiency in producing value added products like edible mushrooms and organic compost from the spent waste for soil enrichment in UAE where the soil is extremely porous and devoid of humus. This can go a long way to further commercializing the technology for agricultural organic waste recycling in the country resulting in the production of high quality mushroom species that are suitable for the arid region to facilitate as one of the potential tools in maintaining the food security of the nation. The spent waste from mushrooms will be chemically tested for the nutritional quality to serve as

manure in enriching the soil for better agricultural production and nutritional ruminant feed.

1.5 Objectives

The objective of the study is outlined as follows.

1. To study the bio efficiency of two different mushroom species in degrading the organic waste materials.

2. Assess the quality of spent waste to utilize it as organic matter for enriching the soil.

3. Assess the quality of spent waste in terms of using as ruminant feed.

4. Assess the comparable quality of mushrooms.

Chapter 2: Materials and Methods

The experiment was conducted simultaneously at the Food and Agriculture college laboratories in the UAE University and Al Foah experimental farm during the summer season of 2015. The initial work commenced in the lab on 26th February, 2015 where the mushroom spawns where produced before inoculating it to the agricultural wastes substrates in Al Foah farm on 6th July, 2015.

2.1 Production of spawns

The fungus *Pleurotus sajor-caju* and *Calocybe indica* were obtained from College of Agriculture, Kerala Agricultural University, India. The fungus cultures were grown on wheat seeds. To enhance more mycelium growth, the spawns were grown in magenta boxes that contain Potato Detox Agarose media (PDA). PDA media was prepared by suspending 39 grams of PDA powder in 1 L of distilled water, heated to boil to dissolve the medium completely. After that it was sterilized by autoclaving at 15 lbs pressure and 121 ℃ for one hour. The media was mixed well before pouring to the magenta boxes. The PDA was left to cool and solidify. After cooling up a small amount of the mushrooms mycelium was inoculated on the media. The magenta boxes were sealed and kept in dark room under 25 ℃ for 14 days until the media was completely colonized with mycelium.

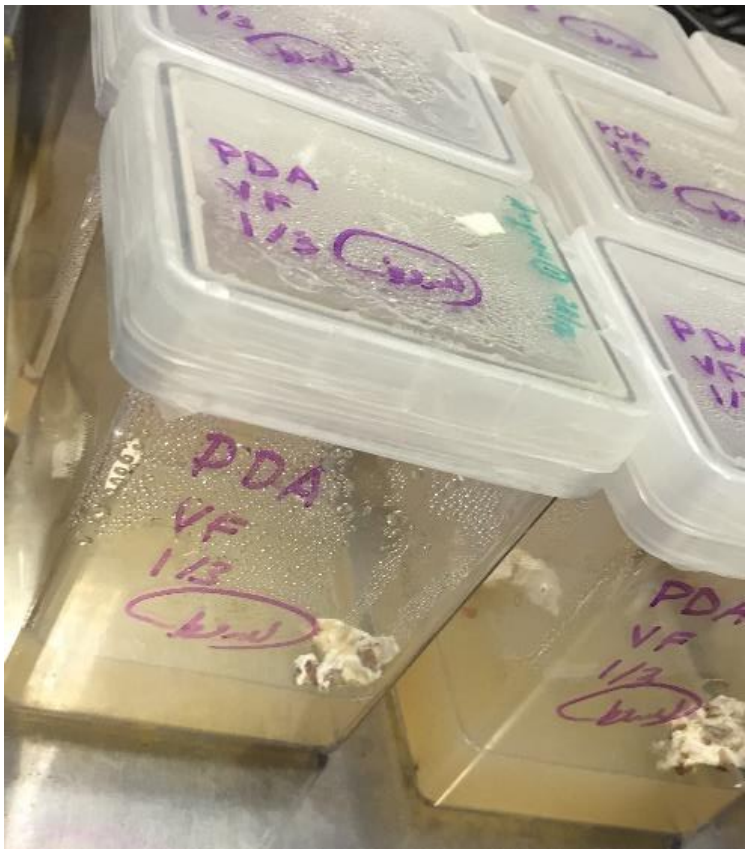

Plate 1: Spawns inoculated into PDA media

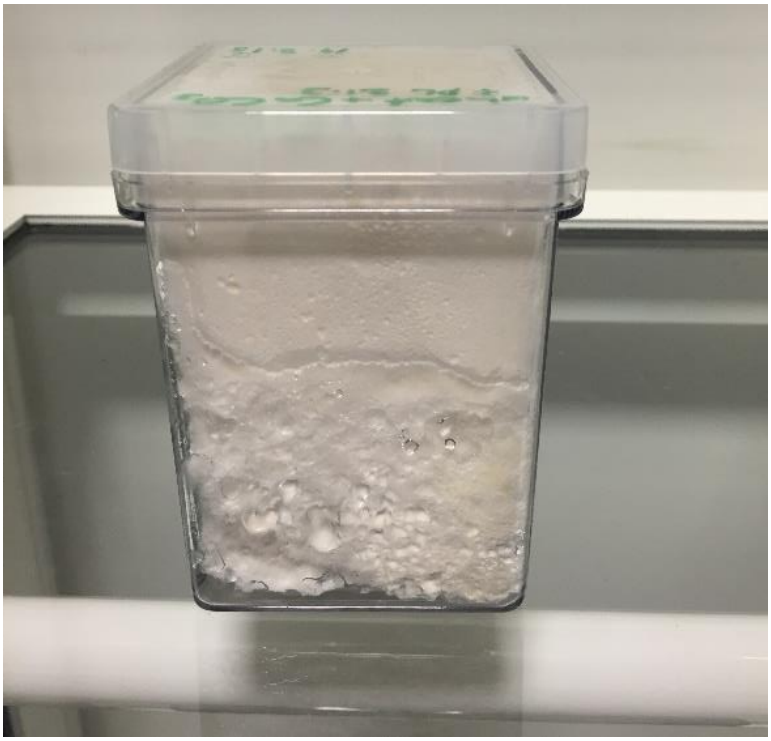

Plate 2: Full mycelial growth in magenta box

During the preparation of spawn, 2 kg of each of wheat, barley and sorghum were washed well with distilled water. The wet seeds were transferred into autoclaved polypropylene bags, each bag contains 500 g of the seeds, and it was autoclaved for two hours under 15 lbs pressure and 121℃.

After the mycelia was grown it was inoculated to the wheat, barley and sorghum seeds after adding $CaCO_3$ 5 g per 1 kg seeds (Theradimani, 2001). The bags were incubated in dark room under 25℃.

After 14 days the spawn running was completed and the mycelium were ready to transfer to the farm.

2.2 Substrate preparation

The substrate used in this experiment were agricultural wastes from date palm bunch waste, date palm leaf waste and mowed turfgrass waste.

These substrates were collected from Al Foah experimental farm. Substrates were dried in sun before chopping them into small pieces using a mechanical chopper. They were then soaked in water for 24 hours before being filled in autoclaved bags and autoclaved under 15 lbs pressure and 121 ℃ for 2 hours. The bags were left to cool until it was ready for the inoculation.

2.3 Fungal inoculation

Two kg of each of the plant wastes were filled in autoclaved bags and the fungal mycelium were inoculated in the substrates as a thin layer, 5 layers of the substrates were filled with 4 layers of mycelium in between.

A greenhouse experiment has been run on shelves that were shaded totally with black polyethylene bags letting 10% of light to enter the experiment area. The greenhouse environment was controlled with stimulated temperature and relative humidity. Accordingly, during the experiment, the greenhouse temperature was maintained at 25± ℃ and relative humidity at around 90%.

The irrigation system was manual irrigation with sprayer 5 times a day. The experiment was carried out with random blocked design (RBD) with 3 replications of each treatment.

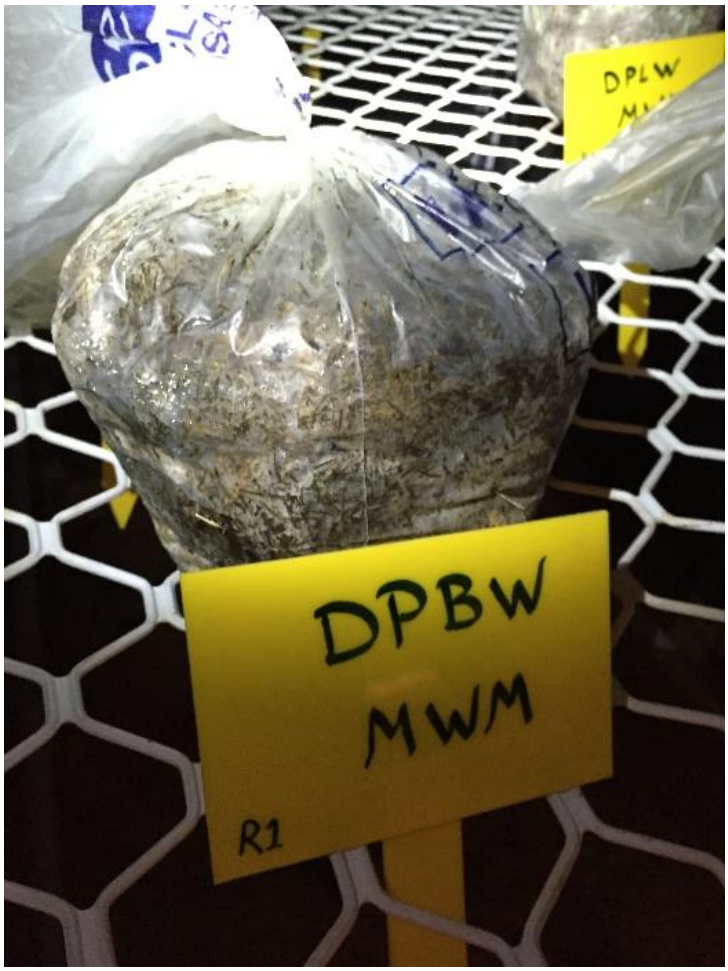

Plate 3: Bags after filling with substrate inoculated with mycelia

2.4 Morphological parameters

2.4.1 Mycelium growing period

The mycelium growth time was calculated at 50% and 100% of the mycelial coverage in the bags.

2.4.2 Fresh weight and dry weight of mushroom fruiting bodies

After harvesting each flush of the mushrooms, the fresh weight of the fruiting bodies was determined by using an electronic balance (Model – XK3190-A7M) and the values were expressed in grams. After taking fresh weight, the mushrooms were dried at 60 ℃ in hot air oven for 24 hours. After drying the weight was measured and the values were expressed in grams.

Plate 4: Measuring the weight of fresh mushrooms

2.4.3 Dry weight of the substrate

After the final harvesting of mushrooms, the substrates were air dried and then weighed by using an electronic balance (Model – XK3190-A7M) and the values were expressed in grams. This weight was used to calculate the biological efficiency.

2.5 Chemical analysis

The chemical analysis of the mushrooms and substrates was carried in the end of the experiment for the macronutrients like: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and Sulfur (S). The analysis also included trace minerals like: manganese (Mn), iron (Fe), copper (Cu), molybdenum (Mo), zinc (Zn), cobalt (Co). The mushrooms and substrates samples were collected and dried in the oven and finely ground to be used for lab estimations.

Total carbon and total nitrogen estimation were carried out via high temperature combustion on an Elementar vario MACRO cube CHNS analyzer that convert the elements into gaseous products. Then the gases are separated by purge and trap chromatography at up to three specific columns and detected at TCD.

The phosphorus content of the mushrooms and substrates was determined calorimetrically. 0.5 gram of the sample was digested in triacid mixture consisting of nitric acid, sulphuric acid and perchloric acid in the ratio of 5:1:2.

Potassium, phosphorus cobalt, copper, iron, manganese, zinc, calcium and magnesium estimation in the mushrooms and substrates were carried out via ICP-OES. Samples were accurately weighed and treated with acids to destroy the organic matter and solubilized the recoverable elements. After cooling, the sample was made up to the volume with deionized water and filtered. The sample solution was aspirated through nebulizer and the resulting aerosol was transported to the plasma torch where

excitation occurs. Element specific emission spectra were produced by radio-frequency inductively coupled plasma. The spectra were dispersed by a grating spectrometer, and intensities of the line spectra were monitored at specific wavelengths by a charged coupled detector.

2.6 Biochemical analysis

2.6.1 Proline

Proline content was estimated following (Bates, 1973)'s method. Five hundred mg of mushroom samples was taken in a pestle and mortar and homogenate with 10 ml of 3 percent aqueous sulfosalicylic acid. Then the homogenized was filtered through Whatman No. 2 filter paper. The residue was re-extracted two times with 3 percent sulfosalicylic acid and pooled. The filtrates were made up to 20 ml with 3 per cent sulfosalicylic acid and used for the estimation of proline.

Two ml of the extract was taken in a test tube with 2 ml of Ninhydrin reagent and 2 ml of glacial acetic acid were added to it. The mixture was incubated for one hour at 110 ℃ in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then, 4 ml of toluene was added to each tube and mixed vigorously using a test tube stirred for 10-20 seconds. The toluene containing the chromophore was separated from the aqueous phase using a separating funnel. The absorbance of proline was measured in a spectrophotometer at 520 nm using an appropriate blank. The proline content was determined from standard curve prepared with proline and the results were expressed in mg/g dry weight.

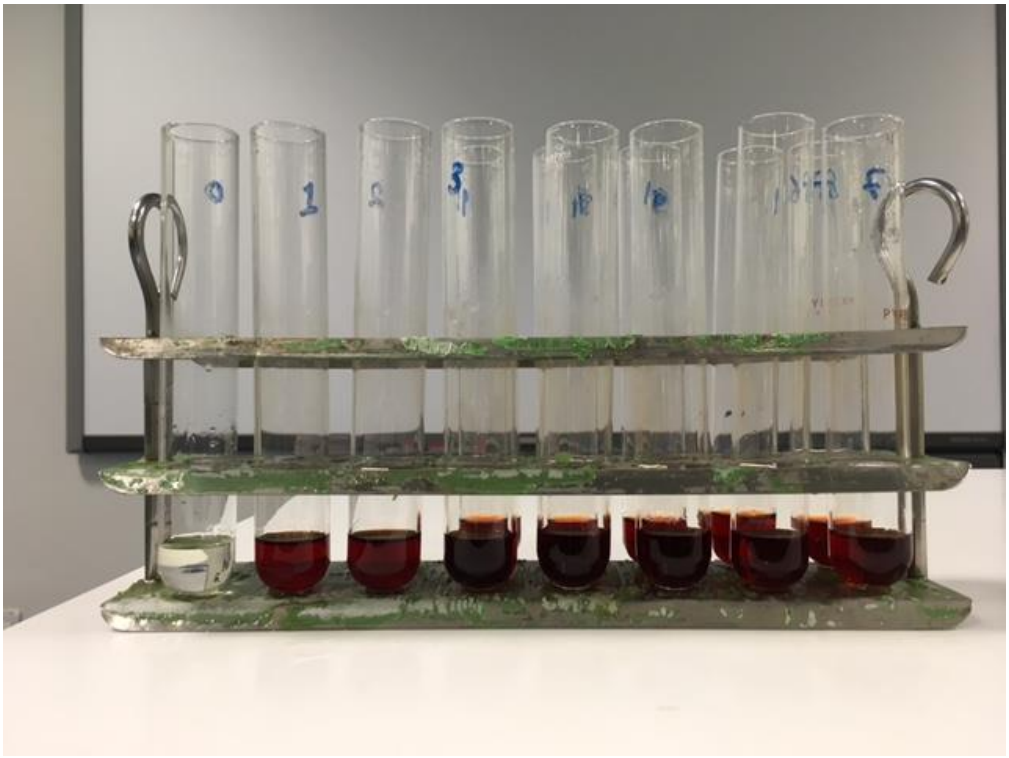

Plate 5: Preparations for proline analysis

2.6.2 Crude Fiber

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the procedure by (Van Soest, Robertson, & Lewis, 1991) using the Ankom220 fiber analyzer (Ankom®, Tech. Co., Fairport, NY, USA).

ADF and NDF estimation was carried out by digesting the samples with H_2SO_4 and CTAB, 0.45-0.55 g of prepared samples were weighed directly in filter bags and sealed completely with a heat sealer. Then the samples bags were placed into the bag suspender and inserted into the fiber analyzer vessel with the heat turned on for 60 minutes. After that bags were soaked in 250 ml of acetone for 3-5 minutes before placing them on a wire screen to air-dry. Then they were oven dried at 102±2 °C within 2-4 hours. Then they were removed from the oven and placed directly into a collapsible

desiccant pouch and flattened to remove air. After cooling to ambient temperature they were weight to measure the crude fiber percentage.

2.6.3 Protein

Protein content of the mushrooms and the substrates was detected using Jones factor (Mariotti et al., 2008) where the nitrogen content is multiplied by 6.25 conversion factor as this method have been used for more than 70 years in measuring protein content in food.

2.7 Statistical Analysis

The statistical analysis has been done through IBM SPSS Statistics 23 program to derive the two-way ANOVA tables. The mean values were compared to test the level of significance with P-value of 0.05%.

Plate 6: Fruiting bodies of Pl mushroom in DPLW substrate

Plate 7: Fruiting bodies of MWM in DPBW substrates

Chapter 3 : Results

The results are presented on the interaction effect of mushrooms and substrate in enriching the substrate to be used as soil ameliorant and as ruminant feed is investigated. The results also show the quality of mushrooms in different substrates as influenced by the substrate.

3.1 Morphological parameters

3.1.1 Mycelium growing period

In the data, when 50% mycelial coverage is considered, it could be observed that there was significant difference between Calocybe (MWM) and Pleurotus (Pl), where took 10.13 days while MWM took 12.33 days.

In case of different substrates there was a significant difference between three substrates, DPLW was the fastest with 9.5 days, DPBW took 11.33 days and MTGW was the slowest with more than 15 days (Table 1)

Table 1: 50% mycelial growing period of mushrooms on different substrates (days)

	MWM	Pl	
DPLW	11.33	7.67	9.5
DPBW	13.33	9.33	11.33
MTGW	-	15	-
	12.33	10.13	

$P < 0.05$

LSD = 2.519

The data didn't show any significant difference between mushroom types at P-value ≥ 0.05 when 100% mycelium coverage is considered.

In case of different substrates, there was no significant difference between DPLW and DPBW with respect to mushroom types. DPLW took 18.50 days when DPBW took 18.67 days. In MTGW there was no full mycelial growth at all (Table 2)

Table 2: 100% mycelial growing period of mushrooms in different substrates (days)

	MWM	PL	
DPLW	18.67	18.33	18.50
DPBW	19	18.33	18.67
MTGW	-	-	-
	18.83	18.33	
P ≥ 0.05			
LSD = 0.167			

3.1.2 Fresh weight and dry weight of mushroom fruiting bodies

Fresh weight of mushroom data showed significant interaction between mushroom and substrate, the highest yield was obtained in MWM in DPBW with a mean production of 466.6 g while Pl in DPBW showed the lowest fresh weight yield with a mean of 252.03 g. In the case of MWM grown in DPLW the results was 340.18 g and 294.82 for Pl in DPLW. (Table 3) (Fig. 1)

Table 3: Fresh weight of mushroom growing bodies (g)

	MWM	PL	
DPLW	340.18	294.82	317.50
DPBW	466.59	252.02	359.30
	403.38	273.42	
P ≤ 0.05			

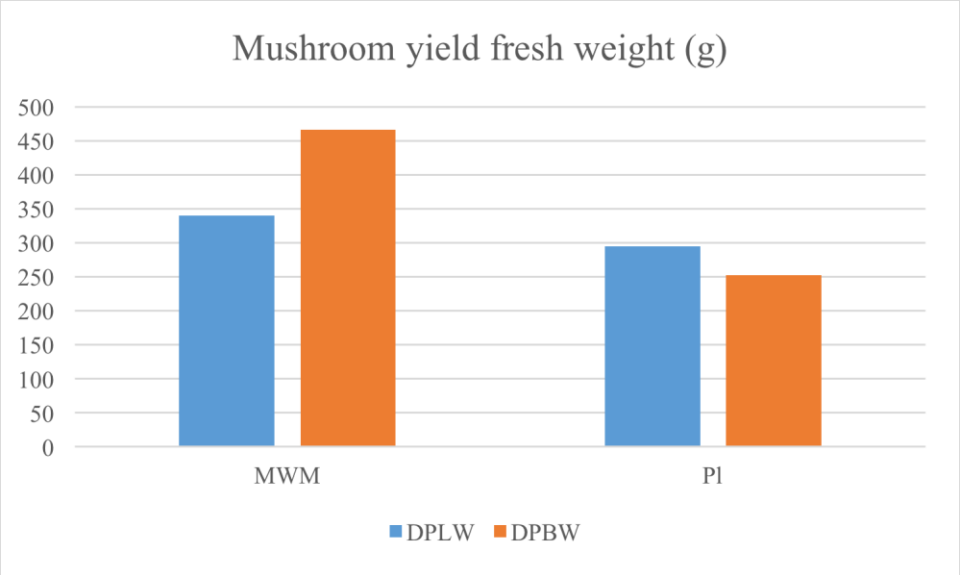

Figure 1: Fresh weight of mushroom yield in different substrates (g)

Dry weight of mushrooms showed a significant difference between two mushroom types where MWM had the highest mean 35.17 g while the mean of Pl was 19.87 g (Fig. 2)

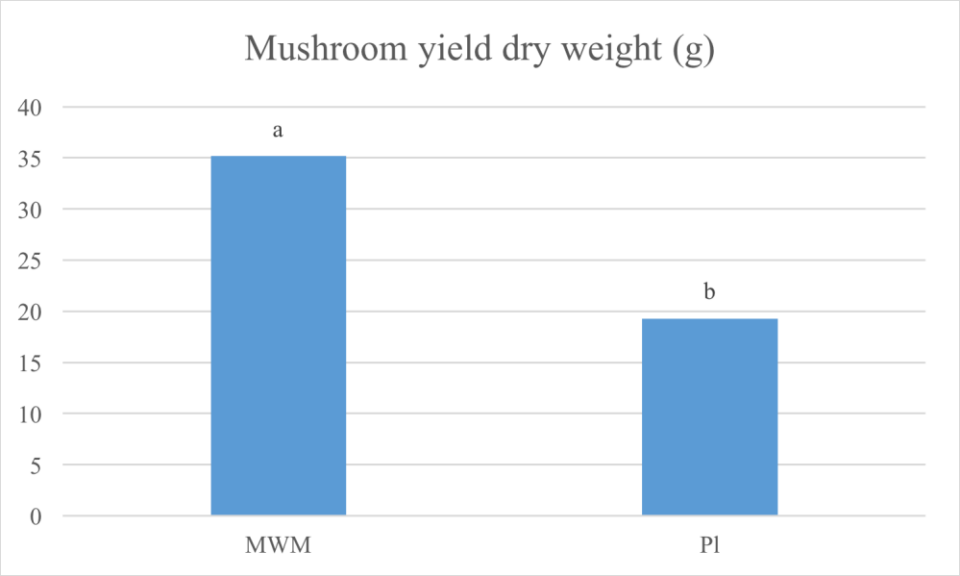

Figure 2: Difference between dry weight of different mushrooms (g)

3.1.3 Biological efficiency

Biological efficiency of the mushrooms was expressed in percentage with dry weight of fruiting bodies divided by the initial dry substrate weight (Bisaria, 1987) (Jwanny, 1995):

$$\%\text{Biological efficiency} = \frac{Weight\ of\ harvested\ fruiting\ bodies}{Weight\ of\ dry\ substrate} \times 100$$

The data showed that there is an interaction between mushroom and substrate where DPBW and MWM had the highest biological efficiency with 34.06%, MWM in DPLW with 27.88%, Pl in DPLW had 19.62% and 15.01% for Pl in DPBW (Table 4). MTGW showed 0% biological efficiency as there was no mushroom yield in this substrate.

Table 4: Biological efficiency of mushrooms grown on different substrates (%)

	MWM	Pl	
DPLW	27.88	19.62	23.75
DPBW	34.05	15.01	24.53
	20.64	11.54	
$P \leq 0.05$			
LSD = 15.46			

3.2 Chemical analysis

3.2.1 Macronutrients

3.2.1.1 Nitrogen, carbon and CN ratio in mushrooms

The data showed that there is no interaction between mushroom and substrate types, no significant difference between mushrooms or substrates at P-value ≥ 0.05 with respect to nitrogen%, carbon% and CN ratio (Table 5).

Table 5: Carbon, nitrogen and CN ratio in mushrooms grown on different substrates

	MWM		Pl	
	DPLW	DPBW	DPLW	DPBW
Nitrogen	5.03±0.27	4.60±0.36	4.78±0.36	4.95±0.14
Carbon	40.21±0.14	40.1667±0.37	39.99±0.04	39.45±0.80
CN ratio	8.00±0.44	8.77±0.63	8.39±0.64	7.97±0.25

($P \geq 0.05$)

NS

3.2.1.2 Phosphorus content in mushrooms

The data showed a significant difference between mushroom types and substrates types. The highest phosphorus content was observed in Pl where it had 12776.88 mg/kg when compared to MWM with 10496.39 mg/kg. In case of substrates, mushrooms that were grown in DPBW had higher phosphorus content than those grown in DPLW (Table 6).

Table 6: Phosphorus content in mushrooms grown on different substrates (mg/kg)

	MWM	Pl	
DPLW	9952.36	12460.53	11206.44
DPBW	11040.43	13093.23	12066.83
	10496.39	12776.88	
$P < 0.05$			

3.2.1.3 Potassium content in mushrooms

The data showed a significant difference between mushroom types and substrates types. The highest potassium content was noticed in Pl with 18430.76 mg/kg compared to MWM with 17001.96 mg/kg. In the substrates, mushrooms that were grown in DPBW showed higher phosphorus content with 18553.65 mg/kg of potassium while those that were grown in DPLW had only 16879.08 mg/kg (Table 7).

Table 7: Potassium concentration in mushrooms grown on different substrates (mg/kg)

	MWM	Pl

DPLW	16056.93	17701.23	16879.08
DPBW	17701.23	19160.30	18553.65
	17001.96	18430.76	
P < 0.05			

3.2.1.4 Calcium content in mushrooms

According to the analysis, it was shown that there was no interaction between mushrooms and substrates, but the data showed a significant difference in calcium level between mushroom types, where Pl had the highest Ca level with 586.60 mg/kg compared to MWM with 327.183 mg/kg (Fig. 3)

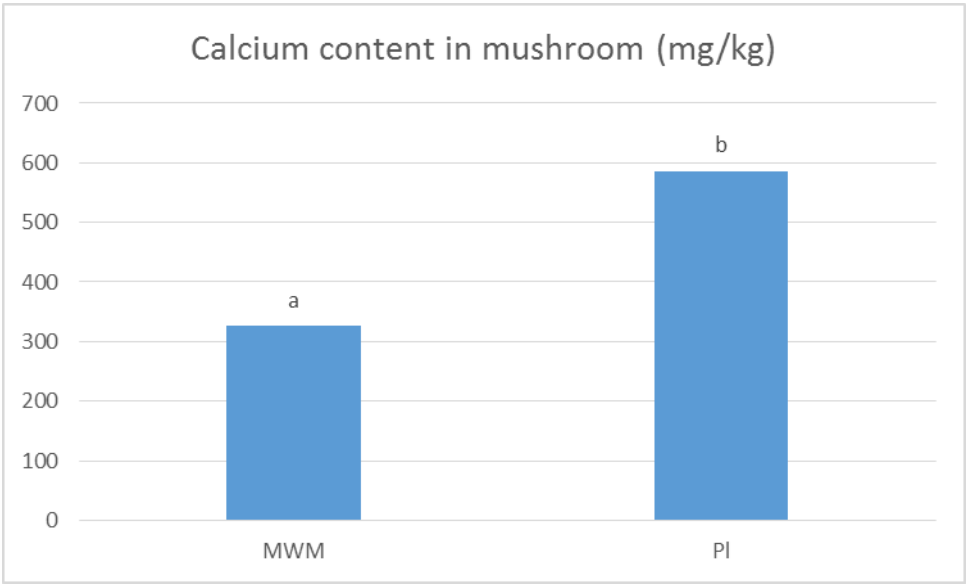

Figure 3: Calcium concentration in different mushroom types (mg/kg)

3.2.1.5 Magnesium content in mushrooms

The data showed a significant difference between mushroom types and substrates types. The highest amount of magnesium was in Pl with 2289.80 mg/kg compared to MWM with 1880.86 mg/kg. In case of substrates, mushrooms that were grown in DPBW were significantly high in magnesium where they had 2187.26 mg/kg of magnesium while those that were grown in DPLW had 1983.39 mg/kg (Table 8).

Table 8: Magnesium concentration in mushrooms grown on different substrates (mg/kg)

	MWM	Pl	
DPLW	1720.76	2246.03	1983.39
DPBW	2040.96	2333.57	2187.26
	1880.86	2289.80	
$P \geq 0.05$			

3.2.1.6 Sulfur content in mushrooms

Sulfur analysis did not show an interaction between mushrooms and substrates. Data also did not show any significant difference between mushroom types and substrate types. (Table 9).

Table 9: Sulfur concentration in mushrooms grown on different substrates (mg/kg)

	MWM	Pl	
DPLW	3357.85	3239.27	3298.56
DPBW	3155.20	3508.23	3331.71
	3256.52	3373.75	
$P \geq 0.05$			
NS			

3.2.1.7 Nitrogen, carbon and CN ratio in the substrate

Nitrogen percentage in the substrates did not show any interaction with mushroom and there was no significant difference between substrate types and control where the substrate with no mushrooms inoculated, at $P \geq 0.750$ (Table 10)

Table 10: Nitrogen content in different substrates with different mushrooms grown on them

Substrates	Control	MWM	Pl
DPLW	0.44	1.17	0.54
DPBW	0.42	0.62	0.63
MTGW	2.19	2.04	1.32
$P \geq 0.05$			
LSD = 0.458			

Carbon percentage is affected by substrate types, where a significant difference was found between the substrates. DPBW showed 40.74% of carbon, DPLW had 40.12 and MTGW had 21.60% (Fig. 4). The mushrooms grown in the substrates had no effect on carbon concentration (Table 11).

Table 11: Carbon concentration in different substrates with different mushrooms grown in them (%)

	Control	MWM	Pl	
DPLW	36.44	44.25	37.22	40.12
DPBW	42.03	40.15	40.90	40.74
MTGW	30.38	24.17	16.00	21.12
P ≥ 0.05 LSD = 4.36				

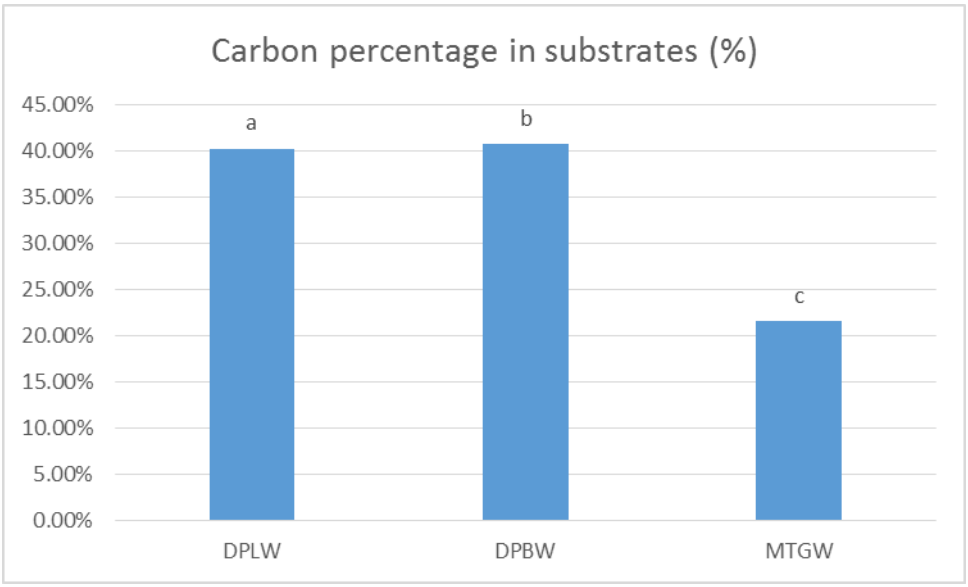

Figure 4: Carbon content in different substrates (%)

The carbon to nitrogen ratio (CN) also did not show an interaction between substrates and mushrooms at ($P \geq 0.05$) (Table 12), but there was a significant difference between substrate types where DPBW had the highest ratio of 76.30, followed by DPLW with 69.55 and MTGW with 12.62 (Fig 5).

Table 12: CN ratio in different substrates with different mushrooms grown in them

	Control	MWM	Pl	
DPLW	82.81	57.16	68.68	69.55
DPBW	99.36	64.58	64.97	76.30
MTGW	13.98	11.81	12.08	12.62
$P \geq 0.05$				
LSD = 7.07				

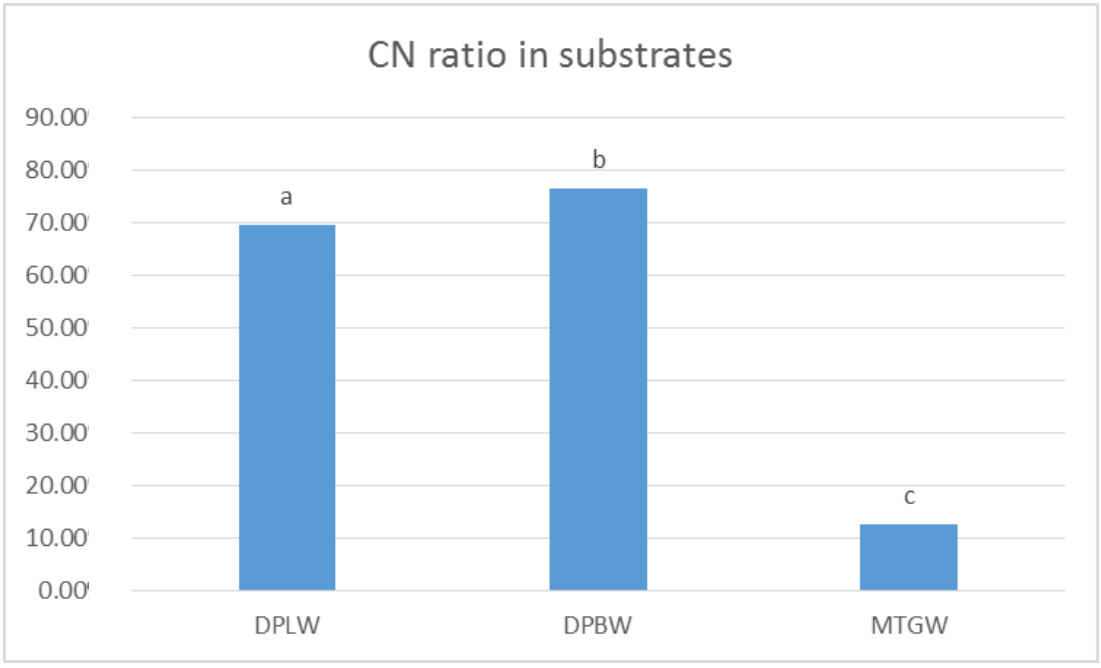

Figure 5: CN ration in different substrates

The data showed no interaction between substrates and mushrooms at ($P \geq 0.05$) and there was no significant difference in phosphorus content between different spent substrates and the controls (Table 13).

Table 13: Phosphorus content in different substrates with different mushrooms grown in them (mg/kg)

	control	MWM	Pl	
DPLW	543.10	625.76	485.41	551.42
DPBW	659.00	624.47	873.68	719.05
MTGW	2828.70	2872.833	2605.743	2769.09
$P \geq 0.05$				
LSD = 181.98				

3.2.1.9 Potassium content in substrates

It was observed after computing the means (Table 14) that there is significant difference between substrate types and their controls (Fig. 6). The data showed that the substrates had a decline in potassium levels where MTGW showed the highest decrease with 1654.12 mg/kg.

Table 14: Potassium concentration in different substrates (mg/kg)

	Control	MWM	Pl	
DPLW	1764.60	1467.11	661.69	1297.80
DPBW	1350.70	833.79	1541.28	1241.92
MTGW	6382.60	4661.56	4690.62	5244.92
$P \geq 0.05$				
LSD = 123.13				

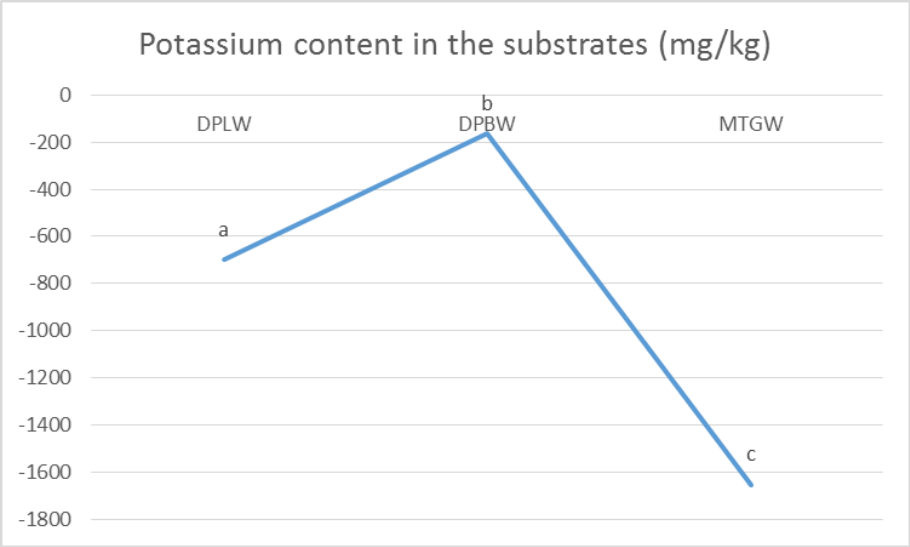

Figure 6: Difference in potassium concentration between different substrates and their controls (mg/kg)

3.2.1.10 Calcium content in substrates

Calcium analysis showed a significant difference between substrates and their controls irrespective of mushrooms as shown in (Table 15) and (Fig. 7) where DPBW showed an increase of 1129.13 mg/kg compared to the control. DPLW showed 1748.75 mg/kg decrease and MTGW showed a decrease of 8678.43 mg/kg.

Table 15: Calcium content in different substrates with different mushrooms grown in them (mg/kg)

	Control	MWM	Pl	
DPLW	50950.13	54413.40	61360.20	19455.16
DPBW	19384.26	18404.23	20643.00	12081.35
MTGW	12668.20	12247.26	11328.60	55574.57
P ≥ 0.05 LSD = 1166.67				

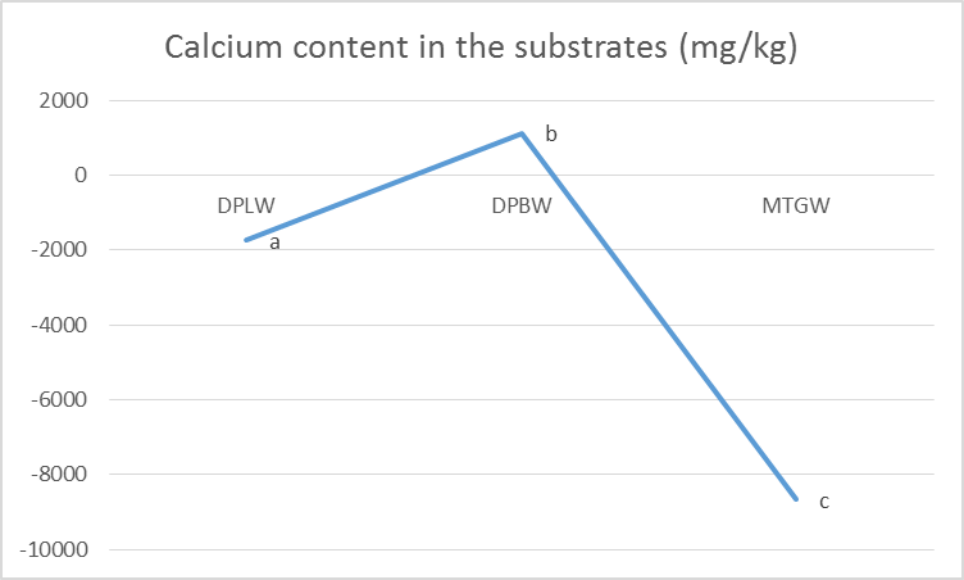

Figure 7: Difference in calcium concentration between different substrates and their controls (mg/kg)

3.2.1.11 Magnesium content in substrates

The data of showed that there is significant differences between substrates and their controls irrespective of mushrooms as shown in (Table 16). (Fig. 8) shows the

decline in magnesium concentration in all substrates where DPLW had the highest decrease with 3968.75 mg/kg.

Table 16: Magnesium content in different substrates with different mushrooms grown in them (mg/kg)

Substrates	Control	MWM	Pl	
DPLW	11818.80	7241.77	8458.31	27518.9
DPBW	5812.39	5220.77	4931.80	15965
MTGW	14582.50	13891.13	14751.50	43225.1
$P \geq 0.05$				
LSD = 2773.75				

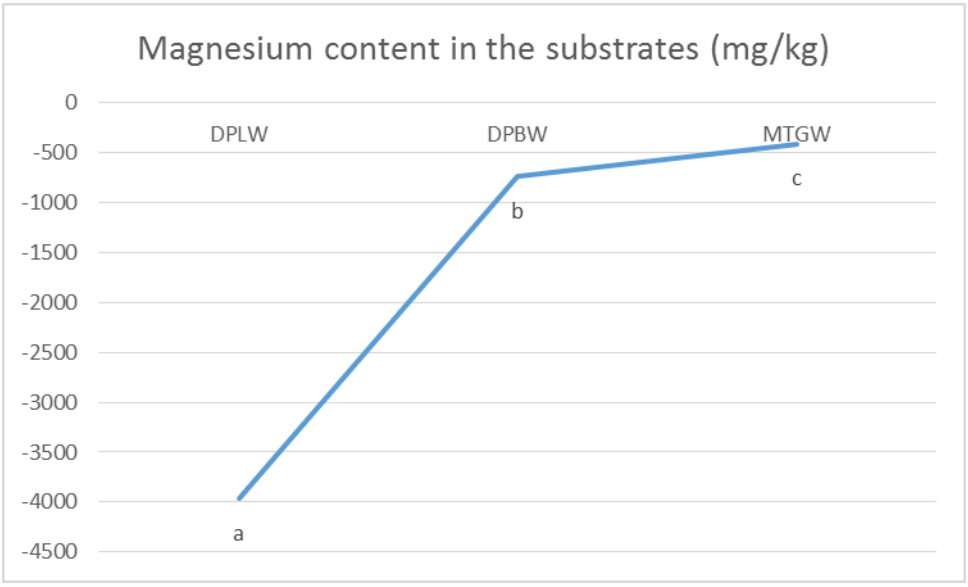

Figure 8: Difference in magnesium concentration between different substrates and their controls (mg/kg)

3.2.1.12 Sulfur content in substrates

It was observed after computing the means (Table 17) that there is a significant difference between substrate types and their controls. Sulfur content increased in MTGW compared to its control while it decreased in DPLW and DPBW (Fig. 9).

Table 17: Sulfur content in different substrates as influenced by different mushrooms (mg/kg)

Substrates	Control	MWM	Pl	
DPLW	1812.27	1834.86	1701.15	5348.28
DPBW	2108.61	1812.67	1653.49	5574.77
MTGW	2475.68	2813.78	2730.89	8020.35
$P \geq 0.05$				
LSD = 34.92				

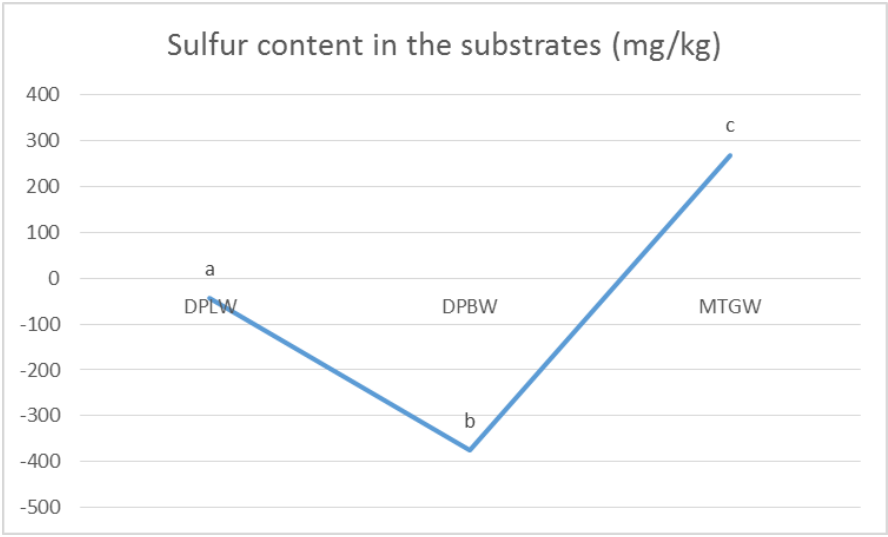

Figure 9: Difference in sulfur concentration between different substrates and their controls (mg/kg)

3.2.2 Trace minerals

3.2.2.1 Copper content in mushroom

The data analysis showed significant interaction between mushroom and substrates as shown in (Table 18) where Pl grown in DPBW had the highest copper content with 14.08 mg/kg and the lowest was MWM grown on DPBW with 10.38 mg/kg. Pl mushroom had higher content of copper than MWM grown on DPBW.

Table 18: Copper concentration in different mushrooms grown on different substrates (mg/kg)

Substrates	MWM	Pl	
DPLW	10.87	12.21	11.54
DPBW	10.38	14.08	12.23
	10.62	13.14	
$P < 0.05$			
LSD = 0.27			

3.2.2.2 Iron content in mushroom

The analyzed data showed no interaction between mushroom and substrate, but there was a significant difference between different mushroom types as shown in (Table 19) and (Fig. 10). Pl mushroom had a mean of 161.37 mg/kg of iron content while MWM got 123.83 mg/kg.

Table 19: Iron concentration in different mushrooms grown on different substrates (mg/kg)

Substrates	MWM	Pl	
DPLW	139.69	168.10	153.89
DPBW	107.96	154.63	131.30
	123.83	161.37	
P ≥ 0.05			
LSD = 7.94			

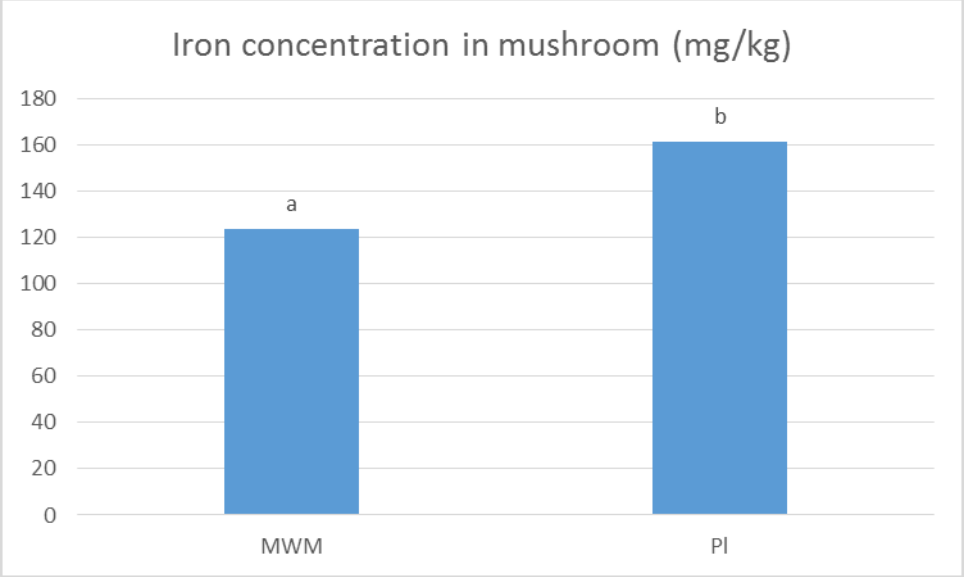

Figure 10: Iron concentration in different mushroom types (mg/kg)

3.2.2.3 Manganese content in mushroom

The data did not show any interaction effect between mushroom and substrate, but a significant difference was observed between mushroom types and also between substrate types (Table 20).

Table 20: Manganese content in different mushrooms grown on different substrates (mg/kg)

Substrates	MWM	Pl	
DPLW	10.27	11.85	11.06
DPBW	8.96	12.44	10.70
	9.62	12.14	
$P \geq 0.05$			
LSD (Mushrooms) = 0.34			
LSD (substrates) = 0.48			

3.2.2.4 Zinc content in mushroom

Data revealed no interaction effect and no significant difference in zinc content between mushrooms and between substrates (Table 21).

Table 21: Zinc content in different mushrooms grown on different substrates (mg/kg)

Substrates	MWM	Pl	
DPLW	77.00	82.57	79.78
DPBW	82.20	89.87	86.04
	79.60	86.22	
$P \geq 0.05$			
NS			

3.2.2.5 Copper content in substrate

Data showed no interaction and no significant difference in copper concentration between substrates and their controls (Table 22).

Table 22: Copper concentration in different mushrooms grown on different substrates (mg/kg)

	Control	MWM	Pl	
DPLW	3.53	3.58	3.48	3.53
DPBW	2.90	2.56	3.25	8.71
MTGW	26.67	26.58	26.79	26.68
$P \geq 0.05$ NS				

3.2.2.6 Iron content in substrates

Iron analysis showed a significant difference between substrates and their controls irrespectively with mushrooms as shown in (Table 23). The substrates showed a decrease in iron concentration compared to their controls, where DPLW showed the highest decrease with 1222.62 mg/kg with that of control and DPBW showed the lowest decrease with 343.54 mg/kg compared to its control (Fig. 11).

Table 23: Iron concentration in different substrates with different mushrooms grown on them (mg/kg)

	control	MWM	Pl	
DPLW	1795.81	1572.72	2018.90	1795.81
DPBW	861.52	821.08	901.96	861.52
MTGW	4650.81	4489.680	4892.50	4677.66
P ≥ 0.05				
LSD = 202.81				

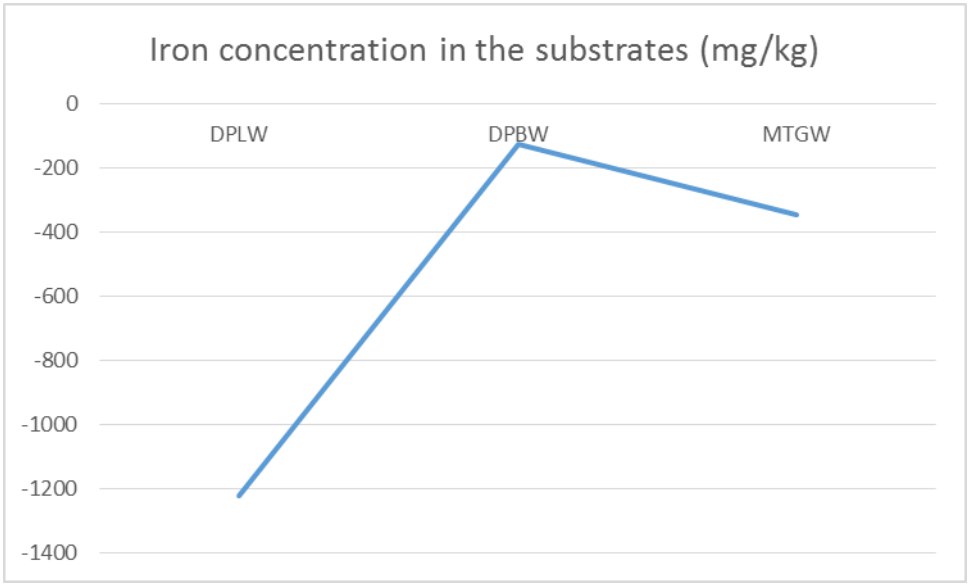

Figure 11: Difference in iron concentration between different substrates and their controls (mg/kg)

3.2.2.6 Manganese content in substrates

The results showed a significant difference between substrates and their controls as shown in (Table 24). DPBW showed 2.90 mg/kg increase in manganese content while DPLW decreased by 14.48 mg/kg. (Fig. 12).

Table 24: Manganese content in different mushrooms grown on different substrates (mg/kg)

	control	MWM	Pl	
DPLW	66.90	47.17	57.73	57.26
DPBW	31.20	34.48	33.66	33.11
MTGW	153.50	144.28	152.97	153.91
P ≥ 0.05				
LSD = 4.59				

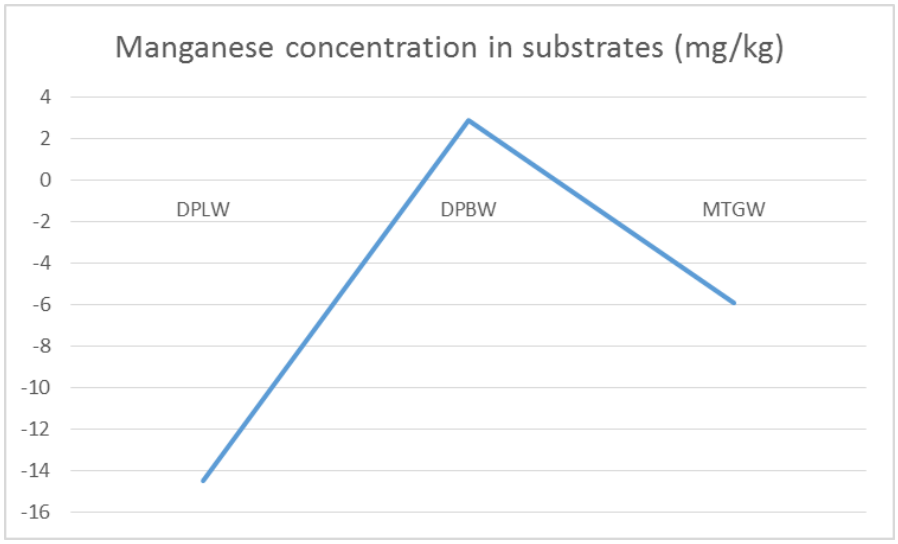

Figure 12: Difference in Manganese concentration between different substrates and their controls (mg/kg)

3.2.2.7 Cobalt content in substrates

Cobalt analysis did not show any interaction between substrates and mushrooms but it showed a significant difference between substrates and their controls irrespective of mushrooms (Table 25) and (Fig. 12) where DPBW showed an increase of 0.11 mg/kg compared to the control, while DPLW showed 1.08 mg/kg decrease and MTGW showed a decrease of 0.13 mg/kg.

Table 25: Cobalt content in different mushrooms grown on different substrates (mg/kg)

	Control	MWM	Pl	
DPLW	3.23	1.75	2.50	2.49
DPBW	1.03	1.05	1.23	1.10
MTGW	5.10	4.79	5.27	5.05
$P \geq 0.05$				
LSD = 0.62				

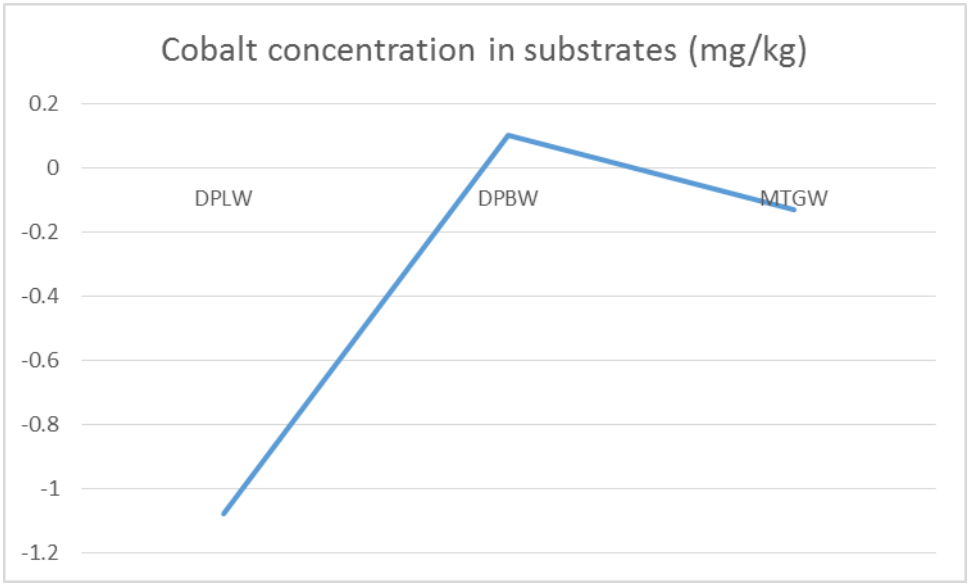

Figure 13: Difference in cobalt concentration between different substrates and their controls (mg/kg)

3.3 Biochemical analysis

3.3.1 Proline

Proline content in mushrooms was between 8.27 mg/g dry and 8.35 mg/g which showed no significant difference between mushroom types in two different substrates. (Table 26)

Table 26: Proline concentration in different mushrooms grown on different substrates (mg/g dry weight)

	MWM		Pl	
	DPLW	DPBW	DPLW	DPBW
Proline	8.30±0.27	8.27±0.17	8.35±0.14	8.32±0.09
$P \geq 0.05$ NS				

3.3.2 Crude Fiber

3.3.2.1 Crude fiber in mushroom

The data of Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) content in the mushroom did not show any significant difference between mushroom types (Table 27).

Table 27: ADF and NDF concentration in different mushrooms grown on different substrates (%)

	MWM		Pl	
	DPLW	DPBW	DPLW	DPBW
ADF	14.80±2.50	15.31±1.20	15.00±2.52	14.83±1.71
NDF	33.13±4.33	33.38±2.51	31.70±1.34	31.53±1.63

3.3.2.2 Crude fiber in substrates

The results of ADF analysis showed an interaction between substrate and mushroom and a significant difference between substrates and their controls, where MTGW with MWM showed the highest increase accumulation in ADF content with a rise of 2.1% compared to the control while DPLW with MWM showed significant decrease with 9.7% (Table 28) (Fig. 13)

Table 28: ADF concentration in different substrates with different mushrooms grown on it (%)

	DPLW			DPBW			MTGW		
	Control	MWM	Pl	control	MWM	Pl	control	MWM	Pl
ADF	54.91	45.24	50.06	49.21	46.56	50.62	43.26	45.36	46.33

($P < 0.05$)

LSD = 0.94

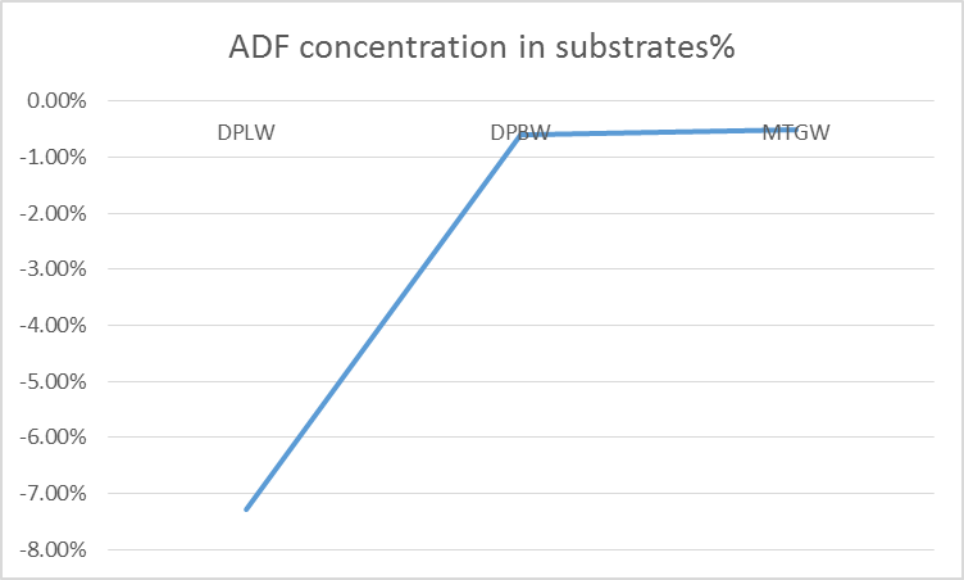

Figure 14: Difference in ADF concentration between different substrates and their controls (%)

In the data on NDF content it could be observed that there is no interaction between substrate and mushroom but there is a significant difference between different substrate types and their controls (Table 29) (Fig. 14).

Table 29: NDF concentration in different substrates with different mushrooms grown on it (%)

	Control	MWM	Pl	
DPLW	63.03	56.42	56.05	58.5
DPBW	58.70	58.66	62.14	59.83
MTGW	51.85	49.33	46.79	49.32
$P \geq 0.05$				
LSD = 4.16				

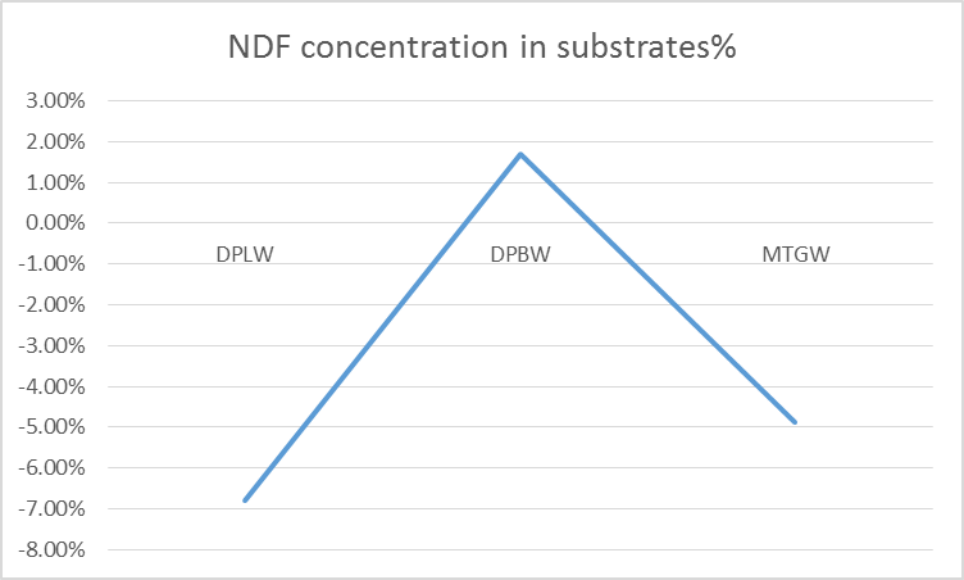

Figure 15: Difference in NDF concentration between different substrates and their controls (%)

3.3.3 Crude protein

3.3.3.1 Crude protein in mushroom

Results did not show any interaction effect between mushroom and substrate P-≥ 0.371 (Table 30).

Table 30: Protein concentration in different mushroom types grown on different substrates (%)

	MWM	Pl	
DPLW	31.46±1.07	29.90±1.07	30.68±0.76
DPBW	28.72±1.07	30.96±1.07	29.84±0.76
	30.09±0.76	30.43±0.76	
P ≥ 0.05			

3.3.3.2 Crude protein in substrate

In substrates, there was a significant difference in crude protein content between substrates and their controls (Table 31) where MTGW showed the highest decrease with 3.18% over the control while DPLW showed the highest increase with 2.6% (Fig. 16).

Table 31: Protein concentration in different substrates with different mushrooms grown on it (%)

	Control	MWM	Pl	
DPLW	2.75	7.32	3.39	4.48
DPBW	2.64	3.89	3.96	3.49
MTGW	13.98	12.78	12.43	13.06
$P \geq 0.05$				
LSD = 2.44				

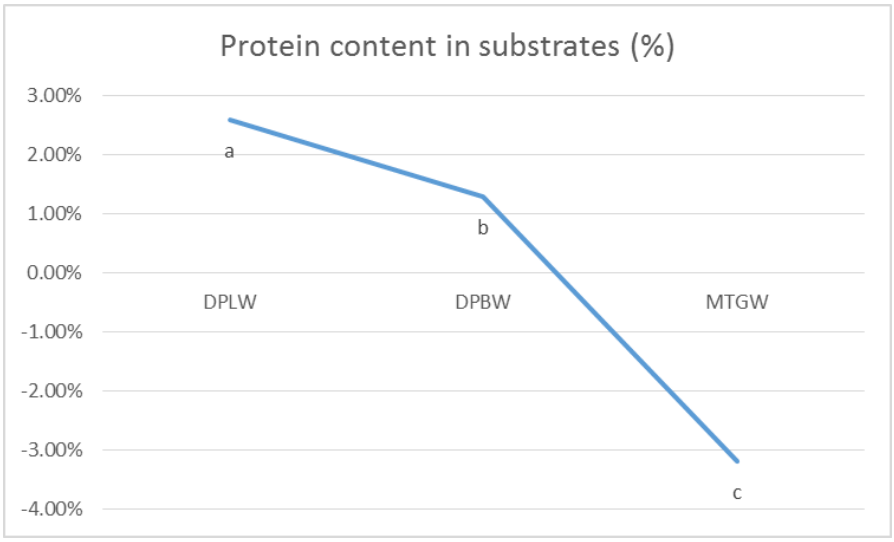

Figure 16: Difference in protein concentration between different substrates and their controls (%)

Chapter 4: Discussion

This study was conducted to determine whether the agricultural wastes are suitable media to grow mushrooms and test if it has the nutritional quality to serve as manure in enriching the soil and as ruminant feed. The results on morphological parameters, chemical and biochemical constituents are discussed hereunder.

4.1 Morphological parameters

4.1.1 Mycelium growing period

The mycelium growing period was affected by the substrate type where date palm bunch waste showed the fastest growth rate followed by date palm leaf waste and mowed turf grass waste came last with only 50% mycelium coverage. The mycelial growth in DPBW and DPLW was slower than the results reported by (Owaid M. N.-S., 2015) which ranged between 13 to 17 days.

4.1.2 Fresh weight and dry weight of mushroom fruiting bodies

Milky white mushroom showed a higher fresh weight results than *Pleurotus*, which affected the biological efficiency of the MWM in a positive way as the biological efficiency has a positive relationship with fresh weight yield (Chang S. T., 1981).

Similarly, in dry weight, MWM showed higher results than *Pleurotus*. Thus the moisture content of *Pleurotus* reached 92.73% while MWM had 91.28% which is higher than the results obtained by (Adejumo & Awosanya, 2005) who reported that high moisture content of mushrooms refers to high perishable ability due to microbial growth susceptibility and high enzyme activity.

4.1.3 Biological efficiency

Biological efficiency of the mushrooms was expressed by the percentage of dry fruiting bodies weight divided by the initial dry substrate weight (Bisaria, 1987 & Jwanny, 1995):

$$\text{Biological efficiency (\%)} = \frac{Weight\ of\ harvested\ fruiting\ bodies}{Weight\ of\ dry\ substrate} \times 100$$

The biological efficiency of S3 was 0% as there was no fruiting bodies due to the high moisture in the substrate which prevented mycelium from growing, while milky white mushroom showed the highest biological efficiency in all substrates especially in date palm bunch waste substrate. Biological efficiency is related to the fresh weight so the highest fresh weight yield got also to highest biological efficiency.

4.2 Chemical analysis

4.2.1 Macronutrients

Nitrogen, carbon and CN ratio and sulfur in the two mushroom types are significantly similar with no effect of the substrate type, while in the substrates there was a negative difference between control and substrates. DPBW had the highest CN ratio which makes it the best substrate to be used for soil enrichment, as reported by (Jordan & Courtney, 2008) that adding spent mushroom substrates increases the carbon content of the soil.

Calcium content in MWM is in accordance with what was reported by (Subbiah & Balan, 2015) while Pleurotus showing higher Ca level.

Potassium, phosphorus and magnesium were higher in Pleurotus that was grown in DPBW while the lowest level was shown in MWM that was grown in DPLW. It

was publishes earlier by (Wang & Suzuki, 2001) that potassium, phosphorus and magnesium are essential minerals for mushroom growth.

In the substrates, the content of N, P and K are high enough to be used as a manure to enhance soil quality (Maher, 1991).

The increase in Ca content of DPBW is due to the decomposition of total carbohydrate, crude fiber, cellulose, hemicellulose which are used by mushroom in the inoculation stage (Patil & Baig, 2010). Ca and Mg are important for fruiting body growth as reported by (Silva, 2002).

4.2.2 Trace elements

Manganese and Iron in Pleurotus showed a significant increase compared to MWM with a values that meet the results shown earlier by (Subbiah & Balan, 2015).

For Copper, the substrate and the mushroom interacted and affected the nutrient level in mushrooms. Pleurotus that was grown in DPBW had the highest Cu content while MWM that was grown in the same substrate had the lowest Cu content. It was known from previous studies that copper is an important nutrient for rigid bone formation, metabolic reaction and transmission of nerve impulses (Adejumo & Awosanya, 2005).

Zinc didn't show any interaction between mushroom and substrate and there was no significant difference in Zn content between two mushrooms.

In the substrate there was a significant difference between control and substrates in iron, manganese and cobalt. Fe showed a decline in all substrates which is similar to the results of a previous study done by (Patil & Baig, 2010). In DPBW

there was an increase in Mn and Co levels while Mn showed a decline in DPLW and Co showed the highest decrease in MTGW.

There was no interaction between substrate and mushroom in copper level and there was no significant difference between substrates types.

4.3 Biochemical analysis

4.3.1 Proline

Proline content in two mushroom types was significantly similar ant it was similar to a previous study that was done by (Chirinang & Intarapichet, 2009).

4.3.2 Crude Fiber

ADF and NDF content in mushrooms didn't show any significant difference between the two types, the results are in accordance with the values reports earlier by (Patil & Baig, 2010).

Growing mushrooms in the substrates improved their quality by reducing the crude fiber content to the value that make those substrates ideal for ruminant feed (Ortega G. M., 1992). DPLW showed the highest decrease in crude fiber content which make it the best substrate for animal feed.

4.3.3 Protein

Protein content in two types of mushrooms was significantly similar, it was between 31.46 and 28.73 which is slightly higher than the results reported by (Silva, 2002) and (Ahmed, 2009) but are in accordance with the national value of protein content in mushroom according to FAO (Food and Agriculture organization of the United Nations).

In the substrate, protein content did not show any significant difference with control and therefore cultivation of mushrooms did not make any difference in protein content of the substrates, even though the protein content is lower than the results reported by (Patil & Baig, 2010) and (Bisaria, 1987).

Chapter 5: Conclusion

The importance of mushroom cultivation has been known for hundreds of years as an edible cultivar that is rich in protein, amino acids, and vitamins. Similarly mushrooms have medicinal values represented in having anti-oxidants, anti-viruses, anti-cancer and anti-microbial properties. The species *Pleurotus sajor caju* and *Calocybe indica* have been cultivated by people using different plants wastes in order to recycle those residues and reduce the pollution.

For the past several years, the amount of plant wastes have been increasing rapidly in the UAE especially the date palm and mowed grass residues since the UAE has more than forty millions of date palm trees and more than 30 million square meter of turfgrass. However, using those wastes in mushroom cultivation is an economical and environmental solution that decrease the pollution and meets the sustainability vision of the UAE government.

In this study three wastes were used: date palm leaf waste (DPLW), date palm bunch waste (DPBW) and mowed turfgrass waste (MTGW), to cultivate two edible mushroom species. The parameters that were tested are: growing period, fresh and dry weight of the yield, biological efficiency, macro-nutrients and trace minerals, proline, crude fiber and protein.

Mycelium growth period in the DPBW was the highest while MTGW did not show a 100% mycelial growth. Fresh weight, dry weight and biological efficiency in MWM showed the highest results.

Proline, crude fiber and protein values in the mushrooms were significantly similar, while trace minerals in Pleurotus where slightly higher than MWM. In

macronutrients, nitrogen and carbon did not show any significant differences between the two mushrooms while Pleurotus was high in calcium, potassium, phosphorus and magnesium.

In the substrates, NPK levels were similar while Ca in DPBW was higher than the other substrates. Iron decreased in all substrates compared to the control. Manganese and cobalt were high in DPBW. The crude fiber in the substrates was reduced compared to the control. While protein content was not affected by mushroom growth.

From the results of this study, it could be concluded that the Pleurotus is better than MWM in the nutritional value. DPBW is the best substrate to be used as a ruminant feed and soil manure.

Further experiments should be done with different temperature, light and moisture regimes to optimize the biological efficiency, and also the role of native mushrooms in degrading the landscape waste materials generated.

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