Use of different spent oyster mushroom substrates as soil amendment on the establishment and growth of *Spinacia oleracea L.* and *Beta vulgaris L.*

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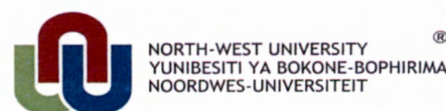

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DEDICATION

To my very own pillar of strength; my mother Ms Winny Gladness Metiso your courage made it easier for me to make it this far in life. It is because of your support, guidance and prayers that I am here today. To my brother Vusane Hlatshwayo, thank you for believing in me and last but not least my very own handsome son Mihla Nkosi. A big hug to you boy for understanding that mommy has to study so she can provide you with stability and security.

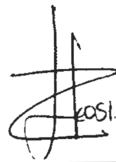
"Education is a key to unlock the golden door of freedom"

By George Washington Carver.

DECLARATION

I declare that this dissertation hereby submitted to the North-West University, for the Master of Science degree in Agriculture (Crop Science) is my own independent work and has not been previously submitted by me to another University, and all sources that have been used or quoted have been correctly acknowledged by means of references.

I further cede copyright of this dissertation in favour of the North West University.



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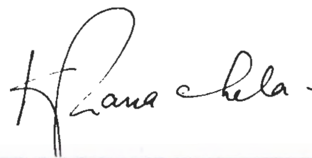

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
DAP	Days After Planting
SAS	Statistical Analysis System
SEM	Standard Error Mean
N	Nitrogen
P	Phosphorus
K	Potassium
Mg	Magnesium
Mn	Manganese
Cu	Copper
Fe	Iron
Zn	Zinc
S	Sulfur
B	Boron
pH	Potential Hydrogen
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalents
SMS	Spent Mushroom Substrate
FYM	Farm Yard Manure
CCI	Chlorophyll Concentration Index
LSD	Least Significant Difference
cm	Centimeters
$\mu g/cm^2$	Microgram per centimeter square

v/v	volume/volume
µL	Microlitre
mL	Mililitre
CPM	Composted poultry manure
CCD	Composted cow dung
CNL	Composted neem-leaf
SMC	Spent Mushroom Compost
Kg	Kilograms
ddH2O	Double-distilled water
HNO_3	Nitric acid
HCL	Hydrochloric acid
NaOH	Sodium hydroxide
H^+	Hydrogen ions
PDA	Potato Dextrose Agar

ABSTRACT

Spent mushroom substrate (SMS) refers to the residual substrate that remains after mushrooms have been harvested. SMS is known to improve the ecological soil status such as improved microbial and biochemical activities when employed as an amendment. A pot experiment was carried out to investigate the growth response of *Spinacia oleracea* and *Beta vulgaris* to three types of spent oyster mushroom substrates and these were; *Urochloa panicoides*; *Zea mays* and *Datura stramonium*. Soil amendments were carried out in the proportion of 60 soil: 40 substrate (v/v) for each of the respective substrates. Three seeds of *S. oleracea* and *B. vulgaris* were directly planted in each respective pot (25cm in diameter) and grown for a period of 12weeks. The experimental treatments were generated from mixtures of soil and SMS of different plant species as follows: (i) *U. panicoides*; (ii) *Z. mays*; (iii) *D. stramonium*; (iv) Substrate mix and (v) Un-amended soil (control) for each vegetable type and these were replicated eight times making a total of forty (40) experimental units for each respective vegetable type. The experimental units were arranged in a Complete Randomized Design (CRD). For both vegetables, results indicated significant differences of different growth media on seedling emergence, seedling height and total root biomass ($P \leq 0.05$). First seedling emergence of *Spinacia oleracea* was observed eight (8) days after planting (DAP) and reaching full emergence on the seventeenth (17) DAP. *S. oleracea* seedling emergence percentages for all four respective amended soils (*U. panicoides, Z. mays, D. stramonium* and substrate mix) was 100% and the un-amended soil had 91.75%, thus were found not significant at $P \leq 0.05$. Similarly *B. vulgaris* also had 100% emergence for seedlings grown on the four amended soils. *U. panicoides* and un-amended soil had 87.63% and 95.87%, respectively and were statistically significantly different ($P \leq 0.05$). The different SMS had a statistically significant influence on seedling height of *S. oleracea* ($P \leq 0.05$). The growth assessments from the respective treatments were as follows: Z. *mays* (27.26cm), *D. stramonium* (25.09cm), substrate mix (21.2cm), un-amended soil (16.53cm) and *U. panicoides* (13.83cm) was the lowest. A similar trend was observed on *B. vulgaris* seedlings; however, the lowest was observed on seedlings grown on un-amended soil (12.06cm). Various SMS had a significant effect on the total dry root biomass of *S. oleracea* and *B. vulgaris* seedlings. The highest total dry root biomass recorded was $8.51g^{-1}$ for *S. oleracea* seedlings grown

on *Z. mays* amended soils and the lowest was seedlings grown on un-amended soil (0.20g^{-1}). For *B. vulgaris,* the highest dry root biomass recorded was on seedlings grown on *D. stramonium* (12.91g^{-1}) and seedlings grown on un-amended soil had the lowest root biomass (0.59g^{-1}).

The *in-vitro* investigation on the suppressive effects of SMS on commonly occurring soil-borne pathogens, such as *Rhizoctonia solani* and *Fusarium oxysporum* was evaluated by assessing the mycelial growth of the two respective pathogens in a PDA infused with respective substrates extracts. The study indicated that mycelial growth of *R. solani* and *F. oxysporum* on *Z. mays* extract infused PDA had the lowest mycelial growth recorded (16mm and 16.2mm). This was probably an indicative of antifungal properties which better inhibited the growth of both *R. solani* and *F. oxysporum* in PDA infused with *Z. mays* extract. The following mycelial diameter growths of *R. solani* were recorded: un-amended soil 72mm; *U. panicoides* 37.25mm; *D. stramonium* 46.50mm and Substrate mix 38mm. Similar results were also noted on mycelial growth of *F. oxysporum*.

The findings of this study have shown that use of spent mushroom substrates as a soil conditioner for horticultural plants improved plant growth and also had suppressive effect on soil-borne pathogens. It was also noted that there was variation between the different substrates in their effect as crop growth enhancement and disease management. It is therefore recommended that different biomass found in various respective farming areas should be evaluated and assessed for their performance as soil amendments and as suppressant for soil-borne pathogens.

Key words: Spent mushroom substrates, soilborne pathogens, *Spinacia oleracea*, *Beta vulgaris, Fusarium oxysporum and Rhizoctonia solani.*

CHAPTER ONE

1.1. General introduction

Vegetables are cultivated for their economic, nutritional and medicinal benefits. It has been reported that consumption of vegetables on a daily basis is important for healthy dietary needs as they are good sources of essential vitamins, minerals and are generally low in fat and calories (Asian Vegetable Research and Development Center, 1990). Some vegetables can be grown under a wide range of conditions while others require certain conditions for optimum growth. These conditions may include the following: optimum temperatures, adequate water and nutrient supply, good aeration and soils free from pathogens (Caoili and De Vera, 1977; Acquaah, 2002). Production of vegetables is generally affected by various constraints and these include: low soil nutrient content, poor soil structure, low and high soil pH and soil-borne disease. In addressing some of these constrains several researchers have identified potential organic materials, such as green and animal manure, which can be utilized as soil amendments in order to improve the soil's physical, chemical and biological properties (Cosico, 1985; Romaine and Holcomb, 2001; Lotter, 2003; Zhang *et al.*, 2012).

In addition to the use of various organic material as soil amendments, several researchers have investigated the use of spent mushroom substrate (SMS) as soil amendments for vegetable production (Iwase *et al.*, 2000; Medina *et al.*, 2009; Idowu and Kadiri, 2013). Results from studies have, however, been variable, thus indicating a need for further research. Spent mushroom substrate refers to the residual substrate that remains after several cycles of mushrooms have been harvested (Sendi *et al.*, 2013). Normally, after harvest, SMS is left abandoned or discarded, causing environmental pollution (Medina *et al.*, 2009). SMS has been reported to be a good source of humus, providing plants with micronutrients, improving soil water holding capacity, improving soil aeration, increasing microbial densities and helps in maintaining soil structure. SMS has also been reported to have antifungal properties which suppress various soil-borne pathogens (Guo and Chorover, 2004; Perez-Piqueres *et al.*, 2006; Segarra *et al.*, 2007; Kardiri and Mustapha, 2010).

As findings by various researchers working on organic soil amendments have been variable, there is a need to investigate different oyster mushroom substrates as soil amendments for the establishment and growth of two selected vegetables.

1.2. Problem statement

A global decline in vegetable production has been noted to be the result of several soil-related factors. These include poor soil structure, poor soil fertility *i.e.*, low soil organic matter, low moisture content, soil-borne diseases and high and low soil pH (Grassbaugh and Bennet, 1998; Goldblat, 2012; Department of Agriculture Forestry and Fisheries, 2013). In addressing issues associated with the physical and chemical composition of soil, organic and inorganic fertilizers have been widely used (Leal *et al.*, 2007). Unlike organic fertilizers, inorganic fertilizers are made from non-living materials which make them environmentally unfriendly. Inorganic fertilizers contain certain compounds which are left in the soil causing a buildup to toxic concentrations of salts creating chemical imbalances (Scialabba and Muller-Lindenlauf, 2010). These imbalances negatively affect plant growth which results in crop yield reduction. Furthermore, inorganic fertilizers are prone to leaching. This leaching often results in contamination of water resources and destruction of the ecosystems, and has been known to contribute to climate change through greenhouse gas emissions (Pretty, 1995; Crain *et al.*, 2009; Li *et al.*, 2010; Scialabba and Muller-Lindenlauf, 2010; IPCC, 2013). In addition, inorganic fertilizers are generally expensive and unaffordable to small scale farmers (Steve and Morrill, 2014).

1.3. Motivation of study

The increasing use of inorganic fertilizers and their detrimental effect on the environment, has recently received considerable attention. Research has been directed towards identifying other natural waste materials which can be employed as amendments to improve crop production. Generally, organic fertilizers are produced from living materials, such as plants, animal waste and powdered minerals. They contain a variety of nutrients that enhance the soil-plant biochemical processes. The benefit of using organic fertilizers over inorganic are that they release their nutrients slowly into the soil, *i.e.*, nutrients are released when the plant needs them and are therefore available to the plants over a longer period. The nutrients are found in complex molecules that do not easily leach with

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the first or heavy rains. They are also less likely to injure the young roots of seedlings (Steve and Morrill, 2014). With the promotion and widespread adoption of oyster mushroom in smallholder farming sector, there is a potential of using SMS as soil amendments for vegetable production. This study, therefore, seeks to investigate the influence of the oyster mushroom substrates on the growth and establishment of the selected two vegetables and their anti-microbial activities on commonly occurring soil-borne pathogens.

1.4. Study Aim and Objectives

1.4.1. Main objective

To investigate the growth response of *Spinacia oleracea* and *Beta vulgaris* to various types of spent oyster mushroom substrates.

1.4.2. Specific objectives

(i) To evaluate the influence of different substrates on the establishment and growth of two selected vegetables.

(ii) To carry out *in-vitro* evaluation of the effectiveness of three substrate extracts in the control of *Rhizoctonia solani* and *Fusarium oxysporum* soilborne pathogens.

1.4.3. Research hypotheses

(i) Spent oyster mushroom substrate will enhance the growth rate of the two selected vegetables by improving soil structure and soil fertility; that is, supplying the vegetables with essential plant nutrients and water in adequate amounts for better plant growth and development.

(ii) Different substrates will vary in their effectiveness to control the fungal growth of *Rhizoctonia solani* and *Fusarium oxysporum*. Their variation in suppressiveness will be more dependent on the presents and/or availability of certain Phytochemicals which are known to possess antifungal properties.

1.5. Literature review

Recently, the utilization of spent mushroom substrate as an organic manure and soil compost in horticulture has received considerable attention by several workers (Rinker, 2002; Santos *et al*., 2005; Machado *et al*., 2007; Azevedo *et al*., 2009; Ribas *et al*., 2009). Typically, mushroom farms generally are desperately looking at ways of disposing the compost that is no longer yielding viable mushroom heads (Zied *et al*., 2011). Development of cost-effective beneficial disposal mechanism through composting would be beneficial to both the ecosystem and horticultural farmers, because this compost residue attracts flies and other insects that can carry diseases and constitutes a potential source of water and air pollution (Zied *et al*., 2011). Although, several workers have investigated SMS as a potential organic material for horticultural crops, there is still insufficient literature on the subject matter. On the other hand, the use of organic manures, such as farm yard waste, household waste and compost made from crop residues have been extensively studied (Ijoyah and Sophie, 2003; Dawuda *et al*., 2011; Dlamini *et al*., 2012). Composting is an aerobic process during which the organic matter is decomposed to humus-like substance. During this decomposition process there is decline in volume and the resulting compost is nutrient rich and more stable than the original material, and can improve soil quality and productivity as well as sustainability of agricultural production (Barral *et al*., 2009; Farrel and Jones, 2009).

Application method of composts

In agriculture, there are two commonly used methods of applying compost and these are incorporation of compost into the soil and application of compost as a mulch (Cogger *et al*., 2008; Bastida *et al*., 2010). Incorporation of compost into the soil involves digging the compost into the top few centimeters of the soil to enable increase accessibility of the humus to soil microbes and also to improve contact with the plant roots. Cogger *et al*. (2008) highlighted that incorporation of compost into the soil provides greater effect on soil C, N and bulk density than mulching. It is generally noted that mulching is mostly practiced in horticulture and agriculture, especially in dry climate areas where water is lost through evaporation (Gonzalez and Cooperban, 2002; Agassi *et al*., 2004; Tu *et al*., 2006). Compared to incorporation, the effect of mulching on the underlying soil is

regarded low as it is limited to soluble compound leaching from mulch layer into soil (Gonzalez and Cooperban, 2002). Course textured compost is considered the best for mulching because it allows water and air to passes through to the soils' underneath. In addition, course textured compost is known to decompose slowly and therefore lasts longer. It is however acknowledged that although fine textured materials can be used as mulch but if applied too densely it can trap water and lower the infiltration rate (Agassi *et al.*, 1998; Paulin and O'malley, 2008).

Effect of compost on soil

Bernal *et al.*, (2009) reported that composts have several advantages compared to plant residues. These advantages include the following: reduced volume of biomass applied, slow mineralization rates and recycling of municipal bio-solid waste. Compost has two main effects on soil, *i.e.*, to replenish soil organic matter and supply plant nutrients, particularly nutrient-deficient soils (Sanchez-Mondero *et al.*, 2004; Tejada *et al.*, 2009b). Organic matter plays a vital role in improving the soil's physical, chemical and biological properties. It is reported that soil structure is improved by the binding between soil organic matter and clay particles through cation bridges and stimulation of microbial activity and root growth (Farrel and Jones *et al.*, 2009; Gao *et al.*, 2010). According to Tisdall and Oades (1982), organic matter can indirectly improve soil structure by increasing microbial activity and thus production of microbial slimes, fungal hyphae and/or roots bind aggregates together. Organic matter is regarded as a significant reservoir of nutrients and is capable of retaining nutrients in a form that they are available to plants (Baidock, 2007). Other beneficial effects of compost include increasing water holding and plant water availability (Curtis and Claassen, 2005; Farrel and Jones, 2009). Furthermore, composts are also known to contribute in the reduction of leaching of nutrients (Gale *et al.*, 2006; Hepperly *et al.*, 2009). Arthur *et al.* (2010) and Gershyny (1994) also highlighted that composts also help to reduce soil erosion and evaporation. They also reported on its beneficial reaction on its plant diseases suppressive effect. However, the application of immature compost can have negative effects on plant growth due to its unpleasant odor. Some compost may contain high salt content such mushroom compost, cow and poultry manures and these can inhibit plant growth. Immature compost very often results in

nitrogen starvation to plants occurring as a result of high rate of microbial activity that sequestrates N making it not to be available to plants (Önal and TopcuoğLu, 2007).

Effect of compost to soil pH

Effect of compost on soil pH is not very well understood. It has been reported that mature compost has neutral to slightly alkaline pH levels. The addition of composts from manures has been reported to both increase or decrease soil pH and has the ability to buffer soil pH depending on composts as well as the soil pH (Johnson *et al.*, 2006; Buttler *et al.*, 2008). soil pH is one of the most important factors that affect metal solubility, plant nutrient uptake and movement; plant growth, microbial activity and many other attributes and reactions (Eptein, 1997; Garcia-Gil *et al.*, 2004). The increase in soil pH following addition of composts made from broiler litter is mainly due to addition of basic cation, ammonification and production of NH_3 during decomposition of added composts (Hubbard *et al.*, 2008). On the other hand, soil pH can decrease after application of composts from rice straw mixed with agro-industrial waste due to the release of H^+ ions through nitrification and/or the production of organic acids during decomposition (Bolan and Hedley, 2003; Rashad *et al.*, 2011).

Effect of compost on plant growth and nutrient uptake

Compost application has been reported by several workers that it enhances plant nutrient availability (Heymann *et al.*, 2005; Kawasaki *et al.*, 2008; Poll *et al.*, 2008). It has however been noted that plant uptake of N and P in compost is lower than that of inorganic N fertilizers. This is because in compost, organic N has to be mineralized before it can be taken up by the plant and furthermore, there is problem of microbial immobilization whereby N is sequestered by saprophytic microbes that are involved in the decomposition of the compost (Ebid, 2008; Odlare and Pell, 2009). Although in the compost amended soil mineralization which results in the release of N is initially slow it however makes it accessible on a slow release form making it available in the next season (Passoni and Bonn, 2009). Curtis and Classasen (2005) in their study, showed that incorporation of soil with farm yard waste (24%v/v) resulted in greater increase in plant water availability Soil organic matter is also said to contain a major component of humic substance, which is

known to have a positive influence on shoot biomass through its hormonal effects on root elongation and plant development (Atiyeh *et al.*, 2002; Zandonadi *et al.*, 2007; Lazcano *et al.*, 2009). Sendi *et al.* (2013) carried out an experiment to investigate possibilities of substituting peat moss (PM) with spent mushroom waste (SMW) for growing Kia-lan (*Brassica oleracea*). The study reported that there was no significant difference in plant height, leaf number and area, SPAD (Silicon Photon Activated Diode) readings and shoot and root masses between PM, SMW and PM mixture with NPK amendment. Previously, Chatterson (2009) worked on the effectiveness of SMS as a soil amendment or as topdressing in landscaping. Results indicated that soil incorporated with SMS gave successful plant establishment than un-amended soil and it was concluded that SMS was as effective as using fertilizers. It was also noted that SMS added organic matter to the soil, suppressed soil-borne pathogens, improved soil structure and maintained and/or increased soil pH and cation exchange capacity. Another study was carried out to evaluate SMS as bio-fertilizer for growth improvement of *Capsicum annuum* and it was reported that analysis of growth in terms of height, number of leaves and yield indicated that SMS of oyster and button mushroom had a positive effect on overall growth of tested plants (Roy *et al.*, 2015).

A study was carried out by Islam *et al.* (2014) where comparative effects of biogas plant residues, poultry manure and inorganic fertilizer on growth and yield of *Abelmoschus esculentus* was investigated. It was noted that full NPK fertilizer did not significantly increase the growth of *A. esculentus* over the control (un-amended soil). Other scientists evaluated the nutritional quality of carrots as influenced by farm yard manure and it was indicated that the yield of carrot was significantly increased by amending the volcanic soil with farm yard manure (Umhoza *et al.*, 2014). A similar study was conducted using chicken manure to investigate its influence on soil chemical properties and response of application rates on the yield of spinach (*Spinacia oleracea*). The results showed that chicken manure was a potential source of plant nutrients and chemical substance (Oagile and Namasiku, 2010). Employment of organic manures as soil amendment not only improves crop yield, but amongst a variety of mode of action, it has also been reported that it contains biochemical compounds that suppress soil borne pathogens (Hortink and Fahy, 1986; Hortink *et al.*, 1997).

Effect of spent mushroom substrate on soil-borne pathogens

Plant pathogens are biological agents that cause plant diseases. These diseases significantly reduce yield and quality of vegetables (Malcolm *et al.*, 2013). Several studies have reported that use of soil amendments, such as composts and manures can suppress soil pathogens. Yohalem *et al.* (1996) reported that, spent mushroom substrate possesses a unique chemical constitution and micro-flora that enhances soil microbial diversity. The actinomycetes, bacteria and fungi inhabiting the compost, not only play a crucial role in further decomposition but also exert antagonism to the normal pathogens surviving and multiplying in the soil ecosystem (Yohalem *et al.*, 1996).

Tuitert *et al.* (1998) investigated the effect of soil amendment with organic household compost and garden waste on the colonization of potting mix by *Rhizoctonia solani* under *in-vivo* and *in-vitro* conditions. The effect of the pathogen infection in the potting mix was obtained by measuring the height where damping-off occurred. Results revealed that compost from two commercial composting facilities suppressed growth of *R. solani* in the potting mixtures. This was further supported by Sabet *et al.* (2013) who investigated cucumber root-rot caused by *Fusarium oxysporum* and the results indicated that amending soil with 25/kg (compost/soil) reduced disease percentage incidence, pathogenic fungi population and resulted in improved yield. Gurama *et al.* (2012) evaluated some physical and chemical properties of three composts namely: composted poultry manure (CPM), composted cow dung (CCD) and composted neem-leaf (CNL) on their efficacy on tomato fusarium wilt. The *in- vitro* results indicated that the extracts of CPM significantly reduced radial growth of *Fusarium oxysporum* to 2.2cm and inhibited growth by 51%. Islam *et al.* (2013) investigated the efficacy of compost tea and poultry litter extract in controlling stem canker of potato under natural infection condition. The results showed that poultry and compost litter extracts decreased stem canker incidence and foliar application of compost tea and poultry litter showed better performance in increasing plant height and yield of potatoes compared with other treatments. An *in vitro* study of Verma (1992) showed that, the anaerobically fermented aqueous extract of the SMS inhibited conidial germination of *Venturia inaequalis*, the causal agent of apple scab. The extract also showed more inhibitory effect on conidial germination of *Cochliobolus*

carborum and *Sphaeropsis sapinea* causing diseases on maize and red pine (*Pinus resinosa*), respectively. Further, SMS is also thought to be a good source of phytochemicals which are often referred to as secondary metabolites which fall into several classes such as polysaccharides, tannis, alkaloids, flavonoids and polyphenols that possess antimicrobial property which can suppress pathogen attack (Harborne, 1973; Rinker, 2002). Among these, polyphenols are a member of aromatic chemical compounds with weakly acidic properties and are characterized by a phenolic hydroxyl (OH) group. However, their presence is considered to be potentially toxic to pathogens (Okwu, 2005). Flavonoids are phenolics that are known to be synthesized by plants in response to microbial infection and have also been found to be effective against a wide range of pathogenic microorganisms (Harborne, 1973).

Effect of available micro-nutrients in spent mushroom substrate on the control of soil-borne pathogens

Micro-nutrients are chemical elements which are required in small amounts by various organisms and are essential for many physiological and biochemical processes (Mertz, 1981). Nutrients such Zn, Cu, Mn, Mo and Fe play an important role in the growth and metabolism of fungi, influencing fungal metabolism by affecting the plant's phenolic and lignin content, and plant cell membrane stability (Graham and Webb, 1991; Blevins and Lukazewski, 1998; Brown *et al.*, 2002). It has been reported that any deficiency or overly concentrated addition of micro-nutrients could have an adverse effect on fungal growth. Several studies have reported that high concentration of such nutrients is toxic to fungi, thus inhibiting their growth (Adiga *et al.*, 1962; Jackson *et al.*, 1989; Cuero *et al.*, 2003 and Cuero and Quellet, 2005).

In **conclusion**, compost addition can increase soil nutrient availability and thereby nutrient uptake by the plant. Their mode of action differs; it can either be direct or indirect. The direct effects can be through nutrient addition with compost, while the indirect effects can be through increased microbial activity, improved soil structure or nutrient and water retention. Nutrient mobilization can be increased by microbial activity, on the other hand may also result in immobilization of nutrients. The improved soil structure and water retention is also known that it stimulates root growth and thus root soil catchment zone.

CHAPTER TWO

Effect of different "spent" oyster mushroom substrates (SMS) on the establishment and growth of Spinach (*Spinacia oleracea* L.)

2.1. Introduction

Spinacia oleracea commonly known as spinach, originates from central and south-western Asia where it was first cultivated by Persians (Boswell, 2010). It is now widely cultivated throughout the world (DAFF, 2010). In South Africa, spinach is manly produced in KwaZulu-Natal, but other provinces, such as North West produce it as well. Spinach is an annual edible flowering plant in the family Amaranthaceae, whose leaves are used as vegetable (LeStrange *et al*., 1999). Spinach have three cultivar types that are generally categorized by their leaf variation and classified as savoy, a plant with wrinkled leaves, semi-savoy, characterized by slight wrinkled leaves and lastly, smooth or flat leaves which have no wrinkled leaves (LeStrange *et al*., 1999; Respondek and Zvalo, 2008).

It has been reported that spinach is a good source of folate and Vitamin A. Vitamin A is vital for normal functioning of the immune system and healthy eye sight. Apart from vitamins, spinach also contains considerable amount of essential minerals of which iron is the most important. In addition, consumption of spinach is said to have some health benefits such as improving blood glucose control in diabetics, lowering the risk of cancer, lowering blood pressure, improving bone health, lowering the risk of developing asthma (Zikakala, 2014).

Spinach can be grown in any soil type provided that it is well-drained and supplied with organic manure. In general, sandy loam soils with an optimum pH between 6 and 7 are considered ideal for its growth (Conte *et al*., 2008; DAFF, 2010). Since spinach needs adequate N supply for rapid growth most farmers rely more on inorganic fertilizers to meet the crop nutritional requirements. It is, therefore, no secret that the use of inorganic fertilizers has made a major contribution to improving crop yield and quality over the years (Wang *et al*., 2008; Fageria, 2009); however, there are concerns that excessive use of inorganic fertilizers has detrimental effects on the ecosystem causing unfavourable

growth conditions which may result in yield reduction (Steve and Morrill, 2014). This study was, therefore, undertaken to evaluate the growth response of spinach grown in soils amended with various SMSs.

Specific objective: To evaluate the influence of *Urochloa panicoides*, *Zea mays*, *Datura stramonium* spent mushroom substrates on the establishment and growth of spinach (*Spinacia oleracea* L.) seedlings.

2.2. MATERIAL AND METHODS

2.2.1. Description of experimental site

The study was conducted at Molelwane, North-West University Research farm located 6.44km away from the Mafikeng Campus University at a geographical location of 25' 47' 54" south; 25' 32' 52" east. Nutrient analysis was carried out at the Animal Health Laboratory and seedling growth studies were carried out in Molelwane under temperature controlled glasshouse.

2.2.2. Description of laboratory analysis

2.2.2.1. Soil preparation and sterilization

One hundred-sixty kilograms (160kg) of Hutton soil was collected from the farm's arable land and autoclaved for thirty minutes at 121°C under hundred-forty (140) KPa pressure. Substrates were not autoclaved to avoid denaturing of bio-active chemical compounds that could possibly have been produced during the growth of the oyster mushroom. After autoclaving of the bulk soil, a proportion of 60% soil: 40% of respective substrate ratio (v/v) was measured and mixed together before 80 pots (25cm in diameter) were filled with the growth medium and the control (un-amended) was filled with 100% soil. Several research workers evaluated different levels of compost incorporation and it was reported that soil incorporation varies depending on the soil type, soil pH and nutrient content (Jones, 2003; Leal *et al.,* 2007; IPNI, 2010). Even though, such factors need to be considered, there is, however, a recommended application rate that ranges from 20 to 50% substrate. For instance; Önal and TopcuoğLu (2007) reported that sandy loam soil incorporation with 50:50% (v/v) resulted in increased plant growth, improved soil bulk

density which promoted water retention and nutrient availability. Paulin and O'malley (2008) study showed that for heavier clay soils a recommended application rate between 40 to 60% (v/v) had significantly improved soil structure, water holding capacity, cation exchange capacity and water infiltration which prompted growth and development of spinach. Although such recommendations were made, when handling composts and manures it is important that precautions should be taken in determining application rates especially when working with immature compost. Immature organic compost are said to contain high salt levels which may negatively affect plant growth and development (Önal and TopcuoğLu, 2007).

2.2.2.2. Soil analysis

50 grams was sub-sampled from the 160kg autoclaved soil and analyzed for macro- and micro elements. Different procedures were used to determine the levels of the following: for N analysis, the procedure by Mulvuney (1996) was used; for K analysis, extractable potassium sulfate ($K_2SO_4^{2-}$) was used; P analysis, Sodium bicarbonate ($NaHCO_3$) extractable P at pH 8.5 was used as described by Olsen, (1952), while for S analysis, a mixture of sodium carbonate (Na_2CO_3) and potassium nitrate (KNO_3) was used (Artiola and Ali, 1990) and for micro-elements analysis ICP- Mass spectrometry was used (Jarvis, 1994a) and soil organic matter was analyzed using the Walkley-Black method (Walkley and Black, 1934).

2.2.2.3. Analysis of macro- and micronutrients in substrates

Samples of SMS were collected from the mushroom dome structures after the mushrooms had been harvested. The following residual mushroom substrates: *U. panicoides, Z. mays* and *D. stramonium* were analyzed for both macro and micro-nutrients. Briefly, One gram of substrate was weighed, placed into crucibles and subjected to dry ashing which was carried out in a muffle furnace at 600°C for 8 h. After ashing, the crucibles were cooled to room temperature and the respective ash samples were dissolved in 8 ml dilute nitric acid (HNO_3) and 2 ml of hydrochloric acid (HCl) and microwaved for 45 minutes (Campbell and Whitfield, 1991; AgriLASA, 1998). Trace elements were then analyzed using NexION 300Q ICP-Mass spectrometer (Williams,

1972). In addition, organic matter was also analyzed using Warncke method (Warncke, 2011).

2.2.2.4. pH analysis

For all three respective SMS and the soil, 20g was weighed out and transferred into four 100 mL beakers. A volume of 40 mL distilled water was added, and the solutions were stirred with glass rod and allowed to stand for 30minutes. Thereafter, the solutions were stirred again immediately before reading. The electrode was immersed into the soil suspension in the beaker and a pH value was read from the pH meter (Soil Analysis Handbook of Reference Method, 1999).

2.3. Experimental design and data collection

The three SMS types (*U. panicoides, Z. mays and D. stramonium*) from the oyster mushrooms were used as experimental treatments. The treatments were as follows: T1= *U. panicoides,* T2= *Z. mays,* T3= *D. stramonium*, T4= Substrate mix (60%soil: *U. panicoides* (13.33%), *Z. mays* (13.33%) *and D. stramonium* (13.33%) and T5= un-amended soil (100%). These respective treatments were amended with a Hutton soil at 60:40 (soil/substrate) ratio and were replicated eight times making a total of 40 experimental units. The 4 individual substrates amended with soil were filled into 40 25cm diameter pots and arranged in a complete randomized design (CRD). In each pot, three seeds were directly planted at a depth of about 20 mm. Experimental units were irrigated at two day intervals with each pot receiving 500 ml. Weeding was carried out once a month.

Seedling emergence evaluations were carried out at a two day interval until full emergence was reached in all the experimental units. Plant height, leaf number and chlorophyll content (CCI) were recorded at 6, 8, 10 and 12 weeks after planting. A 30cm ruler was used to measure the height of the plants, number of leaves were counted per plant and a CCM-200 plus chlorophyll meter (Apogee instruments) was used to assess chlorophyll content on a single leaf which was marked with a red string rope on the petiole per treatment. Assessment of chlorophyll content was undertaken to determine chlorophyll content in intact leaf analysis. Chlorophyll content is a direct indication of plant

health and condition. Fresh biomass of the shoots and roots was determined at harvest using Symmetry PR Precision Scale and the dry biomass was obtained after 72hours of oven drying at 60°C.

2.4. Statistical analysis

The measured growth parameters *i.e.*, emergence, plant height, number of leaves, chlorophyll content and total shoots and roots biomass were subjected to analysis of variance (ANOVA) using the GLM (General Linear Model) procedure of SAS software package, version 9.4 (SAS Institute, Inc. Cary. NC. USA, 2001-2011). The mean comparison of data was interpreted using Least Significance Difference at 5% level of probability.

2.5. RESULTS

2.5.1. Macro- and micronutrients composition of substrates

Statistical analysis of nutrients composition (Table 1) shows that there was no significant difference ($P \leq 0.05$) in K, Ca, Fe, Mn and Cu levels of *Zea mays*, *Datura stramonium*, *U. panicoides* SMS and Substrate mix. However, high levels of N, P, Mg, and Zn were noted on *Zea mays* SMS. The control recorded low levels of both macro- and micronutrients. In regards to organic matter content *Zea mays* had the highest organic matter content when compared with the other substrates.

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Table 1: Macro- and micronutrients composition of different substrates

SMS	N mg/100g Mean ±SD	P mg/100g Mean ±SD	K mg/100g Mean ±SD	Mg mg/100g Mean ±SD	Ca mg/100g Mean ±SD	S mg/100g Mean ±SD	Mn mg/100g Mean ±SD	Cl mg/100g Mean ±SD	Cu mg/100g Mean ±SD	Fe mg/100g Mean ±SD	Zn mg/100g Mean ±SD	O.M (%)
U. panicoides	41.06 ±7.89b	44.69±18.62b	36.93 ± 0.83b	27.37 ± 5.48a	0.39 ± 0.18c	0.22 ± 0.01b	0.16 ± 0.05a	0.13 ± 7.34a	0.15 ± 0.04a	2.52 ±0.53a	0.35 ± 0.52a	36.12
Z. mays	57.24 ±5.89a	67.05 ±17.31a	41.07 ± 1.82a	27.89 ± 8.81a	0. 51 ± 0.19b	0.19 ± 0.07b	0.19 ± 0.13a	0.18 ± 4.47a	0.18 ± 0.03a	2.29 ± 0.75a	0.39 ± 0.11a	68.60
D.stramonium	45.66± 9.06b	63.31±81.85a	31.29 ± 0.72b	18.69 ±13.78b	0.69 ± 0.14b	0.16 ± 0.03b	0.13 ± 0.04b	0.12 ± 2.24a	0.17 ± 0.04a	2.06 ± 0.55a	0.25 ± 0.02b	51.60
Substrate mix	50. 89 ±8.43a	60.74 ±79.96a	32.25 ±1.43b	24.65 ±7.85a	0.57 ±0.37b	0.28 ±0.08b	0.16 ±0.034a	0.15 ±3.52a	0.16 ±0.03a	2.42 ±0.51a	0.22 ±0.03b	56.48
Soil	24.96± 0.57c	37.16 ±13.04b	24.00 ± 3.89c	0.98 ±16.58c	0.88 ± 3.54a	0.32 ± 0.74a	0.00 ±0.00c	0.09 ±0.16b	0.07 ±0.14b	1.76 ±0.48b	0.18 ± 0.41b	1.40

Means followed by the same letter in a column do not differ significantly at P≤0.05.

2.5.2. pH analysis of different substrates

The analysis indicated that SMS *D. stramonium* had the highest pH level of 8.69, followed by *Z. mays* with slightly alkaline levels of 7.83 and *U. panicoides* was neutral with pH level of 7.20. Un-amended soil was found to be acidic with 4.07 pH level (Table 2).

Table 2: pH levels of different spent mushroom substrates and control

Substrates/ Control	pH levels
Soil (control)	4.07
U. panicoides	7.20
Z. mays	7.83
D. stramonium	8.69
Substrate mix	7. 92

2.5.3. Growth response of *Spinacia oleracea* to different substrates

2.5.3.1. Seedling emergence of *Spinacia oleracea*

Assessments of seedling emergence indicated that seedlings grown in *Z. mays* SMS had the highest emergence percentage. This was followed by seedlings grown in *D. stramonium* SMS. The lowest performance was with seedlings grown in un-amended soil (control). Fig.1 shows seedling emergence rates grown in different growth media.

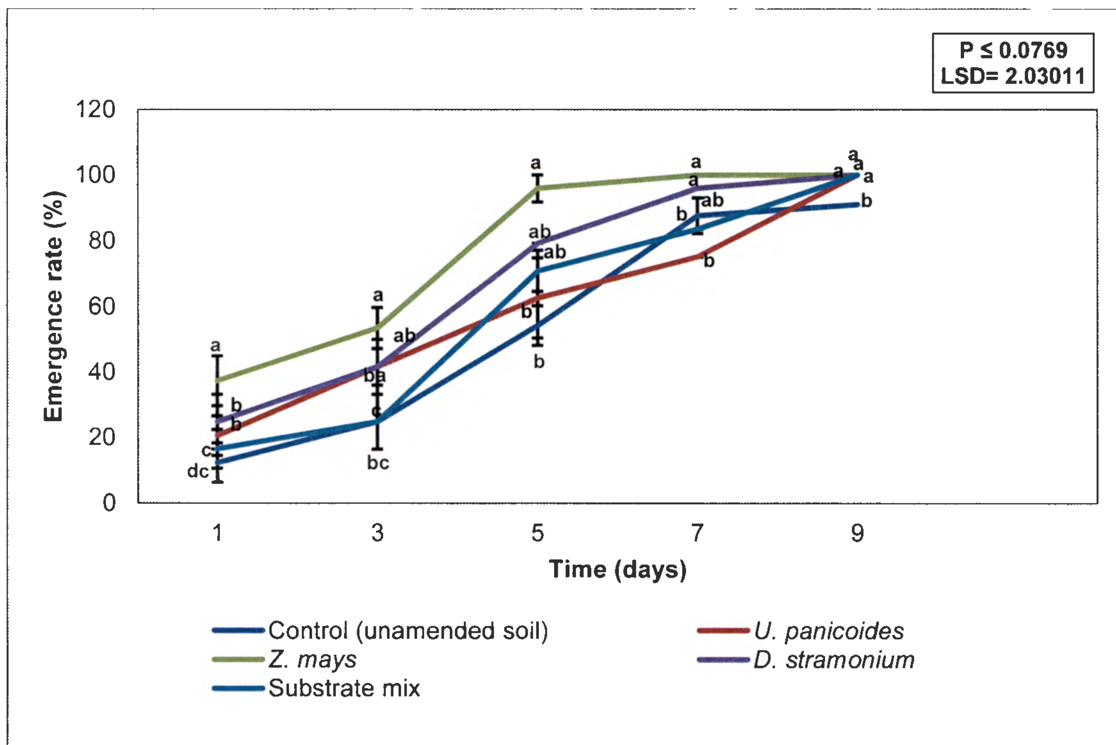

Figure 1: Effect of different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) on the emergence rate of *Spinacia oleracea* eight days after planting at 60 soil: 40 substrate (v/v) potted mix. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

2.5.3.2. Plant height of *Spinacia oleracea* seedlings

Statistical analysis (Table 3) showed that different SMSs had significant influence on plant height of *S. oleracea* seedlings. Seedlings grown on *Z. mays* and *D. stramonium* amended soil gave higher plant height than seedlings grown on substrate mix, *U. panicoides* and un-amended soil throughout the growth period.

Table 3: Effect of different spent mushroom substrates on plant height (cm) of *S. oleracea* seedlings at 6 to 12 weeks after planting

	Growth period				
SMS	6 wk	8 wk	10 wk	12 wk	**Mean**
Control (unamended soil)	12.38 ± 0.67^{b}	13.75 ± 0.58^{c}	14.87 ± 2.18^{c}	16.53 ± 2.43^{b}	**14.38**
U. panicoides	10.55 ± 1.67^{b}	11.96 ± 1.85^{cd}	14.71 ± 2.20^{c}	13.83 ± 3.12^{c}	**12.76**
Z. mays	18.76 ± 1.33^{a}	24.96 ± 1.26^{a}	24.43 ± 1.14^{a}	26.27 ± 1.09^{a}	**23.61**
D. stramonium	18.23 ± 1.11^{a}	20.05 ± 1.21^{a}	23.20 ± 1.03^{a}	25.08 ± 1.25^{a}	**21.64**
Substrate mix	15.25 ± 0.71^{ab}	17.18 ± 0.67^{b}	19.81 ± 0.80^{b}	21.20 ± 0.86^{ab}	**18.36**
$P_{(0.05)}$=	**0.0034**	**0.0012**	**0.0155**	**0.0146**	

Means followed by the same letter in a column do not differ significantly at $P\leq0.05$. SMS, spent mushroom substrate.

2.5.3.3. Number of leaves of *Spinacia oleracea* seedlings

Table 4 shows that the leaf number varied within a narrow range of 4 to 11 leaves $plant^{-1}$ for all seedlings raised on the five respective substrates. Although high leaf production was noted in seedlings grown in *Z. mays*, there was however, no statistical significant difference ($P\geq0.05$) between seedlings raised in *D. stramonium* and *Z. mays* SMS. The lowest performance was noted with seedlings grown in *U. panicoides* amended soil.

Table 4: Effect of different spent mushroom substrates on number of leaves of *Spinacia oleracea* seedlings at 6 to 12 weeks after planting

	Growth period				
SMS	6 wk	8 wk	10 wk	12 wk	**Mean**
Control (unamended soil)	4.12±0.23[b]	4.75±0.25[c]	5.63±0.23[b]	5.88±0.93[d]	**5.09**
U. panicoide	5.00±0.78[b]	5.56±0.87[b]	6.50±0.94[b]	7.37±1.08[c]	**6.11**
Z. mays	7.31±0.50[a]	8.50±0.35[a]	9.00±0.26[a]	11.00±0.35[a]	**8.95**
D. stramonium	6.37±0.30[a]	8.25±0.48[a]	8.75±0.48[a]	9.07±0.59[ba]	**8.11**
Substrate mixture	5.62±0.26[b]	6.50±0.37[b]	6.88±0.35[b]	8.13±0.35[b]	**6.78**
$P_{(0.05)}$=	**0.0014**	**0.0010**	**0.0001**	**0.0150**	

Means followed by the same letter in a column do not differ significantly at P≤0.05. SMS, spent mushroom substrates

2.5.3.4. Chlorophyll Content Index (CCI) of *Spinacia oleracea* seedlings

Figure 4 shows that seedlings grown on *D. stramonium* amended soil had a high CCI (13.2 and 15.21 µg/cm^2) in 6 and 8 weeks after planting respectively. A decline was however noted in week 10 (13.2 µg/cm^2). Though, a decline in CCI was observed in seedlings grown in *D. stramonium* amended soil, there was; however, no statistical significance (P≤0.05) in CCI of seedlings grown in *D. stramonium* and *Z. mays* amended soil, respectively. Seedlings raised in *Z. mays* amended soil showed a gradual increase in CCI when compared with seedlings raised in other substrates.

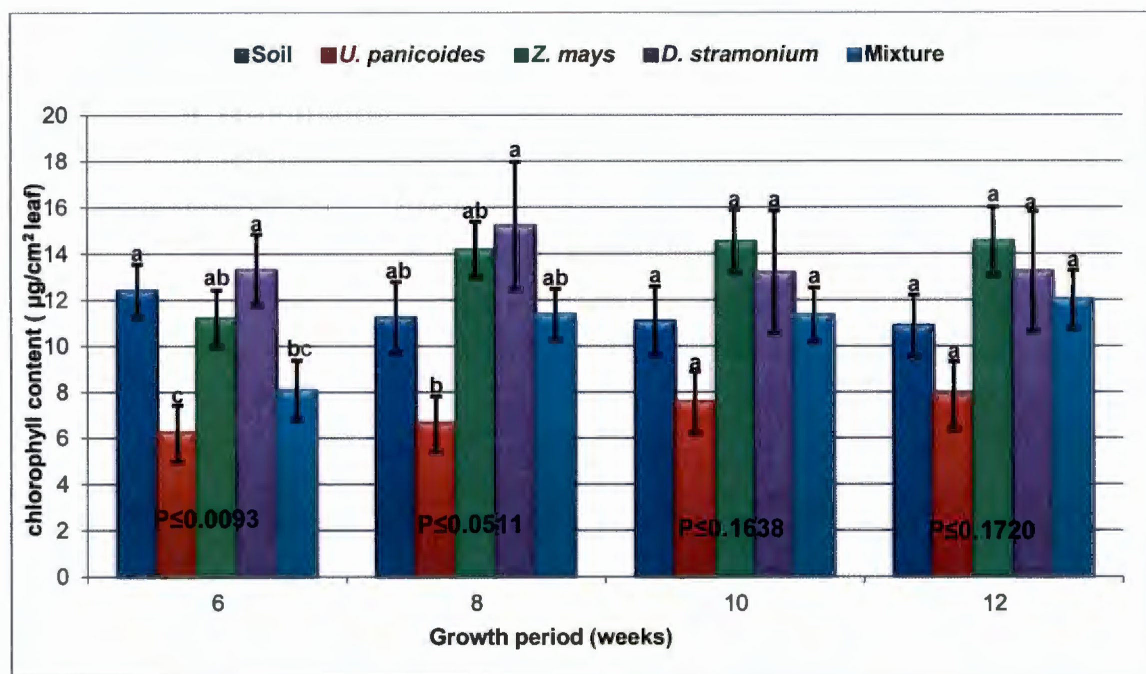

Figure 2: Effect of different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) on the total chlorophyll content index of *Spinacia oleracea* 12 weeks after planting at 60 soil: 40 substrate (v/v) potted mix. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

2.5.3.5. Fresh mass of shoots and roots of *Spinacia oleracea* seedlings

Figure 3a and b shows a statistically significant effect of different substrates on *S. oleracea* seedlings fresh shoots and roots mass. Seedlings grown on *Z. mays*, *D. stramonium* and substrate mixture amended soils attained higher fresh shoots mass than seedlings raised on *U. panicoides* SMS and unamended soils. On the other hand, Fig. 3b showed that seedlings raised on *Z. mays*, *D. stramonium* and substrate mix differ significantly with the highest root mass ($28.84g^{-1}$) recorded on *Z. mays* amended soils. Seedlings raised on substrate mix, *U. panicoides* SMS and *D. stramonium* were found not significant ($P<0.0001$).

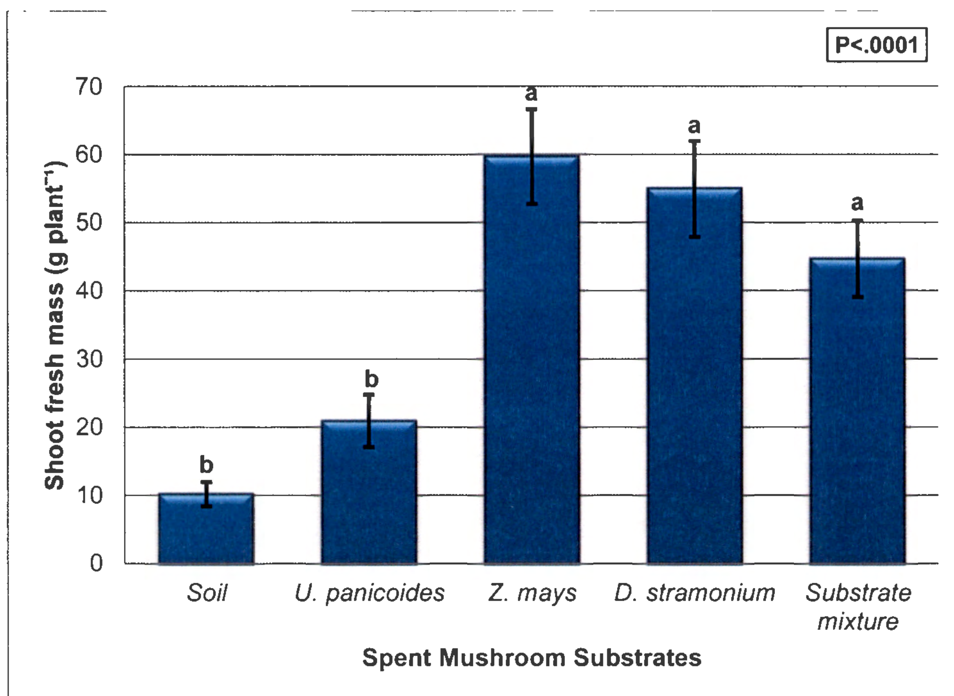

Figure 3a: Effect of different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) on shoots fresh mass of *Spinacia oleracea* 12 weeks after planting at 60 soil: 40 substrate (v/v) potted mix. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.0001.

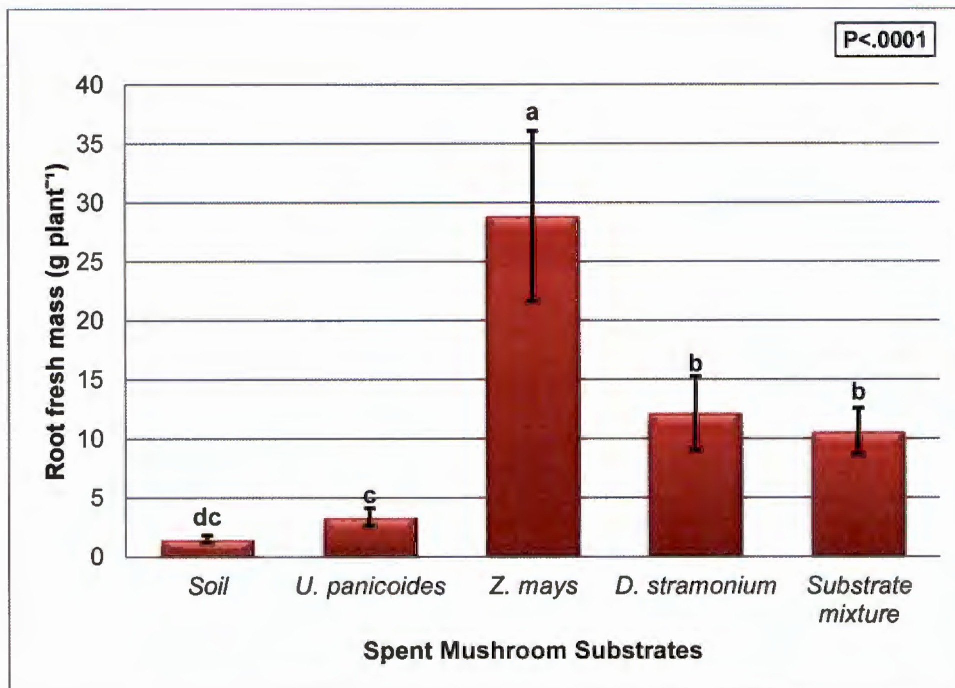

Figure 3b: Effect of different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) on roots fresh mass of *Spinacia oleracea* 12 weeks after planting at 60 soil: 40 substrate (v/v) potted mix. Means (± SE) designated by the same letter(s) are not significantly different at $P \leq 0.0001$.

2.5.3.6. Dry mass of shoots and roots of *Spinacia oleracea* seedlings

Fig. 4a shows that seedlings raised in *Z. mays*, *D. stramonium* and substrate mix amended soil were statistically not different at $P \leq 0.0001$ in terms of shoots dry mass. The lowest shoot dry mass was recorded in seedlings raised in un-amended soil ($3.49g^{-1}$). Fig. 4b showed a strong significant difference among substrates at $P \leq 0.0001$ in root dry mass. Although, the highest root dry mass was recorded on seedlings raised on Z. mays SMS, there was, however, no statistical difference between *Z. mays* and *D. stramonium* SMS seedlings. Similarly, seedlings raised on substrate mix and *U. panicoides* SMS did not differ significantly ($P \leq 0.0001$).

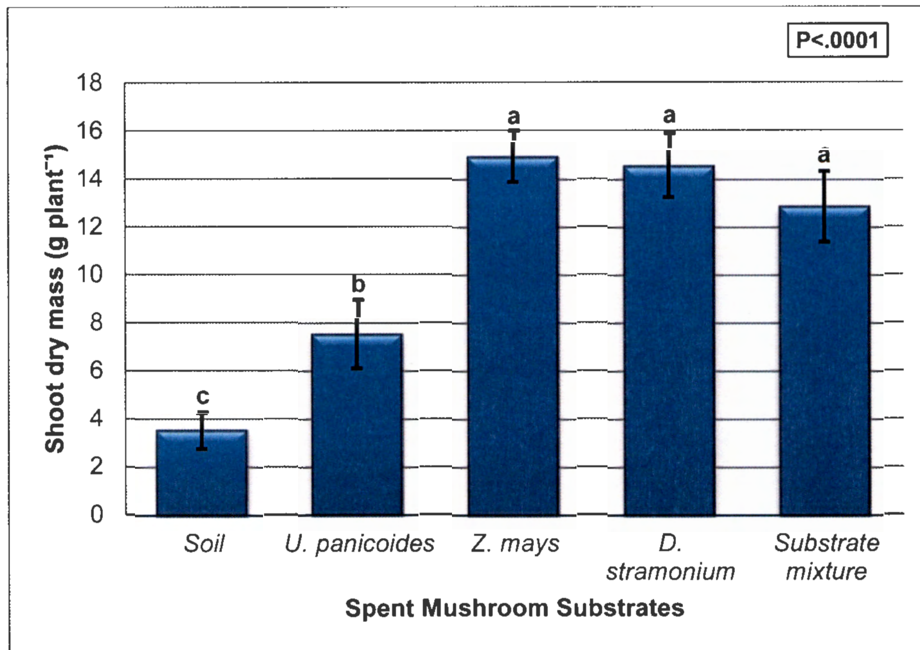

Figure 4a: Effect of different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) on shoots dry mass of *Spinacia oleracea* 12 weeks after planting at 60 soil: 40 substrate (v/v) potted mix. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.0001.

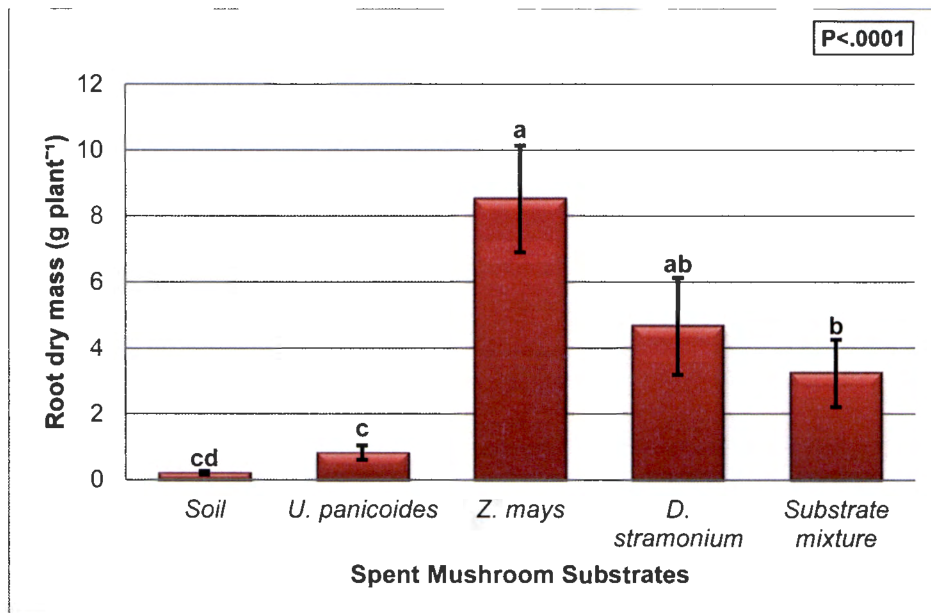

Figure 4b: Effect of different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) on roots dry mass of *Spinacia oleracea* 12 weeks after planting at 60 soil: 40 substrate (v/v) potted mix. Means (± SE) designated by the same letter(s) are not significantly different at $P \leq 0.0001$.

2.6. Discussion

The effect of SMS amended soils on seedling emergence of *S. oleracea* (Fig. 1) indicates that the different growth media used have inherent properties that influenced seedling emergence by enhancing the soils physical and chemical properties. High seedling emergence was noted in seedling growing in *Z. mays* amended soil. This was probably associated with improved soil structure and moisture. Adequate moisture triggers germination process which in turn promotes early seedling emergence. Seedling emergence is influenced by a number of factors of which soil structure is one of them. Soil structure influences seedling emergence and growth rather indirectly. The pores are the controlling factor governing water, air and temperature in the soil, which in turn, governs plant growth. For instance, the effect of soil structure on plant growth is the emergence of seedlings from the soil. Seedlings are responsive to soil physical conditions such as soil compaction so that there is no hindrance to the emergence of seedlings.

After the cotyledon have emerged from the soil, adequate nutrient supply is critical for plant growth and root development. Macro-nutrients, such as N and P were noted to be particularly high in *Z. mays* SMS, *i.e.* N recorded at 57.24mg/100g and P at 67.05mg/100g. The best performance of the seedling growth assessed height, number of leaves and chlorophyll content was noted in seedling raised in *Z. mays* SMS, with highest seedling height recorded at (26.27cm), highest leaf number per plant (11), highest chlorophyll content (14.53ug/cm^2) and total dry root mass of (14.94g^{-1}). The influence of N on the seedling growth could be its role as an essential component of plant growth hormones and enzymes that drive photosynthesis processes which are known to increase production and accumulation of assimilates. Increased assimilates results in an increase in leaf number, seedling height and seedling biomass (El-Gizawy *et al.*, 1992; Ahmed, 2003 and Ahmed *et al.*, 2006). Furthermore, seedlings raised on *Z. mays* amended soils were noted to have a high CCI reading (11.2, 14.18, 14.53 and 14.54ug/cm^2) indicating a high chlorophyll content probably because of the high N supply in the substrate. These readings were higher than CCI readings from seedlings grown in substrate mix, *U. panicoides* and unamended soil (Fig. 2). It could therefore be deduced that a high CCI value indicates high nutrient availability in the soil. This was supported by Papasavvas *et al.* (2008) who observed a linear correlation between SPAD (Silicon Photon Activated Diode) meter readings and the physiological parameters of leaves *i.e.,* photosynthesis. It was concluded that SPAD meter readings may be used as an indirect indicator of nitrogen status since readings were related linearly to NO3-leaf concentration. In addition to high levels of N in *Z. mays* substrate, it was noted that the substrate also had the highest K level. Among many functions of K in the plants physiological processes, it is known that it plays an important role in the transportation of plant growth hormones and sucrose (Prajapati and Modi, 2012). This enhanced translocation of plant growth hormones and sucrose which probably could have had a positive effects on the plant growth rate.

The second best performing growth media were *D. stramonium* and substrate mix amended soil. These growth media also enhanced the growth of *S. oleracea* seedlings, but at a lower rate than *Z. mays* amended soil seedlings (Table 3). This was probably because of the low release of available nutrients by the substrate *D. stramonium*. Table 1 show that this growth medium contains relatively adequate levels of N, P, Mg, S, Ca

and Mn. It is possible that poor availability of these nutrients to seedlings in *D. stramonium* and SMS mix growth medium could have been caused by the substrates' alkaline conditions. *D. stramonium* and SMS mix had pH levels of 8.69 and 7.92 respectively, and these are relative too alkaline for availability of most nutrients to plants. Most nutrients are available to plants at a pH range of 6.5 to 7.5 (International Plant Nutrition Institute, 2010). The high alkaline condition of the two growth media could also have affected availability of P nutrient. Phosphorus is known to be most available to plants at a pH range of 6.5 to 7.5 (IPNI, 2010). Phosphorus is an important macro-nutrient that plays a major role in energy availability through photosynthesis and respiration and is required in large quantities by young cells, such as shoots and roots where the metabolism is high and cell division is rapid (Chida, 2000). Furthermore, good growth rate of seedlings grown in *Z. mays* amended soil could also have been the result of available micro-nutrients, such as Mg, Zn, Fe and Cu which were noted to be relatively higher in *Z. mays* substrate than in other SMS. These micro-elements are required by a plant for adequate growth and development. Even though such elements are required in a small amount they, however, play a vital role in plant nutrition. This was highlighted by Kirkby and Römheld (2004) who reported that the small quantity of such micro-elements are fundamental for growth and development by acting as constituents of the cell wall and membranes, enzyme activators and photosynthesis. It could therefore be deduced that availability of micro-elements in relatively higher levels in *Z. mays* than in other SMS could also have enhanced rapid increase in plant height, leave production, chlorophyll content and root development.

More specifically, Mg is relatively higher in *Z. mays* than in other SMS. Magnesium is part of chlorophyll molecule found in all green plants and is important for photosynthesis. Mg is also a cofactor for numerous plant enzymes required for development (Plaster, 2003; Gardiner and Miller, 2004). This probably could also have contributed to the high chlorophyll content of seedlings grown in *Z. mays* SMS. Other micro-elements such as S and Ca are also known for their various functions. For instance, S is essential in plants for forming proteins (Chida, 2000) and Ca is a form of Calcium pectate which is needed to form rigid cell walls. Rigid cell walls prevent newly emerging leaves from sticking together which cause tearing as leaves expand and uncurl, such as leaves of *S. oleracea*. The low availability of these micro-elements to seedlings grown in *D. stramonium* and

SMS mixture growth media could also have been because of their relatively high pH levels. It is known that soils that have high pH levels are thought to contain certain properties that can severely limit or delay the growth of a plant (Mengel and Kirby, 1987). Plants differ in terms of pH requirement or tolerance. Various studies have highlighted that nutrient availability is pH dependent (Neumann and Römheld 2012). For instance, *S. oleracea* does well under neutral to slightly alkaline soils that are between 6.9 and 7.5 for optimum growth (DAFF, 2010). Hence, an increase in seedling growth was noted in seedlings grown in *Z. mays* amended soil which had a pH of 7.8 which was close to the optimum pH value. In addition, several researchers have reported that most plants grown on alkaline soil are not directly affected by pH, but by poor nutrient supply (Kirby, 1979; Foth and Ellis, 1988; Srivastava, 2000). At high pH levels, the solubility of nutrients such as N, P, Mg, Mn, Zn, Fe and Cu is reduced resulting in nutrients being precipitated as solid materials that plants cannot take up at the time of need even if there is enough in the medium (Mengel and Kirby, 1987). This was explained by the slow plant growth rate and development in seedlings raised in *D. stramonium* SMS and SMS-mix amended soils.

Other than high levels of N, P and K, and adequate microelements observed in *Z. mays* SMS, it was noted that *Z. mays* also had the highest organic matter content of 68.60% compared to the un-amended soil which had the lowest organic matter content of 1.40%. Table 1 shows organic content of the other growth media. Comparative analysis of seedling emergence rates between *Z. mays* and un-amended growth media with their respective organic matter content indicates that growth media with highest organic matter content of 68.60% had highest emergence rate and the lowest organic matter content 1.40% had the lowest emergence rate. It could therefore be deduced that this growth medium characteristic has influence on the seedling emergence rate. This was because of the enhanced microbial activities in the growth media with high organic matter content. Soil microbes such as mycorrhizal fungi require C for their active growth. Healthy mycorrhizal colonization is known to enhance uptake of P which promotes seedling root development (George *et al.*, 1995). Other saprophytic soil microorganisms could also help in breaking down organic matter resulting in the release of nutrients that enhances seedling growth. Furthermore, organic matter is known to improve soil structure, water holding capacity and aeration which promote seedling emergence, root growth and

development increasing nutrient uptake that promotes plant growth (Bot and Benite, 2010).

Table 3, 4 and Figure 3a to 4b shows the effect of other various growth media on different plant growth parameters. The poor growth recorded in seedlings grown in *U. panicoides* amended soil was probably because of nutrients being exhausted by the oyster mushrooms during production. This hypothesis is based on the fact that high mushroom pinning, fruit capsize and yield was observed in *U. panicoides* substrate in other studies (Ramachela and Sihlangu, 2016). Nutrient analysis (Table 1) show that *U. panicoides* substrate had low levels of N, K, S and Ca which are the most required especially during the vegetative growth stage. Stunted growth and yellowing of leaves was observed in seedlings grown in un-amended soil. Low soil pH, low nutrient supply, poor water infiltration rate as a result of compaction could have also contributed to the poor performance of seedlings grown in un-amended soil. Soil pH analysis indicated that un-amended soil was highly acidic at pH 4.07. As mentioned earlier, plant nutrient availability is pH dependent. This was further supported by (McCauley *et al.*, 2009; IPNI, 2010;Liu and Hanlon, 2012) who reported that at low pH nutrients such as Al, Fe, Zn and Mg become toxic to plants and are likely to injure the developing seedling roots. This could possibly result in leaf chlorosis, stunted growth and leaf dieback negatively affecting yield. Nutrient analysis results have also indicated that un-amended soil (control) had low levels of essential nutrients and organic matter percentage which may have greatly negatively influenced the growth of a plant. This most likely caused the yellowing of leaves, poor plant height, poor root development, low CCI readings in seedlings raised in un-amended soils. Findings of this study indicated that *Z. mays* amended soils have the potential to improve emergence, plant growth and development of *S. oleracea* seedlings. Though growth of *S. oleracea* seedlings improved significantly, however a variation was noted between the different substrates in their effect as crop growth enhancement, it is therefore recommended that different biomass found in various respective farming areas should be evaluated and assessed for their performance as soil amendments.

CHAPTER THREE

Effect of different "spent" oyster mushroom substrates (SMS) on the establishment and growth of beetroot (*Beta vulgaris* L.)

3.1. Introduction

Beta vulgaris commonly known as beetroot is a member of Chenopodiaceae family that originates from Europe (Hergert, 2010). Beetroot has many different varieties; however, the most commonly grown varieties are silver beets, sugar beets and fodder beets (Deuter and Grundy, 2004). Although Beetroot is a biennial crop it is however, grown as an annual crop producing green tops and swollen roots during the early stages of growth which serves as a storage organ for nutrients (Daff, 2010). Beetroot has been reported to be a good source of carbohydrates, proteins and has high levels of important vitamins and mineral nutrients that are essential in human diet (Cerne & Vrhovnik, 1999). Beetroot is considered as a vegetable and may have numerous positive impacts on human health (Cerne & Vrhovnik, 1999). For instant, a juice made from Beetroot has recently received increasing attention as a stimulant for the immune system, and as a cancer preventative. Additionally, it has long been considered beneficial to blood, heart and digestive systems (Nottingham, 2004; Ziielinska-Przyjemeska *et al.*, 2009; George *et al.*, 2010).

Beetroot is a cool weather crop that is hardy and tolerates high and too low temperatures. It grows best in spring and autumn, but does well in summer in the Highveld and in winter in the Lowveld. The best quality beetroot are obtained if the crop is grown to maturity in the shortest possible time. The main producing regions in South Africa are North West, Gauteng, Mpumalanga, Kwa-Zulu Natal and Western Cape. The estimated production figures increased from 60 000/annum in 2011 to 80 000tons/annum in 2016 (FAOSTAT, 2014). Moisture and soil fertility are considered as one of the most limiting factors in beetroot production. Hence, hard and compacted soil with poor drainage should be avoided, as this will delay seedling emergence and root development (KZN DAE, 2001; Farming SA & ARC, 2009; DAFF, 2010).

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Specific objective: To evaluate the influence of *Urochloa panicoides*, *Zea mays*, *Datura stramonium* spent mushroom substrates on the establishment and growth of *Beta vulgaris* seedlings.

3.2. Materials and Methods

The study was conducted parallel with that of *Spinacia oleracea* under glass house conditions (Chapter 2). Thus, all aspects related to materials and methods including statistical analysis were similar.

3.3. Results

3.3.1. Growth response of *Beta vulgaris* as influenced by various spent mushroom substrates

3.3.1.1. Seedling emergence of *Beta vulgaris*

Seedlings raised on *D. stramonium* SMS had a significantly ($P \leq 0.05$) faster seedling emergence rate. Seedlings raised on both *D. stramonium* and *Z. mays* amended soils were first to attain full emergence at 17 days after planting (3rd assessment). Fig. 5 shows emergence rates in seedlings raised in respective substrates.

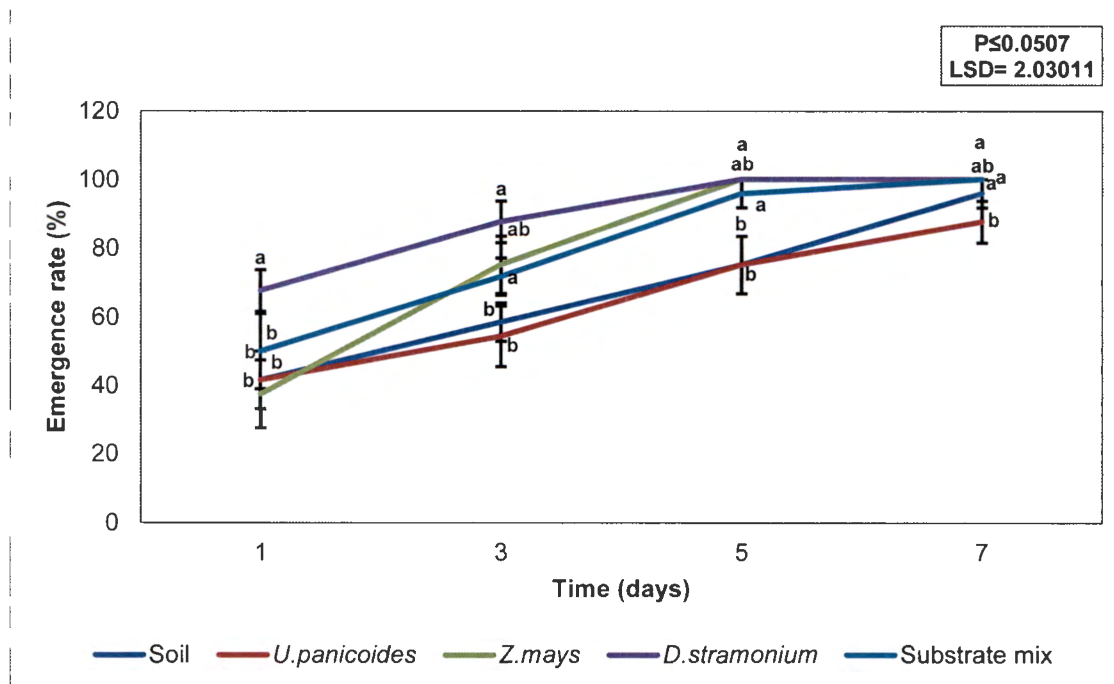

Figure 5: Emergence rate of *Beta vulgaris* seedlings grown for 19 days in different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) amended at 60 soil: 40 substrate (v/v) potted mix. Plotted points are means of 40 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

3.3.1.2. Plant height of *Beta vulgaris* seedlings

The data analysis (Table 5) shows that various SMS had statistically significant effect on plant height of *B. vulgaris* (P≤0.05). The highest plants were measured in seedlings raised on *D. stramonium* and *Z. mays* amended soils.

Table 5: Effect of different spent mushroom substrates on plant height (cm) *Beta vulgaris* seedlings at 6 to 12 weeks after planting

Treatments	Growth period				Mean
	6 wk	8 wk	10 wk	12 wk	
Unamended soil	9.36±1.68^{b}	10.82±1.82^{b}	11.86±2.79^{b}	12.06±2.76^{a}	**11.03**
U. panicoide	9.66±0.60^{b}	11.58±0.68^{b}	14.56±0.80^{b}	16.27±0.80^{a}	**13.02**
Z. mays	12.96±2.48^{ab}	16.10±2.78^{ab}	19.02±3.02^{ab}	21.23±3.28^{a}	**17.33**
D. stramonium	15.33±1.76^{a}	20.48±1.21^{a}	24.21±1.23^{a}	23.67±3.53^{a}	**18.65**
Substrate mixture	14.05±1.28^{ab}	17.42±1.54^{ab}	18.00±2.98^{ab}	21.06±3.36^{a}	**13.18**
$P_{(0.05)}$=	**0.0592**	**0.0018**	**0.0104**	**0.0607**	

Means followed by the same letter in a column do not differ significantly at P≤0.05. SMS, spent mushroom substrates

3.3.1.3. Number of leaves of *Beta vulgaris* seedlings

Seedlings grown on various SMS showed statistically significant effects in leaf production (Table 6). However, the highest number of leaves was noted in seedlings raised on *D. stramonium* SMS.

Table 6: Effect of different spent mushroom substrates on the number of leaves of *Beta vulgaris* seedlings at 6 to 12 weeks after planting

Treatments	Growth period				Mean
	6 wk	8 wk	10 wk	12 wk	
Unamended soil	3.87±0.61^{b}	4.87±0.74^{c}	5.87±0.93^{c}	4.25±1.05^{c}	**4.72**
U. panicoide	4.00±0.33^{ab}	6.00±0.46^{b}	6.75±0.59^{bc}	7.37±0.71^{b}	**6.03**
Z. mays	5.12±0.81^{a}	6.50±1.02^{b}	8.25±1.05^{ba}	9.07±1.17^{a}	**7.23**
D. stramonium	6.12±0.53^{a}	8.12±0.67^{a}	9.37±0.75^{a}	9.50±0.50^{a}	**8.27**
Substrate mixture	5.25±0.56^{a}	6.50±0.46^{b}	7.50±0.33^{b}	7.12±1.14^{b}	**6.59**
$P_{(0.05)}$=	**0.0412**	**0.0401**	**0.1374**	**0.0105**	

Means followed by the same letter in a column do not differ significantly at P≤0.05. SMS, spent mushroom substrates

3.3.1.4. Chlorophyll content index (CCI) of *Beta vulgaris* seedlings

CCM-meter readings presented in Fig. 6 show that the chlorophyll content on leaves of *B. vulgaris* seedlings was influenced by various substrates. There was, however, no statistically significant difference among the treatments. Though, *D. stramonium* was noted to have high chlorophyll reading ranging from 12 to 15 ug/cm² from week 6 to 12. For no explainable reason a decline was however noted in week 8.

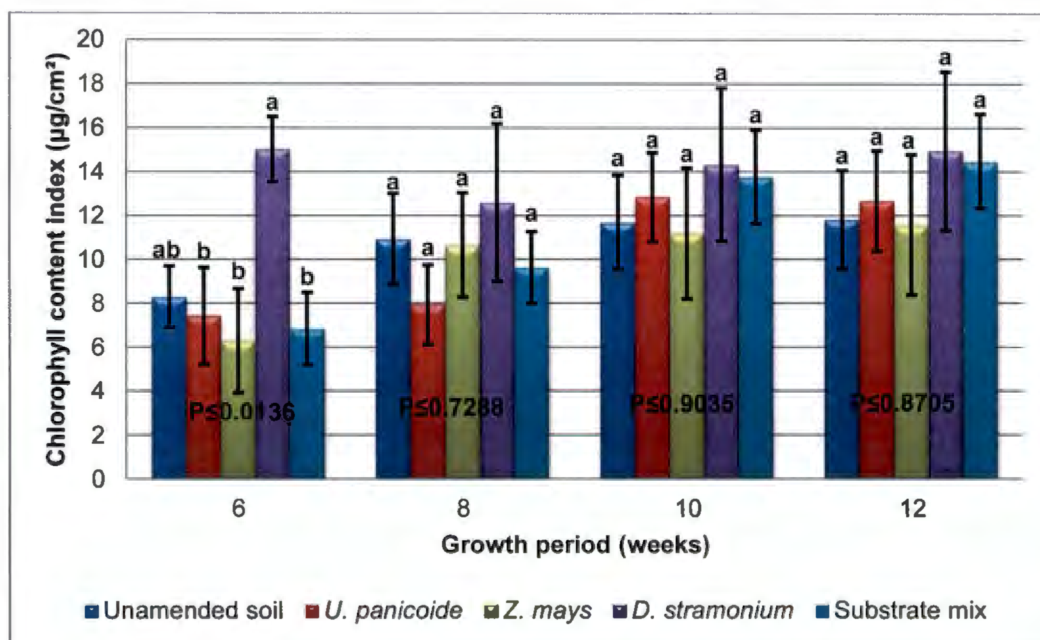

Figure 6: Effect of different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) on the total chlorophyll content index of *Beta vulgaris* 12 weeks after planting at 60 soil: 40 substrate (v/v) potted mix. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

3.3.1.5. Fresh mass of shoots and roots of *Beta vulgaris* seedlings

Fig. 7a shows that seedlings raised on *D. stramonium* amended soil had the highest shoots mass when compared with seedlings grown in the other soil amendments, there was however, no statistical difference (P≤0.05) between seedlings raised on *D. stramonium* and *Z. mays* amended soils. The lowest shoot and storage root biomass was

noted in seedlings raised in un-amended soil. A similar trend was also noted in fresh roots biomass (Fig. 7b).

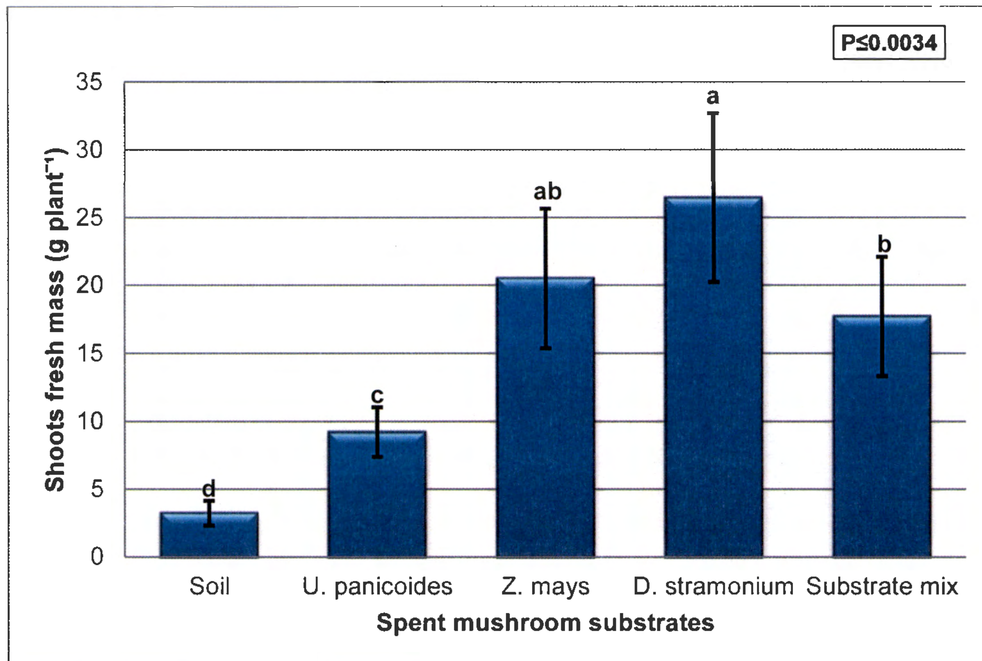

Figure 7a: Shoot fresh mass of *Beta vulgaris* seedlings grown for 12 weeks in different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) amended at 60 soil: 40 substrate (v/v) potted mix. Plotted points are means of 40 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

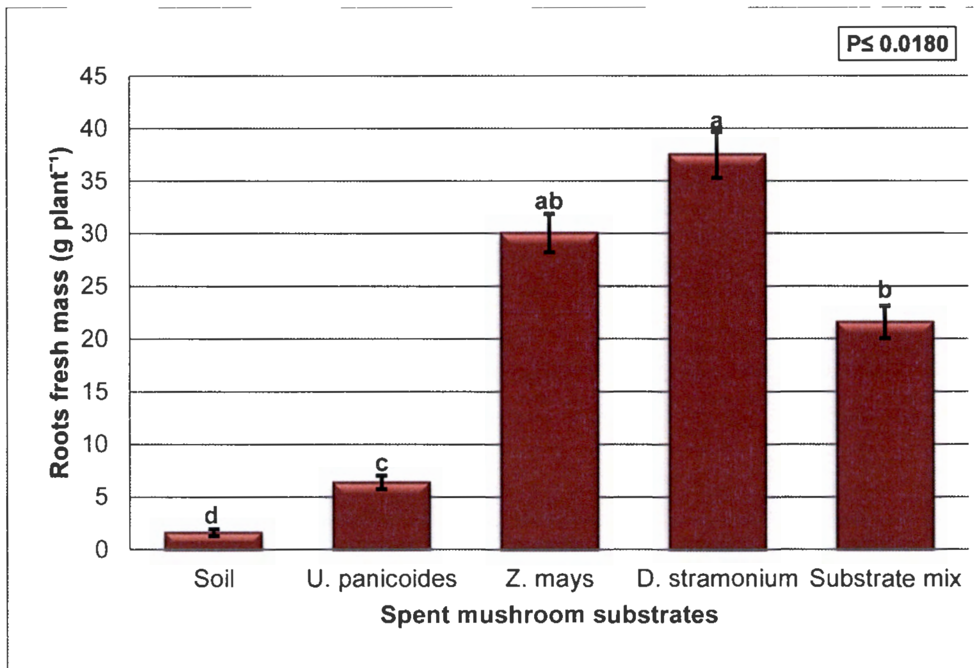

Figure 7b: Roots fresh mass of *Beta vulgaris* seedlings grown for 12 weeks in different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) amended at 60 soil: 40 substrate (v/v) potted mix. Plotted points are means of 40 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at $P \leq 0.05$.

3.3.1.6. Dry mass of shoots and roots of *Beta vulgaris* seedlings

As shown in Fig. 8a and b, seedlings raised in *D. stramonium* SMS had the highest shoot and root dry mass recorded when compared with other substrates, however, seedling raised in *Z. mays* and substrate mix amended soils do not differ significantly ($P \leq 0.05$) with seedlings grown in *D. stramonium* amended soil. On the other hand, seedlings grown in *U. panicoides* SMS and control were found not significant with seedlings grown from *Z. mays* and substrate mix but were statistically significant with seedlings grown on *D. stramonium* SMS.

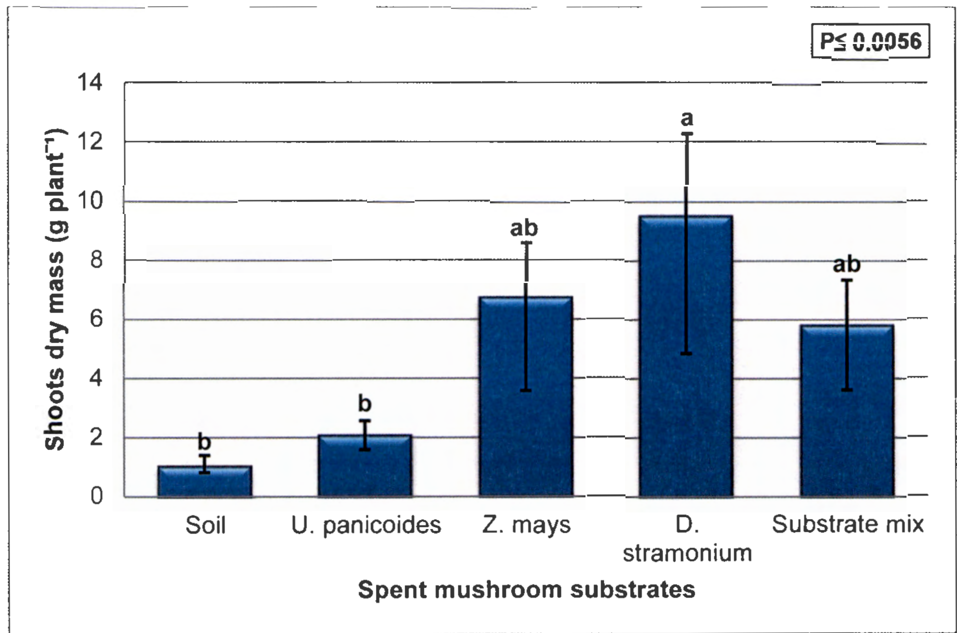

Figure 8a: Shoot dry mass of *Beta vulgaris* seedlings grown for 12 weeks in different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) amended at 60 soil: 40 substrate (v/v) potted mix. Plotted points are means of 40 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at $P \leq 0.05$.

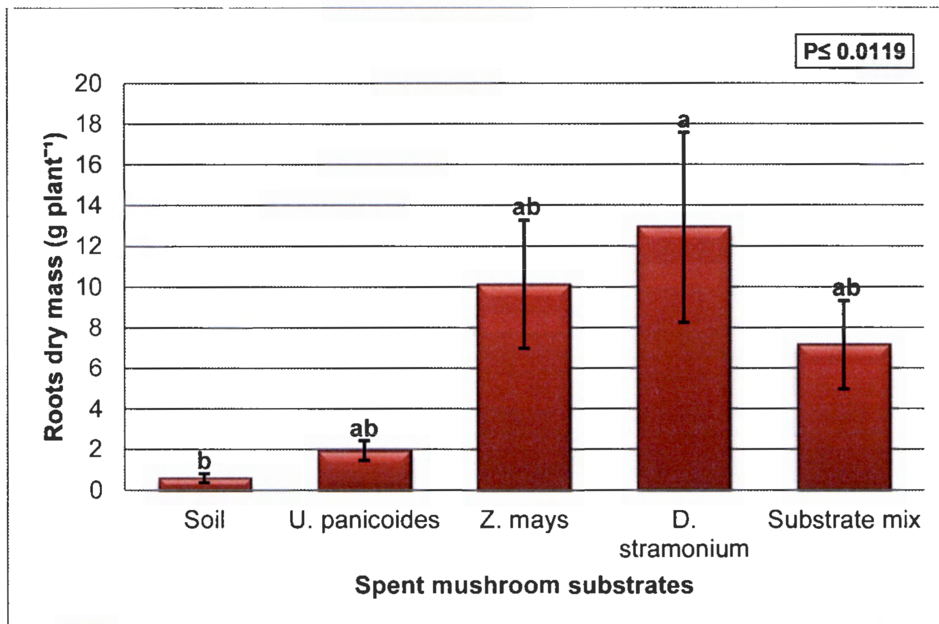

Figure 8b: Shoot fresh mass of *Beta vulgaris* seedlings grown for 12 weeks in different spent oyster mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix) amended at 60 soil: 40 substrate (v/v) potted mix. Plotted points are means of 40 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

3.4. Discussion

First seedling emergence was noted at 11DAP in all five respective substrates. However, seedlings raised on *D. stramonium* and *Z. mays* amended soils were first to attain a 100% emergence 17DAP (Fig.5). Whereas, seedlings raised on *U. panicoides,* substrate mix and un-amended soil reached 100% emergence 21DAP. The increased rate in seedling emergence planted on *D. stramonium* and *Z. mays* amended soils was probably the result of improved soil moisture and soil structure. For germination to take place there should be enough moisture to stimulate breaking of seed dormancy (Finch-savage and Leubner-Metzger, 2006). After seed germinates, the cotyledons needs to emerge from the soil which is influenced by the soil structure. Soil pores are the controlling factors governing water, air and temperature in the soil which in turn governs plant growth. For instance,

the effect of soil structure on plant growth is the emergence of seedlings from the soil. Seedlings are sensitive to soil physical conditions, so that there is no hindrance to the emergence of seedlings there should be optimum soil moisture and aeration. After cotyledon had emerged from the soil, adequate N supply is important at this stage for optimum seedling growth and subsequent canopy development (Amber *et al.*, 2009). For rapid early growth and development, sufficient N needs to be taken up and allocated to the sink organ. Seedlings grown on *D. stramonium* and *Z. mays* amended soil which had readily available N not only achieved early emergence, but had higher plant height, leaf production and chlorophyll content (Table 5, 6 and Fig. 6). Substrate nutrient composition analysis (Table 1) not only indicated high levels of N in the respective substrates but also reflected other essential nutrients which could have enhanced plant growth in seedling raised in these respective substrates. In addition, N is a major constituents of chlorophyll, nucleic acids and amino acids, thus it is necessary for plant metabolism and growth (Epstein and Bloom, 2005). Hence, insufficient N supply at this stage would most likely lead to reduced leaf production, lowered chlorophyll content and decreased photosynthetic rate resulting in lower biomass production and yield (Amber *et al.*, 2009).

Nutrient analysis indicated that *D. stramonium* and *Z. mays* substrates had adequate amount of P, K, Mg, Mn and Zn levels followed by substrate mix (Table 1). *U. panicoides* and un-amended soil had the lowest levels of both macro- and micronutrient composition (Table 1). These nutrients, *i.e.,* P, K, Mg, Mn and Zn are required for optimum growth in *B. vulgaris*. Phosphorus plays an important role in energy transfer, photosynthesis, transformation of sugars, transfer of genetic information and nutrient movement within the plant promoting rapid growth (Marschner, 1995). Potassium is also involved in enzyme activation, charge balance and osmoregulation in plants (Cakmak, 2005). In *B. vulgaris,* K is known to play a significant role in biosynthesis and transfer of sucrose to storage roots, consequently, promoting root development (Winzer *et al.*, 1996). Hence, seedlings grown on *D. stramonium* and *Z. mays* amended soil had the highest dry root mass measured than seedlings planted on the other media (Fig. 8b). Furthermore, Magnesium plays a major role in chlorophyll formation and is, therefore important for photosynthesis (Chida, 2000; Gardiner and Miller, 2004). It could therefore be concluded that as a result

of Mg and N availability, significant increase in CCI was recorded in plants grown on *D. stramonium* amended soil and substrate mix (Fig.6). It has been reported that chlorophyll index and N availability are strongly related (R^2=0.84). High CCI indicates N nutrient status of a plant (Papasavvas *et al.*, 2008).

Furthermore, a significant increase in dry mass of storage roots was recorded in seedlings raised in *D. stramonium*, *Z. mays* and substrate mix than seedlings grown in *U. panicoides* and un-amended soils (Fig.8b). The increase in dry root mass was also probably associated with the high organic matter content recorded on *Z. mays*, *D. stramonium* and substrate mix (Table 1). It has been reported that high organic matter generally favours nutrient retention, organic matter accumulation and humus formation (Carter, 2002). Thus, organic matter has the potential to improve soil structure which enables root growth and development stimulating nutrient availability and uptake with improved active interaction with microorganisms (Bot and Benite, 2005). Microorganisms are known to contribute to the gradual and continuous release of plant nutrients. For instance, in cases where nutrients are available more than what is needed by the plants, they are sequestered by the soil microorganisms. In organic matter depleted soils, however, these nutrients are lost through leaching and runoff, consequently, reducing plant growth and root development (Bot and Benite, 2005). Seedlings raised on *U. panicoides* SMS and un-amended soils performed poorly with regard to plant height, number of leaves, chlorophyll content and shoot and root dry mass (Fig. 7 and 8). The poor seedling growth was probably caused by low nutrient availability, low pH of un-amended soils and poor soil structure. Nutrient analysis in (Table 1) showed that un-amended soil had the lowest organic matter content. A soil with low organic matter indicates poor soil structure restricting root development, water and nutrient absorption (Lotter, 2003; Bot and Benite, 2005).

From the finding of this study it can be concluded that *D. stramonium*, SMS mix and *Z. mays* oyster mushroom substrates are a good source of bio-fertilization as they significantly positively influenced the establishment and growth of *Beta vulgaris*. Use of oyster SMS as an amendment in agricultural soils is a valuable resource for establishment of a sustainable vegetable production system. Despite that, the above mentioned

substrates are a good source of bio-fertilization and other ecological benefit, there is also a need to investigate how these substrates can suppress growth of soil-borne pathogens that are widely known to limit vegetable production.

CHAPTER FOUR

In-vitro evaluation on the effectiveness of different substrate extracts in the suppression of *Rhizoctonia solani* and *Fusarium oxysporum*

4.1 Introduction

Plant pathogenic fungi are the most important of the various groups of organisms that attack plants (Knogge, 1996). Species of *Rhizoctonia solani* and *Fusarium oxysporum* are considered as the most important soil-borne fungal pathogens that cause damping off, root rot and crown rot diseases of a wide range of vegetables (Abu-Taleb *et al.* 2011). Generally, *R. solani* and *F. oxysporum* attack their hosts, while they are still in seedling stage of growth and are commonly found in cultivated soils. The severity of infection of these pathogens varies depending on the vegetable grown, soil type and climatic condition (Ogoshi, 1996; Roberts, 1999). These pathogens have been reported to cause major yield losses ranging from 25% to 100% (Pal and Gardener, 2006).

The control of plant diseases caused by these organisms is difficult due to the various complex biological properties of these pathogens. Their wide host range and versatility has made breeding of resistant cultivars difficult and their capability to adapt to diverse environmental conditions enables the pathogens to overcome various disease control strategies (Baker, 1967; Leach and Garber, 1970). In the control of diseases caused by soil-borne pathogens, foliar applications with fungicides have been extensively used worldwide (Kataria and Gisi, 1996); however, frequent use of fungicides has often resulted in certain fungi developing resistance to the pesticide (Haggag, 2008). This has therefore, necessitated the identification and development of other ecologically friendly disease management strategies. The use of organic amendments, such as manures, composts and compost extracts have been identified as a potential control measure that can be employed in the suppression of these pathogens (Kirkegaard *et al.*, 1996; Hoitink *et al.*, 1997; Paulitz and Belanger, 2001; Ryckeboer, 2001; Bailey and Lazarovits, 2003). This study was, therefore, undertaken to investigate the effectiveness of different spent oyster mushroom substrate extracts on the control of *R. solani* and *F. oxysporum* fungal growth.

Specific Objective: To carry out *in-vitro* evaluation of the effectiveness of *Urochloa panicoides*, *Zea mays*, *Datura stramonium* and substrate mix spent mushroom substrates extracts in the suppression of *Rhizoctonia solani* and *Fusarium oxysporum.*

4.2. Materials and Methods

4.2.1. Description of experimental site

The North West University (Mafikeng) Biochemistry Lab was used to carry out analyses of polyphenols and flavonoids. Crop Science laboratory was used to prepare PDA media, culture fungal isolates, prepare aqueous extracts and autoclaving. The Microbiology Lab was used to carry out *in-vitro* studies where SMS extracts were screened for their efficacy against *R. solani* and *F. oxysporum.*

4.2.2. Description of the experiment

4.2.2.1. Culturing of fungal strains

Pure cultures of pathogenic strains of *R. solani* and *F. oxysporum* isolates from the diseased vegetables were obtained from the Agricultural Research Council (ARC)-Plant Protection Research Institute (PPRI) in Pretoria. In the preparation of a growth medium, Potato Dextrose Agar was prepared following the manufacturer's instructions (Merck-Biology Laboratory). The autoclaved PDA was poured into twenty petri dishes and allowed to cool overnight before refrigeration for later use. A sterilized dissecting pin was used to transfer the isolates of the two respective pathogens into the PDA media. Parafilm was used to seal the petri dishes, and the cultures were placed in an incubator set at 28°C for seven days.

4.2.2.2. SMS Extract preparation

The three respective spent mushroom substrates (SMS) that remained after harvesting oyster mushrooms were air-dried and milled with a blender. Ten grams (10g) of each respective milled SMS was weighed and added to 100ml distilled water. Four 100ml beakers were used to keep the prepared extracts overnight at room temperature (25-26°C). The extracts were then filtered using Whatman qualitative filter paper (No. 4) to

trap any substrate debris before pouring them into the petri dishes. A pipette was used to measure 1mL of each respective substrate extract and dispensed into the petri dishes containing the PDA medium. The petri dishes with PDA that was infused with the respective substrate extracts were kept in a lamina flow and allowed to absorb the extracts. Thereafter, these were kept in a fridge for later use.

4.2.2.3. Mycelial growth assessment preparation and Experimental design

Ten millimeter (10mm) discs from an actively growing *R.solani* culture were placed in the centre of each plate containing the three respective SMS infused PDA. A 10mm disc of *R.solani* mycelia was also similarly placed onto PDA without SMS extract which was used as a control. The four treatments were replicated four times making a total of 12 experimental units. These experimental units were sealed with a parafilm and arranged in Complete Randomized Design (CRD) and incubated in the dark for seven days at 28°C. Fungal growth was determined by measuring growth of the respective 10mm mycelial discs.

Suppressive effect of the respective SMS extracts on both the growth of *R. solani* and *F. oxysporum* was determined by calculating their inhibition ratio by using the following formula (Bekker *et al.*, 2006):

$$\text{Percentage Inhibition} = \frac{(C-T) \times 100}{C}$$

Where C = colony diameter (mm) of the control

T = colony diameter (mm) of the test plate

Percentage inhibition was calculated and analysis of variance of the effect of different treatments on the mycelial growth of the two pathogens was carried out using SAS software package, version 9.4 (SAS Institute, Inc. Cary. NC. USA, 2001-2011).

4.2.2.4. Determination of polyphenols

Total phenolic content was measured using a modified Folin-Ciocalteu colorimetric method (Singelton *et al.,* 1999). 12.5 µL of appropriately diluted sample was added to 50 µL of distilled water. Then, 12.5 µL of Folin-Ciocalteu's phenol reagent was added to the mixture. After 5 min, 125 µL of 7% $NaCO_3$ solution was added to the mixture. Prior to spectrometric analysis, the samples were incubated for 90 min at 25°C. The absorbance of the diluted solution was measured at 750 nm versus a blank consisting of all the reaction agents except the extract using a micro plate reader (Synergy HT, BioTek instruments, USA). A standard curve for total phenolics was developed using gallic acid standard solution. The results are expressed as means (mg gallic acid equivalents g) ±SEM for 3 replications.

4.2.2.5. Determination of flavonoids

A colorimetric assay (Kim *et al.,* 2003) with some modifications was used to quantify total flavonoid content. 25 µL of the diluted sample was added to 125 µL of ddH_2O. Subsequently, 7.5 µL of 5% $NaNO_2$ was added to the mixture. After the mixture was allowed to stand for 5 min, 15 µL of 10% $AlCl_3$ was added. The mixture was incubated at ambient temperature (25°C) for an additional 5 min. Following that and 50 µL of 1 M NaOH was then added to the mixture. The mixture was immediately diluted by the addition of 27.5 µL of ddH_2O and the absorbance of the mixture was measured at 510 nm against a blank prepared with ddH_2O using a microplate reader (Synergy HT, BioTek instruments, USA). (+)-Catechin was used as standard and the results are expressed as means (mg of catechin equivalents g) ±SEM for three replications.

4.2.2.6. Analysis of macro- and micronutrients

Sample of the different spent mushroom substrates *i.e., U. panicoides, Z. mays* and *D. stramonium* were analyzed for both macro- and micro-nutrients. One gram was weighed for each respective substrate, placed into crucibles and subjected to dry-ashing. This was done by use of a muffle furnace at 600°C for 8 hours. After ashing the crucibles were cooled at room temperature. The respective ash samples were dissolved in 8ml dilute nitric acid (HNO_3) and 2ml hydrochloric acid (HCl) concentration and microwaved for 45

minutes (Campbell and Whitfield, 1991; AgriLASA, 1998). Trace elements were analyzed using NexION 300Q ICP-Mass spectrometer (Williams, 1972) and organic matter was also analyzed following the guidelines from Warncke (2011).

4.2.2.7. pH determination

For all the three respective SMS and the soil, 20g samples were weighed out and transferred into four 100 mL beakers. A volume of 40 mL distilled water was added, and the respective solutions were stirred with glass rod and allow to stand for 30minutes. Thereafter, solutions were stirred again immediately before reading and while the pH probe is equilibrating in the soil suspension. The electrode was immersed into the soil suspension in the beaker and a pH value was read from pH meter (Soil Analysis Handbook of Reference Method, 1999).

4.3. Results

4.3.1. Inhibition rate of different SMS extracts on the growth of *Rhizoctonia solani* and *Fusarium oxysporum sp.*

Results in Table 7 shows that *Z. mays* SMS extract had the highest inhibition percentages on both pathogens that is, 76.78% *R. solani* and 77.11% *F. oxysporum* recorded. This was followed by *D. stramonium* and substrate mix SMS extract with zero inhibition percentage recorded on control.

Table 7: Inhibition rate of the different SMS extracts on *Rhizoctonia solani* and *Fusarium oxysporum* growth.

	Rhizoctonia Solani		***Fusarium oxysporum***	
SMS extracts	**Colony diameter (mm)**	**Inhibition %**	**Colony diameter (mm)**	**Inhibition %**
U. panicoides	37.25^{b}	48.61^{b}	40.25^{b}	43.31^{c}
Z. mays	16.72^{c}	76.78^{a}	16.25^{c}	77.11^{a}
D. stramonium	46.5^{b}	35.42^{b}	30.72^{b}	56.73^{b}
Substrate mix	38^{b}	47.22^{b}	24.5^{bc}	65.49^{b}
Control	72^{a}	0^{c}	71^{a}	0^{d}

Means with the same letter within a column, are not significant at P≤0.05

4.3.2. Effect of different spent mushroom substrate extracts on the fungal growth of *Rhizoctonia solani* and *Fusarium oxysporum*

Figure 9 and 10 show that different spent mushroom substrate extracts had significant effect on the fungal growth of *R. solani* and *F. oxysporum.* It shows that PDA infused with *Z. mays* SMS extract was more effective in suppressing the fungal growth of the two respective pathogens than other extracts; however, considering the efficacy of different extracts on the *F. oxysporum,* it shows that PDA infused with substrate mix, *U. panicoide* and *D. stramonium* SMS were not statistically significant (P≤0.05). The control has showed less fungal growth suppression of both pathogens with mean ±SE of 72mm *R. solani* and 71mm *F. oxysporum* recorded.

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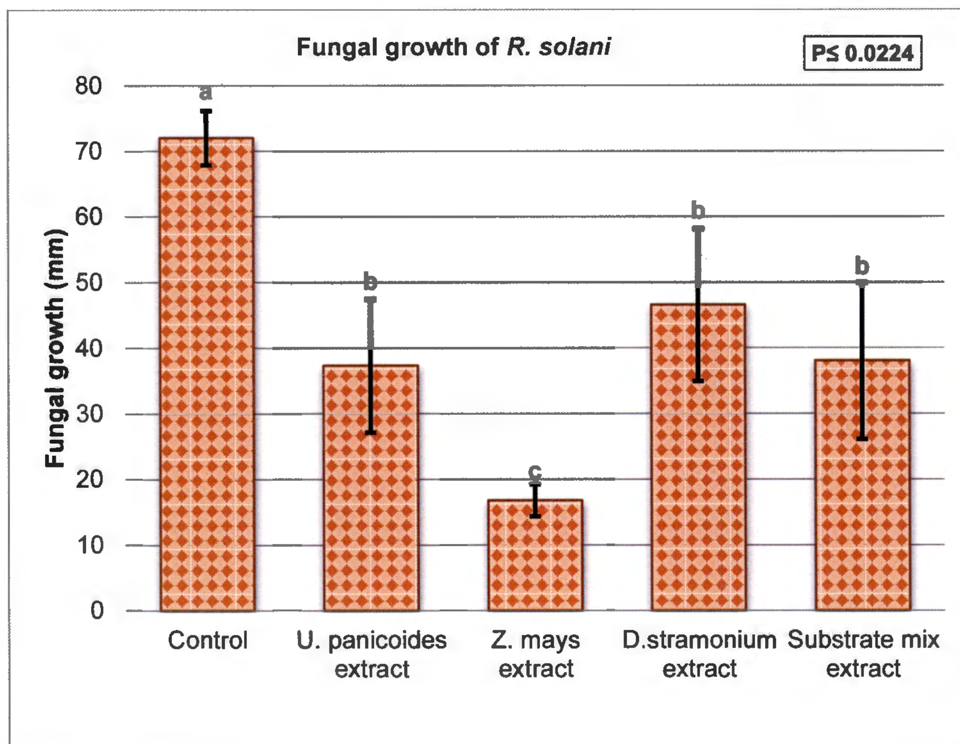

Figure 9: Fungal growth of *Rhizoctonia solani* growing in a PDA media infused with five different SMS extracts after 7 days incubation at 28^0 C. Plotted points are means of 19 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at $P \leq 0.05$.

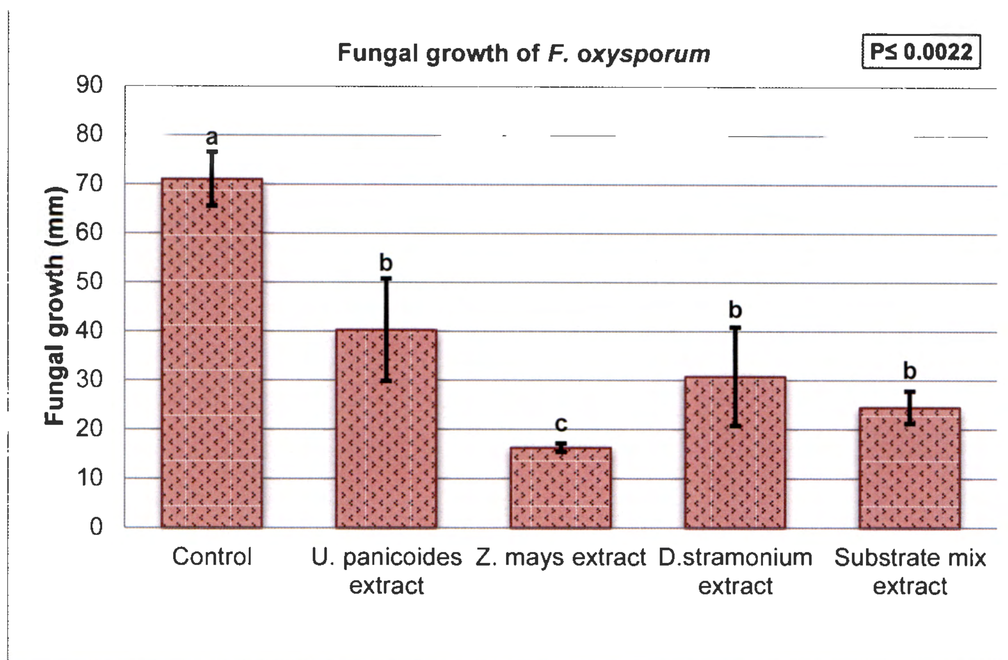

Figure 10: Fungal growth of *Fusarium oxysporum* growing in a PDA media infused with five different SMS extracts after 7 days incubation at 28^0 C. Plotted points are means of 19 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

4.3.3. Total polyphenols concentration in different SMS extracts

Laboratory analysis of the respective substrates showed that the highest polyphenol level was recorded on *Z. mays* extract (52.67mg GAE/g^{-1} sample), followed by *D. stramonium* (43.67mg GAE/g^{-1} sample), Substrate mix (33.67mg GAE/g^{-1} sample) and lowest was *U. panicoides* (29mg GAE/g^{-1} sample).

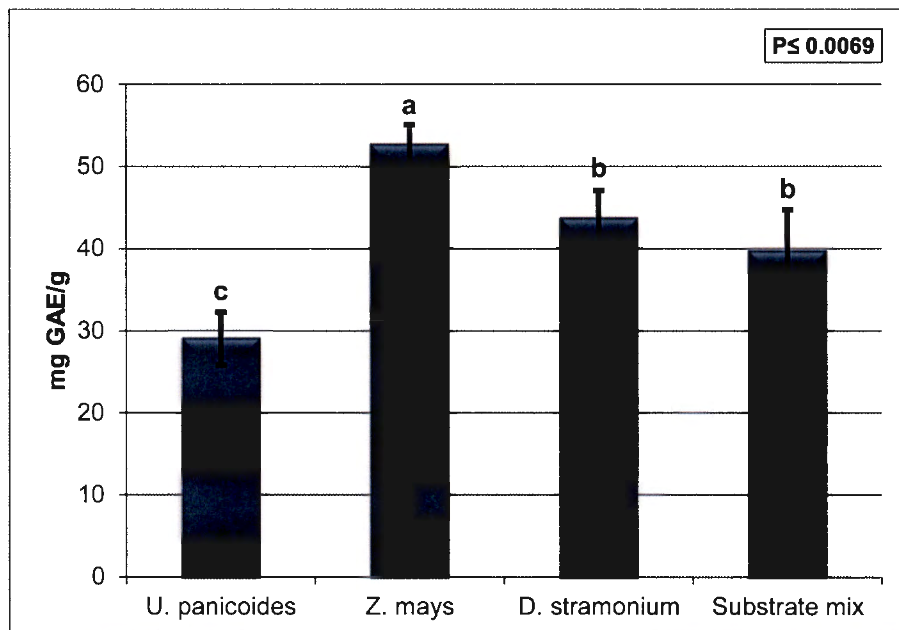

Figure 11: Total polyphenols concentration present in different spent mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix). Plotted points are means of 12 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

4.3.4. Total flavonoids concentration in different SMS extracts

Fig.12 shows analysis of flavonoids indicating that *Z. mays* extract had the highest flavonoids content of 37.33mg QE/g when compared to the other SMS extracts and the lowest content was from *U. panicoides* extract (18.33mg QE/g).

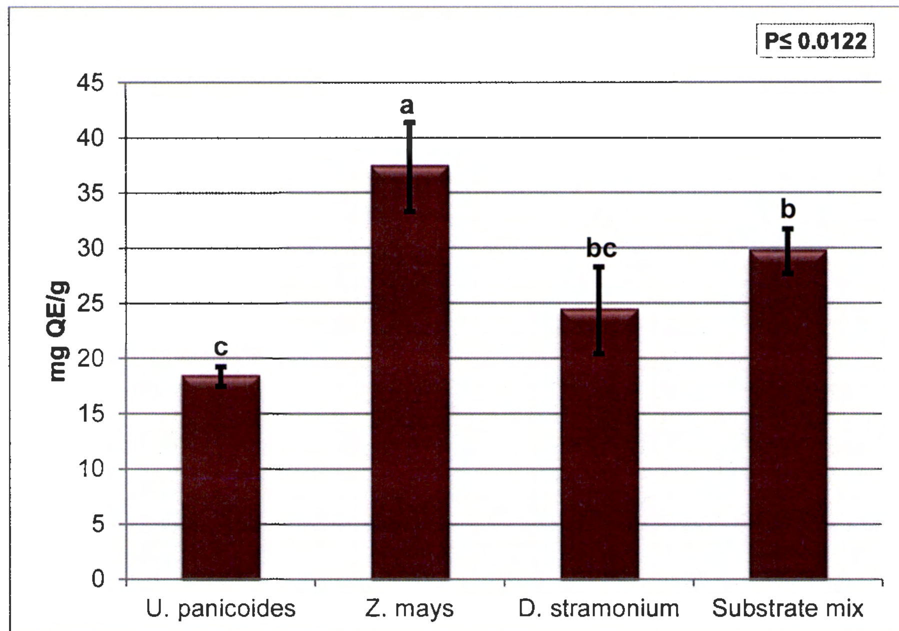

Figure 12: Total flavonoids concentration present in different spent mushroom substrates (*U. panicoides*, *Z. mays*, *D. stramonium* and substrate mix). Plotted points are means of 12 samples, and error bars represent SEM. Means (± SE) designated by the same letter(s) are not significantly different at $P \leq 0.05$

4.3.5. Macro- and micronutrients composition of different substrates.

Nutrient analysis results revealed that *Z. mays* SMS and substrate mix had adequate essential of micro-elements, Zn, Fe, Mn, B and Cl followed by *U. panicoides* and *D. stramonium* the lowest was control (Table 8).

Table 8: Macro- and micronutrients composition of different SMS in mg/100g

Various SMS	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	Mo mg/100g Mean ± SD	Mn mg/100kg Mean ± SD	Cl mg/100g Mean ± SD	Co mg/100g Mean ± SD	Ca mg/100g Mean ± SD	Mg mg/100g Mean ± SD	Cu mg/100g Mean ± SD	B mg/100g Mean ± SD
Urochloa panicoides	2.29 ± 0.72a	0.35 ± 0.052a	2.46 ± 0.19b	0.16 ± 0.05a	0.13 ± 7.34a	0.0026 ± 0.0018b	0.51 ± 0.34b	27.37 ± 5.48a	0.13 ± 0.037b	0.064 ± 0.017b
Zea mays	2.52 ± 0.53a	0.39 ± 0.111a	3.05 ± 1.03b	0.19 ± 0.13a	0.18 ± 4.47a	0.0023 ± 0.0020b	0.69 ± 0.56b	27.89 ± 8.81a	0.17 ± 0.070b	0.071 ± 0.016a
Datura stramonium	2.06 ± 0.55b	0.25 ± 0.061b	4.12 ± 1.28b	0.13 ± 0.04b	0.12 ± 2.24a	0.0046 ± 0.0009b	0.57 ± 1.25b	18.69 ± 13.78b	0.14 ± 0.039b	0.070 ± 0.018a
Substrate Mix	2.42 ± 0.51a	0.27 ± 0.13b	5.01 ± 1.71a	0.16 ± 0.034a	0.15 ± 3.52a	0.0084 ± 0.0032a	0.88 ± 0.37a	24.65 ± 7.85a	0.35 ± 0.012a	0.074 ± 0.011a
Soil	1.76 ± 0.48b	0.18 ± 0.003c	0.02 ± 0.001c	0.00 ± 0.00c	0.09 ± 0.16b	0.0037 ± 0.004b	0.39c ± 3.54a	0.98 ± 16.58c	0.11 ± 0.018b	0.02 ± 0.011c

Means (± SE) designated by the same letter(s) are not significantly different at P≤0.05.

4.3.6. pH levels of different SMS

Results in Table 9 shows that *D. stramonium* was too alkaline (8.69), followed by substrate mix and *Z. mays*, however, *U. panicoides* was found be neutral with pH of 7.2 and soil was found acidic with pH of 4.07.

Table 9: pH levels of different spent mushroom substrates and control

SMS	pH levels after harvest
U. panicoides	7.20
Z. mays	7.93
D. stramonium	8.69
Substrate mix	7.94
Control	4.07

4.4. Discussion

The high inhibitory effect in *Z. mays* extract infused PDA shown in Table 7 was possibly because the substrate had high levels of trace elements such as Zn, Mn, B, Fe and Cu (Table 8). These trace elements are known to have antifungal properties (Huber, 1980). High concentrations of Zn and Cu inhibit fungal growth by enhancing the production of antifungal compounds, such as 2, 4 diacetylphloroglucinol (DAPG) that controls fungal species such as *Fusarium.* It is reported that these compounds inhibit biosynthesis of fusaric acid by *Fusarium sp*. in the plant cell infection process (Thind and Madan, 1977; Blevins and Lukazewski, 1998; Brown *et al*., 2002). Fusaric acid is a biochemical compound that causes the necrosis of the plant cell resulting in the development of a diseases condition. Furthermore, Yuyong *et al*. (2014) working with various other trace elements found that Zn and Cu have better suppressive effect on *F. solani* than Fe. The study highlighted that increasing concentration of Zn and Cu had corresponding suppressive effect on fungal growth. Thind and Madan (1977), working on *F. monilifome* found that at low Zn concentration levels of 0.0001 to 10mg/kg fungal growth was promoted, but when Zn levels were increased to higher levels of 10 to 400mg/kg, this had a suppressive effect on the fungal growth. Another element which may have played a vital role in pathogen suppression is Mn. Manganese is known to suppress fungal growth by inhibiting aminopeptidase induction which is an enzyme that supplies essential amino acids for fungal growth (Dordas, 2008).

Other than trace elements, there are other factors, such as phytochemicals which may have resulted in better inhibitory effect on the mycelial growth of the pathogens grown on PDA infused with *Z. mays* substrate extract. Laboratory analyses of phytochemicals showed that *Z. mays* SMS extract had the highest polyphenol and flavonoids concentration (37.23mgGAE/g and 2.63mgQE/g, respectively) when compared with other SMS extracts (Fig. 11 and 12). Polyphenols and flavonoids are phytochemical compounds that have antifungal properties and thus suppress fungal growth (Okwu, 2004). Polyphenols are reported to be secondary metabolites that are involved in the defense mechanism of a plant against fungal pathogens (Harborne, 1973; Okwu, 2004). The metabolites inhibit spore germination and hyphal growth of fungi by secreting antifungal compounds, such as phytoalexins which produce a broad range of defense metabolites that are toxic to the pathogens (Okwu, 2005). Flavonoids are said to be composed of 15 carbon compounds which are generally distributed throughout the plant kingdom. They are known to be synthesized by plants in response to microbial infection and have also been found to be effective against a wide range of pathogenic microorganisms (Harborne, 1973). Even though statistical analysis indicated that there was no significant difference between effect of PDA infused with SMS mix, *D. stramonium* and *U. panicoides* SMS extracts on the growth of the two fungal pathogens, there was, however, a variation in the inhibition percentages on the two fungal growth of *R. solani* and *F. oxysporum* (Table 7). The variation could have been the result of low to moderate availability of macro- and microelements and phytochemicals in the SMS mix, *D. stramonium* and *U. panicoides*. It has been reported that at certain levels of trace element availability, some cells may be able to reproduce spores which therefore could possibly explain the slight increase in fungal growth in the PDA infused with these extracts thereby exhibiting some degree of tolerance (Lilly and Barnett, 1951). Foster (1939) also highlighted that for pathogenic fungi to be able to reproduce they need to ensure availability of trace elements but at low concentrations. Thind and Madan (1977) further accentuated that some pathogenic fungi can still reproduce spores, but at a low rate, when growing in media with low to moderate levels of trace elements. The slight increase in mycelial disc growth in these three respective SMS extracts could also be explained by the low to moderate levels of Fe, Zn, Cu, Mn and B elements (Table 8).

Furthermore, statistical results also indicated that fungal disc growth on PDA infused with soil extract had a low inhibitory effect on both pathogens with *R. solani* recording 72mm and *F. oxysporum* 71mm in diameter (Fig. 9 and 10). This was probably because the media had high levels of Mg which may have had a stimulatory effect on *F. oxysporum* growth. Najwa (2013) also presented similar results, reporting that there was an increased in colony growth and fungal spores production under high levels of Mg. Another factor which may have contributed to stimulated mycelial growth of the two respective pathogens in PDA infused with soil extract was possibly because of low antifungal trace elements availability (Table 8). Absence of phytochemicals in the soil could also have contributed to the poor fungal growth inhibitory effect. In addition, soil pH range between 4 and 7 is favourable to growth of most fungal species. This could also have contributed to poor suppressive effect of the soil extract which had a pH of 4.07. Rousk *et al.* (2009) reported that at pH levels of 4.06 to 6.5 fungal growth is more prolific. It was observed that conditions which favoured mycelial growth in *in-vitro* included relatively low pH *i.e.,* pH range of 4 to 5.5. Pennanen *et al.* (1998) also had similar observations and highlighted that fungi generally grow well in pH levels ranging from 4.5 to 7 but the optimum is 6.5. On the other hand, a pH of 4.07 (Table 9) was observed in soil extracts which may have promoted spore germination, thus mycelial disc growth of 72mm and 71mm in diameter that was recorded in PDA infused with soil extract (Fig. 9 and 10).

In conclusion, *in-vitro* results have shown that SMS extracts possess antifungal properties that play an important role in the suppression of soil-borne pathogenic fungi. Although, the different substrates showed inhibitory effect on the two pathogens, *Zea mays* SMS extract had, however, the highest suppressive effect than other SMS extracts. *Zea mays* SMS extract has therefore exhibited potential for use in the management of the soil-borne pathogens. There is however need for *in-vivo* studies that would enable to assess not only the effect of SMS on the disease development but their synergistic effect on the seedlings and growth. In addition, the use of organic manure and composts, such as SMS, as soil amendments also has other beneficial effects such as promotion of fungal antagonists that hinder multiplication of pathogenic fungal spores. The effect of antagonists was however, not investigated in this study. There is, therefore, a need for further work to determine antagonistic fungi and bacteria species that can possibly be

found in various SMS. There is therefore potential to isolate and produce these antagonists for use as commercial bio-control agents for the problematic soil-borne pathogens.

5.1. General discussion and conclusion

The study sought to evaluate the influence of three different spent oyster mushroom substrates on the establishment and growth of *Spinacia oleracea* and *Beta vulgaris* seedlings. The study revealed that the use of *Zea mays, Datura stramonium* and substrate mix SMS significantly influenced the morphological and physiological characteristics of seedlings of both *S. oleracea* and *B. vulgaris*. The seedling emergence rate on *S. oleracea* was highest on both *Z. mays* and *D. stramonium* SMS recording 90-100% of seedling emergence at thirteen days after planting (DAP). A similar trend in *B. vulgaris* seedling emergence was observed (Chapter 3). Growing both vegetables on SMS *Z. mays, D. stramonium* and substrate mix significantly increased seedling height and number of leaves. These substrates performed better than the *U. panicoides* and un-amended soil (Table 3, 4, 5 and 6). Results further indicated that seedlings of *S. oleracea* harvested from the *Z. mays* substrate had the highest dry mass of shoots and roots when compared with the other substrates (Fig. 4a and b). On *B. vulgaris*, the highest dry root mass recorded was $12.91g^{-1}$ on seedlings raised on *D. stramonium* SMS (Fig. 8b). The significant increase on seedling growth of *S. oleracea* and *B. vulgaris* could have been the result of variation in water and nutrient retention in the different substrates. There are, however, several other factors such as, temperature and cation exchange capacity which could have had an impact on the growth of the two vegetable crops which were not investigated in the present study. Nutrient analysis results (Table 1) show that *D. stramonium* and *Z. mays* SMS had high nutrient content of N, P, K, Mg, S, Ca, Fe and Zn which are required for optimum plant growth. Several studies have indicated that nutrient-deficient soils do lead to reduced yield and plant quality (Chida, 2000; Jones, 2003 and Zikalala, 2014).

Over and above the growth stimulatory effect of the different SMS, it has been established that these SMS also suppress fungal growth of *Fusarium oxysporum* and *Rhizoctonia solani*. *In-vitro* studies showed that PDA infused with *Z. mays* extracts was more effective than *D. stramonium, U. panicoides* and SMS mix in suppressing mycelial growth of *R. solani* and *F. oxysporum* (Fig. 9 and 10). The suppressiveness effect was possibly because of high levels of phytochemicals (Fig. 11 and 12) and micro-elements (Table 8).

These properties are reported to possess antifungal properties. Even though *in vivo* studies were not carried out in this study, *Z. mays* substrate however, showed great potential in the suppression of soil-borne pathogens and can therefore, be applied as a soil drench in management of soil-borne pathogens. Additionally, *Z. mays* SMS have the potential to improve soil structure by increasing drainage, aeration and nutrient availability which will favour plant growth.

It can therefore be concluded that amending soil with SMS at proportions of 60% soil: 40% substrate had significantly improved the seedling growth and development of *Spinacia oleracea* and *Beta vulgaris*. However, the possibilities of salinity problems as a result of using fresh SMS needs to be taken into consideration. Results of this study indicated that *D. stramonium* SMS was more alkalinity with pH level of 8.69. This is way above the normal suitable pH range for vegetable growth which is between 5.5 and 6.5. Further investigation needs to be undertaken to assess the effect of SMS when applied at different rates and for various soil types on the plant growth response. Additionally, there is a need to carry out *in vivo* studies to evaluate the effectiveness of *Z. mays* SMS as a suppressant for *Rhizoctonia solani*, *Fusarium oxysporum* and various other soil-borne pathogens.

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APPENDICES

Appendix A

PICTURES

Plate 1: *Urochloa panicoide* SMS

Plate 2: *Zea mays* SMS

Plate 3: *Datura stramonium* SMS

Plate 4: *Spinacia oleracea* seedlings

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Plate 5: *Beta vulgaris* seedlings

Plate 6: Chlorophyll content index analysis

Plate 7: Autoclaved PDA media

Plate 8: SMS extracts preparation for PDA infusion

Plate 9: PDA infused with SMS extracts

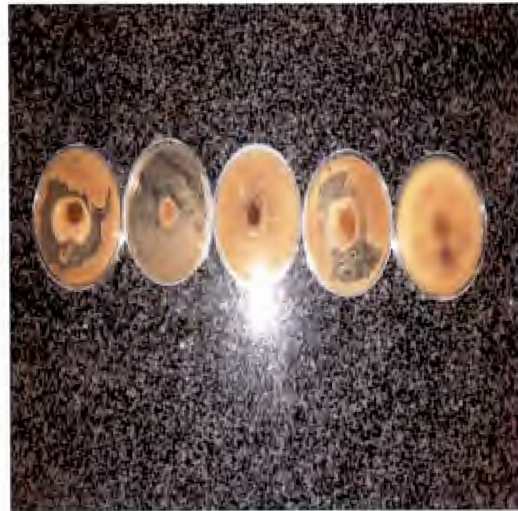

Plate 10: mycelial growth of *F.oxysporum* in SMS PDA infused with different SMS extracts.

Plate 11: mycelial growth of *R. solani* in SMS PDA infused with different SMS extracts.

ANOVA Tables for Chapter 2 (spinach)

Analysis of variance for fresh shoots mass of *Spinacia oleracea* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	14913.68128	3728.42032	15.74	<.0001
Error	35	8289.04907	236.82997		
Corrected Total	39	23202.73035			

Analysis of variance for fresh root mass *Spinacia oleracea* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	3745.225935	936.306484	8.78	<.0001
Error	35	3731.428363	106.612239		
Corrected Total	39	7476.654298			

Analysis of variance for dry shoots mass *Spinacia oleracea* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	796.735750	199.183938	16.60	<.0001
Error	35	419.884450	11.996699		
Corrected Total	39	1216.620200			

Analysis of variance for dry root mass *Spinacia oleracea* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	356.8420900	89.2105225	9.50	<.0001
Error	35	328.7614875	9.3931854		
Corrected Total	39	685.6035775			

ANOVA Tables for Chapter 3 (beetroot)

Analysis of variance for fresh shoots mass *Beta vulgaris* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	2720.660650	680.165162	4.81	0.0034
Error	35	4953.163500	141.518957		
Corrected Total	39	7673.824150			

Analysis of variance for fresh root mass *Beta vulgaris* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	7441.44291	1860.36073	3.44	0.0180
Error	35	18935.50000	541.01429		
Corrected Total	39	26376.94291			

Analysis of variance for dry shoot mass *Beta vulgaris* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	387.220565	96.805141	4.39	0.0056
Error	35	772.171875	22.062054		
Corrected Total	39	1159.392440			

Analysis of variance for dry root mass *Beta vulgaris* seedlings after 12 weeks of growing.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	4	882.194540	220.548635	3.77	0.0119
Error	35	2049.867500	58.567643		
Corrected Total	39	2932.062040			